Analysis of Dioxin and Dioxin-Like Compounds

Eric J. Reiner

This chapter is dedicated to Otto Hutzinger a pioneer in the analysis of dioxin and an inspiration to environmental analytical chemists.

Abstract The analysis of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, and other related dioxin-like compounds requires complex sample preparation and analytical procedures using highly sensitive and selective state-of-the-art instrumentation to meet very stringent data quality objectives. The analytical procedures (extraction, sample preparation), instrumentation (chromatographic separation and detection by mass spectrometry), and screening techniques for the determination of dioxins, furans, dioxin-like polychlorinated biphenyls, and related compounds with a focus on new approaches and alternate techniques to standard regulatory methods are reviewed.

Keywords Analysis, Dioxin-like polychlorinated biphenyls, dlPCBs, Extraction, Fast gas chromatography, Fast GC, Isotope dilution, Mass spectrometry, PCDD, PCDF, Polychlorinated dibenzofurans, Polychlorinated dibenzo-*p*-dioxins, QA/QC, Quality assurance/quality control, Review, Sample preparation, Screening techniques

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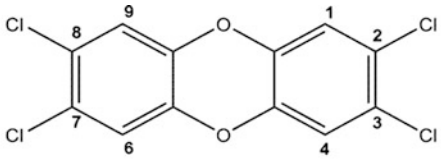
Contents

1 Introduction

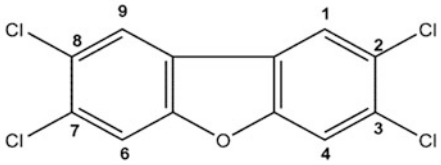
Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are considered among the most toxic groups of chemicals known to man [1, 2]. They are listed in the Stockholm Convention on Persistent Organic Pollutants (POPs) [3] for reduction or elimination. PCDD/PCDFs, as shown in Fig. 1, are planar molecules with two benzene rings connected by either one (furan) or two oxygens (dioxin). They can contain from one to eight chlorines forming a possible 75 different dioxins and 135 different furan congeners. This group of 210 chlorinated compounds is typically called "dioxin" and will be referred to as such in this paper. Congeners containing chlorines in the 2,3,7,8 positions (17 of 210) exhibit dioxin-like toxicity as a result of their preferential binding with the aryl hydrocarbon receptor (AhR) [4]. Toxic effects of dioxins include weight loss, reproductive disorders [5], immune impairment [5], and cancer [6, 7]. A number of other compounds including polychlorinated biphenyls (PCBs) with one or no chlorines in the ortho positions (2 or 2′, 6 or 6′) [8] and some polychlorinated naphthalenes (PCNs) [9, 10] also exhibit dioxin-like character and are classified as dioxin-like compounds (DLCs). DLCs work through a common mechanism, by deregulating the expression of key genes resulting from their interaction with the AhR. As a result, their dioxin relative potency can be compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-T_4CDD), the most toxic dioxin congener [11–13]. The toxic equivalent factor (TEF) is a value assigned to a specific DLC comparing its potency to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [14, 15]. The sum of the concentration of each dioxin-like compound multiplied by its TEF is the toxic equivalent quantity (TEQ) and is the relative amount of DLCs converted 2,3,7,8-T_4CDD equivalents.

$$\mathrm{TEQ} = \Sigma[\mathrm{PCDD}_i \times \mathrm{TEF}_i] + \Sigma\left[\mathrm{PCDF}_j \times \mathrm{TEF}_j\right] + \Sigma[\mathrm{PCB}_k \times \mathrm{TEF}_k] + \Sigma[\mathrm{DLC}_l \times \mathrm{TEF}_l]$$

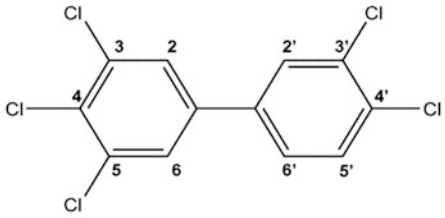
A number of TEF schemes have been reported in the past 30 years. Internationally, accepted TEF schemes have been developed and since 1994 are reevaluated every



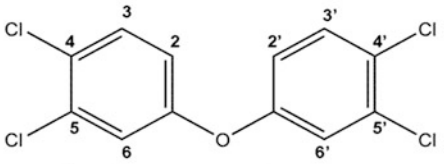
2,3,7,8-tetrachlorodibenzo-p-dioxin



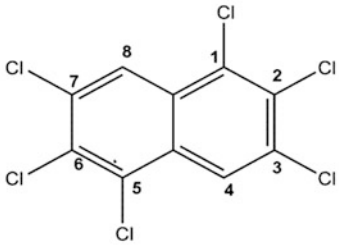
2,3,7,8-tetrachlorodibenzofuran



3,3',4,4',5-pentachlorobiphenyl (PCB-126)



3,3',4,4'-tetrachlorodiphenlyether



1,2,3,5,6,7-tetrachloronaphthalene (PCN-67)

Fig. 1 Structures of dioxin-like compounds: polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, polychlorinated diphenylethers, and polychlorinated naphthalenes

5–10 years by an expert committee designated by the World Health Organization (WHO) [14, 15]. TEF values have also been developed for PCBs and brominated dioxins [14–16] (see Table 1). For other DLC compounds like PCNs, relative

Table 1 Comparison of various toxic equivalent factors (TEF) schemes

	Eadon 1982	Ontario 1984	Germany 1985	California 1986	USEPA 1987	Nordic 1988	NATO-I 1989	WHO 1994	WHO 1998	WHO 2005
PCDDs										
2,3,7,8-TCDD	1	1	1	1	1	1	1		1	1
1,2,3,7,8-PeCDD	1	0.1	0.1	1	0.5	0.5	0.5		1	1
1,2,3,4,7,8-HxCDD	0.03	0.1	0.1	0.03	0.04	0.1	0.1		0.1	0.1
1,2,3,6,7,8-HxCDD	0.03	0.1	0.1	0.03	0.04	0.1	0.1		0.1	0.1
1,2,3,7,8,9-HxCDD	0.03	0.1	0.1	0.03	0.04	0.1	0.1		0.1	0.1
1,2,3,4,6,7,8-HpCDD	0	0.01	0.01	0.03	0.001	0.01	0.01		0.01	0.01
1,2,3,4,6,7,8,9-OCDD	0	0.0001	0.001	0	0	0.001	0.001		0.0001	0.0003
PCDFs										
2,3,7,8-TCDF	0.33	0.5	0.1	1	0.1	0.1	0.1		0.1	0.1
1,2,3,7,8-PeCDF	0.33	0.5	0.1	1	0.1	0.01	0.05		0.05	0.03
2,3,4,7,8-PeCDF	0.33	0.5	0.1	1	0.1	0.5	0.5		0.5	0.3
1,2,3,4,7,8-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
1,2,3,6,7,8-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
1,2,3,7,8,9-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
2,3,4,6,7,8-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
1,2,3,4,6,7,8-HpCDF	0	0.01	0.01	0.03	0.001	0.01	0.01		0.01	0.01
1,2,3,4,7,8,9-HpCDF	0	0.01	0.01	0.03	0.001	0.01	0.01		0.01	0.01
1,2,3,4,6,7,8,9-OCDF	0	0.0001	0	0	0	0.001	0.001		0.0001	0.0003
dl-PCBs										
PCB-077 (3,3′,4,4′-TCB)								0.0005	0.0001	0.0001
PCB-081 (3,4,4′,5-TCB)								0	0.0001	0.0003
PCB-105 (2,3,3′,4,4′-PeCB)								0.0001	0.0001	0.00003
PCB-114 (2,3,4,4′,5-PeCB)								0.0005	0.0005	0.00003
PCB-118 (2,3′,4,4′,5-PeCB)								0.0001	0.0001	0.00003
PCB-123 (2′,3,4,4′,5-PeCB)								0.0001	0.0001	0.00003
PCB-126 (3,3′,4,4′,5-PeCB)								0.1	0.1	0.1
PCB-156 (2,3,3′,4,4′,5-HxCB)								0.0005	0.0005	0.00003
PCB-157 (2,3,3′,4,4′,5′-HxCB)								0.0005	0.0005	0.00003
PCB-167 (2,3′,4,4′,5,5′-HxCB)								0.00001	0.00001	0.00003
PCB-169 (3,3′,4,4′,5,5′-HxCB)								0.01	0.01	0.03
PCB-189 (2,3,3′,4,4′,5,5′-HpCB)								0.0001	0.0001	0.00003

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potenticies [10] can be used for TEQ determination. Most laboratories now only report the seventeen 2,3,7,8-substituted congeners and TEQ values. TEQ values make it easier to compare results between samples, but congener-specific pattern information is lost making it more difficult to identify or indicate sources of contamination. For source tracking, full congener analysis can help identify sources of contamination. This procedure can be time-consuming as the remaining 193 non-2,3,7,8-substituted congeners must be quantified and their relative concentrations recorded [18–21]. Monitoring the impurities formed along with dioxin in samples can be used to track and apportion sources. Parette et al. [22] used tetrachlorodibenzothiopehene formed from the thiophenol impurity in Agent Orange manufacturing to track specific sources of dioxin contamination. The focus of this chapter is on PCDD/PCDFs and dlPCBs. A brief summary of the analytical chemistry of associated DLCs is provided at the end of this chapter.

The goal of any analytical method is to quantitatively and selectively as possible extract target analytes from the sample matrix while removing the desired analytes from the bulk matrix and potential interferences and to detect the analytes of interest using an appropriate selective and sensitive detector. The analysis of dioxin is one of the most challenging procedures in analytical chemistry. Due to the highly toxic nature of dioxin, method detection limits are up to three orders of magnitude lower than for most other organic contaminant Stockholm POPs in order to meet the strict regulatory limits and guidelines ($<$1 ppt (picogram per gram for food based on fat or tissue) per congener [23, 24], $\leq$1 ppt for soils/sediments, $\leq$10 ppq (picograms per liter) for aqueous samples, and less than 1 pg/m^3 for air samples); concentration factors of 10^6–10^9 are needed to meet these detection limits. Levels of many interfering compounds like PCBs and polychlorinated diphenyl ethers (PCDEs) must be removed so that extracts can be concentrated to the very small volumes needed to meet the very low detection limits required to meet regulatory limits or guidelines for the protection of wildlife and human health. For aqueous samples, the dioxin in 1 L of sample is extracted, cleaned, and concentrated to an extract of about 20 μL. The classical method uses a three-stage silica, alumina, and carbon cleanup, and in order to achieve accurate quantification, isotope dilution mass spectrometry (IDMS) with ^{13}C-labeled surrogate standards is necessary. Carbon-13 labeled analogs of dioxins, furans, and dlPCBs are added to the sample prior to extraction to account for analyte loss and instrument variability. Gas chromatography (magnetic sector)-high-resolution mass spectrometry (GC-HRMS) is typically used for detection and quantification because of its superior sensitivity and selectivity.

There are a number of regulatory methods available for the analysis of dioxin and dioxin-like compounds. A number of these methods are summarized in Table 2. Specific details regarding analytical standards, labware, instrumentation, procedures, calibration, and quality control are outlined within these methods.

Table 2 Various analytical methods for dioxin-like compounds

Method	Method description/analytes	Source/year of publication
US EPA 23	Determination of 2,3,7,8-substituted dioxins and furans with congener group totals in incinerator stack gasses by isotope dilution (ID) – GC-HRMS	US EPA 1995
US EPA 1613b	Determination of 2,3,7,8-substituted dioxins and furans with congener group totals in water and wastewater by GC-ID HRMS	US EPA 1994
US EPA 8290 (SW-846)	Seventeen 2,3,7,8-substituted dioxins and furans with congener group totals in materials and waste. Uses isotope dilution – GC-HRMS	US EPA 1994a
US EPA TO-9A	Compendium of methods for the determination of toxic organic compounds in ambient air	US EPA 1999
	Second edition compendium method TO-9A determination of polychlorinated, polybrominated, and brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans in ambient air	
US EPA 1668a	Determination of all 209 PCB congeners. 12 WHO dioxin-like PCBs by GC-HRMS, the remaining 197 by GC-MS	US EPA 1999
ISO 17025	General requirements for the competence of testing and calibration laboratories	ISO 2005
ISO 17858	Determination of 12 WHO dioxin-like PCBs in environmental matrices by GC-ID HRMS	ISO 2006
ISO 18073	Determination of seventeen 2,3,7,8-substituted dioxins and furans with congener group totals in water and wastewater by GC-ID HRMS	ISO 2004
ISO 16780	Determination of polychlorinated naphthalenes (PCN) – method using gas chromatography (GC) and mass spectrometry (MS)	ISO 2015
MOE E3418	Determination of 2,3,7,8-substituted dioxins and furans including congener group totals and 12 WHO dioxin-like PCBs by GC-ID HRMS	MOE 2012
MOE E3431	Determination of polychlorinated naphthalenes (PCNs) in environmental matrices by GC-HRMS	MOE 2012
EU EN 1948	Seventeen 2,3,7,8-substituted dioxins and furans and congener group totals in stationary sources by isotope dilution – GC-HRMS	European Standard 1997
ENV CAN 1/RM/19	Seventeen 2,3,7,8-substituted dioxins and furans and congener group totals in pulp and paper effluents by isotope dilution – GC-HRMS	Environment Canada 1992
JIS K0311	Seventeen 2,3,7,8-substituted dioxins and furans including congener group totals in incinerator stack gasses by isotope dilution – GC-HRMS	JIS 1999a
JIS K0312	Seventeen 2,3,7,8-substituted dioxins and furans including congener group totals in wastewater by isotope dilution – GC-HRMS	JIS 1999b

Note: US EPA methods available from www.usepa.org, ISO methods from www.ISO.org, and MOECC methods from LaboratoryServicesBranch@Ontario.ca

2 Extraction

Quantitative extraction of the analytes from the bulk matrix is critical to obtain quality data. A wide variety of extraction techniques and systems have been used for the extraction of DLCs. Sonication; Soxhlet extraction; pressurized fluid extraction (PLE – also known as accelerated solvent extraction (ASE)); microwave-assisted extraction (MAE), also called microwave-assisted solvent extraction (MASE); solid-phase extraction (SPE); liquid/liquid extraction; and supercritical fluid extraction (SFE) have all been used to extract dioxin and DLCs from bulk matrix. Passive sampling devices such as semipermeable membrane devices, polymer (e.g., polyethylene and polydimethylsiloxane) passive samplers, sorptive stir bars, window films, and tree bark have also been used to extract dioxin-like compounds from the bulk sample matrix. The hydrophobic nature of DLCs makes them very soluable in nonpolar organic solvents. Aromatic solvents like benzene or toluene are preferred, but require extensive safety precautions due to their toxicity and carcinogenic nature. Benzene is a controlled substance in many countries and is now rarely used except for analytical standard preparation. Other solvents like hexane and/or dichloromethane are also used, but are not as effective as toluene which is the solvent of choice for most applications. These extraction methods are detailed in a number of publications [25–28].

The extraction of solid matrices including soil, sediment, sludge, biological tissue, and vegetation has classically been done by Soxhlet extraction. Soxhlet extraction is simpler and less expensive, but more labor intensive when compared to other newer procedures, and is typically the recommended or required procedures in most regulatory methods. Wet samples (sediments, biota, and vegetation) can be Soxhlet extracted with a Dean-Stark device, which separates the water from less dense nonpolar solvent. The method allows the drying step to be skipped. The water is collected in an alternate receiving vessel. The amount of water can be determined volumetrically or gravimetrically enabling moisture content of the pre-extracted sample to be determined [29, 30]. Sonication and agitation – shaking in a paint shaker – have also been used. They can be less efficient than Soxhlet but are much faster.

Automated extraction methods like PLE and MAE can run unattended. PLE is a pressurized filtration method that subjects the sample to heat and elevated pressure [31–33] which can enhance extraction efficiency over room temperature procedures. MAE [34–36] uses microwave energy to produce heat resulting increased pressure for enhancing extraction efficiency. Microwave-assisted extraction requires the use of a polar solvent or a solvent mixture containing a polar solvent like acetone or methanol to convert microwave energy to heat. Because a polar solvent is used, samples do not have to be dried prior to extraction. Both PLE and MAE can be used to reduce extraction times over the classical Soxhlet and sonification procedures.

A number of PLE methods have been developed where column packing materials are inserted in a modified PLE cell so that extraction and sample cleanup can

be completed in the same step [37–39]. This can result in very quick extraction and preparation of relatively clean samples. Highly concentrated and complex samples can severely contaminate or overload the system, and therefore prior knowledge of levels of dioxin and other coextractables in the samples is important when using this method. Biological samples and wet sediment/soil samples can be extracted using matrix solid-phase dispersion (MSPD). Wet samples are ground and mixed with a solid dispersant (e.g., silica, diatomaceous earth, sodium sulfate) and placed in a Soxhlet or PLE cell where the analytes are extracted, while the water remains on the dispersant. Extreme care must be taken in determining moisture content as this can result in significant variability or bias if the sample being analyzed is not homogeneous and the resulting relative dry weights are not calculated correctly. This is also the case for biological samples. Dioxin and other POPs partition into the lipid tissue. Care must be taken to ensure that the sample is homogenized properly to minimize variability and bias. Results for biological samples can be reported on wet-weight or lipid-weight basis. If lipid levels vary greatly between organisms, lipid corrected data can provide better results for comparison [40].

Supercritical fluid extraction (SFE) using pressurized CO_2 [41] has been used to extract dioxins from soils and sediments [42] and marine biota [43]. SFE is a solventless extraction procedure that may need only very small amounts of solvent to elute the analytes from the trapping or packing material (e.g., carbon or C_{18}).

The extraction of liquid samples can be challenging, especially if they contain significant amounts of solid particulate material (SPM). Dioxins and other DLCs have log Kow values that range from 4 to 8; therefore, they tend to adsorb to SPM, and extraction schemes must be able to quantitatively remove analytes from the SPM. The classical dioxin extraction method is liquid/liquid extraction (LLE). The LLE method is very labor intensive; therefore, aqueous and other liquid samples including biological fluids are now often extracted using solid-phase extraction (SPE), a technique that uses a solid stationary phase like C_{18} or Amberlite XAD-2 resin in an extraction cartridge or disk to extract nonpolar dioxin-like compounds and pass through polar or slightly compounds. The classical dioxin procedure for aqueous samples is to filter the sample to collect the particles. The filtered portion is then extracted using liquid/liquid or solid-phase extraction, and the filter is extracted by Soxhlet extraction or PLE. If the amount of SPM is relatively low, e.g., below 2 g/L, direct extraction of samples by SPE is possible [44–46]. The particles are trapped on top of the extraction disk or column bed. Quantitative elution of the particles and disk can be done in a single step, significantly reducing solvent usage and sample preparation time. Erger et al. [47] have reviewed the various solid phases and uses of SPE disks for samples that contain SPM. The SPE disk extraction procedure can be automated if SPM levels are low [48, 49]. Aqueous samples that contain very small amounts or no visible levels of SPM can also be extracted using stir-bar sorptive extraction followed by thermal desorption [50].

Passive samplers have been used for a number years as integrative samplers to assess long-term time-weighted average levels of contaminants in water and air. Semipermeable membrane devices (SPMDs) were some of the first used [51]. Passive samplers for aqueous media include SPMDs (triolein in a semipermeable

membrane housing), silicone rubber (SR), low-density polyethylene (LDPE) strips, and polydimethylsiloxane (PDMS) [52–58]. If performance reference compounds (PRCs are labeled or other internal standards) are spiked into the samplers, their rate of elimination from the samplers can be used to determine relative sample volumes to estimate contaminant concentrations [55]. Sample volumes with passive samplers are not measured directly but are estimated using lab-based constants and PRC behavior which varies from compound to compound. Passive samplers collect only the dissolved fraction of dioxin, and therefore results do not compare directly to those using the whole-water extraction methods described above. Due to their K_{OW}s, the majority of dioxins are present on the particulate phase. The dissolved phase, however, is the bioavailable fraction, and as a result, passive samplers can be used as surrogates for exposure to dioxin and other compounds with low K_{OW}s. Passive samplers are extracted using the same methods that are used for solid samples: Soxhlet, sonication, and PLE.

Passive samplers used for air sampling are typically polyurethane foam (PUF) plugs housed in a metal canister and can include a type of XAD resin. Other sampler designs have included just the XAD resin in a metal housing [59–61]. Passive air sampling has also been carried out using window films, building wipes [62, 63], and tree bark [64, 65]. Passive samplers do not require electrical power and therefore can be deployed for extended periods of time enabling good detection limits to be achieved.

Ambient air samples are typically sampled using high-volume samplers. Air is passed through an 8½ by 11 in. filter paper to trap the particulate fraction containing penta to octa dioxin/furan congeners followed by a PUF to trap the volative (mono to penta) congeners. Some configurations use XAD sandwiched between 2 PUFs. The filters and PUFs are extracted using Soxhlet or large cell PLE systems. See method US EPA TO-9 or MOECC 3418 for more details.

3 Extract Cleanup

The extraction of dioxin-like compounds with nonpolar solvents or media is not very selective. The highly aggressive extraction methods used with nonpolar solvents or nonpolar media for DLCs co-extract many additional POPs and matrix compounds which can interfere in the analysis and must be removed in the sample preparation process. This requires a comprehensive sample preparation scheme to remove bulk matrix coextractables, eliminate any potential interfering compounds, and retain as much of the analytes of interest as possible while achieving the high concentration factors listed above. The classical dioxin cleanup developed over 30 years ago is still basically the same procedure used today. The method originally developed by Nesterick and Lamparski [66] referred to as the “Dow cleanup” was later modified by Smith and Stallings [67] using a multilayered acid/basic silica column to remove acidic/basic polar compounds and silver nitrate/silica to remove any sulfur compounds, especially any chlorinated dibenzothiophenes. Silver nitrate

is more expensive than mercury or copper but much faster and easier to use and not a significant additional cost to the method. Mercury is toxic and now only typically used in emergency response situations. Sulfur removal is less critical nowadays as most laboratories are using HRMS or tandem mass spectrometry (MS/MS) detection systems.

Column chromatography with various combinations of silica, alumina, Florisil, and carbon adsorbents can be used to remove bulk matrix and interfering compounds and to direct specific analytes into desired fractions [68–73]. Due to the ability to extract a variety of analyte groups together (dioxin, PCBs, PBDEs, PCNs, etc), sample preparation schemes have been developed to combine analytical methods and fractionate extracts to eliminate any potential interfering compounds. For example, activated carbon can separate the planar from the nonplanar compounds. Dioxin and coplanar PCBs and PCNs can be collected in one fraction and the nonplanar compounds (ortho-PCBs, PBDEs, PCDEs, chlorinated pesticides) in another. This eliminates a number of the gas chromatographic coelutions, e.g., PCB77/110 and PCB 87/81, and also splits the PCDE a major interference to PCDFs into separate fractions [74]. The PCDEs can fragment in the mass spectrometer ion source to form PCDFs which can result in a significant bias. They are highly bioaccumulative and can be a major interference to PCDFs in biological samples. In all cases, the use of each adsorbent type must be carefully characterized and calibrated to optimize elution volumes of the analytes of interest while minimizing the contribution of interferences.

Alumina and Florisil are used to remove or separate (e.g., ortho-substituted PCBs) some of the less polar compounds as well as residual lipids not removed by the silica column cleanup. Any combination of silica, carbon, alumina, or Florisil can be used to remove potential interferents. The strategy is to use minimal amounts of packing to reduce contamination and possible irreversible adsorption of analytes on the packing material to achieve the highest analyte recoveries possible. For difficult samples, additional amounts of packing materials and cleanup stages may be needed to produce an extract clean enough to inject on the gas chromatograph. For samples with high levels of lipids, e.g., biological tissue or biosolids, gel permeation chromatography (GPC) [75] can be used as a preliminary cleanup step. Additional amounts of acid silica packing can also be used to remove excess lipids. This option is preferred if sample extracts are not also used to analyze acid labile compounds like some of the brominated flame retardants.

Classical open-column cleanup procedures like the Smith/Stallings method (silica, alumina/Florisil, or carbon) can be very labor intensive. It can 3–5 days extract and cleanup a set of 10–20 samples using manual procedures. Each column eluant is concentrated, typically to dryness. This is one of the most important steps in the analytical scheme as aggressive extract concentration can result in analyte loss, low surrogate recovery, and elevated detection limits.

In order to minimize labor costs, a number of automated or semiautomated systems have been developed. Fluid Management Systems Inc. (FMS – Watertown MA, USA) developed the first automated dioxin sample preparation system, the PowerPrep System, that uses a variety of different disposable prepackaged columns

which allows for the ability to prepare extracts for a single group of compounds (e.g., dioxin, PBDEs) or multiple compound groups or a combination of analyte groups together in a single method [46, 76]. Figure 2 shows the FMS automated sample preparation system. In recent years, other systems have become available from a variety of vendors including LCTech (Dorfen Germany) and Miura Co (Ehime Japan, also available from DSP Systems, Erichem Netherlands). Fayez et al. [78] reported that the cost of preparing dioxin extracts using the FMS system is about the same as conventional manual methods; however, a single analyst can prepare double the number of samples in the same period of time. For larger contract labs that analyze many thousands of samples per year, using assembly line processing and manual methods with disposable labware can be more cost-effective.

Fayez et al. [79] have also used the FMS system to extract and cleanup dioxin, PCBs, PCNs, and BDEs in biological tissue in a single run using PLE extraction with combined manual transfer to the FMS system for cleanup. This method reduces the extraction/sample preparation time to 2–3 days significantly reducing analytical time and costs. Doing the four tests separately using manual methods could take 10 days to extract and prepare. The ortho-substituted PCBs and BDEs are collected by forward elution through the activated carbon column, while dioxin, coplanar PCBs, and PCNs are collected in a reverse elution fraction. Focant et al. [80] were able to interface a PLE with the FMS system to automatically extract and cleanup a set of food samples ready to be analyzed in about 10 h.

These automated systems can work very well with samples like biological tissues and food where the matrix is relatively constant and analyte levels do not vary significantly. In cases where high concentrations are expected or there is heavy matrix loading, manual methods may be preferred so that the automated systems are not contaminated to the point that background levels interfere with detection limits.

Cape Technologies (South Portland ME, USA) have developed a semiautomated method using disposable glass columns. The system (Fig. 3) is pressurized with nitrogen gas. A multi-analyte compound group method (dioxin, PCBs, PBDEs, and PCNs) similar to that developed for the FMS system was developed by Yang et al. [77, 81, 82]. The major advantage of the Cape system is that it does not require power, and because the sample extract only makes contact with disposable labware, no rinse cycles and labware cleaning are needed making this a very cost-effective sample preparation method. Because the sample never touches any reusable labware, cross contamination from high-level samples is minimized. Some high production contract labs use disposable labware methods similar to the Cape procedure, but find it more cost-effective to purchase reagents and adsorbents in bulk and pack their own columns.

There have been a number of publications reporting the extraction and cleanup using an ASE or modified ASE or PLE cell. The samples are typically mixed with some type of matrix dispersant like diatomaceous earth or hydromatrix and are placed above a series of column packings like silica or carbon in the extraction cell allowing extraction and sample cleanup to be carried out simultaneously [38, 39, 83–85].

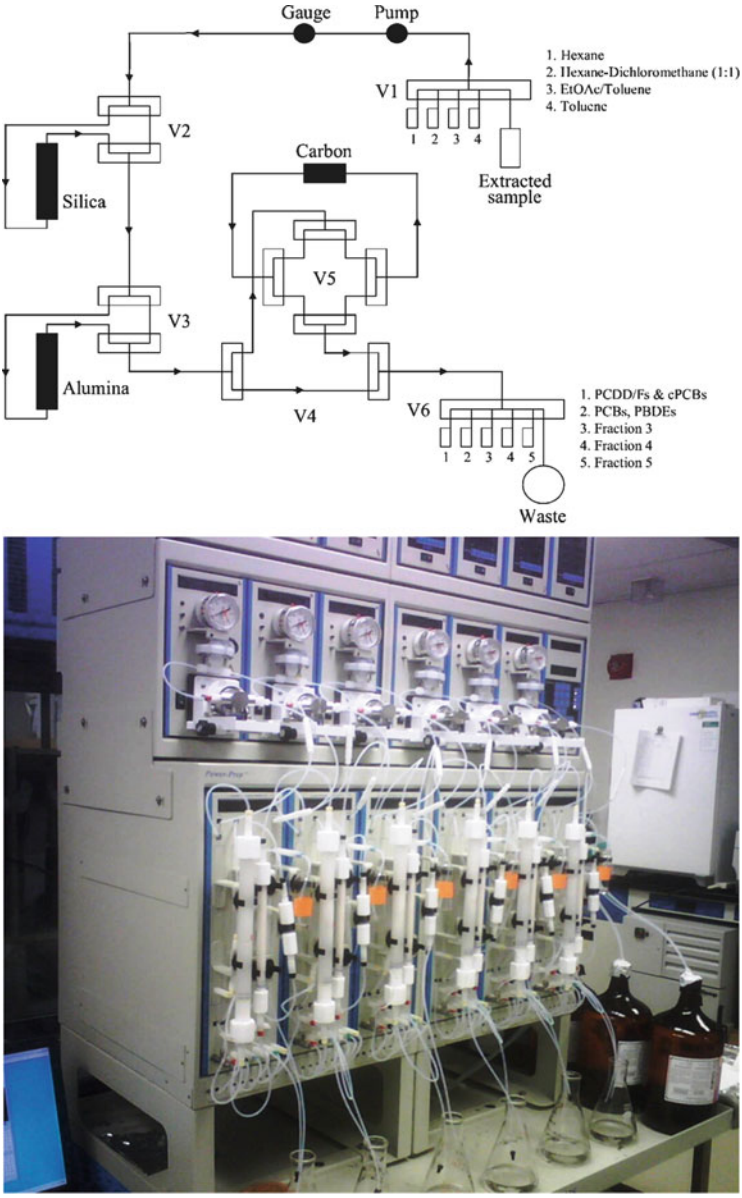

Fig. 2 (**a**) Schematic representation of an automated cleanup system. Reproduced with permission from [76] of GC-MS, (**b**) FMS PowerPrep System

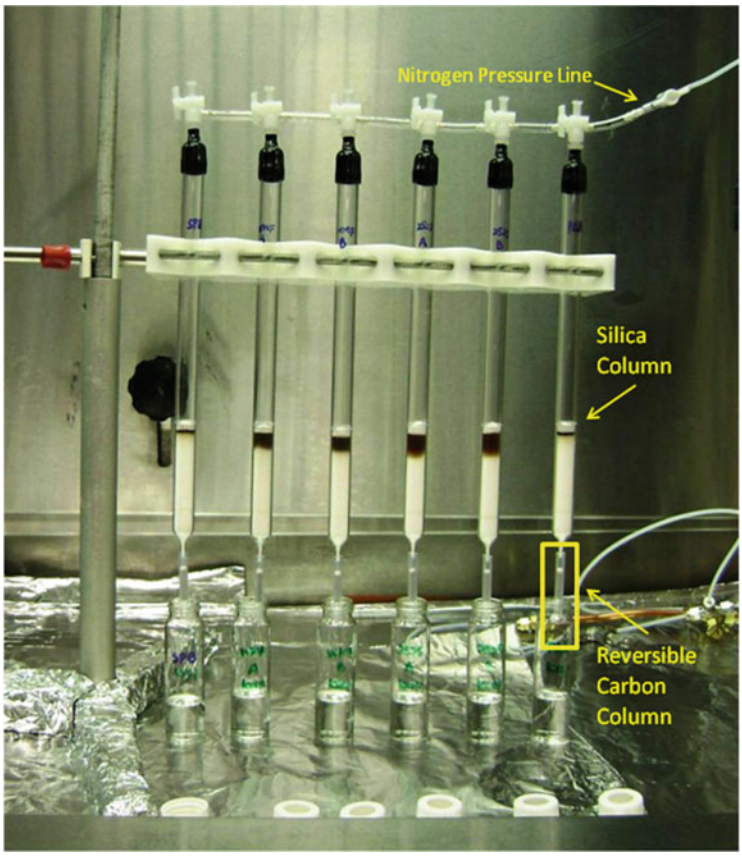

Fig. 3 Cape Technologies Disposable Sample Preparation System (www.Cape-Tech.com)

4 Chromatographic Separation

Dioxins, furans, and PCBs contain many isomers or congeners of similar structure, and as a result of the symmetrical nature of these compounds, the mass spectra of their isomers (e.g., tetra dioxins) are essentially identical. In addition, congeners of higher degrees of halogenation can fragment in the mass spectrometer to form interfering ions at mass-to-charge ratios of their lower halogenated homologues. Because mass spectrometers cannot separate isobaric compounds, it is important that the toxic compounds be separated from the nontoxic ones either physically (by placing them in separate fractions) or chromatographically, especially if accurate TEQ values are required.

The majority of dioxin and furan congeners are not considered toxic, and therefore a chromatographic column or combination of columns able to uniquely separate the 17 toxic congeners from one another as well as the remaining

193 nontoxic congeners is required. Coelutions with nontoxic congeners or other interferences can cause biased results. PCBs can interfere with dioxins, and PBDEs can interfere with PCBs [86]. The Wellington Reference and Handling Guide [87] provides information on a variety of halogenated compounds including exact masses and theoretical isotope ratios that can be used to determine potential interferences.

There currently is no single GC column that can uniquely resolve all of the toxic dioxin congeners from the other 193 nontoxic dioxins or other interfering compounds, and therefore, samples must be analyzed on separate GC phases, or sample extracts must be fractionated in a way that interfering compounds are split into different fractions.

There have been a number of attempts to develop a dioxin-specific column that can separate the seventeen 2,3,7,8-substituted congeners. Many of these attempts have resulted in a column with lower numbers of coelutions than the standard 5% phenyl-methyl phases. DB-dioxin [88] was the first dioxin-specific column developed. Rtx-dioxin-2 [89–91] and BPX-DXN [92] are also dioxin-specific GC columns. A number of reviews have compared the separation of dioxin on 5% phenyl-methyl phases to a number of other polar and analyte-specific phases [93–95]. Table 3 compares the ability of a variety of GC columns to separate each of the seventeen 2,3,7,8-substituted dioxins. None of the columns currently available can uniquely separate all the toxic congeners, and therefore in most regulatory methods, the 5% diphenyl-dimethylpolysiloxane column is still used in conjunction with the DB-225 (50% cyanopropylphenyl-dimethylpolysiloxane). A lower bleed version of the 5% diphenyl-dimethylpolysiloxane column was developed by J&W in the early 1990s. The elution pattern of this phenyl arylene polymer is slightly different from the standard 5% phenyl-methyl columns. Abad et al. [96] and Fishman et al. [95] have reported the relative retention times and coelutions for all of 2,3,7,8-substituted dioxins and furans on these "5" phase-ms columns. The 5-MS column can uniquely separate 2,3,7,8-T_4DCF from the other T_4DCFs eliminating the need for secondary confirmation of 2,3,7,8-T_4DCF. The standard GC columns typically used for confirmation for dioxin analysis completed on a 5% phenyl phase are the DB-225 or SP-2331 columns. Both columns are polar, have very low-temperature maximums, and can suffer from significant bleed. A number of liquid crystal columns have been developed for dioxin analysis [97, 98]. These columns can have very high resolving powers for planar compounds and therefore are very selective to dioxins and other dioxin-like compounds. Unfortunately, they typically have low-temperature maximums. A variety of ionic liquid columns have been developed recently. They have higher temperature limits than the liquid crystal columns but are not as stable as conventional cross-linked columns. Do et al. [99, 100] have reviewed the various column combinations. The combination of a 5-MS and SLB-IL61 column will separate all of the 2,3,7,8-substituted congeners and is an alternative combination to the classical 5% diphenyl-dimethylpolysiloxane and 50% cyanopropylphenyl-dimethylpolysiloxane pair.

The GC-HRMS analysis of dioxin using a conventional 5% phenyl-dimethyl, 60 m, 0.25 mm, 0.25 μm, and GC column is about 50 min long. Prior to analyzing

Table 3 Isomer-specific separation of 2,3,7,8-substituted PCDD/Fs on a variety of stationary phases

Congeners	DB-XLB	βDEXcst	LC-50	SLB-IL61	SLB-IL76	SLB-IL111	Equity-5	DB-5 ms	VF-5 ms	VF-Xms	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	Smectic	RTX Dioxin2	BPX-DXN	5Sil MS
2,3,7,8-TCDF	−−	−−	−−	++	−−	++	−−	+−	++	++	−−	−−	++	+−	++	−−	+−	+−	−−	+−	+−	+−
1,2,3,7,8-PeCDF	++	++	−−	+−	−−	−−	+−	++	++	++	+−	+−	−−	−−	−−	−−	−−	−−	++	++	++	++
2,3,4,7,8-PeCDF	−−	+−	−−	++	++	++	−−	−−	−−	−−	−−	−−	++	++	++	+−	++	++	−−	−−	−−	−−
1,2,3,4,7,8-HxCDF	++	+−	+−	−−	−−	++	−−	++	++	++	−−	−−	+−	−−	+−	−−	−−	−−	++	+−	+−	++
1,2,3,6,7,8-HxCDF	++	+−	+−	++	++	+−	+−	++	++	++	+−	+−	−−	+−	−−	+−	+−	+−	++	+−	++	++
2,3,4,6,7,8-HxCDF	++	+−	++	++	++	++	+−	−−	−−	+−	−−	+−	+−	++	−−	++	++	++	−−	−−	−−	−−
1,2,3,7,8,9-HxCDF	++	−−	++	++	++	++	+−	−−	−−	+−	+−	+−	++	−−	++	++	++	++	++	−−	−−	+−
1,2,3,4,6,7,8-HpCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
1,2,3,4,7,8,9-HpCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
OCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
2,3,7,8-TCDD	+−	−−	++	++	−−	−−	++	++	++	++	+−	++	−−	−−	+−	−−	++	+−	++	+−	+−	++
1,2,3,7,8-PeCDD	−−	−−	++	++	−−	−−	++	+−	++	++	+−	++	+−	−−	−−	++	++	++	++	−−	+−	++
1,2,3,4,7,8-HxCDD	++	−−	−−	++	++	++	+−	+−	++	++	+−	++	++	+−	++	++	++	++	−−	+−	++	++
1,2,3,6,7,8-HxCDD	+−	−−	−−	++	++	++	+−	+−	++	++	+−	+−	++	++	++	++	++	++	−−	+−	++	++
1,2,3,7,8,9-HxCDD	++	++	++	++	++	++	−−	+−	+−	++	−−	−−	++	++	++	++	++	++	++	++	++	++
1,2,3,4,6,7,8-HpCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
OCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

+ +: baseline separation or at least 10% valley. Peak resolution of $R > 1$
+ −: quantifiable result (separation that allows peak resolution of $R \sim 0.8$)
− −: coelution or interference present. Maximum possible concentration
Data compiled from references [93–95, 99, 100]

samples, the instrument must be tuned to 10,000 resolutions and then calibrated. A resolving power (ΔM/M) of 10,000 is the best compromise between selectivity (eliminating most interfering compounds that are not removed by sample preparation) and sensitivity on a magnetic sector instrument. A series of quality control (QC) samples include a column performance check containing the nearest eluting neighbors of 2,3,7,8-TCDD, low-level standard (sensitivity check), continuing calibration standard, laboratory blank, spiked sample or certified reference material (CRM), and duplicate sample. The setup procedure can take 5 h or more. Continuing calibration methods are used to reduce analytical run times because running a five-point calibration curve can add up to another 4 h to the analytical run. Even when using the continuing calibration procedure, only about 15–18 samples can be analyzed in a single day.

If columns of smaller inner diameters and thinner films (inner diameter <0.20 mm and film thickness <0.18 μm, i.e., microbore columns) are used, a technique called fast GC can significantly reduce analysis times. If the phase ratio (the ratio of film thickness to column inner diameter) remains constant, the relative retention times do not change. As the inner diameter and phase thickness are reduced and their ratio is kept constant, retention times decrease, and the chromatoraphic peaks get narrower and taller. Microbore columns have much lower height equivalent theoretical plates (HETP) than classical columns resulting in significantly increased theoretical plates per meter, enabling significantly shorter columns to be used. Microbore columns require higher column head pressures, flow rates, and temperature ramp rates, which results in reduction of analytical run times by up to 50% and an increase of peak heights of up to a factor of five [101–105].

Multidimensional gas chromatographic techniques have been used for a number of years for the analysis of dioxin and related compounds. Comprehensive multidimensional gas chromatograph or GCxGC is an emerging technique that continues to gain interest. It can enhance sensitivity and reduce analysis times and has been used to analyze dioxins and PCBs [106–114]. GCxGC is carried out using two GC columns of different stationary phases that are connected through a device called a modulator. The modulator traps the analytes eluting from the first (primary) column, compresses their column plug, and then reinjects these small plugs or slices into the second (secondary) column where further separation occurs by a different mechanism that happens in the primary column [110]. Figure 4a shows a schematic of a comprehensive GCxGC system. The modulation process produces much narrower and taller chromatographic peaks which can increase signal-to-noise ratios and sensitivity by up to an order of magnitude. Fig. 4b depicts the procedure, whereas peak that is not completely separated on the primary column (X + Y) is cut into "slices" by the modulator and can be separated on the secondary column as a result the different stationary phase. The slices are small very narrow packets of ions (Fig. 4b) where each packet results from a separate modulation cycle – P_M. The slices can be reconstructed as either a three-dimensional plot or a contour plot. Columns of distinctly different phases (depending on temperature compatibility) can be used resulting in significantly enhanced peak capacity. If the main process of separation for each column is different (e.g., boiling point, polarity, shape

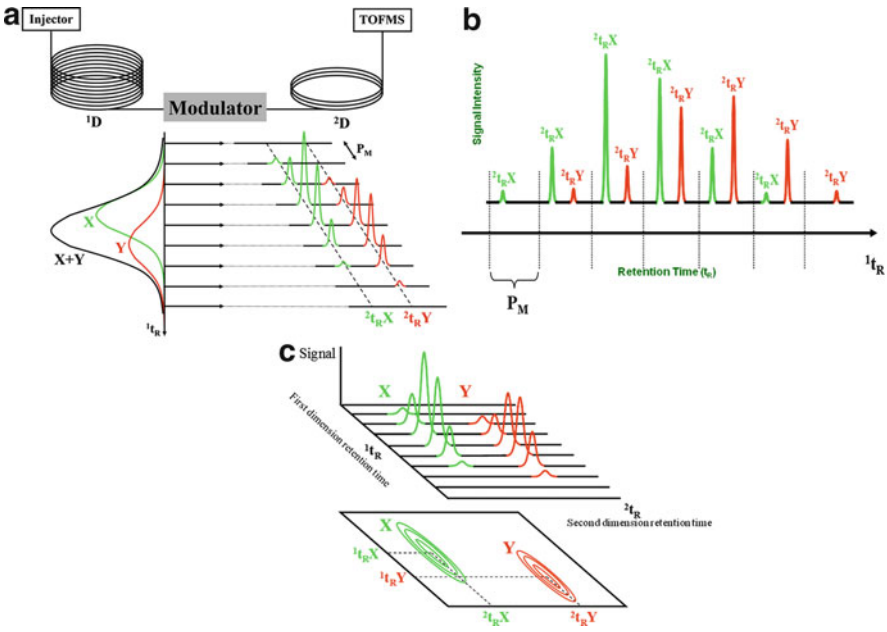

Fig. 4 Schematic of the column coupling in the GCxGC-TOFMS apparatus. (**a**) The modulator allows rapid sampling of the analytes eluting from the first-dimension GC (1D) and reinjection into 2D. The modulation process is illustrated for two overlapping compounds (X and Y) eluting from 1D at a defined first-dimension retention time $^{1}t_R$. As the modulation process occurs during a defined modulation period P_m, narrow bands of sampled analytes enter 2D and appear to have different second-dimension retention times $^{2}t_R(X)$ and $^{2}t_R(Y)$. (**b**) Raw data signal as recorded by the TOFMS through the entire two-dimensional separation process. (**c**) Construction of a two-dimensional contour plot from the high-speed secondary chromatograms obtained in (**b**), in which similar signal intensities are connected by the contour lines. Reproduced with permission from [106]

selection), orthogonal (optimal) separation can be achieved. Orthogonal separations typically result in separation of isomers in bands at an angle of about 45°. The peak capacity in an orthogonal GCxGC system is the product of the peak capacity of the primary and secondary columns and can be typically in the order of 1,000 (approximately 50×20) (first dimension (30 M, 0.25, 0.25) $\times$ second dimension (2 M, 0.10, 0.10)) [115]. The major challenge of this technique is obtaining enough data points across the very narrow peaks produced in the modulation process. GCxGC peaks are in the order of 400 ms wide. In order to accurately define a second-dimension GC peak (7–10 measurements), scan rates of greater than 25 Hz are required. Figure 5 exhibits the power of GCxGC showing a chromatogram of a sediment sample where many analytes can be measured in a single run. In many areas of the chromatogram, there would be multiple coelutions if the extract was analyzed in by regular single-dimensional chromatography.

Electron-capture detectors (ECD) have been used in GCxGC applications because of the significantly enhance separation enables much simpler and less

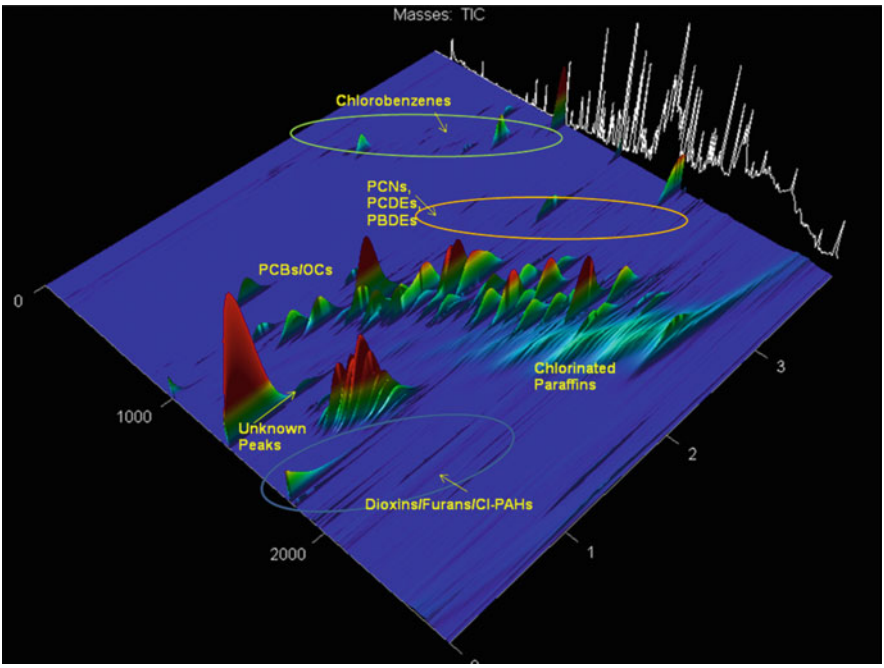

Fig. 5 Three-dimensional GCxGC-ECD plot of a sediment sample, private communication from Alina Muscalu using the method from [116]

selective detectors to be used [116, 117]. The significantly increased peak capacity of this technique enables multiple analyte groups like dioxin, PCBs, organochlorine (OC) pesticides, PCNs, and other organohalogen compounds to be analyzed in the same single run in many cases bypass the need for sample extract fractionation. Cryogenic zone compression modulation (CZC) has been used to enhance sensitivity of the analysis of dioxin in blood samples [118]. In this technique, a single 7 m, 0.1 mm id, and 0.1 μm d_f column is fed through a modulator. A long modulation period of 6–9 s is used to attempt to trap the entire peak into one modulation. An on-column detection limit of 300 attograms which is about an order of magnitude lower than conventional methods can be achieved.

5 Mass Spectrometric Detection

A number of different types of mass spectrometers are capable of analyzing dioxin. The magnetic sector high-resolution mass spectrometer (HRMS) was the first used in the early 1970s and is still the most sensitive and selective instrument for dioxin analysis [119–121]. It is considered the gold standard for dioxin analysis and is the only type of mass spectrometric detector allowed for a number of regulatory

analytical methods for litigation purposes. GC-HRMS is the most technically complex system and requires the most highly skill operators. There are a number of reviews comparing different mass spectrometric system [26, 27, 105, 122–130]. GC-HRMS must be operated in the selected ion monitoring (SIM) mode where only ions from the analytes are scanned. In this process, the magnet is set to a specific mass range, and the accelerating voltage is jumped to pass ions with mass-to-charge ratios only of the ions of interest, because the instrument only spends time directly on desired analytes and not scanning a range of masses that in most cases will not produce a signal, SIM can enhance sensitivity up to two orders of magnitude and is required in order to meet the very low detection limits needed for dioxin analysis. HRMS instruments are operated at a resolution of 10,000. Regulatory methods require the resolution to be confirmed every 12 h. This was a challenge for instruments in the late 1970s and early 1980s. Modern instruments can now hold 10,000 resolutions for days. HRMS instruments require a mass calibrant like perfluorokerosene (PFK) to ensure mass accuracy. PFK is used to tune the mass spectrometer and calibrate the mass scale. It is then infused into the mass spectrometer during the analytical run as a lock mass to ensure there is no mass drift. The lock mass trace is monitored to ensure results are not biased by matrix suppression.

Tandem mass spectrometry using triple quadrupoles [131, 132] has been used for the analysis since the mid-1980s. Recently, tandem quadrupole mass spectrometers have been used and approved in Europe specifically for food testing and food safety purposes [133–135]. Hybrid mass spectrometers (HRMS/quadrupole) [136], ion trap mass spectrometers [137–139], and the orbitrap [140, 141] have also been used for tandem mass spectrometry analysis of dioxin. Hybrid instruments are expensive and even more difficult to set up and operate than HRMS systems and are no longer routinely available commercially. Ion traps have been used mainly for screening purposes because being a trapping-type mass analyzer, ion suppression from coextractable compounds can result in loss of sensitivity or bias. The orbitrap can meet method requirements of regulatory dioxin methods and may also become a replacement for magnetic sector instruments in the future.

Tandem mass spectrometry uses multiple reaction monitoring (MRM) where the molecular ion is selected by the first mass analyzer and is then passed through a collision gas (typically nitrogen or argon) in a second quadruple set to pass all ions. Characteristic fragment ions are then selected by the third quadruple (mass analyzer). Dioxins lose COCl (63 Da) in MRM transitions and are the only group of compounds that lose this specific neutral fragment producing clean mass chromatograms with very few interferences [132].

Most dioxin methods also determine results for the dioxin-like PCBs. Polychlorinated biphenyls do not have a unique MRM fragmentation reaction like the loss of COCl from PCDD/PCDFs. The major fragment reaction for PCBs is the loss of Cl_2. Most polychlorinated compounds lose Cl_2, and therefore MRM chromatograms for PCBs can exhibit a significant number of interferences.

The sensitivity of newer triple quadrupole mass spectrometers can approach that of HRMS instruments using MRM. Applying additional mass selection stages to the

analytes of interest reduces their transmission through the instrument resulting in a reduced signal. The additional mass filtering however reduces the chemical noise at a greater rate than the loss in analyte signal, therefore resulting in net increase in the signal-to-noise ratio. GC-MS/MS has been accepted in a number of dioxin methods as an alternative to HRMS including the analysis of food and feed [23, 24, 134].

A number of adjustments that can be used to increase sensitivity include using larger sample sizes or sample volumes (or PTV/LVI injection) and/or microbore columns. A number of applications using large volume injection (LVI) or programmed temperature vaporization (PTV) have been reported. Programmed temperature vaporization enables a greater portion of the sample extract to be injected into the mass spectrometer increasing sensitivity and allowing smaller sample sizes to be used [142–146]. It also enables minimal sample concentration. Bias resulting from analyte loss for semivolatile compounds like dioxin and PCBs is typically not significant, especially when isotope dilution mass spectrometry is used. The major advantage is time savings from not having to concentrate organic solvents.

5.1 Ionization Techniques

Electron ionization (EI) is the classical ionization method for dioxin. It is available in essentially all GC-MS instrument types. Electron ionization is normally carried out using 70 eV electrons which produce ions with a broad range of internal energies resulting in variety of different fragment ions [147]. When using SIM, the number of possible ions produced should be as few as possible. In order to minimize the degree of fragmentation, an electron energy of about 35 eV should be used. The reduced electron energy decreases the degree of fragmentation and enhances the molecular ion signal which correspondingly enhances sensitivity. The mass spectra of halogenated compounds exhibit multiple isotopic peaks for the molecular ions as well as their corresponding fragment ions. Sensitivity is reduced due to these isotopic ion clusters, because the signal is split over the isotopic ions. This is also an advantage as isotopic ratios are much more stable than fragmentation ratios, and this information can be used for identification and conformation of the analytes of interest. Specific abundance of molecular or fragment ions is monitored and compared to acceptance criteria. This can be important in identifying potential interfering compounds that may be present. If interfering compounds are routinely presents, analysts can select other isotopic peaks which may be free of interferences for quantification.

Chemical ionization (CI) [148] is a soft ionization technique producing mass spectra with little fragmentation where the molecular anion is typically the base peak [149, 150]. Electron-capture negative-ion chemical ionization (ECNI) is a form of NCI using a reagent gas like methane that is to adsorb energy from electrons so they can be more easily attached to analytes of interest. The sensitivity of most polyhalogenated compounds like PCDD/PCDFs increases with increasing

degree of chlorination in ECNI, and for most congeners, the sensitivity in NCI can be much greater than with EI. However, for 2,3,7,8-T_4CDD, as for a number of organochlorine pesticides, the Cl^- anion [151, 152] is the base peak resulting in a 2,3,7,8-T_4CDD molecular anion with much lower intensity in NCI than that of the molecular ion in EI. Due to the presence of many other chlorinated compounds in most sample extracts, the Cl^- anion is common, and therefore monitoring Cl^- is not very selective. Chemical ionization using O_2 as a reagent gas has also been investigated [149, 153, 154]. It can be used to distinguish different congeners, but is much more difficult to use than EI and not sensitive enough to be used for ultra-trace analytical purposes with regular chemical ionization vacuum sources.

Atmospheric pressure ionization is a new technique that has been available for a few years. It ionizes target molecules by charge exchange from the nitrogen present in the source which has been ionized from a corona discharge [155, 156]. The advantage of atmospheric pressure ionization is that it can be matched with other atmospheric liquid chromatographic sources enabling instruments to be used as a more flexible combined GC and LC instrument. There are only a few reports of atmospheric pressure gas chromatography (APGC) for dioxin using tandem quadrupole mass spectrometry [156, 157]. APGC-MSMS is an excellent combination where the ion sources are based on a modified LC source. Both APGC source and the tandem mass analyzer are simple to maintain and operate, resulting in a rugged, sensitive instrument with minimal downtime. Figure 6 shows the sensitivity of this new ionization technique.

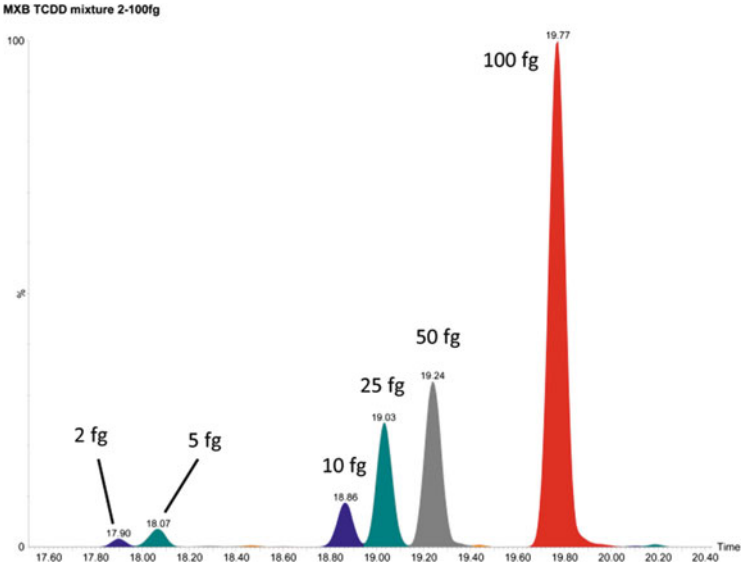

Fig. 6 Chromatogram demonstrating the sensitivity of the APGC-MS/MS. Congener identifications are, from *left* to *right*, 1,3,6,8-, 1,3,7,9-, 1,3,7,8-, 1,4,7,8-, 1,2,3,4-, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Reproduced with permission from [158]

5.2 Calibration and Quantification

Quantification of dioxin and dioxin-like compounds is a challenge because of the large number of congeners and the many cleanup stages required. In order to obtain good-quality data, isotope dilution with ^{13}C-substituted PCDD/PCDF internal standards (surrogates) for quantification of the 2,3,7,8-containing congeners is used. In addition to using mass-labeled internal standards for quantification, they are also used as GC retention time markers to ensure that the correct native toxic isomer is detected. Isotope dilution is the most accurate form of quantification [159, 160]. Adding labeled surrogates at the beginning of the analytical procedure enables results to be corrected for any losses during the multistage sample preparation procedure, sample concentration steps, volumetric transfers, or instrumental variability. Additional labeled internal standards can also be added at different stages. Cleanup standards are added after extraction to evaluate extraction and cleanup efficiency. Injection or syringe standards are added before injection to correct for injection variability and instrumental drift.

A calibration curve with five or more concentrations is typically used. The calibration standards typically contain all 17 native 2,3,7,8-substituted congeners and 16-labeled 2,3,7,8-substituted congeners. Labeled OCDF is not included because the higher mass isotopes of labeled OCDF can interfere with native OCDD. Concentration of the lowest-level calibration standard (CS1) for 2,3,7,8-TCDD is typically 0.5 pg/μL. Concentrations increase by factors of 4 or 5, to a concentration of 200 pg/μL in CS5, the highest-level calibration standard. Levels of the penta to hepta congeners are typically a factor five higher than the tetra concentrations and OCDF and OCDD which are typically 10 times higher. Examples of calibration standard sets can be seen in any of the methods listed in Table 2. A relative response factor (RRF) is calculated between the native analyte and labeled surrogate for each 2,3,7,8 substituted congener except 1,2,3,7,8,9-HxCDD whose labeled standard is used as an injection standard and OCDF. The average RRFs of the other two HxCDD congeners are used to quantify 1,2,3,7,8,9-HxCDD, and the response factor of OCDD is used for OCDF. Relative response factors are used to correct for any difference in response or concentration of the labeled standards relative to the native compounds in the calibration standards and should be close to 1. Non-2,3,7,8-substituted congeners are quantified using the average RRFs determined to their specific congener group.

In most methods, an average response factor over the calibration range is used instead of a response factor from linear regression using a classical calibration curve. Response factors (RFs) for the labeled surrogate standards are determined using internal standard calibration by the ^{12}C-labeled injection standards (1,2,3,4-T_4CDD and 1,2,3,7,8,9-HxCDD). Concentrations of the surrogates are determined and reported as percent recoveries which are used for quality control purposes.

5.3 Detection Limits and Low-Level Quantification

Dioxin is considered the most toxic organic chemical, and as such, analytical methods must be able to report results at sub-picogram (10^{-12}g) levels and well into the femtogram (10^{-15}g) level for food analysis. At these low levels, background levels can easily contribute to contamination resulting in positive values in the laboratory blanks which often is the limiting factor and the determination sample detection limits [161]. This can usually be controlled for dioxins and furans in most cases; however, contamination from PCBs poses a larger problem. The use of PCBs in many building and electrical equipment remains and circulates in the building and laboratory area. Dust control, air filtration, and good laboratory practices can minimize background contamination. If levels are constant, background subtraction can be used [162].

In the analytical scheme, there are a number of detection limits that need to be considered. Instrumental detection limits can be used to determine sample sizes and sample extract volumes needed to obtain required detection limits. Average recoveries and background blank levels also need to be considered [163]. Method detection limits (MDLs) are a statistical determination of detection limits using a set ($n \geq 7$) of low-level spiked samples (spiked at 5–10 times the instrument detection limit) that are taken through the entire method [159, 160, 164] The MDLs are determined by multiplying the standard deviation (σ) of the mean concentration of the sample set by the student's T value. In this method, the value of the MDL of a compound can be elevated if there is contribution from background contamination. Because of the low levels required, many labs calculate the actual detection limit (DL) for each parameter in every sample [165]. In this method, the signal-to-noise ratio of five to one (peak to peak) or three to one (average to peak) is used to estimate the area of a peak that would have been present at the retention time of the target analyte. The estimated area value is in the concentration calculation. The advantage of this method is that it is an actual determination of the detection limit for the target analyte in each sample being analyzed. This method is not always used because it is very time-consuming. The limit of quantification (LOQ) is typically about three times the method detection limit value and is the level where the analyte signal is repeatably identifiable, discrete, and reproducible with a precision of about 20% and an accuracy of ±20% [80, 159, 165]. The LOQ is important parameter for regulatory and litigation situations. In such cases, sample size and concentrations factors should be used such that results are well above LOQ values. In Ontario Canada, LOQ values must be greater than 10% of the regulatory limit if technologically possible.

Any compounds found in laboratory blanks can elevate detection limits and/or result in biased high values. Correction for background contamination can be carried out if blank levels are relatively constant (RSD <30%). This is often the case for low-level sample analysis like biological or human samples [80, 165]. Background contamination in blanks and reduced analyte recovery (as indicated by low recovery of labeled surrogates) can also elevate detection limits. Adsorption to

surfaces or cleanup packing materials is a major source for reduced analyte recovery. Bias can be magnified in low recovery situations (<40%). The correction of values for surrogate standard recovery also magnifies any value by the same amount as the recovery correction including the contribution for background contamination. The main advantage of using isotope dilution methods is that labeled analogs are often spiked into samples at levels significantly greater than those in the sample which can reduce losses due to adsorption effects. For other environmental samples like sediments, sludge, ash, or hazardous waste where levels in samples can vary up to eight orders of magnitude, blank levels can fluctuate significantly. In this case, determining accurate values for blank correction can be very difficult, and this may result in significantly elevated uncertainty values (i.e., >100%).

Other sources of bias include polychlorinated diphenyl ether (PCDE) fragment ions for PCDFs. PCDEs fragment by loss of Cl_2 to form PCDFs in the mass spectrometer ion source. PCDE traces are typically monitored to ensure there is now column break through during carbon column sample preparation.

6 Screening and Alternate Methods

Dioxin analysis is the most complex, time-consuming, and labor-intensive analytical method. Screening methods can be used to increase analytical capacity and reduce costs. They can be very valuable for screening samples like food and feed where dioxins are not expected or contaminate sites where they are used to provide quick results to enhance site cleanup procedures [166, 167].

The high cost of analysis for dioxin has resulted in the development of a number of alternate less expensive and quicker analytical methods. Bioassays [168–173], immunoassay [174–177], and aryl hydrocarbon receptor-based polymerase chain reaction (PCR) assays [178] have been developed. For bioassays to produce accurate results and compare very well with the classical HRMS methods [179, 180], a rigorous dioxin-type cleanup sample cleanup procedures must be used.

Screening approaches include instrumental methods monitoring a reduced number of congeners, reduced resolution methods, and a variety of bioanalytical methods. Schrock et al. [181] have compared the analysis of dioxins in soils using a modified USEPA-1613b GC-HRMS method. Samples were extracted using PLE, extracts outside the calibration range were not diluted and reanalyzed, and no secondary column confirmation (DB-225) was used. The majority of results compared within 10–20% with the classical USEPA-1613b method [30]. In most cases, contribution of compounds like 2,3,7,8-T_4CDF after multiplication by their respective TEF is significantly less than the uncertainty of the method, and their contribution to the TEQ is minimal indicating that confirmation by a secondary column may not be required in most cases.

Bioassay and immunoassay methods do not require complex expensive instrumentation for analyte detection enabling any laboratory with basic equipment to

analyze samples for dioxin. However, it is still critical that proper sample preparation procedures are used. The inability to use labeled internal standards creates challenges with respect to analyte loss during sample preparation, and cross-reactivity from compounds with similar structures can result in biased results. Dindal and Billets [182] compared a number of bioassay/immunoassay methods used in an intercalibration/method validation study. Participating laboratories were typically biased high for dioxin/furan and PCB and less precise when compared to HRMS. Detection limits ranged from approaching those of HRMS to more than two orders of magnitude higher. The number of false positives ranged from 6 to 10%, and the number of false negatives ranged from 0 to 8%. Analytical costs were significantly lower ranging from 5 to 50% of HRMS, and analysis times were less than 10% that of HRMS. Nording et al. [183] compared two bioassays and one immunoassay method with HRMS. The bioassays tended to overestimate results due to contributions from cross-reactivity of other dioxin-like compounds in the sample. If an extensive cleanup was used, no statistical difference between the bioassay and HRMS results was observed.

7 Quality Assurance/Quality Control/Accreditation

The analysis of dioxins and related compounds requires an extensive QA/QC program to ensure that analytical procedures are developed and validated by methods that can produce accurate, precise, and ultra-trace results in order to meet required data quality objectives [184–186].

There are numerous quality control limits and ranges that must be met for each set of samples. These include limits such as:

1. An acceptable range for calibration and continuing calibration checks. The relative standard deviation in the value of RRFs determined as the mean RRF across the calibration curve range should be less than 20%. This is typically the largest contribution to the uncertainty of the analyte. The value for continuing calibration acceptance is also $\pm$ 20% of the mean RRF value for the specific congener.
2. An acceptable range for recoveries of surrogates from 25 to 150% in most regulatory methods [30]. Recoveries of less than 10% to over 200% can be observed resulting from suppression or enhancement in signal for extracts that contain significant amounts of coextractable matrix materials. If this occurs at the retention time of the injection standards and/or labeled internal standards, significant bias in the result for the recovery of the associated internal standards can occur, and analysts should check the lock mass trace for any dips or disturbances. Changes of more than 10% in the level of the lock mass can result in bias, and further cleanup of the sample extract or dilution and reanalysis should be done.

3. Elution of target analytes within the proper elution window as defined by the "Window defining standard" which contains the first and last of every dioxin and furan. This standard is analyzed daily or after any GC column maintenance.
4. Ability to separate the 1237/1238 coeluting pair and 2378-TCDD at a valley of 25% or lower on a 5% phenyl-methyl column. This is the critical pair for 2378-TCDD separation and column performance.
5. Resolution check to ensure the instrument is operating at a resolving power of 10,000 or greater. This check is required least every 12 h for most regulatory methods.
6. GC peak shape of the native dioxin must be Gaussian and eluting within 2 s of its corresponding ^{13}C-labeled standard.
7. The isotopic ratios must be within 15% of their theoretical value. Values and ranges are listed in most regulatory methods.

Text box 1 summarizes the key considerations in the development of an analytical scheme for the analysis of dioxin and other POPs. Due to the ultra-trace nature of dioxin analysis, extra care must be taken to ensure that contamination for background levels and carryover does not result in significant analytical bias. Isotope dilution mass spectrometric procedures, IDMS, enable the correction for losses in the extraction and cleanup stages [187] as well as correction for any variability in the analysis stage. The $^{13}C_{12}$-labeled standards act as time markers for the native analyte being determined to ensure that the 2,3,7,8-labeled congeners are correctly identified even if there is any shift in retention times. The recovery of the surrogate standards effectively turns every sample into its own spiked matrix sample providing important quality control information. Different surrogates can be added at different stages of the sample preparation procedure and can be used to investigate any problems in the analytical procedure. ISO/IEC 17025 lists a number of critical quality control checks required to produce quality data. Key components of a quality assurance program as well as some of these critical quality control checks are listed in Text Box 2. One important consideration is that the analytical methods that are used are validated to ensure the methods are rugged and produces data that are accurate and precise.

Participation in interlaboratory studies and the use of reference materials can be used to assess accuracy and precision [188, 189] Certified reference materials (CRMs) are important tools for performance evaluation to ensure the laboratory is reporting accurate and precise results. A number of different CRMs for Dioxin have been developed in the past few years [190–193].

Van Bavel and Abad [194] reported a significant enhancement of the quality in dioxin data over the previous 15 years. They concluded that through a series of interlaboratory studies, issues with sample preparation, chromatographic separation and instrumental detection had been identified and corrected and that limits for confirming that methods and procedures are in control and "fit-for-purpose" were $\leq$10% RSD for standards, $\leq$15% RSD for samples extracts and $\leq$20% RSD for soil/sediment or ash samples. The uncertainties for dioxin methods are typically in the order of $\pm$20% per congener [123] which agrees very well with the limits

reported by Van Bavel and Abad [194]. The uncertainty of the TEQ tends to be lower (about 10%) than that of the individual congeners because it is the sum of the uncertainties of each of the individual congeners [123].

However, depending on how TEQs are calculated, there can be significant bias or error when comparing TEQs. It is important to identify the specific TEF scheme used and how the TEQs were calculated. TEQs can be calculated a number of different ways using different values for detection limits, e.g., ND = 0, ND = 1/2ND, ND = $ND^{0.5}$ or ND = ND. At lower levels, there can be a significant difference in TEQ values depending on which detection limits (value for ND) used varying up to 50% [162, 194].

Laboratory accreditation is an important requirement for routine analytical laboratories. Accreditation to ISO/IEC 17025 – General requirements for the competence of testing and calibration laboratories [195], is the standard to which labs are deemed technically competent.

Text Box 1: Key Considerations in an Analytical Scheme for POPs Analysis

- Ensure all labware, reagents and analytical instruments are free of contamination and interferences before beginning analysis
- Detect analytes at levels to meet MDLs – e.g., to meet sub-picogram detection limits for dioxin, every piece of labware should be prechecked (<500 fg) or labware segregated for high and low samples
- Determination of a representative sub-sample including gravimetric or volumetric determination for analysis. May require adjusting sample size or replicate analyses
- Addition of all internal and surrogate standards to the sample such that they will behave as the natural analytes in the sample during the analysis minimizing potential bias
- Quantitative extraction of analytes from matrix
- Extract may contain significant amounts of coextractable organic material including: Dioxins, Furans, PCBs PCNs, PCDEs, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCs), brominated flame retardants (BFRs), and many other organic compounds including lipids, humic material and sulfur
- Cleaning of extracts to remove interfering coextractables to the degree where DQOs and QC limits can be met for the required analysis
- Not all coextractable compounds need to be removed, but they should not affect the separation or detection systems with respect to the analytes of interest
- Separation of target analytes from non-target or nontoxic isomers, congeners or interfering compounds (e.g., GC-HRMS for dioxin)
  - There are many congeners per analyte group

(continued)

Dioxins/furans: 210; PCBs: 209, PCNs 75

– Separate and accurately quantify all toxic congeners

Dioxins/furans: 17, PCBs: 12, PCNs: 8–10

- Ensure method and instrument selectivity and sensitivity meet DQOs and QC limits
- Accredited laboratory (to ISO 17025), Quality System, trained/experienced analysts, proper instrument and analytical procedures, validated and documented methods and SOPs, control charting, non-conformance and root cause determination
- Ensure quantitative accuracy
  - Calibration, blanks, spiked samples, CRMs, ILS, Performance Evaluation, surrogates and internal standards, standard validation.
- Other considerations:
  - Toxicity of compounds can range up to six orders of magnitude. For example, T_4CDD toxicity can range from: NOEL = 3 g/kg to $LD_{50} = 1$ μg/kg – must identify correct isomers using proper GC columns and conditions.
  - Range of concentrations – fg/g (10^{-15} g/g) to % levels – potential lab contamination and instrument issues from carryover.
  - Range of sample types and complexities – biota, air, water, soil, hazardous waste which have different matrix dependent and method requirement issues. Use method fit for purpose that has been validated for specific matrix being analyzed.
  - Are the patterns of congeners representative of samples being analyzed, e.g., does the pattern match an Aroclor mixture (PCB) or does an ash sample have a combustion source pattern or does a biota sample have only the toxic congeners present (dioxin)?

Text Box 2: Key Steps for Quality Assurance and Quality Control

A Comprehensive Quality Assurance Program Includes:

- A Quality Management System that includes a Quality Manager, Quality Management group, quality manual and procedures manuals (for non analytical procedures)
- Detailed written analytical methods and standard operating procedures (SOPs)

(continued)

- Document management system to ensure controlled documents and approved procedures are used
- Facilities management – proper working conditions (temperature control, light and power) and supply of water, air (fume hoods), and IT (networks, data system and storage)
- Human Resource management to ensure trained and proficient analysts
- Health and Safety Committee and protocols to ensure safe working conditions
- Sample management – proper reception, logging, storage and distribution to ensure samples do not spoil
- Procurement of equipment and supplies – proper instruments, labware, data systems, software
- Preventative action (maintenance) – procedures to ensure failures are minimized, e.g., change pump oils on regular schedules
- Corrective action – system to review failures or non-conformance and determine root cause to ensure problem will not reoccur
- Standardization and calibration of instruments, volumetric labware, balances, etc
- Research and development, continual improvement
- Performance evaluation/review: intercalibration, round robins to determine accuracy and ruggedness of method
- Accreditation – external review to ensure lab is following proper procedures, e.g., ISO 17025
- Control charting – data trend analysis to ensure data quality
- Determination of uncertainty

Critical Quality Control Checks Include:

- Control standard – to check calibration and accuracy (alternate source standard) – determines accuracy of calibration
- Low level standard – confirm sensitivity of instrument and detection limits can be met
- Column performance check – should contain critical pair showing analytes are resolved chromatographically, e.g., 1237/1238-TCDD and 2,3,7,8-TCDD
- Blanks: laboratory (procedure), field, reagent, instrument blanks – to determine levels of contamination
- Control sample: spiked samples/reference materials – determines overall accuracy of method and data set
- Internal standards/surrogates – determines recovery and accuracy of analytes in samples
- Duplicate/replicate – determines precision

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8 Related Dioxin-Like Pops

There are a number of other compounds that interact with the Ah receptor and have dioxin-like toxicity. These include brominated and mixed halogenated dioxins and furans, polychlorinated naphthalenes and halogenated carbazoles [196–201]. Brominated dioxins/furans can be formed by thermal degradation of PBDEs and, bromo/chloro exchange can occur to form mixed bromo/chloro dioxins/furans [202]. At lower temperatures (between 250°C and 500°C). Rupp and Metzger [203] reported that bromo/chloro exchange occurs on the PBDE molecule prior to dehalogenation and ring closure.

Methods for most halogenated compounds use common extraction and cleanup and techniques [204, 205]. Polybrominated dioxins and furans [198, 206, 207] can be analyzed under similar conditions to those for chlorinated dioxins and furans, however shorter thin film columns should used and care must be taken to limit contamination from PBDEs and degradation from light and heat [207–211].

Mixed halogenated dioxins and furans are the most challenging type of dioxins to analyze. There are 1,436 mixed bromo/chloro dioxins congeners [212–214] and chloro/fluoro dioxins [215, 216] and only a few bromochloro standards are available commercially. Mixed halogenated dioxins and furans are at least as toxic as chlorinated dioxins [217]. Van den Berg et al. [16] reported that the ranges of relative potencies determined from various studies of chlorinated, brominated and mix halogenated dioxins, furans and dioxin-like PCBs overlapped and therefore, TEFs for the most recent chlorinated compounds should be used in TEQ calculations.

Levels of mixed halogenated dioxins are typically an order of magnitude or more lower than the chlorinated PCDD/Fs [218, 219]. The use of GC-MSMS where the loss of COCl and COBr is monitored significantly increases selectivity over HRMS methods [220]. The increased sensitivity of APGC has helped lower detection limits for analysis [156, 158, 221]. Multidimensional (GCxGC) chromatography has also been used [222].

9 Future Trends

There are significant challenges to enhance sensitivity, selectivity and speed for the analysis of Dioxin and dioxin-like compounds while reducing analytical costs. Dioxin sample preparation methods are evolving toward more automated or semi-automated extraction and sample preparation systems that analyze multiple analyte groups of POPs including polybrominated dioxins/furans, PBDEs, and PCNs. The current trend is toward lower detection limits as toxicity and risk is now better understood. Analytical instrument sensitivity is much lower in recent years and blank levels are the limiting factor in reducing detection limits in many cases. The current limitation is the availability of analytical instrumentation that can separate

and detect the many closely related compounds at levels low enough for protection of human health and the environment in a single analytical run. More sensitive multi-analyte group screening techniques that require reduced sample preparation as a result of the advancement of chromatographic techniques like multidimensional chromatography and fast scanning mass spectrometric detectors will greatly aid in the advancement for the analysis of Dioxin and related dioxin-like POPs. The development of full scanning analytical instrumentation with the sensitivity and selectivity of current HRMS instruments could be used to analyze target and non-target compounds a levels low enough to detect Dioxins and provide results for other known or unknown compounds new and emerging compounds.

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References

1. Cairns T, Fishbein L, Mitchum RK (1980) Review of the dioxin problem. Mass spectrometric analysis of tetrachlorodioxins in environmental samples. Biomed Mass Spectrom 7:484–492
2. Huff JE, Moore JA, Saracci R, Tomatis L (1980) Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ Health Perspect 36:221–240
3. Stockholm Convention Secretariat (2001) UNEP. http://chm.pops.int/
4. Okey AB (2007) An aryl hydrocarbon receptor odyssey to the shores of toxicology: the deichmann lecture, international congress of toxicology-XI. Toxicol Sci 98:5–38
5. Yu ML, Guo YL, Hsu CC, Rogan WJ (2000) Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: long-term follow-up interviews of the women from the Taiwan Yucheng cohort. Int J Epidemiol 29:672–677
6. Birnbaum LS, Tuomisto J (2000) Non-carcinogenic effects of TCDD in animals. Food Addit Contam 17:275–288
7. De Roos AJ, Hartge P, Lubin JH, Colt JS, Davis S, Cerhan JR, Severson RK, Cozen W, Patterson DG Jr, Needham LL, Rothman N (2005) Persistent organochlorine chemicals in plasma and risk of non-Hodgkin's lymphoma. Cancer Res 65:11214–11226
8. Giesy JP, Kannan K (2002) Dioxin-like and non-dioxin like effects of polychlorinated biphenyls: implications for risk assessment. Lakes Reserv Res Manag 7:139–181
9. Hayward D (1998) Identification of bioaccumulating polychlorinated naphthalenes and their toxicological significance. Environ Res 76:1–18
10. Puzyn T, Falandysz J, Jones PD, Giesy JP (2007) Quantitative structure – activity relationships for the prediction of relative in vitro potencies (REPs) for chloronaphthalenes. J Environ Sci Health A Tox Hazard Subst Environ Eng 42:573–590
11. Okey AB, Riddick DS, Harper PA (1994) The Ah receptor: mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Toxicol Lett 70:1–22
12. Safe S (1997) Limitations of the toxic equivalency factor approach for risk assessment of TCDD and related compounds. Teratog Carcinog Mutagen 17:285–304

13. Haws LC, Su SH, Harris M, Devito MJ, Walker NJ, Farland WH, Finley B, Birnbaum LS (2006) Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. Toxicol Sci 89:4–30
14. van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106:775–792
15. van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Toyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE (2006) The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93:223–241
16. van den Berg M, Denison MS, Birnbaum LS, DeVito M, Fiedler H, Falandysz J, Rose M, Schrenk D, Safe S, Tohyama C, Tritscher A, Tysklind M, Peterson RE (2013) Polybrominated dibenzo-p-dioxins (PBDDs), dibenzofurans (PBDFs) and biphenyls (PBBs)-inclusion in the toxicity equivalency factor concept for dioxin-like compounds. Toxicol Sci 133:197–208
17. Bhavsar SP, Reiner EJ, Hayton A, Fletcher R, MacPherson K (2008) Converting toxic equivalents (TEQ) of dioxins and dioxin-like compounds in fish from one toxic equivalency factor (TEF) scheme to another. Environ Int 34(7):915–921
18. Rappe C, Andersson R, Bergqvist P-A, Brohede C, Hansson M, Kjeller L-O, Lindström G, Marklund S, Nygren M, Swanson SE, Tysklind M, Wiberg K (1987) Overview on environmental fate of chlorinated dioxins and dibenzofurans. Sources, levels and isomeric pattern in various matrices. Chemosphere 16:1603–1618
19. Rappe C (1994) Dioxin, patterns and source identification. Fresenius J Anal Chem 348:63–75
20. Srogi K (2008) Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review. Environ Chem Lett 6:1–28
21. Shen L, Gewurtz SB, Reiner EJ, MacPherson KA, Kolic TM, Helm PA, Brindle ID, Marvin CH (2008) Patterns and sources of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in surficial sediments of Lakes Erie and Ontario. Environ Pollut 156:515–525
22. Parette R, Pearson WN (2014) 2,4,6,8-Tetrachlorodibenzothiophene in the Newark Bay Estuary: the likely source and reaction pathways. Chemosphere 111:157–163
23. Malisch R, Kotz A (2014) Dioxins and PCBs in feed and food—review from European perspective. Sci Total Environ 491:2–10
24. Hoogenboom R, Traag W, Fernandes A, Rose M (2015) European developments following incidents with dioxins and PCBs in the food and feed chain. Food Control 50:670–683
25. Fidalgo-Used N, Blanco-Gonzalez E, Sanz-Medel A (2007) Sample handling strategies for the determination of persistent organic pollutants from biota samples. Anal Chim Acta 590:1–16
26. Reiner EJ (2010) Analysis of dioxin and related compounds". Mass Spectrom Rev 29 (4):526–559
27. Reiner EJ, Jobst KJ, Megson D, Dorman FL, Focant JF (2014) Analytical methodology of POPs. In: O'Sullivan G, Sandau C (eds) Environmental forensics for persistent organic pollutants. Elsevier, Amsterdam, pp 59–139
28. Rawa-Adkonis M, Wolska L, Namiesnik J (2003) Modern techniques of extraction of organic analytes from environmental matrices. Crit Rev Anal Chem 33(3):199–248
29. Dean EW, Stark DD (1920) A convenient method for the determination of water in petroleum and other organic emulsions. Ind Eng Chem 12(5):486–490
30. US EPA Method 1613 (1994) Revision B: tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS, EPA 821-B94-0059 Office of Water

31. Antunes P, Viana P, Vinhas T, Capelo JL, Rivera J, Gaspar EMSM (2008) Optimization of pressurized liquid extraction (PLE) of dioxins-furans and dioxin-like PCBs from environmental samples. Talanta 75:916–925
32. Ricther BE, Jones BA, Ezzell JL, Porter NL, Avdalovic N, Pohl C (1996) Accelerated solvent extraction: a technique for sample preparation. Anal Chem 68:1033–1039
33. Saito K, Takekuma M, Ogawa M, Kobayashi S, Sugawara Y, Ishizuka M, Nakazawa H, Matsuki Y (2003) Extraction and cleanup methods of dioxins in house dust from two cities in Japan using accelerated solvent extraction and disposable multi-layer silica-gel cartridge. Chemosphere 53:137–142
34. Srogi K (2007) A review: application of microwave techniques for environmental analytical chemistry. Anal Lett 39:1261–1288
35. Sun Y, Takaoka M, Takeda N, Matsumoto T, Oshita K (2005) Application of water as a solvent in microwave-assisted extraction for analysis of PCBs and CBzs in fly ash. J Sep Sci 28:585–588
36. Van Beuzekom AC, Hijman WC, Berkhoff CJ, Stoffelsen BHG, DenBoer AC, Groenemeijer GS, Mooibroek D, Hoogerbrugge R, Broekman MH, Baumann RA, Hogendoorn EA (2004) Fast sample preparation involving MASE and coupled column normal phase liquid chromatography for the rapid trace analysis of dioxins in air-dust samples from fire catastrophe emissions. Talanta 63:1183–1191
37. Wiberg K, Sporring S, Haglund P, Bjorklund E (2007) Selective pressurized liquid extraction of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls from food and feed samples. J Chromatogr A 1138:55–64
38. Do L, Lundstedt S, Haglund P (2013) Optimization of selective pressurized liquid extraction for extraction and in-cell clean-up of PCDD/Fs in soils and sediments. Chemosphere 90:2414–2419
39. Do L, Xuan TH, Lundstedt S, Haglund P (2013) Modular pressurized liquid extraction for simultaneous extraction, clean-up and fractionation of PCDD/Fs in soil, sediment and sludge samples. Anal Methods 5(5):1231–1237
40. Bernert JT, Turner WE, Patterson DG Jr, Needham LL (2007) Calculation of serum "total lipid" concentrations for the adjustment of persistent organohalogen toxicant measurements in human samples. Chemosphere 68(5):824–831
41. Mannila M, Koistinen J, Vartiainen T (2002) Development of supercritical fluid extraction with a solid-phase trapping for fast estimation of toxic load of polychlorinated dibenzo-p-dioxins-dibenzofurans in sawmill soil. J Chromatogr A 975:189–198
42. Miyawaki T, Kawashima A, Honda K (2008) Development of supercritical carbon dioxide extraction with a solid phase trap for dioxins in soils and sediments. Chemosphere 70:648–655
43. Garcia-Rodriguez D, Carro-Diaz AM, Lorenzo-Ferreira RA (2008) Supercritical fluid extraction of polyhalogenated pollutants from aquaculture and marine environmental samples: a review. J Sep Sci 31:1333–1345
44. Taylor KZ, Waddell DS, Reiner EJ, MacPherson KA (1995) Direct elution of solid phase extraction disks for the determination of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in effluent samples. Anal Chem 67:1186–1190
45. Pujadas E, Diaz-Ferrero J, Marti R, Broto-puig F, Comellas L, Rodriguez-Larena MC (2001) Application of the new C_{18} speedisks™ to the analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans in water and effluent sample. Chemosphere 43:449–454
46. Focant JF, DePauw E (2002) Fast automated extraction and clean-up of biological fluids for polychlorinated dibenzo-p-dioxins, dibenzofurans and coplanar polychlorinated biphenyls analysis. J Chromatogr B 776:199–212
47. Erger C, Schmidt TC (2014) Disk-based solid-phase extraction analysis of organic substances in water. Trends Anal Chem 61:74–82

48. Choi JW, Lee JH, Moon BS, Baek KH (2007) Semi-automated disk-type solid-phase extraction method for polychlorinated dibenzo-p-dioxins and dibenzofurans in aqueous samples and its application to natural water. J Chromatogr A 1157:17–22
49. Youn YY, Park DH, Lee YH, Lim YH, Cho HS (2015) High-throughput method of dioxin analysis in aqueous samples using consecutive solid phase extraction steps with the new C18 Ultraflow™ pressurized liquid extraction and automated clean-up. Chemosphere 118:124–129
50. Prieto A, Zuloaga O, Usobiaga A, Etxebarria N, Fernandez LA (2007) Development of a stir bar sorptive extraction and thermal desorption-gas-chromatography-mass spectrometry method for the simultaneous determination of several persistent organic pollutants in water samples. J Chromatogr A 1174:49–49
51. Petty JD, Orazio CE, Huckins JN, Gale RW, Lebo JA, Meadows JC, Echols KR, Cranor WL (2000) Considerations involved with the use of semipermeable membrane devices for monitoring environmental contaminants. J Chromatogr A 879:83–95
52. Kot-wasik A, Zabiegala B, Urbanowicz M, Dominiak E, Wasik A, Namiesnik J (2007) Advances in passive sampling in environmental studies. Anal Chim Acta 602:141–163
53. Vrana B, Mills GA, Allan IJ, Domiiak E, Svensson K, Knutsson J, Morrison G, Greenwood R (2005) Passive sampling techniques for monitoring pollutants in water. Tends Analyt Chem 24:845–868
54. Namiesnik J, Zabigala B, Kot-Wasik A, Partyaka M, Wasik A (2005) Passive sampling and/or extraction techniques in environmental analysis: a review. Anal Bioanal Chem 381:279–301
55. Lohmann R (2011) Critical review of low-density polyethylene's partitioning and diffusion coefficients for trace organic contaminants and implications for its use as a passive sampler. Environ Sci Technol 46:606–618
56. Jacquet R, Miège C, Smedes F, Tixier C, Tronczynski J, Togola A, Berho C, Valor I, Llora J, Barilon B, Marchand P, Coquery M (2014) Comparison of five integrative samplers in laboratory for the monitoring of indicator and dioxin-like polychlorinated biphenyls in water. Chemosphere 98:18–27
57. Addeck A, Croes K, Van Langenhove K, Denison MS, Elhamalawy A, Elskens M, Baeyens W (2014) Time-integrated monitoring of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in urban and industrial wastewaters using a ceramic toximeter and the CALUX bioassay. Chemosphere 94:27–35
58. Friedman CL, Cantwell MG, Lohmann R (2012) Passive sampling provides evidence for Newark Bay as a source of polychlorinated dibenzo-p-dioxins and furans to the New York/New Jersey, USA, atmosphere. Environ Toxicol Chem 31:253–261
59. Lucaciu CM, Fayez L, Reiner EJ, Kolic TM, Boden AR, MacPherson KA, Crozier PW, Wania F, Emerson R (2004) Comparison of dioxin-like PCBs in passive air and vegetation samples surrounding a metal reclamation incinerator. Organohalogen Compd 66:153–156
60. Xie Z, Ebinghaus R (2008) Analytical methods for the determination of emerging organic contaminants in the atmosphere. Anal Chim Acta 610:156–178
61. Vilavert L, Nadal M, Schuhmacher M, Domingo JL (2014) Seasonal surveillance of airborne PCDD/Fs, PCBs and PCNs using passive samplers to assess human health risks. Sci Total Environ 466:733–740
62. Rayne S, Ikonomou MG, Butt CM, Diamond ML, Troung J (2005) Polychlorinated dioxins and furans from the World Trade Center attacks in exterior window films from lower Manhattan in New York City. Environ Sci Technol 39:1995–2003
63. Pleil JD, Lorber MN (2007) Relative congener scaling of polychlorinated dibenzo-p-dioxins and dibenzofurans to estimate building fire contributions in air, surface wipes, and dust samples. Environ Sci Technol 41:7286–7293
64. Clarkson PJ, Larrazabal-Moya D, Staton I, McLeod CW, Ward DB, Sharifi VN, Swithenbank J (2002) The use of tree bark as a passive sampler for polychlorinated dibenzo-p-dioxins and furans. Int J Environ Anal Chem 82:843–850

65. Hermanson MH, Johnson GW (2015) Chlorinated dibenzo-p-dioxin and dibenzofuran congener and homologue distributions in tree bark from Sauget, Illinois, USA. Environ Sci Technol 49:855–862
66. Lamparski LL, Nesterick TJ, Stehl RH (1979) Determination of part-per-trillion concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin in fish. Anal Chem 51:1453–1458
67. Smith LM, Stallings DL, Johnson JL (1984) Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. Anal Chem 56:1830–1842
68. Poole SK, Dean TA, Oudsema JW, Poole CF (1990) Sample preparation for chromatographic separations: an overview. Anal Chim Acta 236:3–42
69. Kloskowski A, Chrzanowski W, Pilarczyk M, Namiesnik J (2007) Modern techniques of sample preparation for the determination of organic analytes by gas chromatography. Crit Rev Anal Chem 37:15–38
70. Krokos F, Creaser CS, Wight C, Startin JR (1997) Congener-specific method for the determination of ortho and non-ortho polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in foods by carbon-column fractionation and gas chromatography-isotope dilution mass spectrometry. Fresenius J Anal Chem 357:732–742
71. Fernandez A, White S, D'Silva K, Rose M (2004) Simultaneous determination of PCDDs, PCDFs, PCBs and PBDEs in food. Talanta 63:1147–1155
72. Djien Liem AK (1999) Basic aspects of methods for the determination of dioxins and PCBs in foodstuffs and human tissues. Tends Analyt Chem 18:429–439
73. Santos FJ, Galceran MT (2002) Application of gas chromatography to environmental analysis. Tends Analyt Chem 21:672–685
74. Huestis SY, Sergeant DB (1992) Removal of chlorinated diphenyl ether interferences for analyses of PCDDs and PCDFs in fish. Chemosphere 24:537–545
75. Norstrom RJ, Simon M, Mulvihil MJ (1986) A gel-permeation/column chromatography cleanup method for the determination of CDDs in animal tissue. Int J Environ Anal Chem 23:267–287
76. Focant JF, Eppe G, Pirard C, DePauw E (2001) Fast clean-up for polychlorinated dibenzo-p-dioxins, dibenzofurans and coplanar biphenyls of high-fat-content biological samples. J Chromatogr A 925:207–221
77. Ontario Ministry of the Environment (2014) The determination of polychlorinated dibenzo-p-dioxins, polychlorinated furans, dioxin-like polychlorinated biphenyls, polychlorinated naphthalenes and polybrominated diphenyl ethers in biota by automated sample extraction/cleanup and gas chromatography high resolution mass spectrometry (GC-HRMS). Environment Ontario Laboratory Services Branch Method E3481. Toronto, ON, Canada
78. Fayez L, Brunato R, Reiner E (2005) Comparison of manual and automated extraction and clean-up of PCDDs, PCDFs and DLPCBs in fish tissue. Organohalogen Compd 67:565–568
79. Fayez LB, Reiner EJ, Kolic TM, Brunato R, Lega R, MacPherson KA, Crozier PW (2008) An automated extraction/sample preparation method for the determination of PCDD/F, DLPCBs, BDEs and PCNs in biota in a single analysis. Organohalogen Compd 70:1296–1299
80. Focant JF, Eppe G, Massart AC, Scholl G, Pirard C, DePauw E (2006) High-throughput biomonitoring of dioxins and polychlorinated biphenyls at the sub-picogram level in human serum. J Chromatogr A 1130:97–107
81. Yang S, Reiner EJ, Kolic TM, Lega R, Harrison RO (2010) A rapid and simple cleanup method for the analysis of dioxins/furans, dioxin-like PCBs, PBDEs and PCNs. Organohalogen Compd 72:1788–1791
82. Yang S, Reiner EJ, Harrison RO, Kolic T, MacPherson K (2013) Determination of dioxins/furans (PCDD/Fs), dioxin-like PCBs, polybrominated diphenylethers (PBDEs) and polychlorinated naphthalenes (PCNs) in clean water using the cape technologies technique. Organohalogen Compd 75:739–742

83. Haglund P, Sporring S, Wiberg K, Björklund E (2007) Shape-selective extraction of PCBs and dioxins from fish and fish oil using in-cell carbon fractionation pressurized liquid extraction. Anal Chem 79:2945–2951
84. Spinnel E, Danielsson C, Haglund P (2008) Rapid and cost-effective analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in soil, fly ash and sediment certified reference materials using pressurized liquid extraction with an integrated carbon trap. Anal Bioanal Chem 390:411–417
85. Nording M, Sporring S, Wiberg K, Borklund E, Haglund P (2005) Monitoring dioxins in food and feedstuffs using accelerated solvent extraction with a novel integrated carbon fractionation cell in combination with a CAFLUX bioassay. Anal Bioanal Chem 381:1472–1475
86. Alaee M, Backus S, Cannon C (2001) Potential interference of PBDEs in the determination of PCBs and other organochlorine contaminants using electron capture detection. J Sep Sci 29:465–469
87. Wellington Laboratories Reference and Handling Guide (2015) http://well-labs.com/docs/gcms_reference_handling_guide.pdf. Accessed 5 Feb 2016
88. Bacher R, Ballschmitter K (1992) Separation of polychlorinated-p-dioxins and dibenzofurans on the new polar stationary phase DB DIOXIN. Chromatographia 34:137–142
89. Dorman F, Reese S, Reiner E, MacPherson K, Focant J, Cochran J (2004) Improved separation and quantification of dioxin and furan congeners using novel low-bleed capillary gas chromatography columns and mass spectrometry. Organohalogen Compd 66:834–837
90. Cochran J, Dorman F, Stidsen G, Reese S, MacPherson K, Kolic T, Reiner E, Ryan J, Bradley J, Craig D, Priest P (2007) Retention time profiling for all 136 tetra- through octachlorinated dioxins and furans on a unique, low-bleed, thermally-stable gas chromatography column. Organohalogen Compd 69:477–480
91. Cochran J, Dorman F, Stidsen G, Reese S, MacPherson K, Kolic T, Reiner E, Ryan J, Bradley J, Craig D, Priest P (2007) Retention and co-elutions for all 136 tetra- through octachlorinated dioxins and furans on a unique, low-bleed, thermally-stable gas chromatography column. Organohalogen Compd 69:1146–1149
92. Otaka H, Shimono M, Hashimoto S (2004) Optimization of solid-phase extraction procedure for determination of polychlorinated dibenzo- *p*-dioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls in humic acid containing water. Anal Bioanal Chem 378:1854–1860
93. Fishman VN, Martin GD, Lamparski LL (2004) Comparison of series 5 gas chromatography column performances from a variety of manufacturers for separation of chlorinated dibenzo-p-dioxins and dibenzofurans using high resolution mass spectrometry. J Chromatogr A 1057:151–161
94. Fishman VN, Martin GD, Lamparski LL (2007) Comparison of a variety of gas chromatographic columns with different polarities for the separation of chlorinated dibenzo-p-dioxins and dibenzofurans by high resolution mass spectrometry. J Chromatogr A 1139:285–300
95. Fishman VN, Martin GD, Wilken M (2011) Retention time profiling of all 136 tetra-through octa-chlorinated dibenzo-p-dioxins and dibenzofurans on a variety of si-arylene gas chromatographic stationary phases. Chemosphere 84:913–922
96. Abad E, Caixach J, Rivera J (1997) Application of DB-5ms gas chromatography column for the complete assignment of 2,3,7,8-substituted polychlorodibenzo-p-dioxins and polychlorodibenzofurans in samples from municipal waste incinerator emissions. J Chromatogr A 786:125–134
97. Chang-Chien GP, Lee WS, Tsai JL, Jeng SH (2001) Liquid crystalline polysiloxane polymer as stationary phase in gas chromatography capillary column for the separation of dioxin/furan compounds. J Chromatogr A 932:97–105
98. Naikwadi N, Britten A, Reiner EJ (2005) Development and application of isomer specific GC capillary columns for PAH and PCDD analysis. Organohalogen Compd 67:404–407

99. Do L, Liljelind P, Zhang J, Haglund P (2013) Comprehensive profiling of 136 tetra-to octa-polychlorinated dibenzo-p-dioxins and dibenzofurans using ionic liquid columns and column combinations. J Chromatogr A 1311:157–169
100. Do L, Geladi P, Haglund P (2014) Multivariate data analysis to characterize gas chromatography columns for dioxin analysis. J Chromatogr A 1347:137–145
101. Matisova E, Domotorova M (2003) Fast gas chromatography and its use in trace analysis. J Chromatogr A 1000:199–221
102. Mastovska K (2008) Recent developments in chromatographic techniques. Compr Analyt Chem 51:175–200
103. Korytar P, Janssen HG, Matisova E, Brinkman UAT (2002) Practical fast gas chromatography: methods, instrumentation and applications. Tends Analyt Chem 21:558–572
104. Reiner EJ, MacPherson KA, Brunato R, Chen T, Bogard MA, Boden AR, Ladwig G (2000) Analysis of persistent organic pollutants (POPs) using microbore columns. Organohalogen Compd 45:17–20
105. Clement R, Reiner EJ (2012) Analysis of persistent halogenated compounds: chlorinated dibenzo-p-dioxins and dibenzofurans. In: Lebedev AT (ed) Comprehensive environmental mass spectrometry. ILM Publications, St Albans, pp 329–352
106. Focant JF, Reiner EJ, MacPherson K, Kolic T, Sjodin A, Patterson DG Jr, Reese SL, DormanFL CJ (2004) Measurement of PCDDs, PCDFs, and non-ortho-PCBs by comprehensive two-dimensional gas chromatography-isotope dilution time-of-flight mass spectrometry (GCxGC-IDTOFMS). Talanta 63:1231–1240
107. Hoh E, Lehotay SJ, Mastovska K, Huwe JK (2008) Evaluation of automated direct sample introduction with comprehensive two-dimensional gas chromatography/time of flight mass spectrometry for the screening analysis of dioxins in fish oil. J Chromatogr A 1201:69–77
108. Adahchour M, Beens J, Brinkman UAT (2008) Recent developments in the application of comprehensive two-dimensional gas chromatography. J Chromatogr A 1186:67–108
109. Bordajandi LR, Ramos JJ, Sanz J, Gonzalez MJ, Ramos L (2008) Comprehensive two-dimensional gas chromatography in the screening of persistent organohalogenated pollutants in environmental samples. J Chromatogr A 1186:312–324
110. Hoh E, Mastovska K, Lehotay SJ (2007) Optimization of separation and detection conditions for comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans. J Chromatogr A 1145:210–221
111. De Vos J, Dixon R, Vermeulen G, Gorst-Allman P, Cochran J, Rohwer E, Focant JF (2011) Comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GCx GC-TOFMS) for environmental forensic investigations in developing countries. Chemosphere 82:1230–1239
112. Shunji H, Yoshikatsu T, Akihiro F, Hiroyasu I, Kiyoshi T, Yasuyuki S, Masa-aki U, Akihiko K, Kazuo T, Hideyuki O, Katsunori A (2008) Quantification of polychlorinated dibenzo-p-dioxins and dibenzofurans by direct injection of sample extract into the comprehensive multidimensional gas chromatograph/high-resolution time-of-flight mass spectrometer. J Chromatogr A 1178:187–198
113. Korytar AP, Parera J, Leonards PEG, DeBoer J, Brinkman UAT (2005) Quadrupole mass spectrometer operating in the electron-capture negative ion mode as detector for comprehensive two-dimensional gas chromatography. J Chromatogr A 1067:255–264
114. Focant JF, Eppe G, Scipo ML, Massart AC, Pirard C, Maghuin-Rogister G, DePauw E (2005) Comprehensive two-dimensional gas chromatography with isotope dilution time-of-flight mass spectrometry for the measurement of dioxins and polychlorinated biphenyls in foodstuffs comparison with other methods. J Chromatogr A 1086:46–60
115. Poole SK, Poole CF (2008) The orthogonal character of stationary phases for gas chromatography. J Sep Sci 31:1118–1123
116. Muscalu AM, Reiner EJ, Liss SN, Chen T, Ladwig G, Morse D (2011) A routine accredited method for the analysis of polychlorinated biphenyls, organochlorine pesticides,

chlorobenzenes and screening of other halogenated organics in soil, sediment and sludge by GCxGC-μECD. Anal Bioanal Chem 401:2403–2413
117. Haglund P, Korytar P, Danielsson C, Diaz J, Wiberg K, Leonards P, Brinkman IAT, deBoer J (2008) GCxGC-ECD: a promising method for the determination of dioxins and dioxin-like PCBS in food and feed. Anal Bioanal Chem 390:1815–1827
118. Patterson DG, Welch SM, Turner WE, Sjödin A, Focant JF (2011) Cryogenic zone compression for the measurement of dioxins in human serum by isotope dilution at the attogram level using modulated gas chromatography coupled to high resolution magnetic sector mass spectrometry. J Chromatogr A 1218:3274–3281
119. Crummett WB, Stehl RH (1973) Determination of chlorinated dibenzo p dioxins and dibenzofurans in various materials. Envrion Health Perspect 5:15–25
120. Baughman R, Meselson M (1973) An analytical method for detecting TCDD (dioxin): levels of TCDD in samples from Vietnam. Envrion Health Perspect 5:27–35
121. Bethem R, Boison J, Gale J, Heller D, Lehotay S, Loo J, Musser S, Price P, Stein S (2003) Establishing the fitness for purpose of mass spectrometric methods. J Am Soc Mass Spectrom 14:528–541
122. Clement RE (1991) Ultratrace dioxin and dibenzofuran analysis: 30 years of advances. Anal Chem 63:1130A–1139A
123. Reiner EJ, Clement RE, Okey AB, Marvin CH (2006) Advances in the analytical techniques for polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin-like PCBs. Anal Bioanal Chem 386:791–806
124. Clement RE, Tosine HM (1988) The gas chromatography/mass spectrometry determination of chlorinated dibenzo-p-dioxins and dibenzofurans. Spectrom, Mass
125. Eppe G, De Pauw E, Focant JF (2006) High-resolution GC coupled to high-resolution MS in the analysis of dioxins and related substances, principles and applications. In: Niessen WMA (ed) The encyclopedia of mass spectrometry, vol 8, Hyphenated methods. Elsevier, Oxford, pp 531–541
126. Focant JF, Pirard C, Eppe G, DePauw E (2005) Recent advances in mass spectrometric measurement of dioxins. J Chromatogr A 1067:265–275
127. Focant JF, Sjödin A, Patterson DG (2006) Human biomonitoring of persistent toxicants using comprehensive Two-dimensional Gas chromatography and time-of-flight mass spectrometry. In: Niessen WMA (ed) The encyclopedia of mass spectrometry, vol 8, Hyphenated methods. Oxford, Elsevier, pp 553–564
128. de Boer J (1999) Capillary gas chromatography for the determination of halogenated micropollutants. J Chromatogr A 843:179–198
129. Eljarrat E, Barcelo D (2002) Congener specific determination of dioxins and related compounds by gas chromatography coupled to LRMS, HRMS, MS/MS and TOFMS. J Mass Spectrom 37:1105–1117
130. Scippo ML, Eppe G, DePauw E, Saegerman C, Scholl G, DePauw E, Maghuin-Rogister G, Focant JF (2008) Persistent organochlorine pollutants, dioxins and polychlorinated biphenyls. Compr Analyt Chemistry 51:457–506
131. Reiner EJ, Schellenberg DH, Taguchi VY, Mercer RS, Townsend JA, Thompson TS, Clement RE (1990) Application of tandem quadrupole mass spectrometry for the ultratrace determination of polychlorinated dibenzo-p-dioxins and dibenzofurans. Chemosphere 20:1385–1392
132. Reiner EJ, Schellenberg DH, Taguchi VY (1991) Environmental applications for the analysis of chlorinated dibenzo-p-dioxins and dibenzofurans using mass spectrometry/mass spectrometry. Environ Sci Technol 25:110–117
133. Ingelido AM, Brambilla G, Abballe A, Domenico A, Fulgenzi AR, Iacovella N, Iamiceli AL, Valentini S, Felip ED (2012) 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. Rapid Commun Mass Spectrom 26:236–242

134. L'Homme B, Scholl G, Eppe G, Focant JF (2015) Validation of a gas chromatography–triple quadrupole mass spectrometry method for confirmatory analysis of dioxins and dioxin-like polychlorobiphenyls in feed following new EU Regulation 709/2014. J Chromatogr A 1376:149–158
135. García-Bermejo Á, Ábalos M, Sauló J, Abad E, González MJ, Gómara B (2015) Triple quadrupole tandem mass spectrometry: a real alternative to high resolution magnetic sector instrument for the analysis of polychlorinated dibenzo-p-dioxins, furans and dioxin-like polychlorinated biphenyls. Anal Chim Acta 889:156–165
136. Tonduer Y, Niederhut WJ, Missler SR (1987) A hybrid HRGC/MS/MS method for the characterization of tetrachlorodibenzo-p-dioxins in environmental samples. BioMed Environ Mass Spectr 14:449–456
137. Plombley JB, Lausevic M, March RE (2000) Determination of dioxins/furans and PCBs by quadrupole ion-trap gas chromatography–mass spectrometry. Mass Spectrom Rev 19:305–365
138. Pirard C, DePauw E, Focant JF (2003) New strategy for comprehensive analysis of polybrominated diphenyl ethers, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by gas chromatography coupled with mass spectrometry. J Chromatogr A 998:169–181
139. Malavia J, Santos FJ, Galceran MT (2008) Comparison of gas chromatography-ion-trap tandem mass spectrometry systems for the determination of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like biphenyls. J Chromatogr A 1186:302–311
140. Peterson AC, McAlister GC, Quarmby ST, Griep-Raming J, Coon JJ (2010) Development and characterization of a GC-enabled QLT-Orbitrap for high-resolution and high-mass accuracy GC/MS. Anal Chem 82(20):8618–8628
141. Peterson AC, Hauschild JP, Quarmby ST, Krumwiede D, Lange O, Lemke RA, Grosse-Coosmann F, Horning S, Donohue TJ, Westphall MS, Coon JJ, Griep-Raming J (2014) Development of a GC/quadrupole-orbitrap mass spectrometer, part I: design and characterization. Anal Chem 86(20):10036–10043
142. Saito K, Ohmura A, Takekuma M, Sasano R, Matsuki Y, Nakazawa H (2007) Application of a novel large-volume injection method using a stomach-shaped inlet liner in capillary gas chromatographic trace analysis of dioxins in human milk and plasma. Anal Sci 23:661–666
143. Zhao Y, Yang L, Wang Q (2007) Pulsed large volume injection gas chromatography coupled with electron-capture negative chemical ionization quadrupole mass spectrometry for the simultaneous determination of typical halogenated persistent organic pollutants. J Am Soc Mass Spectrom 18:1375–1386
144. Eppe G, Focant JF, Pirard C, DePauw E (2004) PTV-LV-GC/MS/MS as screening and complementary method to HRMS for the monitoring of dioxin levels in food and feed. Talanta 63:1135–1146
145. Ho NH, Bugey A, Zimmerli P, Nançoz J, Ortelli D, Edder P (2014) Targeted screening and quantification of dl-PCBs and dioxins in various foodstuffs by programmed-temperature vaporizer large-volume injection coupled to GC–MS. CHIMIA Int J Chem 68(10):732–738
146. Shen H, Guan R, Li J, Zhang L, Ren Y, Xu X, Song Y, Zhao Y, Han J, Wu Y (2013) Determination of dioxin-like polychlorinated biphenyls in 1mL whole blood using programmable temperature vaporization large volume injection coupled to gas chromatogram and high-resolution mass spectrometry. Anal Chim Acta 767:112–117
147. Calder IC, Johns RB, Desmarchelier JM (1970) The mass spectra of dibenzo-p-dioxin and group IV analogues. Org Mass Spectrom 4:121–131
148. Harrison AG (1992) Chemical ionization mass spectrometry. CRC, Boca Raton
149. Chernestsova ES, Revelsky AI, Revelsky IA, Mikhasenko IA, Sobolevsky TG (2002) Determination of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls by gas chromatography/mass spectrometry in the negative chemical ionization mode with different reagent gases. Mass Spectrom Rev 21:373–387

150. Khatymov RV, Muftakhov MV, Schukin PV, Mazunov VA (2004) Specific features of the resonant electron attachment to the chlorodibenzo-p-dioxin molecules. Russ Chem Bull 53 (4):738–741
151. Koester CJ, Harless RL, Hites RA (1992) Comparative analysis of dioxins and furans in ambient air by high resolution and electron capture mass spectrometry. Chemosphere 24:421–426
152. Buser HR, Rappe C, Bergqvist PA (1985) Analysis of polychlorinated dibenzofurans. Dioxins and related compounds in environmental samples. Environ Health Perspect 60:293–302
153. Mahle NH, Shadoff LA (1982) The mass spectrometry of chlorinated dibenzo-p-dioxins. Biomed Mass Spectrom 9(2):45–60
154. Hass JR, Friesen MD (1979) The mass spectrometry of polychlorinated dibenzo-p-dioxins. Org Mass Specrom 14:9–16
155. Reiner EJ, Worrall K, Hancock P, Silcock P, Shen L, MacPherson KA (2010) Analysis of persistent halogenated organics using atmospheric pressure gas chromatography (APGC). Organohalogen Compd 72:1273–1276
156. Myers AL, Watson-Leung T, Jobst KJ, Shen L, Besevic S, Organtini K, Dorman FL, Mabury SA, Reiner EJ (2014) Complementary nontargeted and targeted mass spectrometry techniques to determine bioaccumulation of halogenated contaminants in freshwater species. Environ Sci Technol 48:13844–13854
157. van Bavel B, Geng D, Cherta L, Nácher-Mestre J, Portolés T, Ábalos M, Saulo J, Abad E, Dunstan J, Jones R, Kitz A, Winterhalter H, Malisch R, Traag W, Hagberg J, Ericson Jogsten I, Bletran J, Hernández F (2015) Atmospheric-pressure chemical ionization tandem mass spectrometry (APGC/MS/MS) an alternative to high-resolution mass spectrometry (HRGC/HRMS) for the determination of dioxins. Anal Chem 87(17):9047–9053
158. Organtini KL, Haimovici L, Jobst KJ, Reiner EJ, Ladak A, Stevens D, Cochran J, Dorman FL (2015) Comparison of atmospheric pressure ionization gas chromatography-triple quadrupole mass spectrometry to traditional high-resolution mass spectrometry for the identification and quantification of halogenated dioxins and furans. Anal Chem 87(15):7902–7908
159. Boyd RK, Basic C, Bethem RA (2008) Trace quantitative analysis by mass spectrometry. Wiley, Chichester, England
160. Lavagnini I, Magno F, Seraglia R, Traldi P (2008) Quantitative applications of mass spectrometry. Wiley, Chichester, England
161. Ende M, Spiteller G (1982) Contaminants in mass spectrometry. Mass Spectrom Rev 1:29–62
162. Ferrario J, Byrne C, Dupuy AE (1997) Background contamination by coplanar polychlorinated biphenyls (PCBs) in the trace level high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) analytical procedures. Chemosphere 34 (11):2451–2465
163. Ferrario J, Byrne C, McDaniel D, Dupuy A Jr (1996) Determination of 2,3,7,8-chlorine substituted dibenzo-p-dioxins and –furans at the part per trillion level in United States beef fat using high resolution gas chromatography/high resolution mass spectrometry. Anal Chem 68:647–652
164. US EPA (1997) Guidelines for establishing test procedures for the analysis of pollutants, App B, Part 136, definition and procedures for the determination of method detection
165. Ontario Ministry of the Environment (2014) The determination of polychlorinated dibenzo-p-dioxins, polychlorinated furans and dioxin-like PCBs in environmental matrices by GC-HRMS. Environment Ontario Laboratory Services Branch Method DFPCB-E3418. Toronto, ON, Canada
166. Phua STG, Ashman PJ, Daughtry BJ (2008) Levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in fillets of farmed southern bluefin tuna (thunnus maccoyii). Chemosphere 73:915–922

167. Eichbaum K, Brinkmann M, Buchinger S, Reifferscheid G, Hecker M, Giesy JP, Engwal M, van Bavel B, Hollert H (2014) In vitro bioassays for detecting dioxin-like activity—application potentials and limits of detection, a review. Sci Total Environ 487:37–48
168. Levy W, Brena BM, Henkelmann B, Bernhöf S, Pirez M, González-Sapienza G, Schramm KW (2014) Screening of dioxin-like compounds by complementary evaluation strategy utilising ELISA, micro-EROD, and HRGC-HRMS in soil and sediments from Montevideo, Uruguay. Toxicology In Vitro 28:1036–1045
169. Koh C, Khim JS, Villeneuve DL, Kannan K, Johnson BG, Giesy JP (2005) Instrumental and bioanalytical measures of dioxin-like and estrogenic compounds and activities associated with sediment from the Korean coast. Ecotoxicol Environ Saf 61:366–379
170. Windal I, Denison MS, Birnbaum LS, Van Wouwe N, Baeyens W, Goeyens L (2005) Chemically activated luciferase gene expression (CALUX) cell bioassay analysis for the estimation of dioxin-like activity: critical parameters of the CALUX procedure that impact assay results. Environ Sci Technol 39:7357–7364
171. Windal I, VanWouwe N, Eppe G, Xhrouet C, Debacker V, Baeyens W, DePauw E, Goeyens L (2005) Validation and interpretation of CALUX as a tool for the estimation of dioxin-like activity in marine biological matrixes. Environ Sci Technol 39:1741–1748
172. Van Wouwe N, Windal I, Vanderperren H, Eppe G, Xhrouet C, Massart AC, Debacker N, Sasse A, Baeyens W, De Pauw E, Sartor F, VanOyen H, Goeyens L (2004) Validation of the CALUX bioassay for PCDD/F analyses in human blood plasma and comparison with GC-HRMS. Talanta 63:1157–1167
173. Van Wouwe N, Windal I, Vanderperren H, Eppe G, Xhrouet C, De Pauw E, Goeyens L, Baeyens W (2004) Importance of clean-up for comparison of TEQ-values obtained by CALUX and chemo-analysis. Talanta 63:1269–1272
174. Shan G, Leeman WR, Gee SJ, Sanborn JR, Jones AD, Chang DPY, Hammock BD (2001) Highly sensitive dioxin immunoassay and its application to soil and biota samples. Anal Chim Acta 444:169–178
175. Harrison RO, Eduljee GH (1999) Immunochemical analysis for dioxins – progress and prospects. Sci Total Environ 239:1–18
176. Nichkova M, Park EK, Koivunen ME, Kamita SG, Gee SJ, Chuang J, VanEmon JM, Hammock BD (2004) Immunochemical determination of dioxins in sediment and serum samples. Talanta 63:1213–1223
177. Emon JMV, Chuang JC, Lordo RA, Schrock ME, Nichkova M, Gee SJ, Hammock BD (2008) An enzyme-linked immunosorbent assay for the determination of dioxins in contaminated sediment and soil samples. Chemosphere 72:95–103
178. McAlister DR, Fern MJ, Allen LR (2008) Rapid aryl hydrocarbon receptor based polymerase chain reaction screening assay for polychlorinated dibenzo-p-dioxins and furans in soil and sediment. Talanta 74:992–997
179. Chou IC, Lee WJ, Wang LC, Chang-Chien GP, Lee WS, Lee H (2008) Validation of the CALUX bioassay as a screening and semi-quantitative method for PCDD/F levels in cow's milk. J Hazard Mater 154:1166–1172
180. Fochi I, Brambilla G, De Filippis SP, De Luca S, Diletti G, Fulgenzi A, Gallo P, Iacovella N, Scortichini G, Serpe L, Vinci F, di Domenico A (2008) Modeling of DR CALUX bioassay response to screen PCDDs, PCDFs, and dioxin-like PCBs in farm milk from dairy herds. Regul Toxicol Pharmacol 50:366–375
181. Schrock M, Dindal A, Billets S (2009) Evaluation of alternative approaches for screening contaminated sediments and soils for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. J Environ Manage 90:1289–1295
182. Dindal A, Billets S (2008) Experimental design considerations for verifying the performance of screening technologies for dioxin and dioxin-like compounds in soils and sediments. Chemosphere 73:S66–S71

183. Nording M, Denison MS, Baston D, Persson Y, Spinnel E, Haglund P (2007) Analysis of dioxins in contaminated soils with the calux and caflux bioassays, an immunoassay, and gas chromatography/high-resolution mass spectrometry. Environ Toxicol Chem 26:1122–1129
184. Henkelmann B, Schram K-W, Klim C (1996) Quality criteria for the isotope dilution method for HRGC/MS. Fresenius J Anal Chem 354:818–822
185. Eppe G, Cofino WP, DePauw E (2004) Performances and limitations of the HRMS method for dioxins, furans and dioxin-like PCB analysis in animal feeding stuffs part I: results of and interlaboratory study. Anal Chim Acta 519:231–242
186. Eppe G, Maghuin-Rogister G, DePauw E (2004) Performances and limitations of the HRMS method for dioxins, furans and dioxin-like PCB analysis in animal feedingstuffs part II, does it comply with the European pro-active approach. Anal Chim Acta 519:243–253
187. Kiguchi O, Kobayashi T, Saitoh K, Ogawa N (2004) Loss of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls during nitrogen gas blowdown process for ultra-trace analysis. Anal Chim Acta 546:102–111
188. EURACHEM/CITAC (2000) Guide – quantifying uncertainty in analytical measurement, second edition
189. Busch KL (2007) Mass spectrometry forum: quantitative mass spectrometry: part 1. Spectroscopy 22:20–23
190. Fraser CA, Gardner GJ, Maxwell PS, Kubwabo C, Guevremont R, Siu KM, Berman SS (1995) Preparation and certification of a biological reference material (CARP-1) for polychlorinated dibenzo-p-dioxin and dibenzofuran congeners. Fresenius J Anal Chem 352 (1–2):143–147
191. Poster DL, Kucklick JR, Schantz MM, Porter BJ, Sander LC, Wise SA (2007) New developments in standard reference materials (SRMs) for environmental forensics. Environ Forensics 8:181–191
192. Selliah SS, Cussion S, MacPherson KA, Reiner EJ, Toner D (2001) Development of a reference material for routine performance monitoring of methods measuring polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls. Fresenius J Anal Chem 370:208–212
193. Van Leeuwen SPJ, Cleuvenbergen RV, Abalos M, Pasini A-L, Eriksson U, Cleemann M, Hajslova J, De Boer J (2006) New certified and candidate certified reference materials for the analysis of PCBs, PCDD/Fs, OCPs and BFRs in the environment and food. Tends Analyt Chem 25:397–409
194. Van Bavel B, Abad E (2008) Long-term worldwide QA/QC of dioxins and dioxin-like PCBS in environmental samples. Anal Chem 80:3956–3964
195. ISO/IEC 17025 (2005) General requirements for the competence of testing and calibration laboratories. www.ISO.org
196. Eljarrat E, Barcelo D (2003) Priority lists for persistent organic pollutants and emerging contaminants based on their relative toxic potency in environmental samples. Tends Analyt Chem 22:655–665
197. Falandysz J (1998) Polychlorinated naphthalenes: an environmental update. Environ Pollut 101:77–90
198. Hagberg J (2009) Analysis of brominated dioxins and furans by high resolution gas chromatography/high resolution mass spectrometry. J Chromatogr A 1012:376–384
199. Venkatesan AK, Halden RU (2014) Contribution of polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) to the toxic equivalency of dioxin-like compounds in archived biosolids from the US EPA's 2001 National Sewage Sludge Survey. Environ Sci Technol 48:10843–10849
200. Mumbo J, Henkelmann B, Abdelaziz A, Pfister G, Nguyen N, Schroll R, Munch JC, Schramm KW (2015) Persistence and dioxin-like toxicity of carbazole and chlorocarbazoles in soil. Environ Sci Pollut Res 22:1344–1356
201. Pena-Abaurrea M, Jobst KJ, Ruffolo R, Shen L, McCrindle R, Helm PA, Reiner EJ (2014) Identification of potential novel bioaccumulative and persistent chemicals in sediments from

Ontario (Canada) using scripting approaches with GCxGC-ToF MS analysis. Environ Sci Technol 48:9591–9599
202. Weber R, Kuch B (2003) Relevance of BFRs and thermal conditions on the formation pathways of brominated and brominated-chlorinated dibenzodioxins and dibenzofurans. Environ Int 29:699–710
203. Rupp S, Metzger JW (2005) Brominated-chlorinated diphenyl ethers formed by thermolysis of polybrominated diphenyl ethers at low temperatures. Chemosphere 60:1644–1651
204. Van Leeuwen SPJ, de Boer J (2008) Advances in the gas chromatographic determination of persistent organic pollutants in the aquatic environment. J Chromatogr A 1186:116–182
205. Tang HP (2013) Recent development in analysis of persistent organic pollutants under the Stockholm Convention. Trends Anal Chem 45:48–66
206. Donnelly JR, Munslow WD, Vonnahme TL, Nunn NJ, Hedin CM, Sococool GW, Mitchum RK (1987) The Chemistry and mass spectrometry of brominated dibenzo-p-dioxins and dibenzofurans. Biomed Environ Mass Spectrom 14:465–472
207. Donnelly JR, Grange AH, Nunn NJ, Sovocool GW, Breen JJ (1990) Bromo- and bromochloro- dibenzo-p-dioxins and dibenzofurans in the environment. Chemosphere 20:1423–1430
208. Covaci A, Voorspels S, Ramos L, Neels H, Blust R (2007) Recent developments in the analysis of brominated flame retardants and brominated natural compounds. J Chromatogr A 1153:145–171
209. Kolic TM, Shen L, MacPherson K, Fayez L, Gobran T, Helm P, Marvin CH, Arsenault G, Reiner EJ (2009) The analysis of halogenated flame retardants by gas chromatography – high resolution mass spectrometry in environmental samples. J Chromatogr Sci 47:83–91
210. de Boer J, Wells DE (2006) Pitfalls in the analysis of brominated flame retardants in environmental, human and food samples – including results of three interlaboratory studies. Trends Anal Chem 24:364–372
211. Dirtu AC, Ionas AC, Malarvannan G, Covaci A (2014) Transformation products of brominated flame retardants (BFRs). In: Transformation products of emerging contaminants in the environment: analysis, processes, occurrence, effects and risks. Wiley, Chichester, pp 545–576
212. Chatkittikunwong W, Creasei CS (1994) Bromo-, bromochloro- and chloro- dibenzo-p-dioxins and dibenzofurans in incinerator flyash. Chemosphere 29:559–566
213. Donnelly JR, Munslow WD, Grange AH, Pettit TL, Simmons RD, Kumar KS, Sovocool GW (1991) A gas chromatographic/mass spectrometric isomer specific approach for isomer-specific environmental monitoring of the 1700 bromo-, chloro-, and bromo-chloro-dibenzo-p-dioxins. Biol Mass Spectrom 20:329–337
214. Huang LQ, Paivia A, Tong H, Monson SJ, Gross ML (1992) Application of gas chromatography, high resolution mass spectrometry to the determination of trace monobromopolychlorodibenzo-p-dioxins in environmental samples. J Am Soc Mass Spectrom 3:248–259
215. Weber R, Hagenmaier H (1997) Synthesis and analysis of mixed chlorinated-fluorinated dibenzo-p-dioxins and dibenzofurans and assessment of formation and occurrence of the fluorinated and chlorinated-fluorinated dibenzo-p-dioxins and dibenzofurans. Chemosphere 34(1):13–28
216. Weber R, Schrenk D, Schmitz HJ, Hagemaier A, Hagenmaier H (1995) Polyfluorinated dibenzodioxins and dibenzofurans – synthesis, analysis, formation and toxicology. Chemosphere 30:629–639
217. Weber LWD, Griem H (1997) The toxicity of brominated and mixed brominated dibenzo-p-dioxins and dibenzofurans. J Toxicol Environ Health 50:195–215
218. Wiberg K, Rappe C, Haglund P (1992) Analysis of bromo- chloro- and mixed bromo/chloro dibenzo-p-dioxins and dibenzofurans in salmon, osprey and human milk. Chemosphere 24:1431–1439

219. Hayakawa K, Takatsuki H, Watanabe I, Sakai S (2004) Polybrominated diphenyl ethers (PBDEs), polybrominated dibenzo-p-dioxins/dibenzofurans (PBDD/Fs) and monobromo-polychlorinated dibenzo-p-dioxins/dibenzofurans (MoBPXDD/Fs) in the atmosphere and bulk deposition in Kyoto, Japan. Chemosphere 57:343–356
220. Myers AL, Mabury SA, Reiner EJ (2012) Analysis of mixed halogenated dibenzo-p-dioxins and dibenzofurans (PXDD/PXDFs) in soil by gas chromatography tandem mass spectrometry (GC–MS/MS). Chemosphere 87:1063–1069
221. Organtini KL, Myers AL, Jobst KJ, Reiner EJ, Ross B, Ladak A, Cochran J, Dorman FL (2015) Quantitative analysis of mixed halogen dioxins and furans in fire debris utilizing atmospheric pressure ionization gas chromatography-triple quadrupole mass spectrometry. Anal Chem 87(20):10368–10377
222. Organtini KL, Myers AL, Jobst KJ, Cochran J, Ross B, McCarry B, Reiner EJ, Dorman FL (2014) Comprehensive characterization of the halogenated dibenzo-p-dioxin and dibenzofuran contents of residential fire debris using comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry. J Chromatogr A 1369: 138–146