DETERMINATION OF POLYCHLORINATED BIPHENYLS, ORGANOCHLORINE PESTICIDES AND CHLOROBENZENES IN SLUDGE AND SEDIMENT SAMPLES BY GCxGC-μECD

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ABSTRACT

Gas chromatographic analysis of polychlorinated biphenyls, organochlorine pesticides and chlorobenzenes is one of the most common analyses performed by environmental laboratories. Comprehensive two-dimensional gas chromatography allows simultaneous analysis of different classes of compounds. The objectives of this study were to achieve within- and between-class separations for target contaminants and to quantify them in sludge and sediment samples. With only few coelutions present, the results showed that DB-1xRtx-PCB is a powerful column combination providing excellent chromatographic separation. Reference materials and "real-life" sediments and sludges were analysed and the results were compared to their reference values and previous GC data. This method was shown to be precise and accurate for the standards and reference materials tested as well as a very feasible method for the sediment and sludge sample analysis. Furthermore, this GCxGC method may potentially be used to assess the presence of other compound classes including dioxins, dioxin-like compounds and new emerging contaminants in the environmental samples.

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NOMENCLATURE

PCB	polychlorinated biphenyls
OC	organochlorine pesticides
CB	chlorobenzenes
PCN	polychlorinated naphthalenes
GC	gas chromatography
GCxGC	comprehensive two-dimensional gas chromatography
μ-ECD	micro-electron capture detector
TOF-MS	time-of-flight mass spectrometry
HCB	hexachlorobenzene
DMDT	methoxychlor
POP	persistent organic pollutants
FID	flame ionization detector
HCBD	hexachlorobutadiene
PCA	polychlorinated alkanes
PCDDs	polychlorinateddibenzo-*p*-dioxins or dioxins
PCDFs	polychlorinated dibenzofurans or furans
MAE	microwave assisted extraction
SFE	supercritical fluid extraction
ASE	accelerated solvent extraction
1D	first dimension
2D	second dimension

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Organohalogenated compounds are known environmental contaminants due to their persistence and toxicity. Most of the organohalogenated compounds were commercially produced for use in agricultural, industrial and/or household applications, while others such as dioxins and furans were formed unintentionally during municipal waste incineration, in other combustion and thermal processes or as by-products in the chemical industry. Compounds such as polychlorinated biphenyls (PCB), organochlorine pesticides (OC) and chlorobenzenes (CB) have been identified in diverse environmental samples. Due to their physical and chemical properties these compounds tend to bioaccumulate and biomagnify in the food chain (Bernes, 1998; Saito and al., 2004). These findings emphasize the need to screen environmental samples and the need for development of new fast and accurate multi-analyte methods of analysis.

Sediments and sludge, where most persistent contaminants are found to accumulate, are very complex environmental matrices challenging analysts with sample preparation problems and analytical interferences (e.g. matrix effect and coeluting peaks). In order to remove most of the possible interferences, complex sample preparation including extraction, clean-up and extract fractionation is required prior to multiple instrumental analyses. Classical sample analysis for these compounds employs gas chromatography (GC) coupled with an electron capture detector (ECD) or mass

spectrometry (MS). The ECD is often the choice for PCB, OC and CB detection due to its high sensitivity for halogenated compounds (Jacob de Boer, 1999; Cochran *et al.*, 1999). A major drawback of the ECD is the lack of selectivity between various halogenated compounds, thus requiring chromatographic separation in order to obtain accurate quantitative results.

Conventional GC offers good peak capacity but it fails to separate many individual components in complex environmental samples. The introduction of comprehensive two-dimensional gas chromatography (GCxGC) provided significant increases in separating power, peak capacity and speed of analysis (Dalluge *et al.*, 2003). GCxGC involves a serial column configuration separated by a thermal modulator. Comprehensive two-dimensional gas chromatography increases peak capacity by applying two independent separations to a sample resulting in improved resolution of target compounds in a single analysis. Previous studies involving the GCxGC technique, as presented in recent reviews (Adahchour *et al.*, 2006), have demonstrated its advantages over the classical analysis for the separation of PCBs, OCs and CBs in environmental samples.

1.2 OBJECTIVES

The comprehensive multi-dimensional gas chromatography coupled with micro-electron capture detector (GCxGC-μECD) proved to by a very powerful technique allowing simultaneous analysis of the target halogenated contaminants (Korytar et al., 2003; Korytar et al., 2006). Since the ECD permits only peak recognition without

providing any structural information, further confirmation of the compounds by time-of-flight mass spectrometry (TOFMS) may be required. The objectives of this project were to achieve chromatographic separation of the target compounds in one analysis prior to calibration and quantification; to accurately identify and quantify the PCBs, OCs and CBs present in sludge and sediment samples in a single analytical run by using the GCxGC technique. In addition to PCBs, OCs and CBz, other contaminant classes can be evaluated using the same instrumental set-up: dioxins and furans, toxaphene and polychlorinated naphthalenes. The retention time data for these different classes of compounds can be plotted in a graph representing the chromatographic space and later used for further assessing the presence of these contaminants in environmental samples.

The multi-step approach taken was:

i. The implementation of an environmentally friendly extraction method (e.g. pressurized solvent extraction that uses less solvent) followed by a clean-up step without any fractionation prior to GCxGC analysis;

ii. The identification, separation, calibration and quantification of the target organohalogenated contaminants within-class and between-class, in a single analytical run using GCxGC technique;

iii. The application of the new method to accurately identify and quantify the target analytes in complex environmental matrices such as sludges and sediments.

1.3 PROJECT OUTLINE

The background information relevant to this research is reviewed in Chapter 2, outlining the properties, use and occurrence in the environment of PCBs, OCs and CBz, different sample preparation procedures and instrumental analyses. The second chapter describes the theoretical and practical aspects of the GCxGC technique as well as its advantages over the classical methods.

Chapter 3 outlines the experimental procedures along with the materials and instrumental set-up used in this research. Chapter 4 presents the results of the study in four parts: the separation of the target analytes, the calibration and quantification, the uncertainty calculations followed by the analysis of sludge and sediment samples. A more detailed discussion of the results follows in Chapter 5. Chapter 6 summarizes the conclusion and recommendations of this research.

CHAPTER 2: LITERATURE REVIEW

Chapter 2 of this study is presented in four parts: overview of the PCBs, OCs and CBz as persistent environmental pollutants, sample preparation techniques, instrumental analysis followed by the objectives and hypothesis of the research. The emphasis of the literature review is on GCxGC principles since this technique was used in the research. The sample preparation steps are only briefly reviewed to strengthen the conclusion that overall the time gain, the efficiency and the decrease of solvent use are significantly improved when using the procedures selected for this study.

2.1 ENVIRONMENTAL PERSISTENT ORGANIC POLLUTANTS: PCBS, OCS, CBZ

Halogenated organic compounds have been produced in large volumes in the early 1950s and used for different applications. Most of the organohalogen compounds were commercially produced for use in agricultural, industrial, and/or household applications, while others such as dioxins were formed unintentionally during municipal waste incineration, in other combustion and thermal processes or as by-products in the chemical industry. Compounds such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs) and chlorobenzenes (CBz) have entered the air, water, and soil during their manufacture, use and disposal, from accidental spills and leaks during their transport and from leaks or fires in products containing PCBs. They were identified in environmental samples and are generally known environmental

contaminants due to their persistence, toxicity and their tendency to bioaccumulate and biomagnify (Bernes, 1999).

The "dirty dozen" list of POPs includes three sub-divisions: eight OCs (dieldrin, endrin, aldrin, chlordane, heptachlor, DDT, mirex and toxaphene), two industrial chemicals (hexachlorobenzene - HCB and polychlorinated biphenyls) and two unintentionally produced compounds (polychlorinateddibenzo-*p*-dioxins - PCDDs or dioxins, and polychlorinated dibenzofurans - PCDFs or furans) (van Leewen and Boer, 2008). Even though most of these compounds are not currently produced or have been used for decades now, their presence in the environment is still in considerable levels.

Polychlorinated biphenyls are mixtures of synthetic organic chemicals with the same basic chemical structure and similar physical properties ranging from oily liquids to waxy solids. Due to their non-flammability, chemical stability, high boiling point and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment; as plasticizers in paints, plastics and rubber products; in pigments, dyes and carbonless copy paper and many other applications. The 209 possible PCB congeners were manufactured and sold under many names, the most common were the "Aroclor" series, in many of which a numerical identifier included the percentage of Chlorine (e.g., "Aroclor 1254", with 54 percent Chlorine) (Erickson, 1997; Frame, 1997). PCBs do not readily break down in the environment and thus may remain there for very long periods of time. Some PCBs can exist as a vapour in air that can travel long distances and be deposited in areas far away from the point of release. In water, a small amount of PCBs might remain dissolved, but most stick to organic particles and bottom sediments. PCBs also bind strongly to soil and

have the tendency to bioaccumulate and to concentrate through the food chain (Bernes, 1999; EPA, 2005).

The toxicity of PCBs has been intensively studied. Twelve PCB congeners have similar toxic responses to those caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). These PCB congeners can assume a planar dioxin-like conformation. They have a lower energy barrier to assume the conformation necessary to fit into dioxin receptor. Investigation of the biological effects of PCBs in experimental animals revealed the following syndromes: decreased reproductive efficiency, changes in liver morphology, changes in plasma lipid concentrations, hepatic porphyria, dermatological effects, production of tumors in the liver, and decreased immuno-competence (Erickson, 1997; Kannan, 2000; van den Berg, 2006). In addition to the twelve dioxin-like PCBs, seven PCBs are so-called "indicator PCBs": 28, 52, 101,118, 138, 153 and 180 (from tri- to hepta-chlorination) due to their ubiquity in the environment (van Leewen, 2008).

Organochlorine pesticides such as DDT, dieldrin, endrin, aldrin, lindane (γ-BHC), HCB, chlordane were used as pesticides, very effective and with a broad spectrum of application, as insecticides, on animals and protection of humans (e.g. against malaria). They are found to be toxic for humans and some of them are possible carcinogens. Heptachlor and chlordanes are more readily metabolized, but in the process can convert to metabolites that are continue to exist longer and may be more toxic too: heptachlor-epoxide or oxy-chlordane.

2.2 SAMPLE PREPARATION

The determination of halogenated organic contaminants in complex environmental samples starts with their extraction from the matrix. The extracts undergo a rigorous clean-up procedure to remove the possible interferences present along with the target compounds, followed by the final instrumental analysis.

2.2.1 Extraction Techniques

Complex sample preparation such as extraction, clean-up and extract fractionation is required prior to GC analysis. The choice of extraction technique, solvents, temperature, pressure, time of extraction influence the extraction efficiency and need to be carefully selected. There are different types of extractions of sediment and sludge samples and the most common used is Soxhlet extraction. Accelerated solvent extraction is considered over the classical technique due to its advantages (see section 2.2.1.2 Accelerated Solvent Extraction). Other extraction techniques such as microwave assisted extraction (MAE), sonication and supercritical fluid extraction (SFE) have also been employed for POPs analysis in environmental samples.

2.2.1.1 Soxhlet Extraction

The most common procedure employed for the extraction of trace halogenated compounds from a wide variety of matrices such as sediments, soils and biota is Soxhlet extraction. There are numerous advantages when using this type of extraction: the method is simple and does not require expensive equipment, multiple extractions can be

done at the same time and can be employed for many matrices and classes of contaminants. The extraction efficiency is very high but the drawbacks of the method are the long extraction times required (approximately 6-24h) and use of large amounts of solvent (van Leeuwen, 2008; Focant, Pirard and De Paw, 2004). Thus, more automated and faster extraction methods have been developed. Ultrasonic extraction was one of the techniques potentially to replace Soxhlet extraction. It has comparable extraction efficiency and the advantages of reduced extraction time, decreased volume of solvent and sample and replacement of fragile Soxhlet glassware (Erickson, 1997).

2.2.1.2 Accelerated Solvent Extraction

Different types of extraction have been demonstrated to be suitable for sediment and sludge matrices; pressurized liquid extraction (PLE or Accelerated Solvent Extraction - ASE - Dionex Corporation) being one of the techniques that has drawn attention in recent years. The extraction takes place in a stainless steel cell (of 11, 22 or 33 ml fitted with stainless steel frits and a cellulose filter) that can be heated up to 200°C and pressurized to 3000 psi. Usually one or two static extractions employed. The extraction efficiency of ASE was found to be similar to that of Soxhlet extraction. In addition, ASE has some advantages over traditional techniques: the enhanced extraction efficiency achieved by solvents at high pressures and temperatures uses less solvent volume and much less time (Schantz, 2006). The ASE was successfully applied to biota and sediment samples for PCBs and OCs extraction. The conditions were optimized for high recovery in the extraction procedure for different environmental matrices: extraction time, temperature, and the use of different solvents. Different types of solvents were tried

in order to maximize the recoveries for all compounds. An increase in extraction temperature often leads to higher recoveries, especially of volatile compounds where the temperature is one of the most important parameters. The effect of the pressure on the recovery was studied in the range between 500 and 2500 psi, and did not have any significant influence on the extraction (Saito, 2004; Ramos, Kristenson, Brinkman, 2002; Hubert et al., 2000). Toluene was found to be one of the best extraction solvents for soil and sediments samples (Hubert et al., 2000). A drawback of ASE is the cross-contamination; the cells have many parts and should be cleaned thoroughly.

2.2.2 Cleanup

Prior to the instrumental analysis, the extracts need to be cleaned up and split into multiple fractions. The clean-up of PCBs and OCs is often combined, the non-polar PCBs being separated from the more polar OC by silica or florisil fractionation. The PCBs and CBz are eluted usually with a non-polar solvent (hexane) in the first fraction followed by the elution with a more-polar solvent (ethyl ether and dichloromethane mix) to collect the OCs. Open column chromatography or pre-packed cartridges can be used (Ontario Ministry of the Environment, Method 3270, 2008; Erickson, 1997; van Leeuwen, 2008). The drawback of splitting into multiple fractions is that some of the OCs are eluting in the first fraction along with the PCBs and CBz making the chromatographic analysis more difficult. Some examples of these OCs are p,p'-DDE, o,p-DDT, mirex, photomirex, hexachlorobenzene, octachlorostyrene and trans-nonachlor. Other methods use multilayer silica columns where impregnated acidic and basic silica alternate with regular silica to remove the lipids and other oxidizable compounds present in complex matrices.

Alumina, a classic inorganic adsorbent, was also used for the sediment and soil extracts clean-up. Similar to silica and florisil, some of the OCs were recovered in the first fraction along with the PCBs. When structural separation was required, the separation of coplanar PCBs from the other compounds and interferences, carbon clean-up was employed (Erickson, 1997; Focant et al., 2004; van Leeuwen, 2008).

Recent studies have integrated the clean-up step within the extraction process. For instance, Bjorklund et al. (Bjorklund, Sporring, Wiberg, Haglund and Holst, 2006) has added different adsorbents into the ASE extraction cell and assessed the extraction and clean-up together. He achieved the structural separation of coplanar PCBs when custom made carbon cell-inserts were made.

2.3 ANALYSIS

2.3.1 Gas Chromatography Analysis

Following the sample preparation steps, classical instrumental analysis involves several GCs equipped with different stationary phases able to separate specific classes of contaminants present in the final fractioned extracts. Typically, sample analysis for PCBs, OCs and CBz employs gas chromatography (GC) coupled with electron capture detector (ECD) or mass spectrometry (MS). The electron capture detector is often the choice for PCB/OC/CB detection due to its high sensitivity for halogenated compounds. A major drawback of the ECD is the lack of selectivity between halogenated compounds, therefore requiring chromatographic separation in order to obtain accurate quantitative results. In a capillary gas chromatography review J. de Boer (de Boer, 1999) summarized some of the challenges that analysts encounter when analyzing organochlorine pesticides.

For instance, the chlordane congeners tend to split between the fractions and coelute with PCBs making their analysis difficult. Cochran and Frame (Cochran and Frame, 1999) reviewed the gas chromatography separation of PCB congeners on different stationary phases. They concluded that no single column phase can resolve all the congeners even when advanced detection techniques such as mass spectrometry are involved.

Conventional GC offers good peak capacity but it fails to separate many individual constituents in complex environmental samples. The introduction of comprehensive two-dimensional gas chromatography (GCxGC) provided significant increases in separating power, peak capacity and speed of analysis (Dalluge et al., 2003).

2.3.2 Comprehensive two-dimensional gas chromatography

Comprehensive two-dimensional gas chromatography, a relatively new way to solve separation problems, is successfully used for complex environmental samples. The introduction of comprehensive two-dimensional gas chromatography (GCxGC) provided significant increases in separating power, peak capacity and speed of analysis. In the past few years, many studies have demonstrated the applicability of GCxGC for different environmental matrices. The goal of this review is to summarize the principles and configuration of the GCxGC technique highlighting the applications on halogenated contaminants analysis.

2.3.2.1 Principles and Instrumentation

GCxGC involves two columns coupled directly, where two different separation mechanisms are applied to the entire sample. A thermal modulator serves as the interface

component that couples the two columns. GCxGC is a truly comprehensive technique because the information gained from the separation on the first column is preserved in the second column (Dallüge, Beens and Brinkman, 2003). The peak capacity is increased by applying two independent separations to a sample, resulting in improved resolution of target compounds in a single analysis. Under optimal conditions, comprehensive GCxGC can provide an order of magnitude lower saturation of a chromatogram compared to its 1D counter-part based on similar conditions.

The instrumental set-up involves a serial column configuration usually having a 15-30 m x 0.25-0.32 mm I.D. x 0.1-1 μm film thickness as first dimension (1D) column and a much shorter, narrower 0.5-2 m x 0.1 mm I.D x 0.1 μm film thickness as second dimension (2D) column. The shorter second dimension column is necessary in order to maintain the first column separation and to ensure that the second dimension separation is completed in the run time of the first dimension analysis (Dalluge et al., 2003; Adahchour, Beens, Brinkman, 2008; Marriott and Shellie, 2002). The second dimension run time is in order of 1-10 s compared to 45-120 min first dimension separation (Dalluge et al., 2003). A schematic representation of a GCxGC instrument is presented in Figure 2.1.

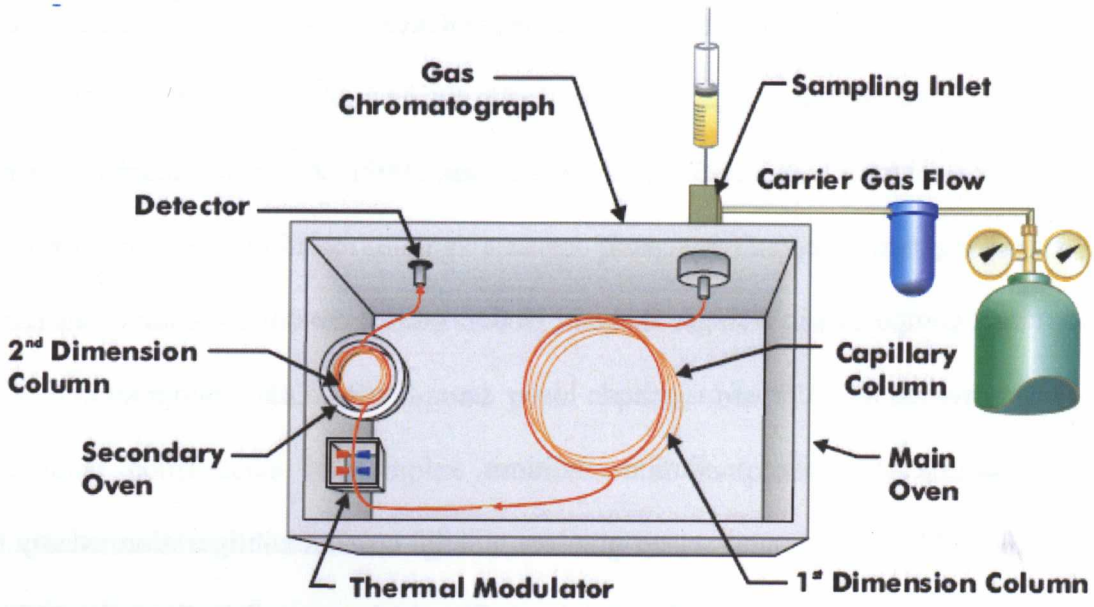


Figure 2.1 Schematic representation of a GCxGC system: 1st dimension column - modulator - 2nd dimension column (from LECO, 2008).

The result of a GCxGC analysis, as presented by Dalluge et al. (Dalluge et al., 2003), consists of a large series of stacked side by side GCxGC chromatograms (Figure 2.2 - step 1. Modulation) that are "transformed" (Figure 2.2 - step 2. Transformation) to form a two-dimensional chromatogram. The chromatograms can be visualized as contour plots in the 2D plane where the colours represent the signal intensity or as three-dimension plots (Figure 2.2 - step 3. Visualisation).

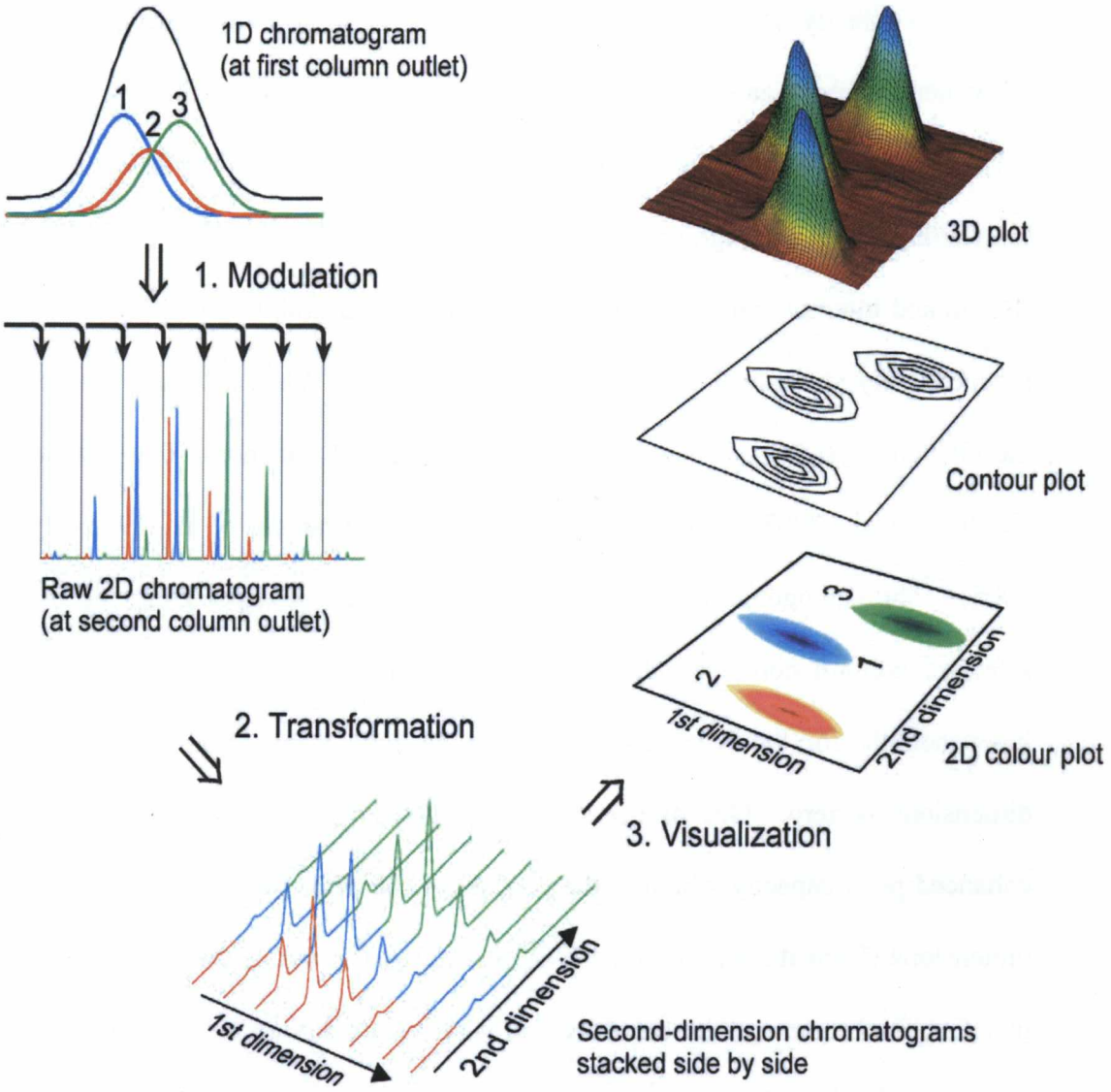


Figure 2.2 The schematic representation of GCxGC chromatogram: generation and visualisation (Dalluge et al., 2003).

2.3.2.2 Column Combinations

As already described, GCxGC involves a serial column configuration separated by a thermal modulator. The role of the primary column is to provide the secondary column with sub-samples of the original sample, and the role of the secondary column is to generate a series of high speed chromatograms. The parameters of the two dimensions (length and internal diameter, column temperature, and mobile-phase linear velocity) are independently chosen (Dimandja, 2004, Pierce, 2008). When selecting the columns for the GCxGC system, one has two choices: orthogonal and non-orthogonal approaches (Dalluge et al., 2003; Adahchour et al. 2006; Venkatramani et al., 1996; Ryan et al., 2005). The orthogonal separation, usually involving a non-polar and polar or shape-selective column combination, is achieved when the separation mechanisms operate independently for the two dimensions and the synentropy (cross information) across dimensions is zero. One of the advantages of using an orthogonal separation is the enhanced peak capacity which is the product of the peak capacities of the two separate dimensions (Venkatramani et al., 1996). Structured chromatograms are distinctly visible in GCxGC chromatograms for structurally related compounds, allowing easier group-type identification. The non-orthogonal approach, the combination of polar column as first dimension and non-polar column as the second dimension, was also studied and some significant separations were reported (Dalluge et al., 2003; Ryan et al., 2005; Haglund et al., 2001; Marriott, Massil, Hügel, 2004), but a comprehensive discussion of these findings is beyond the scope of this study. For more information about the columns used for GCxGC please see Appendix D.

Typically orthogonal separation involves non-polar columns as first dimension (e.g. DB-1, Rtx-1, Rtx-5, HP-5MS, HT-5) and polar or shape-selective columns as second dimension (e.g. DB-17, HT-8, LC-50, Rtx-PCB). Table 2.1 shows some of the column combinations used by different researchers to separate the PCB and organochlorine pesticides in standard mixtures, as well as some of the conditions used for their analysis.

Table 2.1 Column combinations and instrumental GCxGC conditions used for separation of PCBs and OCs in standard mixtures

Column Combinations	PCBs and OCs Resolved	GCxGC Conditions	μECD	References
HT-5xBPX-50 - run time: 20 min	15 PCB 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180	*Oven Programming:* 100°C - hold 1 min 50°/min to 200°C 5°/min to 280°C (hold 1 min) *Modulation:* 5 sec		**Kristenson et al., 2005**
DB-1 x HT8	- ordering based on the number of chlorine	*Oven Programming:* 90°C (hold 2 min) 20°C/min to 170°C 2°C/min to 325°C (hold 20 min) *Modulation:* 8 sec	- 280°C - make-up gas: N_2 - flow rate 150ml/min. - acquisition rate 50Hz	**Korytar et al., 2005**
DB-1x007-210	- little or no selectivity for organochlorinated compounds	*Oven Programming:* 90°C (hold 2 min) 20°C/min to 170°C 2°C/min to 290°C (hold 40 min)	- 280°C - make-up gas: N_2 - flow rate 150ml/min. - acquisition rate 50Hz	**Korytar et al., 2005**
DB-1 x LC50	- better orthogonality than DB-1 x HT8 - separates planar and non-planar PCBs - non-ortho congeners (81, 77, 126, 169), followed by mono- (123, 105, 167, 157, 189), di- (52, 49, 101, 153), tri- (151, 183, 195, 206) and tetra-ortho (155, 201, 207) PCBs	*Oven Programming:* 90°C (hold 2 min) 20°C/min to 170°C 2°C/min to 285°C (hold 40 min) *Modulation:* 9 sec		**Korytar et al., 2005**

Column Combinations	PCBs and OCs Resolved	GCxGC Conditions	µECD	References
DB-1 x 007-65HT	- efficiently separates chlorinated from brominated analogues (PCBs/PBBs and PCDEs/PBDEs) - the best combination for within-class separations of OCs and PCNs	*Oven Programming:* 90°C (hold 2 min) 20°C/min to 170°C 2°C/min to 325°C (hold 20 min) *Modulation:* 8 sec		**Korytar et al., 2005**
DB-1 x VF23ms	- very effective within-class separation for non-aromatic compounds (OCs, Toxaphenes)	*Oven Programming:* 90°C (hold 2 min) 20°C/min to 170°C 2°C/min to 290°C (hold 40 min) *Modulation:* 8 sec		**Korytar et al., 2005**
DB-XLB x LC50	- all WHO-PCBs are resolved and nine out of the 12 WHO-PCBs were also completely separated from all other CBs in the 209-CB mixture - different lengths for the 1st and 2nd dimension columns were analysed	*Oven Programming:* 80°C (2 min) 20°C/min to 160°C 3°C/min to 240°C 30°C/min to 270°C (the upper temperature limit for LC-50) *Modulation:* 5 sec or 6 sec	- 300°C - make-up gas: N_2 - flow rate 150ml/min - acquisition rate 50Hz	**Danielsson et al., 2005**
VF1 x LC-50	- one WHO-PCB pair, CBs 118 and 123, coelutes with each other and with a number of other CBs - 20°C lower elution temperature of VF-1 than DB-XLB	*Oven Programming:* 80°C (2 min) 20°C/min to 160°C 3°C/min to 240°C 30°C/min to 270°C (the upper temperature limit for LC-50) *Modulation:* 8 sec	- 300°C - make-up gas: N_2 - flow-rate 150ml/min - acquisition rate 50Hz	**Danielsson et al., 2005**
HP-1 x BPX-50	90 PCBs analysed: - 71 resolved	*Oven Programming:* 1st oven: 90°C (hold 2 min)	- 300°C - make-up gas: N_2	**Korytar et al., 2002**

Column Combinations	PCBs and OCs Resolved	GCxGC Conditions	μECD	References
	- 9 coelutions: 4/10,28/31/50, 37/44, 61/74, 56/60, 77/136, 118/149, 126/129, 156/171	5°C/min to 110°C 1°C/min to 240°C (hold 25 min) 2nd oven: 20°C offset *Modulation:* 6.5 sec	- flow-rate 60ml/min	
HP-1 x HT-8	90 PCBs analysed: - 78 resolved - 6 pairs coeluted: 4/10, 28/31, 52/69, 74/61, 56/60, 138/163 - all WHO PCBs separated	*Oven Programming:* 1st oven: 90°C (hold 2 min) 5°C/min to 110°C 1°C/min to 240°C (hold 25 min) 2nd oven: 20°C offset *Modulation:* 6.5 sec	- 300°C - make-up gas: N_2 - flow-rate 60ml/min	**Korytar et al., 2002**
HP-1 x SupelcoWax-10	90 PCBs analysed - 84 resolved - 3 pairs coeluted: 28–31, 56–60, 80–88. - little or no ordered structure - all WHO PCBs separated	*Oven Programming:* 1st oven: 90°C (hold 2 min) 5°C/min to 110°C 1°C/min to 240°C (hold 25 min) 2nd oven: 110°C (hold 2 min) 5°C/min to 130°C 1°C/min to 260°C (hold 25 min)	- 300°C - make-up gas: N_2 - flow-rate 60ml/min	**Korytar et al., 2002**
Rtx-1xRtx-PCB	RTX-PCB is highly selective for planar PCBs - dioxin-like PCBs in Aroclor1254 and Aroclor1254/OC mixture were separated	*Oven Programming:* 1st oven: 160°C (hold 0.2 min), 2°/min to 280° 2nd oven: 20°C offset *Modulation:* 8 sec	- 325°C - make-up gas: N_2 - flow rate 148.7ml/min - 50 Hz	**LECO, 2005**
Rtx-5 x DB-17	- 25 OCs from a standard mixture were separated and quantified	*Oven Programming:* 1st oven: 40°C (hold 1 min), 10°C/min to 290°C (hold 1 min) 2nd oven: 10°C offset *Modulation:* 4 sec	- 325°C - N_2 makeup gas - flow rate 148ml/min - 50 Hz	**LECO, 2005**

Column Combinations	PCBs and OCs Resolved	GCxGC Conditions	µECD	References
Rtx-5 x Rtx-200	- OCs: aldrin, α- chlordane, α-hexachlorocyclohexane (HCH), β-HCH, DDD, DDE, DDT, δ-HCH, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, γ-chlordane, γ-HCH, heptachlor, heptachlor epoxide, methoxychlor.	*Oven Programming:* 1st oven: 50°C (hold 0.2 min), 30°C/min to 140°, 5°C/min to 250° 2nd oven: 30°C offset *Modulation:* 6 sec	- 325°C - N_2 makeup gas - flow rate 148ml/min - 50 Hz	**LECO, 2005**

In other studies researchers tried to separate all 209 PCB congeners using GCxGC-TOFMS (Focant et al., 2004) and μECD (Harju, Danielsson, and Haglund, 2003). Harju has achieved the separation of 181 when using DB-XLB x LC-50 column combination in a time frame of 90min (Figure 2.3a). When using DB-XLB column connected to BPX-70, 194 out of 209 PCBs were separated in a time frame of 4 hours (Figure 2.3b). The DB-XLB x SP-2340 was used to analyse halogenated contaminants in seal blubber extract; 64 PCBs were identified and quantified. In addition, p,p'-DDE was found to be very abundant in this sample (Harju et al.). Using different column combinations for the GCxGC-TOF-MS system, DB-1 x HT-8, DB-XLB x HT-8, and HT-8 x BPX-50, Focant (Focant et al., 2004) has successfully resolved 194 PCB congeners. The best column combination was HT-8 x BPX-50 which resolved 194 congeners in 146 min analytical run. An ordered structure was observed in the second dimension for structurally related compounds. The ordered structure of the two dimensional chromatograms provided better information of the group-type separation according to the number of chlorines and ortho-subtitution level.

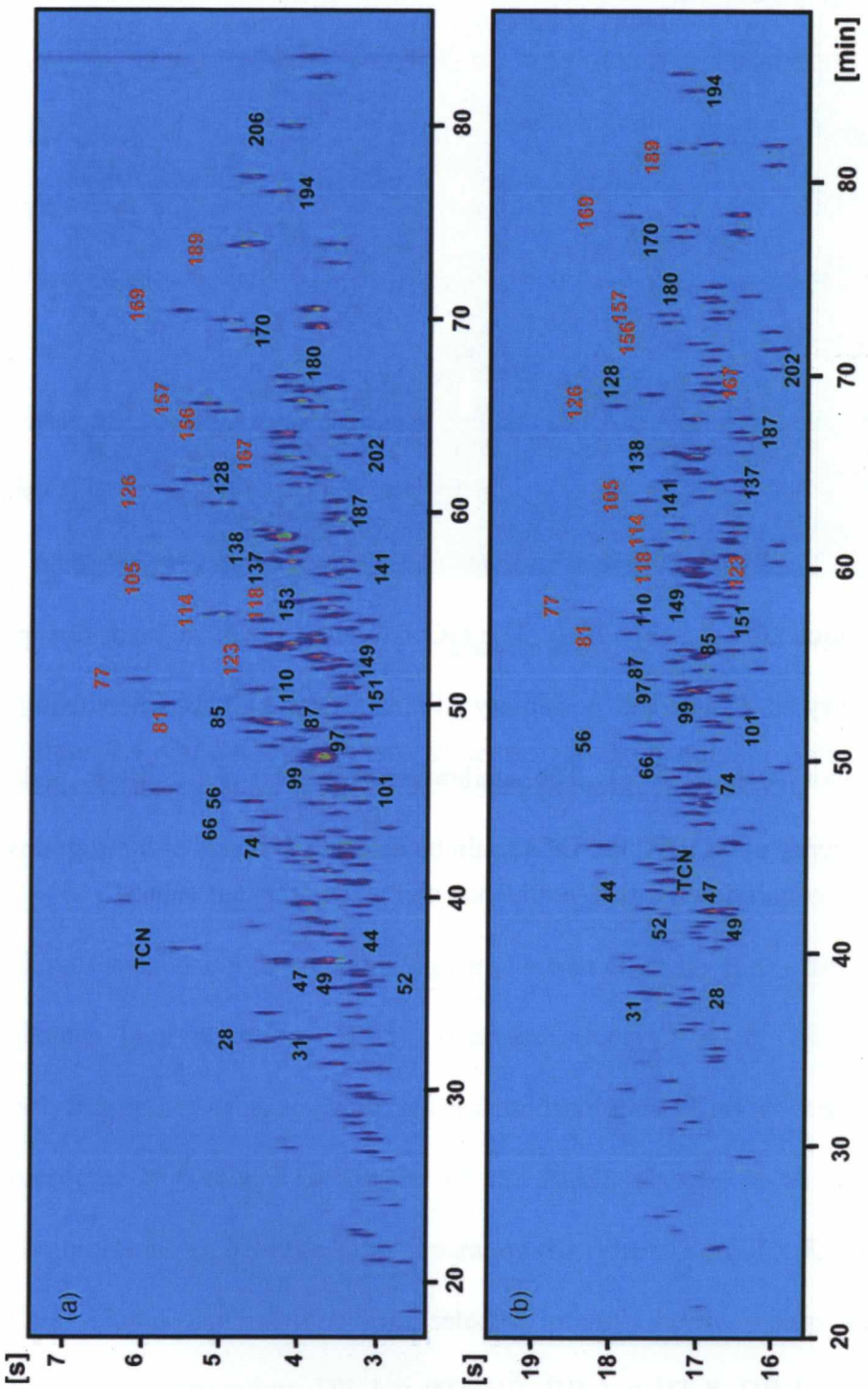


Figure 2.3 Contour plots of the 209 PCBs using the column set: a) DB-XLB x LC-50and b) DB-XLB x SP-2340 (Harju et al, 2003).

Similar to PCBs, Korytar et al. (Korytar et al., 2005) has assessed the separation of organochlorine pesticides by GCxGC-μECD using different column combinations. They concluded that DB-1×007-65HT column combination showed very good distribution of OCs in the 2D plane (Figure 2.4) and solved the *cis*- and *trans*-heptachlor-epoxide coelution present with DB-1×VF23. Additionally, technical notes provided by LECO (LECO, 2005) regarding the separation of OCs (see Table 2.1) also provide information for their linearity and calibration. One of these studies, "OC pesticides by GCxGC" (LECO, 2005), provided a comparison of the classical GC analysis using dual column GC-ECD (Rtx-CLPesticide and Rtx-CLPesticidesII as 1D columns) and the GCxGC technique (Rtx-5 x Rtx-200). The GCxGC was shown to be a very powerful technique, a way to reduce the possibility of quantification bias when using a non-selective detector such as ECD. The calibration responses were linear and due to increased sensitivity of GCxGC the OCs could be detected at levels of femtograms.

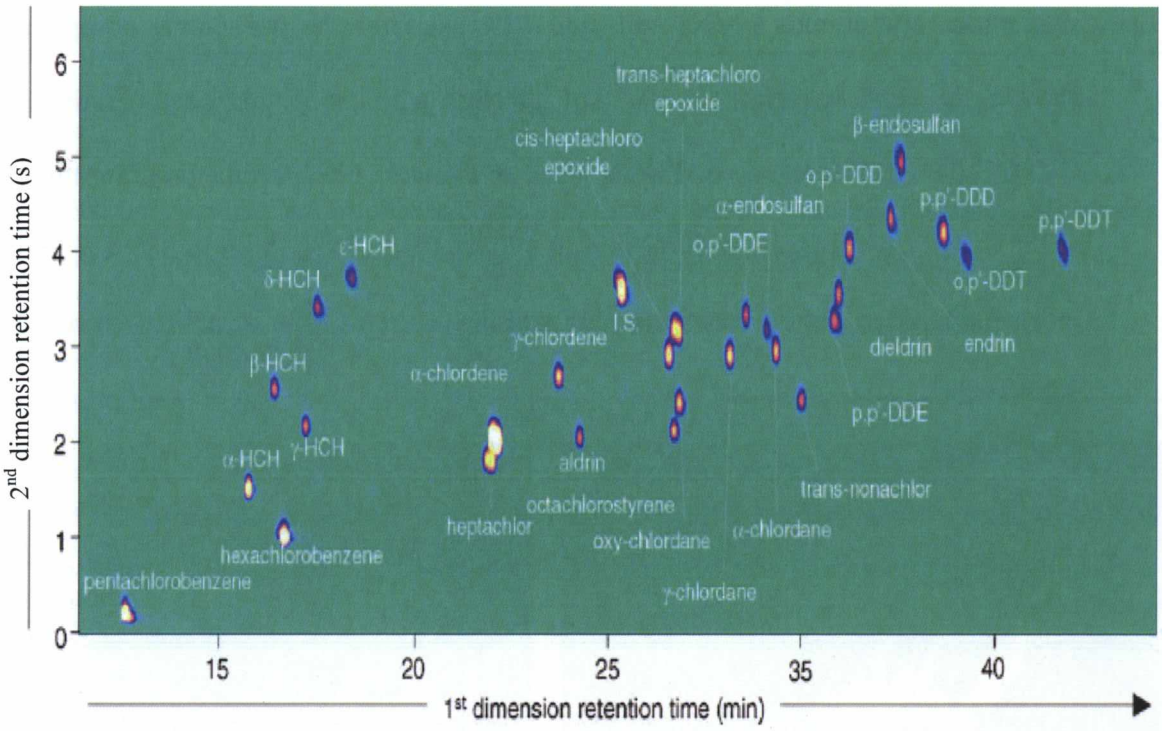


Figure 2.4 GCxGC-μECD two dimensional chromatogram obtained for selected OC pesticides analysed with DB-1×007-65HT (Korytar et al., 2005).

Besides the progress made for within-group separation of different contaminant classes such as polybrominated diphenyl ethers (Korytar et al., 2005), polychlorinated n-alkanes (Korytar et al., 2005), toxaphene (Korytar et al., 2003), 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans (Korytar et al., 2003), and PCBs as presented in Table 2.1 (Korytar et al., 2002), Korytar et al. has evaluated column combinations for between-class separation (Korytar et al., 2005; Korytar et al., 2006). Five column combinations were selected to study between-class and also occasionally within-class separation: DB-1 x 007-210, DB-1 x HT-8, DB-1 x LC-50, DB-1 x 007-65HT and DB-1 x VF-23ms. They concluded that DB-1 x LC-50 column combination was the best choice for between-group separation: three-ring planar compounds (PCDD/Fs, PCDTs and planar PCTs) were most strongly retained, followed by the two-

ring planar compounds (PCNs and planar PCBs), then the non-planar compounds that showed the least retention and did not interfere with the planar ones (Figure 2.5).The GCxGC chromatographic conditions were as follows; temperature programming started at 90°C (hold for 2 min), at 20°C/min to 170°C, then at 2°C /min to 285°C (hold for 40 min); modulation period was 9 seconds and the constant flow of helium carrier gas was 1.2 ml/min.

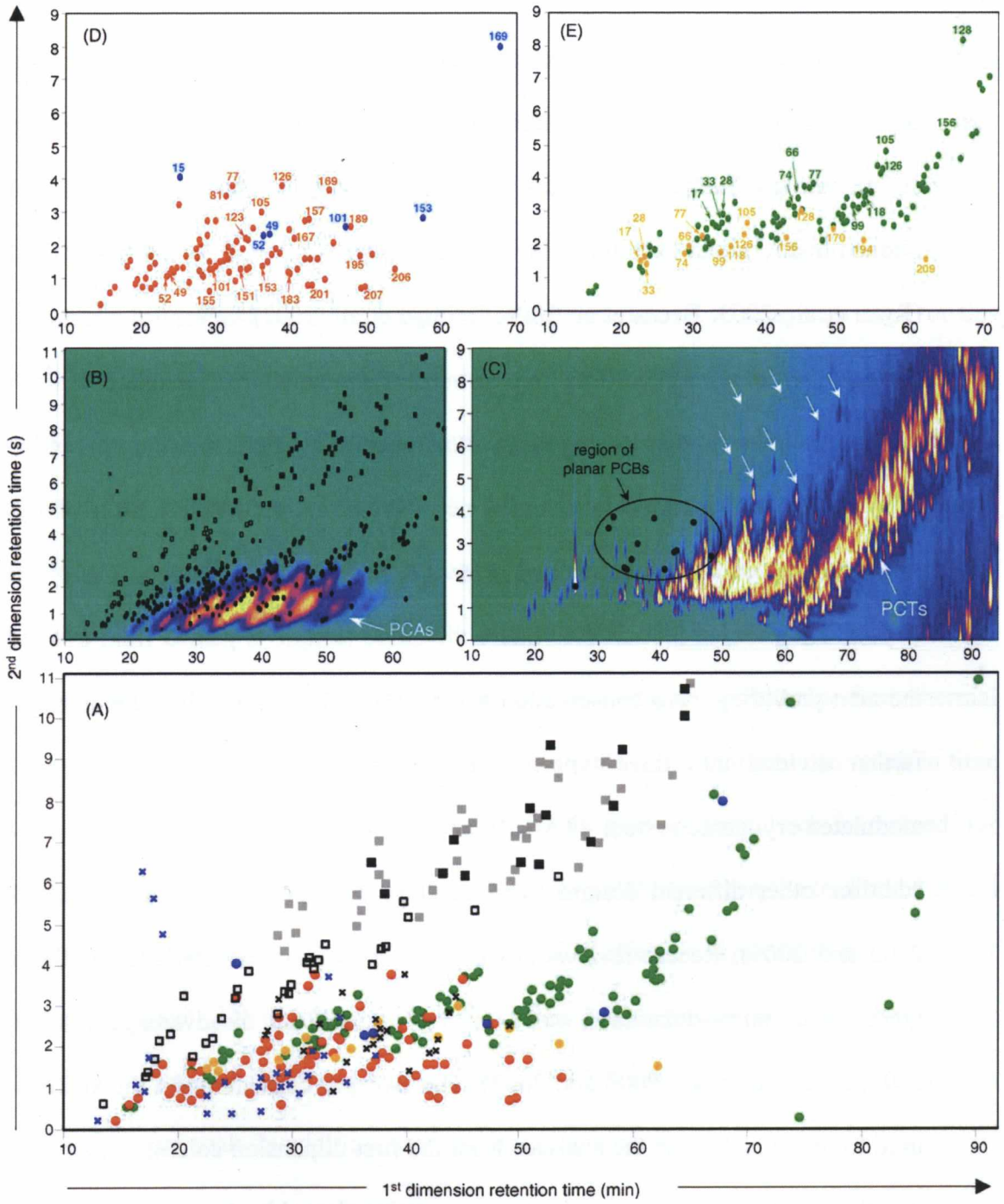


Figure 2.5 Overlaid GC×GC-μECD chromatograms on DB-1×LC-50 column combination of: (A) PCBs, PBBs, PCDEs, PBDEs, PCDTs, PCNs,PCDD/Fs, OCPs, individual toxaphene standards; (B) PCAs (PCA-60) as colour contour plot and other classes as black dots; (C) PCTs (Aroclors5442 and 5460) as colour contour plot and visualized position of dioxin-like PCBs (black dots) and planar PCTs (white arrows); (D) PCBs and PBBs; (E) PBDEs and PCDEs (Korytar et al., 2005).

2.3.2.3 Modulation

The modulator represents the interface component between the two dimension separations. The primary functions of an efficient modulator are to continuously accumulate small adjacent fractions eluting from the first column effluent, to refocus the trapped fractions and to re-inject the focused fractions into the second dimension column (Ryan et al., 2003; Beens et al. 2004; Dalluge et al., 2003). Several different modulator designs can be classified into two groups: flow-switching modulators and thermal modulators. The flow-switching modulators operate as high-frequency diversion valves (0.1–1.0 Hz) and require low maintenance. However, whether this form of GCxGC is truly comprehensive was questioned in several studies (Dimandja et al., 2000; Dimandja, 2004). The thermal modulators, where the entire sample is passed from one column to the next providing mass conservation and resulting in peak amplitude enhancement, are further divided into three types: heat (thermal sweeper), cryogenic (longitudinally modulated cryogenic system - LMCS), and jet-pulsed modulators (Ryan et al, 2003). In addition, other different designs of modulators were proposed (Harynuk and Gorecki, 2002 and 2003). Recent reviews summarized the main characteristics of the different types of thermal modulators as well as their advantages and disadvantages (Dalluge et al., 2003; Adachour et al., 2006 and 2008). The sweeper consisted of a thick-film capillary used to retain and focus the analytes from the first dimension column. Their re-injection into the second column was achieved by a rotating slotted heater which locally heated the capillary column. The disadvantages were the need to move very close to the fragile capillary as well as the requirement of high temperature differences (Adachour et al., 2006). LMCS, the first cryogenic modulator introduced, uses CO_2 (liquid) for trapping

and focusing the eluent from the first column. The trap then moves very fast, exposing the column and the focused fraction to the oven temperature and re-launching the focused analytes into the second column (Haglund et al., 2002). Nowadays, the jet-based modulators with no moving parts and simplified design are used the most (Figure 2.6). Single-, dual- and quad-jet modulators are designed using either CO_2 or liquid N_2 for cooling (Kristenson et al., 2003; Korytar et al., 2006). The GCxGC system used for this study employs a two-stage modulator similar to the one described by Crimi and Snow, 2008. The first dimension eluent is focused with a jet of cryogenically cooled nitrogen gas and then heated with a jet of hot air while a second band is simultaneously focused with a cryogenically cooled liquid nitrogen jet. This cycle is repeated, allowing the re-injection of successive focused bands onto the second column. Recently, LECO Corporation has developed a new dual-jet, quad-stage, consumable-free thermal modulator. The liquid N_2 is not required for cooling the modulator that can result in time and analysis cost savings. The drawback of this type of modulator is noticed for applications that require modulating at extreme low volatility, when the traditional liquid N_2 cooled modulator is required (LECO, 2008). For effective separations the modulator timing is critical. The resulted second-dimension peaks are very narrow (50-600 ms); therefore, the second dimension run-time should be 2-8 s in order to achieve at least three modulations per peak. In addition, very fast detectors are required.

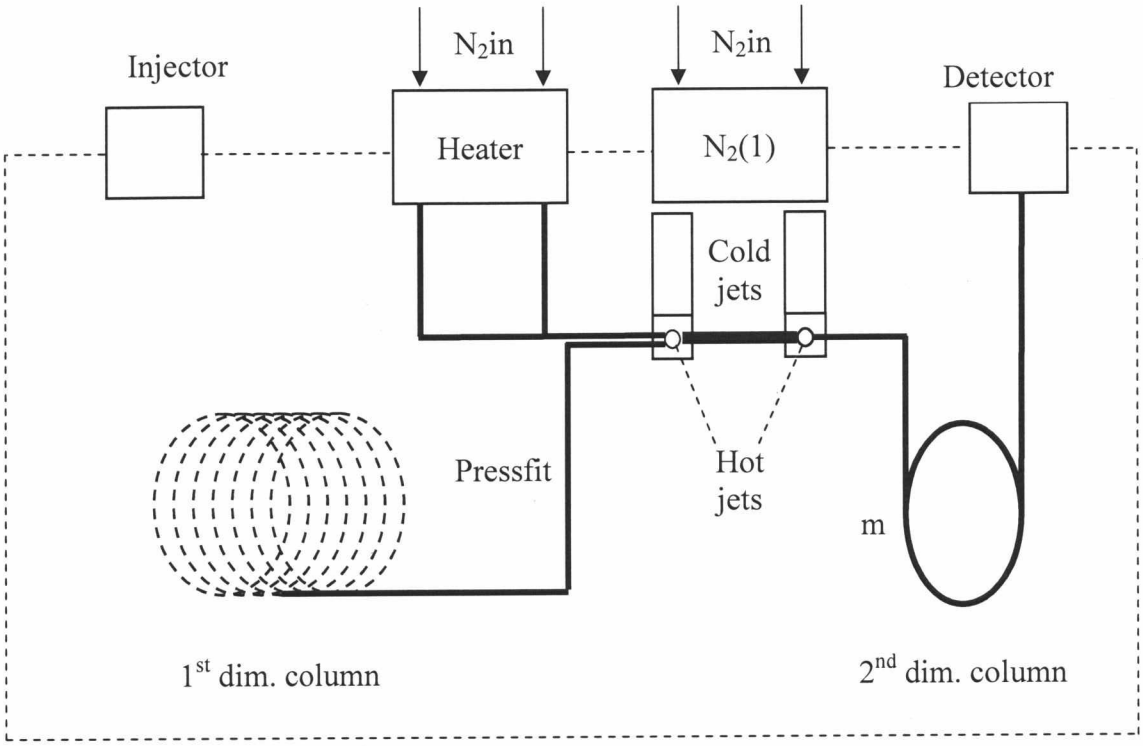


Figure 2.6 Schematic representation of the design of a quad-jet modulator: diagram of the quad-jet N_2 modulator (Adapted from Kristenson et al., 2003).

2.3.2.4 Detection

Due to a very fast separation in the second dimension (peak width 50-600ms) the narrow peaks require fast detectors with a small internal volume and a short detector rise time. Also a high data acquisition rate is required to ensure a proper reconstruction of the second dimension chromatogram.

The detectors used in GCxGC systems are mass spectrometer detectors (TOF-MS) and element-selective detectors such as FID, μECD, SCD, NCD. The element-selective detectors permit only the peak recognition but not structural information; therefore, TOF-MS is indispensable to allow the identification of numerous separated compounds.

The FID detectors, the first detectors applied to GCxGC, have a negligible internal volume and can acquire data at frequencies of 50–200 Hz (von Muhlen, Khummueng, Zini, Caramao and Marriott, 2006).

The focus of this study is to determine trace levels of halogenated contaminants in environmental samples and the μECD is often the choice for PCB/OC/CB detection due to its high sensitivity for these compounds (de Boer, 1999; Cochran and Frame, 1999). The μECD detectors have an internal volume of 30- 150 μl and the data acquisition frequency is typically 50 Hz (LECO Corp., 2005). In order to combine the μECD with the GCxGC system, it is necessary to operate with higher make-up gas flow (Korytar et al., 2002). The best results were obtained when operated at 150ml/min make-up gas flow and temperatures above 300°C (Danielsson et al, 2005).

Since the μECD detector provides peak recognition but not structural information, a mass spectral identification is required. Due to the modulation process, most GCxGC peaks are very narrow, requiring a fast detector. Time-of-flight mass spectrometers (TOF-MS) are the detectors of choice because of their high scanning rate used to ensure accurate characterization of the peaks produced by GCxGC. The TOF-MS is the only detector that can acquire 50 or more mass spectra per second that are required for proper reconstruction of chromatograms and quantification. Using a GCxGC-TOFMS system the ion chromatograms can be used to extract specific groups of compounds based on their unique mass fragmentation patterns and thus to provide an individual analyte identification. Also, TOF-MS allows the mass spectral deconvolution of overlapping peaks when the fragmentation patterns are different (Adahchour et al., 2006; Focant et al., 2004; Dalluge et al., 2003).

2.3.2.5 Applications

PCBs, OCs and CBz are routinely analysed in many sample matrices: fish, fatty food, and environmental samples. The GCxGC technique was successfully used with FID, μECD and TOFMS detectors and applied successfully in many fields: petrochemical (GCxGC–FID, TOF-MS); organic pollutants such as pesticides, PCBs, dioxin, PAHs in food, sediments, biota, and water (GCxGC–FID, μECD, TOF-MS); cigarette smoke characterization (GCxGC–TOF-MS); breath analysis (GCxGC–FID, TOF-MS); blood plasma for pesticides determination (GCxGC–FID, TOF-MS); essential oils and food extracts (GCxGC–FID, TOF-MS). Previously published reviews of the GCxGC technique have summarized the applications in the field (Dalluge et al., 2003; Santos and Galceran, 2003; Panić and Górecki, 2006; Adachour et al., 2006; Adachor et al., 2008). The applications further pointed out in this part of the literature review will be as much as possible related to the environmental contaminants and matrices of interest for this research.

Korytar has studied the PCB separation using different column combinations for the GCxGC system (Korytar et al., 2002) and then applied the technique to a cod liver sample, sediment and dust samples. Figure 2.7 represents a two dimensional chromatogram showing the PCB separation in a cod liver sample using HP-1 as a first dimension column and HT-8 column as second dimension. All 12 priority PCBs along with the most toxic dioxins and furans were separated. The practicability of DB-XLB×LC-50 column set was demonstrated for the PCDD/F fraction of a sediment sample, after fractionation on a carbon column, where a properly tuned GCxGC system could accommodate a very high number of compounds in the 2D plane and could

separate dioxins from co-extractants. Similar, the potential of DB-1×007-65HT was demonstrated for PCA and PBDE determination in dust sample. These findings show the capability of GCxGC to analyse complex environmental samples in a single analytical run.

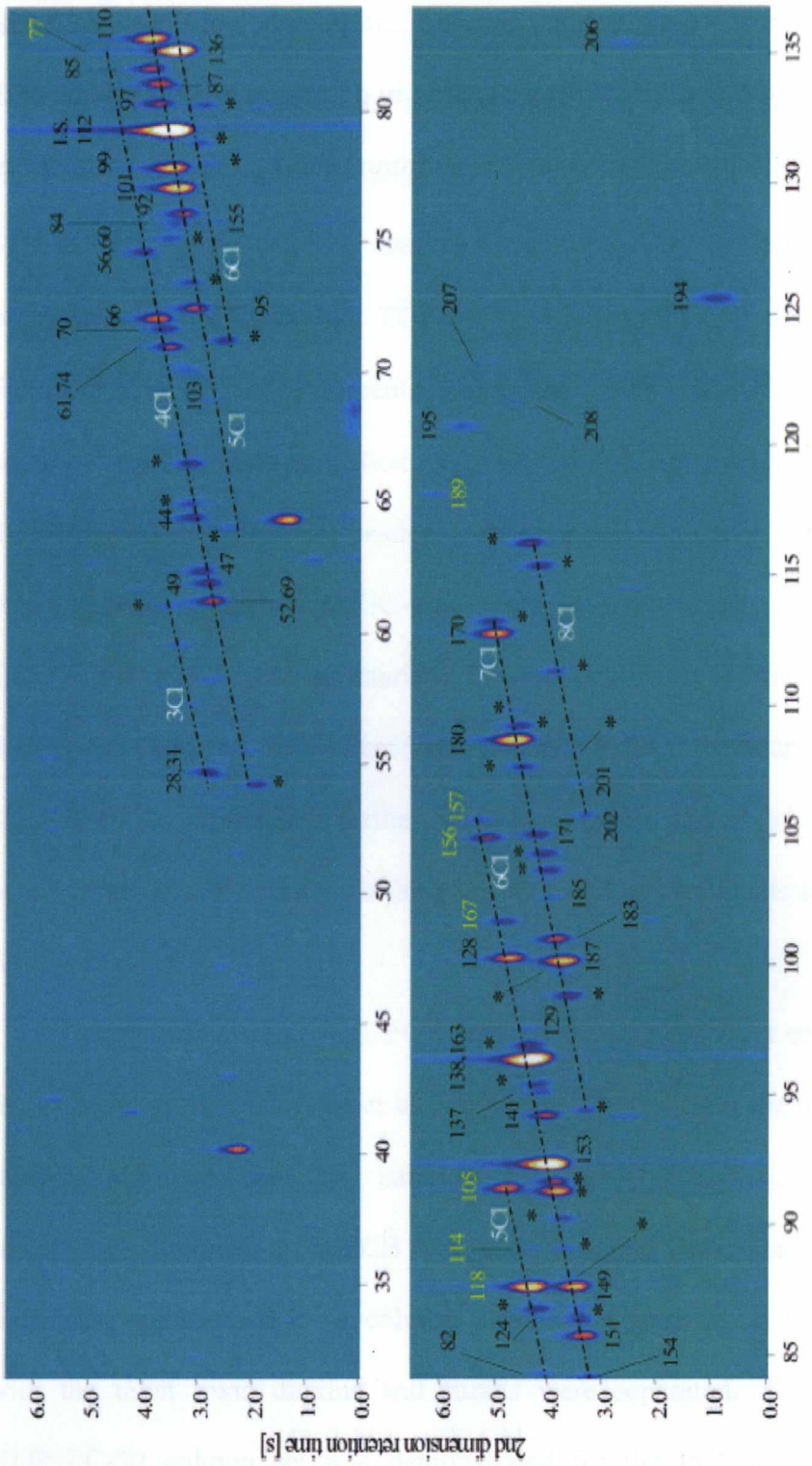


Figure 2.7 GCxGC-ECD chromatogram of a cod liver sample for PCB analysis showing their orthogonal separation (Korytar et al., 2002).

New methods using comprehensive two-dimensional gas chromatography and isotope dilution time-of-flight mass spectrometry (GCxGC-ID-TOF-MS) for the simultaneous measurement of selected polychlorinated biphenyls, organochlorine pesticides, dioxins and brominated flame retardants were presented in serum and milk samples for human monitoring as well as in foodstuffs by Focant et al. (Focant et al., 2004; Focant, Eppe, Scippo, Massart, Pirard, Maghuin-Rogister, 2004). Potential interfering compounds are separated from analytes of interests in the chromatographic GCxGC space due to the increased peak capacity, ensuring sufficient specificity for the low-mass-resolution TOFMS instrument.

Applications of the GCxGC technique, using Rtx-5 x Rtx-200 column set-up, for "real-world" soil extracts were presented in LECO's technical notes. The quantified OC amounts were further compared with classical GC analysis data. The comparison showed how the classical parallel dual-column GC-ECD results were biased high for specific compounds, while the GCxGC analysis further resolved coeluted peaks in the second dimension and significantly reduced the bias. (Figure 2.8) (LECO, Form No. 203-821-244, 2005).

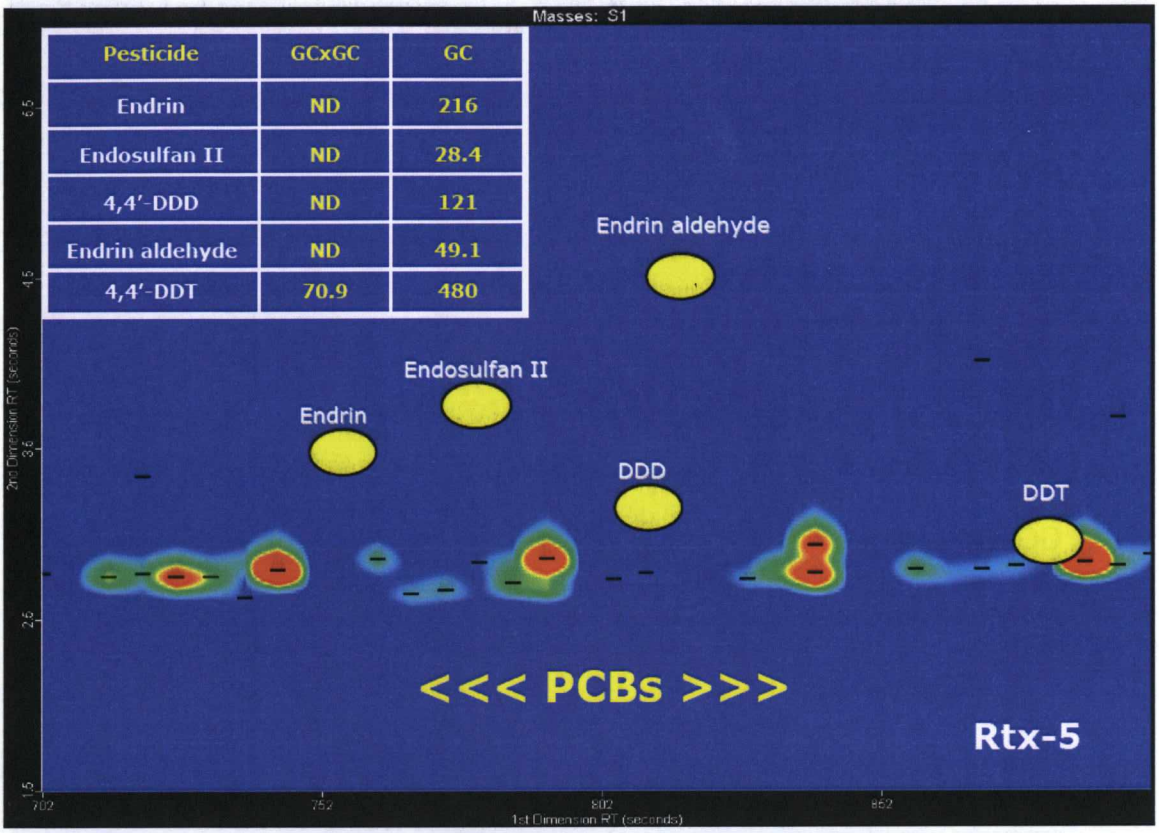


Figure 2.8 Zoomed in contour plot of a "real world" soil extract showing PCBs eluting along a relatively straight line in the first dimension and the OCs (yellow ovals) separated in the 2D and less prone to interference (and high quantitative bias), as seen in the inset table results (LECO, 2005).

A recent review of the GCxGC technique as applied on screening the persistent organohalogenated contaminants in environmental samples, summarized some of the column combinations previously presented in different studies: ZB-5, HT-8, DB-17 and BP-10 as first dimension and combined with columns of increasing polarity in the second dimension, i.e. HT-8, BPX-50 and Carbowax (Bordajandi et al., 2008). The review concluded that although none of the columns assessed in the study allowed a complete separation among the all classes of contaminants investigated, some of the column combinations provided satisfactory separations among selected families: HT-8×BPX-50

for PBDEs and PCDD/Fs, DB-17×HT-8 for PCNs and OCPs, BP-10×BPX-50 for CTT, PCDD/Fs and PBDEs. To further separate all the expected classes of contaminants, one should consider the parallel use of GCxGC equipped with different column combinations.

2.4. PROJECT OBJECTIVES AND HYPOTHESIS

This chapter has given a review of the relevant literature with regards to applicability of GCxGC technique for the PCBs, OCs and CBz analysis. The comprehensive multi-dimensional gas chromatography coupled with micro-electron capture detector has shown to be a very powerful technique allowing simultaneous analysis of the halogenated contaminants. Furthermore, the improved separation power of GCxGC allows the implementation of faster and more environmental friendly extraction and clean-up methods prior to instrumental analysis.

PCBs, OCs and CBz are ubiquitous in the environment and they are routinely analysed by many laboratories following complex sample preparation and fractionation steps. It was shown that GCxGC is a feasible technique for analysing these compounds of interests in one run with excellent separations; however, the applicability of GCxGC was not extensively studied for soil, sediment and sludge samples. The objectives of this project are to accurately identify and quantify the PCBs, OCs and CBs present in sludge and sediment samples in a single analytical run by using the GCxGC technique. The column selection for this study, DB-1 x Rtx-PCB, was based on previous reported data for PCBs and OCs simultaneous separation (LECO, 2005). The first step is to achieve chromatographic separation for all the target compounds in one analysis prior to calibration and quantification. In addition to PCBs, OCs and CBz, other contaminant

classes will be evaluated to "map" their elution in the chromatographic space when using the same instrumental set-up. This might serve as a preliminary assessment of the presence of other contaminant classes in environmental samples. Reference materials along with sediments and sludge samples previously analysed by classical GC analysis (Dioxin and Toxic Organics Section, Ontario Ministry of the Environment) will be analysed with the new GCxGC method and their results compared. The premise is that all target contaminants can be analysed simultaneously without any necessary fractionation prior to GCxGC analysis. Once the method is developed and proved to be precise and accurate it would be expected that the compounds of interest present in real-life samples can be accurately identified and quantified.

CHAPTER 3: EXPERIMENTAL

3.1 GENERAL DISCUSSION

This study involved the development of a new method for the analysis of PCBs, OCs and CBz in sediment and sludge samples using GCxGC-μECD. Prior to instrumental analysis, the extraction and clean-up procedures were optimized to obtain the best recoveries of the target analytes with reduced solvent use and less sample preparation time. While the previous chapter, Literature Review - Chapter 2, described the theoretical aspects of the procedures involved, Chapter 3 describes in detail the equipment, the experimental conditions and chemicals used. This work was conducted at the Ontario Ministry of the Environment, Laboratory Services Branch, Dioxin and Toxic Organics unit and the experimental approach is presented in Figure 3.1.

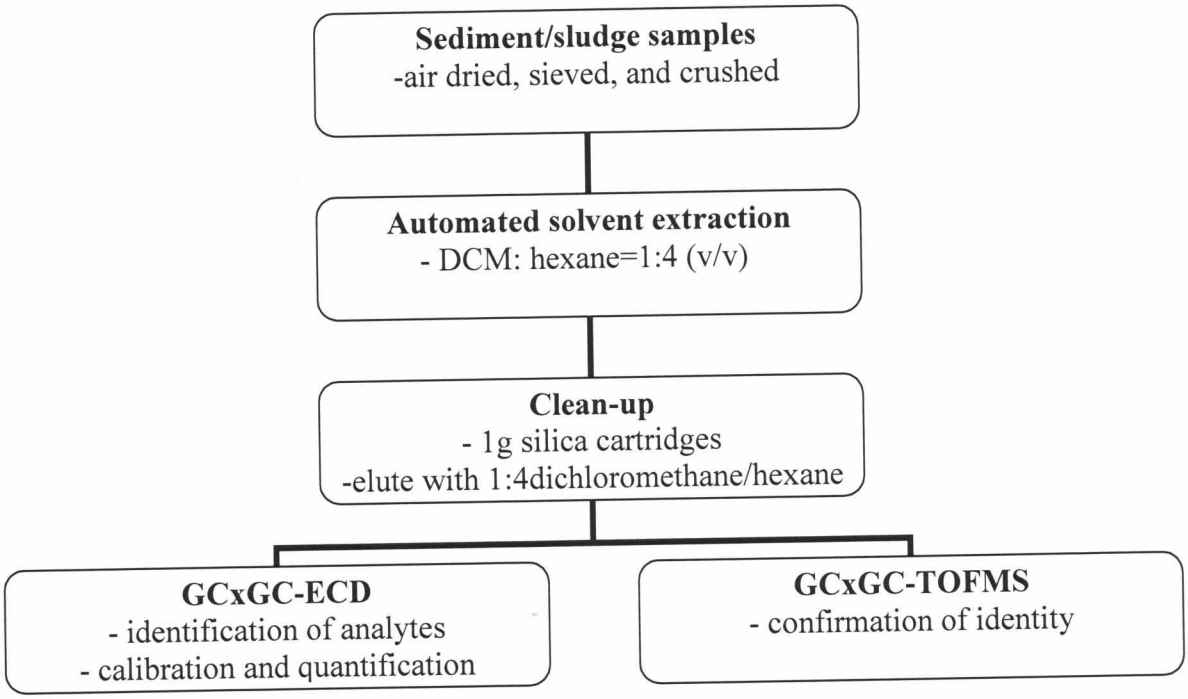


Figure 3.1 Experimental approach of the GCxGC-ECD study.

3.2 REAGENTS AND MATERIALS

3.2.1 Standards and chemicals

Polychlorinated biphenyl standards were obtained from Wellington Laboratories (Guelph ON, Canada) having different congener composition and concentration:

i. PCB BP-MS containing 62 congeners was used for calibration.

ii. PCB BP-EC containing 62 congeners was used for preparing the spiking solution.

iii. PCB BP-MS-PL1, -PL2 and -PL3 were used for identification purposes.

The congeners present in these PCB standards as well as their concentration are listed in Appendix A.

Along with PCBs, CBz standard mixture of 15 chlorobenzenes, OC standard mixture of 23 compounds, decachlorobiphenyl and 1,3,5-tribromobenzene were purchased from UltraScientific (North Kingstown, RI, USA).

Six level calibration standard solutions of PCB/OC/CBz were prepared by mixing the above PCB (BP-MS), OC and CB standards in isooctane with the final concentrations ranging from 1 to 500 ng/mL. Similarly, an OC/CBz spiking solution and decachlorobiphenyl/1,3,5-tribromobenzene surrogate solution was prepared with the final concentration of 500 ng/mL. In addition, 4,4'-dibromooctafluorobiphenyl was used as internal standard for PCB congeners' quantification. Prior to injection, 10 μL/mL of 4,4'-dibromooctafluorobiphenyl standard solution at 1 μg/ml were added in each sample.

The solvents used for extraction, clean-up and stock solutions were distilled-in-glass grade and are listed in Appendix A.

3.2.2 Reference Materials and Sludge/Sediment Samples

The method's accuracy was assessed by analysing standard reference materials for both sediment and sludge matrices. Thus, SRM1944 sediment reference material was purchased from NIST (Gaithersburg, MD, USA), EC-8 sediment reference material was obtained from Environment Canada (National Water Research Institute, Burlington, ON, Canada) and CNS-312 sludge reference material was acquired from RT-Corp. (Laramie, WY, USA).

The sediments and sludges were selected from previously analysed samples by classical GC-ECD analysis. Sediments were obtained from an inter-laboratory study, NY State - ELAP 08-01 Inter-laboratory Study for Solid Waste. The sludges were obtained from a wastewater treatment plant (WWTP) in Ontario.

3.3 SAMPLE PREPARATION

3.3.1 Quality Control Procedures

In order to reach the data quality objectives, a quality control procedure need to be established for each method. All the sediment and sludge samples are processed together with a method blank, appropriate method spikes, duplicates and reference materials. The method blank is a check for any possible contamination during the sample preparation and analysis; it is not expected to have any concentration of the target analytes above the quantification limits. The recoveries of the analytes are checked by using spiked blank sediment which is processed along with the "real" samples.

The sediment blank material was prepared from previously analyzed samples that do not contain detectable amounts of the components under test. The sediment was collected, solvent rinsed for two weeks using a large Soxhlet system and analyzed by classical GC-ECD. The dried material was then placed in a sealed jar (PCB5 type, amber glass) and kept at room temperature.

The samples were spiked as follows:

i. Method Blank (MB1): 100 μL of decachlorobiphenyl/1,3,5-tribromobenzene surrogate solution at 500 ng/mL.

ii. Method Spike (SP1): 100 µl of CB/OC spiking solution all compounds at 500 ng/mL (Appendix A), 50µL of BP-EC at 1-50 µg/mL and 100 µL of decachlorobiphenyl/1,3,5-tribromobenzene surrogate solution at 500 ng/mL.

iii. Samples and duplicates: 100 µL of decachlorobiphenyl/1,3,5-tribromobenzene surrogate solution at 500 ng/mL.

iv. Reference materials used: SRM 1944, EC-8 and CNS312 were not spiked.

3.3.2 Automated Solvent Extraction

As presented in Chapter 2, previous studies showed different methods of extraction using pressurized solvent extraction of PCBs, OCs and CBs along with their clean-up procedure before instrument analysis. Enhanced extraction efficiency can be achieved by solvents at high pressures and temperatures when certain intermolecular bonds can be broken (Ramos et al., 2002).

The sediment, soil or sludge samples analyzed were air dried, crushed and sieved prior to extraction. Using a mortar and pestle the samples were ground, sieved if necessary by using a No. 8 mesh sieve and then homogenized. A weight of one gram from each sample was loaded into an 11 mL stainless steal ASE cell with stainless steel frits and cellulose filters. Ottawa sand (purchased from Anachemia, Montreal, QC, Canada) was used to fill out the dead volume in the extraction cells. Figure 3.2 represents the schematic of an extraction cell.

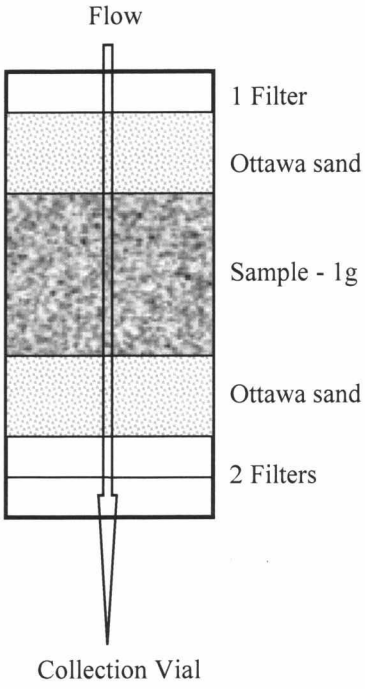


Figure 3.2 Graphical representation of an ASE cell set-up.

Sediment/sludge samples were extracted with 1:4 dichloromethane/hexane (v/v) using automated solvent extraction (ASE 200 - Dionex Corporation, Sunnyvale, CA, USA). The ASE conditions employed in this research were optimized for high recovery in the extraction procedure by selecting the extraction time, the most efficient temperature, and the use of different solvents (Table 3.1).

Table 3.1 The ASE conditions used for the sediment/sludge samples extraction.

Cell volume:	11 mL
Temperature:	100°C
Static time:	5 min
Cycle:	1
Solvent:	1:4 dichloromethane/hexane (v/v)
Heat time:	5 min
Flush volume:	60%
Pressure	1500 psi
Purge time:	90 sec

3.3.3 Clean-up Procedure

Sediment and sludge samples are very complex matrices; therefore a clean-up procedure must be employed prior to instrumental analysis to remove possible interferences (e.g. lipids). The ASE extracts were evaporated to approximately 1mL final volume in isooctane using a Zymark Turbovap LV evaporating system (Zymark Corporation, Hopkinton, MA, USA), applied to 1g silica pre-packed cartridges (Sep-PakTM Plus, Mega Bond Elut HFTM, Varian, Mississauga, ON, Canada) and eluted with solvent.

The following are the steps used in the clean-up procedure:

i. Silica cartridges were conditioned with 5mL of 1:4 dichloromethane/hexane (v/v).

ii. The samples were applied to the pre-conditioned silica cartridges and the extraction vials were rinsed two times with approximately

1.5mL of 1:4 dichloromethane/hexane (v/v). The two rinses were added to the corresponding silica cartridge after the samples were completely adsorbed without letting the adsorbent to get dried.

iii. The cartridges were then eluted with 15 mL of 1:4 dichloromethane/hexane (v/v) and collected in the same vial as the previous rinses. The 15 mL elution solvent was chosen based on previous in-house work (Paul Helm, Ontario Ministry of the Environment, 2007 - personal conversation).

The cleaned-up extracts were evaporated to 1 mL final volume in iso-octane using a Zymark Turbovap LV evaporating system. Copper treatment (10-30 mesh) was applied to all the samples prior to analysis to remove sulphur interferences (MoE LSB Method 3270, 2008).

3.4 ANALYSIS

3.4.1 GCxGC-μECD Instrumental Set-up

The PCBs, OCs and CBs standard solutions along with the sediment/sludge final extracts were analysed using a GCxGC-μECD system provided by LECO Corporation (Benton Harbour, MI, USA). The GCxGC system is equipped with a split/splitless injector, an Agilent Technologies 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA), a stationary quadruple jet dual-stage modulator (LECO Corporation), and μECD detector (Agilent Technologies). The gas chromatograph features a secondary oven and that can be independently controlled, thus different temperature ramps can be

set up for both ovens in the same time. The system is controlled by a computer using the ChromaTOF-FID software, version 3.34, provided by LECO Corporation.

The following chromatographic column combination was used: a 30 m, 0.25mm i.d., 0.25 μm film thickness DB1 (100% dimethylpolysiloxane) from J&W Scientific (Folsom, CA, USA) as the first dimension column, and a 1.6 m, 0.18 mm id, 0.18 μm film thickness Rtx-PCB from Restek Corporation (Bellefonte, PA, USA) as a second dimension column. The connections between the first dimension and second dimension columns were made using a deactivated pres-fit connector (Restek Corporation). The GCxGC-μECD conditions are presented in Table 3.2.

Table 3.2 Instrumental conditions used for the GCxGC-μECD system.

Injector	Temperature: 250°C 1 μL **splitless** injection
Carrier Gas	**He**, 1.5 ml/min flow rate
First Dimension Column	**DB1** – 30 m x 0.25 mm i.d. x 0.25 μm film thickness
Second Dimension Column	**Rtx-PCB** – 1.6 m x 0.18 mm i.d. x 0.18 μm film thickness
Modulation	**4 sec** Hot pulse: 1 sec
μECD Detector	325°C Make-up gas: methane in argon (P5) at **150mL/min** flow

All instrumental conditions such as modulation time, secondary column length, carrier gas flow rates were optimized to avoid wrap-around of the analytes and to achieve the best within- and between-class separations. The μECD was run at a flow rate of

150ml/min as previous studies showed this produced the best results (Korytar et al., 2006).

3.4.2 GCxGC-TOFMS Instrument Set-Up

Besides the GCxGC-μECD analysis, further confirmation by GCxGC-TOFMS (LECO Corporation, Benton Harbour, MI, USA) was employed in the preliminary study to confirm the retention times of the analytes identified by μECD. The GC conditions for this system are the same as the ones presented in Table 3.2; additionally, a mass spectrometer method was created (Table 3.3).

Table 3.3 The GCxGC-TOFMS specifications for mass spectrometer method.

Injector	Temperature: 250°C 1μL **splitless** injection
First Dimension Column	**DB1** – 30m x 0.25mm i.d. x 0.25μm film thickness
Second Dimension Column	**Rtx-PCB** – 1.6m x 0.18mm i.d. x 0.18μm film thickness
Modulation	**4 sec** Hot pulse: 1 sec
TOF-MS Detector	300°C Transfer line temperature 250°C

3.5 STATISTICAL ANALYSIS

Method precision - reproducibility and repeatability - was assessed and statistical calculations were performed using SPSS student v.14 statistical package (SPSS, 2007) and Microsoft Excel for Windows XP. The accepted relative standard deviations (%) for the target compounds should be in within ±25% recovery limits.

The standard reference materials were analysed by GCxGC and the results were compared to their expected values specified in the certificate of analysis. The data was plotted using Microsoft Excel and statistical calculations were employed for assessing the uncertainties of the method.

CHAPTER 4: RESULTS

Chapter 4 of this study is presented in four parts: the separation of the target analytes, the calibration and quantification, the uncertainties calculations of the method and the analysis of sludge and sediment samples. All the data for this research was determined following the sample preparation procedures and the optimized instrumental GCxGC-μECD conditions shown in the previous chapter.

4.1 GCXGC SEPARATION

The introduction of comprehensive two-dimensional gas chromatography provided significant increases in peak capacity, sensitivity and speed of analysis by applying two independent separations to a sample (Dalluge *et al.*, 2003). The enhanced selectivity of GCxGC enabled μECD, a less selective detector, to be used for the analysis of persistent environmental contaminants simultaneously in a single analytical run (Korytar et al., 2003 and 2006). As a result, GCxGC coupled with μECD detector was used to achieve the separation of target organic contaminants in a single analytical run and the obtained data is presented in the following section.

4.1.1 Identification of the target analytes

After optimizing the GCxGC method to obtain the best chromatographic separation of the target analytes, the next step was to identify each of the components present in standard mixes. Since μECD does not provide any mass spectral information, the retention times of each component in the PCB/OC/CB standard mix needed to be

determined. For this purpose, individual constituent standards along with standard mixes containing a small number of components/congeners (e.g., BPMS-PL1, -PL2, -PL3) were analysed using the established GCxGC method and their retention times compared to the ones from the PCB/OC/CB standard mix. The μECD data was based on retention time results only; thus, further confirmation by GCxGC-TOFMS was employed. Target analytes identified by GCxGC-μECD were confirmed by GCxGC-TOFMS. Figures 4.1 to 4.5 represent the final identification of each compound present in the standard mixes analysed.

4.1.2 Within-Class Separation

One of the goals of the study was to separate the target halogenated environmental pollutants within their class using DB-1 x Rtx-PCB. When the PCB, OC, CB standard solutions were analysed separately for each of the individual classes of contaminants, within-class separation was achieved with no coelutions for the OC standard (Figure 4.3) and only one coelution for PCB and CB standards (Figure 4.1 and 4.4).

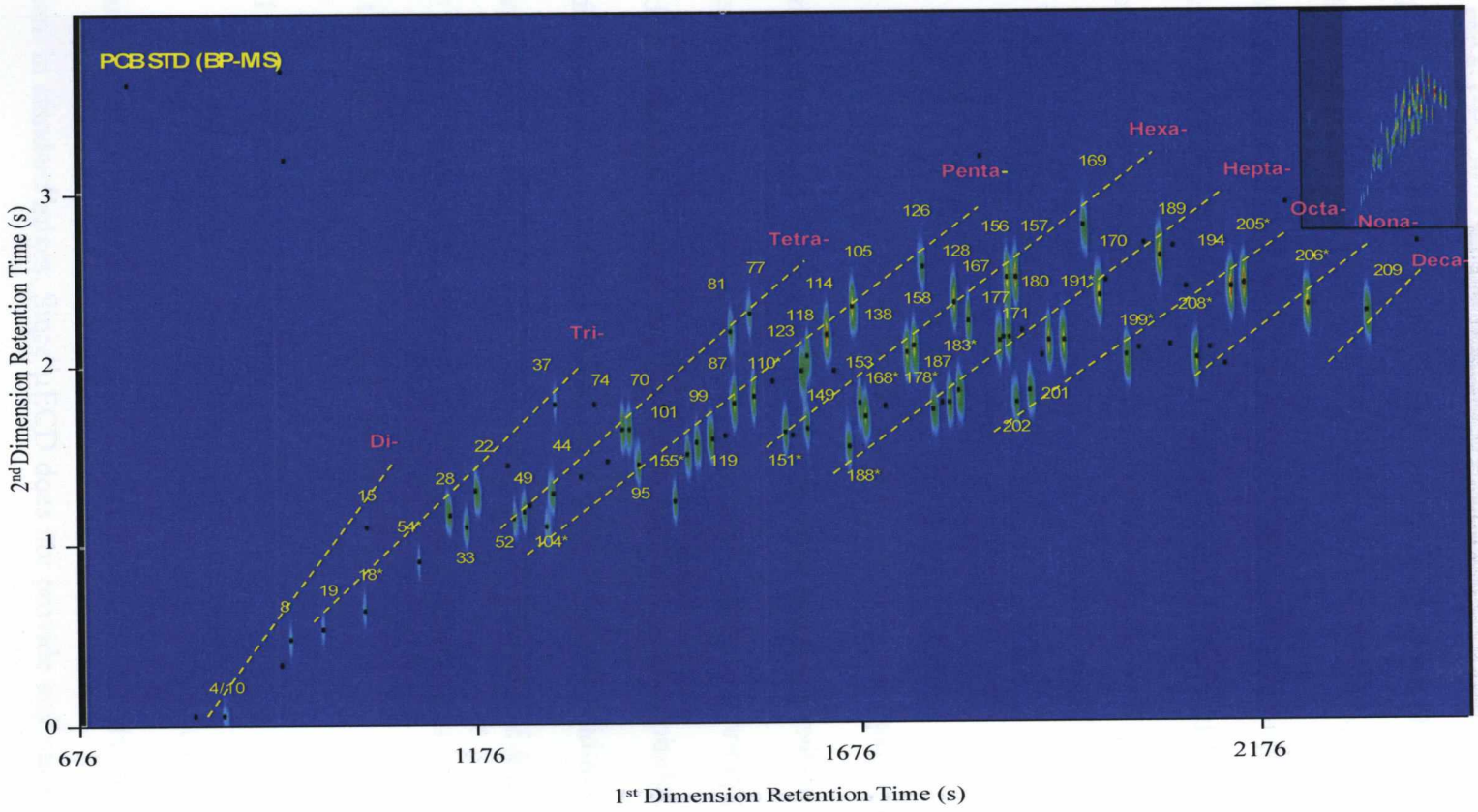


Figure 4.1 GCxGC-μECD two dimensional chromatogram of PCB congeners standard representing their orthogonal separation.

The two dimensional chromatogram representing PCBs, shows that orthogonal separation was achieved when using DB1x Rtx-PCB column combination. An ordered structure is observed in the second dimension for structurally related compounds, in this case PCBs. These findings were similar with the previous published data for different column combinations (Korytar et al., 2003).

The PCB congeners are separated according to their degree of chlorination as well as with their planar structure. Due to the selectivity of Rtx-PCB for the planar compounds (LECO Technical Note, 2005), the non- and mono-ortho PCBs (PCBs 37, 77, 81, 126 and 169) elute later in the second dimension. The dotted lines in Figure 4.1 represent the level of chlorination of PCBs, from mono- to decachlorobiphenyl.

One of the advantages of this technique over the classical GC analysis, as previous studies have shown, is the second dimension separation. Therefore, peaks that coelute on a classical DB1 column in 1D analysis (Frame and al., 1996) are further resolved by Rtx-PCB. Some examples of these coeluting peaks resolved by GCxGC (Figures 4.2 a and b) are PCB77/PCB110 and PCB118/PCB149.

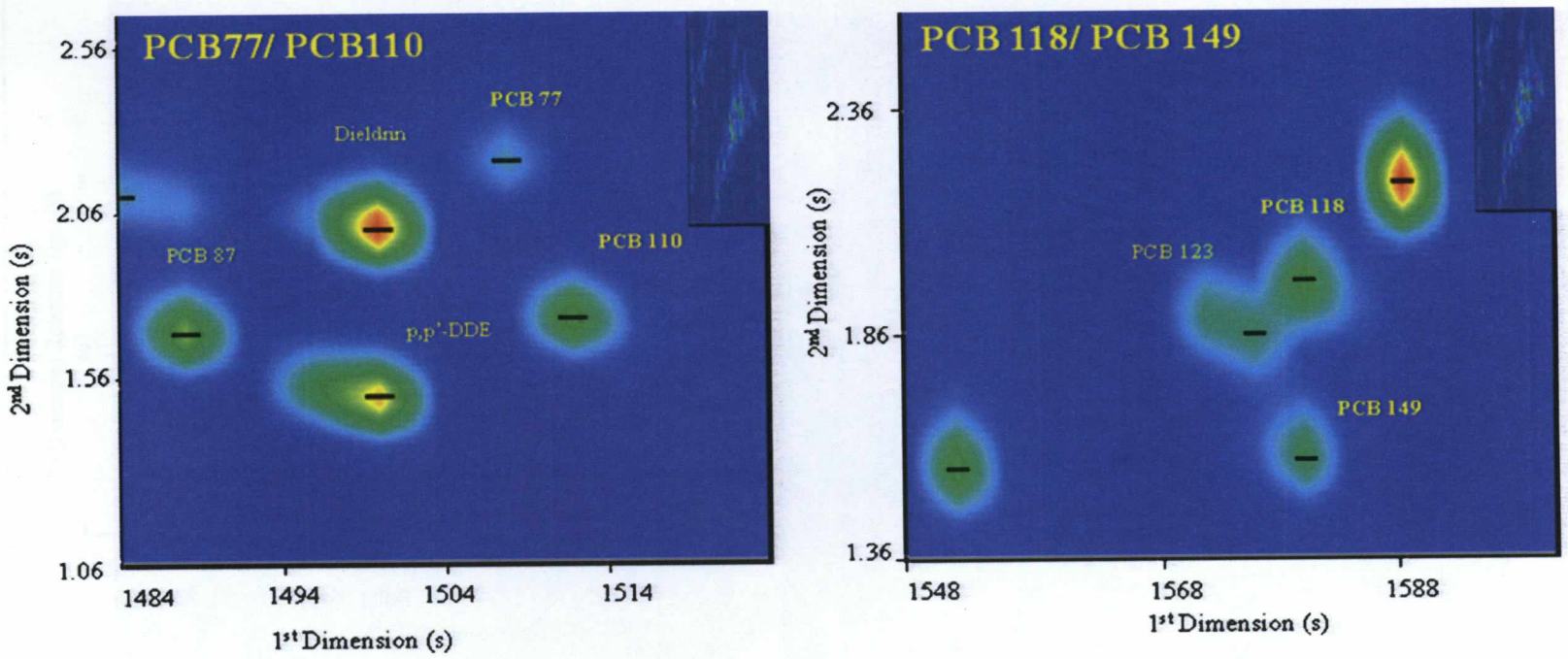


Figure 4.2 Examples of critical PCB pairs resolved in the second dimension by GCxGC, DB1xRtx-PCB column combination: a) PCB77/PCB110 and b) PCB118/PCB149.

Within-class separation was achieved with no coelutions for the OCs standard analysed, all 25 compounds were separated in one analytical run (Figure 4.3).

For the CBz standard analysis by GCxGC-μECD one coelution was found: 1,2,3,5-TCB/ 1,2,4,5-TCB (Figure 4.4).

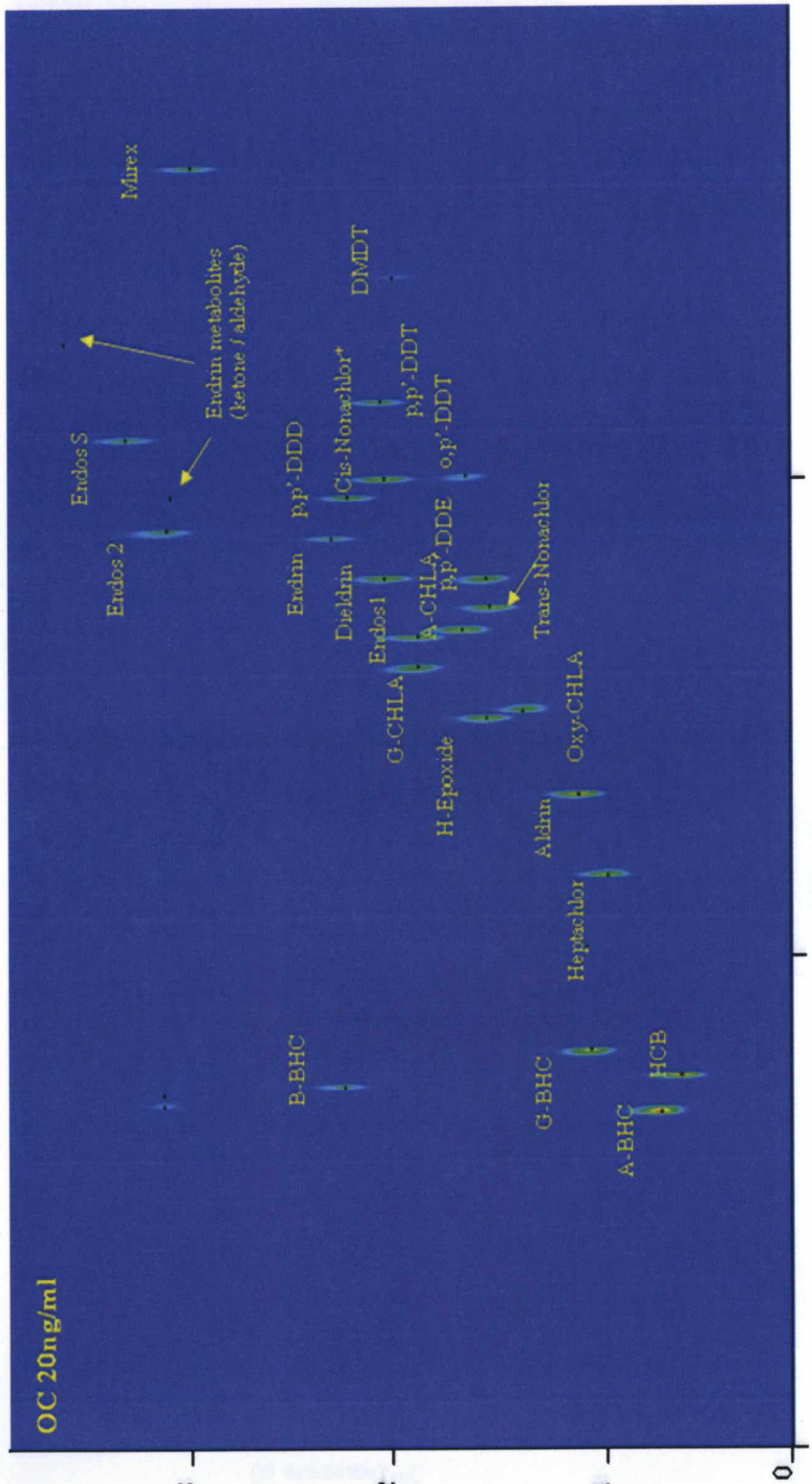


Figure 4.3 GCxGC-μECD two dimensional chromatogram of OC standard representing their separation by DB1-Rtx-PCB column combination.

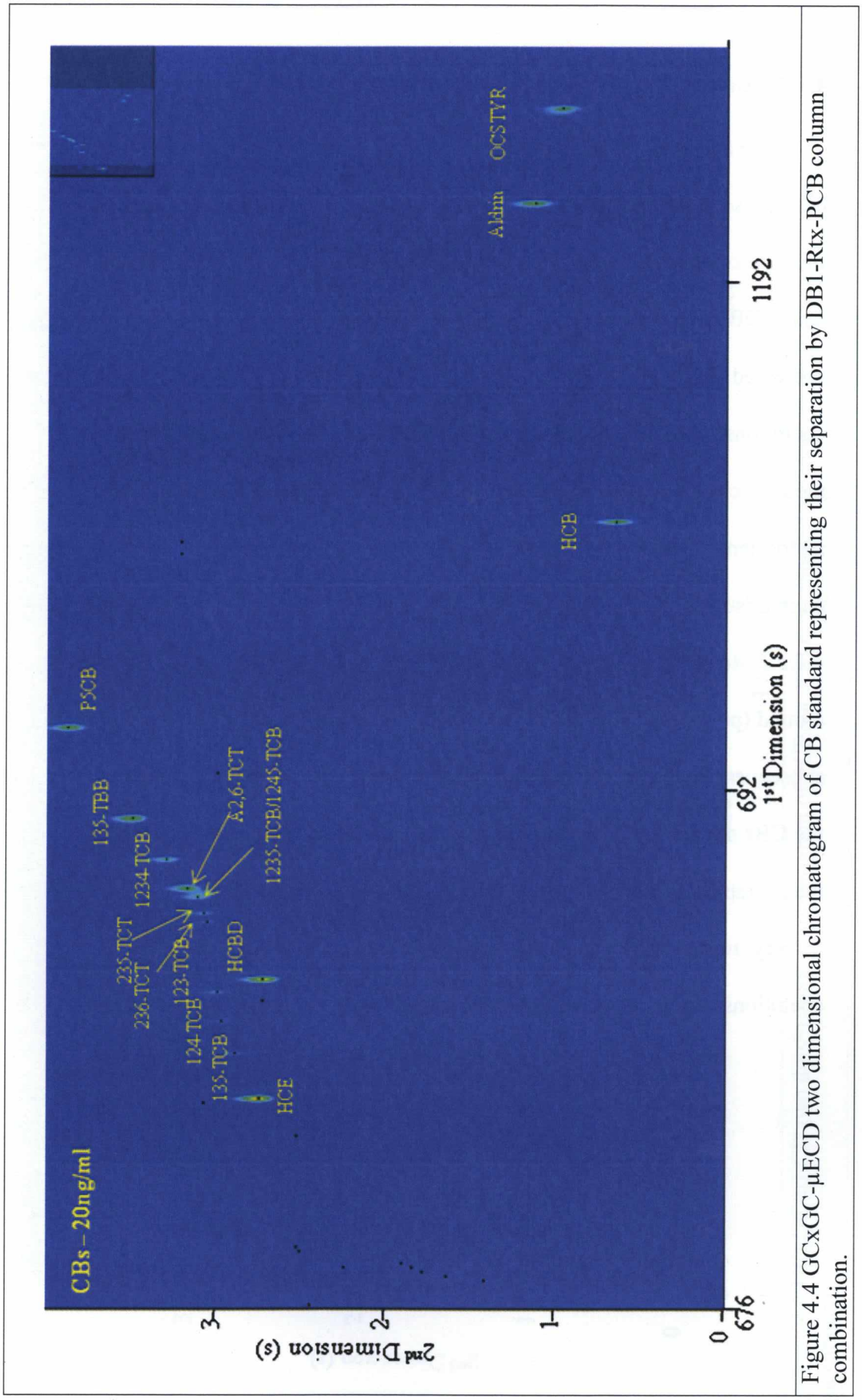


Figure 4.4 GCxGC-μECD two dimensional chromatogram of CB standard representing their separation by DB1-Rtx-PCB column combination.

4.1.3 Between-Class Separation

After assessing the within-class chromatographic separation, the next goal was to achieve separation of all three target classes of compounds in a single analytical run. A mixture containing 62 PCBs, 23 OCs and 15 CBz (96 compounds in total) was analyzed using DB1xRtx-PCB column configuration and the two dimensional chromatogram is presented in Figure 4.5. Separation was achieved with only three between-class coelutions: heptachlor-epoxide/PCB74, cis-nonachlor/PCB114, and methoxychlor/PCB171 (Figure 4.6). The classical GC-ECD analysis involves four instruments that analyze each different compound classe of interest separately. A comparison of the techniques will be described in a different section of this study, section *4.2.2.5 Accuracy*, to emphasize the significance of the research. Additionally, wrap-around (peaks that spend more time in the 2D than one modulation cycle and elute within subsequent modulation cycles) is observed for chlorobenzenes in this separation. Since the CBz do not interfere with any other analytes of interest in the chromatographic space, their wrap-around is not an issue for quantification purposes. Furthermore, the separation is very reproducible between the analytical runs. In addition to GCxGC-μECD, the coelutions were confirmed by GCxGC-TOFMS.

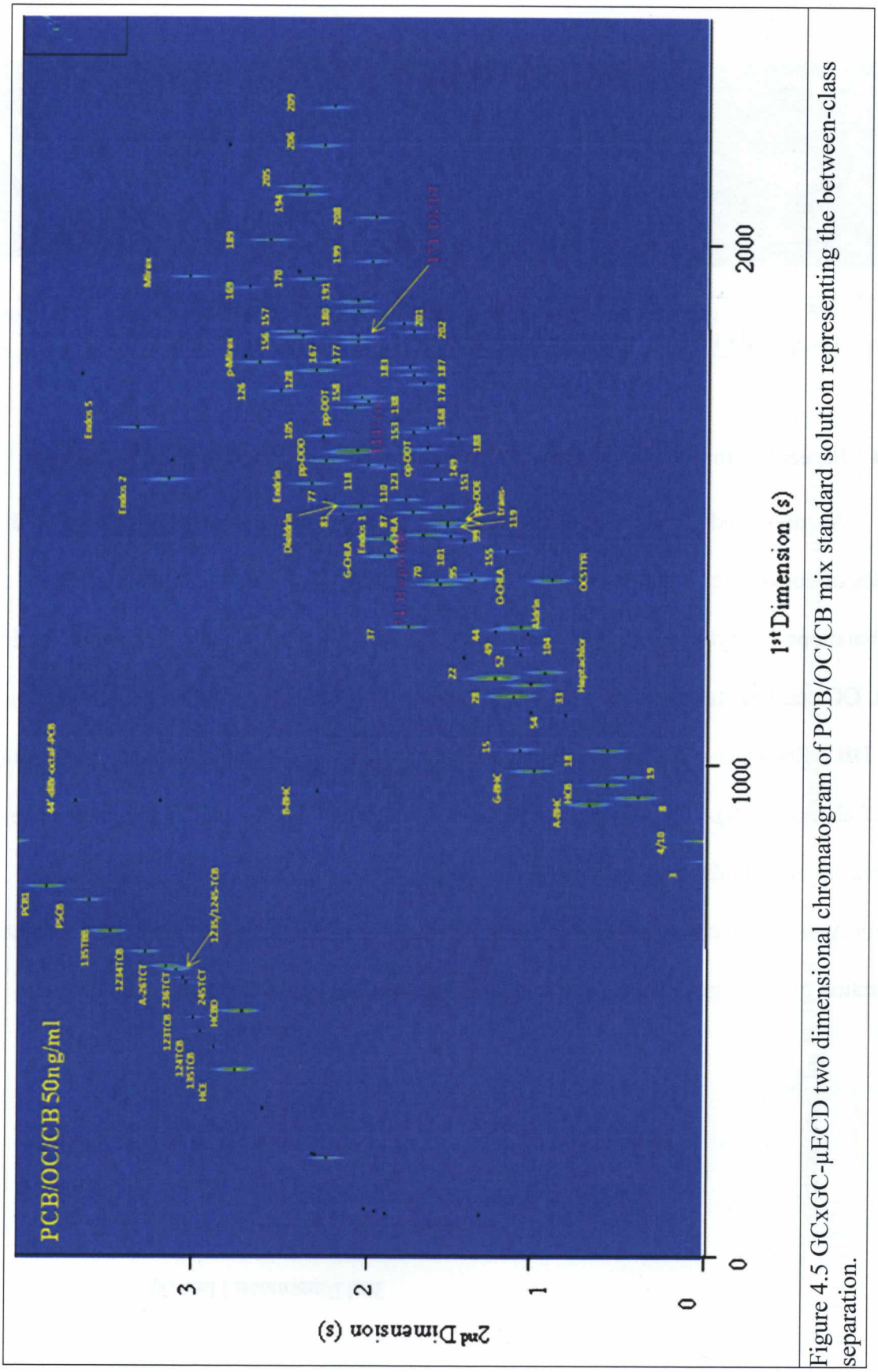


Figure 4.5 GCxGC-μECD two dimensional chromatogram of PCB/OC/CB mix standard solution representing the between-class separation.

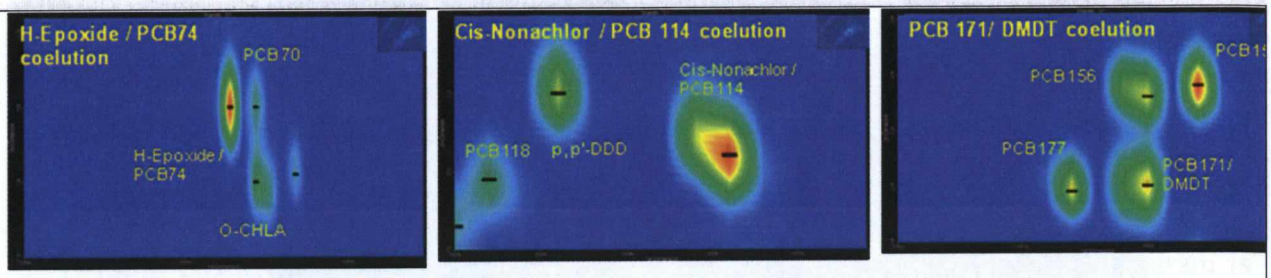


Figure 4.6 Two dimensional chromatograms representing between-class coelutions: a) heptachlor-epoxide/PCB74, b) cis-nonachlor/PCB114 and c) methoxychlor/PCB171.

4.1.4 Screening for PCNs, Dioxins, PCDEs and other persistent organic pollutants

As presented in the literature review section, previous studies showed how different classes of contaminants can be separated in a single analytical run when using different column combinations (Korytar et al., 2002 and 2005; Bordajandi et al., 2008). Therefore, besides the PCB, OC and CB standards previously discussed, other contaminant classes were evaluated for the DB1xRtx-PCB column combination: dioxins/furans, toxaphene and polychlorinated naphthalenes (PCN). Figure 4.7 represents an overlay of GCxGC-μECD chromatograms of all classes of compounds mentioned above.

By applying two independent separations to the sample, GCxGC technique enhanced the separation of the target analytes and increased the peak capacity (Dimandja, 2004).

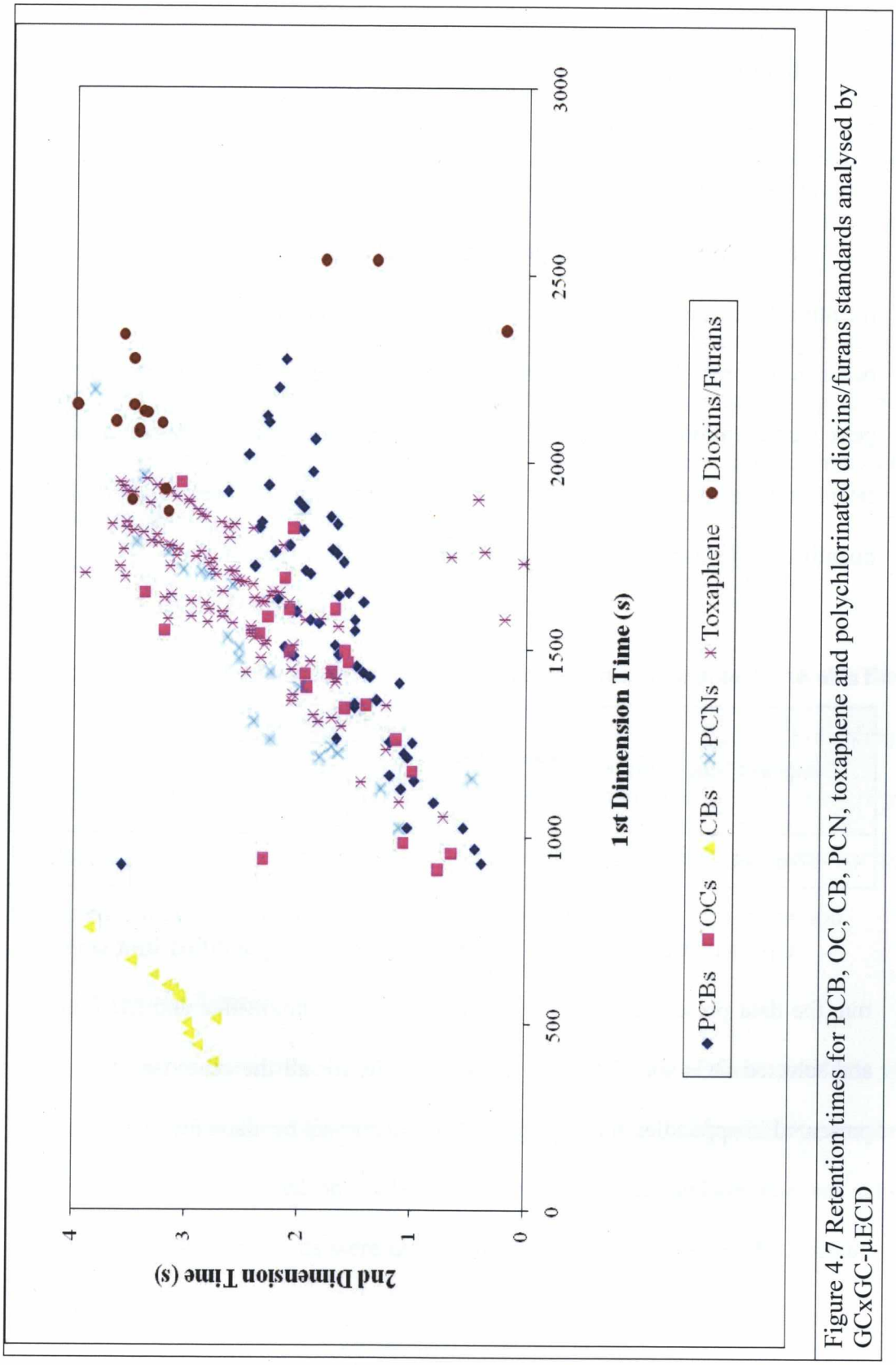


Figure 4.7 Retention times for PCB, OC, CB, PCN, toxaphene and polychlorinated dioxins/furans standards analysed by GCxGC-μECD

4.2 CALIBRATION AND QUANTIFICATION

In order to quantify the target analytes six-level calibration curves were built. An external standard method was used to quantify OCs and CBz and an internal standard method was used for PCB quantification.

In addition to quantification, the internal standard was also monitored to check the retention time stability between analytical runs (Korytar et al., 2006). A small leak can occur in the press-fit connectors and the chromatographic peaks will be shifted. Since the peak identification using an ECD detector is retention time dependent, making sure that retention times are accurate is very important. Thus, the internal standard used for PCBs quantification was monitored for retention time shifts (Table 4.1).

Table 4.1 Data representing the variability of internal standard between analytical runs.

Compound Name - ISTD	Concentration (pg/µL)	R.T.Mean (s) n=10		R.T. SD (s)		Area	
		1D	2D	1D	2D	Mean n=10	RSD (%)
4,4'-dibromooctafluorobiphenyl	10	924	3.652	0.01	0.01	1491364.00	12.07

Given that almost one hundred compounds are quantified in a single analytical run, the data presented in this study will be for the dioxin-like and EU indicator PCBs, and selected OCs and CBz. The detailed results for all the other target analytes will be presented in appendies and appropriate references will be made for each section.

4.2.1 Data Processing Method Parameters

Standards (PCBs, OCs and CBz standard mixes), reference materials and sample extracts were injected in the GCxGC-μECD system and the resulting data was recorded using ChromaTOF-GC software version 3.34 (LECO Corporation). The software was used for data acquisition, instrument control and data analysis (integration, quantification and reporting).

In order to process the chromatographic data, a data processing method was created. The method includes the time selected for baseline tracking, the signal to noise (S/N) that selects which peaks will be picked up for quantification, the calibration tables selected to quantify the compounds of interest.

4.2.2 Method Validation

Method validation defines the analytical requirements and confirms that the new method meets specific requirements (MoE, LSBSOP.027, 2008). Following the identification of the compounds presented in the previous section, the next steps for validating the method were to determine the linearity and establish the dynamic range of the instrument, to confirm accuracy and to establish the precision of the method.

4.2.2.1 Dynamic Range

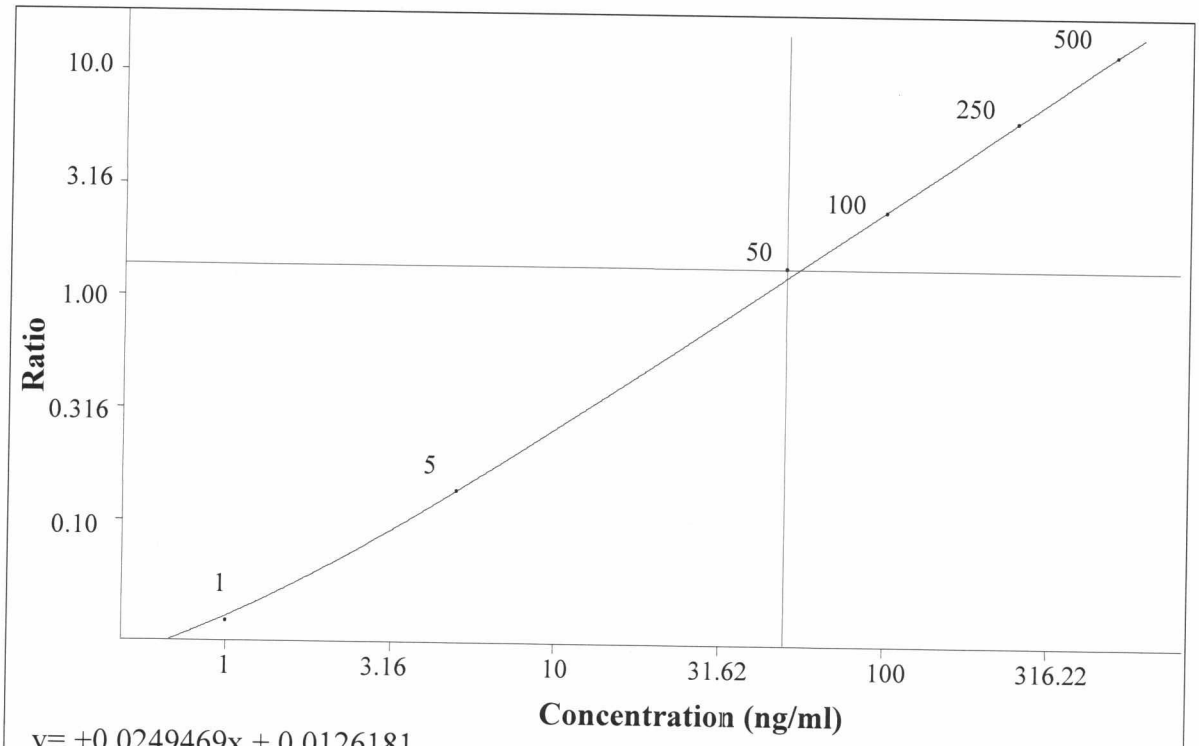
Linearity is determined by a series of injections of five or more standards whose concentrations cover the expected concentration range. The calibration curves for this specific method were based on six-level standards. The intermediate standard solutions prepared from pure standards were used to prepare the standards for the calibration and spiking solution.

Standards PCB/OC/CB mixtures ranging from1-500ng/ml were directly injected in the GCxGC-μECD system and data were recorded using ChromaTOFv.3.34 software (LECO Corporation). Table 4.2 provides the composition and levels of selected standards along with a summary of the calibration settings and results.

Table 4.2 Identification, retention times and within-run repeatability for the selected PCB/OC/CB compounds analysed by GCxGC-μECD

Compound Name	**Retention Time (s)**		**Calibration**			**Repeatability (Std. 50 ng/mL)**		
Dioxin-like PCBs	**1D**	**2D**	**Dynamic Range (pg/μL)**	**R**	**Curve Type**	**Mean (n=10)**	**SD**	**RSD (%)**
PCB77	1508	2.32	1 - 500	0.999	Linear	47.44	1.29	2.72
PCB81	1484	2.22	1 - 500	0.999	Quadratic	54.40	0.28	0.51
PCB126	1724	2.56	1 - 500	0.999	Quadratic	63.18	1.17	1.84
PCB169	1928	2.76	1 - 500	0.999	Quadratic	52.40	0.51	0.97
PCB105	1636	2.34	1 - 500	0.999	Quadratic	52.16	0.30	0.58
PCB114/cis-nonachlor	1604	2.18	2 - 1000	0.999	Quadratic	113.64	1.20	1.06
PCB118	1580	2.06	1 - 500	0.999	Quadratic	55.91	1.57	2.80
PCB123	1572	1.98	1 - 500	0.999	Quadratic	52.55	0.62	1.18
PCB156	1832	2.16	1 - 500	0.999	Quadratic	54.45	0.71	1.30
PCB157	1840	2.52	1 - 500	0.999	Quadratic	54.06	0.40	0.74
PCB167	1780	2.26	1 - 500	0.999	Quadratic	52.17	0.34	0.66
PCB189	2020	2.64	1 - 500	0.999	Quadratic	55.88	1.05	1.89
EU Indicator PCBs								
PCB28 (250ng/mL)	1128	1.22	1 - 500	0.999	Quadratic	262.32	0.75	0.29
PCB52	1212	1.18	1 - 500	0.999	Quadratic	54.62	0.14	0.25
PCB101	1428	1.52	1 - 500	0.999	Quadratic	52.74	0.16	0.30
PCB118	1580	2.06	1 - 500	0.999	Quadratic	55.91	1.57	2.80
PCB138	1704	2.08	1 - 500	0.999	Quadratic	52.54	0.29	0.56
PCB153	1644	1.80	1 - 500	0.999	Quadratic	51.74	1.42	2.74
OC Pesticides								
α-Chlordane	1444	1.72	1 - 500	0.999	Quadratic	51.78	3.77	7.29
γ-Chlordane	1404	1.94	1 - 500	0.999	Quadratic	52.04	0.56	1.07
p,p'-DDE	1500	1.60	1 - 500	0.999	Quadratic	48.75	0.56	1.14
o,p'-DDT	1612	1.70	1 - 500	0.999	Quadratic	50.35	0.71	1.42
p,p'-DDD	1588	2.28	1 - 500	0.999	Quadratic	50.94	0.95	1.87
p,p'-DDT	1692	2.14	1 - 500	0.999	Quadratic	50.77	0.75	1.47
CBs								
HCB	956	0.60	1 - 500	0.999	Quadratic	46.85	0.50	1.06
1,2,4-TCB	472	2.94	1 - 500	0.999	Quadratic	49.29	1.65	3.36
1,3,5-TCB	440	2.86	1 - 500	0.999	Quadratic	48.57	0.48	0.99

Figures 4.8, 4.9 and 4.10 provide examples of calibration curves and calibration tables for selected compounds from each target contaminant classes. All of the 96 compounds analysed had a coefficient of linearity greater than 0.995.

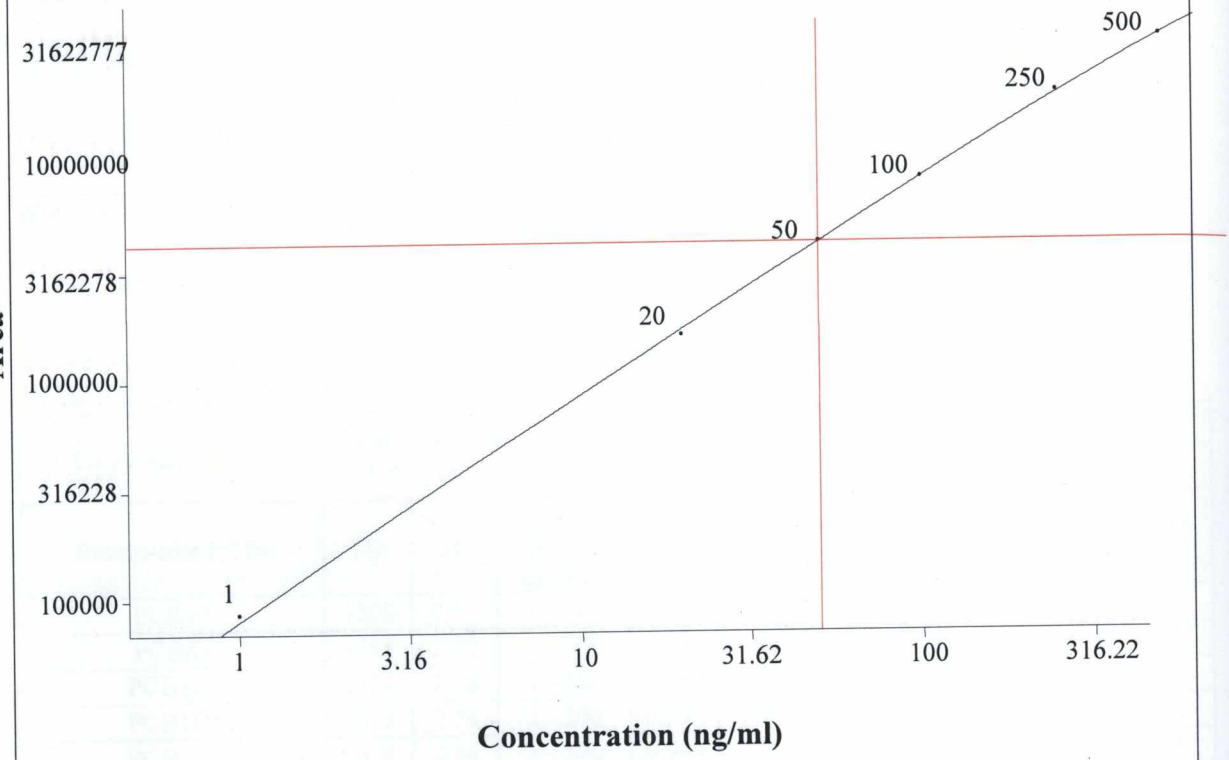


y= +0.0249469x + 0.0126181

r = 0.99994

Standard PCBc (ng/ml)	Height	Area	Calc. Conc.	% Diff. Conc.	Weighting	RF
1	4347.2	47216	0.93	7.13	1	0.358
5	24513	281871	4.99	0.28	0.2	0.274
50	237531	2639629	54.78	9.55	0.02	0.276
100	415987	4553975	98.77	1.23	0.01	0.248
250	1007256	11300009	248.93	0.43	0.004	0.249
500	1740823	20046125	497.61	0.48	0.002	0.249

Figure 4.8 Calibration curve and calibration table generated by ChromaTof 3.34 software for PCB77.

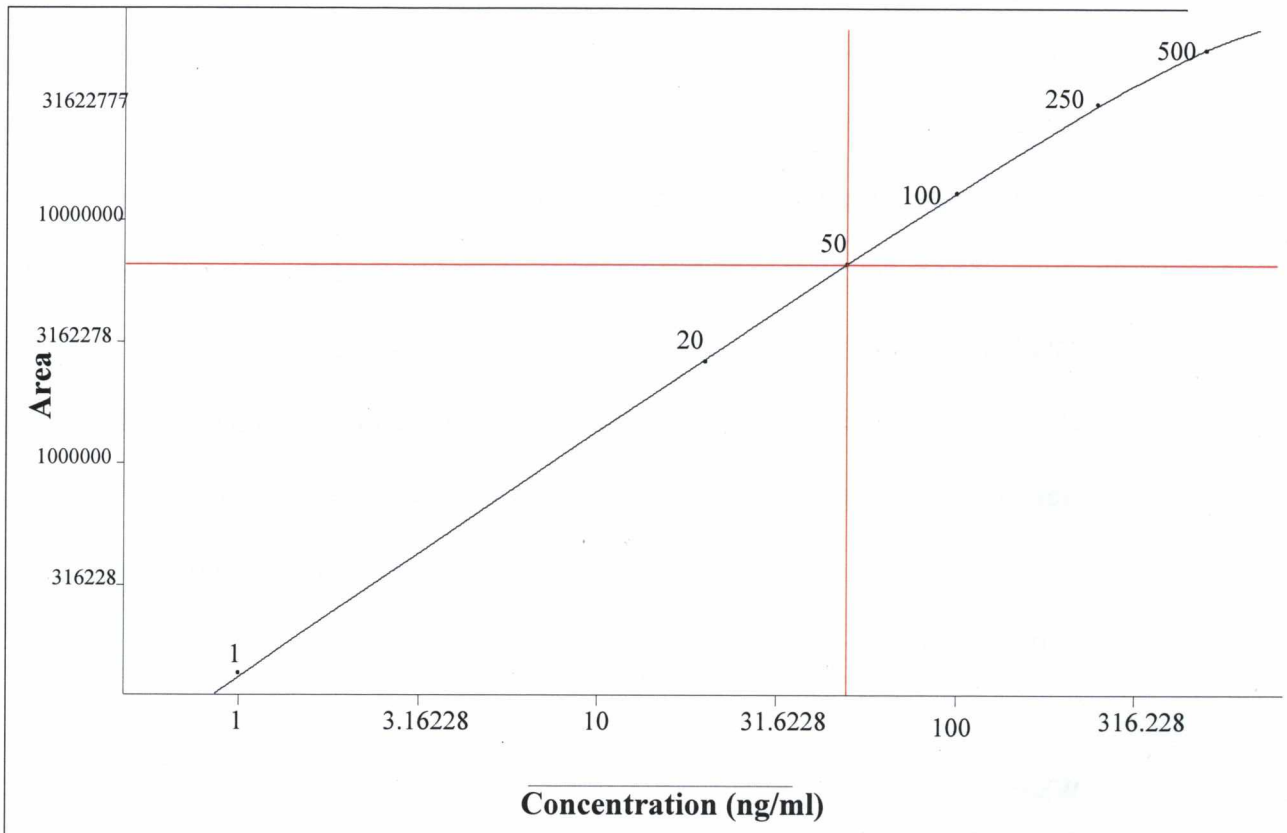


$y = -25.1849x^2 + 86470.8x - 6022.77$

$r = 0.99993$

OC Standard (ng/ml)	Height	Area	Calc. Conc.	% Diff. Conc.	Weighting	RF
1	8838.9	86002	1.07	6.46	1	86002
20	165313	1597101	18.64	6.80	0.05	79855
50	436095	4244292	49.88	0.24	0.02	84886
100	864467	8333770	99.32	0.68	0.01	83338
250	2049354	20356774	254.33	1.73	0.004	81427
500	3540911	36790294	497.67	0.47	0.002	73581

Figure 4.9 Calibration curve and calibration table generated by ChromaTof 3.34 software for o,p'-DDT.



$y = -69.8839x^2 + 136935x - 4053.11$

$r = 0.99994$

CB Standard (ng/ml)	Height	Area	Calc. Conc.	% Diff. Conc.	Weighting	RF
1	15898	137706	1.0358	3.5783	1	137706
20	305432	2633644	19.456	2.7218	0.05	131682
50	778460	6582715	49.344	1.3117	0.02	131654
100	1494677	12904669	99.302	0.69834	0.01	129047
250	3330444	30283225	254.14	1.6572	0.004	121133
500	5382913	50810213	497.29	0.54154	0.002	101620

Figure 4.10 Calibration curve and calibration table generated by ChromaTof 3.34 software for HCB.

From the data obtained and presented in these figures along with the calibration for all the target analytes PCBs, OCs and CBz by GCxGC-μECD were quantified in the range: 1-500 ng. Above 500ng/ml the ECD detector can become saturated for some of the target analytes, e.g. HCB (Figure 4.10).

The quantification by the external standard method for OCs and CBz involved the equations (4.1) and (4.2). The concentration of the target compounds in the sample extract is calculated by the instrument software using the formula (ChemStation Manual, Agilent Technologies):

$$m_x = A_x \times \frac{c_{std_x}}{Astd_x} \qquad (4.1)$$

Where:

m_x = concentration of compound x in the sample extract (ng/mL)

A_x = area count of compound x peak in the sample extract

c_{stdx} = concentration of compound x in the standard (ng/mL)

$Astd_x$ = area count of compound x peak in the standard

The concentration of the target compounds in the original sample (dilution is taken into account) is then calculated from the target compound concentration in the sample extract using the formula:

$$Amount_X = \frac{m_X \times Dilution\ Factor}{Sample\ Weight_X \times Injection\ Volume} \qquad (4.2)$$

Where:

$Amount_x$ = concentration of compound x in the sample (ng/g)

m_x = concentration of compound x in the sample extract (ng/mL)

Dilution Factor = factor converting the final extract volume to the original sample volume/amount

Sample Weight = weight of sample (g)

Injection Volume = the volume of injected sample extract (μL)

The PCB congeners were quantified using the internal standard procedure and the amounts of contaminants were calculated according to the formulas (4.3 and 4.4):

$$RF = \frac{c_{std\ x} \times A_{ISTD}}{c_{ISTD} \times A_{stdx}} \quad (4.3)$$

$$m_x = \frac{A_x}{A_{ISTD}} \times RF_x \times (Actual\ \ Amount\ \ of\ ISTD) \times M \times D \quad (4.4)$$

Where:

C_{ISTD} = concentration of internal standard

A_{ISTD} = area count of compound x peak in the standard

M = multiplier

The other statistics such as mean, standard deviation and percentage relative standard deviation presented further in this study were also calculated. The SPSS software package (SPSS student version 14) and Excel were used for the calculations.

4.2.2.2 Limit of detection and Limit of quantitation

The method detection limits and the estimated limit of quantification were obtained by analyzing eight replicates of a clean sediment matrix spiked (same matrix as the one used for blank) at the lowest level of the analytes in the calibration curve. The lowest level at which accurate quantitation can be achieved must be determined for each matrix. The calculations were based on the following standard equations (4.5, 4.6):

$$MDL = SD \cdot t_{n-1,1-a=0.99} \qquad (4.5)$$

$$LOQ = 3 \cdot MDL \qquad (4.6)$$

Where:

MDL = method detection limits

LOQ = limit of quantification

$t_{n-1, 0.01}$ = student's t value for the 99% confidence level with *n-1* degrees of freedom

The low level spiked (1 ng/mL) clean sediment samples were processed following the sample preparation steps described in the previous chapter and analyzed by GCxGC-ECD. Table 4.3 represents the data obtained and the appropriate statistical calculations for specific compounds. The MDLs varied from 0.06 to 3.5 ng/g while the estimated limits of quantification for PCB/OC/CB were found to be in the range of 1 to 10 ng/g. The results for all the target analytes are presented in Appendix B – Method Validation.

Table 4.3 Results representing MDL and LOQ calculations for selected target analytes.

Compound Name	Expected Concentration (ng/g)	Mean n=8	SD	%RSD	MDL (ng/g)	LOQ (ng/g)
Dioxin-like PCBs						
PCB77	1	1.09	0.14	13.10	0.43	1.28
PCB81	1	1.00	0.12	12.04	0.36	1.08
PCB126	1	1.11	0.08	6.99	0.23	0.70
PCB169	1	1.05	0.15	13.97	0.44	1.32
PCB105	1	1.10	0.08	7.10	0.23	0.70
PCB114/cis-nonachlor	2					
PCB118	1	1.06	0.09	8.54	0.27	0.81
PCB123	1	0.99	0.15	15.31	0.45	1.36
PCB156	1	0.93	0.09	9.54	0.27	0.80
PCB157	1	1.04	0.13	12.41	0.39	1.16
PCB167	1	1.03	0.06	5.76	0.18	0.54
PCB189	1	1.12	0.15	13.53	0.45	1.36
EU Indicator PCBs						
PCB28	1	9.17*	0.72	7.86	2.16	6.48
PCB52	1	2.56*	0.19	7.38	0.57	1.70
PCB101	1	1.24	0.20	16.07	0.59	1.78
PCB118	1	1.06	0.09	8.54	0.27	0.81
PCB138	1	1.26	0.07	5.82	0.22	0.66
PCB153	1	1.29	0.09	6.64	0.26	0.77
OC Pesticides						
p,p'-DDE	1	0.98	0.18	18.32	0.54	1.61
o,p'-DDT	1	1.15	0.17	14.85	0.51	1.54
p,p'-DDD	1	1.21	0.21	17.41	0.63	1.90
p,p'-DDT	1	1.27	0.21	16.15	0.62	1.85
CBs						
HCB	1	1.00	0.04	3.66	0.11	0.33
1,2,4-TCB	1					
1,3,5-TCB	1					

* Interference suspected – possible contamination during the sample preparation steps when using the glassware and evaporators prior used for different methods.

4.2.2.3 Precision: repeatability, reproducibility

Method performance was measured by analyzing ten replicates of a clean sediment sample spiked with the PCB/OC/CB spiking solution. Both within-run (repeatability) and between-run (reproducibility and accuracy) method precision were assessed and the results are presented in this section.

Within-run precision has been calculated from ten replicates processed in the same run (Table 4.4).

Table 4.4 Within-run Method Precision for PCB, OC, CB for ten replicates of spiked sediment samples.

Compound Name	N	Expected amount (ng/g)	Mean	SD	%RSD
Dioxin-like PCBs					
PCB77	10	50	41.73	5.21	12.48
PCB81	10	50	50.03	5.98	11.95
PCB126	10	50	59.56	7.40	12.42
PCB169	10	50	49.87	6.33	12.68
PCB105	10	50	49.02	6.31	12.87
PCB114/cis-nonachlor	10	100 (coelution)	110.39	2.47	2.24
PCB118	10	50	53.28	5.12	9.60
PCB123	10	50	46.85	5.22	11.14
PCB156	10	50	49.42	6.38	12.92
PCB157	10	50	46.29	6.23	13.46
PCB167	10	50	49.11	6.25	12.73
PCB189	10	50	52.46	6.92	13.19
EU Indicator PCBs					
PCB28	10	250	224.02	29.21	13.04
PCB52	10	50	50.24	6.60	13.13
PCB101	10	50	48.48	5.99	12.35
PCB118	10	50	53.28	5.12	9.60
PCB138	10	50	49.26	6.32	12.83
PCB153	10	50	48.89	6.18	12.65
OC Pesticides					
p,p'-DDE	10	50	47.44	1.03	2.18
o,p'-DDT	10	50	52.90	3.46	6.53
p,p'-DDD	10	50	51.96	1.17	2.25
p,p'-DDT	10	50	52.90	3.46	6.53
CBs					
HCB	10	50	38.57	1.18	3.07
1,2,4-TCB	10	50	34.53	4.74	13.72

Compound Name	N	Expected amount (ng/g)	Mean	SD	%RSD
1,3,5-TCB	10	50	32.63	2.76	8.46

Between-run precision has been calculated from eight replicates processed in the same run (Table 4.5).

Table 4.5 Between-run Method Precision

Compound Name	N	Expected amount (ng/g)	Mean	SD	%RSD
Dioxin-like PCBs					
PCB77	6	50	47.64	5.20	10.92
PCB81	6	50	51.12	4.19	8.19
PCB126	6	50	65.00	5.99	9.21
PCB169	6	50	50.45	4.13	8.18
PCB105	6	50	48.8	3.3	6.69
PCB114/cis-nonachlor	6	100 (coelution)	113	8.5	7.49
PCB118	6	50	49.3	2.4	4.78
PCB123	6	50	44.0	4.6	10.42
PCB156	6	50	51.9	2.1	4.03
PCB157	6	50	50.8	2.4	4.68
PCB167	6	50	49.7	3.5	6.98
PCB189	6	50	53.3	3.4	6.37
EU Indicator PCBs					
PCB28	6	250	217	13.9	6.40
PCB52	6	50	49.3	2.0	4.01
PCB101	6	50	47.6	2.1	4.47
PCB118	6	50	49.3	2.4	4.78
PCB138	6	50	49.6	2.2	4.34
PCB153	6	50	50.9	3.4	6.77
OC Pesticides					
p,p'-DDE	8	50	49.66	8.17	16.44
o,p'-DDT	8	50			
p,p'-DDD	8	50	57.34	9.25	16.14
p,p'-DDT	8	50	56.50	10.54	18.66
CBs					
HCB	8	50	39.84	7.99	20.06
1,2,4-TCB	8	50	30.8	16.3	53.01

From the RDS values presented in Tables 4.4 and 4.5 we see that between-run precision was better than within-run precision, oposite to what is usually expected. This

might be because of the small data set (6 and 8 replicates) available when assessing the between-run precision.

4.2.2.4 Accuracy – reference materials quantification

The accuracy of the method was assessed by analyzing reference materials for different matrices. Sediment (SRM1944 and EC-8) and sludge (CNS-312) samples were processed according to the extraction, clean-up and instrumental methods and the calculated analyte amounts were compared to their reference values (Figure 4.11, 4.12a and b). Furthermore, the GCxGC-μECD data for SRM1944 and EC-8 was also compared with previous results obtained from the classical GC-ECD analysis (Figure 4.10a and b). The GC-ECD data was obtained using Ontario Ministry of the Environment, Dioxins and Toxic Organics Section Methods 3412 and 3270 (MoE Method 3412 and 3270, 2008). PCB congeners values for reference materials SRM1944 and EC-8 analysed by GC-ECD were provided by Tony Chen, Dioxin and Toxic Organics, Ontario Ministry of the Environment.

A comparison between the classical GC-ECD and GCxGC-μECD analyses of sediment and sludge samples is required at this point since all the reference materials data will be presented as a comparison of the two methods to illustrate the significance of the GCxGC technique. Table 4.6 points up the major steps of each method.

Table 4.6 Comparison of GC-ECD vs. GCxGC-μECD methods for sediments/sludge samples analysis.

	GC-ECD Methods 3270 and 3412 (MOE, 2008):	**GCxGC-ECD Method**
Extraction	- Sonication and manual extraction using acetone: DCM: hexane mixture - Final volume: 100 mL	- ASE: one static extraction with DCM:hexane - Final volume: approx. 25 mL
Clean-up	- Florisil clean-up: splitting in two fractions - Copper treatment to remove sulphur interferences	- Silica clean-up (pre-packed cartridges) collecting one final fraction - Copper treatment to remove the interferences prior to analysis
Analysis	- 4 GC-ECD instruments: • DB-5 and DB-1701 for PCB congeners determination in the 1st fraction • DB-17 for OCs and PCB total analysis from 1st fraction • Rtx-CLP1 and Rtx-CLP2 for OC determination in the 2nd fraction • DB-1 and DB-1701 for CB determination in the 1st fraction	- GCxGC-μECD analysis: • DB1 x Rtx-PCB - PCBs, OCs, CBs quantified in one single analysis (45 min run)

SRM1944 – Sediment Reference Material (NIST)

SRM 1944, provided by the National Institute of Standards & Technology (NIST, USA), is a mixture of marine sediment collected near urban areas in New York and New Jersey. The purpose of this reference material is to evaluate analytical methods for the

determination of selected polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, and selected polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran congeners in marine sediment and similar matrices. All of the certified compounds were naturally present in the sediment material before processing (SRM1944 – Certificate of Analysis, 2008).

SRM1944 samples were processed following both GC-ECD and GCxGC-μECD sample preparation and analysis steps and the results are presented in Table 4.7. In addition, the data was also plotted and presented in Figure 4.11 to better visualise the outcomes.

Table 4.7 Comparison data of selected PCBs, OCs and CBs analysed by classical GC-ECD, GCxGC-μECD and their reference values for SRM1944.

Name	Certified amount (ng/g)		GCxGC-μECD Mean (n=3)		GC-ECD Mean (n=3)
	Amount (ng/g)	SD	Amount (ng/g)	SD	Amount (ng/g)
PCB 28	80.8	2.7	105.1	6.5	111.0
PCB 52	79.4	2	89.5	2.9	87.8
PCB 101	73.4	2.5	74.3	0.2	74.2
PCB 118	58	4.3	57.2	2.5	55.3
PCB 138	62.1	3	69.8	1.8	67.7
PCB 153	74	2.9	64.1	1.9	62.3
PCB 156	6.5	0.66	7.0	0.2	6.9
PCB 170	22.6	1.4	20.9	0.9	21.4
PCB 180	44.3	1.2	44.7	1.1	43.5
HCB	6.03	0.35	5.7	0.3	5.8
α-chlordane	16.5	0.83	18.7	0.7	18.5
p,p'-DDE	86	12	68.9	2.1	66.7
p,p'-DDD	108	16	105.0	8.8	101.6
p,p'-DDT	119	11	114.6	2.6	117.2

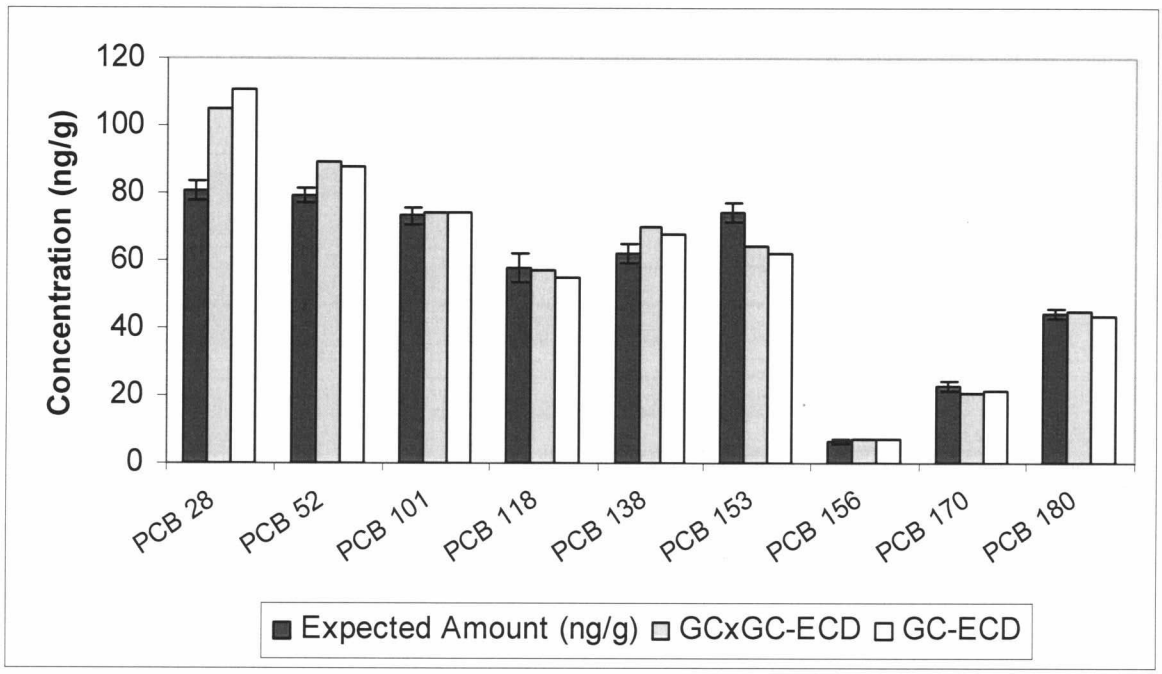


Figure 4.11 Graphical representations of specific PCB congeners amounts in SRM1944 analysed by GC-ECD, GCxGC-μECD and their certified values.

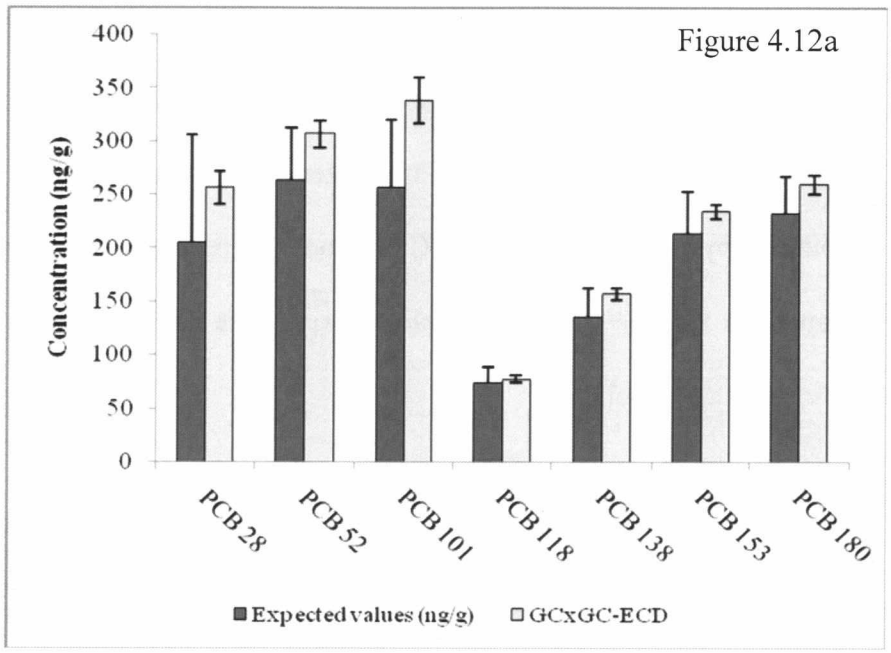
CNS312 – Sludge Reference Material (RTC)

CNS312 is a sludge material collected from a sewage works serving a residential area with light industrial influence, located in the Western United States. According to the provider's certificate of analysis (RT Corp.), the matrix was air dried, sterilized, sieved and then homogenized. These sludge reference materials are "real-world" samples, thus the analyst is challenged by the same analytical problems as for similar matrices received by the laboratory for analysis. Table 4.8 represents the data obtained by GCxGC-μECD analysis; GC-ECD data was not available for CNS312.

Table 4.8 Data comparison of the GCxGC-μECD analysis (8 replicates) and the certified reference values for CNS312 sludge reference material (RTCorp. – CNS312 Certificate of Analysis).

Name	Certified amount (ng/g)	Reference SD	GCxGC-ECD		
			Mean	Std. Deviation	%RSD
PCBs					
PCB28	205	101	256.20	15.53	6.06
PCB52	263	50	306.95	12.88	4.20
PCB101	257	63	338.54	21.19	6.26
PCB118	73.6	15	77.35	3.31	4.28
PCB138	136	26.5	157.23	5.86	3.73
PCB153	214	39	234.53	6.71	2.86
PCB180	232	36	259.50	8.64	3.33
OCs					
2,4-DDT	223	61	221.96	5.13	2.31
4,4-DDD	809	51.7	796.47	31.75	3.99
4,4-DDE	229	93.8	197.82	4.91	2.49
4,4-DDT	23.5	6.17	25.82	0.73	2.85
Aldrin	221	79.1	139.57	2.27	1.63
α-BHC	137	72.3	109.32	4.05	3.71
β-BHC	111	70.4	98.26	11.41	11.62
γ-BHC	578	249	529.18	13.77	2.60
Dieldrin	569	110	497.32	7.89	1.59
Endosulfan1	296	176	331.75	6.42	1.94
Endrin	336	135	446.05	12.47	2.80
HCB	689	277	456.48	12.79	2.80
Heptachlor	197	65.4	160.25	1.37	0.86
Heptachlor-hepoxide	104	50.1	190.35	3.36	1.77

Also, for better visualisation of the results, the data from Table 4.8 was plotted as bar graph and presented in Figures 4.12a and b.



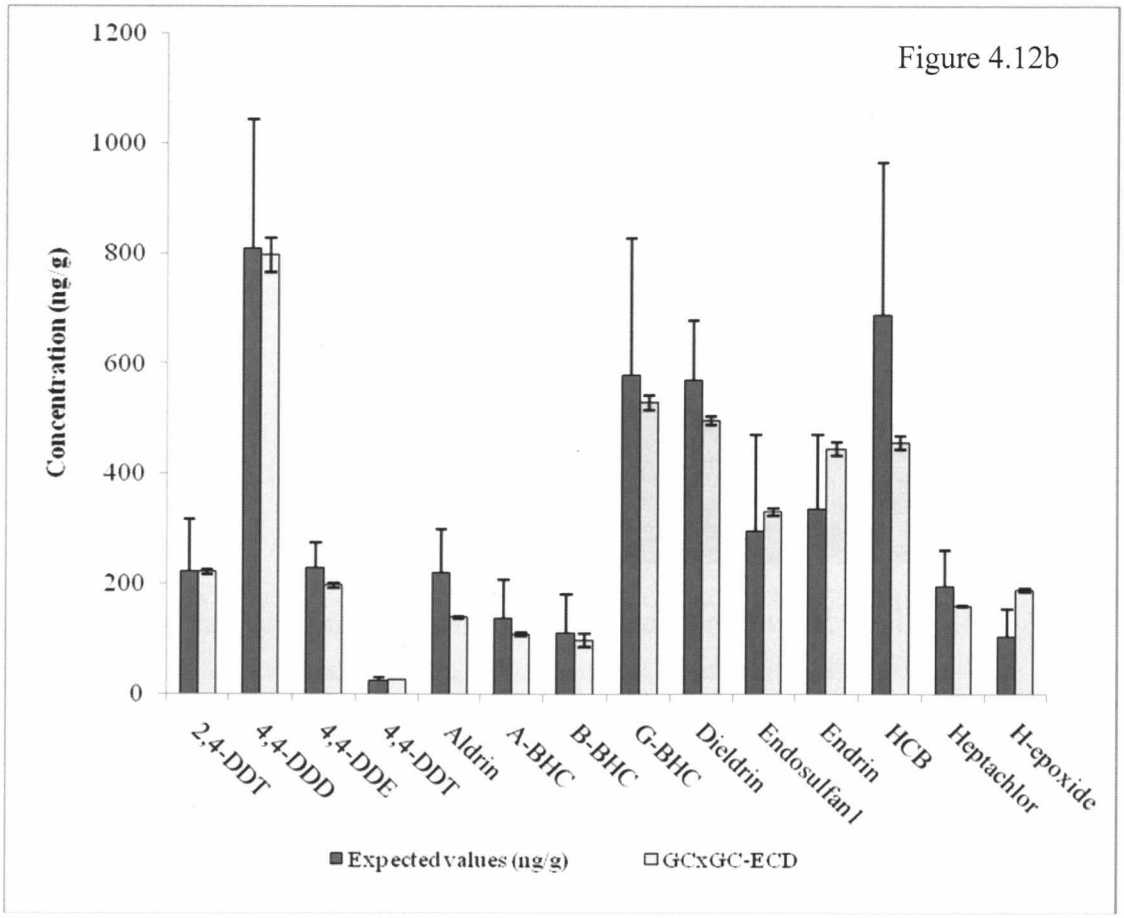


Figure 4.12 Graphical representations of selected compounds in CNS312 analysed by GCxGC-μECD and compared to their certified values: a) PCB congeners and b) OC pesticides.

EC-8 Sediments Reference Material

EC-8 sediment reference material, provided by Environment Canada, is a lake sediment collected from the plume of the Niagara River in Lake Ontario. This sample was analysed to assess the quantification of chlorobenzenes by GCxGC-μECD (Figure 4.13). The data obtained by both GC and GCxGC methods are presented in Figure 4.14. The coeluting compounds 1,2,3,5-/1,2,4,5-tetrachlorobenzene are not resolved by the 2D column and were reported as a coelution.

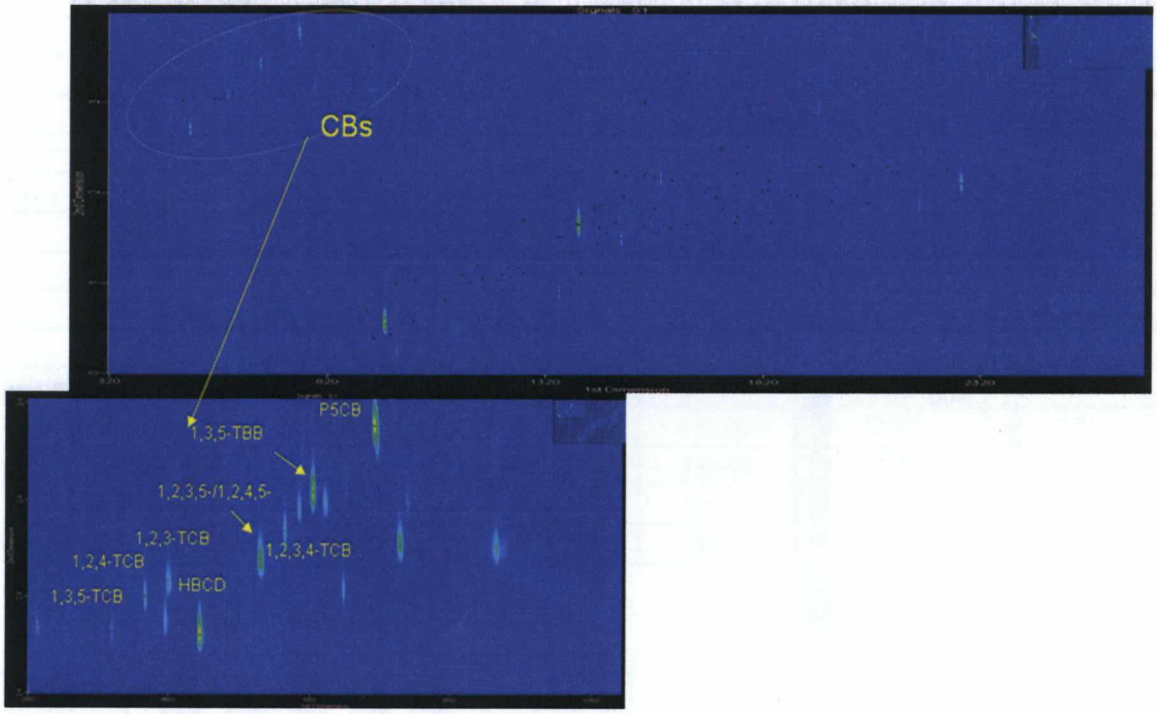


Figure 4.13 Two dimensional chromatograms representing the analysis of CBz in EC-8 sediment reference material.

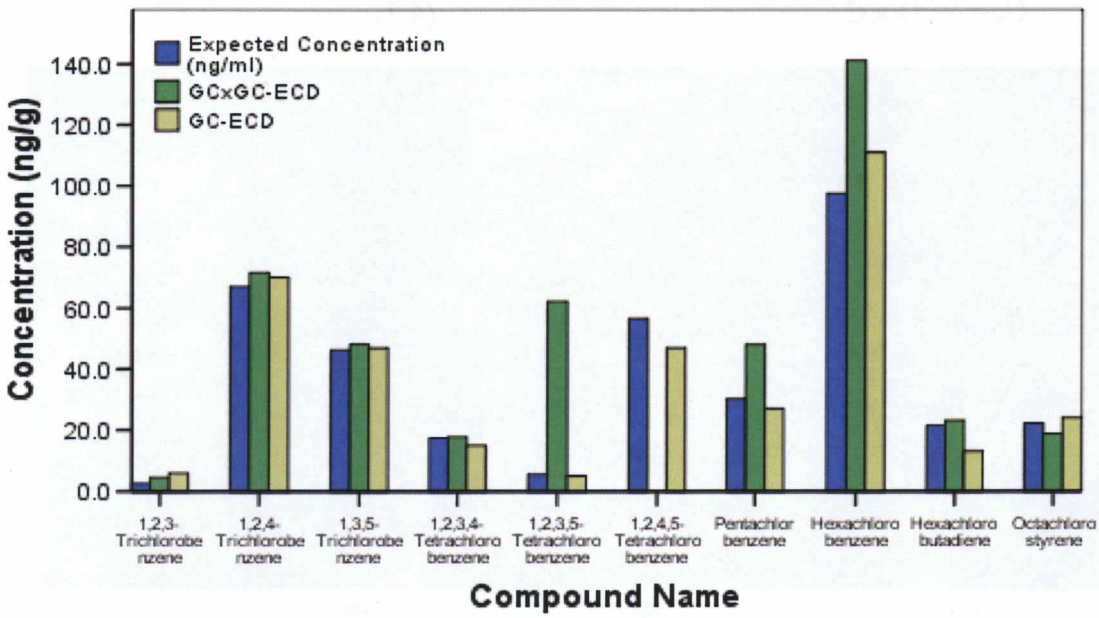


Figure 4.14 Data comparison for CBz determination in sediment reference material EC-8 by both GC-ECD and GCxGC-μECD methods.

As presented in Figures 4.11 to 4.13, almost all of the quantified amounts for PCBs, OCs and CBz are within the specified standard deviation when compared to the reference concentration, demonstrating that the method produces accurate results.

4.3 CALCULATIONS OF UNCERTAINTIES

The uncertainty component (U_x), standard uncertainty expressed as standard deviation (SD), combined standard uncertainty (U_c) and expanded uncertainty (U) were calculated according to Laboratory Services Branch, LSBSOP.030 (MoE, 2008) for all the compounds analysed. These calculations give an estimation of uncertainty for any given result of any given parameter quantified with this method. The formulae 4.7 to 4.10 were used for obtaining the final results that are presented in Appendix C.

$U_x = RSD \times C$ (4.7)

$$Uc = \sqrt{Ux^2 + Uo^2} \quad (4.8)$$

$U_0 = W = MDL/3$ (4.9)

$U = 2 \times Uc$ (4.10)

Where:

U_0 = uncertainty at near zero concentration

W = limit of measurement

4.4 SLUDGE AND SEDIMENT SAMPLES

4.4.1 Sludge Samples

Sludge samples collected from a raw influent of a waste water treatment plant (WWTP) in Ontario have been previously submitted to Dioxin and Toxic Organics Section, MoE, for PCB total and OC pesticides analysis by GC-ECD (Method 3270 presented in Table 4.6). The samples were later re-analysed by GCxGC-μECD following the sample preparation steps and the results were compared to the ones obtained from classical analysis. The two dimensional chromatograms GCxGC-μECD also revealed other classes of compounds present in the samples (Figure 4.14); for instance, patterns of polychlorinated alkanes were seen. Figure 4.15 shows a short chain PCA standard (55.5%chlorinated) analysed with the same method offering a better identification of the unknown bands seen in these specific sludge samples.

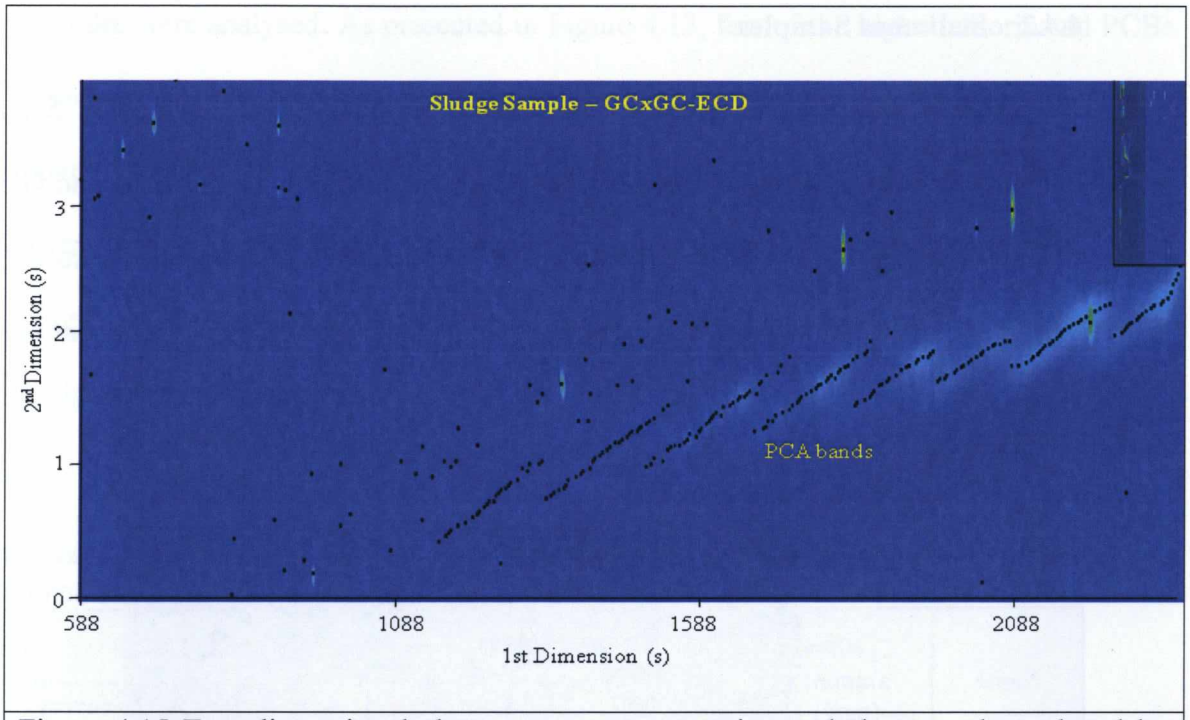


Figure 4.15 Two dimensional chromatogram representing a sludge sample analysed by GCxGC-μECD

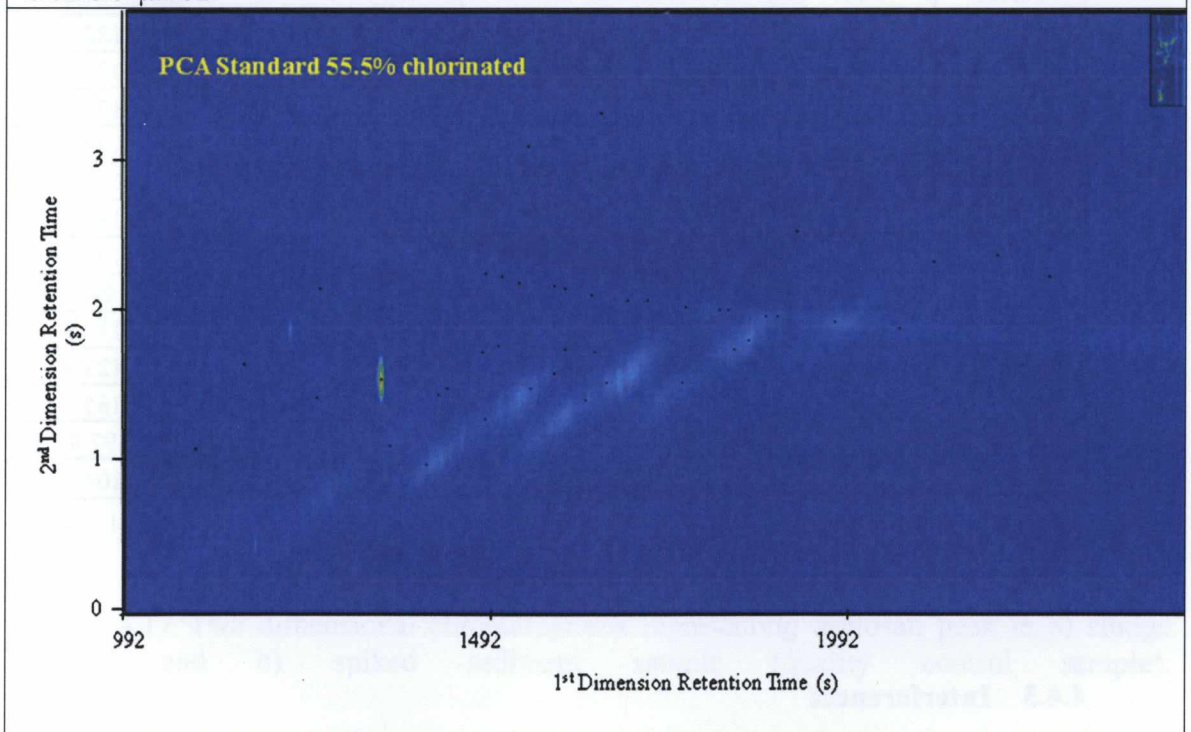


Figure 4.16 Two dimensional chromatogram representing PCA C10-13, 55.5%Cl standard analysed by GCxGC-μECD

4.4.2 Sediment Samples

Sediments sample from an inter-laboratory study (*New York State ELAP 08-01Inter-laboratory Study for Solid Waste*) was examined by both GC-ECD and GCxGC-ECD techniques for the determination of OC pesticides. The quantified results (Table 4.9) are comparable between the classical GC, GCxGC as well as the sediment expected values.

Table 4.9. The comparison of the obtained concentrations of selected OCs analysed by both GC-ECD and GCxGCμECD for New York State ELAP 08-01 sediment sample

Name	Certified amount (ng/g)	GCxGC-μECD		GC-ECD	
		Amount (ng/g)	Recovery (%)	Amount (ng/g)	Recovery (%)
α-BHC	157	186	118.5	200	127.4
β-BHC	325	454	139.7	430	132.3
γ-BHC	185	214	115.7	230	124.3
γ-CHLA	216	296	137.0	280	129.6
Aldrin	221	258	116.7	330	149.3
Endrin	376	413	109.8	510	135.6
Dieldrin	267	335	125.5	320	119.9
Endos 1	225	239	106.2	320	142.2
Endos S	252	251	99.6	250	99.2
Heptachlor	288	223	77.4	380	131.9
p,p'-DDD	234	325	138.9	290	123.9
p,p'-DDE	174	223	128.2	270	155.2
p,p'-DDT	225	273	121.3	220	97.8
DMDT	354	316	89.3	370	104.5

4.4.3 Interferences

The sample preparation and clean-up steps along with the chromatographically separation employed with this method were optimized to allow only the analytes of interest to be detected. However, some interferences were noticed when "real-world"

samples were analysed. As presented in Figure 4.13, few of the higher chlorinated PCBs interfere with PCA bands in some of the sludge samples analysed making their quantification difficult. In addition to PCAs, another unknown compound later identified as triclosan by GCxGC-TOFMS interfered with γ-chlordane in almost all of the samples (Figure 4.17a and b). Although triclosan was present, γ-chlordane was manually re-assigned and re-quantified.

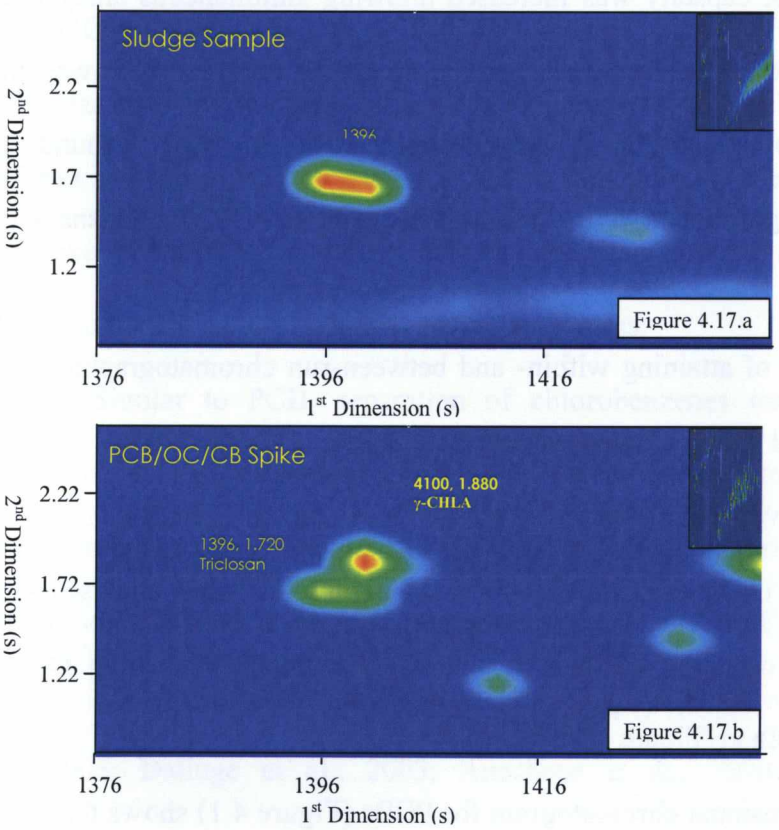


Figure 4.17 Two dimensional chromatograms representing triclosan peak in a) sludge sample and b) spiked sediment sample (quality control sample).

CHAPTER 5: DISCUSSION

5.1 GCXGC SEPARATION

In this study, GCxGC-μECD was observed to be a very powerful technique providing excellent chromatographic separations of the different contaminant classes of interest. By selecting the non-polar (DB1) and shape selective (Rtx-PCB) column combination, the peak capacity was increased allowing simultaneous analysis of more classes of halogenated contaminants in a single analytical run. Rtx-PCB was chosen as second dimension column due to its retentive properties for the compounds that can achieve planar configuration and its unique selectivity for PCB congeners (Stidsen, 2005).

The objective of attaining within- and between-run chromatographic separations was achieved for all three classes of interest: PCBs, OCs and CBs. Within-class separation was achieved with one coelution for PCB standard (PCB4/PCB10 - Figure 4.1) and CB standard (1,2,3,5-TCB/ 1,2,4,5-TCB - Figure 4.4) when analysed separately. No coelutions were observed for any of the 23 compounds present in OC standard analysed with this method (Figure 4.3).

The two dimensional chromatogram for PCBs (Figure 4.1) shows that orthogonal separation was achieved when using the DB1 x Rtx-PCB column combination. An ordered structure is observed and PCBs are seen as bands in the second dimension. The PCB congeners are separated according to their degree of chlorination. The dotted lines in Figure 4.1 represent the degree of chlorination of PCBs, from mono- to decachlorobiphenyl. The mono-ortho and non-ortho PCBs such as PCB37, PCB 77, PCB

81, PCB 126, and PCB 169 elute later in the second dimension due to the selectivity of Rtx-PCB ((LECO, 2005) for the compounds that can achieve a planar configuration. These results are similar to previous data reported for different column combinations by Korytar et al. (2002, 2005 and 2006).

Using comprehensive dual gas chromatography, PCBs that coelute on a classical DB1 column (Frame and al., 1996) are further resolved by the second column (Korytar et al., 2002), in this case Rtx-PCB. Some examples of these coeluting pairs resolved by GCxGC are PCB81/PCB87, PCB77/PCB110, PCB123/PCB149, and PCB105/PCB153. The emphasis of the study was on the separation of the twelve dioxin-like PCBs (WHO PCBs) and the seven EU indicator PCBs (Appendix A: Methods and Materials). With one exception, PCB114 which coelutes with cis-nonachlor, all the others WHO and EU PCBs are resolved both within- and between-class.

Similar to PCB, separation of chlorobenzenes was achieved with only one coelution: 1,2,3,5-tetrachlorobenzene and 1,2,4,5- tetrachlorobenzene (Figure 4.4). These two compounds also coelute in a classical GC-ECD analysis when using DB-1 column (Method3270, MoE, 2008) and were not resolved by GCxGC.

As many studies and reviews have shown before (Korytar et al., 2002; Korytar et al. 2005; Dalluge et al., 2003; Adachour et al., 2006), comprehensive dual gas chromatography is a way to increase peak capacity supporting the hypothesis that more than one class of target environmental pollutants can be separated and quantified in one analysis with one detector for complex environmental matrices. Between-class separation was assessed and, without any necessary splitting into multiple fractions prior to GCxGC analysis, separation was achieved with only three between-class coelutions present:

heptachlor-epoxide/PCB74, cis-nonachlor/PCB114, and methoxychlor/PCB171 (Figure4.6). Additionally, wrap-around is observed for chlorobenzenes in this separation (peaks that spend too much time in the second dimension column and do not elute in their own modulation time). Since the CBz do not interfere with any other analytes of interest in the chromatographic space, the wrap-around is not an issue for quantification purposes of these compounds. Furthermore, the separation is very reproducible between the analytical runs. Thus, the GCxGC method was able to chromatographically resolve 86 out of 96 compounds assessed in a relatively short analytical run (45 min.) using a fast sample preparation method as presented in Chapter 3. In addition to within- and between-class separations, GCxGC using DB-1 x Rtx-PCB column combination has significantly improved the separation of the target analytes from the matrix constituents.

To further emphasize the significance of this research, the GCxGC technique was compared to the classical GC method. While the GCxGC can analyse all the target compounds in a 45 minute analytical run, the classical GC-ECD uses multiple columns and instruments that analyse each different class of interest separately in four analytical runs (Method3412 and Method3270, MoE, 2008). Thus, the time gain is considerable when using GCxGC. Additionally, the sample preparation steps followed in the classical method are more time consuming and involve more solvent use and sample handling. Another advantage of GCxGC is that the non-ortho PCBs, lost in the clean-up procedure in the classical method (split in the second fraction), can now be separated and quantified.

5.2 SCREENING FOR OTHER PERSISTENT ORGANIC POLLUTANTS

Since environmental samples are very complex matrices and may contain more classes of environmental pollutants than target analytes, other contaminant classes were evaluated for the DB1 x Rtx-PCB column combination. As presented in Chapter 4 (Figure 4.7 and Figure 4.14), five other groups were analysed by GCxGC: dioxins and furans, toxaphene, polychlorinated naphthalenes and polychlorinated alkanes. DB1 x Rtx-PCB provided orthogonal separation and ordered chromatograms for structurally related compounds such as toxaphene and PCNs, according to their degree of chlorination and planar configuration. Dioxins and furans were more retained in the second dimension due to their planarity (three-ring planar compounds) and some of them exhibited wrap-around.

A polychlorinated alkanes standard was assessed after unknown clusters of contaminants were seen in the two dimensional chromatograms of some sludge extracts. PCA bands interfere with some of the higher chlorinated PCBs (PCB155, 151, 149, 188, 153, 168, 178, 187, 183, 167, 202, 201, 180, 191, 170, 199, 208 and 209). As for other structurally related compounds, DB1 x Rtx-PCB showed ordered separation of PCA bands into various degrees of chlorination which later can simplify their challenging analysis and quantification.

The practicability of the method was demonstrated when reference samples were analysed and the presence of other classes of contaminants did not interfere with the PCBs, OCs and CBz. Previous studies, as presented in Chapter 2, discussed the separation of different classes of contaminants to demonstrate the advantages of the

technique. However, in this method, not only the separation was achieved but also an accurate quantification of PCBs, OCs and CBz.

5.3 METHOD PERFORMANCE

5.3.1 Quantitative Performance

The method performance data presented in the previous chapter shows that DB1 x Rtx-PCB is a very powerful column combination; thus, GCxGC is a feasible technique for environmental samples analyses of halogenated organics. The calibration curves based on the measurements of six solutions (six-level standards) presented correlation coefficients higher than 0.9995. The method detection limits were calculated and their values varied from 0.06 to 3.5ng/g while the estimated limits of quantification for PCB/OC/CBz were found to be in the range of 1 to 10ng/g (Appendix B). Some of the chlorobenzenes in selected samples were impossible to quantify due to the presence of background interferences; therefore, MDLs were calculated for a lower number of replicates than PCBs and OCs. Higher LODs were also observed for specific PCBs (i.e., PCB8, 18, 52); this can be due to a possible contamination from the lab environment.

The ChromaTof software used for data handling and processing automatically assigns and quantifies the compounds set-up in the calibration tables except for three coeluting pairs that needed manual manipulation: PCB70/oxy-chlordane, PCB99/α-chlordane and PCB44/aldrin. These peaks are not baseline resolved and they need to be determined manually in order to obtain a proper integration and quantification.

Method performance was also assessed by calculating the repeatability (within-run precision) and reproducibility (between-run precision) and expressed as percentage

RSD. The data presented in Appendix B shows that better repeatability than reproducibility was obtained for some of the target compound while for others showed the opposite. This outcome for better between-run precision might be associated with the small number of replicates (6 and 8) available at the time. The percentage relative standard deviation for repeatability falls in the range of 2 to 14% while for reproducibility falls in the accepted ±25% limits proposed for this method.

Along with method precision, the accuracy of the method was confirmed by analysing reference materials for different matrices: sludge and sediment. The final results were compared with both the reference values and previous GC-ECD data that were available. As presented in Figures 4.11 and 4.12, the quantified amounts for PCBs and OCs are within the specified standard deviation when compared to their reference concentration, demonstrating that the method produces accurate results. The GCxGC chromatograms also revealed different classes of compounds present in the reference samples that can be identified and quantified later if required. A previous study with regards to the contaminant composition for NIST reference materials (Wise et al., 2006) showed that other halogenated compounds were present in SRM1944 (e.g. BDEs), confirming the presence of the unknown peaks seen in the two dimensional chromatograms. Thus, this method can assess the presence of other classes of contaminants in environmental samples. It is important to note at this point that none of the other compounds present in these reference samples has interfered with our target analytes.

To further assess the method, the uncertainties were calculated and expressed as standard uncertainty, combined standard uncertainty and expanded uncertainty

(Appendix C). When the percentage relative standard deviation of expanded uncertainty was calculated, the values varied from 2 to 25% with one exception, PCB157 at 27%.

5.3.2 Samples Quantification and Method Interferences

This method was shown to be precise and accurate for the standards and reference materials tested, indicating that it could be a method suitable for "real-life" samples testing. The quantification of sediment and sludge samples obtained from engineered and environmental systems confirmed that GCxGC analysis is a viable procedure for their analysis.

The results obtained for OC pesticides in *New York State ELAP08-01* sediment samples were comparable when examined by both GC-ECD and GCxGC-ECD techniques (Figure 4.15). A second set of sediment samples (Lake Simcoe Sediment Survey, MoE, 2008) previously analysed by GC-ECD for PCB congeners (Method 3412, MoE, 2008) were analysed with the same GCxGC instrumental set-up and the results were compared. The final data for target PCB congeners was comparable (Table 4.10) between the techniques. Also p,p'-DDE was detected at a very low level with both methods. In addition to the PCBs and p,p'-DDE, other "unknown" classes of compounds are seen (chromatogram not shown) which may be identified and quantified later.

The quantified PCBs by GCxGC-ECD for the sludge samples collected from a raw influent of a WWTP were compared to previous data from the GC-ECD analysis. While only p, p'-DDE and very low amount of total PCBs were found by conventional analysis, GCxGC-ECD revealed other classes of compounds present in the samples (Figure 4.13). One of the challenges encountered when analysing these sludge extracts, was the presence of polychlorinated alkanes bands that interfered with some of the higher

chlorinated PCBs (i.e. PCB170, PCB180) making their quantification difficult by μECD only, thus requires TOFMS for accurate identification and quantification. In addition, a more rigorous clean-up procedure can be established. When sludges from a different source were analysed, the PCA bands were not present thus no interferences compromised the results (not presented in this study).

Besides the PCA clusters, another problem was the interference of an unknown compound, later identified as triclosan by GCxGC-TOFMS, with γ-chlordane (Figure 4.16a). A possible source of contamination might be the detergent used for washing the glassware, where triclosan is one of its constituents. In order to eliminate this potential interference a more rigorous cleaning procedure needs to be established. Depending of the background interference, γ-chlordane was properly quantified and chromatographically separated from triclosan. In a "real" sample, the analyst should pay careful attention to γ-chlordane's retention time and compare it with a control sample, such as matrix spike when using ECD as the detection of choice (Figure 4.16b). It was observed that triclosan was quantified as γ-chlordane when the γ-chlordane was not present; thus, manual integration was required to accurately quantify γ-chlordane or to remove the analyte assignment.

5.4 ENVIRONMENTAL SIGNIFICANCE

While classical gas chromatography permits the analysis of target classes of contaminants, GCxGC could be both a target analysis method as well as a screening method. When "real-life" sediment and sludge samples were analysed by GCxGC in this study, the two dimensional chromatograms revealed many others compounds present along with PCBs, OCs and CBs. Unknown classes of compounds at the time of analysis could be possibly identified by using available retention time data also pointed out in this study. The technique could be used as screening method for the determination of dioxins, dioxin-like compounds, and new emerging contaminants in the environment. Previously saved data can be qualitatively and quantitatively interpreted and historical trends can be determined offering several advantages to conventional approaches.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

The objectives of this study were to accurately identify and quantify the PCBs, OCs and CBs present in sludge and sediment samples in a single analytical run by using the GCxGC technique. The increased peak capacity and enhanced resolving power of GCxGC allowed the separation of PCBs, OCs, and CBs without fractionation prior to instrumental analysis. The separation of the three classes of interest was achieved and the method validation results demonstrated that this technique can be used for environmental samples analysis.

The following conclusions can be drawn from the results presented in this study:

i. PCBs, OCs and CBs were separated within- and between- class in a single analytical run when using the column combination DB-1 x Rtx-PCB. With only few coelutions present, this method resolved 86 out of 96 compounds in a 45 minute run.

ii. This method was shown to be precise and accurate for the standards and the reference materials tested. The results obtained by GCxGC are comparable with the reference values as well as with previous GC data.

iii. The quantification of sediment and sludge samples obtained from engineered and environmental systems confirmed that GCxGC analysis is a viable procedure for their analysis. The quantified results compared to previous GC data.

iv. Some other classes of compounds were present in the two dimensional chromatograms and this method can be used for screening potential

contaminants. With the exception PCA clusters found to interfere with higher chlorinated PCBs, no other compounds were found to significantly affect the PCBs, OCs and CBs quantification. When PCAs are present, further TOFMS analysis may be required for accurate analysis.

v. The GCxGC method can result in significant savings in time of analysis.

The sediment and sludge extracts are very complex, and many unidentified compounds were observed in the two dimensional chromatograms obtained. One of the advantages of using comprehensive dual gas chromatography is its increased peak capacity therefore, allowing to more than one class of target environmental pollutants to be separated in one analysis. Some of the groups or compounds might interfere with the target analytes and improvements need to be considered to avoid any unnecessary contamination or background interferences. Others could be possibly identified by using available data also pointed out in this study and the technique could be used as screening method for the determination of dioxins, dioxin-like compounds, and new emerging contaminants in the environment. In addition, any unknown peaks can be identified in the future. Previously saved data can be qualitatively and quantitatively interpreted and historical trends can be determined offering several advantages to conventional approaches.

For the first time, the GCxGC method can potentially replace the classical GC multiple instrumental analysis. This would result in significant time savings and reduction in analysis costs with subsequent increase in data quality. Sediment and sludge samples can be routinely analysed using GCxGC-μECD. The GCxGC-TOFMS may be

required when PCA interferences are present, thus this method needs to be further validated for the TOFMS use.

The recent advances and publications in the field of GCxGC have shown that the technique is applicable to other environmental matrices than sediments and sludges. Since DB-1 x Rtx-PCB column combination selected for the GCxGC system yielded excellent within- and between- class separations, further research might be employed for the analysis of different matrices such as biota and vegetation samples.

CHAPTER 7: REFERENCES

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APPENDIX A: METHODS AND MATERIALS

List of PCB Standards - (Wellington Laboratories, Guelph, ON, Canada)

IUPAC#	PCB Native	BP-EC (µg/ml)	BP-MS (µg/ml)	BP-MS-PL1 (µg/ml)	BP-MS-PL2 (µg/ml)	BP-MS-PL3 (µg/ml)
	Monochlorobiphenyl					
1	2-Monochlorobiphenyl	50	2			
3	4-Monochlorobiphenyl	50	2			
	Dichlorobiphenyl					
4	2,2'-Dichlorobiphenyl	10	2			
8	2,4'-Dichlorobiphenyl	10	2			
10	2,6-Dichlorobiphenyl	10	2			
15	4,4'-Dichlorobiphenyl	10	2			
	Trichlorobiphenyl					
18	2,2',5-Trichlorobiphenyl	5	2			
19	2,2',6-Trichlorobiphenyl	5	2			
22	2,3,4'-Trichlorobiphenyl	5	2			
28	2,4,4'-Trichlorobiphenyl	5	2			
33	2',3,4-Trichlorobiphenyl	5	2			
37	3,4,4'-Trichlorobiphenyl	5	2			
	Tetrachlorobiphenyl					
44	2,2',3,5'-Tetrachlorobiphenyl	1	2			
49	2,2',4,5'-Tetrachlorobiphenyl	1	2		2	
52	2,2',5,5'-Tetrachlorobiphenyl	1	2	2		
54	2,2',6,6'-Tetrachlorobiphenyl	1	2			
70	2,3',4',5-Tetrachlorobiphenyl	1	2	2		
74	2,4,4',5-Tetrachlorobiphenyl	1	2		2	1
77	3,3',4,4'-Tetrachlorobiphenyl	1	2			2
81	3,4,4',5-Tetrachlorobiphenyl	1	2			
	Pentachlorobiphenyl					
87	2,2',3,4,5'-Pentachlorobiphenyl	1	2	2		
95	2,2',3,5',6-Pentachlorobiphenyl	1	2		2	
99	2,2',4,4',5-Pentachlorobiphenyl	1	2		2	
101	2,2',4,5,5'-Pentachlorobiphenyl	1	2	2		
104	2,3,3',4,4'-Pentachlorobiphenyl	1	2			
105	2,2',4,6,6'-Pentachlorobiphenyl	1	2			
110	2,3,3',4',6-Pentachlorobiphenyl	1	2	2		
114	2,3,4,4',6-Pentachlorobiphenyl	1	2			
118	2,3',4,4',5-Pentachlorobiphenyl	1	2			
119	2,3',4,4',6-Pentachlorobiphenyl	1	2			
123	2',3,4,4',5-Pentachlorobiphenyl	1	2			
126	3,3',4,4',5-Pentachlorobiphenyl	1	2			
	Hexachlorobiphenyl					
128	2,2',3,3'4,4'-Hexachlorobiphenyl	1	2			2

IUPAC#	PCB Native	BP-EC (µg/ml)	BP-MS (µg/ml)	BP-MS-PL1 (µg/ml)	BP-MS-PL2 (µg/ml)	BP-MS-PL3 (µg/ml)
138	2,2',3,4,4',5'-Hexachlorobiphenyl	1	2	2		
149	2,2',3,4',5',6-Hexachlorobiphenyl	1	2		2	
151	2,2',3,5,5',6-Hexachlorobiphenyl	1	2		2	
153	2,2',4,4',5,5'-Hexachlorobiphenyl	1	2	2		
155	2,2',4,4',6,6'-Hexachlorobiphenyl	1	2	2		
156	2,3,3',4,4',5-Hexachlorobiphenyl	1	2			
157	2,3,3',4,4',5'-Hexachlorobiphenyl	1	2			
158	2,3,3',4,4',6-Hexachlorobiphenyl	1	2		2	
167	2,3',4,4',5,5'-Hexachlorobiphenyl	1	2			
168	2,3',4,4',5',6-Hexachlorobiphenyl	1	2		2	
169	3,3',4,4',5,5'-Hexachlorobiphenyl	1	2			
	Heptachlorobiphenyl					
170	2,2',3,3',4,4',5-Heptachlorobiphenyl	1	2			
171	2,2',3,3',4,4',6-Heptachlorobiphenyl	1	2		2	
177	2,2',3,3',4',5,6-Heptachlorobiphenyl	1	2	2		
178	2,2',3,3',5,5',6-Heptachlorobiphenyl	1	2			2
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	1	2	2		
183	2,2',3,4,4',5',6-Heptachlorobiphenyl	1	2			
187	2,2',3,4',5,5',6-Heptachlorobiphenyl	1	2			
188	2,2',3,4',5,6,6'-Heptachlorobiphenyl	1	2	2		
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl	1	2			
191	2,3,3',4,4',5',6-Heptachlorobiphenyl	1	2			
	Octachlorobiphenyl					
194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl	1	2			
199	2,2',3,3',4,5,5',6'-Octachlorobiphenyl	1	2			
201	2,2',3,3',4,5',6,6'-Octachlorobiphenyl	1	2	2		
202	2,2',3,3',5,5',6,6'-Octachlorobiphenyl	1	2			2
205	2,3,3',4,4',5,5',6-Octachlorobiphenyl	1	2			
	Nonachlorobiphenyl					
206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	1	2			
208	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl	1	2			
	Decachlorobiphenyl					
209	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	1	2			

List of WHO PCBs and EU indicator PCBs

IUPAC#	PCB
WHO (dioxin-like) PCBs	
77	3,3',4,4'-Tetrachlorobiphenyl
81	3,4,4',5-Tetrachlorobiphenyl
126	3,3',4,4',5-Pentachlorobiphenyl
169	3,3',4,4',5,5'-Hexachlorobiphenyl
105	2,3,3',4,4'-Pentachlorobiphenyl
114	2,3,4,4',6-Pentachlorobiphenyl
118	2,3',4,4',5-Pentachlorobiphenyl
123	2',3,4,4',5-Pentachlorobiphenyl
156	2,3,3',4,4',5-Hexachlorobiphenyl
157	2,3,3',4,4',5'-Hexachlorobiphenyl
167	2,3',4,4',5,5'-Hexachlorobiphenyl
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
EU indicator PCBs	
28	2,4,4'-Trichlorobiphenyl
52	2,2',5,5'-Tetrachlorobiphenyl
101	2,2',4,5,5'-Pentachlorobiphenyl
118	2,3',4,4',5-Pentachlorobiphenyl
138	2,2',3,4,4',5'-Hexachlorobiphenyl
153	2,2',4,4',5,5'-Hexachlorobiphenyl
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl

List of OC Standards (UltraScientific, North Kingstown, RI, USA)

OCs	Concentration (g/mL)	Custom Std. #
α-BHC	100	CUS-3935
β-BHC	100	CUS-3935
γ-BHC	100	CUS-3935/CUS-5641
Heptachlor	100	CUS-3935/CUS-5641
Aldrin	100	CUS-3935/CUS-5641
Heptachlor-epoxide	100	CUS-3935
α-chlordane	100	CUS-3935
γ-chlordane	100	CUS-3935
Oxychlordane	100	CUS-3935
Cis-nonachlor	100	CUS-3935
Trans-nonachlor	100	CUS-3935
Dieldrin	100	CUS-3935
Endrin	100	CUS-3935
Endosulfan 1	100	CUS-3935
Endosulfan 2	100	CUS-3935
Endosulfan sulfate	100	CUS-3935
p,p'-DDE	100	CUS-3935/CUS-5641
o,p'-DDT	100	CUS-3935/CUS-5641
p,p'-DDT	100	CUS-3935
p,p'-DDD	100	CUS-3935
Methoxychlor (DMDT)	100	CUS-3935
Mirex	100	CUS-3935/CUS-5641

List of CB Standards (UltraScientific, North Kingstown, RI, USA)

CBz	Concentration (g/mL)	Custom Std. #
Hexachlorobenzenes	100	CUS-5641/CUS-3935/CUS3939
Hexachloroethane	100	CUS3939
1,3,5-Triclorobenzene	100	CUS3939
1,2,4-Triclorobenzene	100	CUS3939
1,2,3-Triclorobenzene	100	CUS3939
Hexachlorobutadiene	100	CUS3939
2,4,5-Trichlorotoluene	100	CUS3939
2,3,6-Trichlorotoluene	100	CUS3939
1,2,3,5-Tetraclorobenzene	100	CUS3939
1,2,4,5-Tetraclorobenzene	100	CUS3939
1,2,3,4-Tetraclorobenzene	100	CUS3939
A-2,6-Trichlorotoluene	100	CUS3939
Pentachlorobenzene	100	CUS3939
Octachlorostyrene	100	CUS-5641/CUS3939
1,3,5-Tribromobenzene	100	CUS3939

List of solvents/materials used for sample preparation and analysis

Solvents	Provider
Iso-octane	Caledon Lboratories LTD., Georgetown, ON, Canada
Dichloromethane	Caledon Lboratories LTD., Georgetown, ON, Canada
Hexane	Caledon Lboratories LTD., Georgetown, ON, Canada
Copper 20-30 Mesh	J.T.Baker, Phillisburg, NJ, USA

Note: All solvents used are distilled in glass grade.

APPENDIX B METHOD VALIDATION

Calculations of MDL and LOQ for PCBs, OCs and CBs spiked sediment samples analysed by GCxGC-μECD

Name	Expected amount (ng/mL)	N	Avg.	Std. Deviation	%RSD	MDL	LOQ
PCB8	1	8	5.70	0.53	9.35	1.60	4.79
PCB15	1	8	1.79	0.26	14.58	0.78	2.35
PCB18	1	8	9.77	1.10	11.24	3.29	9.87
PCB19	1	8	1.12	0.08	6.97	0.23	0.70
PCB22	1	8	2.52	0.18	7.14	0.54	1.62
PCB28	1	8	9.17	0.72	7.86	2.16	6.48
PCB33	1	8	4.11	0.32	7.71	0.95	2.85
PCB37	1	8	1.46	0.12	8.06	0.35	1.06
PCB44	1	8	1.39	0.10	6.99	0.29	0.87
PCB49	1	8	1.67	0.18	10.76	0.54	1.62
PCB52	1	8	2.56	0.19	7.38	0.57	1.70
PCB54	1	8	1.05	0.12	11.68	0.37	1.10
PCB70	1	8	1.92	0.30	15.61	0.90	2.70
PCB77	1	8	1.09	0.14	13.10	0.43	1.28
PCB81	1	8	1.00	0.12	12.04	0.36	1.08
PCB87	1	8	1.02	0.13	13.24	0.40	1.21
PCB95	1	8	1.01	0.09	8.62	0.26	0.78
PCB99	1	8	1.13	0.07	6.34	0.21	0.64
PCB101	1	8	1.24	0.20	16.07	0.59	1.78
PCB104	1	8	0.90	0.15	17.09	0.46	1.38
PCB105	1	8	1.10	0.08	7.10	0.23	0.70
PCB110	1	8	1.44	0.13	9.11	0.39	1.18
PCB118	1	8	1.06	0.09	8.54	0.27	0.81
PCB119	1	8	1.02	0.09	8.77	0.27	0.81
PCB123	1	8	0.99	0.15	15.31	0.45	1.36
PCB126	1	8	1.11	0.08	6.99	0.23	0.70
PCB128	1	8	1.11	0.07	5.94	0.20	0.59
PCB138	1	8	1.26	0.07	5.82	0.22	0.66
PCB149	1	8	1.04	0.17	16.72	0.52	1.56
PCB151	1	8	1.14	0.06	5.23	0.18	0.54
PCB153	1	8	1.29	0.09	6.64	0.26	0.77
PCB155	1	8	0.97	0.08	8.33	0.24	0.72
PCB156	1	8	0.93	0.09	9.54	0.27	0.80
PCB157	1	8	1.04	0.13	12.41	0.39	1.16
PCB158	1	8	1.11	0.08	7.55	0.25	0.75
PCB167	1	8	1.03	0.06	5.76	0.18	0.54

Name	Expected amount (ng/mL)	N	Avg.	Std. Deviation	%RSD	MDL	LOQ
PCB168	1	8	1.24	0.21	17.32	0.64	1.93
PCB169	1	8	1.05	0.15	13.97	0.44	1.32
PCB170	1	8	1.07	0.18	16.82	0.54	1.62
PCB177	1	8	1.18	0.13	10.69	0.38	1.13
PCB178	1	8	1.05	0.08	7.98	0.25	0.75
PCB180	1	8	1.20	0.07	6.14	0.22	0.66
PCB183	1	8	0.91	0.09	9.49	0.26	0.78
PCB187	1	8	1.11	0.07	6.57	0.22	0.66
PCB188	1	8	0.93	0.09	9.82	0.27	0.82
PCB189	1	8	1.12	0.15	13.53	0.45	1.36
PCB191	1	8	0.83	0.09	11.20	0.28	0.84
PCB194	1	8	1.09	0.16	14.40	0.47	1.40
PCB199	1	8	1.14	0.07	6.26	0.21	0.64
PCB201	1	8	0.93	0.11	11.73	0.33	0.98
PCB202	1	8	0.83	0.09	10.42	0.26	0.78
PCB205	1	8	1.10	0.09	8.47	0.28	0.84
PCB206	1	8	1.13	0.10	8.99	0.30	0.91
PCB208	1	8	1.09	0.19	17.70	0.58	1.74
PCB209	1	8	0.99	0.08	8.10	0.24	0.72
HCB	1	8	1.00	0.04	3.66	0.11	0.33
A-BHC	1	8	1.14	0.05	4.67	0.16	0.48
B-BHC	1	8	0.55	0.03	5.28	0.09	0.26
G-BHC	1	8	1.47	0.03	2.01	0.09	0.27
A-CHLA	1	8	1.21	0.02	1.68	0.06	0.18
G-CHLA	1	8	5.32	2.29	42.99	6.86	20.58
Oxy-CHLA	1	8	1.12	0.04	3.12	0.11	0.32
Aldrin	1	8	0.96	0.02	2.48	0.07	0.21
Endrin	1	8	1.37	0.03	1.88	0.08	0.23
Dieldrin	1	8	1.03	0.13	12.88	0.40	1.19
Endos 1	1	8	1.07	0.12	11.37	0.36	1.09
Endos 2	1	8	0.74	0.11	15.21	0.34	1.02
Endos S	1	8	0.79	0.09	10.96	0.26	0.78
Heptachlor	1	8	1.22	0.03	2.24	0.08	0.25
OCSTYR	1	8	1.15	0.08	7.17	0.25	0.74
Trans-Nonachlor	1	8	1.06	0.16	15.49	0.49	1.47
o,p'-DDT	1	8	1.15	0.17	14.85	0.51	1.54
p,p'-DDD	1	8	1.21	0.21	17.41	0.63	1.90
p,p'-DDE	1	8	0.98	0.18	18.32	0.54	1.61
p,p'-DDT	1	8	1.27	0.21	16.15	0.62	1.85
Mirex	1	8	1.04	0.20	19.31	0.60	1.81
p-Mirex	1	8	1.07	0.22	20.94	0.67	2.02

Within-run repeatability for PCB, OC, CB standards analysed by GCxGC-μECD

Name	Expected amount (ng/mL)	N	% Rec.	Mean	Std. Deviation	%RSD
PCB8	500	10	109.73	548.66	9.51	1.73
PCB15	500	10	110.27	551.34	20.13	3.65
PCB18	250	10	107.41	268.53	7.64	2.84
PCB19	250	10	110.67	276.67	1.79	0.65
PCB22	250	10	105.26	263.15	1.25	0.47
PCB28	250	10	104.93	262.32	0.75	0.29
PCB33	250	10	105.38	263.44	0.90	0.34
PCB37	250	10	107.60	268.99	1.23	0.46
PCB44	50	10	97.04	48.52	2.09	4.31
PCB49	50	10	107.84	53.92	0.08	0.15
PCB52	50	10	109.25	54.62	0.14	0.25
PCB54	50	10	115.81	57.91	1.91	3.29
PCB70	50	10	110.38	55.19	2.39	4.33
PCB77	50	10	94.87	47.44	1.29	2.72
PCB81	50	10	108.79	54.40	0.28	0.51
PCB87	50	10	108.01	54.01	0.33	0.61
PCB95	50	10	108.44	54.22	0.49	0.90
PCB99	50	10	95.85	47.93	1.47	3.06
PCB101	50	10	105.47	52.74	0.16	0.30
PCB104	50	10	109.01	54.51	0.30	0.55
PCB105	50	10	104.31	52.16	0.30	0.58
PCB110	50	10	105.66	52.83	0.23	0.43
PCB118	50	10	111.81	55.91	1.57	2.80
PCB119	50	10	107.88	53.94	0.22	0.41
PCB123	50	10	105.10	52.55	0.62	1.18
PCB126	50	10	126.35	63.18	1.17	1.84
PCB128	50	10	109.45	54.73	0.36	0.66
PCB138	50	10	105.07	52.54	0.29	0.56
PCB149	50	10	89.22	44.61	0.69	1.54
PCB151	50	10	110.27	55.13	0.20	0.36
PCB153	50	10	103.47	51.74	1.42	2.74
PCB155	50	10	112.45	56.23	1.09	1.94
PCB156	50	10	108.91	54.45	0.71	1.30
PCB157	50	10	108.12	54.06	0.40	0.74
PCB158	50	10	108.16	54.08	0.35	0.65
PCB167	50	10	104.34	52.17	0.34	0.66
PCB168	50	10	107.20	53.60	0.76	1.43
PCB169	50	10	104.80	52.40	0.51	0.97
PCB170	50	10	105.40	52.70	0.42	0.81
PCB177	50	10	106.85	53.42	0.37	0.70
PCB178	50	10	106.38	53.19	0.21	0.40
PCB180	50	10	109.00	54.50	0.60	1.10

Name	Expected amount (ng/mL)	N	% Rec.	Mean	Std. Deviation	%RSD
PCB183	50	10	105.14	52.57	0.36	0.68
PCB187	50	10	105.32	52.66	0.30	0.57
PCB188	50	10	107.85	53.93	0.27	0.51
PCB189	50	10	111.76	55.88	1.05	1.89
PCB191	50	10	112.99	56.50	0.54	0.96
PCB194	50	10	97.83	48.91	0.53	1.08
PCB199	50	10	106.46	53.23	0.48	0.89
PCB201	50	10	104.63	52.32	0.39	0.75
PCB202	50	10	110.73	55.36	0.35	0.64
PCB205	50	10	105.65	52.83	0.58	1.10
PCB206	50	10	92.64	46.32	0.60	1.30
PCB208	50	10	100.87	50.43	0.59	1.18
PCB209	50	10	106.74	53.37	0.62	1.16
HCB	50	10	93.69	46.85	0.50	1.06
HCBD	50	10	103.43	51.71	0.70	1.35
HCE	50	10	102.17	51.08	2.44	4.77
1,2,3,4-TCB	50	10	96.21	48.11	0.51	1.05
1,2,3,5-/1,2,4,5-TCB	100	10	92.32	92.32	1.15	1.24
1,2,3-TCB	50	10	93.87	46.94	0.90	1.92
1,2,4-TCB	50	10	98.58	49.29	1.65	3.36
1,3,5-TBB	50	10	97.15	48.57	0.48	0.99
1,3,5-TCB	50	10	98.46	49.23	0.75	1.53
2,3,6-TCT	50	10	90.11	45.05	0.69	1.54
2,4,5-TCT	50	10	95.73	47.87	0.80	1.67
P5CB	50	10	99.86	49.93	0.51	1.01
A2,6-TCT	50	10	95.88	47.94	0.73	1.52
A-BHC	50	10	100.56	50.28	0.50	0.99
B-BHC	20	10	107.22	21.44	0.21	0.97
G-BHC	50	10	98.74	49.37	0.58	1.17
A-CHLA	50	10	103.55	51.78	3.77	7.29
G-CHLA	50	10	104.09	52.04	0.56	1.07
Oxy-CHLA	50	10	108.82	54.41	0.71	1.30
Aldrin	50	10	86.90	43.45	0.49	1.13
Endrin	50	10	121.14	60.57	2.95	4.88
Dieldrin	50	10	104.08	52.04	0.58	1.11
Endos 1	50	10	108.12	54.06	0.59	1.09
Endos 2	50	10	103.57	51.78	0.51	0.99
Endos S	50	10	103.93	51.97	1.57	3.02
Heptachlor	50	10	111.55	55.77	0.69	1.23
OCSTYR	50	10	95.41	47.71	0.56	1.17
Trans-Nonachlor	50	10	98.99	49.50	0.53	1.07
o,p'-DDT	50	10	100.71	50.35	0.71	1.42
p,p'-DDD	50	10	101.89	50.94	0.95	1.87
p,p'-DDE	50	10	97.50	48.75	0.56	1.14
p,p'-DDT	50	10	101.53	50.77	0.75	1.47

Name	Expected amount (ng/mL)	N	% Rec.	Mean	Std. Deviation	%RSD
Mirex	50	10	100.10	50.05	2.27	4.54
p-Mirex	50	10	110.96	55.48	0.60	1.09
DMDT/PCB171	100	10	104.74	104.74	1.56	1.49
Cis-Nonachlor/PCB114	100	10	113.64	113.64	1.20	1.06
H-Epoxide/PCB74	100	10	112.74	112.74	1.47	1.30

Within-run method precision (repeatability) for PCBs, OCs, CBs spiked sediment samples analysed by GCxGC-μECD

Name	Expected amount (ng/mL)	N	% Rec.	Mean	Std. Deviation	%RSD
PCB8	500	9	83.66	418.32	44.19	10.56
PCB15	500	9	86.58	432.91	42.45	9.81
PCB18	250	9	86.63	216.57	21.88	10.10
PCB19	250	9	85.50	213.74	20.21	9.46
PCB22	250	9	89.11	222.77	19.56	8.78
PCB28	250	9	89.34	223.35	19.76	8.85
PCB33	250	9	92.22	230.55	25.75	11.17
PCB37	250	9	92.25	230.62	23.47	10.18
PCB44	50	9	103.40	51.70	5.42	10.49
PCB49	50	9	91.92	45.96	5.12	11.13
PCB52	50	9	98.14	49.07	5.76	11.74
PCB54	50	9	98.46	49.23	5.23	10.63
PCB70	50	9	103.42	51.71	5.17	9.99
PCB77	50	9	85.85	42.93	5.15	12.01
PCB81	50	9	97.93	48.96	5.17	10.56
PCB87	50	9	94.72	47.36	4.80	10.14
PCB95	50	9	96.83	48.41	5.10	10.53
PCB99	50	9	93.72	46.86	3.67	7.83
PCB101	50	9	94.55	47.27	4.89	10.34
PCB104	50	9	93.90	46.95	4.98	10.61
PCB105	50	9	95.44	47.72	5.04	10.56
PCB110	50	9	94.28	47.14	5.21	11.04
PCB118	50	9	105.28	52.64	4.84	9.19
PCB119	50	9	96.98	48.49	5.07	10.46
PCB123	50	9	94.38	47.19	5.01	10.61
PCB126	50	9	114.26	57.13	3.61	6.31
PCB128	50	9	100.94	50.47	5.34	10.57
PCB138	50	9	95.88	47.94	5.04	10.52
PCB149	50	9	91.14	45.57	6.38	13.99
PCB151	50	9	100.70	50.35	5.17	10.28

Name	Expected amount (ng/mL)	N	% Rec.	Mean	Std. Deviation	%RSD
PCB153	50	9	98.33	49.17	5.55	11.28
PCB155	50	9	100.73	50.36	4.80	9.53
PCB156	50	9	96.47	48.23	5.08	10.53
PCB157	50	9	90.45	45.22	5.56	12.29
PCB158	50	9	97.89	48.94	5.11	10.44
PCB167	50	9	95.69	47.85	5.08	10.61
PCB168	50	9	96.76	48.38	4.82	9.97
PCB169	50	9	97.12	48.56	5.07	10.44
PCB170	50	9	97.43	48.72	5.04	10.35
PCB177	50	9	93.65	46.83	5.32	11.36
PCB178	50	9	98.34	49.17	5.05	10.27
PCB180	50	9	101.21	50.60	5.15	10.18
PCB183	50	9	95.44	47.72	5.04	10.55
PCB187	50	9	96.47	48.24	5.08	10.54
PCB188	50	9	99.38	49.69	5.14	10.34
PCB189	50	9	103.21	51.60	5.02	9.73
PCB191	50	9	105.73	52.86	5.46	10.33
PCB194	50	9	92.60	46.30	4.83	10.43
PCB199	50	9	98.93	49.47	5.22	10.56
PCB201	50	9	94.78	47.39	5.03	10.61
PCB202	50	9	99.75	49.87	5.48	11.00
PCB205	50	9	101.77	50.89	5.69	11.18
PCB206	50	9	90.07	45.04	4.64	10.31
PCB208	50	9	95.79	47.90	5.25	10.97
PCB209	50	9	104.36	52.18	5.30	10.16
HCB	50	9	77.58	38.79	1.21	3.11
HCBD	50	9	62.10	31.05	3.64	11.72
HCE	50	9	49.94	24.97	3.70	14.80
1,2,3,4-TCB	50	9	63.93	31.96	2.69	8.41
1,2,3,5-/1,2,4,5-TCB	100	9	60.46	60.46	4.86	8.03
1,2,3-TCB	50	9	52.68	26.34	1.98	7.52
1,2,4-TCB	50	9	69.06	34.53	4.74	13.72
1,3,5-TBB	50	9	65.25	32.63	2.76	8.46
1,3,5-TCB	50	9	59.87	29.93	3.76	12.57
2,3,6-TCT	50	9	57.98	28.99	2.20	7.57
2,4,5-TCT	50	9	61.56	30.78	2.89	9.40
P5CB	50	9	69.34	34.67	2.24	6.47
A2,6-TCT	50	9	69.17	34.58	4.04	11.69
A-BHC	50	9	80.76	40.38	1.14	2.82
B-BHC	20	9	99.60	19.92	0.54	2.69
G-BHC	50	9	86.31	43.15	1.12	2.59
A-CHLA	50	9	104.03	52.01	3.15	6.06
G-CHLA	50	9	108.08	54.04	1.69	3.12
Oxy-CHLA	50	9	104.52	52.26	0.93	1.77
Aldrin	50	9	78.84	39.42	0.97	2.47

Name	Expected amount (ng/mL)	N	% Rec.	Mean	Std. Deviation	%RSD
Endrin	50	9	118.85	59.42	1.97	3.32
Dieldrin	50	9	103.25	51.63	1.18	2.29
Endos 1	50	9	108.16	54.08	1.23	2.27
Endos 2	50	9	94.33	47.16	2.27	4.80
Endos S	50	9	85.30	42.65	2.82	6.62
Heptachlor	50	9	99.02	49.51	1.56	3.14
OCSTYR	50	9	89.68	44.84	1.29	2.88
Trans-Nonachlor	50	9	97.32	48.66	1.15	2.37
o,p'-DDT	50	9	99.84	49.92	0.97	1.93
p,p'-DDD	50	9	103.88	51.94	1.24	2.39
p,p'-DDE	50	9	94.95	47.47	1.11	2.33
p,p'-DDT	50	9	104.76	52.38	3.40	6.50
Mirex	50	9	105.81	52.91	1.10	2.08
p-Mirex	50	9	109.25	54.62	1.39	2.55
DMDT/PCB171	100	9	97.50	97.50	2.65	2.72
Cis-Nonachlor/PCB114	100	9	110.39	110.39	2.47	2.24
H-Epoxide/PCB74	100	9	109.42	109.42	4.47	4.08

Between-run method precision (reproducibility) for PCBs, OCs, CBs spiked sediment samples analysed by GCxGC-μECD

Name	Expected amounts (ng/g)	N	Mean	Std. Deviation	%RSD
PCB8	500	7	416.6	54.4	13.0
PCB15	500	7	437.2	58.1	13.3
PCB18	250	7	218.5	25.4	11.6
PCB19	250	7	209.6	26.2	12.5
PCB22	250	7	222.8	24.0	10.8
PCB28	250	7	218.6	24.6	11.3
PCB33	250	7	232.3	25.8	11.1
PCB37	250	7	227.5	38.9	17.1
PCB44	50	7	40.1	6.0	14.9
PCB49	50	7	47.7	5.2	11.0
PCB52	50	7	50.2	6.9	13.7
PCB54	50	7	51.4	5.5	10.8
PCB70	50	7	52.9	7.0	13.2
PCB77	50	7	46.6	4.5	9.7
PCB81	50	7	48.4	6.4	13.3
PCB87	50	7	47.4	5.8	12.3

Name	Expected amounts (ng/g)	N	Mean	Std. Deviation	%RSD
PCB95	50	7	49.3	6.3	12.8
PCB99	50	7	46.0	8.8	19.2
PCB101	50	7	48.0	5.7	11.9
PCB104	50	7	46.0	6.5	14.2
PCB105	50	7	48.9	6.6	13.5
PCB110	50	7	47.8	6.1	12.7
PCB118	50	7	49.9	6.9	13.8
PCB119	50	7	49.0	6.1	12.5
PCB123	50	7	43.9	6.9	15.7
PCB126	50	7	58.8	7.3	12.4
PCB128	50	7	49.6	6.6	13.3
PCB138	50	7	49.6	5.5	11.1
PCB149	50	7	50.8	6.3	12.3
PCB151	50	7	50.9	5.7	11.2
PCB153	50	7	50.9	5.2	10.2
PCB155	50	7	50.1	5.4	10.8
PCB156	50	7	50.3	6.4	12.6
PCB157	50	7	50.4	6.1	12.1
PCB158	50	7	50.9	7.2	14.1
PCB167	50	7	50.8	3.0	5.9
PCB168	50	7	51.3	4.4	8.6
PCB169	50	7	48.7	6.9	14.2
PCB170	50	7	50.2	6.2	12.4
PCB177	50	7	51.3	5.7	11.1
PCB178	50	7	50.5	5.6	11.2
PCB180	50	7	52.3	6.6	12.6
PCB183	50	7	49.5	6.2	12.5
PCB187	50	7	49.7	5.6	11.2
PCB188	50	7	51.2	5.3	10.4
PCB189	50	7	52.6	7.1	13.5
PCB191	50	7	53.8	6.6	12.3
PCB194	50	7	46.9	6.0	12.7
PCB199	50	7	50.1	5.9	11.8
PCB201	50	7	49.5	5.9	11.9
PCB202	50	7	51.7	5.6	10.8
PCB205	50	7	50.3	6.4	12.8
PCB206	50	7	45.5	5.6	12.2
PCB208	50	7	48.6	6.1	12.6
PCB209	50	7	56.5	5.1	9.1
HCB	50	8	38.3	8.6	22.5
HCBD	50	7	34.7	8.5	24.6
HCE	50	8	30.5	7.7	25.2
1,2,3,4-TCB	50	8	33.2	7.9	23.8
1,2,3,5-/1,2,4,5-TCB	100	8	64.5	14.9	23.1

Name	Expected amounts (ng/g)	N	Mean	Std. Deviation	%RSD
1,2,3-TCB	50	8	28.3	6.8	24.2
1,2,4-TCB	50	8	34.6	8.9	25.6
1,3,5-TBB	50	8	51.1	21.4	41.8
1,3,5-TCB	50	8	31.3	7.6	24.3
2,3,6-TCT	50	8	31.0	6.6	21.3
2,4,5-TCT	50	8	32.1	7.5	23.3
P5CB	50	8	36.5	9.7	26.6
A2,6-TCT	50	8	34.5	7.4	21.4
A-BHC	50	8	40.9	8.0	19.5
B-BHC	20	8	20.6	4.1	19.8
G-BHC	50	8	43.8	7.4	16.8
A-CHLA	50	8	51.1	7.5	14.7
G-CHLA	50	8	55.3	5.9	10.6
Oxy-CHLA	50	8	48.1	4.2	8.7
Aldrin	50	8	44.3	5.9	13.3
Endrin	50	8	64.1	25.5	39.8
Dieldrin	50	8	53.9	7.4	13.7
Endos 1	50	8	54.2	8.9	16.4
Endos 2	50	8	44.4	18.3	41.1
Endos S	50	8	42.1	16.0	37.9
Heptachlor	50	8	51.9	7.6	14.7
OCSTYR	50	8	45.5	5.2	11.3
Trans-Nonachlor	50	8	53.1	7.8	14.6
o,p'-DDT	50	8	52.8	6.0	11.4
p,p'-DDD	50	8	53.5	2.2	4.2
p,p'-DDE	50	8	46.7	2.2	4.7
p,p'-DDT	50	8	51.6	2.9	5.7
Mirex	50	8	52.8	6.0	11.4
p-Mirex	50	8	54.4	4.2	7.8
DMDT/PCB171	100	7	104.2	19.6	18.8
Cis-Nonachlor/PCB114	100	7	114.4	10.0	8.8
H-Epoxide/PCB74	100	7	106.3	6.5	6.1

APPENDIX C: UNCERTAINTIES CALCULATIONS

Uncertainties Calculations for PCB congeners

Name	Expected amount (ng/g)	SD	%RSD	W	U_x	$\sum U_x^2+U_o^2$	Uc	U
PCB8	500	44.19	10.56	0.53	52.82	2790.38	52.82	105.65
PCB15	500	42.45	9.81	0.26	49.03	2404.03	49.03	98.06
PCB18	250	21.88	10.10	1.10	25.26	639.03	25.28	50.56
PCB19	250	20.21	9.46	0.08	23.64	558.84	23.64	47.28
PCB22	250	19.56	8.78	0.18	21.95	481.83	21.95	43.90
PCB28	250	19.76	8.85	0.72	22.11	489.55	22.13	44.25
PCB33	250	25.75	11.17	0.32	27.93	779.97	27.93	55.86
PCB37	250	23.47	10.18	0.12	25.44	647.43	25.44	50.89
PCB44	50	5.42	10.49	0.10	5.24	27.51	5.25	10.49
PCB49	50	5.12	11.13	0.18	5.57	31.03	5.57	11.14
PCB52	50	5.76	11.74	0.19	5.87	34.48	5.87	11.74
PCB54	50	5.23	10.63	0.12	5.32	28.28	5.32	10.64
PCB70	50	5.17	9.99	0.30	5.00	25.04	5.00	10.01
PCB77	50	5.15	12.01	0.14	6.00	36.07	6.01	12.01
PCB81	50	5.17	10.56	0.12	5.28	27.91	5.28	10.57
PCB87	50	4.80	10.14	0.13	5.07	25.74	5.07	10.15
PCB95	50	5.10	10.53	0.09	5.27	27.73	5.27	10.53
PCB99	50	3.67	7.83	0.07	3.91	15.31	3.91	7.83
PCB101	50	4.89	10.34	0.20	5.17	26.77	5.17	10.35
PCB104	50	4.98	10.61	0.15	5.30	28.16	5.31	10.61
PCB105	50	5.04	10.56	0.08	5.28	27.88	5.28	10.56
PCB110	50	5.21	11.04	0.13	5.52	30.50	5.52	11.05
PCB118	50	4.84	9.19	0.09	4.60	21.12	4.60	9.19
PCB119	50	5.07	10.46	0.09	5.23	27.36	5.23	10.46
PCB123	50	5.01	10.61	0.15	5.30	28.16	5.31	10.61
PCB126	50	3.61	6.31	0.08	3.16	9.97	3.16	6.31
PCB128	50	5.34	10.57	0.07	5.29	27.95	5.29	10.57
PCB138	50	5.04	10.52	0.07	5.26	27.67	5.26	10.52
PCB149	50	6.38	13.99	0.17	7.00	48.97	7.00	14.00
PCB151	50	5.17	10.28	0.06	5.14	26.40	5.14	10.28
PCB153	50	5.55	11.28	0.09	5.64	31.81	5.64	11.28
PCB155	50	4.80	9.53	0.08	4.77	22.72	4.77	9.53
PCB156	50	5.08	10.53	0.09	5.27	27.74	5.27	10.53
PCB157	50	5.56	12.29	0.13	6.15	37.79	6.15	12.30
PCB158	50	5.11	10.44	0.08	5.22	27.26	5.22	10.44
PCB167	50	5.08	10.61	0.06	5.31	28.15	5.31	10.61
PCB168	50	4.82	9.97	0.21	4.98	24.87	4.99	9.97
PCB169	50	5.07	10.44	0.15	5.22	27.26	5.22	10.44
PCB170	50	5.04	10.35	0.18	5.18	26.82	5.18	10.36
PCB177	50	5.32	11.36	0.13	5.68	32.26	5.68	11.36

Name	Expected amount (ng/g)	SD	%RSD	W	U_x	$\sum U_x^2+U_o^2$	Uc	U
PCB178	50	5.05	10.27	0.08	5.14	26.39	5.14	10.27
PCB180	50	5.15	10.18	0.07	5.09	25.92	5.09	10.18
PCB183	50	5.04	10.55	0.09	5.28	27.86	5.28	10.56
PCB187	50	5.08	10.54	0.07	5.27	27.78	5.27	10.54
PCB188	50	5.14	10.34	0.09	5.17	26.73	5.17	10.34
PCB189	50	5.02	9.73	0.15	4.86	23.67	4.87	9.73
PCB191	50	5.46	10.33	0.09	5.17	26.70	5.17	10.33
PCB194	50	4.83	10.43	0.16	5.22	27.23	5.22	10.44
PCB199	50	5.22	10.56	0.07	5.28	27.88	5.28	10.56
PCB201	50	5.03	10.61	0.11	5.31	28.18	5.31	10.62
PCB202	50	5.48	11.00	0.09	5.50	30.24	5.50	11.00
PCB205	50	5.69	11.18	0.09	5.59	31.25	5.59	11.18
PCB206	50	4.64	10.31	0.10	5.15	26.57	5.15	10.31
PCB208	50	5.25	10.97	0.19	5.48	30.12	5.49	10.98
PCB209	50	5.30	10.16	0.08	5.08	25.79	5.08	10.16

Uncertainties Calculations for OC pesticides

Name	Expected amount (ng/g)	SD	%RSD	W	Ux	$\sum U_x^2+U_o^2$	Uc	U
A-BHC	50	1.14	2.82	0.05	1.41	1.99	1.41	2.82
B-BHC	20	0.54	2.69	0.03	0.54	0.29	0.54	1.08
G-BHC	50	1.12	2.59	0.03	1.29	1.67	1.29	2.59
A-CHLA	50	3.15	6.06	0.02	3.03	9.18	3.03	6.06
G-CHLA	50	1.69	3.12	2.29	1.56	7.67	2.77	5.54
Oxy-CHLA	50	0.93	1.77	0.04	0.89	0.79	0.89	1.77
Aldrin	50	0.97	2.47	0.02	1.23	1.52	1.23	2.47
Endrin	50	1.97	3.32	0.03	1.66	2.75	1.66	3.32
Dieldrin	50	1.18	2.29	0.13	1.14	1.33	1.15	2.30
Endos 1	50	1.23	2.27	0.12	1.13	1.30	1.14	2.28
Endos 2	50	2.27	4.80	0.11	2.40	5.78	2.40	4.81
Endos S	50	2.82	6.62	0.09	3.31	10.95	3.31	6.62
Heptachlor	50	1.56	3.14	0.03	1.57	2.47	1.57	3.14
OCSTYR	50	1.29	2.88	0.08	1.44	2.08	1.44	2.88
Trans-nonachlor	50	1.15	2.37	0.16	1.18	1.43	1.20	2.39
o,p'-DDT	50	0.97	1.93	0.17	0.97	0.96	0.98	1.96
p,p'-DDD	50	1.24	2.39	0.21	1.19	1.47	1.21	2.43
p,p'-DDE	50	1.11	2.33	0.18	1.17	1.39	1.18	2.36
p,p'-DDT	50	3.40	6.50	0.21	3.25	10.60	3.26	6.51
Mirex	50	1.10	2.08	0.20	1.04	1.12	1.06	2.11
p-Mirex	50	1.39	2.55	0.22	1.27	1.67	1.29	2.59

APPENDIX D: GC COLUMNS

LC-50	50% liquid crystalline-methylpolysiloxane
007-65HT	65% phenyl-methylpolysiloxane
VF-23ms	proprietary (70-90% cyano-containing polymer)
VF-1ms	100% methylpolysiloxane
HT-8	8% phenyl-methylpolysiloxane (carborane)
DB-1/HP-1	100% dimethylpolysiloxane
RTX-5/DB-5	5% diphenyl-dimethylpolysiloxane
DB-Wax	polyethylene glycol
DB-XLB	proprietary
Rtx-PCB	proprietary
SupelcoWax-10	polyethylene glycol
DB-1701	14% (cyanopropyl-phenyl)-methylpolysiloxane
DB-17HT	50% phenyl-methylpolysiloxane
DB-210	trifluoropropylmethyl polysiloxane
BPX-50	50% phenyl-methylpolysiloxane (silphenylene)
007-210	50% trifluoropropyl-methylpolysiloxane

(3) Bk-15-89