Comprehensive two-dimensional chromatographic techniques for ultra-trace quantitative analysis of chlorinated dioxins in South Africa

by

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I DEDICATE THIS TO

My grandmother, Elizabeth Beveridge Scott, né Stevenson, my mother, Jean Crawford Kegge, né Scott, and my brother, William Neil Garth Kegge. Their combined unconditional and uncomplicated love will always abide in me and their memories live on through me.

My husband Phillip, for believing in me, for loving me, his steadfast support and encouragement all the time.

My Father-in-Law, Dirk Cornelius Petrus de Vos, who now lives in the house of the Lord forever. Dadda always asked about my thesis progress and was so proud of me.

My Lord and Savior, Jesus Christ, for saving my soul through His Divine Grace.

I declare that the thesis, which I hereby submit for the degree *Philosophiae Doctor* (Chemistry) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Betty-Jayne de Vos	Date

SUMMARY

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are toxic environmental pollutants formed as by-products of industrial and thermal processes. They are chlorinated compounds that have similar structures and chemical properties that were included in the original United Nations Environment Programme's '*dirty dozen'* and now form part of the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs). As a signatory to the Stockholm Convention, South Africa has the obligation to undertake appropriate research, monitoring, and cooperation pertaining to POPs, and more particularly, PCDD/Fs. Currently there is no established PCDD/F laboratory in South Africa capable of these demanding measurements and alternative approaches must be considered that are more affordable, more robust and more user friendly for a developing economy.

PCDD/Fs are highly toxic, causing a myriad of negative human health effects such as chloracne, carcinogenicity, hepatotoxicity, teratogenicity, endocrine disruption and alterations in neural development. The toxicity of PCDD/Fs is mediated through the aryl hydrocarbon receptor (AhR). PCDD/Fs bind to the AhR and elicit an AhR-mediated biochemical and toxic response. The in-depth information available on the mechanism of toxicity also allows for PCDD/Fs to be analysed using the AhR receptor mediated response in genetically modified cell lines. This led to the development of a bio-analytical screening technique using *in vitro* H4IIE-*luc* reporter gene bio-assay for AhR active compounds for the initial screening of PCDD/Fs in soil and sediment. The intension in this study was to develop an integrated approach to the analysis of PCDD/Fs in the South African environment, considering the associated cost as well as the available instrumentation, expertise and relevance within a developing economy.

Historically, the quantitative confirmatory analysis of these compounds has been achieved by targeted analysis using gas chromatography coupled with high-resolution magnetic sector mass spectrometry instruments (GC-HRMS), the accepted benchmark technology used for determining the level of trace organic environmental contaminants such as PCDD/Fs. However, these methods are time consuming and expensive.

Advances in technology have led to comprehensive two-dimensional gas chromatography - time-of-flight mass spectrometry (GC×GC-TOFMS) methodology that can be used for the analysis of PCDD/Fs in samples with different matrices. This approach is well suited for application in developing economies where access to GC-HRMS and highly skilled personnel is limited. This thesis describes the bio-analytical technique and the method development and analysis of the seventeen toxic PCDD/F congeners using GC×GC-TOFMS methodology. The technique provides the selectivity (added peak capacity of GC×GC) and the sensitivity (focusing effect of the modulator) needed to meet the requirements as mandated for analysis in US EPA Method 1613B. Extracted samples analysed on a GC-HRMS instrument, were re-analyzed using the low-resolution GC×GC-TOFMS instrument and the results confirmed using a high resolution TOF mass spectrometer (HRT). The quantitative results obtained compare well with those obtained using GC-HRMS. Because GC×GC-TOFMS is not a target compound analytical technique (as is GC-HRMS), it is possible to obtain information on numerous other classes of organic pollutants present in the samples in one analytical run, although this information can be sample clean-up dependant.

Preliminary validation of the GC×GC-TOFMS method is investigated using a certified reference material and real South African soil samples. The South African soil samples studied showed extremely high levels of PAHs, aliphatic hydrocarbons and sulphur. The organic content and matrix interference of South African soil samples (and the NIST standard reference material sediment; SRM 1944) provided significant challenges for the validation study.

This study has shown that GC×GC-TOFMS provides a quick, convenient screen for numerous pollutant classes which may be present in environmental samples. Retrospective data mining of archived data (extraction dependant) is possible and has provided key information on other chlorinated and brominated contaminants present in South African waste, soil and sediment samples.

NMISA now has a viable GC×GC-TOFMS dioxin analytical method for low level (ultra-trace) quantitative screening of chlorinated compounds that can be offered to South African analytical laboratories for routine dioxin analysis. The work is relevant

scientifically and is a definitive contribution to the growing compilation of GC×GC methodology, providing efficient methods for this demanding environmental application.

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PUBLICATIONS AND PRESENTATIONS

This thesis is based on the following manuscripts:

Published papers

J. **de Vos**, R. Dixon, G. Vermeulen, P. Gorst-Allman, J. Cochran, E. Rohwer, et al., Comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS) for environmental forensic investigations in developing countries., *Chemosphere*. 82 (2011) 1230–9.

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Awards

Shimadzu Trophy Award for '*Chromatographer of the Year'*, November 2013.

ACRONYMS AND ABBREVIATIONS

A	
AFRIMETS	Inter-Africa metrology system
AhR	Aryl hydrocarbon receptor
AMDIS	Automated mass spectral deconvolution and identification system
Arnt	Aryl hydrocarbon Receptor nuclear translocator
ASE	Accelerated Solvent Extraction
ATP	Adenosine triphosphate
ATSDR	Agency for toxic substances and disease registry
B	
BDEs	Brominated diphenyl ethers
BEQ	Bio-assay equivalents
BIPM	International Bureau of Weights and Measures
bw	Body weight
C	
CALUX	Chemical activated luciferase gene eXpression
CC	Consultative committee
CCQM	Consultative committee for amount of substance: chemistry and biology
CGPM	General Conference on Weights and Measures
CIL	Cambridge Isotope Laboratories
CIPM	International committee for weights and measures
CRS-LV	Concurrent solvent re-condensation of large volume splitless injection
D	
^{1}D	First dimension
^{2}D	Second dimension
DEA	Department of Environmental Affairs
DCM	Dichloromethane
DDT	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
DEEEP	Direct estimation of the ecological effect potential
DL-PCB	Dioxin-like polychlorinated biphenyls
L	
LECO©	LECO Corporation
LOD	Limit of detection
LOQ	Limit of quantitation
M	
MAE	Microwave assisted extraction
MDL	Method detection limit
MS	Mass spectrometry
MOE	Ontario Ministry of the Environment, Canada
M_R	Modulation ratio
N	
NIP	National Implementation Plan
NMISA	National Metrology Institute of South Africa
NWU	North West University
O	
OAWG	Organic analysis working group
OCDD	Octachlorinated dibenzo-*p*-dioxins (octa dioxins)
OCDF	Octachlorinated dibenzofurans (octa furans)
OHR	Optimal heating rate
P	
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCBzs	Polychlorobenzenes
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
PCNs	Polychlorinated naphthalenes
PCPs	Polychlorophenols
PeCDD	Pentachlorinated dibenzo-*p*-dioxins (penta dioxins)
PeCDF	Pentachlorinated dibenzofurans (penta furans)

DR CALUX	Bio detection system for dioxins and/ or dioxin-like polychlorinated biphenyls
DRE	Dioxin responsive DNA element
Dw	Dry weight
E	
EPA	Environmental Protection Agency
EU	European Union
F	
FFP	Folded flight path
FMS	Fluid Management Systems
FSL	Forensic Science Laboratory
FWHM	Full width at half maximum
G	
GC	Gas chromatography
GC×GC	Comprehensive two-dimensional gas chromatography
GC-HRMS	Gas chromatography coupled with high resolution magnetic sector mass spectrometry
GC-MS	Gas chromatography mass spectrometry
GEF	Global environmental fund
GMP	Global monitoring program
GPC	Gel permeation chromatography
H	
H4IIE-*luc*	Recombinant rat hepatoma cell line containing a luciferase reporter gene
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexanes
HRMS	High resolution magnetic sector mass spectrometry
HRT	High resolution time-of-flight mass spectrometer
HVAC	Heating, ventilation and air conditioning
HxCDD	Hexachlorinated dibenzo-*p*-dioxins (hexa dioxins)
HxCDF	Hexachlorinated dibenzofurans (hexa furans)
PFE	Pressurised fluid extraction
PLE™	Pressurized liquid extraction Module
P_M	Modulation period
PFK	Perfluorokerosene
PFTBA	Perfluorotributylamine
POPs	Persistent organic pollutants
POPT	Persistent Organic Pollutants Toxicants Group (NWU)
Power-prep™	Multi-column sample cleanup Module
PTV	Pressurised Temperature vaporising injector
Q	
QISTMS	Quadrupole storage tandem-in-time mass spectrometry
R	
REACH	Registration, evaluation, authorisation and restriction of chemicals
RP	Relative potency
RoHS	Restriction of hazard substances
RQS	Resource Quality Services (Department of Water Affairs)
RSA	South Africa
S	
SADC	Southern African Development Community
SAPS	South African Police Services
SC	Stockholm convention
SCCPs	Short chained chlorinated paraffins
SIM	Selected ion monitoring
SFE	Supercritical fluid extraction
S/N	Signal to noise ratio
SOF	Speed optimised flow
Spectra/ s	Spectra per second
T	
TBT	Technical barriers to trade
TCDD	Tetrachlorinated dibenzo-*p*-dioxins (tetra dioxins)
TCDF	Tetrachlorinated dibenzofurans (tetra furans)

HpCDD	Heptachlorinated dibenzo-*p*-dioxins (hepta dioxins)
HpCDF	Heptachlorinated dibenzofurans (hepta furans)
I	
IFCS	Intergovernmental forum on chemicals safety
ILAC	International laboratory accreditation cooperation
IMEP®	International measurement evaluation programme
INC	Intergovernmental negotiating committee
IPAP	Industrial Policy Action Plan
IPCS	Inter-organization programme on chemicals safety
ISO	International Organisation for Standardisation
I-TEF	International toxic equivalency factor
J	
JCGM	Joint committee for guides in metrology
K	
KCRV	Key comparison reference value
TCQM	Technical committee for amount of substance
TEF	Toxic equivalency factor
TEQ	Total equivalent concentrations
the dti	The Department of Trade and Industry
TOFMS	Time-of-flight mass spectrometry
TRP™	Total rapid preparation system
U	
UNEP	United Nations environmental programme
UoM	Uncertainty of measurement
UP	University of Pretoria
US	United States
V	
VOCs	Volatile organic components (compounds)
W	
WHO	World Health Organisation

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INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous, toxic environmental pollutants formed as by-products of industrial and thermal processes (Fiedler *et al*. 1996 and Pereira 2004). The toxicity of these chemicals and other persistent organic pollutants (POPs) has led to the development of a global treaty to protect human health and the environment from chemicals, the Stockholm Convention (2010). As a signatory to the convention, South Africa has an obligation to undertake appropriate research, monitoring, and co-operation pertaining to POPs, and more particularly, PCDD/Fs (DEA 2011). The list of potentially hazardous chemicals is ever increasing and the lack of analytical services in South Africa to monitor these environmental contaminants is becoming critical. South Africa has limited, if not any information about the accurate levels of hazardous chemicals in the environment (Bouwman 2004). Reliable environmental monitoring is essential to meet consumer demands, to ensure successful enforcing of international environmental regulations and to overcome technical barriers to trade (TBTs); it's a compliance issue (IPAP 2014).

The risks posed by POP compounds are of increasing concern in many countries, resulting in actions being taken or proposed at the national, regional and international level to protect human health and the environment (Langer *et al*. 1973 and Okey *et al*. 1994). With evidence of long-range transport of these substances to regions where they have never been used or produced, and the consequent threats they pose to the global environment (Wania & Mackay 1996), the international community has called for urgent global actions to reduce and eliminate releases of these chemicals. The need for environmental monitoring is essential to ensure

sustainable growth without exposing the population to dangerous toxins (Birnbaum & DeVito 1995).

Currently South Africa has no established gas chromatography – high resolution mass spectrometry (GC-HRMS) facility for dioxin analysis (Batterman et al. 2009). This method of analysis is mandated by the USA EPA for PCDD/F determination. The lack of GC-HRMS facilities in South Africa (and other developing countries) necessitates the development of an alternative solution that is affordable and easy to manage. To be advantageous, the analytical approach must be robust, must provide fast turnaround times and be able to screen for many classes of environmental pollutants in a single analysis. To meet this need, comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) was established as the analytical capability at the National Metrology Institute of South Africa (NMISA) for ultra-trace quantitative analysis and measurement traceability of chlorinated organic compounds, with particular focus given to PCDD/Fs (dioxins).

GC×GC-TOFMS provides both the selectivity (increased peak capacity of GC×GC; Dimandja 2004 and Korytár 2006) and the sensitivity (focusing effect of the modulator leading to improved peak sharpness; Semard *et al*. 2009) needed for this demanding environmental analysis. It is expected that the selectivity lost due to low resolution mass spectrometry as opposed to HRMS will be offset by the higher selectivity of comprehensive gas chromatography. GC×GC-TOFMS acquires full mass range spectral data (Cochran & Frame 1999 and Focant *et al*. 2004b); it is not a target compound technique such as single ion monitoring (SIM), and is ideal for screening for multiple classes of environmental pollutants in a single analysis (Reiner 2010). As such, retrospective data mining of archived data may be possible when future focus turns to new target compounds, e.g., the brominated dioxins.

To refine measurement approaches towards solving biodiversity and environmental issues that are potentially impacting the health and safety of biota and humans in South Africa, instrumental and bio-analytical techniques have been combined for screening of PCDD/Fs in environmental samples. The bio-analytical technique developed for the initial screening of PCDD/Fs in soil and sediment uses the *in vitro*

H4IIE reporter gene bio-assay (Hoogenboom *et al*. 2006 and Whyte *et al*. 2004). Only samples that elicited a response above 20% were selected for instrumental analysis. The GC×GC-TOFMS technique was used as a cost effective quantification method where positive results could later be confirmed using GC-HRMS (Focant *et al*. 2001a), or new confirmatory techniques such as gas chromatography tandem mass spectrometry (GC-MS/MS; L'Homme *et al*. 2015).

NMISA has based development work around time-of-flight mass spectrometers (both GC×GC-TOFMS and high resolution TOF (HRT) since these instruments provide accurate trace quantitative analysis of chlorinated compounds of environmental concern (Hoh *et al.* 2007, Korytár 2006 and Verentchikov *et al.* 2005a). The quantitation of chlorinated dioxins and furans has been governed internationally by US EPA Method 1613B (1994a) or methods derived from it (US EPA 2007d and Kolic & Macpherson 2012). It mandates the minimum levels of quantitation of PCDD/Fs for solid matrices, and specifies the use of GC-HRMS for detection and quantitation of PCDDs/Fs. The detection limits and quantitation levels in the method are usually dependent on the level of interferences rather than instrumental limitations. US EPA Method 1613B does make provision for the use of alternate methodologies like GC×GC-TOFMS as long as the developed method meets the stringent requirements stipulated for the seventeen toxic PCDD/Fs.

This thesis has been written as a narrative, describing the research and linking this to the work published in support of the research. The thesis describes the development of a PCDD/F capability for South Africa, starting with a general introduction, and leading to a preliminary validation of the GC×GC-TOFMS method using a certified reference material and South African soil samples. Conclusions are presented on the viability and robustness of the combined bio-analytical and instrumental techniques as a comprehensive, integrated approach for screening of toxic samples for numerous classes of organic pollutants, and also for the quantitative analysis of the individual compounds in the South African environment.

The various chapters have been structured to explain each step in the method development process. Chapter 2 introduces NMISA and provides an in-depth description of POP compounds, their physical and chemical properties, their possible

sources and formation processes, their toxicity and environmental fate. It also explains the South African situation regarding legislation and the need for a PCDD/F analysis capability. It covers the US EPA Method 1613B requirements and the quantitative analysis of PCDD/Fs using GC-HRMS. Chapter 3 provides a background on GC, multi-dimensionality, comprehensive GC and GC×GC-TOFMS as an analytical tool for the analysis of PCDD/Fs in developing countries, closing with a discussion on the first pesticide results using this technology. Chapter 4 deals with the proposed South African approach to solving analytical challenges for dioxin measurements using combined bio-analytical and instrumental techniques. The work has been described in the papers by De Vos *et al*. (2013a). The sample extraction challenges encountered are explained in chapter 5, which then deals with the various sample extraction methods tested, leading to an improved extraction and clean-up procedure.

GC×GC-TOFMS is an ideal screening tool, and chapter 6 deals with qualitative approaches for screening for chlorinated species using scripting and also describes the isomer specificity separation that underpins the validation criteria for selectivity. The work has previously been published in the papers by De Vos *et al*. (2011a) and De Vos & Gorst-Allman (2013).

Chapter 7 addresses the quantitative determination of PCDD/Fs to meet the minimum low levels as mandated in US EPA Method 1613B. The work has been previously published in the papers by De Vos *et al*. (2011a), De Vos *et al*. (2011b) and De Vos *et al*. (2013b). Chapter 8 details the NMISA pre-validation for the seventeen toxic PCDD/Fs, including the measurement uncertainty, uncertainty budget and traceability statement, and chapter 9 provides the final South African metrological results. Concluding remarks are presented in chapter 10.

2

BACKGROUND

2.1 CONVENTION OF THE METRE

The metric system was first developed during the French Revolution to replace the various measures used at that time. During the nineteenth century the metric system was adopted by both the world-wide scientific community and many countries as the system of measurement. This confirmed the international need to establish a measurement institute for the purpose of coordinating international metrology. The development of the metric system called for a treaty that would coordinate international measurement and establish associated organisations to oversee the running of the institute (BIPM *c*. 2005a, Naughtin 2008).

The Convention of the Metre is a treaty that created the International Bureau of Weights and Measures (BIPM), an intergovernmental organisation under the authority of the International Committee for Weights and Measures (CIPM). The CIPM has established a number of Consultative Committees that ensures the world's experts, in their specified fields, meet as advisers on scientific, technical and administrative matters (BIPM *c*. 2005b). The Metre Convention was signed in Paris in 1875, thus establishing a permanent organisational structure for member governments to act in mutual agreement on all matters relating to units of measurement (BIPM *c*. 2005c).

A '*Mutual Recognition Arrangement'* framework document (CIPM MRA), designed for national measurement standards and for calibration and measurement certificates issued by National Metrology Institutes (NMIs), was signed in Paris in response to a growing global need for reliable quantitative information on the comparability of

national metrology services and to provide the technical basis for wider agreements negotiated for international trade, industry and regulatory activities (BIPM 2003).

The task of the BIPM is to ensure world-wide uniformity of measurements and their traceability to the International System of Units (SI) under the auspices of the Metre Convention. The BIPM operates through the Consultative Committees (BIPM *c.* 2005d), whose members are the national metrology institutes (NMIs) of the Member States of the Convention, and through its own laboratory work.

2.2 ACCURATE MEASUREMENT IN SOUTH AFRICA

The National Metrology Institute of South Africa (NMISA), is mandated by an Act of Government, Act No. 18, 2006 (Act of Parliament 2006), to provide for the use of measurement units of the International System of Units and certain other measurement units.

In August 1964, South Africa became the 40th signatory to the Metre Convention and signed the CIPM MRA in October 1999 on behalf of the Department of Trade and Industry (**the dti).** NMISA was initially established in 1947 as the National Physics Laboratory in the Council for Scientific and Industrial Research (CSIR). It was later renamed the National Metrology Laboratory (McDowell 1997). Under the Measurement Act of 2006, NMISA was promulgated in May 2007 as an independent public entity, forming part of the Technical Infrastructure of **the dti**.

2.2.1 Why is Measurement Important?

Accurate measurement is paramount for fair trade, competitive manufacturing, efficient health care and effective environmental monitoring and law enforcement (**the dti** 2014). NMISA links the South African and regional measurement system to the international measurement system through its participation in the Convention of the Metre and its organs, the CIPM and the BIPM. The expanding global trade and pressure to eliminate technical barriers to trade (TBTs) creates a constant demand for greater accountability and demonstrated competence in NMIs and plays a leading role in the development of a sound metrology infrastructure in Africa, especially in support of South Africa's immediate neighbours in the Southern African Development Community (SADC). The brief outline described above for the establishment of the

Metre Convention and the BIPM is intended to inform the reader of the measurement framework within which NMISA functions and to better understand the role of chemical metrology in NMISA to provide measurement traceability for analytical measurements for the South African industry and for measurement collaboration in Africa.

2.2.2 Metrology in Chemistry

For chemistry, at the international level, measurement traceability is coordinated by the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM), of which the NMISA Chemistry Division is a full member. The CCQM was established in 1993. Present activities include the development and optimisation of reference measurements using primary methods for measuring amount of substance, participation in international comparisons to benchmark analytical capabilities and the establishment of international equivalence between national laboratories (BIPM *c.* 2005d).

Metrology is defined as the science of measurement and its application (JCGM-VIM 2012: viii) and a '*metrologist*' is a scientist that performs metrology work that requires precise and accurate measurement of the true value of the measurand with its associated uncertainty (JCGM-VIM 2012). Participation in international activities are imperative for NMISA as an NMI. These interactions serve to benchmark South Africa's capability to compete in measurement equivalence that directly impacts the ability to disseminate traceability for the country.

2.2.3 Why is Measurement Important in South Africa?

Economic prosperity, population health, efficient monitoring of the environment and successful law enforcement are directly influenced by the measurement infrastructure and services in a country. Without adequate quality control measures in place, measurement results are often of an unsatisfactory quality. Measurement errors not detected in time are compounded, leading to poor quality goods, which in turn, can inhibit fair trade (Miller & Miller 2010 and IPAP 2014).

The NMISA Chemistry Division was established to provide measurement traceability to industry through reference analyses, the development of reference methods, as well

as the preparation and certification of reference materials and standards through a comprehensive suite of techniques using primary methods and/ or methods of higher-order measurement, and the provision of proficiency testing schemes. Projects are aligned with **the dti's** Industrial Policy Action Plan (IPAP; **the dti** 2014), and include environmental monitoring of persistent organic pollutants (POPs) and contaminants, air pollution monitoring and food safety. Although NMISA is mandated to maintain and disseminate the national measurement standards (NMS), the research underpinning the NMS involves collaborative associations and projects with government, science institutes and academia and sourcing external revenue through these collaborative associations and projects where possible.

In an attempt to contribute to current knowledge pertaining to POPs in the South African environment, NMISA needed to investigate the nature of the chemicals being analysed and to understand their physio-chemical properties, environmental fate, formation and the main sources that contribute to their levels within the environment. Analyses for various pesticides and polycyclic aromatic hydrocarbons (PAHs) are being conducted, but there was no established capability for the analysis of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs or dioxins) and polychlorinated dibenzofurans (PCDFs or furans); therefore one of the main focus areas became the development of a measurement technique and capability to determine the presence and levels of PCBs and PCDD/Fs in abiotic matrices.

2.3 PERSISTENT ORGANIC POLLUTANTS

POPs are organic compounds of natural or anthropogenic origin that resist photolytic, chemical and biological degradation (Baeyens *et al*. 2004, Bernard *et al*. 2002, Bornman *et al*. 2007, Corsolini *et al*. 2005 Pereira 2004 and Safe 1995). They are typically characterized as having low water and high lipid solubility. Properties such as their high melting point, low vapour pressure, good stability, affinity for non-polar conditions, accumulation and bio-magnification in the food chain, resistance to metabolic degradation and semi-volatility make them an important environmental concern (Van Overmeire, *et al.* 2001). These compounds are also strongly adsorbed onto particulate matter which impacts their fate in the environment and assists with

deposition and removal from the atmosphere, which is beneficial from an air pollution perspective (Van Overmeire, *et al.* 2001; McKay 2002 and Breivik & Alcock 2002). The semi-volatile nature of these compounds enables them to move long distances in the atmosphere (and in low concentrations by the movement of fresh and marine waters), causing wide spread distribution across the earth, including regions where they have never been used or generated, thus exposing both humans and environmental organisms to POPs around the world and in many cases, for an extended period of time (Baeyens *et al*. 2004, De Boer & Fiedler 2013, Prevedouros *et al*. 2004 and Wania & Mackay 1996).

The risks posed by POPs have become of increasing concern in many countries, resulting in actions to protect human health and the environment being taken or proposed at the national, regional and international levels. Decision 18/32 (UNEP 1995) was adopted at the May 1995 Governing Council meeting of the United Nations Environmental Programme (UNEP), which invited the Inter-Organisation Programme on Chemicals Safety (IPCS) and the Intergovernmental Forum on Chemicals Safety (IFCS), to initiate an assessment process. The February UNEP Governing Council agreed on decision 19/13C (UNEP *c.* 1997 and UNEP *c.* 2014) which called for the establishment of an Intergovernmental Negotiating Committee (INC) to develop a global instrument to address POPs.

The inherent global risk posed by POPs culminated in the development of the Stockholm Convention after the signing of the treaty in May 2001 in Sweden and came into effect in May 2004 (Fiedler 2007). The main aim of the Stockholm Convention is to protect humans and the environment from chemicals that are persistent, bio-accumulate, and tend to become geographically widely distributed. The convention deals with the phasing out of the production and use, as well as the waste management of POPs (Stockholm Convention 2010). Originally the Stockholm Convention focused on twelve chemicals, the so called '*dirty dozen'*, which comprised pesticides, industrial chemicals and unintentionally produced organic chemicals (Bouwman 2004 and Stockholm Convention 2014). Nine new POPs were included in the Stockholm Convention in May 2009 and one additional group, technical endosulfan sulphate and its related isomers, was included in in May 2011. There are still chemicals being proposed for listing under the convention and in 2014 another listed

chemical was included in the convention (Table 2.1). Further discussion will focus on PCBs and the unintentionally produced PCDD/F organic compounds.

Table 2.1. *Persistent organic pollutants as identified by the Stockholm Convention for priority action.*

Initial POPs (Dirty Dozen)		
Aldrin	Chlordane	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT)
Dieldrin	Endrin	Heptachlor
Hexachlorobenzene (HCB)	Mirex	Toxaphene
Polychloronated biphenyls (PCBs)	Polychlorinated dibenzo-*p*-dioxins (PCDDs)	Polychlorinated dibenzofurans (PCDFs)
New POPs		
Included 2009		
Chlordecone	Alpha-Hexachlorocyclohexane (α-HCH)	Beta-Hexachlorocyclohexane (β-HCH)
Lindane (γ-HCH)	Pentachlorobenzene (PeCB)	Hexabromobiphenyl
Selected brominated diphenyl ethers (tetraBDE, pentaBDE, hexaBDE, heptaBDE)	Perfluorooctane sulfonic acid (PFOS) and its salts	Perfluorooctane sulfanyl fluoride (POSF)
Included 2011	*Included 2014*	
Technical endosulfan (including α-endosulfan and β-endosulfan)	Hexabromocyclododecane (HBCD)	
Chemicals proposed for listing		
Short chained chlorinated paraffins (SCCPs)	Polychlorinated naphthalenes (PCNs)	
Hexachlorobutadiene	Pentachlorobutadiene	

2.3.1 PCBs, PCDDs (Dioxins) and PCDFs (Furans)

PCBs are ubiquitous contaminants of the environment due to their large scale production (until the end of the 1980s) and use in numerous applications (Batterman *et al*. 2009, Davy 2004 and Pereira 2004). PCBs were first synthesised in the late 1920's (global estimated production since then is almost 10^6 tons; Jones & De Voogt 1999) for a variety of industrial uses such as dielectrics in transformers and capacitors,

heat exchange fluids, paint additives, in plastics, coolants and insulating fluids for transformers and capacitors, stabilizing additives in PVC coatings, pesticide extenders, cutting oils, flame retardants, hydraulic fluids, sealants, adhesives, wood floor finishes, and carbonless copy paper. PCBs were supposed to be confined to the industrial environment, but can be found in virtually all global ecosystems (Batterman *et al*. 2009, Focant 2003 and Jones & De Voogt 1999).

PCDD/Fs constitute two classes of structurally related chlorinated aromatic hydrocarbons that are both highly toxic and produced as by-products (Batterman *et al*. 2009, Davy 2004, De Voogt 1999, Korytár 2006, Langer *et al*. 1973, Okey *et al*. 1994 and Pereira 2004). Due to their hydrophobic character and resistance to metabolic degradation, these substances are usually found together in the environment as complex congener mixtures (Focant 2003, Korytár 2006 and Naile *et al*. 2011). Almost every living being has been exposed to PCDD/Fs and studies have shown that exposure at high enough levels may cause a number of adverse health effects, including cancer (Pereira 2004). The health effects associated with PCDD/Fs depend on a variety of factors including the actual human exposure and the level, duration and recurrence of the exposure (Hanberg 1996, Jones & De Voogt 1999, Lonati *et al*. 2007 and Wu *et al.* 2008). These compounds can consequently be considered environmental indicators of anthropogenic activities since their occurrence can always be linked to human activities (Pereira 2004 and Wu *at al*. 2008).

2.3.2 Physical and Chemical Properties

Over the past two decades measurement interest has been focused on the toxicology of PCBs, particularly on those congeners that express the same type of toxicity as PCDD/Fs. Theoretically, there are 209 different PCB congeners, each differing from the other by level of chlorination and substitution position (De Boer *et al*. 1993, Jones & De Voogt 1999 and Korytár 2006). Technical formulations such as Aroclors and Clophens contain about 140 of these congeners that have been found in environmental samples (Korytár 2006:25). PCBs and PCDD/Fs therefore constitute three classes of structurally related chlorinated aromatic hydrocarbons. PCDDs are a group of 75 related chemical compounds that differ in the number and location of

chlorine atoms on a basic three ringed structure comprising carbon, hydrogen and oxygen atoms (Figure 2.1 and Table 2.2).

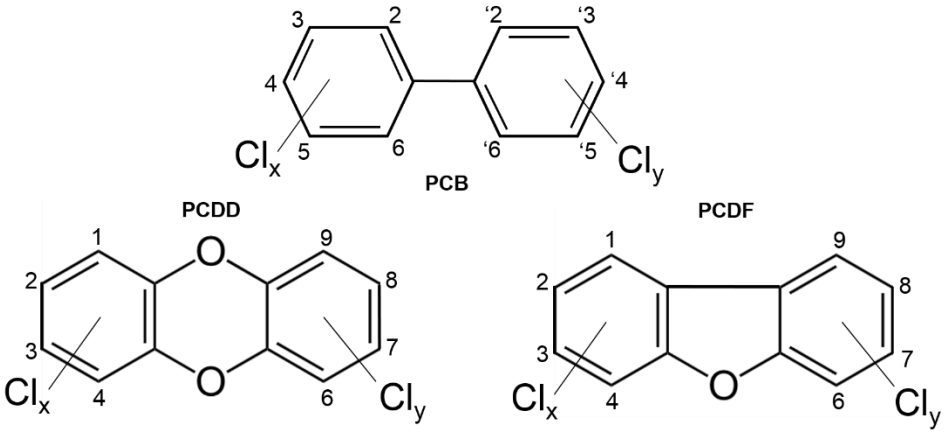


Figure 2.1. *PCB, PCDD (Dioxin) and PCDF (Furan) structures*

Table 2.2. *Number of possible PCDD, PCDF and PCB congeners based on chlorine number*

Number of Chlorine atoms	Number of Isomers PCDDs	PCDFs	PCBs
1	2	4	3
2	10	16	12
3	14	28	24
4	22	38	42
5	14	28	46
6	10	16	42
7	2	4	24
8	1	1	12
9	-	-	3
10	-	-	1
Number of Congeners	75	135	209

PCDFs are a group of 135 related chemical compounds that have up to eight chlorine atoms attached to the carbon atoms of the parent chemical, dibenzofuran. The compounds that contain chlorine atoms at the 2,3,7,8-positions of the dibenzofuran molecule (likewise for the PCDDs) are known to be especially harmful. PCDFs do not dissolve in water easily and occur as solids. There is no known use for these chemicals, other than for research purposes (Jones and de Voogt 1999, Korytár 2006, Mackay 2002, Pereira 2004 and Reiner 2010).

The Agency for Toxic Substances and Disease Registry's (ATSDR) toxicological profile succinctly characterises the toxicological and adverse health effect information for the PCDD/Fs as summarised in Table 2.3. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicological property (ATSDR 1994 and ATSDR 1998, Focant 2003, McKay 2002, Sinkkonen & Paasivirta 2000, Shlu *et al*. 1988[5] and Van den Heuvel & Lucier 1993).

Table 2.3. *Typical physical and chemical properties of PCDD/Fs*[*]

Parameter	Empirical Formula/ Symbol	No of Cl atoms and Chemical Information
Formula PCDDs[1,2,5]	$C_{12}H_xCl_yO_2$	X = 1-7 Y = 1-8
Formula PCDFs[1,2,5]	$C_{12}H_xCl_yO_1$	X = 0-4 Y = 4-8
Vapour pressure[3,5]	Pa at 20-25 ºC	0.5 – $1x10^{-10}$
Melting point[5]	ºC	89 – 330
Boiling point[5]	ºC	374 - 537
Flash point[1]	ºC	No data
Thermal stability (up to)[2]	ºC	800
Destruction (above)[2]	ºC	1300
Water solubility (hydrophobic and lipophilic)[1,4]	mg/ l	1.2 x 10^{-4} – 7.4 x 10^{-8}
Log K_{ow}[1,3,4]		6.2 – 8.8
Half life in humans[2,6]		5-10 years
Half life in environment[2]		10-12 years
Half life in atmosphere[1,3]		Several days

**ATSDR 1994[1]; Focant 2003[2], McKay 2002[3], Sinkkonen & Paasivirta 2000[4], Shlu et al. 1988[5] and Van den Heuvel & Lucier 1993[6].*

Even though these PCDD/F compounds are highly persistent, and degradation occurs over an extended period, the levels and environmental fate thereof has never specifically been studied under South African conditions. Deposition fluxes of PCDD/Fs show seasonal changes with deposition levels being higher in winter than in summer (Moon *et al*. 2005). The causality for this difference can be due to increased formation during colder months because of increased combustion activity (Lohmann & Jones 1998 and Moon *et al.* 2005,), as well as meteorologically driven variations in the magnitude of deposition, revolatilisation and degradation (Backe *et al*. 2004, Lohmann & Jones 1998 and Moon *et al*. 2005). South Africa's meteorological conditions are markedly different to that of the northern hemisphere where most

studies concerning these chemicals have been done. The South African Highveld has extended summers, low winter precipitation, comparatively high temperatures and elevated light intensity throughout the year (Tyson *et al*. 1996 and UNEP 2006). This is combined with unique air-current circulation over the central part of South Africa which represents the industrial heartland of the country (UNEP 2006). During the summer months, higher levels of photo-degradation, scavenging by plants, and reactions with OH^- radicals occur that leads to the decomposition of dioxin-like chemicals. The elevated temperature and light intensity will cause these PCDD/F compounds to react differently in the South African climate with an expected shorter half-life in summer than in winter conditions (Doering & Saey 2014 and Tyson *et al*. 1996).

2.3.3 Possible Sources, Formation Processes and Fate

PCDD/Fs can be formed in a variety of industrial and thermal processes, with combustion processes being the largest contributor of PCDD/Fs to ambient air levels (Fiedler 1996 and Vanden Heuvel & Lucier 1993). According to Pereira (2004) there are three main categories for sources of PCDD/Fs: stationary (thermal processes, chemical industries), diffuse (fuel burning, fires) and secondary sources (bio-compost, sewage sludge). PCDD/Fs are formed as trace by-products from chemical processes such as in the chlorochemical industries, paper and pulp industry, chlorophenol usage, or accidents such as Seveso, or in high temperature processes such as combustion. Anderson & Fisher (2002) postulate that four processes or sources exist from which PCDD/F compounds can be released (Table 2.4).

Table 2.4. *Typical Sources for PCDD/Fs*

Formation sources	Examples
Chemical production processes	Chloro-chemical industries and chlorine bleaching in the paper and pulp industry
Thermal and combustion processes	Municipal solid waste incinerators, power generation and metal production, combustion of leaded gasoline, accidental burning of PCB-containing electrical equipment, incineration of coal, peat and wood, cigarette smoke
Biogenic processes	Formation of dioxins from precursors such as pentachlorophenol
Reservoir sources	Historical pesticide stores, dumps and contaminated sites

The US-EPA has estimated that 70% of all quantifiable environmental emissions were contributed by air emissions from three source categories: municipal waste incineration, backyard waste burning and medical waste incinerators (Fierens 2006, Fréry *et al*. 2007a, Fréry *et al*. 2007b, Lee *et al*. 2002, Tuppurainen *et al*. 1998, Van Overmeire *et al.* 2001 and Zhang *et al.* 2011). The formation of PCDD/Fs requires chlorine, oxygen and the presence of a metal catalyst and can be formed in a two-step condensation reaction from alkali salts of 2,4,5-trichlorophenol (sodium pentachlorophenate) at elevated temperatures (Firestone 1973, Hays & Aylward 2003, Hashimoto *et al*. 1995, Kearney *et al*. 1973 and Langer *et al*. 1973). The incineration of various wastes or the combustion of various materials containing chlorine leads to the formation and emission of polychlorobenzenes (PCBzs), polychlorophenols (PCPs), PAHs, PCBs and PCDD/Fs (Lauridsen 2008 and Lavric *et al*. 2005).

Although human activity is the main source of PCDD/Fs in the environment, these PCDD/Fs and dioxin-like compounds can be formed through natural processes. This has been confirmed as these dioxin-like compounds have been measured in earth and marine sediment cores that predate large-scale manufacturing and the wide-spread use of chlorinated chemicals (Prevedouros *et al.* 2004). These compounds also undergo long-range atmospheric transport that leads to relatively-high concentrations in remote areas with minimal human activity (Hashimoto et al. 1995, McKay 2002, Pereira 2004, Prevedouros *et al.* 2004 and Wania & Mackay 1996). Even though these substances are likely to be formed through biochemical and geochemical processes and natural combustion processes (forest fires, volcanoes), there has been a meaningful increase in environmental levels coinciding with increased industrial development (Hays & Aylward 1993 and McKay 2002).

PCDD/Fs can be formed through biological processes and formation has been noted in sewage sludge and compost under normal environmental conditions. These substances are not only transported through air, but also through water and by the transport of products and waste. PCDD/Fs and dioxin-like compounds are long-lived in the environment, and the global journey of a dioxin-like molecule may take decades from its initial point of release until it is permanently trapped in an environment (Breivik & Alcock 2002 and Wania & Mackay 1996).

The combustion of carbonaceous fuels in incineration systems results in the emission of the products of incomplete combustion, including PCDD/Fs and dioxin-like compounds. There are two elevated temperature windows in which PCDD/Fs can be formed that describe both a homogenous route (pyrolytic rearrangement of chlorinated precursors, such as chlorophenols and chlorobenzenes in the gas phase, and are formed at high temperatures between 500 and 800 ºC) and heterogeneous route (a catalysed reaction that takes place on the ash or soot particles present in combustion systems at temperatures between 200 and 400 ºC) (Stanmore 2004 and Tuppurainen *et al*. 1998). Formation may be in the vapour phase or on solid surfaces such as soot or ash particles (Stanmore 2004). A third route, the '*de novo'* formation, occurs at lower temperatures between 250-350 ºC and involves the oxidation and chlorination of any unburned carbon in the particles present (Hoekstra *et al*. 1999). The reaction pathway is based on the presence of pre-existing macro-molecular structures such as 3-ring carbon skeletons. Oxygen is essential for the '*de novo'* synthesis to occur (Tuppurainen *et al*. 1998 and Stanmore 2004).In a thermal system, the final dioxin emission will result from the difference between the rates of formation and thermal degradation. The degradation of dioxins is an important consideration in the overall formation of PCDD/Fs. The degradation temperatures for PCDD/Fs are higher than those for formation, thus illustrating the importance of a sufficiently high operating temperature (Addink & Olie 1995 and Stanmore 2004).

PCDD/F emissions from combustion sources can also occur due to dioxin contamination of the raw fuel (Huang & Buekens 1996). The formation of PCDD/Fs in these systems can be controlled by upgrading the plants and with the addition of systems to reduce pollutant emissions. Incinerators with high quality air pollution control systems (Addink & Altwicker 2004) have reduced emissions of PCDD/Fs through end-of-pipe removal techniques, the use of chemical inhibition, control of waste composition, improvement of combustion conditions and prevention of formation in the post-combustion zone which can all lower the PCDD/F emissions (Ruokojärvi *et al*. 2004).

Soil acts as a conservative matrix and processes, such as volatilisation and degradation of PCDD/Fs, do not play a significant role; rather the soil reflects cumulative PCDD/F deposition over long periods of time (Domingo *et al*. 2001). After

being released into the environment, PCDD/Fs can be deposited on environmental media where they are subject to bio-accumulation due to their low mobility, high resistance to degradation and persistence (Pereira 2004). A process termed '*aging*' is used to describe the change undertaken in the availability of certain organic compounds when they have resided in soil for an extended period. Bio-availability is influenced by the process of aging, as well as the chemical and biological characteristics of a substance (Reid *et al*. 2000). Field and laboratory studies have both shown that persistent compounds have a trend of declining availability to microorganisms with residence time in the soil (Alexander 1995 and Domingo *et al*. 2001).

PCDD/Fs are poorly water-soluble and they tend to associate strongly with carbon-rich matrices such as soils and sediments (Baeyens *et al*. 2004, Bernard *et al*. 2002, Bornman *et al*. 2007, Corsolini *et al*. 2005, Fiedler 1996 and Wania & Mackay 1996). Wet deposition transports atmospheric compounds to the surface by precipitation and has been shown to be a major pathway responsible for the deposition of the higher chlorinated PCDD/Fs to soil; however, dry deposition of PCDD/Fs to soil particles does increase at cooler temperatures (Lohmann & Jones 1998). PCDD/F deposition shows seasonal fluctuations with higher deposition levels in winter than in summer. This difference could be ascribed to increased formation during colder months due to increased combustion activity, as well as atmospheric driven variations in the magnitude of deposition, re-volatilisation and degradation (Moon *et al*. 2005, Backe *et al*. 2004 and Lohmann & Jones 1998). The deposition to soil depends on the variable characteristics of the soil such as organic carbon content, moisture content, texture, structure and porosity (Backe *et al*. 2004). Characteristics such as pH play a negligible role when looking at dioxin-like compounds and since these compounds are non-polar and non-ionic, their abundance will not be strongly affected by this characteristic (Brzuzy & Hites 1995).

The majority of dioxin-like chemical emissions are transported hundreds of kilometres from their formation site and therefore not deposited locally (Fiedler 1996 and Pereira 2004). The exceptions are emissions from waste incinerators, medical waste incinerators and vehicles. These sources tend to have a greater fraction of their total dioxin-like chemicals deposited locally and one of the reasons for this phenomenon is

the higher percentage of dioxin-like chemical sources associated with larger particles that will settle close to the point of origin.

Lohman & Seigneur (2001) studied the levels of PCDD/Fs in the vicinity of a municipal waste incinerator. The fraction of dioxin-like chemical deposited in an area is dependent upon the particle size, distribution, congener profile, source characteristics, meteorological conditions and the land-use of the area. Land-use can be an important factor in the concentration levels of PCDD/Fs found in the agricultural areas where the soil tends to have fewer sources of pollutants when compared with urban and industrial areas. There is speculation that the tillage and erosion of agricultural soil can play a role in the destruction or dilution of PCDD/Fs and dioxin-like compounds in soil (Rogowski & Yake 2005). All these factors have to be considered when studying the distribution, transport and fate of PCDD/Fs and dioxin-like compounds.

2.3.4 Toxicology and Health Aspects

The toxicity of these PCDD/Fs is usually restricted to those congeners with four or more chlorine atoms in the molecule occupying the 2,3,7,8- positions (Hahn 2002, Pereira 2004, Shimba *et al*. 1998, Stanmor 2004 and Vanden Heuvel & Lucier 1993; Figure 2.1, Table 2.2). Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) causes a variety of toxic responses in humans including chloracne, tumor promotion, thymic involution, hydronephrosis, and cleft palate and wasting syndrome (Shimba *et al*. 1998). After 2,3,7,8-TCDD has been deposited into the adipose tissue where this chemical accumulates, it inhibits glucose transport, lipoprotein lipase activity and fatty acid synthesis (Shimba *et al*. 1998). The expression of adipose differentiation-specific transcription factors is inhibited in the presence of 2,3,7,8-TCDD (Shimba *et al*. 1998). The US-EPA (among others) has confirmed that PCDD/Fs are a cancer hazard and exposure can cause severe reproductive and developmental problems in humans and animals (Bornman *et al*. 2007 Hays & Aylward 2003 Hoh *et al*. 2008, McKay 2002, Okey *et al*. 1994 and Sanderson & Van den Berg 2003). One of the reproductive influences these PCDD/Fs and dioxin-like compounds have, is to lower the male/ female sex ratio of birth in the offspring of humans exposed to high levels of 2,3,7,8-TCDD (Mocarelli *et al.* 2000). A foetus before birth and a baby when breast feeding, is at highest risk in the human food chain subjected to PCDD/Fs and dioxin-like PCBs,

consuming the most concentrated amounts in its daily fat intake. Due to an infant's high risk of exposure and probable sensitivity, a breast-fed baby is regarded as the primary risk group for these toxins (Hanberg 1996, Koppe 1995 and Vandentorren *et al.* 2011). Since dioxin-like compounds have a long half-life in the human body (greater than seven years in adults for certain congeners), body burdens do not change rapidly in response to changes in intake exposure levels (Hays & Aylward 2003).

The primary pathways for these lipophilic dioxin-like compounds to enter the food chain is 'air-to-plant-to-animal', and from 'water/ sediment-to-fish' (Corsolini *et al*. 2005 and Van Overmeire *et al*. 2001). Significant dioxin-like activity has been observed in the eggs of birds as well as birds at different stages of development (Giesy *et al*. 2002), showing that these dioxin-like compounds have found their way into the higher levels of the food chain. The amount of PCDD/Fs and PCBs that is capable of entering the food chain depends on the bio-availability (accessibility of a pollutant to an organism) of these substances. Once a pollutant has entered the food chain, direct human exposure to contamination is bound to occur as humans are exposed to toxic PCDD/F congeners daily through their diet (Corsolini *et al*. 2005, Hays & Aylward 2003 and Kitamura *et al*. 2004). Since the gastrointestinal permeability and diffusion capability across membranes correlate with the lipophilicity of a substance (Dybing *et al.* 2002), the uptake of dioxin-like compounds through the food chain is a serious concern. Other pathways through which people can be potentially exposed to dioxin-like compounds include the intake of contaminated soil, inhalation of re-suspended particles and dermal absorption (Meneses *et al*. 2004).

Available information from industrialised countries indicates that the daily intake of PCDD/Fs and dioxin-like compounds during the last few decades has varied roughly between 2-10 pg WHO-TEQ.kg^{-1} body-weight (bw) per day for a 60 kg adult. However, with stringent laws concerning formation and release in European countries, a significant decrease in intake has been reported (Baeyens *et al*. 2004, Cao *et al*. 2013 and Hays & Aylward 2003).

Values set for the regulation of these substances are often based on Total Toxic Equivalency (TEQ) concentrations, including emission limits that will eventually

determine the amount of PCDD/Fs and dioxin-like compounds that populations are exposed to (Dyke & Stratford 2002, Van den Berg *et al*. 1998 and Van den Berg *et al*. 2006). In this approach, the biological or toxic potencies of a mixture of PCDD/Fs and dioxin-like compounds are expressed relative to a benchmark dioxin, usually 2,3,7,8-TCDD, since it is the most potently toxic congener (Hahn 2002). The TEQ approach is an attempt to provide an integrated assessment of the toxic potential of an environmental mixture and thus represents the total 2,3,7,8–TCDD-toxic equivalent potency of the mixture of dioxin-like compounds (Schwirzer *et al*. 1998, Van Overmeire *et al*. 2001 and Hahn 2002).

Table 2.5. *WHO-TEFs for humans (Van den Berg et al. 2006).*

PCDDs and PCDFs			PCBs			
Structure	WHO TEF		IUPAC No	Structure	WHO TEF	
	1998	2005			1998	2005
Dioxins			**Non-ortho (planar) substituted chlorobiphenyls**			
2,3,7,8-TCDD	1	1				
1,2,3,7,8-PeCDD	1	1	77	3,3',4,4'-TCB	0.0001	0.0001
1,2,3,4,7,8-HxCDD	0.1	0.1	81	3,4,4',5-TCB	0.0001	0.0003
1,2,3,6,7,8-HxCDD	0.1	0.1	126	3,3',4,4',5-PeCB	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1	169	3,3',4,4',5,5'-HxCB	0.01	0.03
1,2,3,4,6,7,8-HpCDD	0.01	0.01	**Mono-ortho substituted chlorobiphenys**			
OCDD	0.0001	0.0003				
Furans			105	2,3,3',4,4'-PeCB	0.0001	0.00003
2,3,7,8-TCDF	0.1	0.1	114	2,3,4,4',5-PeCB	0.0005	0.00003
1,2,3,7,8-PeCDF	0.05	0.03	118	2,3',4,4',5-PeCB	0.0001	0.00003
2,3,4,7,8-PeCDF	0.5	0.3	123	2',3,4,4',5-PeCB	0.0001	0.00003
1,2,3,4,7,8-HxCDF	0.1	0.1	156	2,3,3'4,4',5-HxCB	0.0005	0.00003
1,2,3,6,7,8-HxCDF	0.1	0.1	157	2,3,3',4,4',5-HxCB	0.0005	0.00003
1,2,3,7,8,9-HxCDF	0.1	0.1	167	2,3',4,4',5,5'-HxCB	0.00001	0.00003
2,3,4,6,7,8-HxCDF	0.1	0.1	189	2,3,3',4,4',5,5'-HpCB	0.0001	0.00003
1,2,3,4,6,7,8-HpCDF	0.01	0.01				
1,2,3,4,7,8,9-HpCDF	0.01	0.01				
OCDF	0.0001	0.0003				

TEQs can be calculated by multiplying the relative potency for the specific assay or the International Toxic Equivalency Factor (I-TEF) by the concentration of the specific congener, giving the total sum TEQ per mass unit (Hilscherova *et al*. 2000). The relative potencies of samples are calculated as the amount of standard (2,3,7,8-TCDD) giving the same response as the sample, based on the amount of sample

required to produce 50% of the maximal standard response and has only been established by use of *in vitro* bio-assays for a few alkyl phenolic compounds and PAHs (Giesy *et al*. 2002). The World Health Organisation (WHO) held an expert meeting in Stockholm in 1997 to derive consensus toxic equivalency factors for PCDD/Fs and dioxin-like PCBs based on different assays and analyses; these values are suitable for risk assessment (Van den Berg *et al*. 1998). Currently there are two sets of Toxic Equivalency Factor (TEF) values, the I-TEF and the WHO-TEF. The more recent values are the WHO-TEF values that include values for the dioxin-like PCBs and distinguish between species with different values for humans/ mammals, fish and wildlife (Fiedler 2003). The TEF consensus is based on the assumption that the combined effects of the different congeners are concentration additive. The different WHO-TEF values are shown in Table 2.5.

The TEQ concentration can be determined by summing the products of multiplying the concentrations of various molecules for which a TEF has been assigned (Equation 2.1). It has been implemented as a risk assessment tool dedicated to approximate the toxic potency of exposure to a mixture of PCDD/Fs and dioxin-like compounds and not to produce precise estimate of risk (Van den Berg *et al*. 1998 and 2006 and Van Leeuwen 1999).

$$TEQ = \sum_{n1}[PCDD_i \; x \, TEF_i] + \sum_{n2}[PCDF_i \, x \, TEF_i] + \sum_{n3}[PCB_i \, x \, TEF_i] \quad \text{Equation 2.1}$$

The TEQ approach is very important when dealing with PCDD/Fs and dioxin-like compounds. Humans that are exposed to PCDD/Fs and dioxin-like PCBs are usually exposed to a mixture of these dioxin-like compounds (Focant 2003 and Maruyama *et al*. 2003). The overall toxicity of a sample containing a mixture of PCDD/Fs and PCBs is thus indicative of the toxicity of the sample (Reiner 2010, Van den Berg *et al*. 1998 and Van Leeuwen 1999).

Several countries have put much effort into PCDD/F research programs with the aim of assessing the risks associated with PCDD/F exposure, to identify PCDD/F sources and to quantify their contribution to environmental emissions, environmental fate, human exposure and to develop measures to reduce emission and exposure (De Boer *et al*. 2008, Poland & Knutson 1982, Prevedouros *et al*. 2004 and Reiner 2010).

The clear evidence of long-range transport of these substances to regions where they have never been used or produced and the consequent risks they pose to the global environment, the international community rightly has called for urgent global actions to reduce and eliminate releases of these chemicals and South Africa is not immune to the risks associated with PCDD/Fs and dioxin-like compounds.

2.4 SOUTH AFRICAN SITUATION

South Africa is party to numerous environmental conventions and is thus bound by those regulations. In essence, South Africa has committed to reducing the level of environmental impact through anthropogenic activity in such a way that ensures the safety of not only South African citizens, but ensures the environmental integrity of our region. This includes the monitoring of environmental toxins that would negatively impact biota (**the dti** *c* 2001 and Quinn 2010).

Environmental conventions that directly impact South African monitoring of biota include the Stockholm Convention (2010), various biological conventions (Biological Diversity 1992, Bonn 1979 and Ramsar 2013) and the South African Environmental Conservation Act (Environment Conservation Act No. 73 of 1989). The release of environmental pollutants with long range transport potential and thus international implications are regulated under the Stockholm, Basel (1989), Rotterdam (2004) and climate change conventions (UNFCCC 1992), and control the release of chemicals such as greenhouse gasses and 'The Montreal Protocol' for the control of trans-boundary air pollution (UNEP 2012).

South Africa has signed the Stockholm Convention on POPs and thus has the set responsibility, according to Article 11, to undertake appropriate research, development, monitoring and cooperation pertaining to POPs (De Boer & Fiedler 2013, Fiedler 2007, Stockholm Convention 2010, and Proclamation No. R.76 of 2000, **the dti** *c* 2001 and UNEP 2000:2). The continued lack of data in this regard is the main motivation for undertaking research into the status of POPs and emerging POPs within the South African context (Bouwman 2004). Although many of these compounds have been banned or their use seriously restricted, POPs are still present and detected in environmental media (Hilscherová *et al*. 2000 and Quinn 2010).

The effect of environmental and industrial toxins on trade and industry includes countries and trade regions that impose regulations and directives to trade goods and protect the health of people and the environment. The list of potentially hazardous chemicals is ever increasing and the lack of analytical services to monitor these environmental toxins is of concern in South Africa. There is a shortfall in quality analyses for these toxic compounds on and in goods that are exported to foreign markets (including mineral and industrial exports). Potential problems exist in the export of goods, especially food produce and manufactured products (IPAP 2014), due to the ever more strict legislation and initiation of environmental programs that are being applied globally (Commission Regulations 2006a, b and c, 2009, 2012a, b and c and 2014, EFSA 2010, European Commission 2000, 2001 and 2011, EFSA 2010 and Slabbert 2004). Compliance is becoming critical in trade negotiations and overcoming technical barriers to trade (TBTs; Batterman 2009, Bouwman 2005, Fiedler 2007 and IPAP 2014).

According to the provisions of the Stockholm Convention, each party is expected to develop a plan for the implementation of its obligations under the Stockholm Convention. South Africa, as a party to the Convention, must establish measures and report on its efforts to meet the objectives of the Convention.

2.4.1 National Implementation Plan

Dioxin-like compounds are only a very small group of the many thousands of chemicals that are formed unintentionally or used in commerce and are routinely analyzed or regulated (Fiedler 2007 and Reiner 2010). South Africa has signed the Stockholm Convention, making the analysis of some environmental pollutants a legal obligation. South Africa has drafted a National Implementation Plan (NIP) that outlines the legal and administrative actions that are required to reduce or eliminate the production and release of POPs into the environment. The South African NIP was developed based on Article 7 of the Stockholm Convention (Department of Environmental Affairs 2011). After the NIP is submitted to the Stockholm secretariat, South Africa can then be deemed eligible for support and funding through the Global Environmental Fund (GEF) as facilitated through the Department of Environmental Affairs (DEA 2011).

The NIP has far-reaching effects on both industry and research. It has identified areas that will increase the responsibilities of industries concerning the control and elimination of these compounds. The Stockholm Convention and NIP have both highlighted the need to establish at least one centralised analytical laboratory capable of the analysis of persistent environmental pollutants, equipped with 'state-of-the-art' measurement instrumentation and infrastructure, not only as mandated through the increased monitoring requirements, but through the renewed interest in priority pollutant compounds.

In 1997, South Africa produced some 538 million tons of hazardous waste per year, and in excess of 418 million by the end of 1998 (Oelofse & Musee 2008) and has also authorised the import of hazardous waste from abroad (Basel Action Network 2000). Ninety five per cent of South African waste is disposed of on land, much of it in the province of Gauteng (GroundWork 2006). The potential harm to trade, should an incident similar to the Belgian dioxin chicken contamination event (Bernard *et al*. 2002) happen here (or anywhere in Africa with no access to such an analytical facility to independently check claims made by consumers overseas) would be devastating. The worst effect of such a disaster is the potential future loss in export markets as consumers lose confidence in a country's products. South Africa faces a major crisis in compliance testing for very toxic substances such as PCDD/Fs and dioxin-like compounds as there is currently no established routine facility to measure PCDD/Fs. It would take just one such calamity to turn the first world consumer against '*contaminated, poisonous'* African produce; a reputation, once earned, that may never be resolved.

UNEP have Global Environment Facility (GEF) programs aimed at supporting and assisting Western Africa and Eastern/ Southern Africa in the implementation of their global monitoring plan (GMP) and to build institutional capacity to fulfil the obligations under the Stockholm Convention. A UNEP Amsterdam workshop report was compiled to discuss preliminary results of a Global Air Monitoring Program that was established to fulfil the Stockholm Convention in Africa (Leslie & De Boer 2011). Unfortunately, South Africa was not one of those partner countries.

Professor Jacob de Boer (Institute for Environmental Studies, VU University Amsterdam) has been supporting and assisting laboratories in Africa (De Boer *et al*. 2008 and Lal *et al*. 2013) with POP analyses. The inference from this investigation revealed constraints such as: lack of high-resolution instrumentation, bureaucratic inertia, lack of analytical procedures, inability to obtain or access chemical consumables and standards, especially those classed as hazardous, lack of hazardous chemical waste disposal samples and lack of structured investment in POP analyses and trained analysts. The emphasis for that sorely needed capacity building and expertise in the analysis of POPs should be given priority and be supported by government. This is stressed and acknowledged in some African countries, but regrettably not currently in South Africa. There is at this time no tangible evidence for properly structured or adequately funded POP collaboration programs in South Africa, either by private institutions or government (Batterman *et al*. 2009, Bouwman 2004 and DEA 2011).

Cao *et al*. (2013) undertook an economic status study to determine national PCDD/F releases and the implications for reduction of PCDD/F releases. The annual releases of PCDD/Fs from 68 countries/ regions were investigated by correlating quantitative emissions with economic status of the nations by applying the UNEP Toolkit (ten source groups that report releases to five vectors; UNEP 2005); thus establishing a methodology for PCDD/F burden reduction. Open burning has been confirmed as the most predominant PCDD/F source and its proportion decreases along with economic development. The approach taken by this study can be used by governments to improve international PCDD/F reduction which implies that all countries/ regions are liable to establish PCDD/F inventories (Fiedler 1996 and, Pereira 2004). South Africa was not specifically included in this study, but is by no means exempt.

Monitoring POPs, in particular PCDD/Fs, PCBs, PAHs and chlorinated pesticides, involves complex measurement techniques and instrumentation (extraction and analysis). The analytical techniques applied for the analysis of the extensive list of POP compounds is well established and many optimised analytical methods for their separation and quantification are cited in the literature (Baughman & Meselson 1973, Baugros *et al*. 2008, Becher *et al*. 2004, Boden & Reiner 2005, Bodner 2005, Bordajandi *et al*. 2008, Cochran & Frame 1999, Cochran 2002, Crummett & Stehl

1973, Danielsson *et al*. 2005, Dasgupta *et al*. 2010, De Boer *et al*. 1993, Do *et al.* 2013, Eppe *et al*. 2006, Focant *et al*. 2002a, Focant, Sjödin *et al*. 2004d, Focant, Pirard *et al*. 2005a, Focant 2008, Focant 2014, Harju *et al*. 2003a, Hasegawa *et al*. 2007, Hilton *et al*. 2010, Hoh *et al*. 2007, Kolic & Macpherson 2012, Konieczka & Namieśnik 2008, Korytár *et al*. 2002, Korytár *et al*. 2004, Korytár *et al*. 2005, Korytár *et al*. 2006, L'Homme *et al.* 2014, Malavia *et al*. 2007, Marriott *et al*. 2003, Mondello *et al*. 2007, Mühlberger *et al*. 2004, Myers *et al*. 2010, Nosheen *et al*. 2013, Patil *et al*. 2009, Pehlivan & Pehlivan 2001, Pirard *et al*. 2003, Reiner *et al*. 2006, Reiner 2010, Sandra & David 2002, Shunji *et al*. 2008, Song *et al*. 2012, Tranchida *et al*. 2007, van Cleuvenbergen *et al*. 2005, van der Lee *et al*. 2008, Van Leeuwen & De Boer 2008, Van Mispelaar *et al*. 2003, Vogt *et al.* 2007, Weber *et al*. 1995, Wu *et al.* 2008, etc.).

To assist South African laboratories trying to establish such a capability, using a cheaper, but still accurate, alternative for monitoring these pollutants, NMISA has established analytical expertise in the analysis of PCDD/Fs and other POPs using GC ×GC-TOFMS. The analytical methods have been validated against GC-HRMS (high resolution magnetic sector mass spectrometry) techniques as applied in international laboratories (De Vos *et al*. 2011a and 2011b). Collaboration with international institutions has resulted in various lectures and peer-reviewed publications that have placed South Africa in the international arena as having a PCDD/F analytical capability. The outcome of this collaboration has been the development of a cost effective, integrated bio-analytical and instrumental analysis approach to POP analysis and in particular, PCDD/Fs (De Vos *et al*. 2013a). This collaborative effort (discussed in Chapter 4) will unfortunately not address the urgent need for an internationally benchmarked routine facility to provide the compliance testing needed to safeguard South African and regional agricultural exports.

2.5 PCDD/F ANALYTICAL REQUIREMENTS

An important goal in analytical chemistry is to use the most appropriate extraction, preparation, and instrumental techniques such that the accuracy and uncertainty of the technique is acceptable to meet the required data quality objectives and to ensure that the analytical method used is suitable for its intended purpose. Sensitivity, selectivity, speed of analysis and cost (the four key method attributes) must be

considered when selecting the most suitable analytical method. The data produced from the method must allow appropriate decisions to be made or answer specific questions relating to the compounds/ analytes of interest, the levels at which the compounds/ analytes are present, the matrix and possible interferences (Mondello *et al*. 2008, Reiner 2010 and Reiner *et al*. 2014).

All organic POP methods follow the same basic analytical scheme: extraction of analytes from the matrix, separation of analytes from interfering matrix co-extractables, and analysis of cleaned extract using chromatographic separation followed by instrumental detection. Selecting the correct analytical approach is critical in order to obtain the best quality data possible. The US Environmental Protection Agency Method 1613B (1994a) was developed by the United States Environmental Protection Agency's Office of Science and Technology for isomer-specific determination of the 2,3,7,8-substituted, tetra through octa-chlorinated PCDD/Fs in solid and tissue matrices by isotope dilution, high resolution capillary column gas chromatography (HRGC)/ high resolution mass spectrometry (HRMS).

2.5.1 US EPA 1613B and US EPA 8290A Requirements

US EPA Method 1613B (1994a) extends minimum levels of quantitation of PCDD/Fs into the low parts-per-quadrillion range for aqueous matrices and the low parts-per-trillion range for solid matrices. The method is specific for the seventeen 2,3,7,8-substituted PCDD/Fs and specifies the use of GC-HRMS for detection and quantitation of PCDDs/Fs and each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure. The detection limits and quantitation levels in the method are usually dependent on the level of interferences rather than instrumental limitations. The Method Detection Limit (MDL) for 2,3,7,8-TCDD has been determined as 4.4 pg/ L (parts-per-quadrillion) in water and 1 pg/ g (parts per trillion) for solids and as 0.5 pg/ µl (parts per billion) for sample extracts using this method.

The method's use of isotope dilution techniques, internal standard calibration, including the US EPA 1600 series method quality control protocols, results in improved sensitivity, precision and accuracy. These improvements have been validated through both intra-laboratory and inter-laboratory validation studies.

US EPA Method 1613B (1994a) is 'performance-based' and permits advances in technology and reductions in the cost of analyses by allowing the use of alternate extraction and clean-up techniques. The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all method equivalency and performance criteria are met. By allowing the use of alternate extraction procedures that reduce the solvent volumes required during analysis, US EPA Method 1613B (1994a) supports the environmental protection agency's pollution prevention initiative. US EPA Method 8290A (2007d) is based on GC-HRMS, although it is more applicable to highly contaminated environmental and matrix samples.

2.5.2 GC-HRMS

A comprehensive synopsis on the development of the first mass spectrometer instrument in 1913 is well documented by Burgoyne & Hieftje (1997) and El Aneed *et al*. (2009). Mass spectrometry was first described by physicists in the late 1880s. In 1886, Eugen Goldstein observed rays in gas discharges under low pressure that traveled away from the anode and through channels in a perforated cathode, in the opposite direction of the negatively charged cathode rays (that travel from cathode to anode). Goldstein called these positively charged anode rays *'Kanalstrahlen'* or '*canal rays*'). Wilhelm Wien determined that strong electric or magnetic fields deflected the canal rays and, in 1899, constructed a device with parallel electric and magnetic fields that separated the positive rays according to their charge-to-mass ratio (Q/m). Wien found that the charge-to-mass ratio depended on the nature of the gas in the discharge tube and later, English scientist Sir Joseph John Thomson, improved on the work of Wien by reducing the pressure to create the mass spectrograph (Bertsch 1978a and 1978b, El Aneed *et al*. 2009 and Klee & Blumberg 2002).

Mass spectrometry is an analytical technique that produces spectra of the masses of ions derived from the atoms or molecules or molecular fragments comprising a sample of material and considered suitable for stable isotope studies. A mass spectrometer consists of an ion source, a mass analyzer and a detector. Commercial mass spectrometers first appeared on the market in the 1940s. Gas chromatography was invented in 1952 as a separation tool for organic compounds and Holmes and Morrell pioneered the first coupling of gas chromatography with mass spectrometry (GC-MS)

in 1957 to provide a powerful diagnostic tool to detect and analyse these compounds (Bertsch 1978a and 1978b, El Aneed *et al*. 2009 and Reiner 2010).

Since the development of the first mass spectrometers ('spectrographs') by Thomson, Dempster and Aston, many additional mass spectrometers have been designed and built (Burgoyne & Hieftje 1997). In the early 1970's a number of top scientists met to try to determine the best instrumentation for PCDD/F analysis. The American Association of Science Team was put together in 1970 to determine the detection limits required to eliminate risks to dioxin exposure and determined the levels of dioxin risk exposure at 1 part per trillion (ppt) (Reiner 2010). Column chromatography using packed columns and electron capture detection (ECD) could only achieve detection levels of 50 parts per billion (ppb). Gas chromatography coupled with high resolution mass spectrometry (GC-HRMS) was the only technique capable of reaching this target level and it was decided to use the magnetic sector high resolution mass spectrometer (HRMS) instrument to meet the 1 ppt detection limit for the protection of the environment and human health (Reiner 2010). Since then magnetic sector instruments have become much more sensitive (sub parts per quadrillion level and lower); well below the 500 femtograms (fg) on column limit of detection mandated for 2,3,7,8-TCDD, thus easily meeting US EPA 1613B (1994a) requirements, and there is no other instrument that can measure up in both sensitivity and selectivity. The history of GC-HRMS is detailed in a book by Warrant Crummett (Crummett 2002).

Until about ten years ago, GC-HRMS was the only option and became the 'gold standard' for detecting PCDD/Fs and dioxin-like compounds. Since 1970, it has been estimated that more than US$1 billion has been spent on determining the toxicity of PCDD/Fs in samples (Behnisch *et al*. 2001 and Reiner 2010). The first GC-HRMS method for the analysis of dioxins and furans was performed in 1973 (Baughman & Meselson 1973 and Crummett & Stehl 1973).

2.5.3 Dioxin Analysis using GC-HRMS

To analyse all seventeen toxic PCDD/F congeners requires highly sensitive and selective analytical methods. GC-HRMS is currently the only accepted method for the instrumental analysis of PCDD/Fs and related analyses that use isotope dilution where the concentrations are determined by measuring the ratio of the analyte to the

appropriate isotopically labeled internal standard (Cochran *et al*. 2004 and Focant, Sjödin *et al*. 2004a). GC-HRMS easily measures organic compounds at these ultra-low levels and is capable of detecting 2,3,7,8-TCDD at levels as low as 10 fg of material on column, and is considered the method of choice for regulatory and litigation purposes in the analysis of PCDD/Fs providing improved sensitivity; the sensitivity gain is partially due to improved mass resolution (Reiner 2010).

The methods routinely used for the analysis of PCDD/Fs and dioxin-like PCBs using GC-HRMS have been well described and sufficiently optimised in a number of publications (Alaee *et al*. 2008, Bianco *et al*. 2008, Cochran & Frame 1999, Eppe *et al.* 2004, Eppe *et al*. 2006, Eppe *et al.* 2008a, Focant *et al*. 2001a, Focant *et al*. 2002a, Focant *et al*. 2002b, Focant & De Pauw 2002, Focant 2003, Focant, Pirard *et al*. 2005a, Focant, Pirard *et al*. 2005b, Focant *et al*. 2006a, Focant *et al*. 2006b, Krumwiede *et al*. 2011, Konieczka & Namieśnik 2008, Nording *et al*. 2007, Reiner 2010, etc).

With GC-HRMS, there is much less interference on an ion signal (e.g., when mass 468.1342 is plotted, as opposed to 468). This allows increased sensitivity for low intensity signals, as the noise associated with the exact mass is extremely small, or non-existent. The majority of the sensitivity advantages are expensive, in the region of US$ 500,000 or more. Magnetic sector instruments are research grade instruments that include higher quality components, resulting in increased cost of manufacture and concurrent increase in purchase price (Armbruster & Pry 2008, Dorman *et al.* 2008a, Focant, Eppe *et al*. 2005a and Reiner & Keller 2003). The resolution of a mass spectrometer defines its ability to distinguish between ions of differing mass. For the analysis of most halogenated POPs, a resolution of 10,000 is sufficient. As a consequence of isomeric similarities of PCDD/F compound peaks, chromatographic separation must be done before the analytes of interest are detected by the mass spectrometer. There is a further benefit to the mass resolution argument; for PCDD/F analyses, the PCDD/Fs are frequently found in the presence of PCBs. The chlorine patterns of these analytes overlap, so accurate mass becomes very useful in differentiating ions resulting from PCDD/Fs and ions resulting from PCBs (Crummett 2002, Focant, Pirard *et al*. 2005a, Mondello *et al*. 2008, Reiner 2010 and Volmer & Sleno 2005).

The other sensitivity advantage of a magnetic sector instrument is that it can be run in '*Selected Ion Monitoring* (SIM)' mode. With scanning instruments, significant signal intensity is lost through the scanning process. As an example, while counting ions at mass 100, all other ions arriving at the detector during that time are lost. Consider an acquisition rate of one spectrum per second. If the instrument is acquiring a range of masses (50 to 550 amu) in that one second, the detector spends 1/500th of a second counting the signal for each individual mass. In SIM mode, three ions may be selected for monitoring purposes, so 1/3rd of a second is spent counting each mass. This allows more signal to be measured and the sensitivity for those three ions increases considerably. More signal is counted because the instrument spends more time counting it (Crummett 2002, Focant, Pirard *et al*. 2005a, Mondello *et al*. 2008, Reiner 2010 and Volmer & Sleno 2005).

With SIM, the analyte identification is based upon detecting ions that are recorded in the right ratio at the right retention time. Many other analytes may have those same ions in similar ratios and this creates a false positive report for an identified PCDD/F that may well be, e.g., a PCB. There can be interferences on one of the ions that would alter its intensity. This distorts the ratio and the system could report the analyte as not found. This is a false negative. The analyte is actually present, but it is co-eluting with another analyte that has an interfering ion in its spectrum leading to an incorrect assumption that a PCDD/F is not present because of a co-eluting PCB that distorts the ion ratios in SIM (Focant, Pirard *et al*. 2005a, Mondello *et al*. 2008, Reiner 2010 and Volmer & Sleno 2005).

Accurate mass SIM removes the interferences in most cases that would cause a false negative, solving some problems for SIM accuracy, hence the high cost for this instrumentation. The measurements require meticulous calibration of the mass axis throughout the acquisition process. The dynamic range of these calibrations is very small (around two orders of magnitude). In order to measure 100 fg of 2,3,7,8-TCDD it is necessary to calibrate with no more than 1 pg of calibration material; a very small amount and can lead to potential errors (Dorman *et al.* 2008a, Focant *et al*. 2004b , Focant, Pirard *et al*. 2005a and Reiner 2010).

Although GC-HRMS is deemed the 'gold standard' for PCDD/F analysis, there is a drawback to SIM achieving the limits of detection and quantification necessary for the analysis of the targeted PCDD/Fs. This is not a limitation of the method itself, but is a disadvantage if a sample contains other compounds that are not the SIM target ions for analysis. The confirmation of analyte identity is achieved by selecting specific ions; compounds that do not contain these ions are not detected, and then precious spectral information is lost. This may be avoided by using deconvolution procedures (Mondello *et al*. 2007). From a developing nation analytical perspective, it would be advantageous to be able to screen samples for a broader range of POPs simultaneously, which would be less expensive and faster, while simultaneously identifying compounds that require further investigation without having to repeat the analysis.

For certain classes of POPs, only GC-HRMS instrumental analysis provides the sensitivity and selectivity required for the determination of individual congeners as is the case with PCDD/Fs (US EPA 1994a). This has led to a situation in which samples suspected of containing PCDD/Fs have to be sent overseas for analysis. This is not only time consuming and expensive, but leads to situations where members of the population are placed at risk while analytical results are anticipated. Large amounts of money are spent on samples for permits, transportation, currency exchange and the greater relative cost of overseas human resources.

GC-HRMS is a prohibitively expensive investment for the average South African laboratory as it also requires a highly trained and skilled operator, as well as a specialised laboratory infrastructure, including clean room facilities for environmental (very low level) samples. With a GC-HRMS instrument costing in the region of $575 000, and adding the cost for building a suitable laboratory infrastructure, plus resource considerations and purchasing very expensive sets of isotopically labelled standards, the price tag increases significantly. It is not an investment that can be taken lightly and until regulations are actually enforced, there is no urgency to budget for such a highly specialised laboratory, amidst many other pressing priorities (Batterman *et al*. 2009, De Vos *et al*. 2011a, De Vos *et al*. 2011b and DEA 2011). On the other hand, a GC×GC-TOFMS system costs approximately $300 000 and can be used within a routine laboratory infrastructure.

2.5.4 Alternative Approach

GC×GC-TOFMS was selected as an alternative quantitative technique having been used previously for PCDD/F analysis, though little attention has been focussed on combining the accurate quantitative capability of GC×GC-TOFMS with broad level priority pollutant screening (Reiner *et al*. 2006, Focant *et al*. 2004a, Focant *et al*. 2004b, Focant et al., 2004c, Focant *et al*. 2004d and Focant *et al*. 2004e). Requirements for PCDD/F analysis are selectivity, sensitivity, and speed. GC-HRMS compromises on speed, but is optimal for sensitivity and selectivity. GC×GC-TOFMS provides a compromise for these three important parameters (Reiner 2010).

GC×GC-TOFMS is robust and easy to use. The system provides powerful software algorithms, such as *'Spectral Deconvolution'* and *'Peak Find'*, and does not require cleaning of the ion source. As such there is minimal downtime associated with the instrument, which is ideal for environmental analysis, where fast response is frequently necessary (Mondello *et al*. 2007 and Reiner 2010). No sensitivity advantage is gained, similar to SIM acquisition, as all the ions are pulsed down the flight tube at the same time and all the ions are counted. There is no advantage to counting 1 ion over counting 995 ions in terms of the intensity of signal measured; the entire signal is counted. While SIM may give a sensitivity enhancement, it does forfeit many characteristic ions in the mass spectrum that may be necessary for definitive analyte identification. Acquisition of full mass range data thus ensures that all the information will always be available for further data analysis if required for unknown analytes. An additional advantage of GC×GC-TOFMS is the ability to fully characterize the matrix Adahchour *et al*. 2006a, Bertsch 1978a, Dimandja 2004, Dorman *et al.* 2008a, Hilton *et al*. 2010, Marriott & Shellie 2002, Marriott *et al*. 2003, Mondello *et al*. 2007 and Reiner 2010) and so it is worthy of consideration as an alternative approach for PCDD/F quantification (Hoh *et al*. 2007).

NMISA, in collaboration with several other institutions, both locally and internationally, has been developing this methodology so that it can be used to screen samples for several classes of POPs, including PCDD/Fs. These methods will not only screen for a variety of potentially harmful compounds in various matrices, but can be used to accurately quantify various POPs at the levels imposed by statutory organisations in first world countries (European Commission 2000 and 2001, US EPA 1994a and

2007d, etc). Such methodologies must be more affordable, less complicated and be robust, since in addition to limited funding, there is a shortage of qualified personnel that can routinely conduct these analyses in South Africa. Therefore the need exists to further develop GC×GC-TOFMS as an alternative solution for the analysis of various POPs, particularly PCDD/Fs in South Africa, and the technique will be detailed in Chapter 3.

3

GC×GC-TOFMS

3.1 GAS CHROMATOGRAPHY

3.1.1 Introduction

The invention of chromatography and its importance was recognized by Tswett some thirty years' before it became a widely accepted laboratory technique (Ettre 2003). The development of modern gas chromatography (GC) is generally attributed to James and Martin (1952) in their publication following a presentation in October 1950 at a meeting of the Biochemical Society, with wide interest generated by a lecture at a Society of Chemical Industry meeting in Oxford in 1952. In fact, the origin of GC lies in a sentence overlooked by other researchers at the time in a 1941 publication (Martin & Synge 1941) in which Martin, with Synge, first described liquid phase partition chromatography; '*Very refined separations of volatile substances should be possible in a column in which permanent gas is made to flow over gel impregnated with a non-volatile solvent'.*

Early GC was carried out on packed columns, typically 1-5 m long and 1-5 mm internal diameter and filled with particles, each of which was coated with a liquid or elastomeric stationery phase (Martin & Synge 1941). The resolution of packed columns is limited by their length, which is itself restricted by the pressure drop resulting from the resistance to gas flow. This restriction was removed by the invention of the capillary column, suggested by Martin in 1956 and realised by Golay in 1957, who laid out the theory of operation and demonstrated its use in 1958 (Dandeneau 1989, Ettre 1981 and 1987, Mondello *et al*. 2008 and Perkin Elmer 1998).

3.1.2 Capillary Columns

The principles and theoretical background of capillary columns have been described and understood since at least the early sixties (Bertsch 1978a, Bertsch 1978b, Dorman *et al.* 2008a and Mondello *et al*. 2008). It is a mature technique providing high separation efficiency (Cochran & Frame 1999, Dorman *et al.* 2008a, Marriott *et al*. 2012 and Reiner 2010). In a capillary column, the stationary phase is coated on the inner wall, either as a thin film (wall coated open tubular) or impregnated into a porous layer on the inner wall (porous layer or support coated open tubular; James & Martin 1952).

Capillary columns have the advantage over packed columns of greatly increased separation efficiency. They are more effective at a lower temperature and provide improved separation in equal time or the same separation in shorter time (Bertsch 1999, Bertsch 2000 and James & Martin 1952). Initially, and until 1980, capillary columns were manufactured from laboratory glass tubing. Progress with glass columns was difficult due to their fragility and activity towards highly polar analytes. These problems were to a large extent overcome by the invention of fused silica columns (Ettre 1981).

Although one dimensional capillary GC (1D-GC; Marriott *et al*. 2012) is a giant leap forward from packed column GC, researchers analysing more and more complex samples experienced growing difficulties with peak co-elution. A capillary column has a limited peak capacity (the number of resolved peaks that may be eluted in the available one-dimensional space). This results in overlapping of compounds in the chromatogram making it very difficult to identify unknown compounds of low concentration that are obscured by other components eluting at the same time (Bertsch 1978a, Davis & Samuel 2000 and Mondello *et al*. 2008). This precipitated the invention of a more effective way to enhance resolving power using two different chromatographic phases known as two dimensional gas chromatography. Two-dimensional (2D-GC; Marriott *et al*. 2012) separations are those techniques in which a sample is subject to two independent (orthogonal) displacement processes (Giddings 1984).

3.2 TWO-DIMENSIONAL CHROMATOGRAPHY

3.2.1 Heart-cutting (2D-GC)

Full resolution of all compounds of interest in the minimum time span is the primary objective in one dimensional GC. However, the technique does not always provide sufficient separation for a complete quantitative analysis even when identification/ confirmation techniques such as those based on mass spectrometry (MS) are used (Dorman *et al.* 2008a). Even after extensive sample preparation, complex extracts still contain high concentrations of matrix constituents that can easily obscure the analytes of interest (Adahchour *et al*. 2005, Bertsch 1978a, Blumberg *et al*. 2008 and Mondello *et al*. 2008). For these cases, multi-dimensional gas chromatography (MDGC) provides a simple and direct solution.

The transfer of one or more selected groups of overlapping compounds eluted from a primary GC column onto a second column is referred to as 'heart cutting' or 'cutting' (Deans 1968). The development of multi-dimensional GC (MDGC, GC-GC or 2D GC heart cutting) is thus an effective way to enhance resolving power (Ong & Marriott 2002). MDGC instrumentation enables the transfer of material to the second column by means of an interface (either a switching valve or a 'Deans' switch; Dunn *et al* 2004 and Mondello *et al*. 2008). The principle advantage of the technique is that the transferred fractions are subjected to a further separation on a full-length conventional column. The main drawbacks include the high time costs, the instrumentation complexity, and the operational expertise requirements. Continuous transfers applied to the entire sample can cause considerable overlapping of compounds previously resolved in the first dimension and is considered a further drawback (MacNamara *et al.* 2004, MacNamara *et al.* 2014 and Mondello *et al*. 2008).

An alternative approach, which avoids many of these pitfalls, is comprehensive two-dimensional GC (GC×GC), where all compounds eluting from the first column are passed to the second column by using a modulator (interface between the two columns that samples narrow bands from the eluate of the first column for fast re-injection into the second column) for further separation (Marriott *et al*. 2012).

3.2.2 Comprehensive Two-Dimensional GC (GC×GC)

Comprehensive two-dimensional gas chromatography (GC×GC) is a technique that uses two mutually independent separation mechanisms in which all components eluting from a first dimension column (^{1}D) are continuously introduced onto a second column that has different retention characteristics (Adahchour *et al*. 2005, Bertsch 1978a, De Geus *et al*. 1996, Dimandja 2004, Marriott *et al*. 2012, Sandra & David 2002 and Schoenmakers *et al*. 2003). Between the two columns is a thermal modulator which traps portions of eluent from the first column and re-injects them onto the second column Dimandja, *et al*., 2003, Focant *et al*., 2003, Focant *et al*., 2004c and Semard *et al*., 2009). The technique was described by Liu & Phillips in 1991. The entire sample is introduced onto the second column thus greatly enhancing peak capacity (Mondello *et al*. 2008). The separation mechanism in the second dimension separation (^{2}D) must therefore be fast enough to preserve the information obtained in the first dimension separation (Adahchour *et al*. 2005, Beens *et al*. 1998a and Beens *et al*. 1998b).

GC×GC can be applied to essentially all GC amenable complex samples containing various analyte classes (Beens & Brinkman 2004, De Geus *et al*. 1996 and Giddings 2003). The technique provides increased resolving power and enhanced sensitivity through solute band re-concentration, suited to trace-level component detection as compounds are fully resolved from background matrix or interfering analytes (Adahchour *et al*. 2005). A further advantage is the grouping of chemically similar compounds in the second dimension chromatographic space, thus providing considerably more structured information than one dimensional chromatography (Dimandja 2004 and Mondello *et al*. 2008). This aids in tentative identification of unknown compounds or group-type identification when no standards for those compounds are available and in the absence of corresponding MS library spectra (Bertsch 1978a, Dallüge *et al*. 2003, Giddings 1995, Hilton *et al*. 2010 and Phillips & Beens 1999).

3.2.3 GC×GC Design Considerations

Where 2D-GC typically requires two columns connected in series, through a flow-switch interface, GC×GC allows the entire first dimension effluent to be sampled onto

the second column without compromising the integrity of the chromatographic separation in either dimension (Dimandja 2004, Dallüge *et al*. 2003, Davis & Samuel 2000, De Geus *et al*. 1996, Focant *et al*. 2006a, Górecki *et al*. 2004 and Mustafa *et al*. 2012). Figure 3.1 provides a schematic to illustrate how the ^{1}D and ^{2}D columns are connected, with the modulator positioned between the two columns.

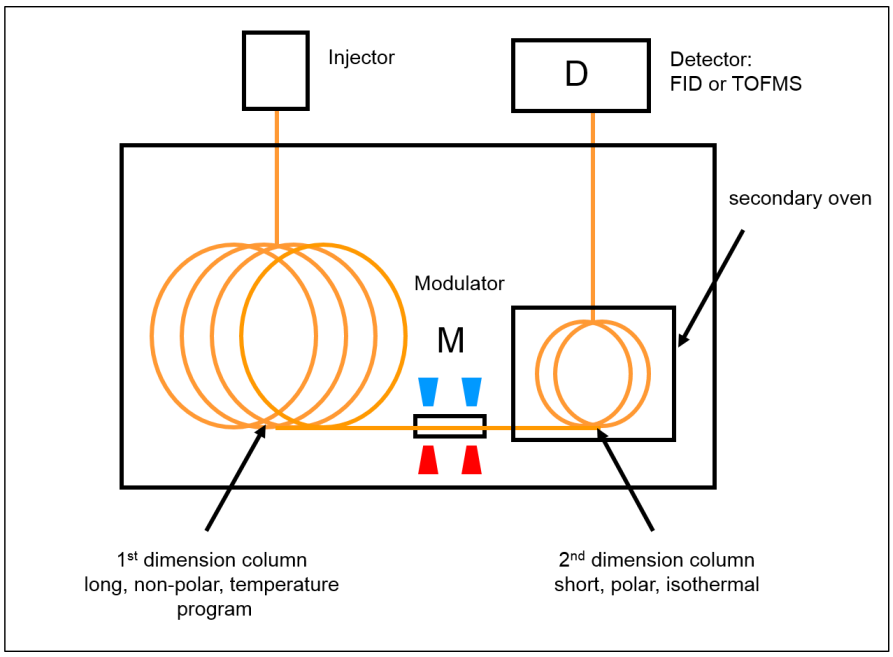


Figure 3.1. *A schematic showing the ^{1}D column connected to the ^{2}D column (usually using a press-tight connector) with the modulator (quad-jet design) positioned between the two columns. Adapted courtesy of LECO Corporation.*

Figure 3.2 illustrates how a GC×GC chromatogram is mapped. The modulator functions as the interface between the first and second dimension separations. During the separation, peaks eluting from the ^{1}D column may be sampled more than once onto the ^{2}D column, depending on the width of the peaks in the ^{1}D separation relative to the selected modulation period (Dimandja 2004). Modulation (sampling) occurs continuously during the ^{1}D separation and the total GC×GC run time is the same as for classical GC (Focant *et al*. 2006a, LECO 2009b and Mondello *et al*. 2008).

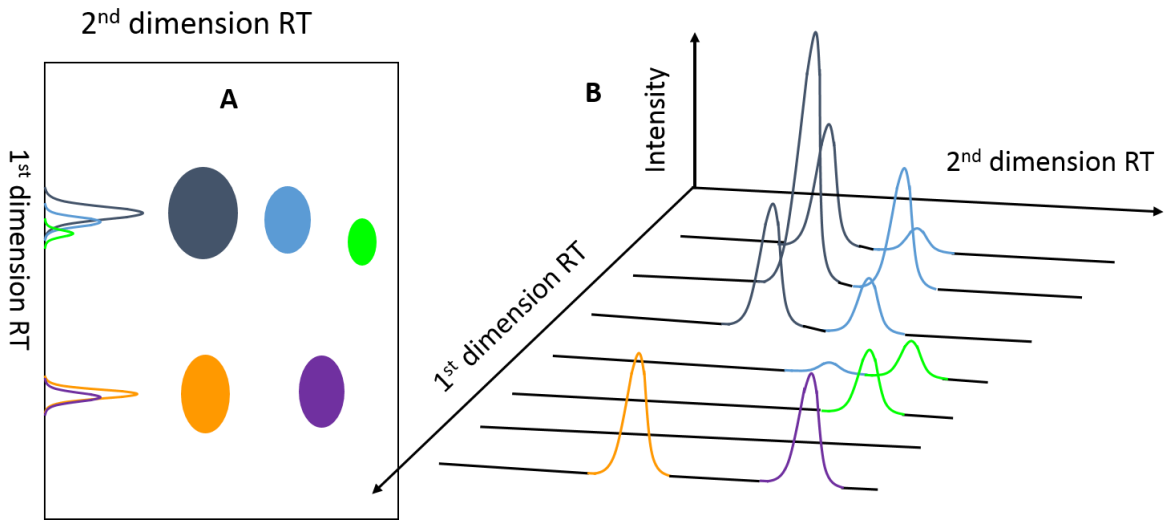


Figure 3.2. *Animated comprehensive two-dimensional (^{2}D) gas chromatographic separation showing how sample material eluting from the ^{1}D column is re-injected onto the ^{2}D column via the modulator as a 3-D surface plot. Adapted courtesy of LECO Corporation.*

GC×GC does result in lower resolution power in the second dimension, as compared with 2D-GC, as shorter columns with thinner films have been used to satisfy the high speed requirements for the secondary separation. However, the cumulative effect of the succession of high-speed secondary separations throughout the GC×GC run produces chromatograms with increased peak capacity over 1D GC (Blumberg *et al.* 2008, Dallüge *et al.* 2003, Dimandja 2004, Giddings 1995, Ryan & Marriott 2003 and Tranchida et al. 2011).

Detailed information on the theoretical considerations and operation of GC×GC is very well described in the literature (Adahchour *et al.* 2006a-d, Adahchour *et al.* 2008, Beens & Brinkman 2004, Beens & Brinkman 2005, Bertsch 1978a, Bertsch 1978b, Bertsch 1999, Bertsch 2000, Blumberg *et al.* 2008, Cochran 2002, Cochran & Frame 1999, Dallüge *et al.* 2003, Davis & Samuel 2000, De Geus *et al.* 1996, De Geus *et al.* 1998, De Geus *et al.* 2000, Dorman *et al.* 2008a, Focant *et al.* 2006a, Fraga *et al.* 2001, Giddings 1995, Górecki *et al.* 2004, Harynuk & Gorecki 2002, Klee & Blumberg 2002, Kristenson *et al.* 2003, Korytár 2006a, Liu *et al.* 1995, Marriott & Shellie 2002, Mondello *et al.* 2007, Ong & Marriott 2002a, Pierce *et al.* 2008, Phillips

& Beens 1999, Reiner *et al*. 2006, Ryan & Marriott 2003, Sandra & David 2002, Semard *et al*. 2009, Western & Marriott 2003, etc.).

The addition of a third mass spectrometric dimension to a GC×GC system generates the most powerful analytical tool today for volatile and semi-volatile analytes (Mondello *et al*. 2008 and Peroni *et al*. 2013), although this will not be further discussed.

3.2.4 Modulators and Modulation

Efficient chromatography is dependent on the effectiveness of the modulation process. The modulator is positioned between the two columns and its function is to collect, re-concentrate and re-inject portions of material (De Geus *et al*. 1998, Focant *et al*. 2003, Kristenson *et al*. 2003 and Tranchida *et al*. 2011). This process occurs repeatedly, so that all the compounds from the sample are subjected to two distinct separations (Dimandja 2004 and Gaines & Frysinger 2004). In addition, the sensitivity of the GC×GC system is improved approximately tenfold relative to ^{1}D GC by the focusing effect of the modulator (Harvey & Shellie 2011, Korytár 2006, Mondello *et al*. 2008 and Semard *et al*. 2011). The modulation rate (or time) is the period of time the output of column one is stored before releasing it onto column two. During the second dimension run time (equal to the modulation period), the modulator reverts to trapping the next fraction eluting from the first dimension column. A modulation period that is longer than the retention time of any component on the second column is considered standard practice and the second column's dimensions are usually chosen so that component retention times are only a few seconds (Dorman et al 2008).

There are various modulator design types, occupying two distinct categories, namely thermal modulators and valve based and pneumatic (pulsed-flow) modulators (Harvey & Shellie 2011). A Longitudinally Modulating Cryogenic System (LMCS) was invented by Kinghorn & Marriott (1998) to improve the robustness of the technique, a feature that had not been available when using the rotating heated modulator as the mechanism for achieving comprehensive chromatography. Phillips *et al*. (1999) developed the first robust dual-stage thermal-desorption modulator. De Geus *et al*. (2000) describes a rotating thermal desorption modulator. Ledford Jr &

Billesbach (2000) describe a jet cooled thermal modulator, later commercialised by Zoex. Dimandja 2004 discusses cryogenic modulators, jet-pulsed modulators and dual stage modulators in some detail, as do Kristenson *et al*. 2003, Pursch *et al*. 2002 and Shellie *et al.* 2003. Tranchida *et al*. (2011) provides a comprehensive summary, detailing the various types of modulators developed since 1991 (Liu & Phillips 1991).

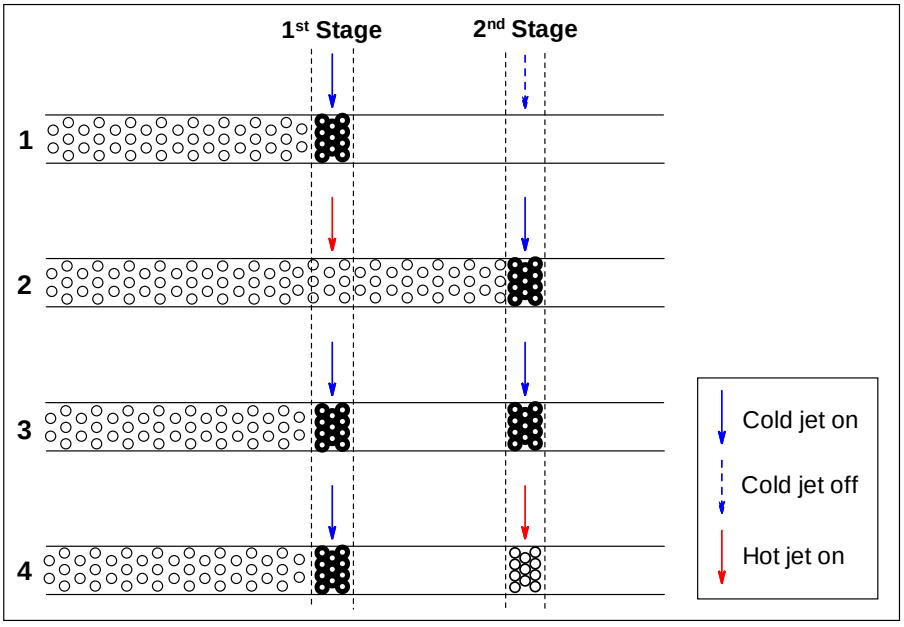


Figure 3.3. *A schematic of the quad-jet dual stage thermal modulator; overview of one modulation period (LECO 2011). Adapted courtesy of LECO Corporation.*

Cryogenically cooled quad-jet dual stage thermal modulation is very effective and is used with the LECO Pegasus® 4D GC×GC-TOFMS (Figures 3.1 and 3.3). The modulator consists of two sets of dual (cold and hot) jets that produce two distinct trapping stages at the beginning of the second column. The cold jets are generated by pulsed flows of nitrogen (cooled by passing through liquid nitrogen) and the hot jets by heated synthetic air, respectively. The eluent from the first column is transferred in slices (modulations) onto the shorter second dimension (^{2}D) column. An independently heated secondary oven is used for optimization of the second dimension separation. (Focant, Sjödin *et al*. 2004d, Harvey & Shellie 2011, LECO 2009a, LECO 2009b and Mustafa *et al*. 2012).

The cycle repeats continuously, sampling slices of the order of a peak width from the first dimension column effluent. The entire transfer process of one portion of material from the first column is known as one modulation period (P_M – the sampling duration of the first column effluent) and produces one ^{2}D slice. The focusing of the broader ^{1}D peak through modulation into narrower ^{2}D peak slices increases detection sensitivity. The sampling rate can be effectively described by the modulation ratio (M_R), which is defined as the ratio of four times the first column peak standard deviation divided by the modulation period (P_M) and is fully described by Khummueng *et al*. (2006), Harvey & Shellie (2011) and Marriott *et al*. 2012.

3.2.5 GC×GC Column Sets and Orthogonality

GC×GC column sets are obtained by coupling conventional GC columns, based on volatility, polarity, and shape selectivity (Dimandja 2004, Haglund *et al*. 2001 and Korytár 2006a). In the majority of all studies a non-polar stationary phase is used as the first dimension column providing separation of a large number of compounds and volatility is then the only parameter of interest; consequently, a boiling-point based separation is obtained. With all other column phases, separation will be primarily governed by the specific interaction(s) of the analyte(s) with the selected column phase. Shape selectivity plays a significant role in the separation of targeted analytes (Haglund *et al*. 2001 and Harynuk *et al* 2005). Because of the fast, almost isothermal second-dimension separation, there is no boiling-point contribution to the separation in that dimension (Adahchour *et al*. 2008).

If the separation process in one dimension is independent of the separation process in the second, then the system can be termed orthogonal (Korytár 2006a and Liu *et al.* 1995). True orthogonality in a two-dimensional separation may be achieved when elution times for each dimension can be treated as statistically independent (Ryan *et al*. 2005, Sinha *et al*. 2004 and Western & Marriott 2003). Ryan *et al*. (2005) further describes the theory and experimental parameters required to achieve true orthogonality and to utilise the chromatographic space. Dallüge *et al*. 2002b, Sinha *et al*. (2004), Skoczyńska *et al*. 2004 and Watson *et al.* (2007) describes the mathematical procedure to 'quantify' orthogonality, using equations that provide the basic premise for applying ^{2}D separations to obtain more information than from a

more traditional ^{1}D separation. An advantage provided by orthogonal GC×GC separations is that ordered continuous bands or groups now occur in most GC×GC chromatograms for structurally related homologues, congeners and isomers.

Not using an orthogonal approach to GC×GC can potentially lead to retention correlation across both dimensions reducing the maximum peak capacity and providing a multidimensional separation that is essentially a one-dimensional separation with peaks distributed along a diagonal. Venkatramani *et al.* 1996 approached orthogonality by combining techniques that utilise very different chemistries or mechanisms for separation and achieving orthogonal structure by varying or tuning the separation conditions of the second dimension as a function of the progress of the first dimension.

The concept of selecting the correct GC×GC column phases for separation of PCDD/Fs and DL-PCBs will be discussed in the section dealing with targeted analysis. Haglund *et al*. (2001), Korytár (2006), Mustafa *et al* 2012 and Ong *et al.* (2002) have demonstrated the use of shape-selective columns as primary columns for the enhanced separation of halogenated congeners such as PCB mixtures. This aspect, along with orthogonality considerations will be applied to PCDD/F separation and analysis.

3.3 GC×GC TOFMS

Mass spectrometry (MS) is a powerful tool for both qualitative and quantitative analysis. It relies on the formation of gas-phase positively (or negatively) charged ions that can be separated electrically (or magnetically) based on their mass-to-charge ratio (m/z) providing important analyte structural information, including purity and composition (El Aneed *et al*. 2009, Burgoyne & Hieftje 1997, Mühlberger *et al*. 2004 and Volmer & Sleno 2005).

Time-of-flight analysers (TOFs) were first described in the 1950s, but only in the 1990s were they were utilised in commercial instruments. TOF relies on the movement of ionised molecules in a field free flight tube from source to detector. The velocity can be directly correlated to the mass-to-charge ratio (m/z) of the ions so that their arrival time at the detector is dependent on their masses. TOF has the

advantage of being able to detect ions of very high mass. Resolution is dependent on ions entering the flight tube having identical kinetic energies. This requirement has been met in the first LECO Pegasus® GC×GC-TOFMS instruments (May 2000, LECO Corporation, St Joseph, USA) by the use of the *'reflectron'* (electrostatic ion mirror); an ion optic device that modifies the path of the ions (El Aneed *et al*. 2009, LECO 2009b and LECO 2011).

As a result of the modulation process, analyte bands elute as very sharp peaks into the second column and very narrow peaks ranging from 50 to 500 milliseconds (ms) wide are produced. These narrow peak widths require a detection system that is capable of collecting data at rates of 100 Hz or more in order to adequately characterize the shape of the chromatographic peak. TOFMS, with continuous full-range mass spectral acquisition rates up to 500 spectra/ s, is the only MS fast enough to provide the data density necessary to address the requirements of GC×GC separation for proper reconstruction of the chromatogram (very fast second dimension peaks) and to identify unknown and/ or target analytes whenever complex samples require analysis and quantitation (Adahchour *et al*. 2006b Dallüge *et al*. 2002b, Dimandja 2004, Dorman *et al.* 2008a, Focant *et al*. 2006a, Mondello *et al.* 2008 and Zrostĺíková *et al.* 2003).

3.3.1 Detection, Data Processing and Reporting

TOFMS spectra are generated by the summation of single snapshots of the contents of the ion source (they are not recorded by a scanning mass analyser) and are therefore not 'skewed' by changes in concentration in time, as for example during the GC elution of a compound. This means that the relative intensities of the ions in the mass spectra across a chromatographic peak are constant. This allows for better implementation of data-processing algorithms such as deconvolution that can be used to mathematically separate co-eluting peaks in samples for which 'chromatographic' separations are incomplete (Peters *et al.* 2013). The mathematical algorithms that describe GC are well known, and are easy to apply in the one-dimensional case. Although the theory remains the same for GC×GC, optimisation and translation are more difficult for GC×GC than ordinary 1D-GC irrespective of the detector used (vacuum TOF or FID; Dorman *et al.* 2008a).

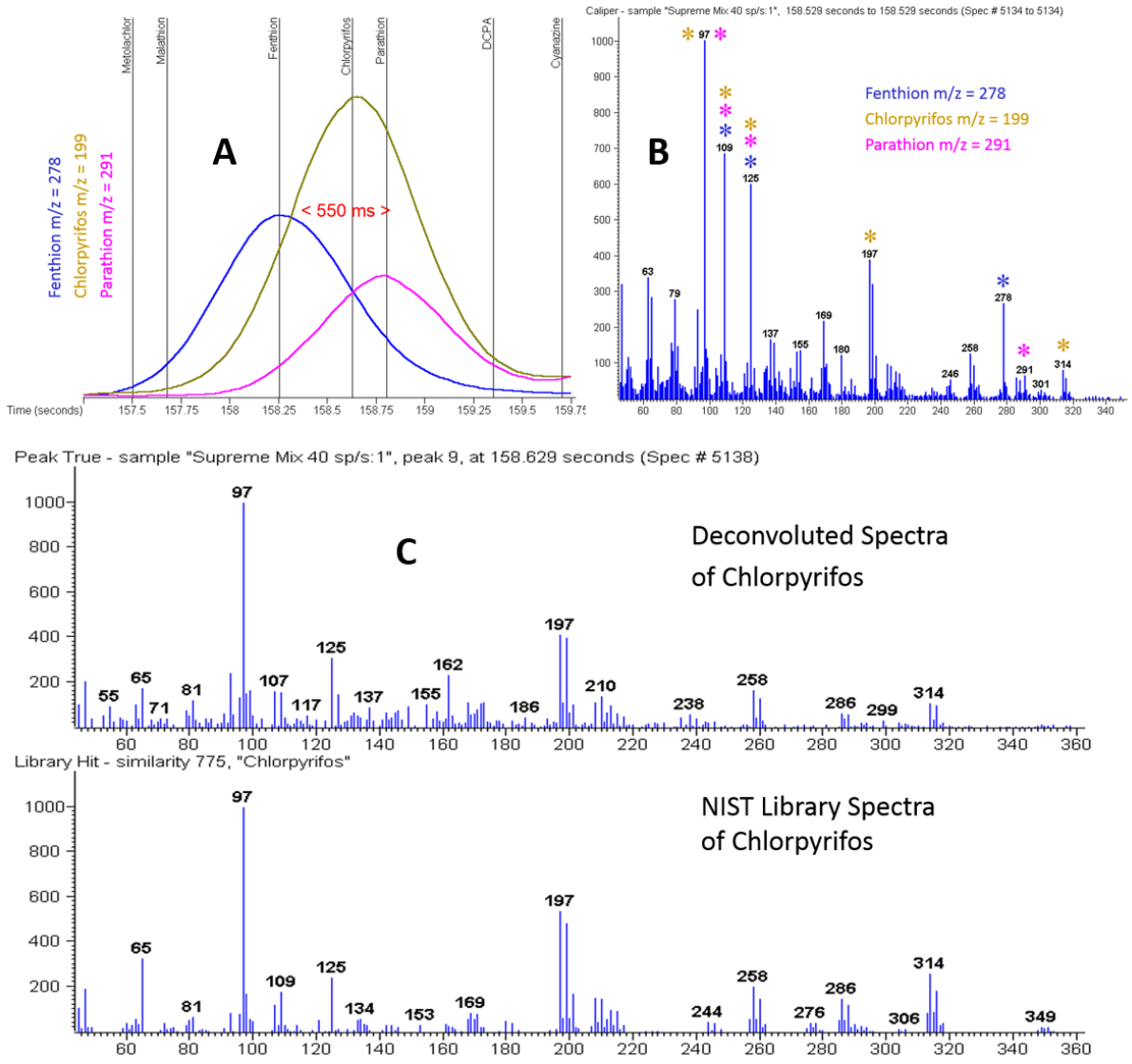


Figure 3.4. *Schematic to demonstrate spectral deconvolution:* ***A*** *denotes three co-eluting pesticides and each coloured trace represents a unique fragment ion.* ***B*** *gives the mixed/ co-eluted mass spectrum for chlorpyrifos at* $^{1}t_R$ *= 158,529 and* ***C*** *gives the unique mass spectrum for chlorpyrifos as identified by the MS deconvolution software. Mass spectra courtesy of LECO Corporation.*

An important characteristic of the LECO Pegasus® GC×GC-TOFMS instrument is the ability to deconvolute unresolved peaks on the basis of mass spectral differences; a 10 ms (or 5 spectra) retention time difference is sufficient for the deconvolution algorithm to locate and identify co-eluting analytes based on the constancy of ion ratios across a GC peak, thereby overcoming insufficient chromatographic

separation (Adahchour *et al.* 2008). In other words, spectral deconvolution is possible because TOFMS achieves high-speed full-spectrum acquisition, without mass spectral skewing (Mondello *et al.* 2008).

An example of what can be achieved with modern deconvolution software is shown in Figure 3.4 for three closely eluting pesticides, namely fenthion, chlorpyrifos and parathion (data collected at 40 spectra/ s). The unique fragment ions plotted for each pesticide using the LECO Corporation ChromaTOF® '*peak find*' software feature shows the peak apices in a portion of the chromatogram which is only 550 ms wide (A). The unprocessed mass spectrum at 158.629 s is shown in (B). This is a mixed, or co-eluted mass spectrum. The colored asterisks (***) show some of the co-eluting m/z ions (e.g. m/z = 97, 109, 125). Trying to library search a spectrum like this generates poor matches, with ambiguous results. Deconvolution (C) eliminates the overlap of shared masses and library searching the deconvoluted mass spectrum confirms the NIST library match for chlorpyrifos. This is an extremely powerful method of resolving overlapping peaks into their individual components.

Data files acquired by TOFMS at high acquisition rates are large, thus making detection and data presentation complex and time-consuming. Powerful software is required to handle large amounts of generated data. The new-generation LECO ChromaTOF® software allows direct presentation of total ion current (TIC) and extracted ion chromatogram ^{2}D plots without the need for external data conversion and presentation software (Adahchour *et al.* 2008 and Dallüge *et al.* 2002a).

GC×GC-TOFMS fulfils the requirements for unparalleled selectivity (three separation dimensions, related to volatility, polarity, and mass), high sensitivity (through band compression), enhanced separation power, and increased speed (comparable to ultra-fast GC experiments, if the number of peaks resolved per unit of time is considered; Mondello *et al.* 2008). It is uniquely suited to detecting all or most of the compounds (sample preparation method dependant) in complex samples at or near the levels needed for priority pollutant determination in a single analysis (US EPA 1994a). It can provide rapid and comprehensive identification of compounds present that are harmful to human and animal health and can be used to obtain quantitative

data for the seventeen toxic PCDD/Fs and dioxin-like PCBs (Focant *et al*. 2004b and Hoh *et al*. 2007).

To address the need to have a dioxin capability in South Africa and to be able to analyse real world samples for the presence of PCDD/Fs at ultra-trace levels amidst challenging high levels of other more prevalent contaminants, the selectivity needed to be improved through different column combinations. To investigate and understand the limit of detection (instrument and method) of the GC×GC-TOFMS and to understand the levels that can be reliably quantified in the presence of interferences is important. This will require developing and optimising sample preparation techniques to eliminate matrix interference as far as possible. To assess the accuracy of the GC×GC-TOFMS method, it is also necessary to compare results obtained with those already confirmed using GC-HRMS. Finally, it needs to be shown that the individual congeners can be separated and identified for accurate validation of the method with associated uncertainty of measurement.

3.4 TARGET COMPOUND ANALYSIS USING GC×GC-TOFMS

Over the years POPs have been widely analysed by conventional GC techniques, generally with selective detection and/or MS detection. The analysis of PCDD/Fs, PCBs and other related persistent organic pollutants is analytically challenging and the potential of GC×GC, specifically when combined with TOFMS detection, to unravel the composition of a variety of complicated samples provides an analytical solution well suited to target compound analysis (Adahchour *et al*. 2008, Dallüge *et al*. 2002a and Reiner 2010). Among these toxic analytes, the separation of the 209 PCB congeners has proven to be a very challenging task. PCBs can be classified into ten homologue groups on the basis of their chlorine content and, due to their similar chemical-physical properties, the analytical difficulties encountered are not surprising (Mondello *et al.* 2008). PCDD/Fs are monitored in the environment because of the toxicity of congeners having chlorine substitution at positions 2,3,7 and 8 (Van den Berg *et al*. 1998). US EPA Methods 1613B and 8290A define parameters to accurately quantify the seventeen toxic PCDD/F congeners by separating them from the 136 total possible congeners with at least 4-chlorine substitution (US EPA 1994a and 2007d). In order to achieve separation

requirements, these methods generally require an initial analysis using a 5% diphenyl/ 95% dimethyl polysiloxane stationary phase for the primary analysis with a further confirmatory analysis using a stationary phase that has been shown to separate these congeners from the less toxic congeners (Dorman *et al.* 2004 and Reiner 2010).

As part of his Doctoral thesis, Korytár (2006) examined many column combinations used to address co-elutions of environmentally occurring non-2,3,7,8-substituted congeners with the toxic congeners. Dorman *et al.* 2004 have addressed the potential need for new stationary phases, namely a primary analysis column that has improved separation and higher thermal stability, and a confirmation column that has improved lifetime and thermal stability. Restek has developed the Rtx®-Dioxin2 capillary column with a proprietary stationary phase that has a thermal stability in excess of 340 ºC. The Rtx®-Dioxin2 capillary column has been demonstrated to separate all seventeen toxic PCDD/F congeners from each other, and from all of the environmentally significant congeners found in the sample extracts (Cochran *et al*. 2010 and 2012).

GC×GC with micro electron-capture detection (μECD) has been optimised for the separation of PCB congeners with emphasis on the separation of 12 toxic non- and mono-*ortho* chlorinated biphenyls (CBs), namely, CBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189 (Korytár *et al*. 2002). A complete separation of all twelve priority substituted CBs was obtained with ordered structures for the *ortho* and non-*ortho* substituted PCBs thus assisting with congener identification. Focant, Sjödin *et al*. (2004b) improved the separation of the 209 PCB congeners using GC×GC-TOFMS and four thermally stable column combinations. Similarly, Korytár *et al*. (2004) separated the seventeen 2,3,7,8-substituted PCDD/Fs and twelve dioxin-like PCBs using GC×GC coupled with electron-capture detection (μECD) and GC×GC-TOFMS (Korytár 2006). Chromatographic conditions, in particular the elution temperature of analytes and the average gas velocity, influence the elution order of POP analytes in the first and second dimensions. Careful consideration of the operating conditions and understanding column dimensions can lead to a better understanding of GC separation of these toxic congeners (Ong *et al*. 2002).

3.4.1 Analysis of PCDD/F using GC×GC-TOFMS Principles

Focant *et al*. (2004b) used isotope-dilution (ID) TOFMS to separate the seventeen priority PCDD/Fs and the four non-*ortho* PCBs using an Rtx®-Dioxin2/ Rtx®-500 column combination. The 60 m x 0,25 mm x 0,25 µm ^{1}D Rtx®-Dioxin2 column was selected for its good resolution of PCDD/Fs and its high thermal stability. The Rtx®-500 (2 m x 0,18 mm x 0,10 µm) phase provided a clean separation of the target analytes from the bulk of the matrix interferences and from each other in 35 minutes. This separation was required to separate the target analytes from biogenic material having similar masses. GC×GC yielded a baseline separation of all target analytes except the 2,3,7,8-TCDD/ PCB-126 pair.

Hoh *et al*. (2007) optimized GC×GC–TOFMS conditions for the analysis of the seventeen priority PCDD/Fs in the presence of potentially interfering co-planar dioxin-like PCBs using a Rtx®-Dioxin2 (60 m x 0,25 mm x 0,25 µm) and Rtx®-PCB (2 m x 0,18 mm x 0,18 µm) column set. Under optimum conditions, all PCDD/Fs could be separated in less than 60 minutes. Method parameters for modulation time, maximum recommended temperature and more efficient ionization to ensure in-phase (by adjusting the modulation period, column heating rates and gas flow rates to elute peaks in a single slice to ensure maximum sensitivity) second-dimension slice distribution for TCDD are detailed in the publication.

Boden & Reiner (2005) have developed a GC×GC-TOFMS method for the simultaneous determination of substituted PAH compounds, PCBs, organochlorine pesticides and PCDD/Fs in environmental matrices. The best overall separation was achieved using a combination of a DB17-HT ^{1}D column (30 m x 0,25 mm x 0,15 µm) coupled with an Rtx®-PCB ^{2}D column (2 m x 0,18 mm x 0,18 µm). This 40 minute 'mega method' can be used as a screening method for target compounds, effectively reducing the sample preparation time and the number of instruments and analytical runs required.

There are many interesting applications cited in the literature that detail various optimised methods and column combinations for PCDD/F separation by GC×GC-TOFMS, (Adahchour *et al*. 2006c, Cochran 2002, Cochran *et al*. 2004, Cochran *et al*. 2007a, Cochran *et al*. 2007b, Dallüge *et al*. 2002a, Danielsson *et al*. 2005, Dasgupta

et al. 2010, Dorman *et al.* 2004, Dorman *et al.* 2008a, Dorman *et al.* 2008b, Focant *et al*. 2003, Focant *et al*. 2004a, Focant *et al*. 2004b, Focant, Pirard *et al*. 2004a, Focant, Pirard *et al*. 2004b, Focant, Sjödin *et al*. 2004a, Focant, Sjödin *et al*. 2004b, Focant, Sjödin *et al*. 2004c, Focant, Sjödin *et al*. 2004d, Focant, Eppe *et al*. 2005a, Focant, Eppe *et al*. 2005b, Focant, Pirard *et al*. 2005a, Focant *et al*. 2006a, Focant *et al*. 2006b, Focant 2008, Focant 2014, Haglund *et al*. 2001, Harju *et al*. 2003a, Harju *et al*. 2003b, Hilton *et al*. 2010, Hoh *et al*. 2007, Hoh *et al*. 2008, Klee & Blumberg 2010, Klee *et al*. 2015, Korytár *et al*. 2002, Korytár *et al*. 2003, Korytár *et al*. 2004, Korytár *et al*. 2005, Korytár, Parera *et al*. 2005a, Korytár, Parera *et al*. 2005b, Korytár 2006, Korytár *et al*. 2006, Kristenson *et al*. 2005, Liu *et al.* 1995, Marriott & Shellie 2002, Marriott *et al*. 2003, Marriott *et al*. 2004, Mondello *et al*. 2007, Mondello *et al*. 2008, Mühlberger *et al*. 2004, Ong *et al.* 2002, Ong & Marriott 2002, Pascal *et al*. 2007, Patil *et al*. 2009, Peroni *et al*. 2013, Reiner *et al*. 2004, Reiner *et al*. 2006, Reiner 2010, Ryan & Marriott 2003, Ryan *et al*. 2005, Semard *et al*. 2009, Shellie *et al*. 2002, Shellie *et al*. 2003, Shunji *et al*. 2008, Sinha *et al*. 2004, Skoczyńska *et al*. 2004, Tranchida *et al*. 2007, Tranchida *et al*. 2011, Van der Lee *et al*. 2008, Van Leeuwen & de Boer 2008, Van Mispelaar *et al*. 2003, Van Stee & Brinkman 2011, Venkatramani *et al.* 1996, Vogt *et al.* 2007, Welthagen *et al.* 2003, Western & Marriott 2003, Zeng *et al.* 2012, Zeng *et al.* 2014, Zhu *et al.* 2006 and Zrostľíková *et al.* 2003, etc), but the work done by Boden & Reiner (2005), Dorman *et al.* (2004) Focant *et al*. (2004b) and Hoh *et al*. (2007) have formed the basis of the initial method development work for the analysis of the seventeen toxic PCDD/F congeners and twelve dioxin-like PCBs at NMISA.

3.4.2 PCDD/F and Dioxin-like PCB Applications

The application of various methods, mosty using GC-HRMS and GC×GC-TOFMS, for the determination of the levels and congener distribution of dioxin like compounds in various matrices is of interest. A few are briefly mentioned, as not every method can be captured in detail. Van Leeuwen & de Boer (2008) have assessed pollutants in the aquatic environment, while De Boer *et al*. (1993) have analysed non-ortho and mono-ortho substituted PCBs and PCDD/Fs in marine and freshwater fish and shellfish from the Netherlands. Corsolini *et al*. (2005) have looked at POPs in edible fish and Fayez *et al*. (2005) have compared manual and automated extraction and clean-up of PCDD/Fs and dioxin-like PCBs in fish tissue. Lo *et al*. (2005) have performed preliminary studies of immunoassay for PCCD/Fs in dietary fish and Lott & Baker (1993) did a comparison of a matrix solid phase dispersion and classical

extraction method for the determination of chlorinated pesticides in fish muscle. Hasegawa *et al*. (2007) have determined PCDD/Fs and dioxin-like PCBs in fish oils for feed ingredients by congener-specific chemical analysis and CALUX bioassay, while Hoh *et al*. (2008) evaluated automated direct sample introduction for the screening analysis of PCDD/Fs in fish oil. Lindström *et al*. 2002 have compared world-wide analytical data for PCDD/Fs and non-ortho PCBs in samples of chicken, butter and salmon. Some interesting studies to determine the levels of co-planar polybrominated-chlorinated biphenyls of fish from the Great Lakes in Canada was undertaken by Alaee *et al*. (2008) and Geeraerts *et al*. (2008 and 2010) have determined levels of PCDD/Fs and dioxin-like PCBs in Belgian river eel specimens. Harju *et al*. (2003b) have investigated atropisomeric and planar PCBs and their enantiomeric fractions and tissue distribution in grey seals.

Pesticide residues in food, food extracts, grapes, wine and fruit has been very comprehesively studied (Dallüge *et al*. 2002a, Dasgupta *et al*. 2010, Pascal *et al*. 2007 and Zrostĺíková *et al.* 2003). Trace analysis of PCDD/Fs in food and foodstuffs and assessing food, diet and dietary intake has been investigated (Dybing *et al*. 2002, Esposito *et al.* 2010, Focant *et al*. 2002a, Focant, Pirard *et al*. 2004b and Focant, Eppe *et al*. 2005a). Becher *et al*. (2004) undertook a world-wide comparison on the quality of analytical determinations of PCDD/Fs and dioxin-like PCBs in food and Malavia *et al*. (2007) analysed PCDD/Fs in vegetable oil samples. Fish oil from herring, spiked cows' milk, vegetable oil and an eel extract were analysed by Danielsson *et al*. (2005). Pirard *et al*. (2003 and 2004a) developed a new strategy for the analysis of polybrominated diphenyl ethers, PCDD/Fs and PCBs in beef, soil and eggs, while Sandra & David 2002 assessed PCBs and fatty acid methyl esters in food samples (chicken, pork fat, eggs) following the Belgian dioxin crisis, while Bernard *et al*. 2002 evaluated the health risks associated with the contamination of the food chain and Windal *et al.* (2010) assessed the dietary intake of PCDD/Fs and dioxin-like PCBs of the Belgian population.

PCBs in soils, sediments, sludge and milk have been well documented (Backe *et al*. 2004 and Rogowski & Yake 2005). Batterman *et al* (2009) undertook a study to quantify PCBs in air, soil and milk in industrialised and urban areas of KwaZulu

Natal, South Africa, and Nieuwoudt *et al*. (2009) focused on dioxin-like chemicals in soil and sediment from residential and industrial areas in central South Africa.

As early as 1973, Baughman & Meselson and Crummett & Stehl have developed analytical methods for detecting TCDD levels in samples and materials. PCDD/Fs have since then been analysed in water, fertilizers, soils, soil conditioners and sewage sludge and marine sediment in various countries, but not in South Africa (Baugros *et al*. 2008, Bonn 1998, Bordajandi *et al*. 2008, Cochran *et al*. 2004, Dumortier *et al.* 2011, Kristenson *et al*. 2005, Konieczka & Namieśnik 2008, Naile *et al*. 2011 and Pehlivan & Pehlivan 2001).

Hilton *et al*. (2010) have looked at a method for rapid, non-targeted screening for environmental contaminants in household dust. Jordaan *et al*. (2007) have examined the contribution of dioxin-like compounds from platinum mining and processing samples in the South African context. Korytár *et al*. (2006) have analysed micro-contaminants and Moon *et al*. (2005) have studied atmospheric deposition of PCDD/Fs in urban and suburban areas of Korea.

Open burning of household waste, waste processing plants and municipal solid waste from developing countries and incinerators contribute numerous PCDD/Fs contaminants into the environment, potentially impacting on human health (Domingo *et al*. 2001, Eppe *et al.* 2008b, Lonati *et al*. 2007, Lundin *et al*. 2013, Solorzano-Ochoa *et al*. 2012, Zeghnoun *et al.* 2007a and Zhang *et al.* 2011). This has led to studies to determine PCDD/F and dioxin-like PCB levels and congener distribution in serum, human milk and biological matrices (Croes *et al*. 2013, Eppe *et al.* 2007, Eppe *et al.* 2008a Eppe & De Pauw 2006, Fréry *et al*. 2007a, Fréry *et al*. 2007b, Fierens 2006, Focant, Sjödin *et al*. 2004a, Focant, Sjödin *et al*. 2004c, Misselwitz *et al*. 2011, Pirard *et al*. 2004b, Vandentorren *et al.* 2011 and Zeghnoun *et al.* 2007b).

The brief description provided for the development of GC, leading to the development of multi-dimensional and comprehensive two-dimensional capillary gas chromatography, has outlined the need for the application of GC×GC-TOFMS in the analysis of PCDD/Fs and dioxin-like compounds. The numerous papers by experts in their respective fields support this view, with the quantification of real world samples being a significant challenge to analytical scientists monitoring POPs.

NMISA has acquired analytical equipment to be used for the separation, detection and quantitation of POP compounds by GC×GC-TOFMS. Establishing such a capability will minimise the costs associated with sending samples for analyses overseas, and curtail the analysis of negative samples. This was a significant consideration in the development and optimisation of methods for ultra-trace target analysis of PCDD/Fs at NMISA. Only positive samples will then be sent for quantitative analysis using GC-HRMS, if required.

3.4.3 Analytical Benchmarking in South Africa using GC×GC-TOFMS

National Metrology Institutes (NMIs) generally do not use nominal mass techniques, such as GC×GC-TOFMS, for traceable analytical results. NMISA has acquired this instrumentation and has investigated this analytical approach for measurement accuracy. During 2005 initial work was started for the quantification of polycyclic aromatic hydrocarbons and chlorinated pesticides using GC-TOFMS. NMISA participated in two CCQM pilot studies (not the key comparisons) for selected PAHs and chlorinated pesticides in calibration solutions. The PAH samples were analysed and quantified using a GC-TOFMS system (LECO Pegasus® III GC-TOFMS). These were the first results reported by an NMI for measurement equivalence capability using TOFMS. The results for the chlorinated pesticides in solution were analysed and quantified using a classic quadrupole system (Agilent GC-MSD and GC-μECD). The pesticide work was then repeated using the LECO Pegasus® 4D GC×GC-TOFMS and these results were discussed at the '*Analitika 2006*' conference held in South Africa.

A GC×GC-TOFMS method was developed as it was anticipated that a freeze-dried mussel tissue sample would be extracted and analysed for chlorinated pesticides using NIST SRM 2261 (chlorinated pesticides in hexane) as the traceable calibrant. This was the first attempt at extracting a complex matrix without access to proper extraction capability. It was one of the reasons why NMISA only submitted results for the calibration solutions and not for the homogenized freeze-dried mussel tissue extract.

Detector linearity and response were assessed between the classical quadrupole mass selective detector and the TOFMS detector. Repeatability and relative

standard deviation of repeat injections of standards and real samples for target analytes in solution by GC-TOFMS was undertaken. Lindane, p,p'-DDE and p,p'-DDT were selected as the target analytes in the CCQM study. The repeatability study using GC-TOFMS produced a relative standard deviation (%RSD) of less than 1 % for 1 µg/ ml at 1750V. For 20 ng/ ml at 1750V, the %RSD was less acceptable (mean %RSD = 8.9). The uncertainty then becomes too large and therefore the detector voltage was increased from 1750V to 1950V. This improved the signal to noise (S/N) ratio and improved the area calculation that brought the % RSD to within more acceptable levels (mean %RSD = 2.8).

The GC×GC-TOFMS instrumental conditions are captured in Table 3.1. The analysis could have been conducted using only the GC-TOFMS and directly compared with the GC-MSD results. Sample extraction and clean-up for POP analytes from complex matrices is not mature at NMISA and GC×GC provides improved separation of target analytes from matrix and non-target analyte interferences since samples are not always conveniently simple calibration solutions with little to no matrix interferences.

Table 3.1. *GC×GC-TOFMS conditions for the chlorinated pesticide study*

Instrument	LECO Pegasus 4D GC×GC-TOFMS
^{1}D column	Rtx®-5ms *low polarity phase* (30m x 0,25mm x 0,25 µm)
^{2}D column	Rtx®-200 *mid-polarity phase* (2m x 0,18 mm x 0,20 µm)
^{1}D Temperature program	100 ºC for 1 min, 10 °C/ min to 280°C, hold 1 min
^{2}D Temperature program	105 °C for 1 min, 8 °C/ min to 285 °C, hold 1 min
Helium flow rate	1 ml/ min
Injector temperature	Agilent 6890N S/SL injector @ 250 ºC
Injector mode	1 µl injected splitless
Modulator temperature offset	+ 5°C (the modulator temperature follows the primary oven)
Modulation period	6 s
Acquisition rate & mass range	100 spectra/ s (35 – 450 u)
Transfer line temperature	250 ºC
Ion source temperature	200 ºC
Detector voltage	1950 V

The GC×GC-TOFMS results for the chlorinated pesticide study compared well with the gravimetric value, although biased slightly high for the p,p'-DDT and p,p-DDE. The submitted values for the GC-MSD results were biased low, but were still in good agreement with the CCQM key comparison reference value (KCRV) for the '*CCQM-K39/ P31c.1: Determination of chlorinated pesticides in solution'* study as reflected in Table 3.2.

Table 3.2. *Results for the chlorinated pesticides with associated uncertainties for the GC-MSD, GC×GC-TOFMS, gravimetric mean for the sample and the CCQM KCRV (consensus value of all NMIs participating in the key comparison).*

	GC-MSD (ng/ g)	GC×GC-TOFMS (ng/ g)	CCQM-P31c.1 (ng/ g) (gravimetric mean)	CCQM-K39 KCRV value (ng/ g)
Lindane	33 ± 0.5	34.6 ± 0.6	33.7 ± 0.6	33.9 ± 0.9
p,p'-DDE	306 ± 1.6	354 ± 15.8	318.7 ± 2.4	318.0 ± 5.4
p,p'-DDT	54 ± 0.5	61.1 ± 2.8	56.8 ± 0.8	56.8 ± 1.7

These were the first NMISA results submitted as participants in an international pilot study for chlorinated compounds using GC-MSD. The repeat analysis using GC×GC-TOFMS clearly indicated that in order to obtain precise and accurate results to be included in the consensus value, the methods will require further development and optimisation, improved accuracy and investigation of associated uncertainties. This exercise formed the basis for further development of the GC×GC-TOFMS technique for traceable and accurate analysis of PCBs, PCDD/Fs and polybrominated diphenyl ethers (PBDEs).

Sample preparation and clean-up forms an integral part of any analysis and if not approached with the necessary care and understanding, can lead to major uncertainties in the quantitation of analytical results. It is challenging and requires a lot of patience and expertise with sample matrix extraction. This will be examined in Chapter 5.

4

SOUTH AFRICAN APPROACH TO DIOXIN ANALYSIS

NMISA, in collaboration with the Persistent Organic Pollutant and Toxicant Research Group (POPT), School of Biological Sciences, Zoology, North-West University (NWU), Potchefstroom, South Africa, has been implementing methods to screen for various classes of POPs in South Africa. To address these challenges, the approach taken has been to actively investigate alternative techniques to the accepted GC-HRMS methods used for the analysis of PCDD/Fs (De Vos *et al.* 2011a, US EPA 1994a, Reiner 2010 and Stockholm Convention 2010).

One approach is the use of bio-analytical techniques based on *in vitro* transactivation assays with whole H4IIE-*luc* cells for aryl hydrocarbon receptor active compounds. Studies by Safe *et al*. (1986) have demonstrated rank-order correlations between biochemical measurements in H4IIE cells (e.g., enzyme induction, receptor binding) and whole-animal effects, suggesting that cell culture systems could be used as a rapid, inexpensive tool to estimate the toxic potency of dioxins and their mixtures (Whyte *et al*. 2004).

By screening a large number of samples from a site, the most contaminated locations are identified. Samples with a bio-assay equivalent (BEQ) above a pre-determined level were then sent abroad for extraction and analysis by GC-HRMS for congener-specific quantification. The bio-assay is a non-selective assay; any compound that can bind to the aryl hydrocarbon receptor (AhR) receptor will elicit a positive response for dioxin-like activity, so the criteria for selection was based on the concentration of dioxin-like chemicals determined by comparing a sample signal to that of the positive

control, 2,3,7,8-TCDD, and is reported as BEQ_{20}; the values were calculated at the point equivalent to a 20% response elicited for 2,3,7,8-TCDD. This criteria was selected as none of the samples analysed elicited a response above 50% (Giesy *et al*. 2002, Hoogenboom *et al*. 2006 and Vromman *et al.* 2012).

Sending samples abroad for analysis led to a 'black-box' effect where analytical problems, such as low recoveries, went unnoticed. During 2008, an ecological emergency was experienced that involved sudden mass crocodile mortalities (Bouwman *et al*. 2014 and Huchzermeyer *et al*. 2013), threatening one of the largest naturally occurring Nile crocodile populations in Southern Africa. The laboratory data from the first mass deaths were only received as the second seasonal *inc*idence in 2009 occurred, nearly derailing the project from an analytical stand point.

This highlighted the need for locally available analytical capability independent of overseas facilities, and led to the collaboration between NMISA and NWU for GC×GC-TOFMS analytical confirmation of results obtained by bio-assay. The bio-assay work discussed is not directly related to the analytical work covered in this thesis, but is important to mention as this two-step process saves money and improves the accuracy, reliability, and scientific basis of quantitative assessment of environmental health risks, proving to be a practical and viable method for quantitation of PCDD/Fs in South Africa (De Vos *et al*. 2013a).

4.1 BIOANALYTICAL (BIO-ASSAY) APPROACH

Bio-analytical techniques, including reporter gene bio-assays, are based on a measurable phenotypic response, distinguishable from a specific gene 'output', to the presence of a chemical group (Naylor 1999 and Vromman *et al.* 2012). The toxicity of dioxin-like chemicals, including PCBs and PCDD/Fs, is mediated through the AhR. These chemicals bind to the AhR and elicit a biochemical and toxic response (Behnisch *et al*. 2001, Bock & Köhle 2005, Giesy *et al*. 2002, Janošek *et al*. 2006, Mandal 2005 and Rivera *et al*. 2002).

The chemical activated luciferase gene expression (CALUX) bio-assay is a screening method for dioxins and dioxin-like PCBs in food and feed, and the only assay accepted in routine monitoring programs (Hoogenboom *et al*. 2006, Whyte *et al*. 2004). This

reporter gene bio-assay uses genetically-modified rat or mouse hepatoma cells. These cells are stably transfected with a firefly luciferase gene that is under transcriptional control of the dioxin responsive element (DRE). When an AhR ligand binds to the receptor, transcription of the reporter gene, luciferase (*luc*), is initiated (Giesy *et al*. 2002 and Hilscherova *et al*., 2000). A luminescent signal proportional to AhR-active compounds in the sample is produced once luciferin, salts and ATP are added. The concentration of dioxin-like chemicals is determined by comparing its signal to that of the positive control, 2,3,7,8-TCDD, and is reported as bio-assay equivalents (Hilscherova *et al*. 2000, Sanctorum *et al*. 2007 and Schwirzer *et al*. 1998).

During the bio-analytical approach information is obtained on the overall potency specific to the class of compounds of interest as any compound with the same mode of action is assessed by the bio-assay and therefore provides little to no information on the concentrations of individual compounds responsible for the effects. Therefore, subsequent instrumental analysis must be conducted to determine the causative agents and in so doing identify possible sources of toxicants (Nieuwoudt *et al*. 2009). When combined with instrumental analysis it is an ideal research tool to determine possible toxicological impacts of novel contaminants as well as contaminant mixtures present in environmental samples.

4.2 ANALYTICAL MEASUREMENT APPROACH

NMISA is currently not a reference laboratory equipped for the analysis of all POP compounds, but it is vital to establish the methods required to become such a reference laboratory for POP analyses. ID-HRMS capability may become necessary if South Africa, as a signatory to the Stockholm Convention, is to comply with the high level analytical capability needed for these types of analyses (Stockholm Convention 2010).

While GC-HRMS was initially the only available technique that could reach the low levels required by US EPA Method 1613B, the advancement of instrument technology has since given rise to alternative approaches. GC×GC-TOFMS, although not a high resolution technique, can be used as a screening tool for the analysis of POPs, including PCDD/Fs compounds (Cochran 2002, Focant *et al*. 2004b, Focant, Sjödin *et*

al. 2004b and Focant, Sjödin *et al*. 2004d) and as such, NMISA has implemented method development utilising this technique.

4.3 COMBINED APPROACHES

A number of studies have reported combined approaches to POP analyses including bio-assay techniques and these are reveiwed in this section.

Houtman *et al*. (2006) have identified various PAH compounds in sediment by GC× GC-TOFMS, using a bio-assay directed fractionation approach with the '*in vitro*' estrogen and dioxin responsive reporter gene assays and DR-CALUX. Focant *et al.* (2001a) have investigated a simple and time-effective analytical strategy for monitoring PCDD/Fs using quadrupole storage tandem-in-time mass spectrometry (QISTMS), HRMS and polyclonal antibody immunoassay.

The integration of PCBs into the EU regulations has helped the DR-CALUX assay become the screening method of choice for global toxicity evaluation at a moderate cost. Both GC×GC-ID-TOFMS and GC–ID-QIST-MS/MS have proved to be complementary alternative methods to GC–ID-HRMS that are capable of describing PCB and PCDD/F congener profiles with reasonable precision when source identification is required for contamination tracking (Focant, Eppe *et al*. 2005b and Lo *et al*. 2005).

Combining two screening techniques was thus considered an appropriate solution: the H4IIE-*luc* bio-assay and GC×GC-TOFMS. The former is fast, user-friendly and inexpensive; the latter is unique in its ability to detect all compounds in complex samples at the levels needed for priority pollutant determination in a single analysis. It can provide quantitative data and is capable of reaching the low levels stipulated in US EPA Method 1613B for PCDD/F analysis (US EPA 1994a). This study is the most comprehensive dioxin environmental screening and determination to date in South Africa.

4.3.1 Experimental

4.3.1.1 Extraction and clean-up procedures for the H4IIE-luc bio-assay and GC×GC-TOFMS analysis

Soil and sediment samples were collected from diverse regions of South Africa to incorporate various land-uses and anthropogenic impacts, theoretically representing a spectrum of PCDD/F concentrations. Sediment was collected from major rivers (Vaal River, including tributaries) throughout South Africa, while soil was collected mainly from industrialised regions (Vaal triangle area, central South Africa), and also from agricultural and less-developed (non-industrialised) areas. The main aim of the study was to perform a more comprehensive investigation of dioxin-like persistent organic pollution in the Vaal Triangle area. The Water Research Commission report by Roos *et al.* (2011) details the extensive investigation, number and location of samples taken and the outcomes of the study, excluding the PCDD/F results. The PCDD/Fs were not originally targeted for analysis and form the basis for the collaborative study with NMISA.

Calibration and verification standards, as stipulated in US EPA Method 1613B (1994a), were obtained from Wellington Laboratories *Inc.,* USA, and included calibration and verification solutions (EPA-1613CVS), labelled calibration solutions (EPA-1613LCS), internal standard spiking solution (EPA-1613ISS) and clean-up standard stock solution (EPA-1613CSS). Twenty seven soil (including sample blank) and twenty nine sediment (including blank) were selected for PCDD/F analysis. The soil and sediment samples were extracted and underwent clean-up procedures using US EPA Methods 3545A, 3620C, 3640A and 3660B (US EPA 1994a, 2007a, 2007b and 1996d) for instrumental and biological analysis. Extraction and clean-up procedures for chemical and biological analysis were the same with the exception of the use of internal standards (IS). When using bio-assays, an IS cannot be used since the bio-assay response cannot differentiate between labelled and native compounds, but will react without bias to both. Therefore, a separate extraction, with [$^{13}C_{12}$] labelled IS (EPA-1613 LCS mix, Wellington Laboratories *Inc.*, USA) was performed for the instrumental analysis of dioxins.

The samples were taken from storage, air-dried, homogenized and sieved (0.5 mm). After thorough mixing, 40 g of soil and an equal amount of Na_2SO_4 were combined and spiked with 10 µl of [$^{13}C_{12}$] labelled internal standard (100 ng/ ml, EPA-1613CSL). Samples were extracted with a mixture of high-purity hexane and dichloromethane (DCM) in an accelerated solvent extraction (ASE) apparatus (US EPA 2007a). Prior to clean-up, extracts were spiked with 10 µl of EPA-1613 CSS clean-up standard ($^{37}Cl_4$-2,3,7,8-TCDD). Extracts were collected in 250 ml glass ASE bottles and activated copper shavings were added to the ASE bottle with the extract and placed in an orbital shaker for at least one hour. The extracts were inspected periodically and if the copper was fully oxidised more activated copper was added to the extract. The use of copper shavings was crucial to ensure efficient removal of sulphur (US EPA 1996d). The extract was removed and the remaining copper shavings were washed three times with hexane. The extracts were evaporated under a gentle stream of nitrogen at 60˚C to reduce the volume to approximately 2 ml dichloromethane, followed by gel permeation chromatography (GPC, US EPA 1994b) clean-up using Waters *Envirogel* columns and acid digestion with concentrated sulphuric acid. This step was followed by sodium chloride and potassium hydroxide washes to remove co-eluting substances, such as polyaromatic hydrocarbons (PAHs) and lipids (US EPA 1996c). Thereafter, samples were filtered through pre-cleaned glass wool covered with sodium sulphate to remove residual water and evaporated to a volume of 0.5 ml in iso-octane. An Internal standard (1 µl, EPA-1613 ISS) was added to each extraction before injection and analysis by GC×GC-TOFMS.

The H4IIE-*luc* bio-assay method was adapted from the procedure described by Whyte *et al*. (2004) and the application is described in the paper by De Vos *et al.* (2013a). As the bio-assay extraction is not the focus of this thesis, additional detail can be obtained from the PhD thesis by Quinn 2010 and the paper by Roos *et al* (2011).

4.3.2 Instrumental Analysis

A LECO Pegasus® 4D GC×GC-TOFMS (LECO Corporation, St Joseph, MI, USA), Agilent 6890N GC and Agilent 7683B auto-sampler with a secondary oven mounted inside the primary GC oven fitted with a quad-jet dual stage thermal modulator was used for all qualitative and quantitative analyses (described in Chapter 3, Section

3.2.4). Instrument parameters are summarised in Table 4.2. The system was tuned using the m/z = 414 ion for optimisation instead of the m/z = 69 ion usually selected for the conventional perfluorotributylamine (PFTBA) mass calibration tune (Hoh *et al*. 2007). This is different from the standard tuning procedure and is an attempt to improve the signal intensity at the higher mass range. The resolution mass remained set at m/z = 219. All instrument functions and data processing were managed with the *LECO ChromaTOF®* software (version 4.22). Manual review of all peak identifications and integrations was performed using the *True Signal Deconvolution®* and *Automated Peak Find* algorithms. Library searching was performed using an in-house PCB and PCDD/F *user* library compiled from the dioxin-like PCBs (WP-CVS and WP-STK, Wellington Laboratories *Inc.,* USA) and seventeen toxic PCDD/F native and labelled standards.

Chromatographic separation was achieved on a low polarity Rxi®-XLB (30 m x 0.25 mm x 0.25 µm) primary first dimension (^{1}D) column coupled with a ^{2}D Rtx®-200 cross-bond trifluoropropylmethyl polysiloxane (1.1 m x 0.18 mm x 0.18 µm) column in the second dimension (Restek Corp., Bellefonte, PA; Restek 2014). The columns were connected using a deactivated universal glass press-tight connector. A GC multi-step temperature program was developed to facilitate separation and quantification of the maximum number of POP compounds that could be present in environmental samples in a single analysis, thus benefitting from the added selectivity of GC×GC and the full range mass spectra generated by TOFMS (Table 4.1).

The 0,18 µm film thickness was selected to mitigate the concentration effect (zone compression that occurs when using thermal modulators) of the focused ^{1}D band that may lead to overloading of a 0,10 µm ^{2}D column even when the ^{1}D column is not overloaded (Mustafa *et al*. 2012). Harynuk *et al*. (2005) studied the influence the ^{2}D column internal diameter on the ^{2}D peak width using narrow-bore columns in the second dimension and concluded that it might not provide as great an advantage as is commonly thought. When speed of the separation is prioritized over high resolution, thin-film ^{1}D columns and narrow-bore ^{2}D columns might be optimal. However, when high resolution is desired, thicker film ^{1}D columns and larger diameter ^{2}D columns could be a better combination (Harynuk *et al*. 2005).

Table 4.1. *GC×GC-TOFMS instrument conditions for the Rxi®-XLB/ Rtx®-200 column set.*

Parameter	**GC×GC-TOFMS Modified method** *(Hoh et al. 2007, Figure 4.2)*	**GC×GC-TOFMS NMISA method** *(Figure 4.3)*	**GC×GC-TOFMS NMISA Method** *(De Vos et al. 2011b and 2013b, Figure 4.4)*
^{1}D column	Rxi®-5SilMS (30 m x 0.25 mm x 0.25 µm)	Rxi®-5SilMS (30 m x 0.25 mm x 0.25 µm)	Rxi®-XLB (30 m x 0.25 mm x 0.25 µm)
^{2}D column	Rtx®-PCB (1,1 m x 0.18 mm x 0.18 µm)	Rtx®-200 (1,1 m x 0.18 mm x 0.20 µm)	Rtx®-200 (2 m x 0.18 mm x 0.20 µm)
Amount injected	1 µl splitless	2 µl splitless	2 µl splitless
Inlet temperature	250 °C	275 °C	250 °C
Helium flow	1.0 ml/ min constant flow	1.0 ml/ min constant flow	1.4 ml/ min constant flow
Primary oven	140 °C (hold 1 min), ramp at 20 °C/ min to 180 °C, at 3 °C to 295 °C, hold 3,67 min	120 °C (hold 1 min), ramp at 20 °C/ min to 180 ºC, at 2 °C to 250 °C, at 20 °C/ min to 300 °C, hold 20 min	80 °C (hold 1 min), ramp at 20 °C/ min to 220 °C, no hold, 2 °C/ min to 240 °C, no hold, 1 °C/ min to 250 °C, no hold, 5 °C/ min to 260 °C, no hold, 1°C/ min to 270 ºC, no hold, 5 °C/ min to 310 °C, hold 2 min
Total run time	59,83 min	59,43 min	50,33 min
Secondary oven offset	20 °C	20 °C	20 °C
Transfer line temperature	270 °C	270 °C	270 °C
Modulation period	5 s (1,2 s hot pulse)	4 s (1s hot pulse)	4 s (1 s hot pulse)
Modulator temperature offset	30 °C	35 °C	30 °C
Ion source	250 °C	250 °C	250 °C
Mass range	100 - 520 amu	100 - 520 amu	100 - 520 amu
Acquisition delay	600 s	600 s	600 s
Acquisition rate	50 spectra/ s	100 spectra/ s	50 spectra/ s
Mass defect setting	-40 mu/ 100u	-40 mu/ 100u	-40 mu/ 100u
Electron energy	80 eV	70 eV	70 eV
Calibration	PFTBA	PFTBA	PFTBA

4.3.3 PCDD/F Method Development using GC×GC-TOFMS

Hoh *et al.* (2007) published a paper describing the optimization of separation and detection conditions for GC×GC-TOFMS analysis of PCDD/Fs. Their method formed the basis for the NMISA method for separating and identifying the seventeen toxic PCDD/Fs. At the time, a method had been developed for the analysis of chlorinated

pesticides and PCBs, experimenting with different column sets for ^{1}D and ^{2}D separation. The PCB congeners were well separated using a 30 m Rtx®-PCB column in the first dimension and with a 1,1 m Rxi®-50 column in the second dimension and this method provided a starting point for developing a method for analysing PCDD/Fs. Various column combinations were selected based on an initial review of Dr Peter Korytár's Doctoral thesis (Korytár 2006a and Restek 2014) and columns available in the NMISA laboratory (Table 4.2).

Instrumental parameters mostly followed the method by Hoh *et al*. (2007). A modulation period of 5 seconds was initially selected to ensure enough second dimension chromatographic space and to assess the separation of the PCDD/Fs. To determine the modulation period, 20-25% of the second dimension time (5 s) was considered for the hot pulse and then adjusted. A hot pulse that is too short fails to release all the analytes from the modulator, leaving elution to the next modulator cycle and resulting in peak tailing along the first dimension. Long hot pulses lead to correspondingly short cold trapping times. If the cold pulse is too short, volatile analytes are not trapped efficiently. The added peak capacity of GC×GC provides the selectivity needed for PCDD/F analysis, and the focusing effect of the modulator enhances the sensitivity, so that sub- to low picogram levels can be determined.

Table 4.2. *Different Restek® column combinations used for selectivity optimisation of PCDD/Fs*

First Dimension Column	Second Dimension Column
Rtx®-Dioxin 2 (60 m x 0.25 mm x 0.25 µm)	Rtx®-PCB (2.0 m x 0.18 mm x 0.18 µm)
Rtx®-5SilMS (30 m x 0.25 mm x 0.25 µm)	Rtx®-PCB (1.1 m x 0.18 mm x 0.18 µm)
Rtx®-5MS (30 m x 0.25 mm x 0.25 µm)	Rtx®-200 (1.1 m x 0.18 mm x 0.18 µm)
Rtx®-5SilMS (30 m x 0.25 mm x 0.25 µm)	Rtx®-200 (1.1 m x 0.18 mm x 0.18 µm)
Rxi®-XLB (30 m x 0.25 mm x 0.25 µm)	Rtx®-200 (1.1 m x 0.18 mm x 0.18 µm)
Rtx®-Dioxin 2 (40 m x 0.18 mm x 0.18 µm)	Rxi®-17SilMS (1.0 m x 0.15 mm x 0.15 µm)

4.3.3.1 Column Combinations

Selectivity starts with good chromatography (Do *et al.* 2013, Marriott *et al.* 2003, Marriott *et al.* 2004 and Marriott *et al.* 2012). The choice of column combinations for GC×GC-TOFMS based on orthogonality has been detailed in the literature (Dorman *et al.* 2008, Focant *et al.* 2002a, Fraga *et al.* 2001, Ryan *et al.* 2005, Sinha *et al.* 2004, Watson *et al.* 2007, Venkatramani *et al.* 1996 and Zaiyou *et al.* 1995). The method described by Hoh *et al.* (2007) used a 60 m Rtx®-Dioxin2 column (cross bond phase for isomer specificity of PCDD/Fs, Restek 2014) for the first dimension (^{1}D) separation, and a 2 m Rtx®-PCB column (shape selectivity polymer, Restek 2014) in the second dimension (^{2}D), with a total run time for the analysis not longer than 60 minutes. This approach was used to benchmark the separation of the seventeen toxic PCDD/Fs (Figure 4.1).

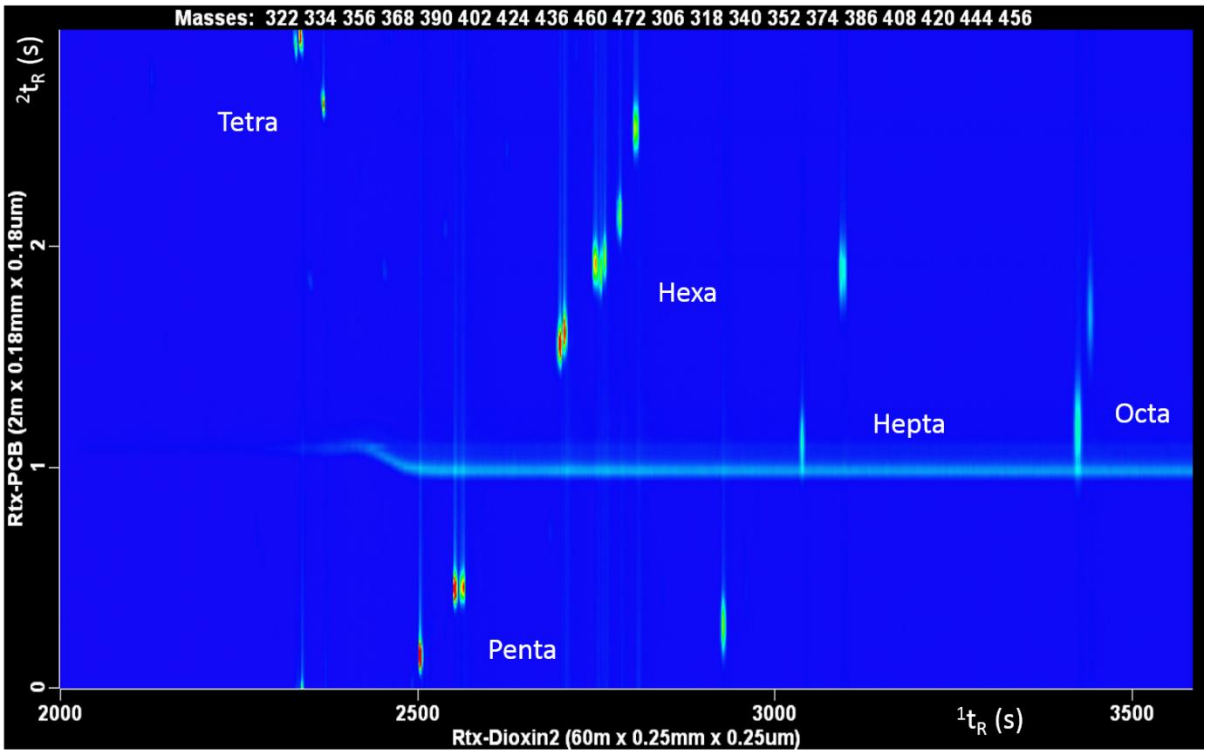


Figure 4.1. *^{2}D selected ion contour plot for the 17 priority PCDD/Fs using the method by Hoh et al. 2007 showing significant wrap around. Possible problems that can be encountered with wrap around are peak broadening of the later eluting compounds and interference from column bleed and matrix. The 1,2,3,4-[$^{13}C_{12}$]-TCDD labelled compound accounts for the 18 peaks that are visible (De Vos et al. 2011b).*

After analysis of the data, it was evident that considerable wrap-around (Marriott *et al.* 2014 and Zaiyou *et al.* 1995) of the dioxin compounds (hepta and octa) had occurred (Marriott *et al.* 2012 and Watson *et al.* 2007), as can be seen in the contour plot for the separation (Figure 4.1). Wrap-around happens when compounds are eluted from the second column, but not in the same modulation period as that in which they were injected; but in a subsequent modulation period. When this method was used for the investigation of heavily contaminated samples, it was found that several of the target analytes were wrapped around into regions of heavy interference. This could have an impact on any attempted quantitation as broader peaks (resulting from wrap-around) are more difficult to quantify at low levels (500 fg for 2,3,7,8-TCDD as stipulated in US EPA Method 1613B; US EPA 1994a), and it is possible that target compounds could wrap-around into areas of high matrix contamination.

The Rtx®-Dioxin2, the only column available at the time, proved very fragile and broke during use. This raised concerns about the fragility and costs involved for 60 m columns. A second combination comprised an Rtx®-5SilMS (low polarity crossbond® silarylene phase) as the ^{1}D column coupled to a shortened (1,1 m) Rtx®-PCB column. By shortening the ^{2}D column relative to the length used by Hoh *et al.* 2007, it was hoped to prevent the wrap-around that had proved problematic earlier. This did indeed prove to be the case, and no wrap-around was observed.

With this column set, difficulty was experienced in assigning peak areas for the HxCDD/F compounds, as there was considerable overlap of 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD in both the first and second dimension. An alternative ^{1}D and ^{2}D column set combination was necessary for further analysis and method validation of PCDD/Fs.

The choice of ^{2}D columns is restricted by the high temperature demands of the analysis. A combination of a ^{1}D Rtx®-5 (crossbond® diphenyl dimethyl polysiloxane) and ^{2}D Rtx®-200 column (crossbond® trifluoropropylmethyl polysiloxane) was then employed (Restek 2014). With this combination, the two HxCDFs (1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF) and the two HxCDDs (1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD) still showed overlap in the second chromatographic dimension, but the three

problem compounds in the separation using the Rtx®-5SilMS/ Rtx®-PCB combination were more clearly resolved (Figure 4.2, Table 4.2); namely 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD.

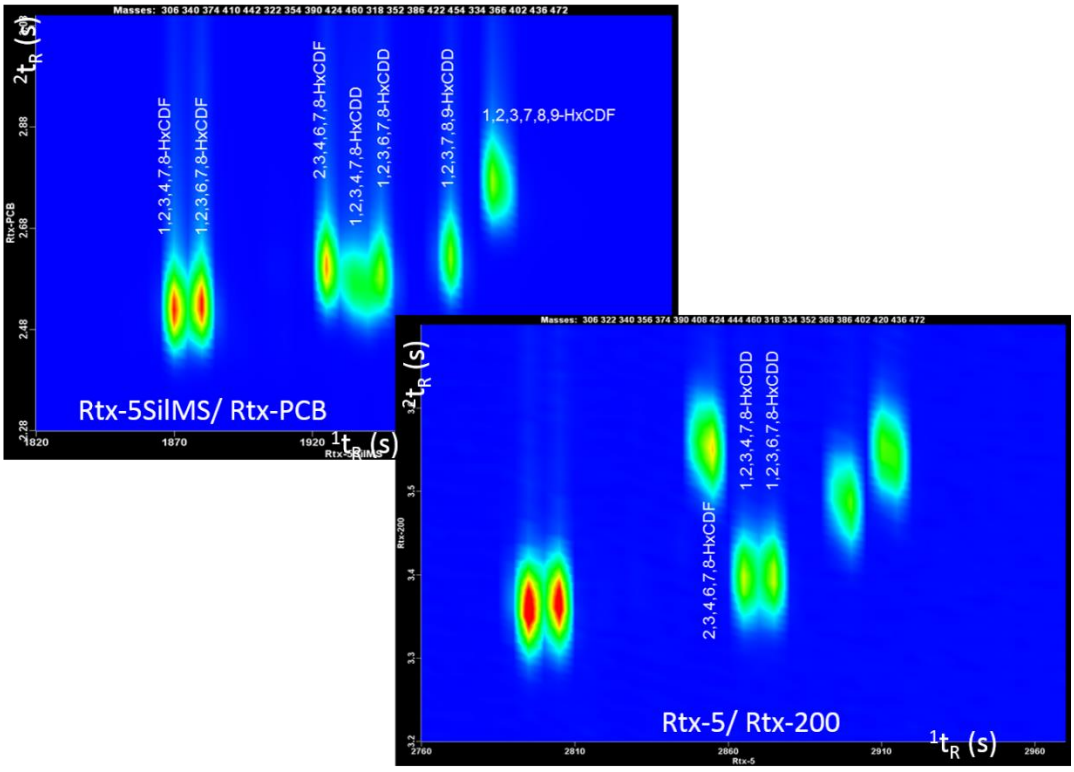


Figure 4.2. *GC×GC-TOFMS chromatogram of the HxCDD/F region using the Rtx®-5SilMS/ Rtx®-PCB and Rtx®-5/ Rtx®-200 column combinations showing improvement in the separation for the 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDDs using the Rtx®-200 column in ^{2}D, but still not enough separation for accurate quantitation (De Vos et al. 2011a).*

The Rtx®-5SilMS and Rtx®-5 ^{1}D column separation was very similar, so it was easy to combine the Rtx®-5SilMS with the Rtx®-200 instead of the Rtx®-PCB. Very good separation was achieved with this column combination, but it was evident that the actual GC parameters needed to be improved to fully realise the separation power of GC×GC and make better use of the chromatographic space (Figure 4.3, Table 4.2).

Since improved separation was achieved using the Rtx®-200 in the second dimension, another column combination was considered. An Rxi®-XLB column (low polarity proprietary phase, Restek 2014) with unique selectivity for pesticides, PCB congeners

and PAHs, was selected for first dimension separation, in the absence of an Rxi®-Dioxin2 column. This column was then coupled to the mid-polarity Rtx®-200 phase in the second dimension. It was hoped to improve the separation of the HxCDD/F compounds, especially the 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDD/F doublets. This indeed proved to be the case, with improved ^{1}D separation for the OCDD/F-compounds too. Method development using the Rxi®-XLB/ Rtx®-200 column combination is detailed in (de Vos *et al*. 2011b; Table 4.2).

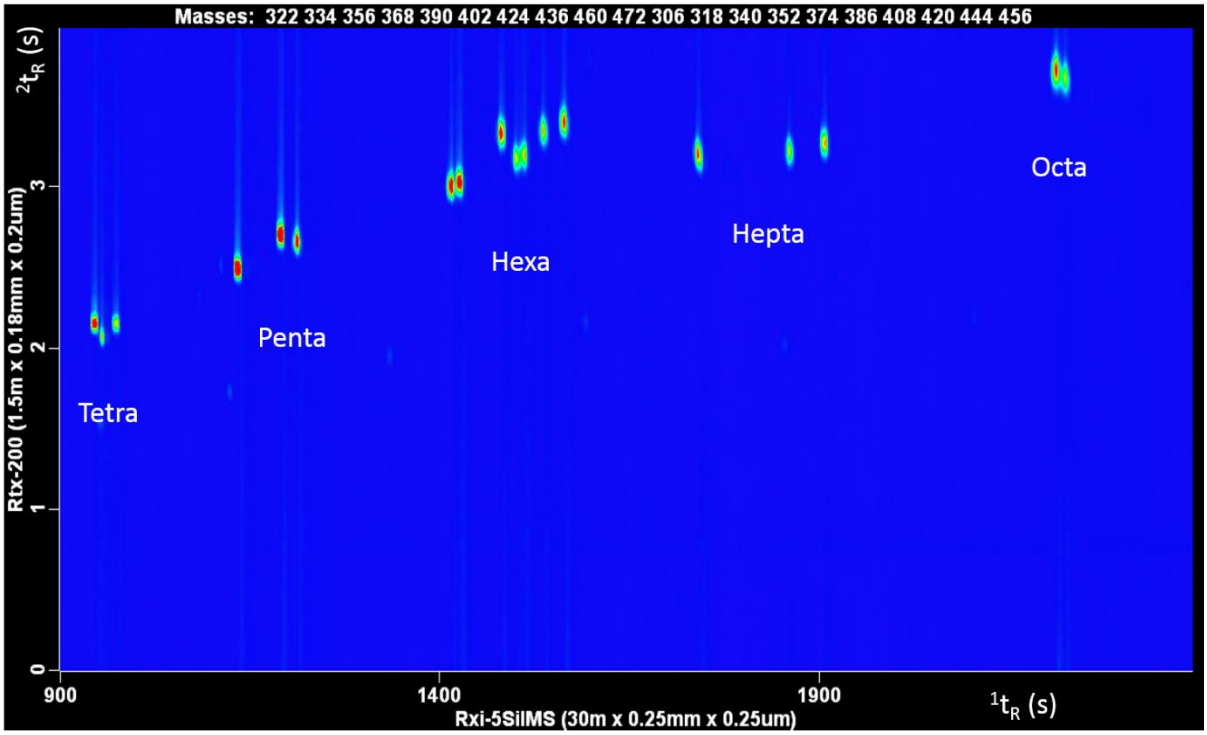


Figure 4.3. *^{2}D selected ion contour plot for the 17 priority PCDD/Fs using the Rxi®-5SilMS/ Rtx®-200 column combination (including 1,2,3,4-[$^{13}C_{12}$]-TCDD). The PCDD/Fs are well resolved from each other. The method is optimized for the separation of all the priority pollutants, not just the 17 target PCDD/F compounds (De Vos et al. 2011b).*

The wrap-around, which had been a problem with the analysis using the ^{2}D Rtx®-PCB column (Figure 4.1), did not occur when using a 2m ^{2}D Rtx®-200 column (Figure 4.4). The HxCDD/F separation is critical for exact measurement and accurate quantitation of all seventeen PCDD/Fs. It is the accepted practice to report the combined results as ng WHO-TEQ per kg (van den Berg *et al*. 2006, Chapter 2 section 2.3.4), but for metrology purposes, accurate analysis of the isomer-specific concentrations of the

seventeen congeners is required to assign a correct TEF value to each component to assess each congener's contribution to the overall toxicity; regulatory decisions are based on accurate reporting of TEQ results.

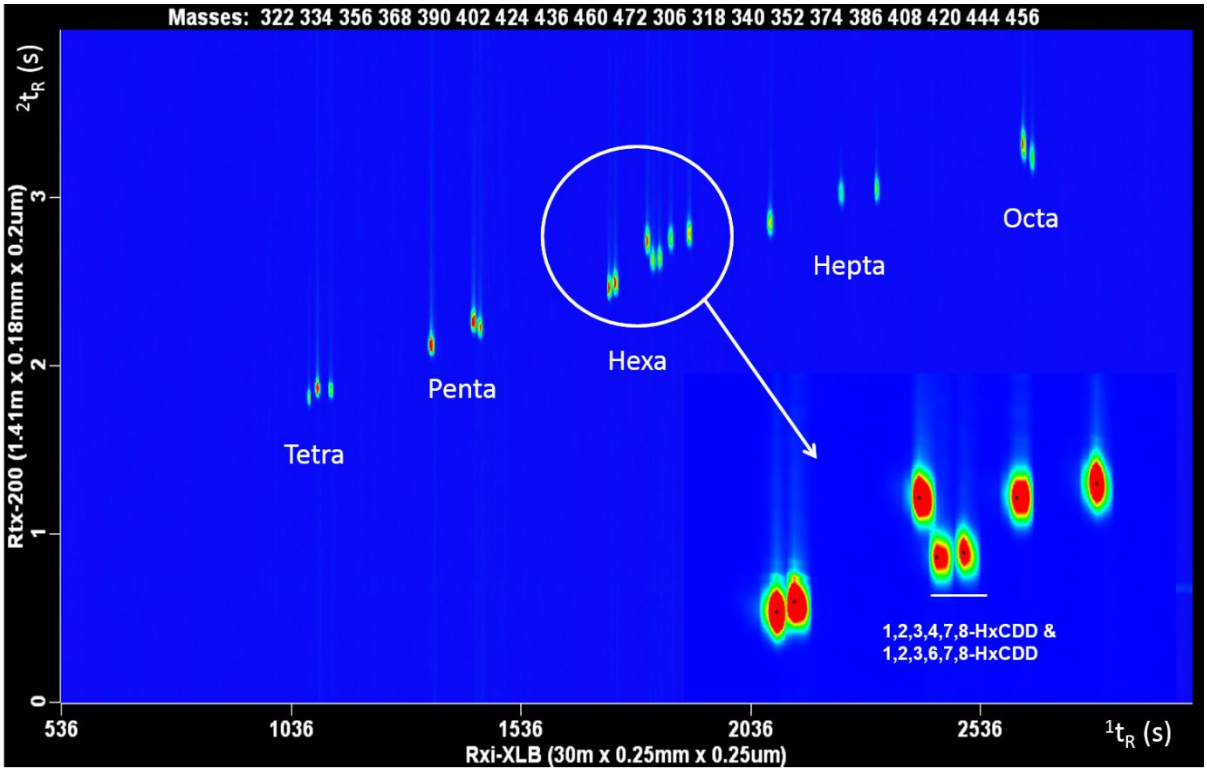


Figure 4.4. *^{2}D selected ion contour plot for the 17 priority PCDD/Fs using the Rxi®-XLB/ Rtx®-200 column combination. The PCDD/Fs are again well resolved, especially the 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD isomers (De Vos et al. (2011b).*

Although GC parameters will be detailed in Chapters 7 and 8, mention must be made of the first GC separation parameters. Although the multi-step parameters were criticised by a reviewer in the publication De Vos *et al*. (2011b), the reason for developing the multi-step temperature programme was to baseline separate the seven HxCDD/F congeners. The initial temperature was 80 ºC and held for 1 min. A fast ramp rate of 20 ºC/ min was selected to accelerate the elution of the tetra PCDD/Fs. The ramp rate was reduced to 2 ºC/ min and then to 1 ºC/ min (Table 4.2). This is the area of the chromatogram where the HxCDD/Fs elute and was an attempt to improve the separation of the individual HxCDD/F congeners, specifically 1,2,3,4,7,8 and 1,2,3,6,7,8-HxDD/F (the dioxin and furan doublets; Figure 4.4). This would allow for

improved quantitation of each individual congener contributing to the overall summed TEQ value.

Figure 4.5 shows a surface plot for a heavily contaminated soil sample using the Rxi®-XLB/ Rtx®-200 column combination. Plotting the selected ions for 2,3,7,8-TCDF/ 2,3,7,8-[$^{13}C_{12}$]-TCDF (m/z = 306, 318) and 2,3,7,8-TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD (m/z = 322, 334) highlights how the multi-step method (Table 4.1) has managed to move the 2,3,7,8-TCDD and 2,3,7,8-TCDF away from more abundant matrix components containing similar ions that would interfere in the analysis. This accentuates the separation power in the second dimension and even with the interference that is present, the target peaks are well resolved and can easily be quantified, providing the added selectivity and sensitivity needed to reach the low levels at which these PCDD/F compounds occur in environmental samples.

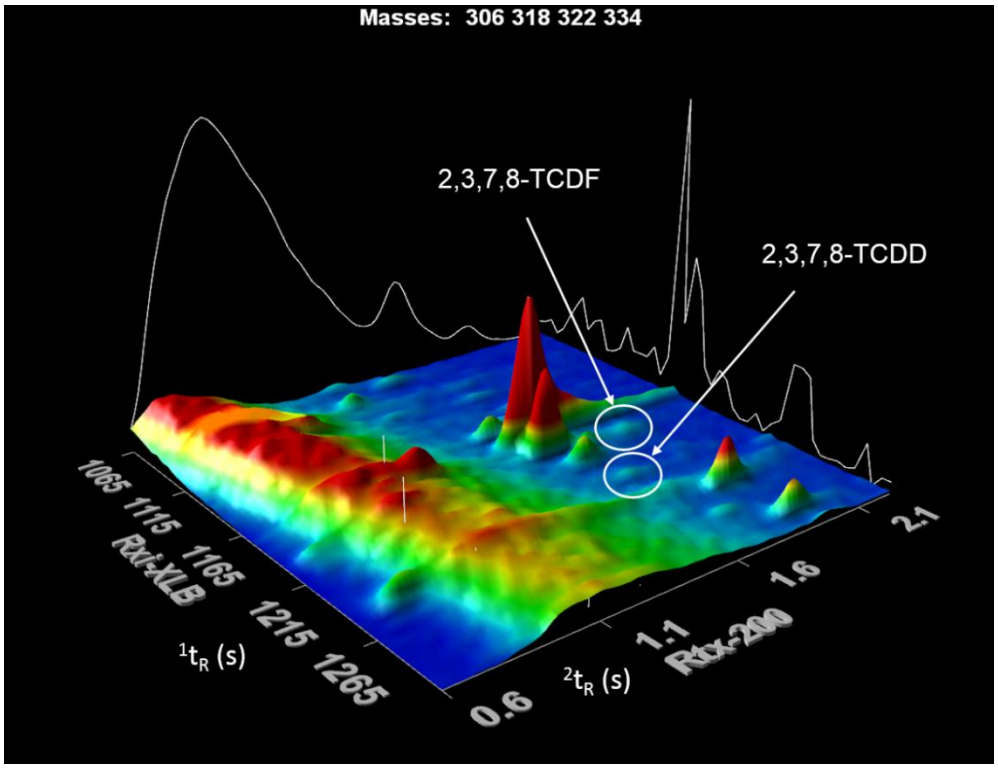


Figure 4.5. *Surface plot showing the position of the 2,3,7,8-TCDF and 2,3,7,8-TCDD in the presence of matrix interference. In this chromatogram it is evident that the TCDD/F peaks would not be detected in a ^1D separation; the peaks would be buried under the matrix interference (heavily contaminated soil). The ^2D separation clearly removes the target analytes from the matrix interference, allowing for improved quantitation when needed (De Vos et al. 2011b).*

If the sample matrix was even more complex, or if the sample clean-up steps caused analyte losses, then it is possible that the TCDD/Fs analytes would be obscured and accurate quantitation would be compromised, resulting in a lack of confidence in the analytical results. This highlights the need for improved sample extraction and adequate clean-up to prevent unacceptable peak overlap, which is limited to some degree by the superior chromatographic resolution of GC×GC. GC method and instrument parameters can only be assessed with real samples to anticipate the level of clean-up required for accurate quantitation of the PCDD/F congeners at or near the limit of detection (LOD) of the instrument. Sample preparation will be discussed in detail in Chapter 5. When using GC-HRMS SIM, the unresolved interferences are less critical, depending on the resolving power of the instrument and the nature of the interference. This aspect will be covered in more detail in Chapter 8.

4.3.3.2 Limit of Detection and Quantitation

A prime consideration in method development, is the accurate determination of small concentrations of 2,3,7,8-TCDD. The method specific detection limit is the minimum concentration of a substance that can be measured with '*reasonable statistical certainty*' and is matrix (and method) dependent (Armbruster & Pry 2008, Konieczka & Namieśnik 2008 and VIM 2012). Instrument detection limit (IDL) is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise by a particular instrument. The limit of detection (LOD) or detection limit of an analyte may be described as that concentration which gives an instrument signal (Y) *significantly different* from the 'blank' or 'background' signal. There is no full agreement among researches as to the exact definition of LOD, but it is accepted as the analyte concentration giving a signal equal to the blank signal, Y_B , plus three standard deviations of the blank, S_B:

$$LOD = Y_B + 3S_B$$

Detection limits are highly dependent on the instrument conditions, including the matrix, method, and the specific target analyte (Miller & Miller 2010). Additional factors to consider include sample size, effectiveness and selectiveness of the sample extraction and clean-up and the sensitivity of the system employed for the actual

measurement, in this case GC×GC-TOFMS (Fraga *et al*. 2001, Gaines & Frysinger 2004, Harvey & Shellie 2011, Harynuk *et al*. 2005, Marriott *et al*. 2004, Shellie *et al*. 2002).

Instrument calibration is an essential stage in most measurement procedures as it establishes the relationship between the response of an instrument and the accepted values of the calibration standards. This requires the preparation of a set of standards containing a known amount of the analyte of interest, measuring the instrument response for each standard and establishing the relationship between the instrument response and analyte concentration (Barwick 2003). In order to determine whether GC×GC-TOFMS has the sensitivity to calibrate 2,3,7,8-TCDD down to 0.5 pg/ µl as required by US EPA Methods 1613B and 8290A, a calibration curve was constructed using the EPA-1613 CVS standard calibration set. This standard set covers a large dynamic range from 0.5 pg/ µl to 200 pg/ µl (2,3,7,8-TCDD/F). The two lower level extended calibration standards; 0.1 and 0.25 pg/ µl, were not included. Duplicate measurements at each concentration level were made as this allows the precision of the calibration process to be evaluated at each concentration level. The uncertainty in a result can never be less than the uncertainty in the standard(s) used (US EPA 1994a, US EPA 2007d and Miller & Miller 2010).

Quantitation was performed by measuring peak area ratios (native/ labelled material) and then using either the calibration curve or the relative response factor (RRF) to quantify the results. As this was the first attempt at quantifying the PCDD/Fs, the RRF calculation was used: RRF = (Area ^{12}C/ Area ^{13}C x [^{13}C]/ [^{12}C]); where [^{12}C] and [^{13}C] are the concentrations of native and labelled material, respectively. This RRF value is a measure of the different detector responses obtained for the native and labelled compounds. The RRF value is then used to calculate the concentration of unknown material in the sample to be quantified using the formula: [^{12}C] = ((Area ^{12}C/ Area ^{13}C x [^{13}C]/ RRF) – BC)) x (1/ sample weight), the result is given as ng/ kg (or as pg/ g). During the calculation a correction for a blank (BC) can be made if required, but was not attempted in this case.

Figure 4.6 shows the linear calibration curve obtained for the 2,3,7,8-TCDD, the crucial compound to detect, with an average RRF of 1.08 for the calibration set. .The

achievable method detection limit will depend critically on the matrix interference, so the lowest level detected will vary from sample to sample. The capability of the method to achieve the required level of quantitation for the lowest standard was established using the low-level CS1 standard (0,5 pg/ µl).

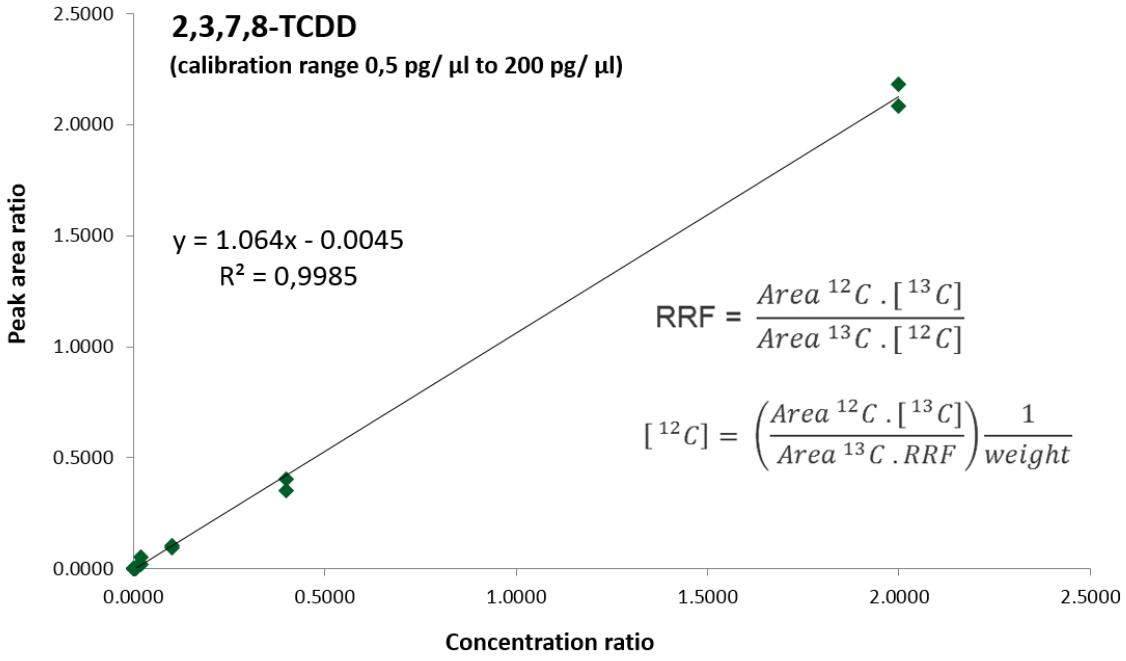


Figure 4.6. *Calibration curve for 2,3,7,8-TCDD (0,5 pg/ µl to 200 pg/ µl). Duplicate measurements at each concentration level were made and the average taken for the regression calculation. This allows the precision of the calibration process to be evaluated.*

The relevant chromatographic peaks used for the quantitation of the 2,3,7,8-TCDD standard at a concentration of 0,5 pg/ µl, together with the corresponding labelled standard at a concentration of 100 pg/ µl are shown in Figure 4.7. Even for the lowest level standard at 0,5 pg/ µl, the chromatographic peak for the ion at m/z = 322 was easily discernible and could be accurately quantified (the labelled standard has been minimised to scale). As an additional step, the S/N ratio for the ion of m/z = 322 for 2,3,7,8-TCDD was calculated using the method stipulated in US EPA Method 8290A. The value measured was 20, which is well above the ratio (> 10) set by US EPA Methods 1613B and 8290A (US EPA 1994a and 2007d).

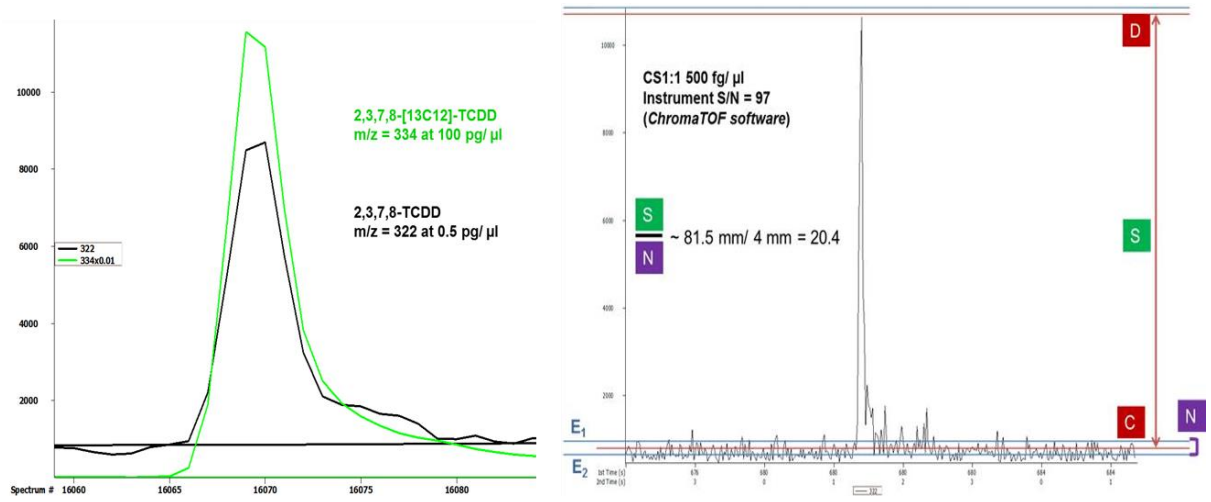


Figure 4.7. *Extracted ion profiles for the native (m/z = 322) and labelled (m/z = 334) 2,3,7,8-TCDD showing the sensitivity that can be achieved for the lowest standard (0.5 pg/ µl), meeting US EPA Method 1613B criteria. In order to compare these two concentration levels, the labelled peak was reduced to scale (x 0,01). (De Vos et al. 2011b).*

In order to establish the method detection limit, two sterilized soil samples were spiked with native PCDD/Fs at concentrations of 500 pg and 2 000 pg for 2,3,7,8-TCDD. After adding labelled material and using accelerated solvent extraction, with silica and alumina clean-up, the detection limits for the PCDD/Fs could be calculated. For the 2,3,7,8-TCDD, the LODs obtained were 350 and 320 fg on column respectively. These results are consistent and indicate that the method can attain the low levels for 2,3,7,8-TCDD required by US EPA 1613B (US EPA 1994a). These calculations were made by determining the S/N for the ion of m/z = 322 and extrapolating linearly to a S/N of 3:1. Results were consistent with the detection limit determined from the EPA-CS1 calibration standard (0,5 pg/ µl), thus providing assurance that the method has the necessary sensitivity. 500 fg is near the detection limit of the instrument and the influence of matrix interference from complex soil samples on this detection limit needed to be considered. When working close to the detection limit, baseline stability becomes crucial. This stability is reliant upon clean extracts with minimal interfering sample constituents present, emphasising the need for a reproducible extraction and clean-up method.

4.4 RESULTS AND DISCUSSION

4.4.1.1 H4IIE-luc Bio-assay Results

The H4IIE-*luc* bio-assay detected dioxin-like activity in 22 percent of the sediment samples (BEQ_{20}; n=96) and 58 percent of the soil samples (BEQ_{20}; n=66). BEQ_{20} indicates a sample extract that gave a 20 percent response to the dioxin (2,3,7,8-TCDD) positive control. Soil had a detection rate three times greater than that of sediment. For sediment, the sites that were impacted by industry contributed more than 80% to the total number of sites testing positive for the presence of dioxin-like activity, while residential, mixed residential and agricultural sites contribute less than 10%. For soil, the greatest concentrations were observed in industrialised areas with agricultural and residential areas having concentrations at or near the LOD.

All the samples that tested positive and six samples that tested negative in the bio-assay analysis were then analyzed by GC×GC-TOFMS for PCDD/Fs. Of these, 23 percent of positive *soil* samples and 41 percent of positive *sediment* samples were found to be false positives (the bio-assay is not PCDD/F specific). The extraction procedure followed for the bio-assay is reported in Roos *et al.* (2011). The extracts were not checked for PAH interference, however, the extraction protocol did include a concentrated sulphuric acid and potassium hydroxide wash that is generally believed to remove all PAH interferences, hence the need for GC×GC-TOFMS analytical confirmation. These results are further discussed in the paper De Vos *et al.* (2013a).

4.4.1.2 GC×GC-TOFMS Results

Soil and sediment sample extracts were analysed for PCDD/Fs using the Rxi®-XLB/ Rtx®-200 column combination (as described in Section 4.3.3.1) and GC×GC-TOFMS method described in Table 4.2. Isotope dilution is an accepted tool for accurate low level quantitation (Vogl & Pritzkom 2010). Isotopically labelled compounds are used as internal standards that provide additional confirmation of the presence of target compounds in an unknown sample with similar GC behaviour. Data processing becomes more involved because of the presence of closely related PCDD/F compounds and the retention times for the native and labelled compounds are almost identical; the mass spectra are mixed and quantitation is based on the extracted ions for each native and labelled compound. Neat standards for each isotopically labelled

PCDD/F and native PCDD/Fs are very expensive; hence the reason that blended mixtures are commercially available for PCDD/F analysis. The EPA 1613 CVS calibration set (0,5 pg/ µl to 200 pg/ µl) was used to construct the calibration curves for each of the seventeen toxic PCDD/Fs, as shown in Figure 4.6 for 2,3,7,8-TCDD.

Table 4.3. *Standard table calculated from calibration table results for the EPA 1613CVS standard set*

Standard (pg/ µl)	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Ion Ratio % Diff.	Calc.Ion Ratio	Expected Ion Ratio	RF	Quant S/N
CS1 (0,5-5,0)	1552	0.50	0.54	7	8.33	0.453	0.494	**1.198**	12
CS2 (2-20)	5893	2.00	2.02	1	1.24	0.500	0.494	**1.126**	40
CS3 (10-100)	27947	10.00	9.76	2	9.18	0.539	0.494	**1.089**	223
CS4 (40-400)	106259	40.00	34.97	13	10.4	0.546	0.494	**0.975**	698
CS5 (200-2000)	575557	200.00	184.33	8	7.44	0.531	0.494	**1.028**	5823
							AveRF	**1.083**	

Quantitation was performed using the relative response factor (RRF) approach to quantify the results. The RRF values can be calculated using the ChromaTOF® software and are shown in Table 4.3. An overall average RRF of 1,08 was calculated for all the PCDD/Fs to estimate the validity of the calibration linearity, although the average RRF for each congener is used to calculate the final results before conversion to TEQ.

A review of the results obtained for the soil and sediment extracts indicated that the extraction procedure followed was inadequate for heavily contaminated samples. Recovery of the labelled internal standard was not consistent from sample to sample. The loss of internal standard indicated a problem arising during the extraction and clean-up procedures, rather than with the standard itself. GPC clean-up was identified as an area of concern; the large sample portions (40g) caused blockages and some samples had to be diluted prior to injection to compensate for the high viscosity and high level of suspended solids present. Consequently, the losses incurred compromised the PCDD/F results for these samples and could not be reliably finalised

and is also why a blank correction was not made. There were a vast number of compounds present in the sample extracts that could mask the PCDD/Fs, including PAHs and halogenated aliphatics, with retention times similar to the target molecules (Figure 4.5). In South Africa, PAHs are generally present at levels an order of magnitude higher than the PCDD/Fs, and this is most likely the cause of the many false positive results from the bio-assay.

As a result of the extraction problem, recoveries were not calculated and the rest of the results were handled qualitatively rather than quantitatively. The primary objective of this investigation was to develop an in-house method for the analysis of dioxin-like chemicals. Only the samples that tested positive for the presence of dioxin-like compounds with the H4IIE-*luc* bio-assay were compared to results obtained for samples that tested positive for the presence of PCDD/Fs through GC×GC-TOFMS analysis. All the other samples were below the LOD for the method. Figure 4.8 shows the linear relationship that is obtained for the two methods, confirming the presence of PCDD/Fs in the few positive samples confirmed. The straight line is also indicative of the fact that the results are close to the LOD. The H4IIE-*luc* bio-assay proved to be a useful screening tool, reducing the need for instrumental analysis by more than 50%. Analysis by GC×GC-TOFMS indicated that there were problems associated with the clean-up procedure in the original methodology. Although acid digestion techniques should be strong enough to remove PAHs (Focant, Sjödin *et al*. 2004b), the relatively high concentrations of these compounds present in the original samples rendered this technique unsuccessful. The increased AhR-response was very likely due to compounds such as PAHs still present in the extracts.

Acknowledging that improved extraction was required, a small experiment was undertaken to determine whether smaller sample aliquots would improve sample results for the South African samples, if correctly spiked with minimal isotope losses. Soil samples of 10g each, known to come from highly industrialised areas in the Vaal Triangle region of South Africa (VD-8, VD-14 and VD-27) were spiked with 40 pg/ µl EPA 1613-LCS labelled standards. The extraction and clean-up was done using the newly acquired Total Rapid Prep™ system (TRP-2) system (Fluid Management Systems Inc., Waltham, MA, USA). Average RRFs were determined using a new calibration curve and TEQ results calculated as ng WHO-TEQ.kg^{-1}. No attempt was

made at this point to follow proper statistical validation methods; the purpose was to determine whether there were PCDD/Fs present in any of these highly contaminated South Africa soil samples.

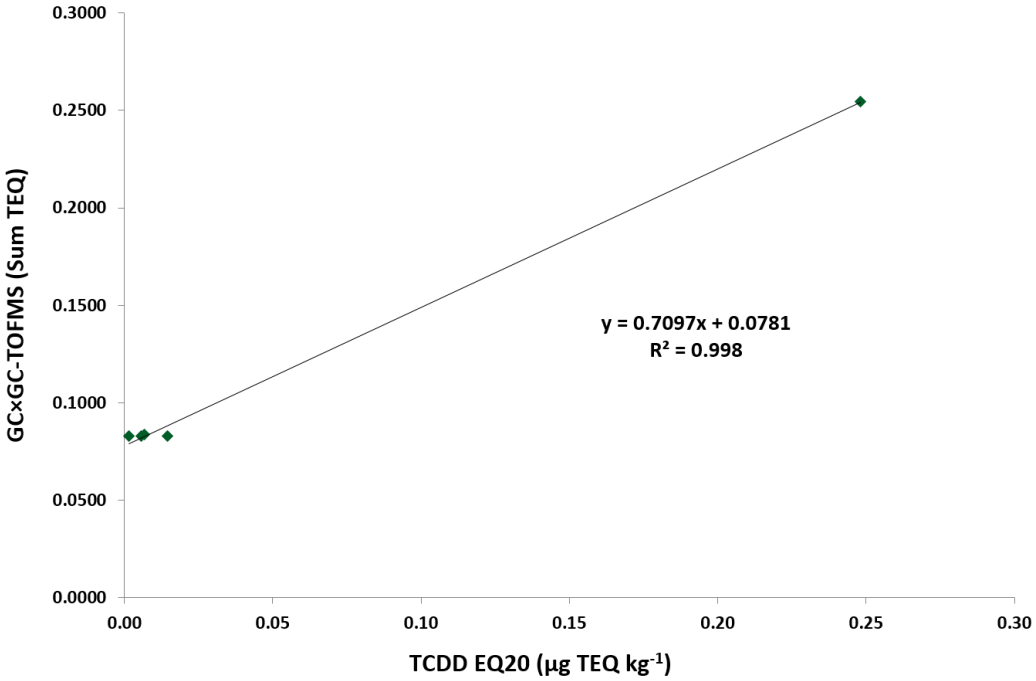


Figure 4.8. *Linear relationship obtained for the bio-assay results and the GC×GC-TOFMS qualitative results*

Table 4.4 represents the GC×GC-TOFMS results for the three repeat soil sample extracts. A duplicate extraction and analysis was done for sample VD-8. What is important to note from these results are the extremely low TEQ levels for PCDD/Fs. Samples from the same Vaal Triangle region in South Africa have been previously analysed by GC-HRMS and these results corroborate the low TEQ levels of PCDD/Fs in these samples. This approach might seem unusual to laboratories that are experienced at handling routine dioxin analysis, but these results were the first South African results obtained for PCDD/Fs and provided assurance that further method optimisation and sample validation would be worthwhile.

Table 4.4. GC×GC-TOFMS TEQ Results for South African soil samples (ng WHO-TEQ.kg^{-1})

PCDD/F Compounds	VD-8 TEF	VD-8b TEF (duplicate)	VD-14 TEF	VD-27 TEF
2,3,7,8-TCDD	0.18	0.19	0.10	0.09
1,2,3,7,8-PeCDD	0.14	0.16	0.20	0.09
1,2,3,4,7,8-HxCDD	0.02	0.01	0.03	0.01
1,2,3,6,7,8-HxCDD	0.02	0.02	0.02	0.01
1,2,3,7,8,9-HxCDD	0.01	0.01	0.00	0.01
1,2,3,4,6,7,8-HpCDD	0.03	0.02	0.00	0.00
OCDD	0.00	0.00	0.00	0.00
2,3,7,8-TCDF	0.01	0.01	0.01	0.01
1,2,3,7,8-PeCDF	0.00	0.01	0.00	0.01
2,3,4,7,8-PeCDF	0.04	0.04	0.04	0.02
1,2,3,4,7,8-HxCDF	0.01	0.01	0.02	0.01
1,2,3,6,7,8-HxCDF	0.01	0.01	0.02	0.01
2,3,4,6,7,8-HxCDF	0.01	0.01	0.02	0.01
1,2,3,7,8,9-HxCDF	0.02	0.01	0.01	0.00
1,2,3,4,6,7,8-HpCDF	0.00	0.01	0.00	0.00
1,2,3,4,7,8,9-HpCDF	0.00	0.00	0.00	0.00
OCDF	0.00	0.00	0.00	0.00
TOTAL PCDD/F TEQs	**0.52**	**0.48**	**0.27**	**0.53**

4.5 CONCLUDING REMARKS

NMISA has incorporated a combined approach for the analysis of PCDD/Fs in soil and sediment samples. It uses the H4IIE *luc* bio-assay as a preliminary screening tool, reducing the need for unnecessary instrumental analysis and driving down the cost per sample. The bio-assay, which is not PCDD/F specific, gives a good indication of the overall toxicity of the sample and reports the combined effect of all compounds capable of binding to the receptor. It provides limited information on concentration and can be seen as a screening technique only. The bio-assay is performed at NWU by qualified NMISA staff as part of the on-going collaboration between NMISA and NWU.

GC×GC-TOFMS analysis of samples perhaps positive for PCDD/Fs is possible at the levels stipulated in US EPA Method 1613B (1994a). The GC×GC-TOFMS analyses are mainly performed at NMISA and occasionally at the LECO Separation Science laboratory, University of Pretoria, depending on the project and allocation of resources. Since GC×GC-TOFMS is not a target compound technique, it is capable of providing information on multiple compound classes present in the sample in one run, giving a comprehensive picture of the toxic potential of the samples.

The combined approach is more robust, more user friendly and aligned to our needs and provides an affordable alternative to high resolution magnetic sector analysis. The end result is a method that is time-efficient, financially viable, and suitable for use in developing economies where instrumental availability, skills and finances are often limited.

Further work is needed to address the identified hurdles still existing in the extraction and clean-up method to try and improve quantitation close to the detection limit of the instrument (Focant, Pirard *et al*. 2004a, Focant *et al*. 2009 and Patterson Jr *et al*. 2009). The GC×GC-TOFMS results did indicate that the main interferences included aliphatic hydrocarbons and PAHs. Additional sample extraction and clean-up procedures using the Total Rapid Prep™ (TRP-2) system (FMS Inc., Waltham, MA, USA) are being developed to attempt to resolve these issues, together with the determination of appropriate concentration spike levels and minimisation of recovery standard losses, and will be discussed in Chapter 5.

5

SAMPLE PREPARATION

Sample extraction techniques provide extracts that contain the analyte of interest, but may also contain matrix components such as lipids as well as other organic compounds that are co-extracted during the extraction procedure. Additional steps are required to remove the bulk of the co-extracted matrix components, to separate PCDD/Fs and then to concentrate the final extract to appropriate volumes of solvent allowing the detection of the analytes at the ultra-trace levels at which they usually occur. The sample extraction, clean-up and GC-MS method together determine the ultimate specificity and selectivity required for accurate measurement (Reiner *et al*. 2010).

5.1 GENERAL SAMPLE PREPARATION FOR POP ANALYSIS

For PCDD/Fs, PCBs and other related compounds, extraction, sample preparation/ fractionation and analysis can be accomplished using accepted analytical procedures (Van Leeuwen & de Boer 2008). These compound groups are simultaneously extracted and extracts can be cleaned and fractionated, depending on the desired analytes to be quantified. This helps reduce analytical time and cost. When selecting a method for the determination of PCDD/Fs, a number of key method attributes (sensitivity, selectivity, speed and cost) must be reviewed and the optimum method selected (Reiner 2010).

Soxhlet or liquid-liquid partitioning have frequently been replaced by automated techniques allowing effective and efficient extraction and clean-up of multiple samples in less time (Van Leeuwen & de Boer 2008). More sophisticated instrumentation such as pressurised liquid extraction (PLE), supercritical fluid extraction (SFE) or microwave-assisted extraction (MAE) is now used. PLE achieves shorter extraction

times since the system operates at increased pressures that allow extraction to be performed at temperatures above the boiling points of conventional organic solvents (Bjorklund *et al*. 2006 and Wiberg *et al.* 2007).

Sample clean-up uses florisil, silica and alumina adsorption chromatography sorbents (Storr-Hansen *et al*. 1992, US EPA 1996a, US EPA 1996b, US EPA 2007b, US EPA 2007c). Combinations of these and carbon adsorbents or size exclusion materials (e.g., gel permeation chromatography) can be used to remove matrix and interfering compounds (US EPA 1994b). For some applications, these sorbents have been modified with silver nitrate or sulphuric acid (US EPA 1996e). Silver nitrate effectively removes sulphur and acidic silica removes all acid sensitive compounds while digesting lipids. The removal of these interferences is crucial as co-extracted lipids, sulphur and other interferences can disturb GC separation and detection, leading to unreliable results (US EPA 1996d). This is followed by acid-basic-neutral silica that removes any remaining acid sensitive compounds. The variation in pH assists with the removal of remaining interferences (US EPA 1996c), such as the majority of PAHs and other aliphatic/ oxidisable compounds. Basic alumina removes most pesticides and finally the carbon removes most of the remaining organic compounds with only the planar compounds, such as PCDD/Fs and dioxin-like PCBs remaining in the extract.

Fluid Management Systems, Inc (FMS) have patended the Total Rapid Preparation TRP™ (PLE™ - Pressurized Liquid Extraction, Power-Prep™ – Multi-column Sample Clean-up and Evaporation) modules to process multiple samples simultaneously using disposable columns. The system greatly reduces sample preparation time as it is capable of extraction and clean-up of ten samples in parallel in less than two hours (Focant *et al*. 2002b, Focant & De Pauw 2002 and Focant *et al*. 2010). Methods for the sample extraction for PCDD/Fs have been described in the literature (Eppe *et al.* 2008a, Pirard *et al*. 2004a, Focant, Pirard *et al*. 2005b, Focant *et al*. 2007, Focant *et al*. 2009, Focant *et al*. 2010, Focant *et al*. 2011 and Patterson Jr *et al*. 2009).

5.2 PLE/ SOXHLET APPROACH IN SOUTH AFRICA

An opportunity to improve sample extraction capability in South Africa, following the extraction difficulties discussed in Chapter 4, arose when the South African Police Services Forensic Science Laboratory (SAPS-FSL) approached LECO Africa Separation Science Laboratory, NMISA and NWU, to assist with the extraction and analysis of hazardous waste samples. A hazardous waste treatment facility had been investigated by the SAPS-FSL for illegally disposing toxic materials in contravention of the South African Environment Conservation Act (Act 73 of 1989). These materials included toxic chemical residues generated in the course of manufacturing assorted chemicals such as pesticides and herbicides.

The SAPS-FSL is equipped with extraction and analytical instruments for chromatographic separation and analysis, but they did not have appropriate methods or capability for the complex sample preparation and analysis required for PCDD/Fs. Professor Jean-François Focant, University of Liège, Belgium, was approached to assist with sample extraction. The waste samples were sub-divided, with one half being sent to Belgium for extraction (Focant *et al*. 2001b and Focant *et al*. 2002b) and instrumental analysis by GC-HRMS (further described in Chapter 6; Eppe *et al.* 2004) and the second half sent to NWU for extraction and sample clean-up. PCDD/F analysis was performed at NMISA using GC×GC-TOFMS on both sets of extracts (Focant *et al*. 2003, Focant *et al*. 2004a, Focant *et al*. 2004b and Hoh et al. 2007) as described in a paper by De Vos *et al*. (2011a).

5.2.1 Soxhlet Extraction of Waste Samples

The first sub-set of waste samples was extracted using a Soxhlet apparatus at the University of Liége as described in papers by Focant *et al*. (2001b and 2002b).

5.2.2 ASE Extraction of Waste Samples

The second sub-set of waste samples was extracted using PLE (Dionex ASE® 100) according to the procedure outlined in US EPA Method 3545A (2007a). After mixing 1 gram of soil (sample size based on the information that the samples were heavily contaminated, otherwise a 5 - 10g sample would have been used as recommended in the literature; Focant *et al*. 2004a and 2004b) with an equal amount of Na_2SO_4 and

spiking with 10 µl of $^{13}C_{12}$ labelled internal standard (EDF-4144 solution from CIL), the samples were extracted with a mixture of hexane and dichloromethane (DCM) (1:3). The obtained extract was filtered through pre-extracted glass wool with Na_2SO_4 added to remove any residual water. The clean-up standard (CSS) was added and the extract was evaporated under a gentle stream of nitrogen at 60˚C to reduce the volume to approximately 0.5 ml. The extract was then reconstituted with 2 ml of dichloromethane prior to GPC partition to eliminate lipids and dispersed high-molecular weight compounds. After GPC treatment the extract was evaporated and reconstituted in 10 ml hexane. The extract then underwent acid digestion using a minimum of three concentrated H_2SO_4 aliquots followed by a 0.2% KOH and a 5% NaCl solution wash. The extract was filtered through pre-extracted glass wool with Na_2SO_4 added to remove any residual water and a florisil SPE clean-up was performed. The final extract was then evaporated to dryness and reconstituted in iso-octane to 50 µl prior to analysis.

Previously 40 grams of material was extracted for the soil and sediment survey as described in Chapter 4, section 4.3.1. However, the larger sample size proved impractical. With the larger extraction mass, although more of the analyte becomes available for concentration, there is a proportionately larger concentration of co-extracted material and the exhaustive clean-up becomes economically unrealistic. A 1 gram sample size is more practical as the clean-up has a greater efficiency and is more economical with respect to solvent, labelled standards, and ease of handling; caution must be taken to avoid homogeneity issues which could result from smaller sample masses. Sample size is influenced by the possible levels of actual PCDD/F contamination present.

Both sets of sample extracts (University of Liège, Belgium and NWU) were analysed qualitatively by GC×GC-TOFMS at NMISA to compare the effectiveness of the NWU extraction. After analysis, it was evident that the clean-up method used in Belgium (multilayer silica column comprising $AgNO_3$, 22% acid, 44% acid, basic, with the final concentrated extract passed through a Pasteur pipette type acidic silica column prior to injection) had not removed all the PAHs as expected and there was a broad band of branched phenolic compounds present (Figure 5.2). This complicates PCDD/F identification in the presence of other chlorinated and interfering compounds. GC-

HRMS is a target analysis technique; the high resolution mass measurement providing added selectivity, so the clean-up required is possibly not as exacting as for GC×GC-TOFMS.

The NWU ASE extraction appeared to be 'cleaner', although the recovery of PCDD/F analytes was not as efficient, possibly due to the additional steps added in the manual GPC clean-up process; 5 – 10% of the extract is lost. This was not pursued further as it is very expensive to investigate sample extraction, especially with isotopically labelled internal standards. These experiments convinced NMISA to invest in sample extraction and clean-up equipment and to establish an extraction and clean-up capability, together with the GC×GC-TOFMS analytical capability.

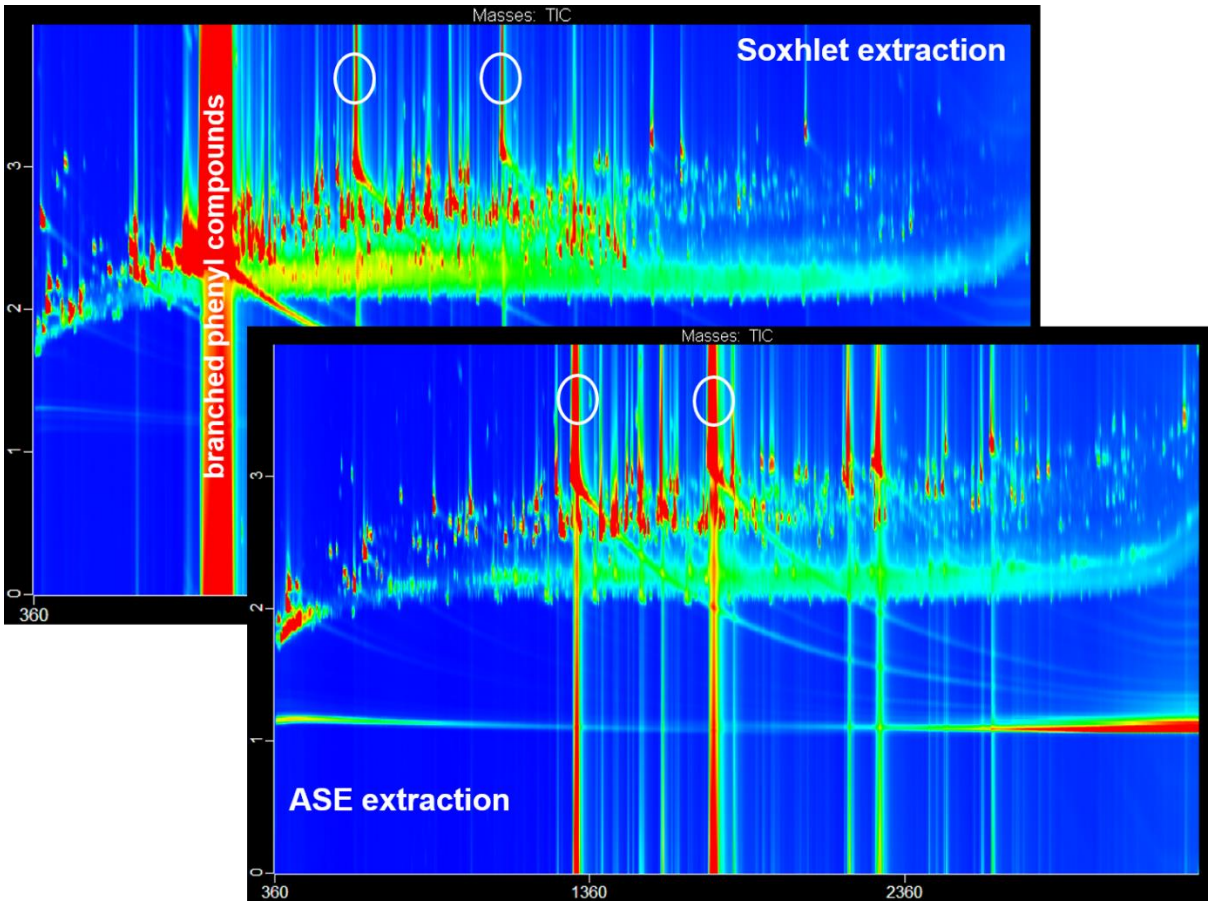


Figure 5.1. *Soxhlet and ASE extraction of South African toxic waste sample 3 (AIC chromatogram, LECO ChromaTOF© contour plot). The white circles indicate the presence of PAHs remaining in both extracts. Waste sample 3 was the most heavily contaminated (fly ash levels).*

5.3 AUTOMATED PREPARATION APPROACH

The TRP™ system combines three sample preparation processes into one automated system. It increases sample throughput while reducing manual errors, thereby improving recoveries. The TRP™ was used to implement and optimise the sample extraction and preparation method for PCDD/Fs in soil and sediment at NMISA (TRP™ *c* 2010).

5.3.1 Sample Extraction

Samples were extracted using the PLE™ Module. The extraction is based on a 5 – 10 g sample size, utilising two extraction cycles with toluene as extraction solvent. Matrix is mixed with the Hydromatrix™ drying agent and then packed into stainless steel extraction cells. All samples are spiked prior to extraction with [$^{13}C_{12}$]-labelled PCDD/F standards. The PLE™ cell was heated to 120 ºC and pressurised to 1700 psi. The stainless steel extraction cell has a 250 ml volume and the fill time and flush time of the packed cell is approximately 1 minute. The extraction cycle was repeated and the eluent then collected in a 250 ml evaporation tube. The toluene volume is approximately 250 ml. From an environmental perspective, the large volumes of toluene are a concern, but from a cost perspective, the silica, alumina and carbon clean-up columns are a more important cost consideration.

The eluents are evaporated to dryness under a gentle flow of nitrogen gas at 35 °C in the evaporation module and then spiked with the clean-up recovery standard (2,3,7,8-$^{37}Cl_4$-TCDD) and reconstituted in hexane for compatibility with the silica clean-up step.

5.3.2 Clean-up

The Power-Prep™ uses valve driven modules connected to the pump and pressure modules responsible for solvent flow and clean-up is performed at low pressure (5 - 30 psi). The extracts are subjected to multi-column (silica, alumina and carbon) chromatographic separation, fractionation and clean-up prior to instrument analysis using the Power-Prep™ module (Figure 5.2). The clean-up is designed to remove the majority of interferences while targeting only PCDD/Fs. Chemicals structurally similar to these compounds will be present in the final extracts and it is therefore essential to monitor these interferences during analysis. If a high level of interferences is still

shown during analysis, the extraction procedure should be repeated and a high capacity acidic silica column included in the clean-up procedure.

After the extraction, clean-up and evaporation steps have been completed the final extract is transferred to a GC vial and evaporated to dryness. The extract is then reconstituted in 25 µl *n*-nonane (end volume of analysis) containing the recovery internal standards (1,2,3,4-[$^{13}C_{12}$]-TCDD and 1,2,3,7,8,9-[$^{13}C_{12}$]-HxCDD), also referred to as the syringe standard, used to determine the recovery rates of the labelled standard. The loss of labelled standards represents the total loss during the extraction, clean-up and evaporation procedures. As with the labelled standard, the internal standard must mirror the concentration of the standards used in the calibration. The extracts are stored at 4 °C prior to analysis by GC×GC-TOFMS.

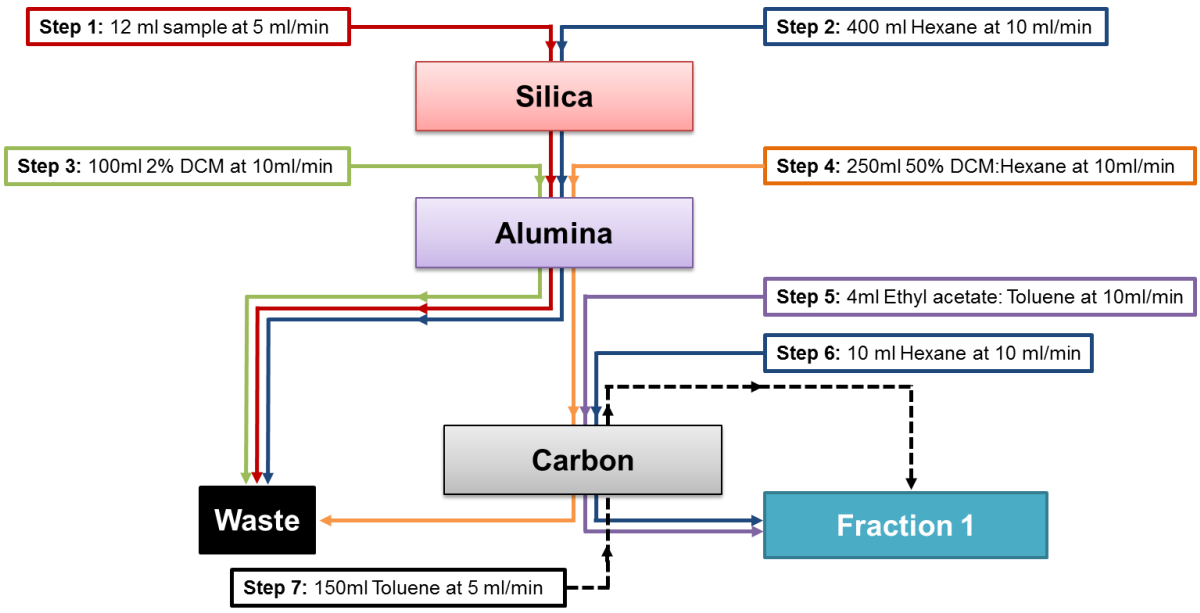


Figure 5.2. *Schematic flow chart for the automated clean-up system.*

5.4 NMISA EXTRACTION METHOD FOR PCDD/FS

5.4.1 Sample Preparation

A bilateral comparison was organised between NMISA and The Laboratory Services Branch, Ontario Ministry of the Environment, Canada (MOE). The study material

comprised South Africa soil samples and results were to be compared against a NIST Standard Reference Material (SRM 1944): New York/ New Jersey Waterway Sediment. The two participating laboratories each received five soil samples and the NIST SRM 1944 sediment for the comparison. Each laboratory used their standard methods for extraction and analysis of the seventeen toxic PCDD/Fs. NMISA participated with quantification by GC×GC-TOFMS and MOE using GC-HRMS. The validation is discussed in Chapter 8, with final results presented in Chapter 9.

The soil samples selected formed part of the study conducted at NWU in 2007 (described in Chapter 4). Samples were collected using pre-cleaned stainless steel or glass equipment and stored in the dark at 4 ºC (Commission Regulation 2006b). The samples were air dried for a minimum of three days in a dark room, manually ground using a mortar and pestle, and sieved three times (0,5 mm) to ensure sample homogeneity. US EPA Method 3545A (2007a: 1.4) does not recommend drying and grinding samples containing PCDD/Fs due to safety concerns, but US EPA Method 8290A (2007d) does provide clear safety information on handling hazardous samples and these were followed. Samples were weighed into 50 g portions in pre-cleaned amber bottles with Teflon-lined lids. An aluminium foil layer was placed between the cap and soil to prevent possible contamination from the melamine lid. Bottles were then sealed to prevent tampering, packed and shipped by courier to MOE. Samples analysed at NMISA were stored at room temperature. Two sub-sample sets were analysed by the participants and reported without correction or normalisation for water or carbon content. The soil and sediment samples were extracted at MOE according to the procedures outlined in the accredited method MOE 3418 (Kolic & Macpherson 2012, pg 75 appendix II). A summary of the methods applied by the two laboratories is provided in Table 5.1.

Table 5.1. *Summary of methodologies used for inter-comparison study between NMISA and MOE.*

Methodology	MOE	NMISA
Sample size	5 g soil and 8 g sediment	5 g soil and sediment
Extraction method	Soxhlet	PLE
Extraction solvent	Toluene	Toluene

Methodology	MOE	NMISA
Clean-up method	Multi-column solid phase extraction	Multi-column solid phase extraction
Analytical instrument	GC-HRMS	GC×GC-TOFMS
Chromatographic column(s)	Rtx®-Dioxin2 or DB-225	Rtx®-Dioxin2 and Rxi®-17SilMS
Method of quantification	Response factor	Linear regression
Type of calibration	External	External

5.4.2 Chemicals

For quantitation of the soil and sediment samples, US EPA Method 1613B calibration and verification solutions (EPA-1613CVS), labelled calibration solutions (EPA-1613LCS), internal standard spiking solution (EPA-1613ISS) and clean-up standard stock solutions (EPA-1613CSS) were selected for spiking and calibration purposes. These solutions were purchased from Wellington Laboratories *Inc.*, USA, and contained the seventeen native and corresponding ^{13}C-labelled PCDD/F congeners in *n*-nonane.

All solvents (acetone, hexane, methylene chloride, *n*-nonane and iso-octane) were high purity grade purchased from Burdick and Jackson (Honeywell International *Inc.*, USA). Sodium sulphate (Na_2SO_4) anhydrous, sodium chloride (NaCl) and potassium hydroxide (KOH) were purchased from Sigma Aldrich (Chemie GmbH, Germany). Liquid nitrogen and chromatographic pure grade helium gas (99.999%) were purchased from Air Products (Kempton Park, South Africa). The packing material used for sample extraction recovery was Chem Tube Hydromatrix® from Varian *Inc.* (supplied via SMM Instruments, South Africa). The consumables for the recovery determinations using the TRP™ system were purchased from FMS (Watertown, Massachusetts, USA) and included a 3 g '*in-cell*' clean-up column (packed with Na_2SO_4, and Hydromatrix®) for pressurized fluid extraction. Further sample clean-up included high capacity PCB-free Silica (#PCB HCDS), acid base neutral Silica (#PCBS: ABN), Alumina (#PCBA: BAS) and Carbon (#PCBC: CCG).

All apparatus and glassware (excluding the evaporation flasks) were pre-cleaned, air dried and solvent rinsed with high purity acetone and hexane prior to use and stored in pre-cleaned aluminium foil. The evaporation flask was treated with dichromic acid, manually washed, air dried and solvent rinsed. The Hydromatrix™ and Na_2SO_4 used during extraction was pre-cleaned following the US EPA Method 3540A (1996) Soxhlet extraction procedure to eliminate contaminants prior to use. The extraction was performed using toluene and carried out for 24 hours by maintaining a percolation rate of three cycles per hour. After extraction, the Hydromatrix™ and Na_2SO_4 were oven dried at 100 ºC overnight and stored in air tight pre-cleaned amber flasks.

5.4.3 Method Summary

An extraction procedure was developed and implemented for the mass fraction (pg/g) determination of the seventeen toxic incurred PCDD/Fs using the TRP™ system, following the methods outlined in US EPA Method 3545A (2007a) and the TRP™ operating manuals (TRP™ *c* 2010). Figure 5.3 provides a summary of the final extraction and clean-up method used at NMISA for the analysis of PCDD/Fs in soil and sediment using GC×GC-TOFMS.

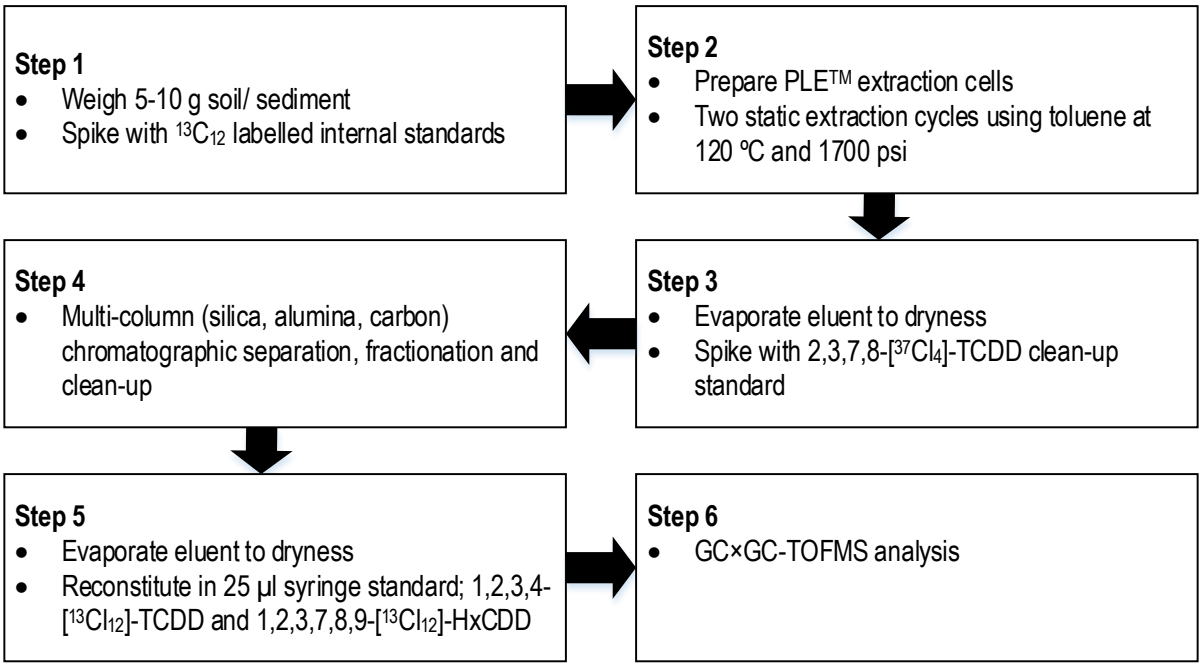


Figure 5.3. *Flow chart summarising the extraction and clean-up process.*

6

SCREENING AND SELECTIVITY

Since GC×GC-TOFMS is not a target compound technique, it is ideal for screening for multiple classes of environmental pollutants in a single analysis (Cochran & Frame 1999, Focant *et al*. 2004b and Reiner 2010). This is one of the advantages of TOFMS over other forms of mass spectrometry, in that it is capable of providing full mass range spectral data even at very low levels. Chapter 6 provides an overview of qualitative screening for chlorinated compounds, thus providing information on the compound classes present in complex sample extracts that can be used to effectively determine whether PCDD/Fs are actually present and whether expensive quantitative analysis is really necessary.

Selectivity (specificity) is a quantitative indication of the extent to which a method can distinguish between the analyte of interest and any interfering substances remaining in the sample extract and is one of the key criteria for the evaluation of an analytical method (Van Zoonen *et al.* 1998). To this end, isomer specificity of the GC columns employed for the determination of 2,3,7,8-TCDD and 2,3,7,8-TCDF will be discussed. Elements of this work have been published in the papers by De Vos *et al*. (2011a) and De Vos & Gorst-Allman (2013). The isomer specificity using the Rtx®-Dioxin2/ Rxi®-17SilMS column combination is still to be published.

6.1 SCREENING WITH GC×GC-TOFMS

6.1.1 Toxic Waste Disposal

The disposal of toxic waste is of enormous concern to authorities in South Africa and other developing economies (Batterman *et al.* 2009, Fiedler 2007 and Fiedler 2013). Facilities are not as rigorous or as well maintained as those in more developed

countries, and poorer countries are sometimes driven to import toxic waste for disposal as a means of raising revenue (Birnbaum and de Vito, 1995 and Schecter *et al*, 2006). Monitoring procedures and methods are generally poor and legal controls are not in place to govern disposal and handling. This allows unscrupulous operators to profit by waste disposal that is criminally negligent (Bouwman 2004, GroundWork 2006 and Lauridsen 2008).

The accepted confirmatory methodology for the qualitative analysis of priority pollutants of environmental concern, and the specific quantitation of PCDD/Fs and dioxin-like non-*ortho* substituted PCB compounds in hazardous waste is GC-HRMS (Cochran & Frame 1999, Eppe *et al*. 2004, Focant *et al*. 2001a, Kolic & Macpherson 2012, Korytár *et al*. 2002, Reiner *et al*. 2006 and US EPA 1994). An alternative approach using GC×GC-TOFMS as an emerging technology that can be used to analyse complex samples has been implemented for the analysis of the seventeen toxic WHO PCDD/Fs (Cochran 2002 and Dorman *et al*. 2008b and Focant and Pirard *et al*. 2005a).

The Forensic Science Laboratory of the South African Police Services (SAPS) has investigated several cases involving hazardous and toxic waste dumping and disposal in South Africa. One such toxic waste case was the reason Dr Jean-François Focant, University of Liège, Belgium, Dr Peter Gorst-Allman, LECO Africa Separation Science Laboratory and NMISA became involved with developing a screening method for PCDD/Fs in collaboration with the SAPS. At the time there was no established extraction capability available and the toxic waste samples were sent to Dr Focant for extraction and analysis using GC-HRMS. The sample extracts were sent back to South Africa for screening and quantitation at NMISA. The study contributed to a first publication on PCDD/Fs (De Vos *et al*. 2011a).

GC×GC is known for increased sample peak capacity, making it an ideal tool for screening a complex toxic waste samples (Dimandja *et al*. 2003, Dorman *et al.* 2008a, Focant, Sjödin *et al*. 2004b, Marriott & Shellie 2002 and Mondello *et al*. 2008). The sensitivity is enhanced due to the focussing provided by the modulator (Dimandja 2004 and Shellie *et al*. 2003). The use of GC×GC-TOFMS as a non-

targeted screening tool to determine the presence of chlorinated compounds will be examined.

Isomer specificity (congener distribution and separation) will also be discussed as it directly impacts the individual congener contribution of the seventeen toxic PCDD/Fs to the total toxicity of a sample. Regulatory decisions are based on risk management outcomes that rely on accurate reporting of TEQ results (Reiner *et al.* 2006) and therefore accurate analysis of the isomer-specific concentrations of the seventeen congeners is required to assign a correct TEF value to each component (Van den Berg *et al*. 2006).

6.2 NON-TARGETED SCREENING

6.2.1 Scripting and Classifications

Scripting is an automatic screening technique that can be effectively used to identify components in a sample based on mass spectral characteristics, e.g., chlorine isotopes (Hilton *et al*. 2010, LECO 2008a, LECO 2008b and Skoczyńska *et al*. 2004 and van Stee & Brinkman 2011). The technique can be used to screen a large quantity of spectral data for characteristics consistent with halogenated compounds.

Preliminary analysis of the toxic waste samples indicated that the incineration process had not been sufficient to completely destroy harmful organic compounds present (Fiedler 2007, Stanmore 2004 and Tuppurainen *et al*. 1998). This raised concerns that many toxic compounds were not being identified in complex samples by one dimensional chromatography, and that the disposal process was improperly carried out at too low temperatures, leading to the creation of more toxic compounds, such as PCDD/Fs. The advantage of the GC×GC-TOFMS approach is that it is possible to locate and identify numerous additional compounds in a single run using full range mass spectra to confirm compound identity.

The toxic waste samples were analysed using the LECO Pegasus® 4D GC×GC-TOFMS (described in Chapter 4) and the data processed using the LECO ChromaTOF® software *True Signal Deconvolution®* and *Automated Peak Find* algorithms to locate and identify compounds and to provide clean, library searchable spectra (LECO 2009a, LECO 2011 and Peters *et al*. 2013). GC×GC-TOFMS is

capable of handling complex samples, but the data generated is very complex and data processing routines are required if the full potential of GC×GC-TOFMS is to be realized. GC×GC chromatographic data is structured and structurally similar compounds will elute in the same areas of the chromatogram under the right conditions and can be grouped or classified. Another feature of the software that can be used to further search for chlorinated compounds is *Mass Spectral Filtering* (selected ion plots), which can be used to selectively view certain compound groups in a contour plot. Further features of the software that can be used to facilitate the detection of hazardous compounds are known as '*Classification'* and '*Scripting*.' *Classifications* can be used to define areas of the two dimensional chromatogram where compound classes occur, and *Scripting* uses *VBScript* programming to establish rules for filtering spectral data to locate compounds with defined spectral properties (Cochran *et al*. 2005, Korytár *et al*. 2005, LECO 2008c and Mondello *et al*. 2007). An interesting study by Zeng *et al.* (2012) also indicates the use of component correlation to achieve similar results.

To illustrate what is possible with the *Scripting* technique, a number of figures will demonstrate how compound classes can be located by plotting chromatograms displaying selected ions that are characteristic of the compound class. The contour plot of the '*Analytical Ion Chromatogram'* (AIC) provides an overall picture of the number of compounds present in the sample (extraction dependant). The AIC plot is similar to the '*Total Ion Chromatogram'* (TIC), but shows only the located peaks without column bleed ion interference; it therefore serves as a type of filter. This ChromaTOF® software feature is shown in Figure 6.1 for one of the toxic waste samples. The second dimension retention time reflected is from 1,6 to 4,0 s to enlarge the area that contains many compounds (over 7360 were located at a unique ion mass with a signal to noise of 100) and it is evident that it will be both tedious and difficult to manually sort all the compounds present for targeted priority pollutant classes and structural features in such a complex chromatogram, and also to identify specific chlorinated compounds of interest. In addition to PCDD/Fs, the toxic samples analyzed contained numerous other environmental pollutants. Among these were PCBs, PAHs, polychloro naphthalenes (PCNs), terphenyls, chlorinated

terphenyls, chlorinated furans and chlorinated pyrenes, as well as several other chlorinated and brominated aromatics.

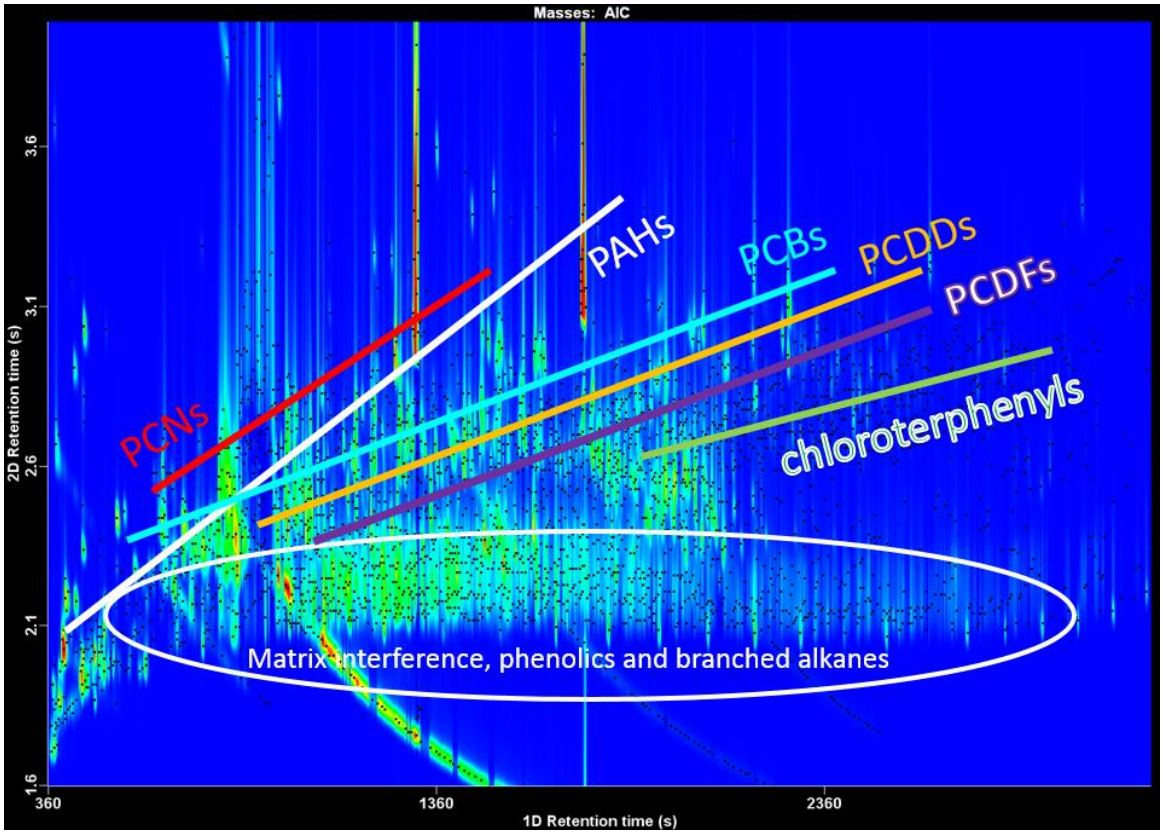


Figure 6.1. *Analytical Ion Chromatogram contour plot showing approximate areas where selected priority pollutant classes occur. Extracted masses would assist with identifying compound classes, but takes up valuable analytical time when searching specifically for chlorinated species (De Vos et al. 2011a).*

To simplify such a complex chromatogram to an extent, extracted masses can help to visualise compound groups and to establish the presence of homologue classes in the absence of standards. As an example, Figure 6.2 shows the chloroterphenyl compounds present in the sample. By using the extracted masses for the chloroterphenyls, it is possible to identify a homologue series that can assist in identifying compound classes actually present in the sample. This form of screening is useful if the analyst is familiar with the masses characteristic of the various priority pollutant compound classes, but it is time consuming (Bouwman 2004, De Boer *et al*.

2008, Fiedler 2007, Focant *et al*. 2002a, Focant, Sjödin *et al*. 2004b and Stockholm Convention 2010).

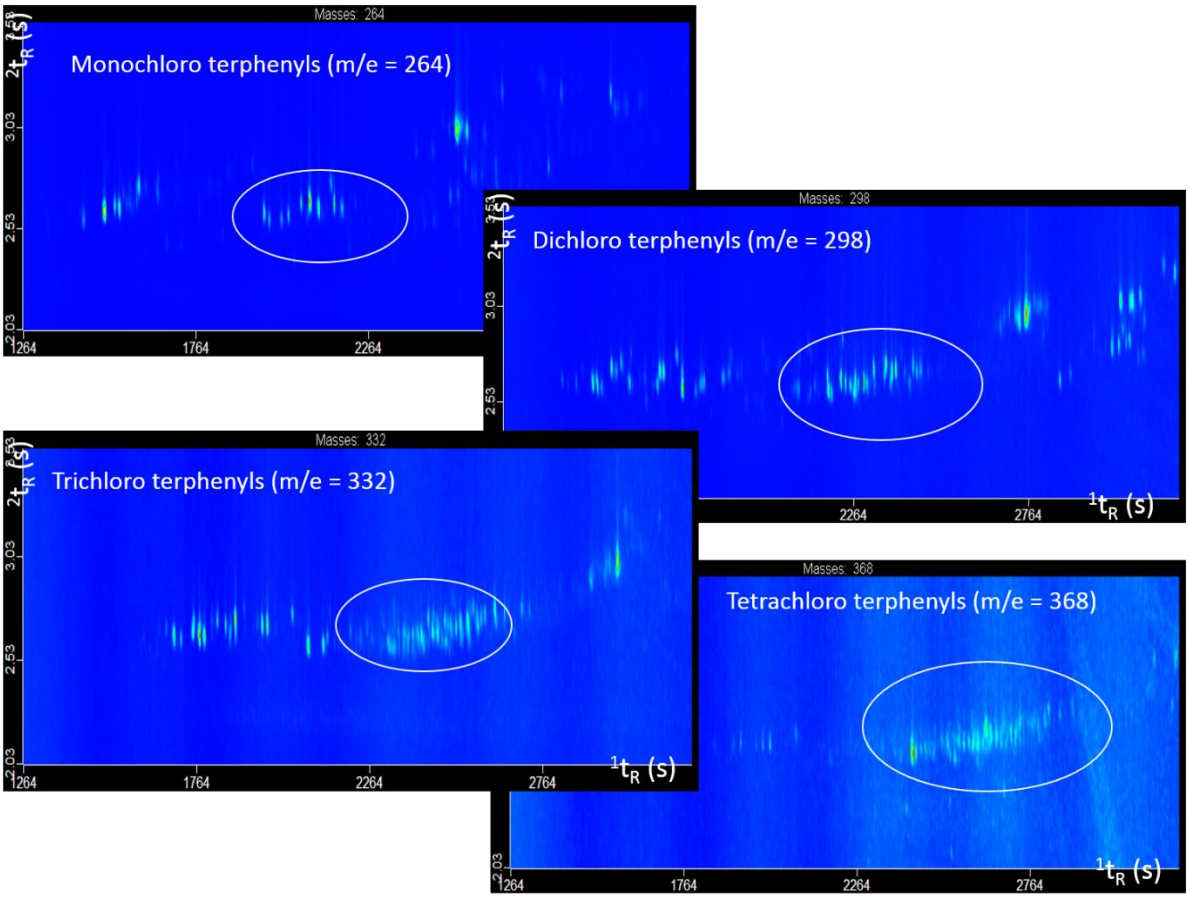


Figure 6.2. *Contour plots showing extracted masses for chloroterphenyl compounds.*

Classifications and *Scripting* are particularly useful in searching for compounds that contain either chlorine or bromine atoms. The isotope patterns found in the mass spectrum of a compound containing these atoms (Cl, Br, S …) are characteristic and easily located using a *Script* function. *Scripting* allows prediction of isotope patterns for specific atoms, then searches for these predicted patterns in the spectra recorded for a sample. This can then be used to refine *Classification* results, by removing compounds, which although contained in a spatial region, do not rightfully belong to the classification group. Many highly hazardous compounds are chlorinated and the ability to locate these quickly and efficiently enhances sample evaluation.

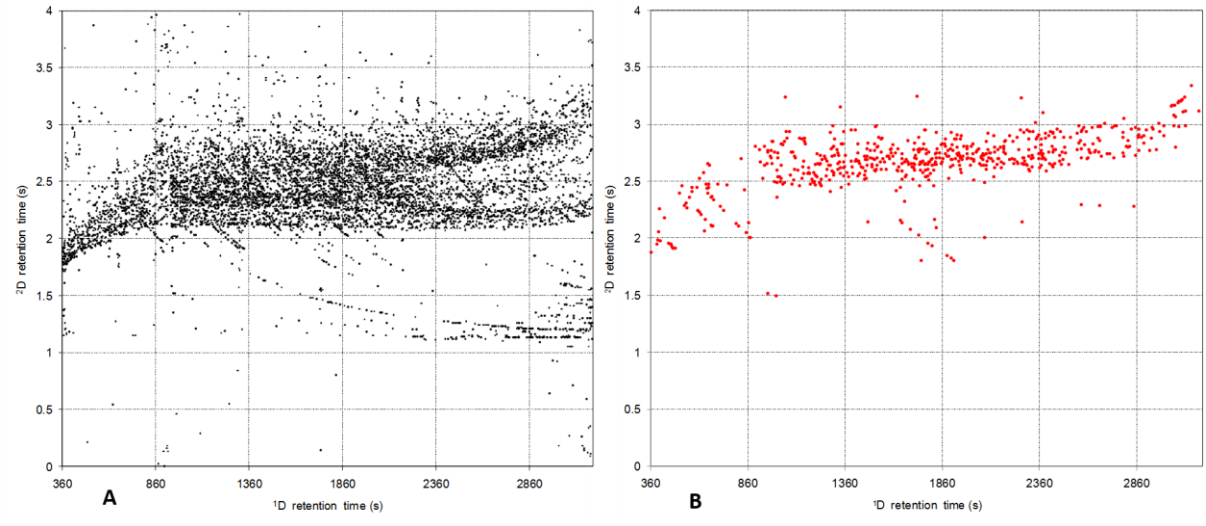


Figure 6.3. *Microsoft Excel apex plot showing all the compounds in the sample (A) and the chlorinated compounds located using the scripting software (B) present in the sample, processed at signal to noise (S/N) of 100 (De Vos et al. 2011a).*

Figure 6.3 shows the same data as presented in Figure 6.1, but in the form of an *Apex Plot* generated using *Microsoft Excel*. Using this simplified presentation of the data, only the position of the different compounds is shown and no intensity data is presented. The *Apex Plot* shows the results of the automated spectral search for chlorinated compounds in the sample using the *Script* as described (*the script used was "AllChlorine", obtained as part of the ChromaTOF® software package*) in which chlorinated compounds have been located using mass spectral filtering of the data, searching for multiple chlorine isotope ratio patterns. The majority of the chlorinated compounds lie in a restricted area across the chromatogram, and this approach allows a fast, convenient screen to determine the extent of chlorination in the sample (extremely high in this case). The number of located compounds is greatly reduced, from 7368 to only 603, permitting many potential chlorinated priority pollutants to be located.

By selecting appropriate ions it is possible to highlight some of the individual compound classes present in the sample, as is shown in the *Apex Plot* chromatogram in Figure 6.4. Detailed information can be obtained in this manner about complex samples, and the samples are easily screened for numerous pollutant

classes. In addition, the data can be presented in a simple format that is easy to visualize, providing information on the chlorinated compounds contained in the sample.

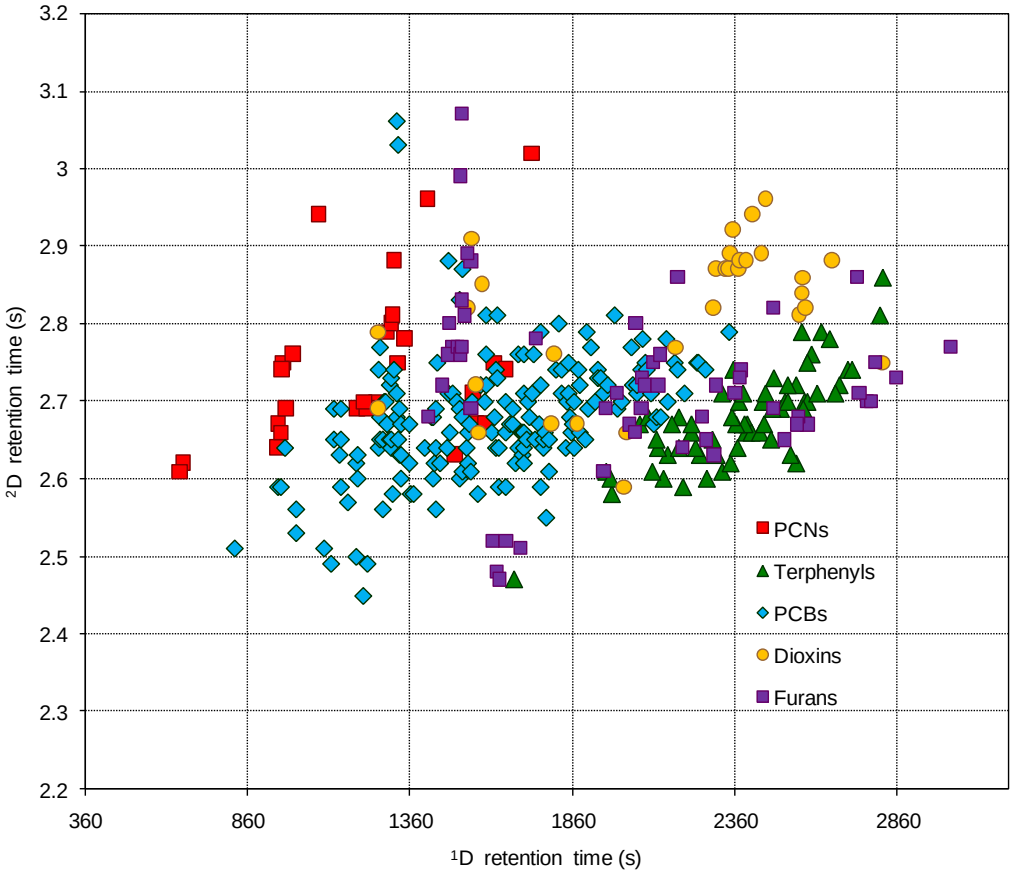


Figure 6.4. *Microsoft Excel Apex Plot of selected chlorinated extracted ions (De Vos et al. 2011a). The ^2t_R is focused between 2,2 and 3,2 s to enlarge the area for the chlorinated compounds. This can be compared to the area at ^2t_R = 2,6 s in Fig 6.1 and Fig 6.3 B.*

Table 6.1 identifies some of those priority pollutant classes detected in the toxic waste sample. This approach provides an indication based on filtering for chlorinated compounds using *Scripts*. Mass spectra of individual compounds would still have to be inspected to verify the isotope pattern (cluster) matches. This can be seen when looking at the location of the PCDFs (purple squares); not every purple square is a PCDF.

Table 6.1. *Priority pollutant classes identified in a toxic waste sample.*

Symbol	Compound Class
	Polychlorinated naphthalenes (PCNs) are characterized by major ions at m/z = 162, 196, 230, 266, 300, 334, 368 and 404.
	Similarly, by plotting the major characteristic ions for the chlorinated terphenyls (m/z = 264, 298, 332, 368, 402, 436, 470 and 506), the mono- to octa- chlorinated terphenyls can be located in the sample.
	Polychlorinated biphenyl compounds (PCBs) with m/z = 188, 222, 256, 292, 326, 360, 394, 428, 464, and 498 can also be found.
	PCDDs with m/z = 218, 252, 286, 322, 356, 390, 424, and 460 are clearly present in the sample.
	PCDFs with m/z = 202, 236, 270, 306, 240, 374, 408 and 444 are also present in the sample

The acquisition of full range mass spectra for all the compounds in the toxic waste sample means that in one run the sample can be examined for all the pollutants of interest. It provides a quick and effective snap shot of the chlorinated compounds present in the sample. This screen is only as effective as the GC and MS parameters selected and the sample extraction and clean-up approach followed. As soon as a sample is targeted for PCDD/Fs, the other classes of priority pollutants will no longer be located. The approach is ideally suited to a quick appraisal of a sample and not intended as a targeted extraction. This saves valuable analytical time when a sample is screened for chlorinated compounds.

6.3 ISOMER SPECIFICITY/ SELECTIVITY

A very important quality criterion of an analytical method is its capability to deliver signals that are free from interferences and provide accurate results. The ability to discriminate between the analytes of interest and interfering components is expressed as the selectivity of a method and measurement system. Selectivity refers to the degree to which the method can be used to determine particular analytes in mixtures and/ or matrices without interference from other components of

similar structure (Cochran *et al*. 2012, Marriott *et al*. 2012, Reiner 2010, Storr-Hansen *et al*. 1992).

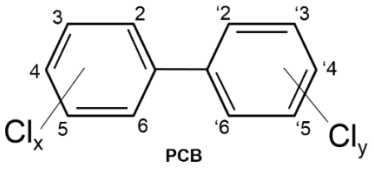
As described in Chapter 2, section 2.3.2, PCDD/Fs and dioxin-like PCBs represent a class of organic compounds that exhibit potential risks for human health. The GC separation and determination of individual PCDD/F's and dioxin-like PCBs is complex because of the potential co-elution of structurally related compounds (isomers), which cannot be distinguished with selected ion monitoring. The mono-*ortho*-chlorine substituted PCB congeners may adopt a coplanar conformation and become isosteric with the toxic PCDD/F congeners with chlorines arranged in the 2,3,7,8-position. The more congeners present, the higher the risk of positive bias due to co-elution with structurally related compounds. Of the 209 PCB congeners, the WHO assigned dioxin toxic equivalency factors (TEF) to twelve of the PCB congeners that might contribute to the total dioxin-like toxicity (Van den Berg *et al* 1998 and 2006). These congeners consist of eight mono-*ortho* and four non-*ortho* chlorinated biphenyl congeners (Table 6.2; Focant, Sjödin *et al*. 2004b and Korytár 2006a).

In addition to possible PCB interferences, PCDD/F congener co-elutions also have to be considered. PCDD/F congeners consist of up to eight chlorine atoms positioned on the respective structures, resulting in 75 PCDDs and 135 PCDFs. Of these 210 compounds, only 49 PCDDs and 87 PCDFs consist of four to eight chlorine atoms. In this group of 136 PCDD/F compounds, the congeners with chlorines arranged in the 2,3,7,8-position are considered to be the most toxically significant. By definition, in order to calculate the total TEQ of a mixture, the concentration of each dioxin-like component in the mixture must be known and hence the need for sensitive and reliable congener-specific chemical analyses.

In South Africa, the analysis of these seventeen PCDD/F congeners is challenging when analysing real samples due to the relatively low concentrations prevalent in the environment. The analysis is further complicated when the PCDD/Fs must be detected and quantified in the presence of other priority pollutants that occur at higher concentrations; sometimes more than ten orders of magnitude higher. The presence of other possible PCDD/F congeners must also be considered when

targeting only the seventeen toxic congeners, including known co-eluting compounds like the PCBs.

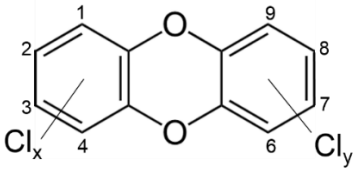
Table 6.2. *Structural formula and number sequence of the 12 toxic WHO dioxin-like PCBs and tetra through octa- chlorinated PCDD/Fs.*



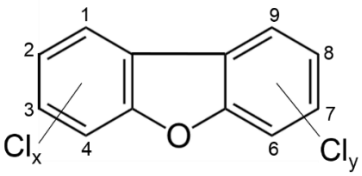
PCB

PCB Number	Structure	PCB Number	Structure
PCB 77	3,3′,4,4′-Tetrachlorobiphenyl	PCB 126	3,3′,4,4′,5-Pentachlorobiphenyl
PCB 81	3,4,4′,5-Tetrachlorobiphenyl	PCB 156	2,3,3′,4,4′,5-Hexachlorobiphenyl
PCB 105	2,3,3′,4,4′-Pentachlorobiphenyl	PCB 157	2,3,3′,4,4′,5′-Hexachlorobiphenyl
PCB 114	2,3,4,4′,5-Pentachlorobiphenyl	PCB 167	2,3′,4,4′,5,5′-Hexachlorobiphenyl
PCB 118	2,3′,4,4′,5-Pentachlorobiphenyl	PCB 169	3,3′,4,4′,5,5′-Hexachlorobiphenyl
PCB 123	2′,3,4,4′,5-Pentachlorobiphenyl	PCB 189	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl

PCDD



PCDF



Chlorines (x+y)	Acronyms	No. of PCDD isomers	No. of PCDF isomers
4	TCDD/F	22	38
5	PCDD/F	14	28
6	HxCDD/F	10	16
7	HpCDD/F	2	4
8	OCDD/F	1	1
Total		**49**	**87**

For reliable dioxin analysis, US EPA Method 1613B (1994a) stipulates that the isomer specificity for the GC columns employed for the determination of 2,3,7,8-TCDD/F must be demonstrated. To determine the congener separation for GC×GC-TOFMS, an isomer-specificity test standard (CIL EDF-4147 *GC Retention Time Window Defining Solution and Isomer Specificity Test Standard,* Cambridge Isotope Laboratories, Massachusetts, USA), containing the most closely eluting isomers separated on a 5% : 95% diphenyl : dimethylpolysiloxane column phase, was

selected since the ^{1}D separation is crucial for the success of the analytical separation.

The GC parameters, as stipulated in the certificate for the CIL EDF-4147 test standard, were followed in order to mimic the US EPA Method 1613B separation as closely as possible. This would then serve as the point of departure for further evaluation of the NMISA GC parameters with regard to isomer specificity (selectivity) as detailed in Chapter 4. The TCDD/F congeners included 1,3,6,8-TCDD, 1,2,8,9-TCDD, 2,3,7,8-TCDD, 2,3,7,8-TCDD ($^{13}C_{12}$, 99%), 1,2,3,7/1,2,3,8-TCDD, 1,2,3,9-TCDD, 1,3,6,8-TCDF, 1,2,8,9-TCDF, 2,3,7,8-TCDF, 2,3,7,8-TCDF ($^{13}C_{12}$, 99%), 2,3,4,7-TCDF and 1,2,3,9-TCDF at a nominal concentration of 200 ng/ ml in *n*-nonane. The penta, hexa and hepta PCDD/Fs congeners in the test mix were also separated, but are not discussed in detail since the aim here was to achieve an optimal separation of the 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners using the Rxi®-XLB ^{1}D column and to then optimise the ^{2}D separation using the Rxi®-XLB/ Rtx®-200 column set. The column performance of the XLB phase (low polarity proprietary phase) is similar to a 5% : 95% diphenyl : dimethylpolysiloxane phase for dioxin analysis, so it is reasonable to assume that this standard would provide a good test for the Rxi®-XLB phase too.

6.3.1 One-Dimensional Separation

The ^{1}D separation obtained using the CIL EDF-4147 test standard, following the temperature programming conditions provided in the certificate of analysis, is shown in Figure 6.5-A. An Rxi®-5SilMS column was used for the ^{1}D separation since the CIL certificate method stipulated a DB-5 column or equivalent. The separation of all the TCDD/F is not shown in Figure 6.5, only the area around the 2,3,7,8 TCDF/ 2,3,7,8-[$^{13}C_{12}$]-TCDF and 2,3,7,8 TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD native and labelled compounds, which is enlarged to demonstrate the ^{1}D separation for these two compounds (segments overlaid as each column method is applied). Chromatographic overlap is evident for the 2,3,7,8-TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD compounds by other co-eluting TCDD isomers and one TCDF isomer. The four dioxin isomer peaks (1,2,3,7/ 1,2,3,8, 1,2,3,9 and 2,3,7,8-TCDD) co-elute to such a

degree that it would not be possible to accurately quantify 2,3,7,8-TCDD with this ^{1}D GC method.

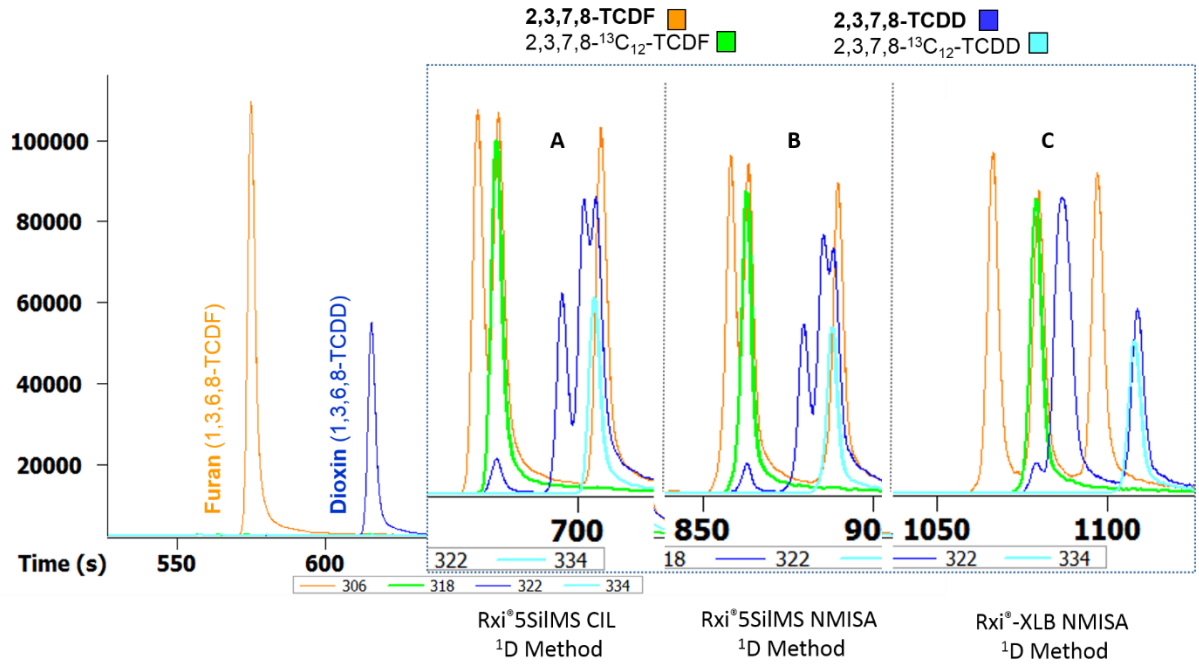


Figure 6.5. *^{1}D separation on the Rxi®-5SilMS column indicating $^{1}t_R$ (s). Each separation is then superimposed, first using the temperature programming parameters given in the certificate of analysis (**A**), then using the multi-step temperature programme (Table 4.1; **B**) and finally the multi-step method applied to the Rxi®-XLB column (**C**). The 2,3,7,8 TCDF/ 2,3,7,8-[$^{13}C_{12}$]-TCDF isotope is well separated from the nearest furan and dioxin; as are the 2,3,7,8 TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD pair.*

Using the same Rxi®-5SilMS column, but with the NMISA GC ^{1}D method parameters produced the separation shown in Figure 6.5-B. As with the previous results, the co-elution with 2,3,7,8-TCDD is still apparent, indicating that accurate quantitation of this compound would not be possible using a DB-5 or Rxi®-5SilMS ^{1}D column phase and either CIL or NMISA GC method parameters, but it does confirm compliance with the US EPA Method 1613B (1994a) requirements for using a DB-5 equivalent phase.

Figure 6.5-C shows a clear improvement in the ^{1}D separation of the 2,3,7,8-TCDF/ 2,3,7,8-[$^{13}C_{12}$]-TCDF and 2,3,7,8-TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD native and labelled compounds with the NMISA multi-step temperature programme described in Chapter

4, Table 4.1) and the Rxi®-XLB column phase. Separation from the other isomers contained in the standard mix was achieved, although co-elution of other TCDD isomers has occurred. The separated native and labelled 2,3,7,8 TCDD/F peaks can be quantified with the ChromaTOF® software deconvolution algorithm using the extracted masses for the respective peaks.

The ^{1}D separation demonstrates the isomer selectivity conformance with the requirements for using a DB-5 phase or equivalent for PCDD/F separation. However, the ^{1}D separation using GC-TOFMS cannot reach the low levels (500 fg on column for 2,3,7,8-TCDD) required in US EPA Method 1613B (1994a). In addition, when analysing environmental sample extracts, both matrix interference and any column bleed present can interfere with the detection and quantitation of the target analytes in these samples. With improved separation established with the Rxi®-XLB phase, the added resolving power of GC×GC-TOFMS was then investigated to ensure that accurate quantitation of the PCDD/F analytes can be achieved.

6.3.2 Two-dimensional (GC×GC) Separation

Figure 6.6 shows the contour plots for the ^{2}D separation of the TCDFs and TCDDs respectively, using the Rxi®-5SilMS/ Rtx®-200 and Rxi®-XLB/ Rtx®-200 column combinations. From the chromatograms, it can be seen that the 2,3,7,8-TCDF/ 2,3,7,8-[$^{13}C_{12}$]-TCDF and the 2,3,7,8-TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD separation with the Rxi®-XLB phase has been achieved with no overlapping interference from the other TCDD/F isomers present. The black dots visible in the contour plots are de-convoluted peak apexes for the extracted ions representing the TCDD/F isomers. The extracted masses for TCDF (m/z = 306, 308) will show the peak apexes for the TCDFs, but also the position in the TCDD peaks in the TCDF contour plot and vice versa. The ^{1}D and ^{2}D separation was described in the paper by De Vos *et al.* (2011b).

The 2,3,4,7-TCDF co-elutes with 2,3,7,8-TCDF/ 2,3,7,8-[$^{13}C_{12}$]-TCDF when using the Rxi®-5SilMS column phase, but is clearly separated from the 2,3,7,8 native and labelled peaks when using the Rxi®-XLB column phase (Figure 6.6).

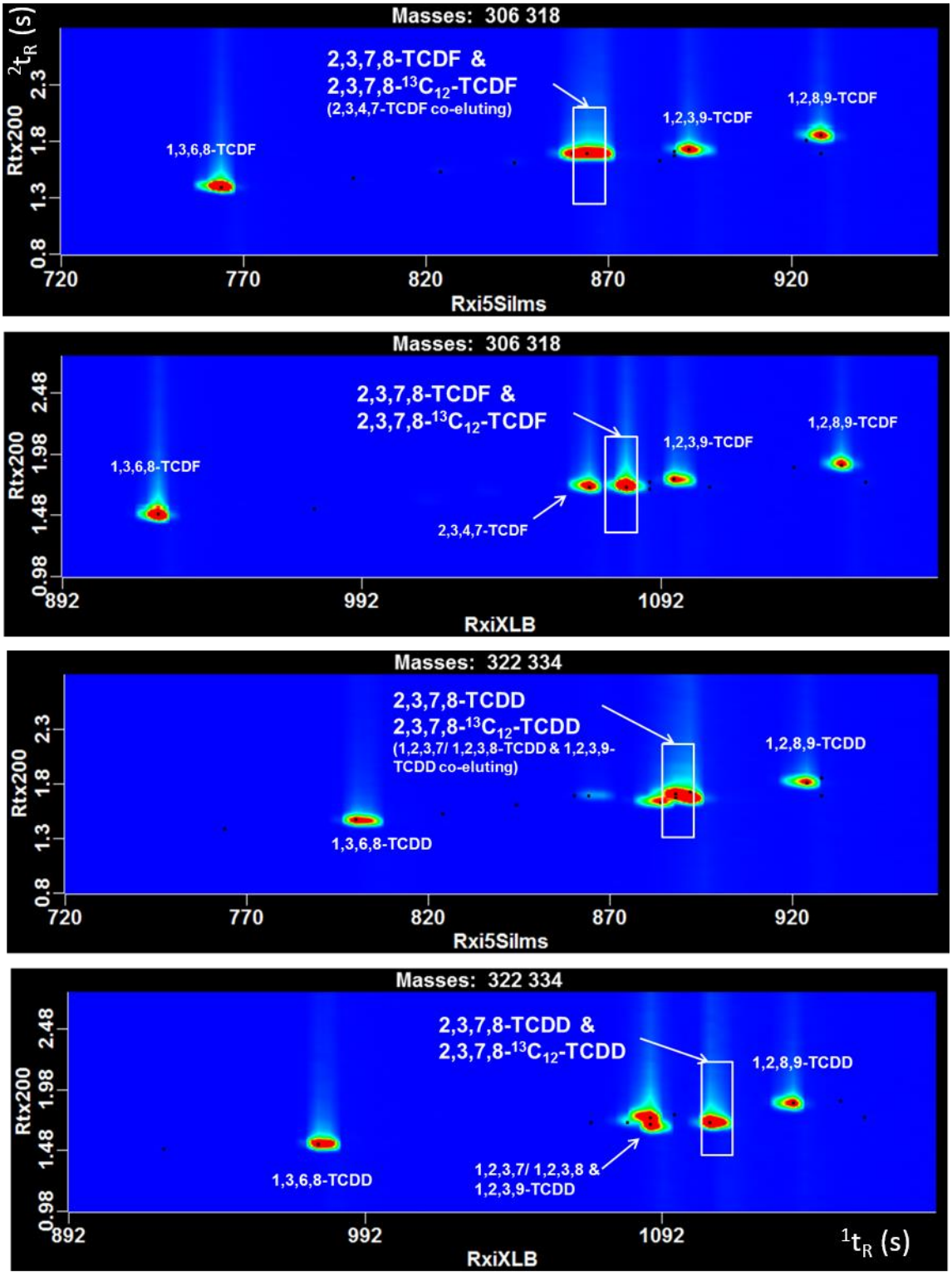


Figure 6.6. *^{2}D TCDF and TCDD separation using the Rxi®-5SilMS/ Rtx®-200 and the Rxi®-XLB/ Rtx®-200 column combinations. The isomer co-elutions are resolved using the Rxi®-XLB/ Rtx®-200 column combination, clearly separating the 2,3,7,8-TCDF and 2,3,7,8-TCDD (De Vos et al. 2011b).*

The co-elution of the 1,2,3,7/ 1,2,3,8 and 1,2,3,9-TCDD isomers with 2,3,7,8-TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD using the Rxi-5SilMS column phase was also resolved. With the Rxi®-XLB phase, the elution order has shifted and the isomer co-elution of 1,2,3,7/ 1,2,3,8 and 1,2,3,9-TCDD with 2,3,7,8-TCDD/ 2,3,7,8-[$^{13}C_{12}$]-TCDD is resolved, thus ensuring that the 2,3,7,8-TCDF and 2,3,7,8-TCDD congeners are separated from the other isomers and can be accurately quantified using the ChromaTOF® software deconvolution algorithm and the Rxi®-XLB phase.

6.3.3 Optimal Flow and Heating Rate for Isomer Separation

Having establised good GC×GC-TOFMS separation for the TCDD/Fs using the Rxi®-XLB phase, further method development with the Rxi®-XLB/ Rtx®-200 combination could have continued to fully establish its use for accurate quantitation, but a decision was taken to once again utilise the Rtx®-Dioxin2 column and move away from the multi-temperature program that would provide improved separation based on sound GC principles. Any given separation requires a combination of speed, capacity and resolution (Reiner *et al*. 2010), with analytical goals or requirements dictating which attributes are favoured over the other. The isomer specificity evaluation using the Rxi®-XLB/ Rtx®-200 combination will be revisited at a later stage as this column combination is potentially still a viable combination for PCDD/F separation.

Blumberg and Klee (2000) published a paper describing aspects for optimal heating rate (OHR) in GC. To increase the speed of GC analysis, it is possible to increase the carrier gas flow rate; and this in turn is achieved by increasing the temperature program heating rates, using a faster carrier gas (such as hydrogen), reducing the column length, the column diameter, the thickness of the stationary phase and using a detector that operates at a lower outlet pressure. Giddings (1984) proposed the existence of an optimal heating rate in GC in the early 1960s but could only suggest semi-empirical formulae for its calculation. An increase in the heating rate causes a reduction in peak capacity, so it is important to carefully consider underlying GC principles. Optimal heating rate provides an adequate separation of the analytes in the shortest possible time.

The ideas on speed optimized flow (SOF) were also developed by Blumberg and Klee (2002). Both speed optimised flow and optimal heating rate are aimed at maximizing peak capacity under relatively fast analysis conditions. SOF is the flow value which gives the shortest analysis time for a given plate number (number of theoretical plates or column efficiency) in a column of a given diameter. Smaller diameter columns are a means of speeding up analyses, so by reducing the diameter, a higher efficiency per column length is produced. A shorter column can be used to give the same separation. When the diameter is reduced, the optimal linear velocity is also faster. This leads to a shorter void time and a shorter analysis time with the same separation power. The penalties to be paid are a much lower sample capacity and much higher carrier gas pressures required to perform a run. Applying this concept provides no difference in separation efficiency, but analysis time is reduced. By using optimised flow and column heating rate, the peaks are kept narrow and this improves sensitivity. Klee *et al*. (2015) have further investigated conditions that yield a near theoretical maximum in peak capacity gain.

The approach taken by Hoh *et al.* (2007), using the Rtx®-Dioxin2 column, was revisited, but taking SOF and OHR into consideration. Instead of a 60 m x 0,25 mm x 0,25 µm first dimension column, a shorter column with narrower diameter and film thickness (40 m x 0,18 mm x 0,18 µm) was selected, with a different column phase for the second dimension. Instead of an Rtx®-PCB ^{2}D column, an Rxi®-17SilMS was selected for its affinity for PAH separation and to move PAH compounds away from the PCDD/Fs. This separation capability is needed for South African samples as the PAH concentration is extremely high relative to the PCDD/Fs. The Rtx®-Dioxin2/ Rxi®-17SilMS column combination was optimised (Table 6.3) to ensure separation of the seventeen toxic PCDD/Fs and to meet the requirements for isomer specificity as stipulated by US EPA 1613B (1994a).

6.3.4 Selectivity using a Dioxin Specific Column

Cochran *et al*. 2007b determined the retention times and co-elution results for all tetra- through octa- chlorinated dioxins and furans using the Rtx®-Dioxin2 column. A retention time of at least three seconds was used to describe separation, but this criterion will not ensure baseline separation of all the TCDD/Fs. The separation was

performed using a GC-HRMS system (Waters AutoSpec Ultima HRMS, UK). It provides the elution order to expect in the absence of individual congeners to confirm each target PCB, PCDD/F and co-eluting isomers. It is also well documented in the literature that PCB-126 co-elutes with the most toxic 2,3,7,8-TCDD congener (Cochran 2002, Cochran *et al*. 2007a, Dorman *et al.* 2004, Do *et al.* 2013, Focant, Sjödin *et al*. 2004b, and Storr-Hansen *et al*. 1992). To confirm the selectivity, all 136 PCDD/F and twelve PCB compounds were analysed, identified and the possible interferences reported (Focant, Sjödin *et al*. 2004b and Harju *et al*. 2003a). This is the first NMISA attempt to characterise all 136 tetra- through octa chlorinated PCDD/Fs using GC×GC-TOFMS.

6.3.4.1 Standard preparation

Individual solutions (25 ng/ ml in *n*-nonane) of the 136 PCDD/F congeners with chlorines arranged in the 2,3,7,8-position were purchased from Cambridge Isotope Laboratories (CIL, Andover, MA, USA). These were combined into 14 mixtures consisting of about 10 compounds per mixture in order to allow assignment of retention times. 25 µl aliquots of each solution were combined, evaporated to dryness under a steady stream of nitrogen and reconstituted in 25 µl of *n*-nonane to achieve a concentration of about 25 ng/ ml. After analysis, a mixture of all 136 PCDD/F congeners and $^{13}C_{12}$-labelled isotopes was prepared by combining all 14 mixtures with 25 µl of the EPA 1613 CS4 standard (taken from the EPA 1613CVS calibration and verification solutions kit, in *n*-nonane from Wellington Laboratories *Inc.*, USA). The mixture was evaporated to dryness under a steady stream of nitrogen and reconstituted in 25 µl of *n*-nonane. To evaluate the known possible PCB interferences, a calibration solution containing 18 labelled and 17 unlabelled PCDD/F compounds was spiked with the twelve WHO dioxin-like PCBs and was also analysed. The concentration of the 'old' PCB standard used (WP-STK, Wellington, Laboratories *Inc.*, USA) was 2000 pg/ µl. The standard had evaporated, and was therefore reconstituted to the original volume of 1,2 ml using *n*-nonane and then diluted to 200 pg/ µl (same concentration range as CS4 (40 – 200 pg/ µl). The final concentration of the blend was 50 pg/ µl for the TCDD/Fs, 250 pg/ µl for the PeCDD/F, HxCDD/F and HpCDD/Fs, 500 pg/ µl for the OCDD/F and approximately 100 pg/ µl for the PCBs.

6.3.4.2 GC×GC-TOFMS conditions

Analysis was performed using the LECO Pegasus® 4D GC×GC-TOFMS system with GC oven conditions adapted from Hoh *et al.* (2007); detailed in Table 6.3. The instrument conditions are detailed in Chapter 4.

Table 6.3. *LECO Pegasus® GC×GC-TOFMS conditions adapted from Hoh et al. 2007.*

Instrument Parameters	Hoh *et al.* (2007)	Adapted NMISA method
^{1}D column	Rtx®-Dioxin2 (60 m x 0,25 mm x 0,25 μm)	Rtx®-Dioxin2 (40 m x 0,18 mm x 0,18 μm)
^{2}D column	Rtx®-PCB (2 m x 0,18 mm x 0,18 μm)	Rtx®-17SilMS (1 m x 0,15 mm x 0,15 μm)
Temperature program	140 ºC for 1 min, 30 °C/ min to 180°C, 2 °C/ min to 250°C, 20 °C/ min to 300°C, hold 20 min	140 ºC for 2 min, 30 °C/ min to 180°C, 2 °C/ min to 250°C, 5 °C/ min to 320°C, hold 5 min
Secondary oven offset	20 ºC	20 ºC
Total run time	59.83 min	57.33 min
Helium flow rate	2 ml/ min for 40 min, ramp at 0,5 ml/ min to 2,5 ml/ min	1.4 ml/ min
Injector temperature	Agilent 6890N S/SL injector @ 275 ºC	Agilent 7890N S/SL injector @ 275 ºC
Injector mode	1 μl injected splitless	1 μl injected; pulsed splitless
Modulator temperature offset	35 ºC	20 ºC
^{2}D Separation time	3 s (0.8 s hot pulse)	2.5 s (0.7 s hot pulse)
Acquisition rate & mass range	50 spectra/s (160 – 520 u)	100 spectra/ s (160-520 u)
PFTBA tune	m/z = 414	m/z = 414
Filament electron Voltage	80 eV	70 eV
Transfer line temperature	270 ºC	300 ºC
Ion source temperature	250 ºC	250 ºC
Detector voltage	1750 V	1750 V

6.3.4.3 Data processing

The instrument control and data processing were controlled through the LECO ChromaTOF® software. It is critical to specify the correct ^{1}D and ^{2}D peak widths as this is used in the deconvolution of the raw data. Data processing included automatic peak find using MS deconvolution in both ^{1}D and ^{2}D chromatograms. In addition, a calibration method was created to allow qualitative identification of the congeners based on truncated spectra and retention times. This data processing

was useful for target analysis. Manual review of each peak slice identified was made to ensure correct peak identification and confirmation of the mass spectra was achieved by library searching against a user defined library specifically compiled using native and labelled PCDD/Fs standards.

6.3.4.4 Results and discussion

As shown in Figure 6.7, the Rtx®-Dioxin2/ Rxi®-17SilMS column combination used for the GC×GC separation was optimised to provide adequate separation of the seventeen PCDD/Fs mandated for analysis by US EPA method 1613B (1994a). The separation was optimised for the HxCDD/F congeners which confirmed that all seventeen individual congeners could be properly separated and identified for accurate quantification. Recovery of individual PCDD/F analytes does impact the final TEQ value that is required for regulatory reporting, hence the importance of ensuring that each individual congener is properly identified and separated from interfering compounds. Due to concentration differences, the ions have been scaled to show all the ions in one chromatogram.

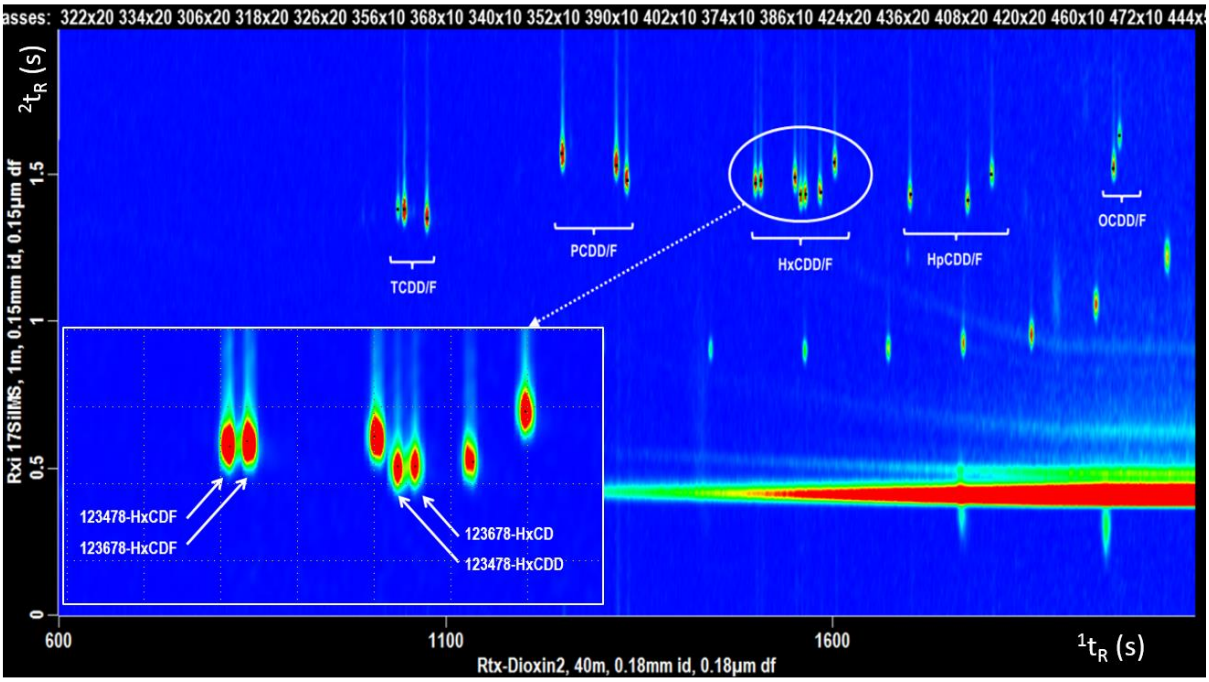


Figure 6.7. *^{2}D selected ion plot for the 17 toxic 2,3,7,8-substituted PCDD/F, including 1,2,3,4-[$^{13}C_{12}$]-TCDD using the Rtx®Dioxin2/ Rxi®-17SilMS column set. The method was optimised to ensure good separation of all the compounds, specifically focusing on the HxCDD/F separation.*

Previous studies have highlighted the co-elution of 2,3,7,8-TCDD and PCB-126 (Focant *et al*. 2004b, Harju *et al*. 2003a and Korytár 2006), and this could not be solved through the MS deconvolution process as the base ion for PCB-126 (m/z = 326) also appears in the 2,3,7,8-TCDD isotope pattern. To investigate this, a diluted EPA 1613-CS5 standard was spiked with the twelve WHO PCBs and analysed using the same GC×GC conditions. It was evident from the data (Figure 6.8) that there was co-elution of the 2,3,7,8-TCDD and PCB-126 and the unit mass resolution of the instrument did not offer the mass resolution to differentiate between these two compounds.

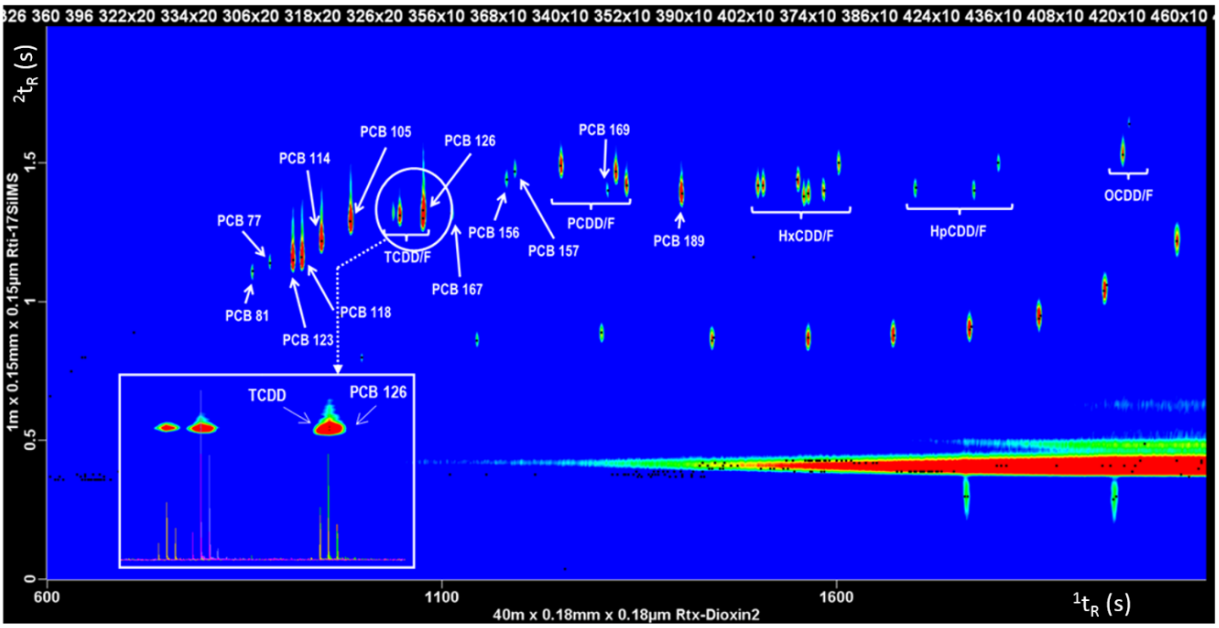


Figure 6.8. *Two-dimensional selected ion contour plot of the 17 toxic 2,3,7,8-substituted PCDD/Fs, including 1,2,3,4-[$^{13}C_{12}$]-TCDD and the 12 WHO PCB's, using the Rtx®Dioxin2/ Rxi®-17SilMS column set. The insert shows that there is still co-elution occurring between 2,3,7,8-TCDD and PCB 126.*

The chromatographic separation was acceptable, but inconsistent peak areas were obtained for 2,3,7,8-TCDD and there was an increase in intensity for the ion at m/z = 326 (base ion for PCB-126; Figure 6.9). GC oven conditions were again optimised to separate the 2,3,7,8-TCDD and PCB-126 congeners. The NMISA GC oven parameters (120 ºC for 2 min, 20 °C/ min to 240°C, 2 °C/ min to 265 °C, 5 °C/ min to 320 °C, hold 3 min) were altered to almost mimic the GC oven parameters as

proposed by Hoh *et al*. (2007); but the GC×GC and modulator conditions were set to accommodate the narrower ^{1}D column and different ^{2}D column phase (Rxi®-17SilMS) and still ensure similar separation without wrap-around (Table 6.3).

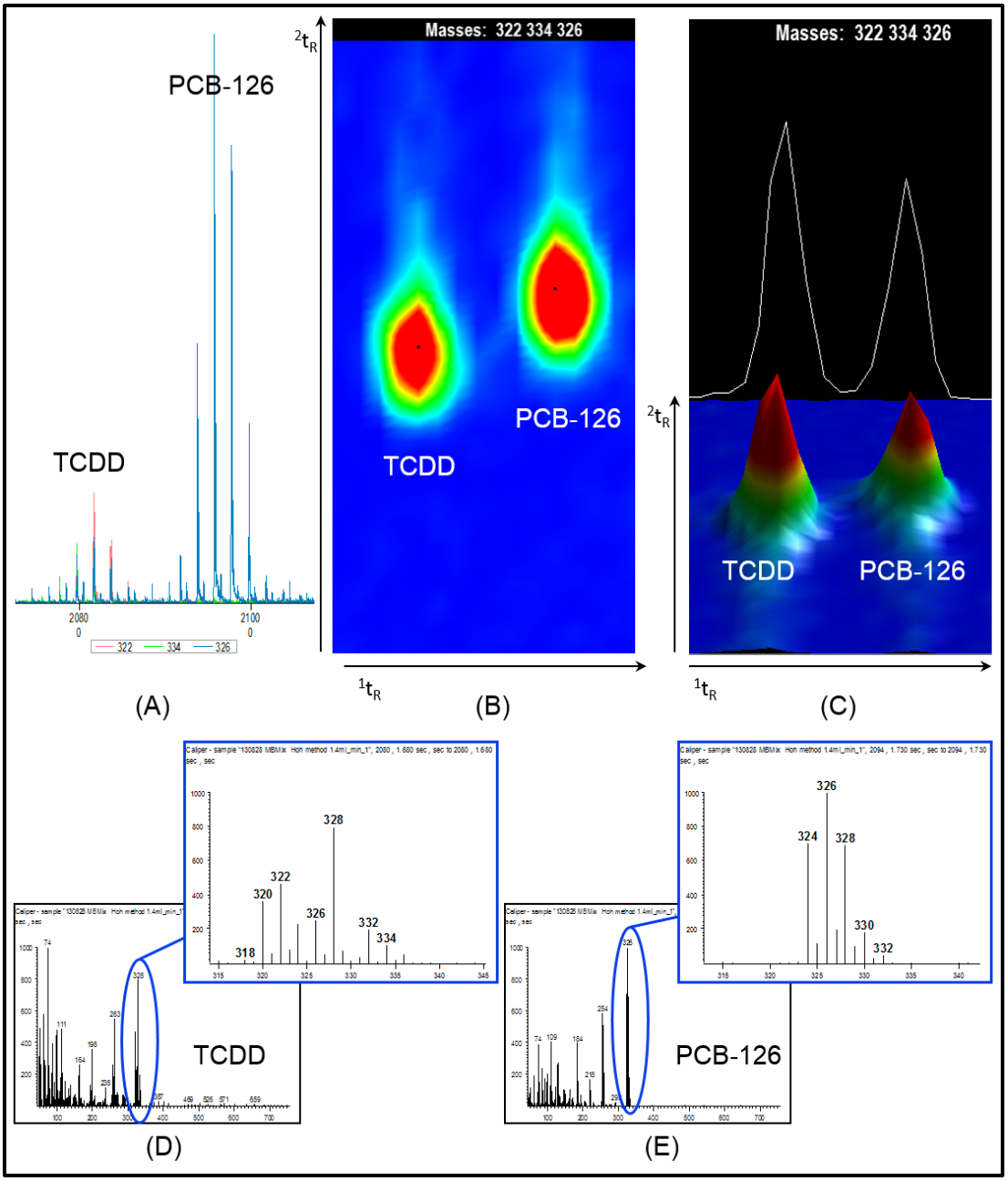


Figure 6.9. *The selected ion plot showing good resolution between PCB 126 (m/z = 326) and 2,3,7,8-TCDD and 2,3,7,8-[$^{13}C_{12}$]-TCDD (m/z = 322 and 334). Plot (**A**) is the ^{2}D chromatogram showing the 2,3,7,8-TCDD and PCB-126 separation; Plot (**B**) is a contour plot of the same compounds, Plot (**C**) is a surface plot, Plot (**D**) is the mass spectrum for 2,3,7,8-TCDD and Plot (**E**) is the mass spectrum for PCB-126.*

The 2,3,7,8-[$^{13}C_{12}$]-TCDD (m/z = 334) and 2,3,7,8-[$^{37}Cl_4$]-TCDD (m/z = 328) isomers also co-elute with 2,3,7,8- TCDD (m/z = 322), which gives even more reason to separate the PCB-126 from 2,3,7,8-TCDD.

Figure 6.9 shows the separation achieved for 2,3,7,8-TCDD and PCB-126 and the mass spectrum for 2,3,7,8-TCDD now has m/z = 326 in the correct ratio to m/z = 322, so incorrect assignment of m/z = 326 from PCB-126 no longer interferes. For quantitation, m/z = 328 is selected for 2,3,7,8-[$^{37}Cl_4$]-TCDD and m/z = 322 for 2,3,7,8-TCDD. The Rxi®-XLB/ Rtx®-200 column combination was also able to move the PCB-126 away from 2,3,7,8-TCDD, so it is a matter of selecting the correct GC parameters and modulator settings to ensure adequate separation.

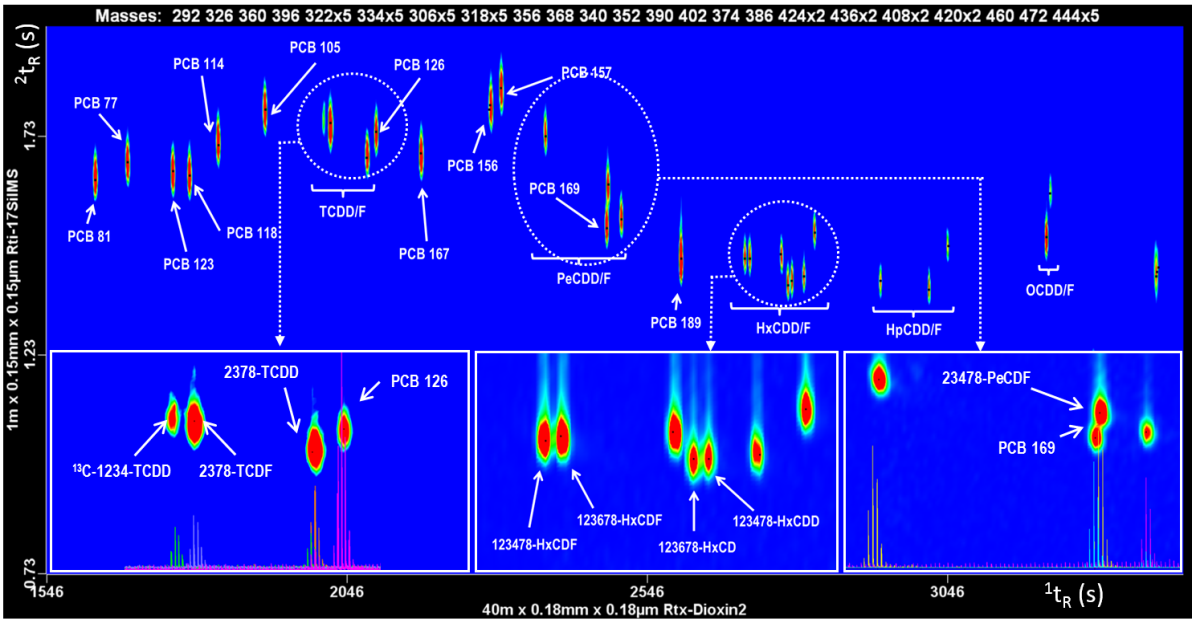


Figure 6.10. *^{2}D selected ion plot of the 17 toxic PCDD/Fs, including 1,2,3,4-[$^{13}C_{12}$]-TCDD and the 12 WHO PCBs, using the Rtx®Dioxin2/ Rxi®-17SilMS column set. The inserts clearly show the separation achieved for PCB 126 from 2,3,7,8-TCDD and PCB 169 from 2,3,4,7,8-PeCDD.*

Figure 6.10 provides very good separation of the seventeen toxic PCDD/Fs and the twelve WHO PCBs with no significant co-elution. The HxCDD/Fs show the same separation as previously and PCB-126 is baseline separated from the 2,3,7,8-TCDD. Although PCB-169 is not baseline separated from 2,3,4,7,8-PeCDD in ^{1}D, the

method was able to separate them in ^{2}D, allowing for 2,3,4,7,8-PeCDD quantification without any significant interference from the PCB-169, and demonstrating orthogonal separation in ^{2}D.

With the PCB co-elutions resolved, the next step was to investigate the separation of the seventeen toxic PCDD/Fs from their structurally related isomers. The 14 separate mixtures, containing about 10 compounds each to ensure that all 136 congeners are separated and identified, were individually analysed. A final sample mixture containing all 136 congeners combined was then analysed and the peaks identified were confirmed by comparing the ^{1}D and ^{2}D retention times obtained for the analysis of the individual groups. The results were then compared and confirmed against previous work done using the same ^{1}D column with GC-HRMS (Cochran *et al*. 2007b and Dorman *et al*. 2004) and GC×GC-TOFMS separations using various column phases.

Figure 6.11 summarises the separation achieved for the various congener groups where congeners could possibly affect the quantitation of the seventeen priority PCDD/Fs. Although the method was not optimised for the separation of the 136 PCDD/F congeners, the data shows good chromatographic separation of all the TCDD/F congeners (Tables 6.4 and 6.5). The only notable TCDD/F co-elution is the 1,2,3,6-TCDD with 2,3,7,8-TCDF. Due to the spectral stability of the TOFMS, the co-elution can easily be resolved by deconvolution of the unique masses selected for quantitation (1,2,3,6-TCDD, m/z = 322 and 2,3,7,8-TCDF, m/z = 306). This co-elution should however still be noted as an interference because the 2,3,7,8-[$^{13}C_{12}$]-TCDF (m/z 318) isotope pattern can be influenced by the presence of the 1,2,3,6-TCDD congener. Possible co-eluting compounds are indicted in red in Tables 6.4 to 6.6.

There are no significant co-eluting congeners near 1,2,3,7,8-PCDF, but there is a significant co-elution between 1,2,3,6,7-PeCDD and 1,2,3,7,8-PeCDD (Table 6.5). The data processing method used to establish the ^{2}D retention time criteria (coupled with ^{1}D and MS considerations) is also used to decide whether slices should be combined (i.e., whether the slices belong to the same peak), or if the slices should not be combined (i.e., different peaks). The ^{2}D criteria dictates that the ‘x+1’ slice

should have a ^{2}D retention time that is the same as, or less than the retention time of 'x' slice, meaning that the slice can arrive early, but not late, in order to be combined. This is coupled with the ^{1}D criterium (slices must occur in consecutive modulations if they are the same compound), and mass spectral matching to decide whether the slices should be combined. The data processing method in this case is not able to clearly distinguish between these two isomers.

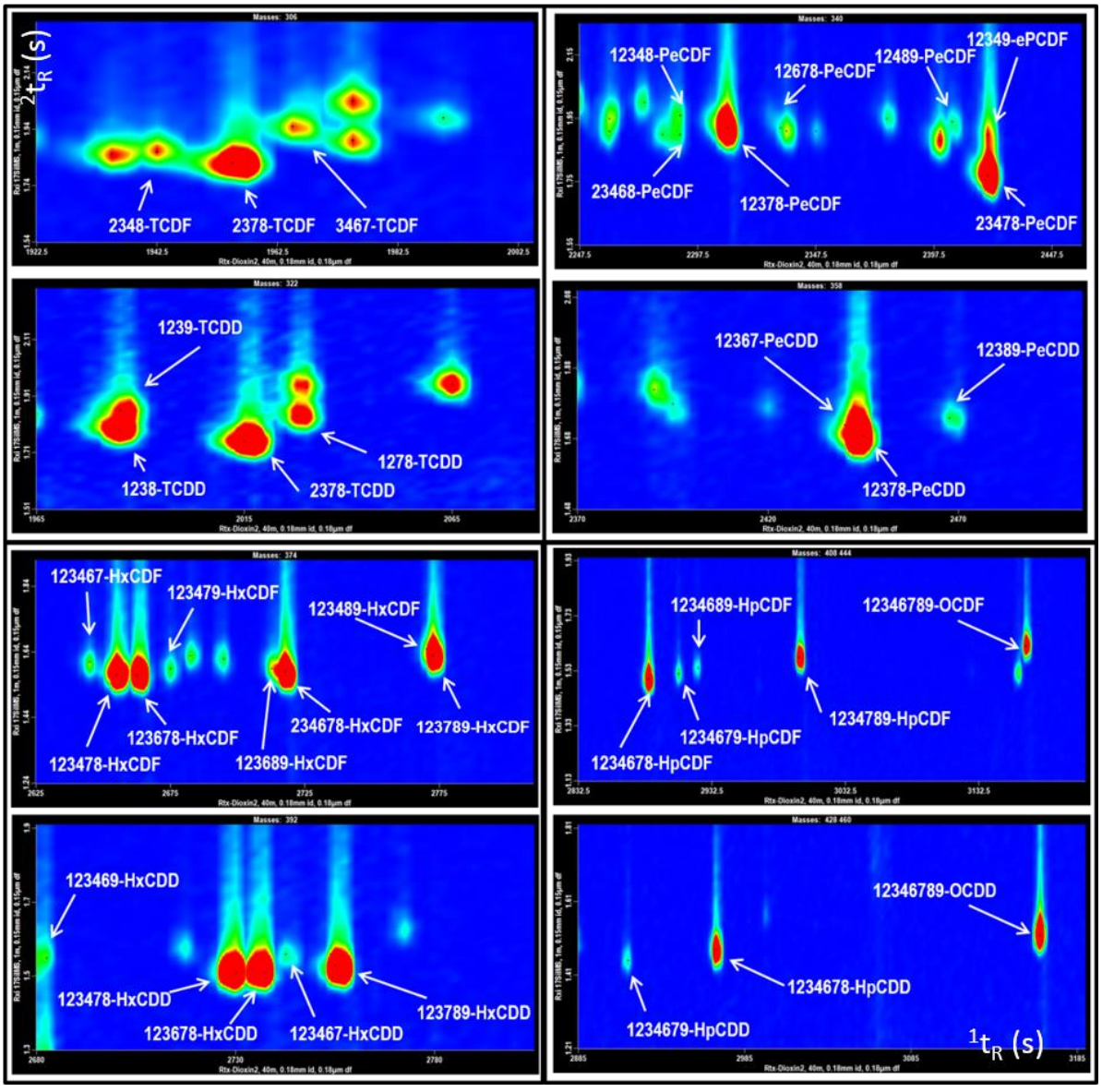


Figure 6.11. *Contour plots for the enlarged area of the GC×GC chromatograms showing the congener elution orders close to the 2,3,7,8-TCDD and 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,7,8-PeCDF, 1,2,3,7,8-PeCDD and the HxCDD/F, HpCDD/Fs and OCDD/Fs, using the Rtx®Dioxin2/ Rxi®-17SilMS column set.*

Table 6.6 gives the retention times for the remaining HxCDD/F, HpCDD/F and OCDD/F congeners. The 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF congeners do show the same ^{2}D retention time separation, but from the enlarged GC×GC contour plots (Figure 6.11), it is clear that there is no co-elution from either the 1,2,3,4,6,7-HxCDF or 1,2,3,4,7,9-HxCDF congeners. The 2,3,4,6,7,8-HxCDF does show significant co-elution with 1,2,3,6,8,9-HxCDF, and 1,2,3,7,8,9-HxCDF with 1,2,3,4,8,9-HxCDF. The identification of these congeners was supported by the presence of the 2,3,4,6,7,8-[$^{13}C_{12}$]-HxCDF and 1,2,3,7,8,9-HxCDF; the native and labelled congeners have the same retention times. The HxCDDs of interest do not show any interferences from the other HxCDD congeners.

It is important also to note that, when dealing with [$^{13}C_{12}$]-labelled and native compounds, the ChromaTOF® software assigns the same number of ^{2}D slices for both labelled and native compounds, which are included for quantification and qualification. This has to occur since the labelled and native compound ^{2}D retention times are identical. This feature also assists with the identification of trace levels of native compounds in the presence of matrix interferences or interfering congeners.

The remaining HxCDD/F, HpCDD/F and OCDD/F congeners do not show any co-elutions and could easily be identified (Table 6.6). Although the OCDD and OCDF elute close together, the unique masses associated with these congeners and the adequate ^{2}D separation allows acceptable integration (Figure 6.11).

Table 6.4. *^{1}D and ^{2}D retention times for all 60 TCDD/F congeners. Congeners shown in red indicate possible co-eluting compounds.*

TCDF Congeners			TCDD Congeners		
Congener	1st Dimension RT (s)	2nd Dimension RT (s)	Congener	1st Dimension RT (s)	2nd Dimension RT (s)
1368-TCDF	1662.5	1.64	1368-TCDD	1780	1.64
1468-TCDF	1685	1.73	1379-TCDD	1800	1.67
1346-TCDF	1732.5	1.78	1369-TCDD	1817.5	1.75
2468-TCDF	1732.5	1.79	1469-TCDD	1860	1.86
1378-TCDF	1737.5	1.7	1246-TCDD	1872.5	1.82
1246-TCDF	1740	1.81	1247-TCDD	1877.5	1.81
1347-TCDF	1747.5	1.72	1248-TCDD	1880	1.75
1247-TCDF	1755	1.72	1249-TCDD	1880	1.77
1348-TCDF	1757.5	1.79	1268-TCDD	1900	1.70
1248-TCDF	1765	1.78	1378-TCDD	1905	1.79
1379-TCDF	1767.5	1.75	1478-TCDD	1927.5	1.80
1367-TCDF	1772.5	1.73	1279-TCDD	1930	1.81
1268-TCDF	1787.5	1.81	1234-TCDD	1945	1.83
1467-TCDF	1792.5	1.82	1269-TCDD	1947.5	1.90
1478-TCDF	1807.5	1.77	1236-TCDD	1955	1.82
1369-TCDF	1827.5	1.82	1237-TCDD	1962.5	1.85
1237-TCDF	1830	1.80	1238-TCDD	1985	1.79
1678-TCDF	1837.5	1.85	1239-TCDD	1985	1.86
1234-TCDF	1842.5	1.85	**2378-TCDD**	**2015**	**1.75**
2467-TCDF	1845	1.84	1278-TCDD	2027.5	1.84
1238-TCDF	1847.5	1.84	1267-TCDD	2030	1.94
1469-TCDF	1847.5	1.92	1289-TCDD	2065	1.95
1236-TCDF	1855	1.85			
2368-TCDF	1867.5	1.75			
1278-TCDF	1872.5	1.85			
1349-TCDF	1875	1.92			
1267-TCDF	1892.5	1.93			
1249-TCDF	1907.5	1.95			
2346-TCDF	1915	1.92			
1279-TCDF	1917.5	1.93			
2347-TCDF	1935	1.85			
2348-TCDF	1942.5	1.86			
2378-TCDF	**1955**	**1.82**			
3467-TCDF	1965	1.95			
2367-TCDF	1975	1.90			
1269-TCDF	1975	2.04			
1239-TCDF	1990	1.98			
1289-TCDF	2075	2.09			

Table 6.5. *^{1}D and ^{2}D retention times for all 48 PeCDD/F congeners. Congeners shown in red indicate possible co-eluting compounds.*

PCDF Congeners		
Congener	1st Dimension RT (s)	2nd Dimension RT (s)
13468-PeCDF	2080	1.81
12468-PeCDF	2087.5	1.81
13678-PeCDF	2197.5	1.84
13467-PeCDF	2202.5	1.89
12467-PeCDF	2205	1.90
14678-PeCDF	2212.5	1.88
13478-PeCDF	2215	1.88
12368-PeCDF	2217.5	1.87
12478-PeCDF	2222.5	1.86
13479-PeCDF	2232.5	1.90
13469-PeCDF	2245	1.99
12479-PeCDF	2260	1.91
12346-PeCDF	2260	1.95
12469-PeCDF	2275	2.00
12347-PeCDF	2282.5	1.90
23468-PeCDF	2290	1.90
12348-PeCDF	2292.5	1.96
12378-PeCDF	**2310**	**1.92**
12678-PeCDF	2332.5	1.92
12367-PeCDF	2335	1.91
12379-PeCDF	2347.5	1.91
12679-PeCDF	2377.5	1.95
12369-PeCDF	2400	1.88
23467-PeCDF	2400	1.91
12489-PeCDF	2405	1.94
23478-PeCDF	**2420**	**1.77**
12349-PeCDF	2420	1.90
12389-PeCDF	2485	1.85

PCDF Congeners		
Congener	1st Dimension RT (s)	2nd Dimension RT (s)
12468-PeCDD	2230	1.81
12479-PeCDD	2232.5	1.81
12469-PeCDD	2270	1.91
12368-PeCDD	2327.5	1.79
12478-PeCDD	2345	1.78
12379-PeCDD	2350	1.78
12467-PeCDD	2362.5	1.85
12369-PeCDD	2365	1.80
12489-PeCDD	2365	1.84
12346-PeCDD	2390	1.82
12347-PeCDD	2395	1.78
12367-PeCDD	2440	1.72
12378-PeCDD	**2445**	**1.69**
12389-PeCDD	2467.5	1.74

Table 6.6. *^{1}D and ^{2}D retention times for all 26 HxCDD/F, eight HpCDD/F and two OCDD/F congeners. Congeners shown in red indicate possible co-eluting compounds.*

HxCDF, HpCDF, OCDF Congeners		
Congener	1st Dimension RT (s)	2nd Dimension RT (s)
123468-HxCDF	2555	1.61
124678-HxCDF	2565	1.60
134678-HxCDF	2565	1.62
134679-HxCDF	2582.5	1.61
124679-HxCDF	2600	1.62
124689-HxCDF	2620	1.62
123467-HxCDF	2645	1.61
123478-HxCDF	**2655**	**1.57**
123678-HxCDF	**2662.5**	**1.57**
123479-HxCDF	2675	1.59
123469-HxCDF	2682.5	1.63
123679-HxCDF	2695	1.62
123689-HxCDF	2712.5	1.59
234678-HxCDF	**2717.5**	**1.58**
123489-HxCDF	2767.5	1.65
123789-HxCDF	**2772.5**	**1.63**
1234678-HpCDF	**2885**	**1.50**
1234679-HpCDF	2907.5	1.52
1234689-HpCDF	2922.5	1.54
1234789-HpCDF	**2997.5**	**1.58**
12346789-OCDF	**3167.5**	**1.62**

HxCDD, HpCDD, OCDD Congeners		
Congener	1st Dimension RT (s)	2nd Dimension RT (s)
124679-HxCDD	2610	1.54
124689-HxCDD	2610	1.55
123468-HxCDD	2655	1.52
123679-HxCDD	2672.5	1.53
123689-HxCDD	2675	1.52
123469-HxCDD	2682.5	1.55
123478-HxCDD	**2730**	**1.51**
123678-HxCDD	**2737.5**	**1.51**
123467-HxCDD	2742.5	1.56
123789-HxCDD	**2755**	**1.53**
1234679-HpCDD	2915	1.45
1234678-HpCDD	**2967.5**	**1.48**
12346789-OCDD	**3162.5**	**1.52**

6.4 CONCLUDING REMARKS

The GC×GC-TOFMS screening approach was intended as an investigative tool to evaluate samples containing significant levels of a number of classes of compounds detrimental to human and animal health. This approach provides a quick convenient screen for numerous pollutant classes that may be present in samples arising from toxic waste disposal where incineration practices are badly designed or poorly executed. This can then be used as a possible test for determination of incineration efficiency and safety and was the first attempt to analyse such complex samples in South Africa. Software features present in the ChromaTOF® software can be used to enhance and speed up the evaluation process.

The NMISA GC×GC-TOFMS method using the Rxi®-XLB/ Rtx®-200 column configuration provided satisfactory separation of the 2,37,8-TCDD/F native and labelled pairs, but did cause co-elutions between some of the remaining TCDD/F congeners in the CIL EDF-4147 standard test mix not experienced with the DB-5/

Rxi®-5SilMS column phase. By changing column combinations and optimising the analytical parameters, the Rtx®-Dioxin2/ Rxi®-17SilMS configuration (Table 6.3) provides good separation of the seventeen toxic PCDD/Fs. All twelve WHO PCBs were separated from each other and the method ensured adequate separation of the target PCDD/Fs from the PCBs. The most toxic congener, the 2,3,7,8-TCDD, found in trace levels in the environment, has been separated from PCB-126. The Rxi-17SilMS ^{2}D column phase and the capability of GC×GC permits accurate identification and quantification, but any co-elution with 2,3,7,8-TCDD would affect the mass spectra and could end in a biased analytical result. The separation also meets the minimum criteria as stipulated in US EPA Method 1613B (1994a) to demonstrate isomer specificity for the GC columns employed for the determination of 2,3,7,8-TCDD/F. The data compares well with the separations achieved using GC-HRMS (Cochran *et al*. 2007b) and some of the remaining few co-elutions reported could most probably be separated with further optimisation of the method or using the thicker column phases as described by Hoh *et al* (2007).

The GC×GC-TOFMS can also be used to obtain quantitative data for the quantitation of the seventeen toxic PCDD/F (Focant, Eppe *et al*. 2005a, Focant, Sjödin *et al*. 2004a and Hoh *et al.* 2008). The focusing effect of the modulator provides adequate sensitivity and the selectivity gain from the two-dimensional chromatographic separation effectively compensates for that lost by the lower resolution mass spectrometer. Accurate quantitation using GC×GC-TOFMS will be investigated in Chapter 7 to confirm that the methodology is capable of reaching the minimum low levels as stipulated in US EPA Method 1613B (1994a) for PCDD/Fs. The Rtx®-Dioxin2/ Rxi®-17SilMS column combination and GC method will be used for the NMISA validation study discussed in Chapter 8.

7

QUANTITATIVE ANALYSIS

In the previous chapter, the qualitative screening, congener identification and isomer separation was addressed. In this chapter, quantitative determination will be discussed. The work has previously been published in the papers by De Vos *et al*. (2011a), De Vos *et al*. (2011b) and De Vos *et al*. (2013b). The samples used for this initial comparison were all provided by the collaborating laboratories since NMISA did not have extraction capability at this time and it was important to establish the GC×GC-TOFMS method and compare the results with those obtained using GC-HRMS.

7.1 QUANTITATIVE ANALYSIS

NMISA, in collaboration with various institutions (Mass Spectrometry Laboratory, Biological and Organic Analytical Chemistry, University of Liège, Belgium, Environmental Protection Agency (EPA) in Taiwan, LECO Africa Separation Science Laboratory, University of Pretoria, South Africa, and The Laboratory Services Branch, Ontario Ministry of the Environment, Canada and Restek, USA), initiated a project to develop a quantitative screening method for multiple classes of POPs, in particular PCDD/Fs, using GC×GC-TOFMS in South Africa. Sample extracts were provided by the collaborators to NMISA in South Africa for low resolution (nominal mass) GC×GC-TOFMS and high resolution accurate mass TOF analysis (HRT). The results obtained for the quantitation of PCDD/F compounds in the various samples were compared with results obtained for the same sample sets using GC-HRMS to confirm the validity of the quantitation. This is the first time such a comprehensive environmental screening and PCDD/F determination has been done in South Africa using GC×GC-TOFMS.

There are numerous challenges that have to be overcome with this approach: firstly the separation of the PCDD/F congeners from each other and from matrix interference, and secondly the sensitivity considerations to allow accurate quantitative measurement down to 500 fg for 2,3,7,8-TCDD as required by US EPA Method 1613B (1994a). In South Africa, PCDD/Fs usually exist at very low levels in the presence of much higher matrix and analyte interferences. The selectivity and sensitivity of GC-HRMS makes it possible to reach these very low levels. GC×GC-TOFMS sensitivity was discussed in Chapter 4 and selectivity was demonstrated in Chapter 6. The ultimate goal was to establish accurate quantitation of the seventeen toxic PCDD/Fs using GC×GC-TOFMS and benchmarked against confirmatory GC-HRMS results.

The quantitative results will be described in three sets: toxic waste sample extracts provided by the University of Liège, Belgium; soil and sediment sample extracts received from EPA Taiwan and soil sample extracts provided by The Laboratory Services Branch, Ministry of the Environment (MOE), Canada.

7.1.1 Materials, Standards and Samples

Three South African toxic waste samples were extracted by Dr Jean-Fransçois Focant, University of Liège, Belgium. All solvents, extraction consumables and chromatographic pure grade helium gas used for extraction and analysis by GC-HRMS are described in De Vos *et al*. (2011a). The internal standard solution for the seventeen 2,3,7,8-chloro-subsituted [$^{13}C_{12}$] labelled PCDD/Fs congeners (EDF-4144), the calibration standard solution (EDF-4143), and the syringe (recovery) standard (EDF-4145) were purchased from Cambridge Isotope Laboratories (CIL, Andover, MS, USA). These standards were added during the sample extraction process in Belgium. The internal standard solutions, labelled congeners, calibration standard solutions for the non-*ortho* PCBs and PCDD/Fs, as well as the ten-point calibration solution and recovery standards and the GC-HRMS standards are described in De Vos *et al*. (2011a), Focant *et al*. 2001b and Pirard *et al*. (2003).

The calibration solutions selected for the GC×GC-TOFMS quantitation of the Belgium extracts were the European Method EN-1948 standards. This choice was based on expected concentration levels and availability. These solutions were

purchased from Wellington Laboratories *Inc*. (Guelph, Canada) and contained the seventeen native PCDD/Fs and the corresponding [$^{13}C_{12}$]-labelled standards, sampling standards and syringe standards. Additional PCDD/F and PCB calibration standards were obtained from Cambridge Isotope Laboratories (CIL, Andover, Massachusetts, USA) and included the internal standard and the recovery standard for these compounds (EDF-4143, EDF 4144 and EDF 4145) that were used for the extraction in Belgium. For quantitation purposes, only the PCDD/Fs and the non-*ortho* substituted dioxin-like PCBs were assessed.

To establish a PCDD/F capability for South Africa, additional calibration and verification standards, as stipulated in US EPA Method 1613B (1994a), were obtained from Wellington Laboratories and included calibration and verification solutions (EPA-1613CVS), labelled calibration solutions (EPA-1613LCS), internal standard spiking solution (EPA-1613ISS) and clean-up standard stock solution (EPA-1613CSS). Details of all solvents, consumables, liquid nitrogen and gas standards used for sample extraction and clean-up are described in De Vos *et al*. (2011b).

Four sample extracts (two sediment, one fly ash and one check standard) were provided by Dr David Peng, the Environmental Protection Agency (EPA) in Taiwan through the NMISA collaboration with LECO Africa. Extraction, clean-up and GC-HRMS analysis of these samples were performed by the same laboratory according US EPA Method 1613B (1994a) using the Wellington Laboratory calibration and verification solutions (EPA-1613CVS), labelled calibration solutions (EPA-1613LCS), internal standard spiking solution (EPA-1613ISS) and clean-up standard stock solution (EPA-1613CSS).

Six soil extracts were provided by Professor Eric Reiner, Laboratory Services Branch, The Ontario Ministry of the Environment, Canada. All samples were prepared according to *Ministry of the Environment* Method E3418 for determination of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls (DL-PCBs) in environmental matrices by gas chromatography-high resolution mass spectrometry (GC-HRMS; Kolic & Macpherson 2012). Native stock standard, containing all seventeen toxic 2,3,7,8-

TCDD/Fs and certified and Precision and Recovery Standard (PAR) from Cambridge Isotope Laboratories *Inc.*, and $^{13}C_{12}$-Labeled PCDD/F stock standard, containing six 2,3,7,8-substituted dioxins and nine 2,3,7,8-substituted furans, (EPA-1613LCS), from Wellington Laboratories *Inc.*, were used for extraction and analysis. Injection Stock Standards, 1,2,3,4 [$^{13}C_{12}$]-T_4CDD and 1,2,3,7,8,9-[$^{13}C_{12}$]-H_6CDD, (EPA-1613-ISS_ST), Wellington Laboratories *Inc.*, were also used. The extracts were analysed by GC-HRMS in Canada, evaporated to dryness and shipped to South Africa. Mr Jack Cochran and Professor Eric Reiner then coordinated the analysis of the reconstituted extracts at NMISA by GC×GC-TOFMS during a training visit (October 2012) to our laboratory. The same extracts were then analysed by Dr Gorst-Allman and myself at the LECO Africa Separation Science laboratory, University of Pretoria, using the LECO Pegasus High Resolution TOFMS (GC-HRT) after the GC×GC-TOFMS runs were completed at NMISA. All results obtained at NMISA using GC×GC-TOFMS were directly compared with the GC-HRT (University of Pretoria) and GC-HRMS results (De Vos *et al*. 2011a, De Vos *et al*. 2011b, De Vos *et al*. 2013a, De Vos *et al*. 2013b).

The sample preparation, extraction and clean-up procedures for the various samples has been detailed in Chapter 5.

7.1.2 Analytical Systems

7.1.2.1 GC-HRMS systems

The validity of the results was established by first analysing the sample extracts using GC-HRMS. The South African toxic waste sample extracts for the PCDD/Fs and non-*ortho* PCBs were measured on a Waters Micromass *Autospec Ultima* high resolution mass spectrometer (Micromass, Manchester, United Kingdom) coupled to an *Agilent* 6890N series (Palo Alto, CA, USA) gas chromatograph equipped with an A200SE auto-sampler (CTC Analytics AG, Zwingen, Switzerland) and using a VF-5MS (50 m x 0.20 mm x 0.33 µm) column (Varian *Inc.*). The mass spectrometer instrument conditions are detailed in Table 7.1. Additional GC and HRMS parameters, including performing conditions, isotope ratio checks, quantifying ions and quality controls for the measurements were as described previously by Eppe *et al.* (2004) and Focant *et al*. (2004b).

The two sediment, one fly ash and check standard samples were analysed at the EPA in Taiwan using a Waters Micromass *Autospec Ultima* high resolution mass spectrometer using a DB-5 (60 m x 0.32 mm x 0.25 µm) column (J&W Scientific); software information was not mentioned. The six Canadian soil samples were analysed using a Waters Micromass *Autospec Premier™* magnetic sector mass spectrometer (with TargetLynx™ Application Manager software), interfaced to an HP6980 gas chromatograph (Agilent Technologies) using a DB-5 (40 m x 0,18 mm x 0.18 µm) column (J&W Scientific), GC-HRMS (EI at lower eV, 10,000 Resolution, SIM). The analysis was performed at The Laboratory Services Branch, Ministry of the Environment, Canada. The mass spectrometer instrument conditions are detailed in Table 7.2.

7.1.2.2 GC×GC-TOFMS system

A LECO Pegasus® 4D GC×GC-TOFMS (LECO Corporation, St Joseph, MI, USA), Agilent 7890N GC and Agilent 7683B auto-sampler with a secondary oven mounted inside the primary GC oven fitted with a quad-jet dual stage thermal modulator was used for all qualitative and quantitative analyses. The GC system, instrument parameters and settings are fully described in Chapter 4, Section 4.3.3. All instrument functions and data processing for the GC×GC–TOFMS were managed with the LECO ChromaTOF® software version 4.44.

7.1.2.3 LECO HRT system

The high resolution time-of-flight mass spectrometer system was a Pegasus HRT (LECO Corporation) using *Folded Flight Path* (FFP) technology (Verentchikov *et al.* 2005a and 2005b). The system included an Agilent 7890N GC equipped with an Agilent 4513A auto-sampler. The HRT instrument conditions are detailed in Table 7.2. The instrument has been designed for high performance MS capabilities, including acquisition speed, mass accuracy and improved isotopic abundance and mass resolution. LECO have incorporated FFP technology to enable mass resolution of 50,000 with mass accuracies of less than 1 ppm and acquisition rates up to 200 spectra per second. NMISA was able to use the HRT for confirmation of mass accuracy for the extended study, providing South Africa with access to high

resolution capability. All instrument functions and data processing for the Pegasus HRT system were managed using LECO ChromaTOF® software version 1.61. Manual review of all peak identifications and integrations was performed using this software. Library searching was performed using a PCDD/F user library compiled from the PCDD/F standards.

7.1.3 Column Selection

Column selection and method optimisation has been discussed in Chapter 4 and 6. Most of the PCDD/F method development work at NMISA has been centered on the Rxi®-XLB/ Rtx®-200 column combination, ensuring accurate identification of each PCDD/F congener to meet separation and analytical requirements stipulated in US EPA 1613B (1994a). In order to achieve the separation requirements these methods generally require an initial analysis using a 5%-diphenyl/95%-dimethyl polysiloxane stationary phase. When this primary analysis detects the presence of 2,3,7,8-substituted congeners, most methods require a confirmatory analysis using a stationary phase that has been shown to separate these congeners from the less toxic congeners. More recently, the Rxi®-Dioxin2/ Rxi®-17SilMS combination has been applied at NMISA. The motivation for moving back to the Rxi®-Dioxin2 column is that it uses a new proprietary stationary phase that has improved separation capability for PCDD/Fs and higher thermal stability (maximum operating temperature of 340 ºC). The ^{2}D Rxi®-17SilMS low bleed mid-polarity crossbond® phase (maximum operating temperature of 340/ 360 ºC), has higher thermal stability when compared with similar phases (Dorman *et al*. 2004). It has excellent inertness and selectivity for active environmental compounds, such as PAHs. South African soil samples have extremely high levels of PAHs and method development must include separating the target PCDD/F congeners away from any remaining PAHs in a soil or sediment sample.

7.1.4 GC-HRMS and GC×GC-TOFMS Instrument Methods

Table 7.1 details the initial instrument parameters used for the analysis of the toxic waste sample extracts and Table 7.2 details the instrument parameters for the extended study.

Table 7.1. *GC-HRMS and GC×GC-TOFMS instrument conditions for the analysis of the toxic waste sample extracts and GC×GC-TOFMS instrument conditions for the analysis of the sample extracts from Taiwan (GC-HRMS parameters were not provided).*

Parameter	GC-HRMS (Toxic waste extracts) *(Eppe et al. 2004)*	GC×GC-TOFMS (Toxic waste extracts) *(De Vos et al. 2011a)*	GC×GC-TOFMS (Taiwan extracts) *(De Vos et al. 2011b)*
^{1}D column	VF-5MS (50 m x 0.20 mm x 0.33 µm)	Rxi®-5SilMS (30 m x 0.25 mm x 0.25 µm)	Rxi®-XLB (30 m x 0.25 mm x 0.25 µm)
^{2}D column	-	Rtx®-PCB (1,1 m x 0.18 mm x 0.18 µm)	Rtx®-200 (2 m x 0.18 mm x 0.20 µm)
Amount injected	1 µl ramped using a programmed temperature vaporising (PTV) injector	1 µl splitless (split/ splitless injector)	2 µl splitless (split/ splitless injector)
Inlet temperature	40 ºC (hold 3 min), ramp at 720 ºC/ min to 320 ºC (hold 4 min), at 720 ºC/ min to 330 ºC (43 min)	250 ºC	250 ºC
Helium flow	1.0 ml/ min constant flow	1.0 ml/ min constant flow	1.4 ml/ min constant flow
Primary oven	60 ºC (hold 1,5 min), ramp at 70 ºC/ min to 200 ºC, at 3,2 ºC to 235 ºC, hold 1,5 min, at 3,2 ºC to 270 ºC (hold 10 min), at 15 ºC/ min to 310, hold 13 min	140 ºC (hold 1 min), ramp at 20 ºC/ min to 180 ºC, at 3 ºC to 295 ºC, hold 3,67 min	80 ºC (hold 1 min), ramp at 20 ºC/ min to 220 ºC, no hold, 2 ºC/ min to 240 ºC, no hold, 1 ºC/ min to 250 ºC, no hold, 5 ºC/ min to 260 ºC, no hold, 1ºC/ min to 270 ºC, no hold, 5 ºC/ min to 310 ºC, hold 2 min
Secondary oven offset	-	20 ºC	20 ºC
Total run time	53 min	45 min	50,33 min
Transfer line temperature	275 ºC	270 ºC	270 ºC
Modulation period	-	5 s (1,2 s hot pulse)	4 s (hot pulse 1 s)
Modulator temperature offset	-	30 ºC	30 ºC
Ion source	270 ºC	250 ºC	250 ºC
Mass range	SIM (10 000 resolution)	100 - 520 amu	100 - 520 amu
Acquisition delay	-	600 s	600 s
Acquisition rate	-	50 spectra/ s	50 spectra/ s
Mass defect setting	-	-40 mu/ 100u	-40 mu/ 100u
Electron energy	40 eV	80 eV	70 eV
Calibration	Perfluorokerosene (PFK)	Perfluorotributylamine (PFTBA)	Perfluorotributylamine (PFTBA)

Table 7.2. *GC-HRMS, GC-HRT and GC×GC-TOFMS instrument conditions for the soil sample extracts provided by MOE, Canada for PCDD/F analyses (De Vos et al. 2013b).*

Parameter	GC-HRMS	GC-HRT	GC×GC-TOFMS
^{1}D column	DB-5 (40 m x 0.18 mm x 0.18 µm)	Rtx®-Dioxin2 (40 m x 0.18 mm x 0.18µm)	Rtx®-Dioxin2 (40 m x 0.18 mm x 0.18 µm)
^{2}D column	-	-	Rxi®-17SilMS (1 m x 0.15 mm x 0.15 µm)
Amount injected	1 µl	2 µl	2 µl
Inlet temperature	280 ºC	280 ºC	250 ºC
Helium flow	0.8 ml/ min	1 ml/ min	1.4 ml/ min
Primary oven	140 ºC (hold 1 min), ramp at 52 ºC/ min to 200 ºC, at 2,9 ºC to 235 ºC, hold 3 min, at 3 ºC to 267 ºC, at 7 ºC/ min to 310, hold until OCDD elutes	140 ºC (hold 1 min), ramp at 50 ºC/ min to 200 ºC, at 3 ºC/ min to 260, at 1 ºC/ min to 280 ºC, 6 ºC/ min to 310 ºC, hold 5 min	120 ºC (hold 2 min), ramp at 20 ºC to 200, at 5 ºC/ min to 320, hold 3 min
Secondary oven offset	-	-	5 ºC
Total run time	39 min	52,2 min	57,33 min
Transfer line temperature	280 ºC	300 ºC	320 ºC
Modulation period	-	-	2,5 s
Ion source	280 ºC	280 ºC	250 ºC
Start mass	SIM amu	140 amu	45 amu
End mass	SIM amu	520 amu	750 amu
Acquisition rate	-	3 spectra/ s	100 spectra/ s
Mass defect setting	-	-	-40 mu/ 100u
Electron energy	35 eV	50 eV	70 eV
Calibration	Perfluorokerosene (PFK)	Perfluorotributylamine (PFTBA)	Perfluorotributylamine (PFTBA)

The initial method development for analysing the toxic waste sample extracts was based on columns already available in the laboratory and some experimentation with the analysis of PCBs, and has been described in Chapter 4. As part of the extended study, extracted samples that had already been analysed by GC-HRMS, were compared with results obtained using GC×GC-TOFMS and high rResolution TOFMS (HRT).

7.2 SAMPLE RESULTS

7.2.1 GC×GC-TOFMS Analysis of Waste Sample Extracts

Prior to sample quantitation, calibration curves were generated for the seventeen PCDD/Fs required by US EPA Method 1613B (1994a). This required the calculation of a relative response factor (RRF) according to the formula:

$$RRF = \frac{(Peak\ area\ native\ \ x\ \ Concentration\ carbon\ label)}{(Peak\ area\ carbon\ label\ \ x\ \ Concentration\ native)} \qquad \text{Equation 7.1}$$

The RRF is then used to calculate the concentration of unknown material in the sample to be quantified, after first adding a known amount of the labelled internal standard, using the formula:

$$Analyte\ concentration = \frac{\left(\frac{Area\ native}{Area\ carbon\ label}\right) x \left(\frac{Concentration\ carbon\ label}{RRF}\right)}{Mass\ sample} \qquad \text{Equation 7.2}$$

To confirm the analytical results, an external calibration method was used and the PCDD/F results were calculated according to the formula:

$$Analyte\ Mass = \frac{\left(Mass\ carbon\ label\ x \frac{\left(\frac{Area\ native}{Area\ label}\right) - intercept}{Slope}\right)}{Mass\ sample} \qquad \text{Equation 7.3}$$

The ion ratio masses used for quantitation included 306/ 318, 340/ 352, 374/ 386, 408/ 420, 444/ 456 for the PCDFs and 322/ 334, 356/ 368, 390/ 402, 424/ 436, 460/ 472 for the PCDDs. During the calculation, a correction for a blank (BC) can be implemented, but this was not done during this study.

The first sample results assessed were the toxic waste extracts received from the University of Liége, Belgium. These results have been previously reported in the paper by De Vos *et al.* (2011a).

An example of the calibration curve obtained for 1,2,3,7,8-PeCDF with a correlation co-efficient of 0,9998 is shown in Figure 7.1. The linearity obtained in the calibration

curves was very good for the low level compounds, especially for the penta to octa congeners. The EN1948 CS1 standard has a concentration of 0,4 pg/ µl for the 1,2,3,7,8-PeCDF and 1.6 pg/ µl for the EN1948 CS2 standard. The correlation achieved ranged from 0.9999 for the 2,3,7,8-TCDD to 0.9996 for OCDD over the entire calibration. The 2,3,7,8-TCDD/F native standards were at concentrations 0,2 pg/ µl (CS1) and 0,8 pg/ µl (CS2) respectively. The CS1 standard at 0,2 pg/ µl was not quite as easily detected, but was still included; the standard is very near the limit of detection for a nominal mass instrument. Standards provide an idealized case in which no interference is present in the sample, but still the ease with which the low levels can be detected indicates that the method can achieve the very low levels needed for persistent organic pollutant analysis. Accurate quantitation should be possible at 500 fg on column (0,5 pg/ µl), as required by US EPA Method 1613B (1994a).

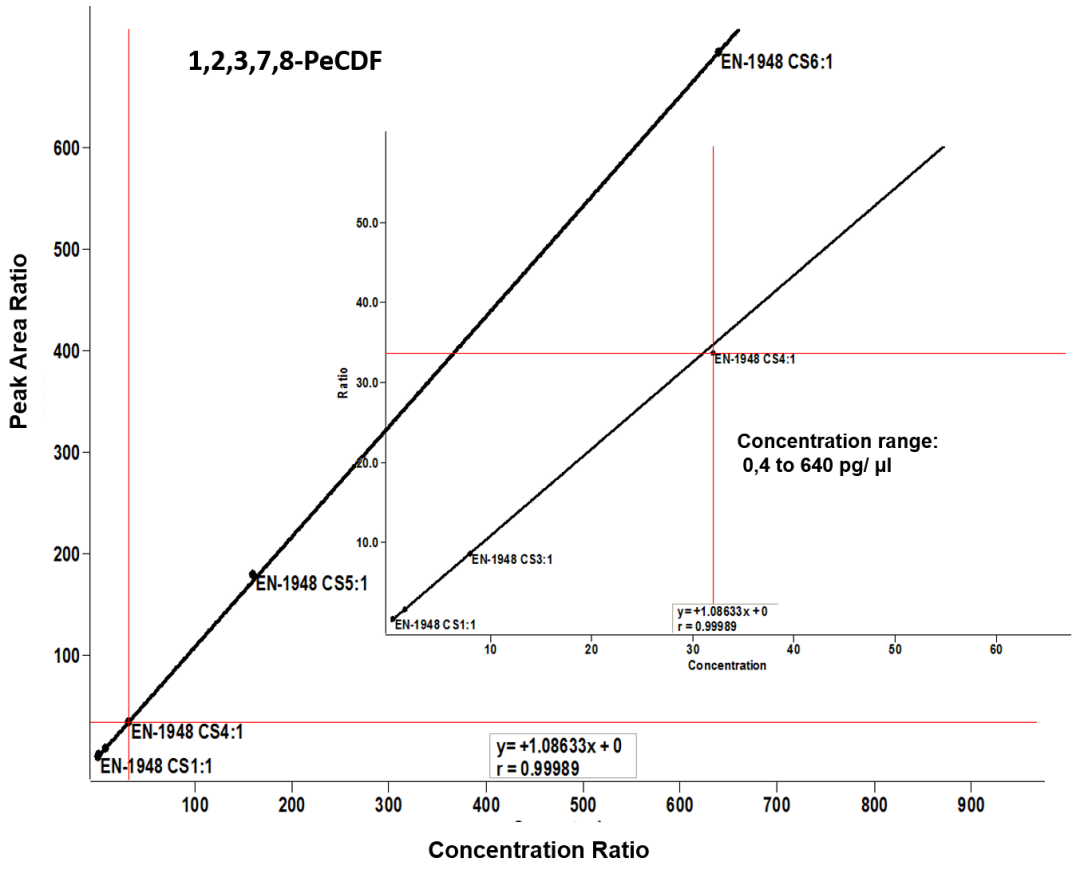


Figure 7.1. *Calibration curve (including expanded insert) for 1,2,3,7,8-PeCDF. These are neat calibration standards obtained from CIL; EDF 4144 - 0,4 pg/ µl to 640 pg/ µl (De Vos et al. 2011a).*

Table 7.3 provides the results obtained for the three toxic waste samples compared against the results obtained using GC-HRMS.

Table 7.3. *Quantitative results obtained for the 17 PCDD/Fs and dioxin-like non-ortho PCBs (pg/ g) in the toxic waste sample extracts (De Vos et al. 2011a).*

	Waste Sample 1		Waste Sample 2		Waste Sample 3	
PCDD/F and DL-PCB Compounds	**TOFMS**	**HRMS**	**TOFMS**	**HRMS**	**TOFMS**	**HRMS**
2,3,7,8-TCDD	ND*	ND*	ND*	ND*	590	510
1,2,3,7,8-PeCDD	18	2.7	7.5	2.8	940	770
1,2,3,4,7,8-HxCDD	ND*	5.5	ND	2.1	430	400
1,2,3,6,7,8-HxCDD	ND*	6.8	4.4	4.1	710	700
1,2,3,7,8,9-HxCDD	23	5.6	ND*	ND*	440	440
1,2,3,4,6,7,8-HpCDD	98	52	7.6	8.9	2500	2300
OCDD	180	110	13	14	2800	2200
2,3,7,8-TCDF	130	120	71	69	10800	8800
1,2,3,7,8-PeCDF	41	30	14	18	4450	3800
2,3,4,7,8-PeCDF	60	69	64	47	5100	4400
1,2,3,4,7,8-HxCDF	36	48	18	18	3100	3200
1,2,3,6,7,8-HxCDF	43	43	15	15	2600	2900
2,3,4,6,7,8-HxCDF	54	53	13	15	2900	2900
1,2,3,7,8,9-HxCDF	23	20	8.9	6.4	840	850
1,2,3,4,6,7,8-HpCDF	250	240	39.46	29.17	7400	7550
1,2,3,4,7,8,9-HpCDF	160	90	12	8.8	2300	1800
OCDF	290	300	84	79	5200	4900
PCB 81	65300	100300	46000	62000	18400	80500
PCB 77	3000	1900	1300	950	2700	2600
PCB 126	320	240	300	280	9000	9000
PCB 169	ND*	ND*	7.1	6.6	830	390

**ND: Not detected*

Table .7.4. *Quantitative results reported as ng WHO-TEQ.kg^{-1} for the toxic waste sample extracts (De Vos et al. 2011a).*

	Waste Sample 1		Waste Sample 2		Waste Sample 3	
PCDD/F and DL-PCB Compounds	**TOFMS TEF**	**HRMS TEF**	**TOFMS TEF**	**HRMS TEF**	**TOFMS TEF**	**HRMS TEF**
2,3,7,8-TCDD	ND*	ND*	ND*	ND*	590	510
1,2,3,7,8-PeCDD	18	2.7	7.5	2.8	940	770
1,2,3,4,7,8-HxCDD	ND*	0.5	ND	0.2	42	40
1,2,3,6,7,8-HxCDD	ND*	0.7	0.4	0.4	71	70
1,2,3,7,8,9-HxCDD	2.3	0.6	ND	ND	44	44
1,2,3,4,6,7,8-HpCDD	0.9	0.5	0.1	0.1	25	23
OCDD	0.1	0.1	0.05	0.05	0.8	0.7
2,3,7,8-TCDF	13	12	7.1	6.9	1080	880
1,2,3,7,8-PeCDF	1.2	0.9	0.4	0.5	130	110
2,3,4,7,8-PeCDF	18	21	19	14	1500	1320
1,2,3,4,7,8-HxCDF	3.6	4.8	1.8	1.8	300	330
1,2,3,6,7,8-HxCDF	4.3	4.3	1.5	1.5	260	300
2,3,4,6,7,8-HxCDF	5.4	5.3	1.3	1.5	290	290
1,2,3,7,8,9-HxCDF	2.3	2.0	0.9	0.6	80	85
1,2,3,4,6,7,8-HpCDF	2.5	2.4	0.4	0.3	74	75
1,2,3,4,7,8,9-HpCDF	1.6	0.4	0.1	0.1	23	18
OCDF	0.1	0.1	0.02	0.02	1.6	1.5
TOTAL PCDD/Fs	**80**	**60**	**40**	**30**	**5500**	**5000**
PCB 81	19	30	14	19	5.5	24
PCB 77	0.3	0.1	0.1	0.1	0.3	0.3
PCB 126	32	24	32	28	890	890
PCB 169	ND*	ND*	0.2	0.2	25	12
TOTAL DL-PCB	**50**	**50**	**50**	**50**	**930**	**930**
TOTAL TEQs	**130**	**110**	**90**	**80**	**6400**	**5800**
% Difference	**15%**		**11%**		**9%**	

**ND: Not detected*

The sum of the concentration of each individual dioxin-like compound multiplied by its TEF, gives the toxic equivalent (TEQ) concentrations and this is the value of an equivalent 'dioxin' concentration for the dioxin-like compounds in the toxic waste samples and is reported as 2,3,7,8-TCDD equivalents (Reiner 2010 and Van den Berg *et al*. 2006). Table 7.4 represents the TEQ values obtained for the PCDD/Fs and non-*ortho* PCBs in the toxic waste samples.

In nearly all cases the values obtained from the GC×GC-TOFMS are in good agreement with those obtained using GC-HRMS. In general, the GC×GC-TOFMS values are slightly higher than those obtained with GC-HRMS. In the case of the GC×GC-TOFMS results, no background subtraction was performed. This may slightly influence the results even though blank runs were made between each standard and sample run. There was no evidence of carry-over or any build-up of interference between runs. The TCDD/Fs were not detected with GC-HRMS and also not with GC×GC-TOFMS in the blanks. Matrix detection limits were not calculated for this study, although an assessment of achievable detection limits was calculated to be approximately 320 to 350 fg on column (discussed in Chapter 4, section 4.3.3.2).

The sample complexity could lead to interference on a quantitation mass, and produce artificially high values for the compound being determined. This is a possibility when assessing the consistently higher value obtained for PCB-81 (possible interference from PCB-87). The low TEF value obtained for PCB-81 (PCB-81 = 0.0003, Van den Berg *et al*. 2006) means that the contribution to the overall sample TEQ value is actually negligible.

Table 7.4 provides a better evaluation of the differences between the GC×GC-TOFMS and GC-HRMS results. The TEQ values are comparable between methods, although the GC×GC-TOFMS results are slightly higher. For a screening method this is not undesirable, as this guarantees that samples sent for further investigation will be biased high so that low positives will not be excluded. The TEQ values are high (especially waste sample 3; 6400 ng WHO-TEQ.kg^{-1}), which is to be expected as these samples resulted from incomplete combustion in a furnace operating at inadequate temperatures (Chapter 5), and are not typical soil samples taken from

industrialised areas of the country, where the TEQ values were less than 1 ng WHO-TEQ.kg^{-1} (Chapter 4, Table 4.4).

7.2.2 GC×GC-TOFMS Analysis of Taiwan Extracts

Method development and optimization has to be followed by method validation. The laboratory of the EPA in Taiwan is equipped with both GC-HRMS and GC×GC-TOFMS systems and it was possible to compare the results provided from a set of samples analysed by the standard GC-HRMS method with the ones obtained using GC×GC-TOFMS. Although the GC×GC-TOFMS method was developed in Taiwan, the raw analytical data collected was analysed at NMISA to obtain the GC×GC-TOFMS results for this study and compared with the GC-HRMS data. Tables 7.5 and 7.6 show an example of the results for two sediment extracts, one fly ash extract and one check standard. There is good agreement between the GC-HRMS and GC×GC-TOFMS indicating that the GC×GC-TOFMS method is capable of handling this complex dioxin analysis for environmental samples (Table 7.1). The TEQ values provided with the GC-HRMS results, as per regulation, were reported using the 1998 TEQs and not converted to the 2005 TEQs for result consistency (Van den Berg *et al.* 1998 and 2006).

The Taiwan sediment TEQ values were similar to those obtained for the South African toxic waste samples; TEQ values were calculated for the PCDD/Fs and did not include the non-*ortho* PCBs. The Taiwan fly ash TEQ values are 640 ng WHO-TEQ.kg^{-1} and the toxic waste sample no. 3 was an order of magnitude higher at 6400 ng WHO-TEQ.kg^{-1}. The preliminary PCDD/F levels obtained for the soil and sediment samples extracted at North West University (NWU) were not much above 10 ng WHO-TEQ.kg^{-1} (described in Chapter 4). The additional extracts of the Vaal Triangle samples had levels below 1 ng WHO-TEQ.kg^{-1} (also described in Chapter 4). These environmental samples provide an indication of the levels of contamination to be expected in and around South Africa, emphasizing the need to make accurate measurements for these highly toxic contaminants in the South African environment at extremely low concentration levels, although it must be noted that the information pertaining to PCDD/F environmental levels is based on one sample set. Not all samples are taken from contaminated waste sites that actually

contain high levels of PCDD/Fs and analyses must be done to verify the levels and to provide an accurate baseline.

Table 7.5. GC×GC-TOFMS and GC-HRMS PCDD/F results for the Taiwan extracts (pg/ g)

	Sample 1 (fly ash; pg/ g)		**Sample 2 (sediment; pg/ g)**		**Sample 3 (sediment; pg/ g)**		**Sample 4 (standard; pg/ µl)**	
PCDD/F Compounds	**GC×GC TOFMS**	**GC HRMS**	**GC×GC TOFMS**	**GC HRMS**	**GC×GC TOFMS**	**GC HRMS**	**GC×GC TOFMS**	**GC HRMS**
2,3,7,8-TCDD	**13**	**16**	**69**	**64**	**242**	**268**	**18**	**19**
1,2,3,7,8-PeCDD	78	69	4.5	4.1	45	26	77	75
1,2,3,4,7,8-HxCDD	66	68	7.2	6.1	24	19	52	49
1,2,3,6,7,8-HxCDD	80	84	13	13	64	81	56	51
1,2,3,7,8,9-HxCDD	47	60	10	10	45	51	41	54
1,2,3,4,6,7,8-HpCDD	383	610	117	186	413	675	35	69
OCDD	1196	1407	889	943	3413	3515	102	96
2,3,7,8-TCDF	**105**	**123**	**45**	**44**	**86**	**49**	**42**	**37**
1,2,3,7,8-PeCDF	227	212	13	19	31	38	48	50
2,3,4,7,8-PeCDF	642	531	232	203	67	91	49	51
1,2,3,4,7,8-HxCDF	497	461	222	205	575	634	52	50
1,2,3,6,7,8-HxCDF	522	524	16	20	102	117	45	50
2,3,4,6,7,8-HxCDF	865	979	15	18	30	59	44	50
1,2,3,7,8,9-HxCDF	144	160	6	7.6	39	47	66	72
1,2,3,4,6,7,8-HpCDF	2050	1993	698	632	2377	2260	48	47
1,2,3,4,7,8,9-HpCDF	236	290	12	16	142	160	43	52
OCDF	1320	1154	869	802	6805	6742	103	97

GC-HRMS results provided courtesy of EPA Taiwan (De Vos et al. 2011b).

Table 7.6. GC×GC-TOFMS and GC-HRMS TEQ results (ng WHO-TEQ.kg^{-1})

	Sample 1 (fly ash)		Sample 2 (sediment)		Sample 3 (sediment)		Sample 4 (standard)	
PCDD/F Compounds	**GC×GC TOFMS (TEF)**	**GC HRMS (TEF)**	**GC×GC TOFMS (TEF)**	**GC HRMS (TEF)**	**GC×GC TOFMS (TEF)**	**GC HRMS (TEF)**	**GC×GC TOFMS (TEF)**	**GC HRMS (TEF)**
2,3,7,8-TCDD	**13**	**16**	**69**	**64**	**242**	**268**	**18**	**19**
1,2,3,7,8-PeCDD	78	69	4.5	4.1	45	26	77	75
1,2,3,4,7,8-HxCDD	6.6	6.8	0.7	0.6	2.4	1.9	5.2	4.9
1,2,3,6,7,8-HxCDD	8.0	8.4	1.3	1.3	6.4	8.1	5.6	5.1
1,2,3,7,8,9-HxCDD	4.7	6.0	1.0	1.0	4.5	5.1	4.1	5.4
1,2,3,4,6,7,8-HpCDD	3.8	6.1	1.2	1.9	4.1	6.7	0.4	0.7
OCDD	0.1	0.1	0.1	0.1	0.3	0.4	0	0
2,3,7,8-TCDF	**11**	**12**	**4.5**	**4.4**	**8.6**	**4.9**	**4.2**	**3.7**
1,2,3,7,8-PeCDF	11	11	0.7	0.9	1.5	1.9	2.4	2.5
2,3,4,7,8-PeCDF	321	266	116	102	33	46	25	25
1,2,3,4,7,8-HxCDF	50	46	22	20	57	63	5.2	5.0
1,2,3,6,7,8-HxCDF	52	52	1.6	2.0	10	12	4.5	5.0
2,3,4,6,7,8-HxCDF	86	98	1.5	1.8	3.0	5.9	4.4	5.0
1,2,3,7,8,9-HxCDF	14	16	0.6	0.8	3.9	4.7	6.6	7.2
1,2,3,4,6,7,8-HpCDF	20	20	7.0	6.3	24	23	0.5	0.5
1,2,3,4,7,8,9-HpCDF	2.4	2.9	0.1	0.2	1.4	1.6	0.4	0.5
OCDF	0.1	0.1	0.1	0.1	0.7	0.7	0	0
TOTAL PCDD/Fs TEQs	**680**	**640**	**230**	**210**	**450**	**480**	**162**	**165**
% Difference	**6.8%**		**8.7%**		**6.4%**		**1.9%**	

GC-HRMS results provided courtesy of EPA, Taiwan, using the 1998 World Health Organization (WHO) Toxic Equivalency Factor (TEF) values (De Vos et al. 2011b).

7.2.3 Extended Study Results

As part of an extended study, soil samples extracted according to US EPA Method 1613B (1994a) and *Ministry of the Environment* Method E3418 (Kolic & Macpherson 2012), and analysed using GC-HRMS, were then courried to South Africa for repeat

analysis by GC×GC-TOFMS and confirmatory analysis by GC high resolution TOF (HRT). These results have been reported previously (De Vos *et al.* 2013b).

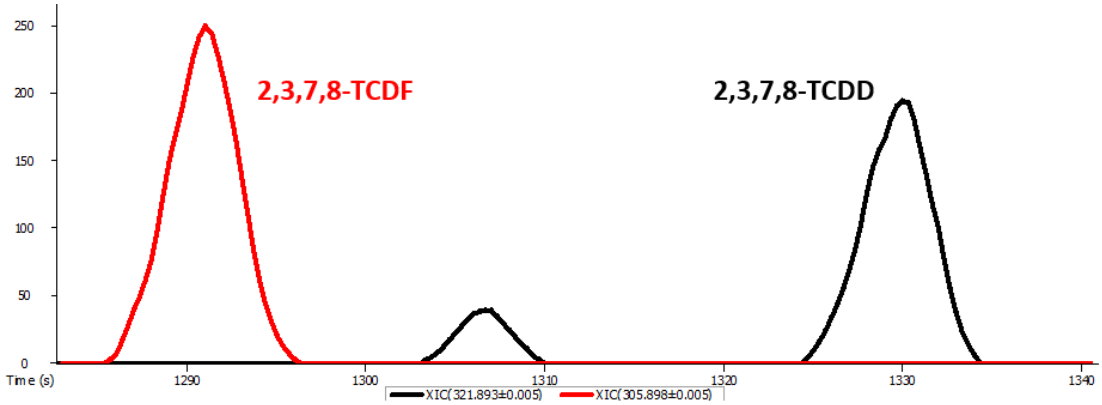


Figure 7.2. *Extracted Ion Chromatogram (XIC) for 2,3,7,8-TCDD (black trace, m/z = 321,893) and 2,3,7,8-TCDF (red trace; m/z = 305,898) at 500 fg/ µl (CS1 standard, Wellington Laboratories Inc.) on the GC-HRT system.*

As previously mentioned, the selectivity and sensitivity for GC×GC-TOFMS is provided by the increased peak capacity and focussing by the modulator. For the GC-HRT, selectivity is provided by the accurate mass capability (< 1 ppm), and sensitivity by the removal of chemical noise due to the high mass resolution. Modern high resolution mass spectrometers show little chemical noise on the plot of an exact mass ion. This is demonstrated in Figure 7.2, which shows a plot of the ion at m/z = 321.893 for 2,3,7,8-TCDD, and 305.898 for 2,3,7,8-TCDF at 500 fg/ µl confirming that the compound is readily detected at the lowest level required by US EPA Method 1613B (1994a), providing assurance that the instrument has the sensitivity for low level dioxin determination.

GC-HRMS results have been plotted against the results obtained using the GC-HRT to demonstrate that the GC-HRT produces similar results. If the instruments produce results that correlate exactly, then the results should all be on a straight line graph. Good agreement (regression correlation: R^2 = 0.9671 over the full concentration range for all PCDD/Fs for the six sediment samples, and R^2 = 0.9963 for 2,3,7,8-TCDD) was achieved, even though the sample extracts were not

analysed simultaneously, thus corroborating the use of GC-HRT for confirmation of analysis results obtained by GC×GC-TOFMS in South Africa (Figures 7.3 and 7.4).

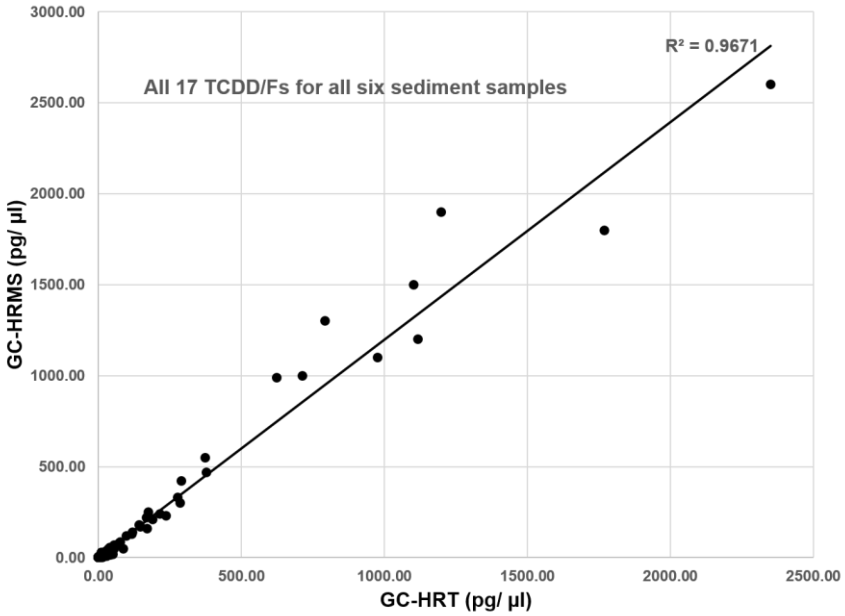


Figure 7.3. *GC-HRMS and GC-HRT comparison for the PCDD/Fs for all six sediment sample results. The correlation is not exactly linear, but adequate to demonstrate suitability for using the GC-HRT for dioxin analysis.*

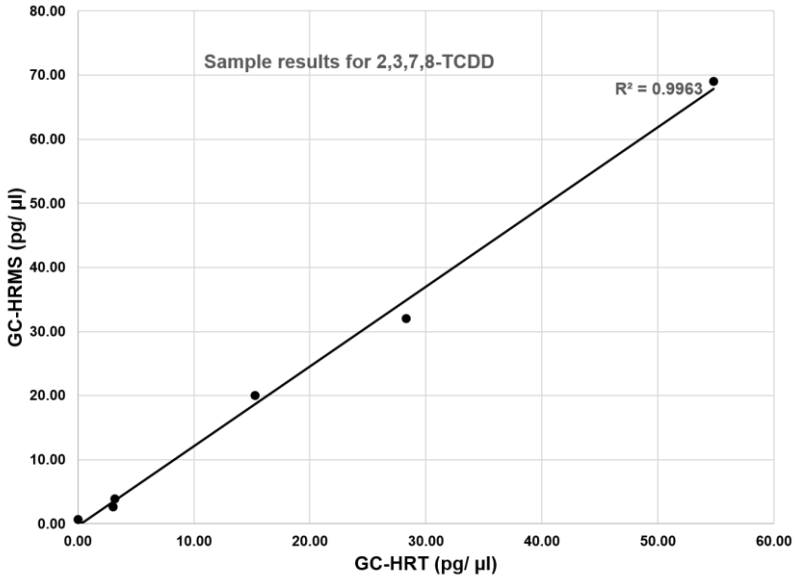


Figure 7.4. *2,3,7,8-TCDD results for the six sediment samples indicating very good correlation between the two instruments.*

Values obtained using the three different instruments correlate well, considering the low levels analysed and the fact that the extracts were not all analysed simultaneously; the extracts were reconstituted for the GC×GC-TOFMS and GC-HRT analyses. The TEQ values were calculated using the WHO 1998 values (Van den Berg *et al.* 1998) so that these results can be compared with previous results obtained (Tables 7.4 and 7.6). The percentage difference for the GC×GC-TOFMS and GC-HRT results were each calculated against the GC-HRMS results.

At the lowest levels, the GC×GC-TOFMS is not capable of detecting analytes, but this can be partially offset using the concurrent solvent re-condensation technique for large volume (CRS-LV) splitless injection as described by Magni & Porzano (2003). It can be done using an unmodified split/ splitless injection port, provided a guard column is used to protect the analytical column, and that the matrix is clean to minimize fouling of the inlet and columns (Misselwitz & Cochran 2011). For dioxin analysis, where the sample has been subjected to considerable clean-up, this latter condition is met, and the technique is advantageous when trying to analyze the trace-level PCDD/Fs in the sample on the low resolution system. The CRS-LV results can be seen in Table 7.7. The TEQ values were calculated against the CRS-LV results. The percentage difference is larger when using this approach, but it could just be an interference on the 2,3,7,8-TCDD peak area, although this was not confirmed.

When compared, the results from the two high resolution systems are generally in good agreement, providing added assurance that the GC-HRT is also capable of achieving the mandated requirements for dioxin quantification according to US EPA Method 1613B (1994a). The results obtained for the six sediment samples, calculated in pg/ g and in ng WHO-TEQ.kg^{-1}, are shown in Tables 7.7 to 7.12.

Table 7.7. GC×GC-TOFMS, GC-HRMS and GC-HRT *sample results for sediment A1 in pg/ g and ng WHO-TEQ.kg^{-1} (Van den Berg et al. 1988).*

PCDD/F Compounds	Sediment Sample A1 (pg/ g)			Sediment Sample A1 (ng.WHO TEQ.kg^{-1})		
	GC×GC-TOFMS	GC-HRMS	GC-HRT	GC×GC-TOFMS	GC-HRMS	GC-HRT
2,3,7,8-TeCDD	**ND (5.6)**	**2.6**	**3.0**	**5.6**	**2.6**	**3.0**
1,2,3,7,8-PeCDD	ND (ND)	1.1	1.5	0	1.1	1.5
1,2,3,4,7,8-HxCDD	ND (ND)	0.8	ND	0	0.1	0
1,2,3,6,7,8-HxCDD	ND (6.3)	1.7	3.1	0.63	0.2	0.3
1,2,3,7,8,9-HxCDD	ND (ND)	1.6	ND	0	0.2	0
1,2,3,4,6,7,8-HpCDD	31 (31)	24	33	0.3	0.2	0.3
OCDD	160 (170)	170	150	0.02	0	0.02
2,3,7,8-TeCDF	**ND (3.5)**	**3.7**	**3.5**	**0.4**	**0.4**	**0.4**
1,2,3,7,8-PeCDF	ND (6.6)	1.7	2.7	0.3	0.1	0.1
2,3,4,7,8-PeCDF	ND (12)	5.6	4.5	6	2.8	2.3
1,2,3,4,7,8-HxCDF	ND (5.7)	1.7	1.7	0.6	0.2	0.2
1,2,3,6,7,8-HxCDF	11 (9.8)	5.4	2.2	1.0	0.5	0.2
2,3,4,6,7,8-HxCDF	ND (ND)	0.6	ND	0	0.1	0
1,2,3,7,8,9-HxCDF	ND (6.5)	1.5	ND	0.7	0.2	0
1,2,3,4,6,7,8-HpCDF	ND (ND)	15	12	0	0.2	0.1
1,2,3,4,7,8,9-HpCDF	16 (16)	1.5	ND	0.2	0	0
OCDF	ND (31)	24	19	0	0	0
TOTAL TEQs				16		8
% Difference from GC-HRMS				44%	9	4%

**ND: Not Detected; value in brackets is for 5 µl Concurrent Solvent Re-condensation-Large Volume Splitless Injection (Magni & Porzano 2003). GC-HRMS results provided courtesy of MOE, Canada, using the 1998 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values.*

Table 7.8. GC×GC-TOFMS, GC-HRMS and GC-HRT *sample results for sediment A2 in pg/g and ng WHO-TEQ.kg^{-1} (Van den Berg et al. 1988).*

PCDD/F Compounds	Sediment Sample A2 (pg/ g)			Sediment Sample A2 (ng.WHO TEQ.kg^{-1})		
	GC×GC-TOFMS	GC-HRMS	GC-HRT	GC×GC-TOFMS	GC-HRMS	GC-HRT
2,3,7,8-TeCDD	**5.8**	**3.9**	**3.2**	**5.8**	**3.9**	**3.2**
1,2,3,7,8-PeCDD	14	4.7	5.1	14.0	4.7	5.1
1,2,3,4,7,8-HxCDD	23	4.1	5.1	2.3	0.4	0.5
1,2,3,6,7,8-HxCDD	14	9.9	13	1.4	1.0	1.3
1,2,3,7,8,9-HxCDD	18	8.1	20	1.8	0.8	2.0
1,2,3,4,6,7,8-HpCDD	66	70	65	0.7	0.7	0.7
OCDD	190	220	170	0.0	0	0.0
2,3,7,8-TeCDF	**48**	**59**	**52**	**4.8**	**5.9**	**5.2**
1,2,3,7,8-PeCDF	130	140	120	6.5	7.0	6.0
2,3,4,7,8-PeCDF	36	55	40	18.0	28	20
1,2,3,4,7,8-HxCDF	210	210	190	21.0	21	19
1,2,3,6,7,8-HxCDF	210	330	280	21.0	33	28
2,3,4,6,7,8-HxCDF	100	50	86	10.0	5.0	8.6
1,2,3,7,8,9-HxCDF	75	28	8.9	7.5	2.8	0.9
1,2,3,4,6,7,8-HpCDF	1000	1000	710	10.0	10	7.1
1,2,3,4,7,8,9-HpCDF	460	470	380	4.6	4.7	3.8
OCDF	5100	5200	6500	0.5	0.5	0.7
TOTAL TEQs				130		112
% Difference from GC-HRMS				1%	129	13%

***ND: Not Detected**
GC-HRMS results provided courtesy of MOE, Canada, using the 1998 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values.

Table 7.9. GC×GC-TOFMS, GC-HRMS and GC-HRT *sample results for sediment B1 in pg/g and ng WHO-TEQ.kg^{-1} (Van den Berg et al. 1988).*

PCDD/F Compounds	Sediment Sample B1 (pg/ g)			Sediment Sample B1 (ng.WHO TEQ.kg^{-1})		
	GC×GC-TOFMS	GC-HRMS	GC-HRT	GC×GC-TOFMS	GC-HRMS	GC-HRT
2,3,7,8-TeCDD	**48**	**32**	**28**	**48**	**32**	**28**
1,2,3,7,8-PeCDD	ND	2.8	2.8	0	2.8	2.8
1,2,3,4,7,8-HxCDD	ND	3.7	10	0	0.4	1.0
1,2,3,6,7,8-HxCDD	ND	9.3	13	0	0.9	1.3
1,2,3,7,8,9-HxCDD	ND	6	14	0	0.6	1.4
1,2,3,4,6,7,8-HpCDD	230	160	170	2.3	1.6	1.7
OCDD	1500	1900	1200	0.2	0.2	0.1
2,3,7,8-TeCDF	**22**	**26**	**20**	**2.2**	**2.6**	**2.0**
1,2,3,7,8-PeCDF	18	18	21	0.9	0.9	1.1
2,3,4,7,8-PeCDF	20	19	21	10	9.5	11
1,2,3,4,7,8-HxCDF	40	35	32	4.0	3.5	3.2
1,2,3,6,7,8-HxCDF	90	85	76	9.0	8.5	7.6
2,3,4,6,7,8-HxCDF	ND	2.3	7.9	0.0	0.2	0.8
1,2,3,7,8,9-HxCDF	32	17	23	3.2	1.7	2.3
1,2,3,4,6,7,8-HpCDF	410	300	280	4.1	3	2.8
1,2,3,4,7,8,9-HpCDF	120	47	55	1.2	0.5	0.6
OCDF	2400	2600	2400	0.2	0.3	0.2
TOTAL TEQs				85		67
% Difference from GC-HRMS				19%	69	3%

***ND: Not Detected**
GC-HRMS results provided courtesy of MOE, Canada, using the 1998 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values.

Table 7.10. GC×GC-TOFMS, GC-HRMS and GC-HRT *sample results for sediment B2 in pg/g and ng WHO-TEQ.kg^{-1} (Van den Berg et al. 1988).*

PCDD/F Compounds	Sediment Sample B2 (pg/ g)			Sediment Sample B2 (ng.WHO TEQ.kg^{-1})		
	GC×GC-OFMS	GC-HRMS	GC-HRT	GC×GC-TOFMS	GC-HRMS	GC-HRT
2,3,7,8-TeCDD	**ND**	**0.7**	**ND**	**0**	**0.7**	**0**
1,2,3,7,8-PeCDD	ND	1.9	ND	0	1.9	0
1,2,3,4,7,8-HxCDD	ND	2.9	ND	0	0.3	0
1,2,3,6,7,8-HxCDD	10	5.8	ND	1.0	0.6	0
1,2,3,7,8,9-HxCDD	15	3.9	14	1.5	0.4	1.4
1,2,3,4,6,7,8-HpCDD	78	75	71	0.8	0.8	0.7
OCDD	480	550	370	0	0.1	0
2,3,7,8-TeCDF	**43**	**50**	**40**	**4.3**	**5.0**	**4.0**
1,2,3,7,8-PeCDF	43	40	35	2.2	2.0	1.8
2,3,4,7,8-PeCDF	30	31	24	15	16	12
1,2,3,4,7,8-HxCDF	47	48	35	4.7	4.8	3.5
1,2,3,6,7,8-HxCDF	120	120	100	12	12	10
2,3,4,6,7,8-HxCDF	38	4.7	17	3.8	0.5	1.7
1,2,3,7,8,9-HxCDF	29	20	23	2.9	2.0	2.3
1,2,3,4,6,7,8-HpCDF	400	420	290	4.0	4.2	2.9
1,2,3,4,7,8,9-HpCDF	90	79	72	0.9	0.8	0.7
OCDF	1200	1200	1100	0.1	0.1	0.1
TOTAL TEQs				53		41
% Difference from GC-HRMS				3%	52	20%

***ND: Not Detected**
GC-HRMS results provided courtesy of MOE, Canada, using the 1998 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values.

Table 7.11. GC×GC-TOFMS, GC-HRMS and GC-HRT *sample results for sediment C1 in pg/ g and ng WHO-TEQ.kg^{-1} (Van den Berg et al. 1988).*

PCDD/F Compounds	Sediment Sample C1 (pg/ g)			Sediment Sample C1 (ng.WHO TEQ.kg^{-1})		
	GC×GC-TOFMS	GC-HRMS	GC-HRT	GC×GC-TOFMS	GC-HRMS	GC-HRT
2,3,7,8-TeCDD	**73**	**69**	**55**	**73**	**69**	**55**
1,2,3,7,8-PeCDD	21	3.1	10	21	3.1	10
1,2,3,4,7,8-HxCDD	18	5.2	17	1.8	0.5	1.7
1,2,3,6,7,8-HxCDD	41	14	18	4.1	1.4	1.8
1,2,3,7,8,9-HxCDD	32	8.6	28	3.2	0.9	2.8
1,2,3,4,6,7,8-HpCDD	210	230	240	2.1	2.3	2.4
OCDD	990	1300	790	0.1	0.1	0.1
2,3,7,8-TeCDF	**40**	**46**	**48**	**4.0**	**4.6**	**4.8**
1,2,3,7,8-PeCDF	32	14	22	1.6	0.7	1.1
2,3,4,7,8-PeCDF	150	180	140	75	90	70
1,2,3,4,7,8-HxCDF	46	21	37	4.6	2.1	3.7
1,2,3,6,7,8-HxCDF	210	240	220	21	24	22
2,3,4,6,7,8-HxCDF	36	1.5	13	3.6	0.2	1.3
1,2,3,7,8,9-HxCDF	41	14	42	4.1	1.4	4.2
1,2,3,4,6,7,8-HpCDF	950	990	620	9.5	9.9	6.2
1,2,3,4,7,8,9-HpCDF	58	17	51	0.6	0.2	0.5
OCDF	980	1100	980	0.1	0.1	0.1
TOTAL TEQs				229		188
% Difference from GC-HRMS				8%	210	11%

***ND: Not Detected**
GC-HRMS results provided courtesy of MOE, Canada, using the 1998 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values.

Table 7.12. GC×GC-TOFMS, GC-HRMS and GC-HRT *sample results for sediment C2 in pg/ g and ng WHO-TEQ.kg^{-1} (Van den Berg et al. 1988).*

PCDD/F Compounds	Sediment Sample C2 (pg/ g)			Sediment Sample C2 (ng.WHO TEQ.kg^{-1})		
	GC×GC-TOFMS	GC-HRMS	GC-HRT	GC×GC-TOFMS	GC-HRMS	GC-HRT
2,3,7,8-TeCDD	**21**	**20**	**15**	**21**	**20**	**15**
1,2,3,7,8-PeCDD	ND	2.5	5.2	0	2.5	5.2
1,2,3,4,7,8-HxCDD	7.8	2.9	5.8	0.8	0.3	0.6
1,2,3,6,7,8-HxCDD	10	8.3	10	1.0	0.8	1.0
1,2,3,7,8,9-HxCDD	ND	5.2	12	0.0	0.5	1.2
1,2,3,4,6,7,8-HpCDD	110	130	120	1.1	1.3	1.2
OCDD	1400	1500	1100	0.1	0.2	0.1
2,3,7,8-TeCDF	**15**	**18**	**12**	**1.5**	**1.8**	**1.2**
1,2,3,7,8-PeCDF	21	19	16	1.1	1.0	0.8
2,3,4,7,8-PeCDF	18	17	17	9.0	8.5	8.5
1,2,3,4,7,8-HxCDF	28	28	30	2.8	2.8	3.0
1,2,3,6,7,8-HxCDF	59	73	71	5.9	7.3	7.1
2,3,4,6,7,8-HxCDF	15	2.7	6.7	1.5	0.3	0.7
1,2,3,7,8,9-HxCDF	17	14	7.2	1.7	1.4	0.7
1,2,3,4,6,7,8-HpCDF	220	250	180	2.2	2.5	1.8
1,2,3,4,7,8,9-HpCDF	31	38	30	0.3	0.4	0.3
OCDF	1900	1800	1800	0.2	0.2	0.2
TOTAL TEQs				50		49
% Difference from GC-HRMS				3%	52	6%

***ND: Not Detected**
GC-HRMS results provided courtesy of MOE, Canada, using the 1998 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values.

7.2.4 Kruskal-Wallis Test

Tables 7.7 to 7.12 provide an overview of the GC×GC-TOFMS and GC-HRT results compared with the GC-HRMS results, but it is still not easy to see whether there is any significant difference discernable between the three approaches. The Kruskal–Wallis test is a way to compare the results obtained from the three instruments and assess whether they are in agreement (Abdollahi & Sajjadi 2009, Bonn 1979 and Kruscal & Wallis 1952).

The Kruscal-Wallis ANOVA (Kruscal & Wallis 1952) is used as a non-parametric alternative to analysis of variance tests such as ANOVAs. The test is used to determine if there is a statistically significant difference between data that is not normally distributed, when a sample set is too small or if the measurement scale is variable. The Kruscal-Wallis ANOVA can be used to compare three or more samples. The principles of this ANOVA are similar to those of a classical one-way ANOVA, however, the samples are compared on the basis of rank rather than that of group medians (Bonn 1979 and Kruscal & Wallis 1952). The smallest number gets a rank of 1 and the largest number gets a rank of n, where n is the total number of values in all the groups. The discrepancies among the rank sums are combined to create a single value called the Kruskal-Wallis statistic or the H-value; the larger the H-value the larger the discrepancy among rank sums (Kruscal & Wallis 1952). The p-value is the calculated probability of the null hypothesis being rejected when considering the inherent variability of the population being tested. The p-value is similar to a sigma value. For this analysis the p-value was calculated to be significant with a confidence level of 95%, therefore if p was equal to or smaller than 0.05, then there is a significant difference (StatSoft Inc 2013).

This test was used to determine the significant difference ($p < 0.05$) between the data obtained by GC×GC-TOFMS, GC-HRMS and GC-HRT (Figure 7.5), and provides a visual picture for quick appraisal of the six sets of results. The box indicates the minimum and the maximum concentration values of all values tabled (Tables 7.7 to 7.9), with the symbol [A1: KW-H (2,51) = 1.2579] indicating the medial value for the group. The box plot does not represent the variation in the measurement, but the variation in the concentration levels of the pollutants in the

various samples analysed. The Kruscal-Wallis ANOVA results indicate that there is no statistically significant difference between the data obtained using the three instruments ($p > 0.05$). However, this data should be used with caution due to the small sample set analysed.

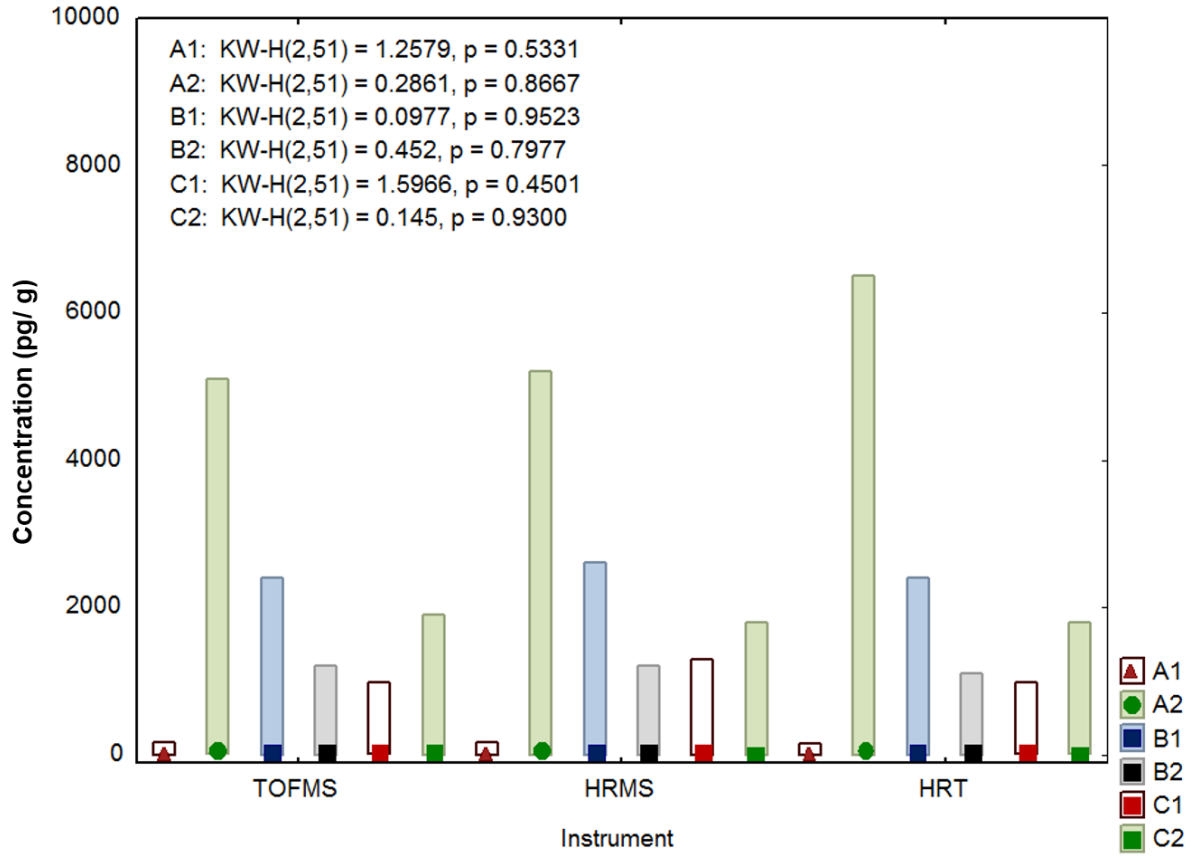


Figure 7.5. *Kruscal-Wallis test to show that there is no significant difference between the three sets of data obtained using GC×GC-TOFMS and GC-HRT compared against GC-HRMS.*

7.3 CONCLUDING REMARKS

GC×GC-TOFMS is a viable tool for PCDD/F screening and quantitation, making it suitable for environmental applications especially in cases where PCDD/F levels are greater than 1 ng/ kg. The technique is ideal for application in developing countries where GC-HRMS is not available, and can be used to minimise costs by selecting only positive samples for further overseas analysis by GC-HRMS, if required.

The GC×GC-TOFMS methods described are rapid (similar to GC-HRMS run times, Tables 7.1 and 7.2), convenient and produce accurate results. The close correlation between values obtained using the GC×GC-TOFMS approach and the established GC-HRMS method confirms the validity of this technique to quantify these compounds at levels required by regulatory bodies. Although the values with the GC×GC-TOFMS method are consistently higher than those obtained with the established technique, the differences are within permissible levels, considering that the analyses were performed in different laboratories and this was the first attempt to quantify such complex sample extracts for PCDD/Fs in South Africa.

GC-HRMS analysis of samples known to contain PCDD/Fs requires a targeted approach to achieve the required low limits of detection. The technique cannot locate and identify other priority POPs that may be present in the samples in the same run. Time-of-flight mass spectrometry, implemented either as a high resolution accurate mass instrument, or as a low resolution instrument coupled with comprehensive two-dimensional GC, provides an alternative to the traditional GC-HRMS approach. TOF systems have the sensitivity to achieve the low detection levels mandated by regulatory authorities for this analysis, and in addition provide the flexibility to locate and identify other priority pollutants in the same analytical run, leading to considerable savings of time and money. The TOF systems (GC×GC-TOFMS and HRT) were able to detect PCDD/Fs across a broad concentration range and successfully achieve detection at the mandatory lower limit required by US EPA Method 1613B (1994a).

The preliminary PCDD/F levels obtained for the soil and sediment samples extracted at North West University (NWU) were extremely low (10 ng TEQ.kg^{-1}) indicating that the levels of contamination to be expected in and around the Vaal Triangle of South Africa are not significant, but this is just one set of data and would have to be corroborated. It cannot be seen as representative of all PCDD/F levels in the country. Developing a capability to accurately measure ultra-trace levels for PCDD/F would then provide an opportunity to do a follow up analysis by taking different samples from more highly contaminated (heavily industrialised) areas. If these analyses had not been undertaken, the extremely low PCDD/F levels obtained would not have been known.

South Africa has little influence at the negotiation and decision-making level of the Stockholm Convention on candidate POPs due to the lack of analytical infrastructure and available data and is not in a position to address emergencies concerning POPs in the environment. These shortcomings could lead to a negative influence on human and environmental health and on trade and industry. The need to develop a local analytical capability that will employ regionally relevant methods and generate internationally acceptable results is now a real possibility based on the outcome of the extended study for the accurate quantitation of PCDD/Fs. As a developing country, the resources needed to make available these measurements must be carefully considered, but the need to have the capability cannot be emphasised enough; should it become apparent that all South African PCDD/F levels are low, then the analytical focus can be shifted to other POP compounds, using the same methodology, to asses contaminant levels that are extremely high, such as the total petroleum hydrocarbons and PAHs, but ultimately, a baseline must be established.

Validation of the method by GC×GC-TOFMS is required to ensure that South Africa has the capability to screen and quantify these contaminants and provide input into regulatory decision making. The validation method is described in Chapter 8.

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Dr Laura Quinn, Organic Analysis Laboratory, NMISA, Pretoria, South Africa, for the Kruskal-Wallis test results.

8

METHOD VALIDATION

The validation of an analytical method uses independent procedures to determine whether the method meets set requirements and specifications that fulfill an intended purpose. The criteria for the validation involves assessing specific parameters, including: selectivity/ specificity; accuracy/ trueness; precision (repeatability, reproducibility); limit of detection (LOD); limit of quantification (LOQ); sensitivity; working range and linearity; ruggedness/ robustness; recovery; uncertainty of measurement and traceability (Miller & Miller 2010 and Van Zoonen *et al.* 1998). The combination of: 1) competent laboratory, 2) validated methods, 3) measurement uncertainty and 4) metrological traceability; together, ensure the accuracy and comparability of measurement results.

Metrological traceability is the '*property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty*' (Barwick & Wood 2010, Meyer 2007 and JCGM-VIM 2012). The uncertainty of measurement is defined as '*a quantitative measure of accuracy*' and must be fit for the intended purpose of the data being generated. For higher order reference measurements, such as those used to assign values to certified reference materials (CRMs), it is important that the measurement uncertainty be as small as possible. This uncertainty will be imparted to the reference measurement or CRM being produced. Traceability is achieved by using high purity or neat CRMs to transfer trueness to calibrators, and traceability to the SI unit for mass (the kilogram), by incorporating the relationship between the measured value and the value of the CRM through the measurement equation (Shehata & Tahoun 2010 and Wise *et al.* 2012).

To perform a realistic estimation of the measurement uncertainty of an analytical method, as required under ISO/IEC 17025 (Ellison *et al.* 2012 and SANS 2005), the parameters influencing the measurement result, as well as the magnitude of their effect, need to be determined. This information is, in-part, obtained during method validation and the method validation data is used in the initial estimation of the measurement uncertainty (Ellison & Williams 2012, Miller & Miller 2010 and JCGM-VIM 2012).

Accuracy determination is indispensable when developing any analytical procedure, especially when analysing organic compounds at ultra-trace levels (Ellison & Williams 2012 and Miller & Miller 2010). Methods commonly used to determine accuracy and precision include the analysis of matrix matched certified reference materials and participation in either an inter-laboratory study or a proficiency testing scheme (PT). During the development and pre-validation of the NMISA procedure for the quantification of PCDD/Fs, it was essential to assess the accuracy of data obtained and this was achieved through an inter-laboratory comparison (Ellison & Williams 2012, Konieczka & Namieśnik 2008, Meyer 2007 and Reiner & Keller 2003).

8.1 NMISA VALIDATION APPROACH

A GC×GC-TOFMS method has been developed to screen for PCDD/Fs in soil and sediment, using the Rxi®-Dioxin2/ Rxi®-17SilMS column combination and the instrument parameters described in Chapter 6, Table 6.3. The selectivity/ specificity of the method to distinguish between the analytes of interest, co-eluting analytes and interfering substances on the basis of signal-to-noise has been described in Chapter 6. To address method accuracy and the remaining validation parameters, it was necessary to participate in an inter-laboratory comparison using South Africa soil samples and to analyse a matrix CRM where measurement results could be compared against reference values, in this case against a NIST Standard Reference Material (SRM 1944): New York/ New Jersey Waterway Sediment (Wise & Watters Jr *et al.* 2008) comprising selected polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, PCDD/Fs and trace elements in marine sediment and similar matrices.

Professor Eric Reiner, Laboratory Services Branch, The Ontario Ministry of the Environment, Canada (MOE), agreed to participate in a bilateral study as a continuation of the project initiated to develop a quantitative screening method for multiple classes of POPs using GC×GC-TOFMS in South Africa as discussed in Chapter 7.

South African soil has proved to be a challenging matrix for PCDD/F analysis (Chapter 4); as there are generally high levels of aliphatic compounds, pesticides and PAH compounds present that interfere in the ultra-low level PCDD/F analysis. Previously analysed soil samples were selected that indicated a positive BEQ_{20} response to 2,3,7,8-TCDD (5,70 to 52,32 ng WHO-TEQ.kg^{-1}). The samples, described in Chapter 4, represented residential, natural and agricultural areas. These were not representative of heavily contaminated soil, but were expected, due to their positive BEQ_{20} response, to have quantifiable levels of PCDD/Fs and enough of the sample material was available to allow for a homogenised sample to be prepared. The two participating laboratories each received five soil samples as well as the NIST SRM 1944 sediment for the comparison. During the comparison, each laboratory used their standard methods for extraction and analysis of the seventeen toxic PCDD/Fs. NMISA performed quantitation using GC×GC-TOFMS and MOE using GC-HRMS. The NMISA sample extraction is described in Chapter 5, with final results presented in Chapter 9.

Method development was based on US EPA Method 1613B (1994a), US EPA Method 8290A (2007d) and MOE 3148 (Kolic & Macpherson 2012) and data validation and review guidelines for PCDD/Fs using Method 1613B, and SW846 Method 8290A (US EPA 2014). The sensitivity of the method is dependent on the instrumental response at low levels and the level of interference within a given matrix. The method that is employed must be appropriate for the analytes of interest (PCDD/Fs), in the matrix of interest, and at the levels of environmental concern. The calibration range selected was thus applicable (0,5 to 200 pg/ µl 2,3,7,8-TCDD/F) to our pre-validation study for a 10 g soil sample.

8.2 REGRESSION ANALYSIS

The statistical procedures used with instrumental analysis methods must provide information on the precision and accuracy of the measurements. Regression analysis provides instrument response plotted against a series of solutions of known concentration. The calibration standards are measured under the same conditions as the unknown samples and a graph is drawn to plot concentration against response from which analysis results are calculated and the errors evaluated (Barwick 2003 and Miller & Miller 2010).

The algebraic form (regression formula) of a straight line is given as $y = bx + a$, where 'a' is the intercept of the line with the y-axis and 'b' is the slope. The slope is the measure of the sensitivity of the procedure; the steeper the slope, the more sensitive the instrumental response to a concentration change as plotted on the x-axis. The correlation between 'x' and 'y' is expressed by the correlation coefficient, 'r', as a measure of the fit of the points to a straight line, expressed as r^2, the coefficient of determination/ variation. Using regression statistics the measurement equation is determined and various validation parameters are investigated; including linearity, sensitivity, as well as the LOD and LOQ (Ellison *et al.*2012, Miller & Miller 2010 and Van Zoonen *et al.* 1998).

The correlation coefficient 'r' can be calculated using Equation 8.1 and the information reported in Table 8.1. The correlation coefficient, r, is often expressed as r^2 and the advantage is that, when multiplied by 100, it indicates the percentage of variation in 'y' associated with the variation in 'x'. The regression analysis can also be calculated in Microsoft excel and the summary output can be seen in Figure 8.1 for 2,3,7,8-TCDD.

Determining linearity, LOD and LOQ of PCDD/Fs in soil and sediment requires calculation of the analyte standard mass ratio (mass native; 2,3,7,8-TCDD to mass labelled; 1,2,3,4-[$^{13}C_{12}$]-TCDD) and analyte standard peak area ratio (peak area for 2,3,7,8-TCDD to peak area for 1,2,3,4-[$^{13}C_{12}$]-TCDD) using Equation 7.1 (Chapter 7). The random error (standard deviation) in 'a' and 'b' are important factors that can impact the uncertainty associated with the measurement result. Table 8.1 summarises the errors using ŷ-residuals for 2,3,7,8-TCDD. Once the residuals are known, the standard deviation in the y-direction can be calculated, which estimates the errors in

the y-direction ($S_{y/x}$) (Equation 8.2). This standard deviation can be used to calculate the standard deviation in the y-intercept and slope using Equations 8.3 and 8.4.

The standard concentration (x_i) is the ratio of the mass [$^{12}C/^{13}C$] in the standards of the calibration curve and the signal (y_i) is the ratio of the peak areas [$^{13}C/^{12}C$] in the standards of the calibration curve and the regression line equation can be used to calculate the concentration x_0 from a given signal y_0. Because the calculation is now moving from a y-value to an x-value, the error in x must be determined, and it is now necessary to find the error associated with this concentration estimate, namely, s_{x0}. This is done using Equation 8.5 to determine the standard error in x_0.

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}}$$

Correlation coefficient — Equation 8.1

$$s_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}}$$

Standard deviation of ŷ residuals — Equation 8.2

$$s_b = \frac{s_{y/x}}{\sqrt{\sum_i (x_i - \bar{x})^2}}$$

Standard deviation of the slope — Equation 8.3

$$s_a = s_{y/x} \sqrt{\frac{\sum_i x_i^2}{n \sum_i (x_i - \bar{x})^2}}$$

Standard deviation of the intercept — Equation 8.4

$$s_{x_0} = \frac{s_{y/x}}{b} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x})^2}}$$

Standard uncertainty for the value of the analyte (*m* duplicate injections) — Equation 8.5

Table 8.1. *Standard mass concentration ratios, peak area signal ratios and ŷ-residuals used to calculate the calibration error for 2,3,7,8-TCDD.*

Cal std	Conc ratio (x_i)	Signal (y_i)	Ave conc $\bar{x}_i$	Ave signal $\bar{y}_i$	$(x_i - \bar{x})$	$(x_i - \bar{x})^2$	$(y_i - \bar{y})$
CS0.1_1	0.0005	0.0024	0.0005	0.0024	-0.5045	0.2545	-0.5586
CS0.1_2	0.0005	0.0028	0.0005	0.0028	-0.5045	0.2545	-0.5582
CS0.2_1	0.0010	0.0022	0.0010	0.0022	-0.5040	0.2540	-0.5588
CS0.2_2	0.0010	0.0037	0.0010	0.0037	-0.5040	0.2540	-0.5572
CS0.5_1	0.0025	0.0048	0.0025	0.0048	-0.5025	0.2525	-0.5562
CS0.5_2	0.0025	0.0043	0.0025	0.0043	-0.5025	0.2525	-0.5567
CS1_1	0.005	0.0066	0.005	0.0066	-0.5000	0.2500	-0.5544
CS1_2	0.005	0.0077	0.005	0.0077	-0.5000	0.2500	-0.5533
CS2_1	0.02	0.0228	0.02	0.0228	-0.4850	0.2352	-0.5382
CS2_2	0.02	0.0538	0.02	0.0538	-0.4850	0.2352	-0.5072
CS3_1	0.10	0.0921	0.10	0.0921	-0.4050	0.1640	-0.4688
CS3_2	0.10	0.0906	0.10	0.0906	-0.4050	0.1640	-0.4703
CS4_1	0.40	0.4270	0.40	0.4270	-0.1050	0.0110	-0.1340
CS4_2	0.40	0.4481	0.40	0.4481	-0.1050	0.0110	-0.1129
CS5_1	2.0	2.2526	2.0	2.2526	1.4950	2.2350	1.6917
CS5_2	2.0	2.2083	2.0	2.2083	1.4950	2.2350	1.6473
Sum (Σ)	**5.050**	**5.6096**	**0.5050**	**0.5609**	**0.0000**	**5.7906**	**0.0000**

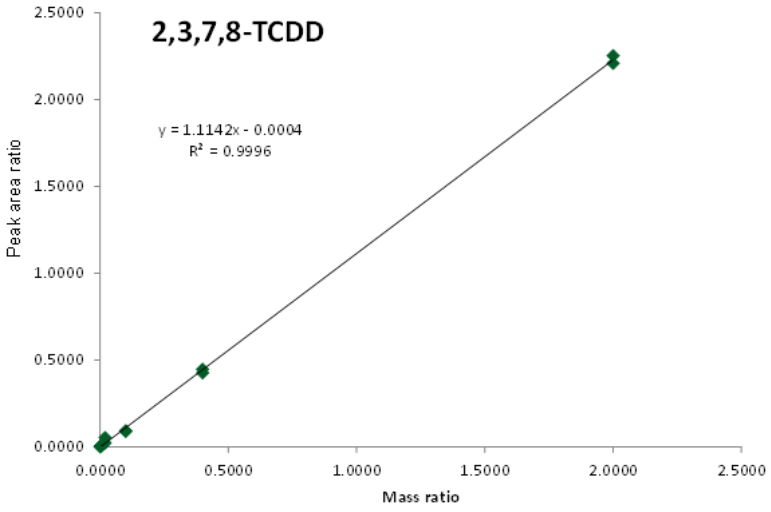
Cal std	$(y_i - \bar{y})^2$	$(x_i - \bar{x})(y_i - \bar{y})$	x_i^2	$\hat{y}_i$	$(y_i - \hat{y}_i)$	$(y_i - \hat{y})^2$
CS0.1_1	0.3120	0.2818	0.0000	-0.002	0.004	0.0000
CS0.1_2	0.3116	0.2816	0.0000	-0.002	0.005	0.0000
CS0.2_1	0.3122	0.2816	0.0000	-0.001	0.003	0.0000
CS0.2_2	0.3105	0.2808	0.0000	-0.001	0.005	0.0000
CS0.5_1	0.3093	0.2795	0.0000	0.000	0.004	0.0000
CS0.5_2	0.3099	0.2797	0.0000	0.000	0.004	0.0000
CS1_1	0.3073	0.2772	0.0000	0.003	0.003	0.000
CS1_2	0.3061	0.2766	0.0000	0.003	0.004	0.000
CS2_1	0.2896	0.2610	0.0004	0.020	0.003	0.000
CS2_2	0.2572	0.2460	0.004	0.020	0.034	0.001
CS3_1	0.2198	0.1899	0.010	0.109	-0.017	0.000
CS3_2	0.2212	0.1905	0.010	0.109	-0.019	0.000
CS4_1	0.0179	0.0141	0.16	0.444	-0.017	0.000
CS4_2	0.0127	0.0119	0.16	0.444	0.004	0.000
CS5_1	2.8618	2.5291	4.0	2.229	0.024	0.001
CS5_2	2.7137	2.4628	4.0	2.229	-0.020	0.000
Sum (Σ)	**7.2075**	**6.4589**	**8.3409**	**5.610**	**0.000**	**0.003**

The data summarised for the regression analysis for 2,3,7,8-TCDD in Figure 8.1 indicates a good general precision; S_a and S_b are smaller than $S_{y/x}$. The calibration can be considered sensitive with significant linearity fitting a straight line model as the slope has an R^2 = 0.9996. The uncertainty in the intercept is smaller than the uncertainty in the slope. This indicates that the working range should be wider to

include more points between the limit of quantitation and the maximum calibration point.

The same approach in evaluating the calibration curves was applied to each of the seventeen PCDD/F congeners. Once the regression analysis has been done, the concentration of the 2,3,7,8-TCDD for the remaining sixteen PCDD/Fs can be calculated using Equation 7.3.

a	-0.000386994
b	1.114242939
No. of Stds	16
DF	14
$s_{y/x}$	0.0150
s_a	0.0016
s_b	0.0058
Theoretical LOD (pg/ g)	0..011
Theoretical LOQ (pg/ g)	0. 014



Regression Statistics	
Multiple R	0.999810782
R Square	0.9996216
Adjusted R Square	0.999594572
Standard Error	0.015044051
Observations	16

ANOVA

	df	SS	MS	F	Significance F
Regression	1	8.370326402	8.370326402	36983.91009	2.32731E-25
Residual	14	0.003168528	0.000226323		
Total	15	8.37349493			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept (a)	-0.000386994	0.0041833	-0.0925093	0.927604163	-0.009359281	0.008585292	-0.0093593	0.008585292
X Variable 1 (b)	1.114242939	0.005793933	192.3120123	2.32731E-25	1.101816188	1.12666969	1.10181619	1.12666969

Figure 8.1. *Regression analysis summary output for 2,3,7,8-TCDD.*

The estimated standard deviation for the value of 2,3,7,8-TCDD is then determined using Equation 8.5. Just as the calibration curve parameters can be subject to errors, the measurements of unknowns can also be subject to errors. All the errors need to be combined to establish the total error in the final measurement result for each PCDD/F. In Equation 8.5, y_0 is the experimental value from which the concentration x_0 is to be determined, 'n' is the number of calibration solutions, 'm' duplicate injections made on the sample to obtain y_0, and S_{x0} is the estimated standard deviation (standard error) of x_0. The results are shown in Table 8.2.

Table 8.2. *Confidence limits and random error in the results for 2,3,7,8-TCDD.*

Samples (Duplicate)	y_0	x_0	$(x_o-\dot{x})^2$	S_y	Lower CL_{y0}	Upper CL_{y0}	S_{x0}	+/-
NIST SRM 1944	0.544	0.489	0.030	0.00389	0.536	1.693	0.0130	0.0279
Soil 10	0.005	0.005	0.097	0.00417	-0.003	-0.002	0.0132	0.0282
Soil 16	0.007	0.007	0.096	0.00417	-0.002	0.003	0.0132	0.0282
Soil 21	0.012	0.011	0.093	0.00416	0.003	0.019	0.0131	0.0282
Soil 23	0.007	0.007	0.096	0.00417	-0.002	0.003	0.0132	0.0282
Soil 42	0.000	0.000	0.100	0.00418	-0.009	-0.019	0.0132	0.0282

From the standard error calculated for the 2,3,7,8-TCDD calibration, the uncertainty can be calculated in each sample using the t-value of the Student's t-distribution (degrees of freedom and 95% confidence limit; Miller & Miller 2010). Table 8.3 shows the calculation to convert the concentration [$^{12}C/^{13}C$] to ng using the average ^{13}C isotope added to the soil samples (2.6434 ng). The final results for the seventeen PCDD/Fs will be discussed in Chapter 9.

Table 8.3. *The uncertainty calculated from the standard error when considering the number of calibrants in the calibration curve at the 95% confidence level.*

Samples	Error uncertainty (+/-), from Table 8.2	Average ^{13}C label added to the samples (ng)	Uncertainty (ng)
140120_Nist1944-A_1	0.0279	2.6434	0.0738
140120_Soil10-A_1	0.0282	2.6434	0.0746
140120_Soil16-A_1	0.0282	2.6434	0.0746
140120_Soil21-A_1	0.0282	2.6434	0.0745
140120_Soil23-A_1	0.0282	2.6434	0.0746
140120_Soil42-A_1	0.0282	2.6434	0.0746

Regression analysis only determines the relative error uncertainty on the calibration curve and the equation used to calculate the concentration in an unknown sample. In order to include all the uncertainty contributors, a full uncertainty budget for the analysis of PCDD/Fs in soil and sediment must be calculated.

8.2.1 Uncertainty of Measurement

Uncertainty of Measurement (UoM) is a statistical parameter that describes the possible fluctuations of the result of a measurement. It can be determined by the addition of the variances of the individual steps followed during a test procedure. When estimating UoM, all sample results obtained from the NIST SRM 1944 analysis were used. The precision of the analytical method has to be considered through repeatability (all parameters remain the same: analyst, reagents and the repeats are done on the same day) and reproducibility (the same method is used, but all other parameters are changed: different analysts, different solvents, etc.) studies. The accuracy is the closeness of agreement between the result and the accepted reference value of the analyte and is assessed through CRMs. Spiked samples alone do not provide much information on accuracy, as the recovery is always better than with incurred materials. However, they do provide information on areas where recovery can be improved and can also assist in determining if the measurement equation is correct.

The equation for the calculation of the quantity (x) or analytical result/ measurand (metrological terminology, Table 8.4), must be noted in detail, together with any other available relevant information (Equation 7.3). It is necessary to develop and record a list of sources of uncertainty relevant to the analytical method. It is often useful to structure this process, both to ensure comprehensive coverage and to avoid uncertainty overestimation. Identifying all the effects on the result is summarised in a '*cause and effect diagram'* (referred to as an Ishikawa or 'fishbone' diagram; Ellison *et al.* 2012 and Meyer 2007). Every parameter that can affect the measurement result should appear in the diagram (Figure 8.2). Although such a diagram can be drawn by free association, it should afterwards be compared with the equation (Equation 7.3), as many factors can be accounted for in single parameters. An example is the extraction efficiency, clean-up and concentration uncertainty that can be assessed as a single bias parameter through the analysis of CRMs. If required, this parameter can then be included in the measurement equation as a correction factor, although this is not recommended. Precision describes random error, whereas bias describes systematic error, and the accuracy (closeness to the true value of a single

measurement or a mean value) incorporates both types of error and these sources of error need to be assessed.

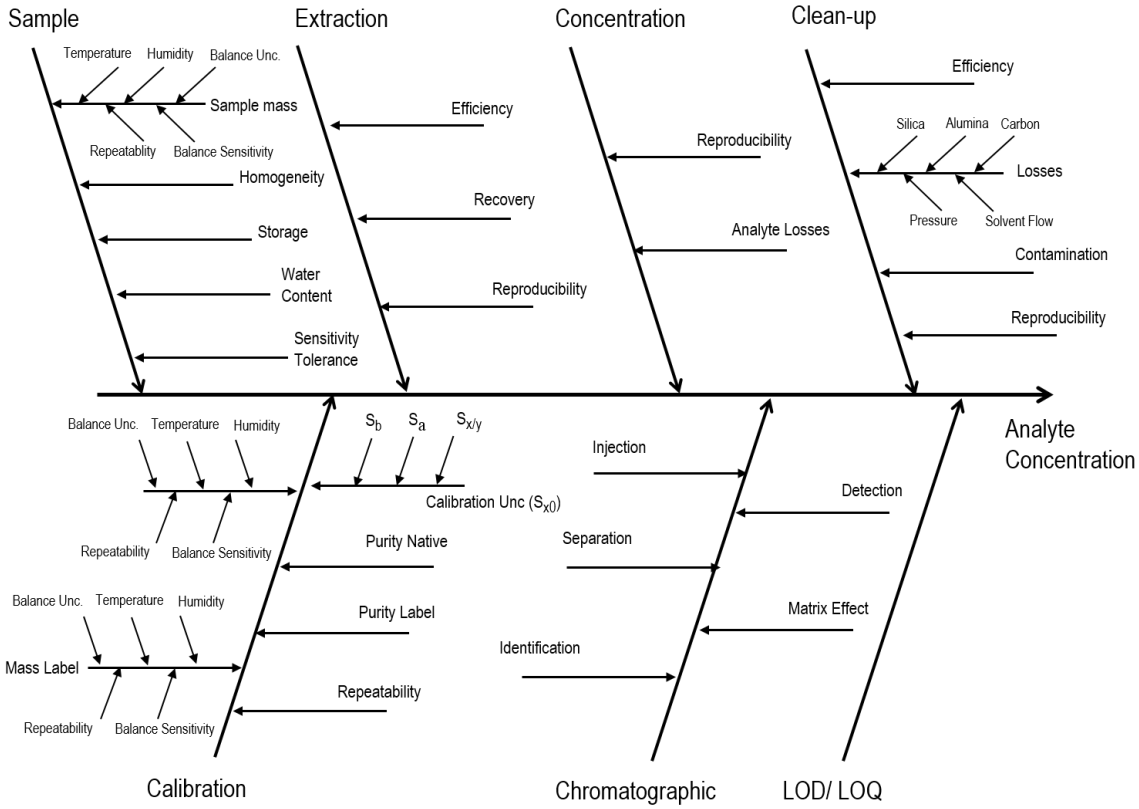


Figure 8.2. *Cause and effect diagram (Ishikawa or 'fishbone' diagram) showing the uncertainty sources of the measurement uncertainty of the analysis.*

Table 8.4. *Simplified uncertainty budget for the analysis for PCDD/Fs in soil and sediment*

Uncertainty contributor	Description	Quantity (x)	Measurement uncertainty (U)
Purity native	As stated on certificate of analysis	Purity mass fraction	Considering reported uncertainty and coverage factor; no information then use rectangular distribution
Recovery	Imported from matrix matched CRM analysis	Average extraction recovery (mass fraction)	Standard deviation of recovery or the estimated standard deviation of the mean
Precision	Precision of multiple analysis	Average obtained for sample analysed	Relative standard deviation of repeat measurements
Reproducibility	Imported from matrix matched CRM analysis	Average concentration of the analyte obtained for CRM on repeat analysis	Relative standard deviation of repeat measurements
S_{x0}	Error of the calibration curve	Average obtained for sample analysed	S_{x0} of calculated calibration curve

The measurement uncertainty described can be substantially simplified due to the procedure used during analysis and the method used to quantify the results (Table 8.4) and must be accompanied by a traceability statement (Figure 8.3).

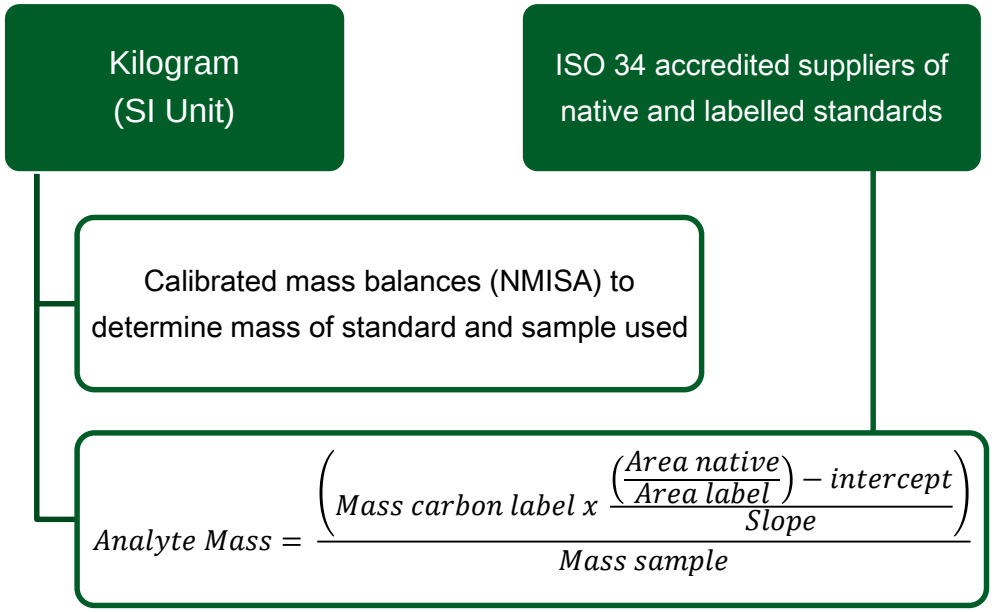


Figure 8.3. *Traceability Statement for the analysis of PCDD/Fs in soil and sediment*

There are currently no PCDD/F certified reference materials available from National Metrology Institutes. The traceability statement for the analysis of PCDD/Fs in soil and sediment includes all standards purchased from ISO 34 accredited suppliers and measurement traceability is to the SI Unit for the kilogram (Ellison *et al.* 2012).

8.2.2 Limit of Detection and Quantitation

There are many accepted methods for the determination of LOD and LOQ that can be determined either experimentally or calculated mathematically. The LOD is defined as the lowest analyte concentration that can be repeatedly and reliably distinguished from instrumental background (Armbruster & Pry 2008). The LOD is usually expressed as the analyte (PCDD/F) concentration that corresponds to the sample blank plus three sample standard deviations. When working with complex matrices it is prudent to determine the LOD within the matrix and not using solvent. Matrix interferences and suppression effects can negatively impact the LOD of the method and should be accounted for. The LOQ can be defined as the lowest concentration of

the analyte that can be accurately quantified within an acceptable and predefined level of uncertainty (Van Zoonen *et al.* 1998). As with the LOD, the LOQ is not only impacted by instrumental sensitivity, but also by the quality of the extract and analysis selectivity.

Factors such as significant losses during the analysis (system leaks, incomplete SPE elution, drying down losses) must be considered when working at levels close to the instrument sensitivity. These losses result in the respective peak areas of the isotope and analyte falling below the LOD or LOQ of the instrument. Quantitation close to the LOD/ LOQ of an instrument results in a high measurement uncertainty due to a decrease in measurement precision (Rocke *et al*. 2003). The estimated uncertainty of measurement (UoM) improves significantly for samples with incurred levels of PCDD/F well above the LOD and LOQ, and where the peak areas fall within the prepared calibration range. According to the ISO GUM, gross errors should be resolved and should not be included in the UoM estimate (JCGM-GUM 2008 and Miller & Miller 2010).

US EPA 8290A (2007d) stipulates that all signal intensities must be ≤ 2.5 times the noise level for positive identification of an unlabelled PCDD/F compound or a group of co-eluting isomers and describes the procedure to be followed for the manual determination of the S/N; labelled analytes must have a S/N ≥ 10. When calculating LOQ for GC×GC-TOFMS, the classical 3 x LOD should be applied with caution. During GC×GC analysis a single peak is divided into slices and it is difficult to determine the true noise value at a specific point within the ^{2}D chromatogram. The second approach used is a mathematical extrapolation using three times the value of the intercept (S_y) calculated from the calibration curve. However, since a non-matrix matched calibration curve is used for the quantification of PCDD/Fs, this value can be well below the realistic LOD achievable within an analysed sample. The instrument response to the concentration level is thus more repeatable in the measurable range (Table 8.1, between calibration standards CS1 and CS2). At concentrations below 0,5 pg/ µl, detection of the peaks is forced and the uncertainty increases exponentially. The regression formula is also assessing the repeatability of the concentration at the higher concentrations (400 x more concentrated than the lower levels); the curve is forced and ignores the lower limits, providing skewed results that cannot be accurately

reported. Matrix interference increases the limit detectable on a single extracted ion for quantitation and the calibration standard (CS1 = 0,5 pg/ µl) is too close to the LOD of the instrument in this instance.

An alternative mathematical extrapolation approach, is to use the uncertainty of the calibration curve as represented by the standard error ($S_{y/x}$) of the linear regression. This error takes both the uncertainty of the slope and the intercept into account and if repeat measurement of the calibration curve is used, it will also take the reproducibility of the signal in response to the concentration into account. In general the standard error ($S_{y/x}$) is greater than the uncertainty in the slope as well as the uncertainty in the intercept as illustrated by the 2,3,4,8-TCDD regression analysis in Figure 8.1.

8.2.2.1 Factors affecting LOD/ LOQ

Many parameters in an experimental procedure can impact the LOD and LOQ that can be obtained for a specific compound. A study of all the parameters that could affect the LOD/ LOQ during GC×GC-TOFMS analysis was undertaken. The data was taken from the various calibration curves generated for sample extracts provided by the collaborating laboratories (University of Liége, EPA Taiwan and Ministry of the Environment (MOE), and various extracts from NMISA. The data was at times rather limited, so extensive interpretation was not possible. This was undertaken so as to ensure a large enough set of data for comparison as there is not extensive data to analyse and interpret. Since all the extracts were made using either Wellington Laboratories *Inc.* or Cambridge Isotope Laboratories *Inc.*, native and labelled standards (described in Chapters 4 and 7), and following US EPA 1613B (1994a) methodology, the extraction was not considered to have any effect on results obtained since the LOD and LOQ were determined from the calibration solutions obtained from suppliers. The LOD/ LOQs for the seventeen toxic PCDD/Fs were corrected for the mass extraction and the nominal concentration effect in the reconstitution process. The parameters focussed on during this study included:

The column combinations

Column combinations were selected to obtain both the most effective congener separation of the seventeen toxic PCDD/Fs, and separation from sample matrix. During method development, various column combinations were assessed and the

analytical methods optimised to obtain the best possible separation (Chapters 4, 6 and 7). These optimised results were then used to calculate the combined LODs and LOQs. The combinations tested included the Rxi®-5SilMS/ Rtx®-200, the Rxi®-XLB/ Rtx®-200 and Rtx®-Dioxin2/ Rxi®-17SilMS columns (Chapter 4, Table 4.1).

Data processing

Two approaches were used during initial data processing using ChromaTOF® software. The first method, referred to as the '*old data processing method* (OP),' used the optimised software data processing method with data review using the actual calibration software. During data processing, standards that appeared to be 'outliers' were removed to improve the RRF. The second method, referred to as the '*new data processing method* (NP),' also used an optimised software data processing method, but was combined with a manual review of each of the seventeen native and labelled congener peak slices. Any peak slices that were incorrectly assigned using the old data processing method, were corrected with the new data processing method.

The calculation used to determine the LOD/ LOQ

Numerous methods were used to determine the LOD/ LOQs for the analysed PCDD/Fs. These methods were based on standard laboratory protocols as well as more conservative metrological techniques. The methods compared included:

(1) Routine linear regression approach using the uncertainty on the y-intercept (S_a), where the LOD = y-intercept + (3 x S_a), and the LOQ = y-intercept + (10 x S_a), referred to as 'MC_N' (Ellison *et al.* 2012 and Miller & Miller 2010);

(2) Conservative linear regression approach using the uncertainty on the y-intercept and slope ($S_{y/x}$), where the LOD = y-intercept + (3 x $S_{y/x}$) and the LOQ = y-intercept + (10 x $S_{y/x}$) referred to as 'MC_O' (Dóka *et al.* 2013 and Konieczka & Namieśnik 2008);

(3) The response factor (RF) method which is similar to MC_O as it also considers $S_{y/x}$ (US EPA 1994a), and

(4) Relative Peak Area Percentage (PAR) that calculated the LOD as 3 x the peak area of noise in a blank sample. The LOQ was calculated as 10 x the peak area of noise in a blank sample (not a recommended method).

Outlier removal of data points generated using repeat injections

Initial data review was not based on statistical outlier removal. Inspection of the calibration curves obtained as generated by LECO ChromaTOF® software, necessitated the removal of one or more repeat injections that clearly indicated an instrumental error, referred to as 'OR'. These were developmental results and the reasons were not investigated at the time. As the method was optimised, an assessment of the effect of 'outlier' removal on the LOD/ LOQs using ŷ-residuals was performed.

8.2.2.2 Statistical analysis

Variations in the calculated LODs and LOQs were assessed using a one-way analysis of variance (ANOVA) in *Statistica* (StatSoft 2013). All statistical analyses were done on untransformed data. When using one-way ANOVA, the group sample sizes do not have to be equal and when a statistical difference is identified between the '*means*' of different groups, the post-hoc '*Tukey'* test (an honest significant difference test) was used to identify which '*means*' were responsible for this difference (Fowler *et al.* 1998 and Tukey 1949). The statistical analysis was performed on a congener specific basis as the congener chemistry could vary significantly over the range of PCDD/Fs analysed, taking physical and chemical characteristics into consideration, the column interactions and resulting separation that was achieved. The large box and whiskers do not represent the variation in the measurement, but the variation in the concentration levels of the pollutants in the various samples analysed.

Tables 8.5 to 8.13 have been included at the end of the Chapter 8 for review. Tables 8.5 to 8.8 summarise the LODs and LOQs obtained using various columns, processing and calculation methods. Tables 8.5 and 8.6 indicate large discrepancies in some of the LODs observed; one example being the 1,2,3,4,7,8-HxCDF that jumps from 9,02 pg/ g using the new processing method to 1848 pg/ g using the old processing method. The latter value was one of the earlier South African extracts that required improved

sample clean-up. The separation between the 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF is based on the ^{1}D retention time only as these two compounds have the same ^{2}D retention time and the same mass, which results in the overlap of peak slices in the same modulation. The deconvolution software cannot distinguish between these two peaks, and consequently an incorrect assignment of slices results in the wrong values, especially if there are still matrix interferences present.

8.2.2.3 The effect of column type on the LOD/ LOQ

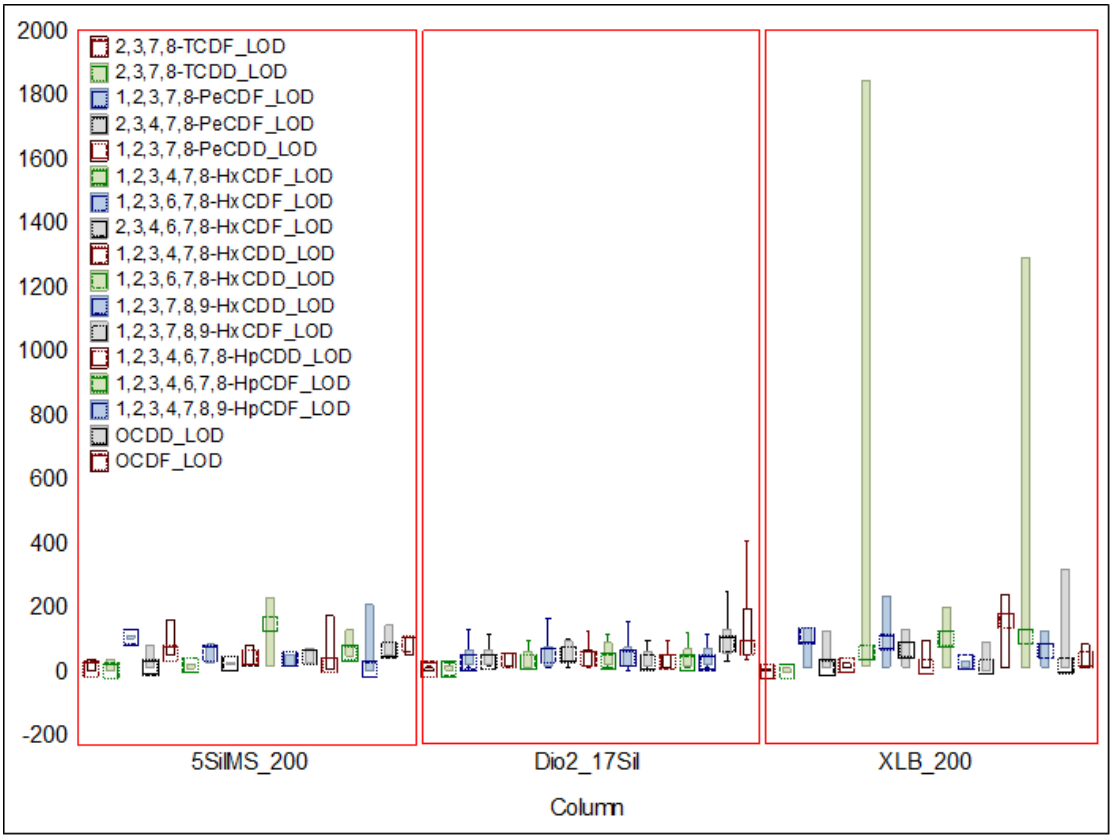


Figure 8.4. *Differences in the LOD/ LOQ obtained using various column sets during method development. The Rxi®-XLB/ Rtx®-200 column combination has a larger variability than the other column sets analysed. The 'y-axis' is the concentration in pg/ g.*

For a one-way ANOVA, a value of $p \geq 0.05$ indicates no significant difference obtained between the different column sets. The only statistically significant effect was on the LOQ for 1,2,3,7,8-PeCDF (possible interferences that did not affect the LOD significantly; $p = 0,1$) and on the LOD/ LOQ for 1,2,3,4,6,7,8-HpCDD (Figure 8.4 and Table 8.9).

The post-hoc '*Tukey'* test indicated a significant improvement in the 1,2,3,7,8-PeCDF LOQ obtained on the Rtx®-Dioxin2/ Rxi®-17SilMS combination as compared to the Rxi®-5SilMS/ Rtx®-200 combination (Table 8.13). There was a significant improvement in the LOD and LOQ for 1,2,3,4,6,7,8-HpCDD using the Rtx®-Dioxin2/ Rxi®-17SilMS when compared with the Rxi®-XLB/ Rtx®-200 combination. This observation was not expected, but could be a result of the multi-step temperature program, the inadequate sample extraction method and the comparatively small sample size used with the Rxi®-XLB/ Rtx®-200 column set (Chapter 4, Table 4.1), although not verified.

8.2.2.4 The effect of processing on the LOD/ LOQ

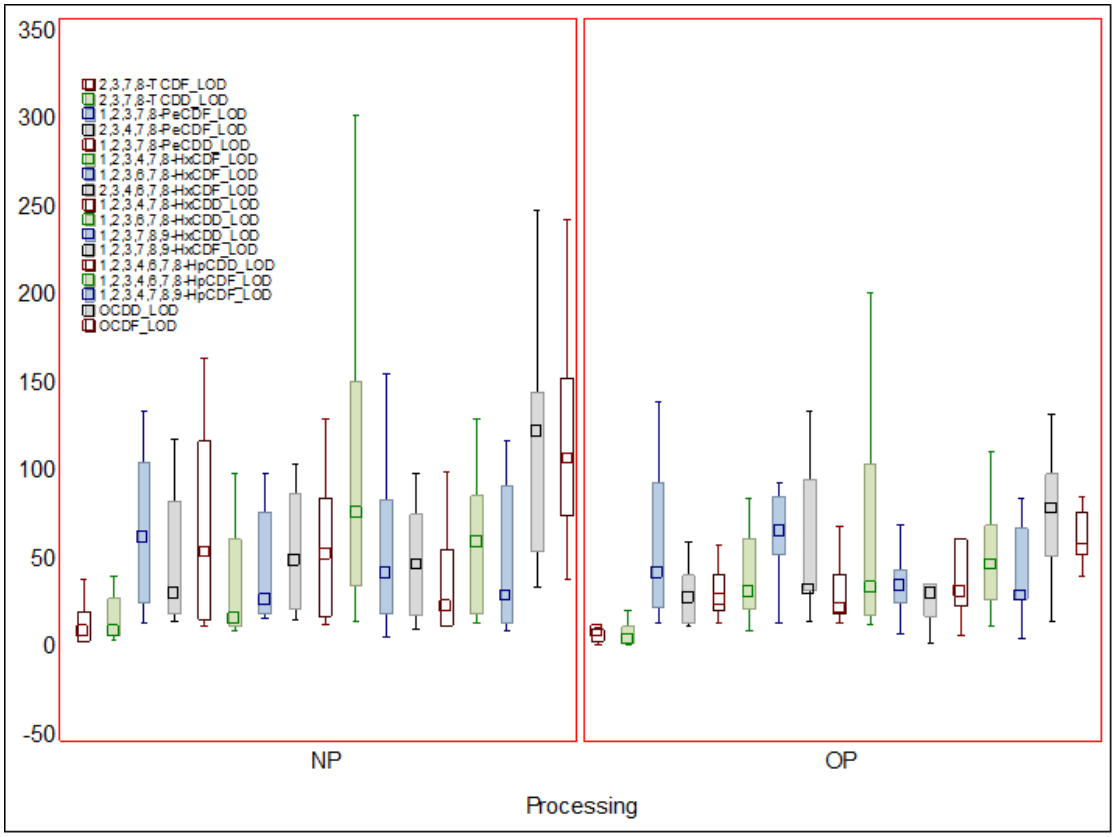


Figure 8.5. *Differences in the LOD/ LOQ obtained using different processing and review methods: New Processing Method with manual review of each ^{2}D peak (NP) and Old Processing Method using RF review (OP) were evaluated. The 'y-axis' is the concentration in pg/ g.*

As expected, there was no statistically significant difference in the processing used (instrument software only or manual integration) to obtain the peak areas for the analysed PCDD/Fs (Figure 8.5 and Table 8.10). The use of manual integration would be highly dependent on the skill of the operator and may not be feasible under routine laboratory conditions as it invariably leads to greater variation.

8.2.2.5 The Effect of the calculation method used for LOD/ LOQ

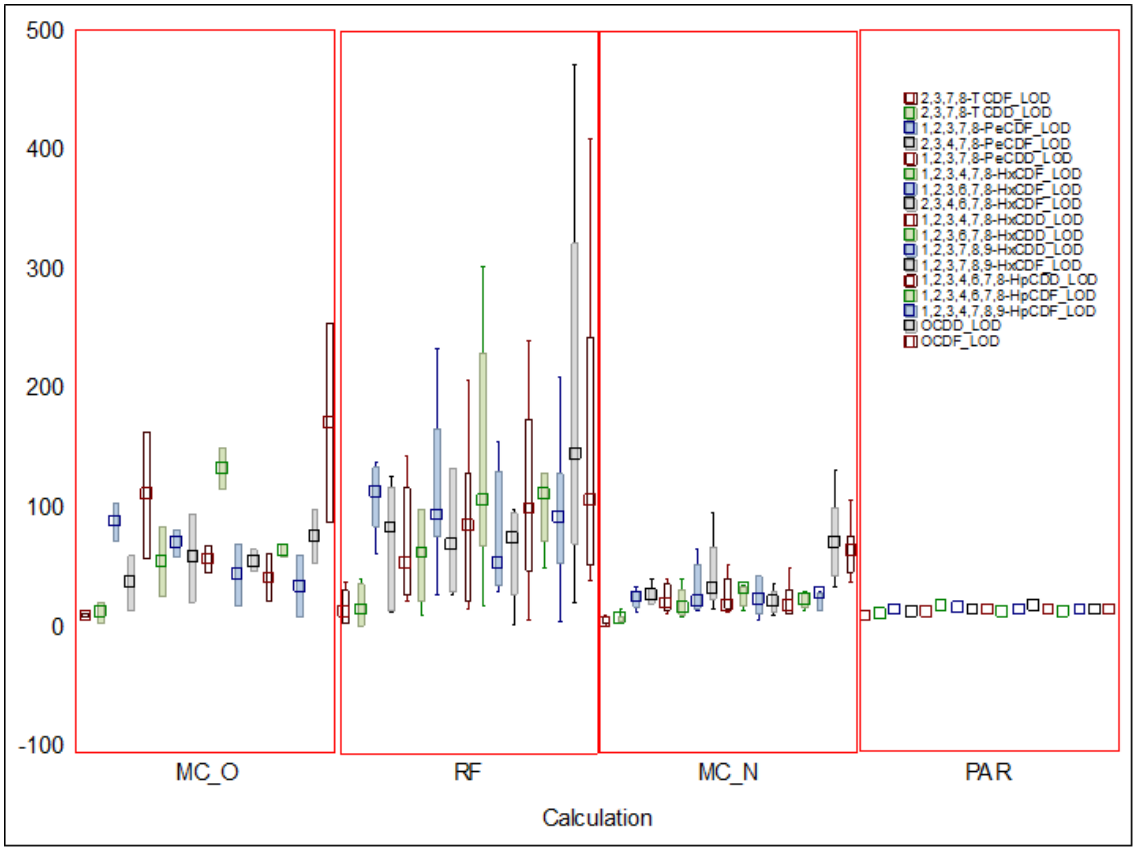


Figure 8.6. *Differences in the LOD/ LOQ obtained using different calculations (nominal concentration vs mass) for determining LOD/ LOQs. Conservative linear regression approach (MC_O), routine approach (MC_N), Relative Response Factor method (RF), and relative 'Peak Area Percentage (PAR)' used to calculate LOD/ LOQ. The 'y-axis' is the concentration in pg/ g.*

The calculation method used had the greatest effect on the LOD/ LOQ obtained. The following congeners showed a significant difference ($p < 0.05$) in the obtained LOD/LOQ depending on the calculation used (Figure 8.6 and Table 8.11): 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF,

2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,7,8,9-HxCDF, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF and 1,2,3,4,7,8,9-HpCDF.

Applying the post-hoc '*Tukey'* test (summarised in Table 8.13), the variations were congener specific and were mainly between MC_N (uncertainty on the y-intercept; S_a), MC_O (uncertainty on the y-intercept and slope; $S_{y/x}$), and RF, where the LOD/ LOQs for MC_N results were statistically lower than the other calculation methods. The PAR calculations were only performed once, so these results should be used with caution as they are significantly lower for 1,2,3,7,8-PeCDF when compared to the other calculation methods.

8.2.2.6 The effect of the outlier removal on LOD/LOQ

For outlier removal, there was a statistically significant improvement in both the LOD and LOQ for 2,3,7,8-TCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD (Table 8.12). This might indicate inconsistency in instrumental response at ultra-trace levels, but the effect was not validated statistically and is therefore not the correct approach to take when outliers are observed.

8.3 CONCLUDING REMARKS

The method pre-validation study has provided large amounts of data to corroborate the NMISA method for the analysis of PCDD/Fs in soil and sediment samples with the associated uncertainty calculated for each of the seventeen toxic congeners. The regression analysis results have confirmed that the GC×GC-TOFMS results are affected by the sample extraction since matrix effects will influence the LOD/ LOQ possible as only one mass, which may be subject to interference, is selected for quantitation. This is not a limitation of the GC×GC-TOFMS method; it indicates that many more factors must be considered when performing ultra-trace analysis with nominal mass instrumentation.

The factors affecting LOD and LOQ were evaluated to gauge the effect of the different column combinations, processing methods and calculation methods used. The calibration curves created for the quantitation results for the toxic waste extracts

discussed in Chapter 7 did have one or two outliers in the duplicate injections. The outliers were removed to ensure an R^2 as close to 1.000 as possible. This was before it was understood that all data must be statistically checked to ensure whether a specific duplicate injection can be excluded or not. The one-way ANOVA statistical analysis does indicate that the LOD and LOQ could be improved by changing various parameters in the instrumental analysis, but this should be applied with caution. The LOD and LOQ should remain a true reflection of the instrumental capabilities while analysing a real sample.

Statistical evaluation of the instrument detection limit has been assessed. Due to the small sample size, a definitive conclusion on which approach would provide a more sensitive result is not possible. However, column type, processing method and calculation approach does affect LOD and LOQ.

LOD is described as the concentration at which an instrument signal is obtained that is significantly different from the background signal; effectively “can it be seen or not”. LOQ is whether the signal can be quantified. LOD becomes very important in trace analysis where the concentration being reported is really present in the sample. Care must be taken not to report the analyte absent, when it is actually present.

A measurement cannot be reliably made at the LOD, and a lower limit for precise measurement would have to be defined. It is possible that the calculated LOD for a particular regression line is higher than a sample with a known analyte concentration that was measured reasonably accurately. The LOD would then be ‘at least’ the concentration of the known analyte concentration.

The final NMISA results obtained for the NIST SRM 1944 sediment sample and 5 soil samples will be discussed in Chapter 9 and compared against GC-HRMS results for the same data set.

Table 8.5. *Summary of LODs obtained using various columns, processing and calculation methods (TCDD/F to HxCDD/F). Calculations are performed using an adequate number of significant figures.*

Parameters possibly affecting LOD					Detection Limit, LOD (pg/ g)								
Dataset	All/ outlier	Calc.	Process -ing	Column	2,3,7,8 -TCDF	2,3,7,8 -TCDD	1,2,3,7,8-PeCDD	2,3,4,7,8-PeCDF	1,2,3,7,8-PeCDD	1,2,3,4,7,8 -HxCDF	1,2,3,6,7,8-HxCDF	2,3,4,6,7,8 -HxCDF	1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDD
Taiwan	All	MC_O	NP	5SilMS/200	9.26	3.61	104.95	14.50	164.25	25.84	58.82	21.53	45.61	150.99
W_1	All	RF	NP	5SilMS/200	38.63	40.57	112.81	83.00	58.68	10.06	27.21	27.10	84.62	230.49
Set_1	All	RF	NP	Dio2/17Sil	30.96	15.42	84.99	88.19	117.14	99.12	166.63	103.95	129.77	107.14
Set_2	All	RF	NP	Dio2/17Sil	13.49	36.37	62.45	117.99	53.87	61.37	98.27	60.66	67.85	68.64
Set_3	All	RF	NP	Dio2/17Sil	20.06	27.63	134.39	75.67	143.78	65.18	76.79	345.47	208.07	302.56
Set_1	All	MC_N	OP	Dio2/17Sil	9.79	4.87	26.87	27.88	37.03	31.33	52.68	32.86	41.02	33.87
Set_2	All	MC_N	NP	Dio2/17Sil	3.51	9.46	16.25	30.70	14.01	15.97	25.57	15.78	17.65	17.86
Set_3	All	MC_N	NP	Dio2/17Sil	5.04	6.95	33.78	19.02	36.14	16.38	19.30	86.85	52.31	76.06
Set_1	OR	MC_N	OP	Dio2/17Sil	9.79	4.87	26.87	27.88	20.77	31.33	52.68	32.86	41.02	33.87
Set_2	OR	MC_N	NP	Dio2/17Sil	2.94	9.46	13.53	21.53	12.07	9.04	16.31	15.78	14.52	14.79
Set_3	OR	MC_N	NP	Dio2/17Sil	3.30	5.25	25.16	19.02	15.96	12.00	16.29	48.88	13.11	34.92
Set_2	All	MC_N	OP	Dio2/17Sil	8.60	15.01	57.94	40.84	41.41	40.95	65.29	96.95	18.91	31.80
Set_2	OR	MC_N	OP	Dio2/17Sil	1.48	8.42	18.77	34.47	19.80	9.57	13.90	31.99	18.91	18.24
Set_1	All	MC_O	OP	Dio2/17Sil	11.19	20.86	73.20	60.09	58.19	84.90	82.36	95.47	68.66	115.74
FMS	OR	RF	OP	XLB/200	3.38	1.02	139.28	126.34	22.39	61.5	234.52	70	97.02	104.21
W_2	OR	RF	OP	5SilMS/200	4.2	1.97	ND	13.59	52.98	21.61	85.53	29.86	21.61	17.9
NWU_1	OR	RF	OP	XLB/200	3.41	1.27	114.26	13.41	26.98	1848.21	93.78	133.66	16.21	201.38
NWU_2	All	PAR	OP	XLB/200	10.30	11.61	13.87	12.41	13.76	18.81	15.80	14.46	13.9	13.2

NP	New processing method with manual review of each 2nd dimension peak
MC_O	Conservative linear regression approach to LOD/LOQ
RF	RF used to calculate LOD/LOQ, this approach is similar to conservative linear regression calculations
ALL	All data points included in calculations
Taiwan	Taiwan sample extracts
W1 & W2	Toxic waste sample extracts from Belgium (NMISA and NWU)
FMS	NMISA LOD sample set using clean soil

OP	Old processing method using RF review
MC_N	Routine approach to LOD/LOQ
PAR	Relative peak area percentage used to calculate LOD/LOQ
OR	Outliers removed as determined through y-residuals
SET	NMISA validation sample sets for NIST SRM 1944
NWU_1	South African sample extracts
NWU_2	Large volume South African sample extracts

Table 8.6. *Summary of LODs obtained using various columns, processing and calculation methods (HxCDD/F to OCDD/F). Calculations are performed using an adequate number of significant figures.*

Parameters possibly affecting LOD					Detection limit, LOD (pg/ g)					
Dataset	All/ outlier	Calc.	Process -ing	Column	1,2,3,7,8,9-HxCDD	1,2,3,7,8,9-HxCDF	1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,8-HpCDF	1,2,3,4,7,8,9-HpCDF	OCDD	OCDF
Taiwan	All	MC_O	NP	5SilMS/200	18.74	46.97	22.70	59.23	9.25	54.35	88.04
W_1	All	RF	NP	5SilMS/200	53.51	75.48	174.28	129.89	209.56	145.13	106.70
Set_1	All	RF	NP	Dio2/17Sil	130.36	96.09	99.83	85.98	91.75	248.67	242.97
Set_2	All	RF	NP	Dio2/17Sil	83.87	57.60	55.48	72.40	54.12	472.14	409.66
Set_3	All	RF	NP	Dio2/17Sil	155.47	98.63	47.97	120.33	117.65	136.08	152.89
Set_1	All	MC_N	OP	Dio2/17Sil	43.70	30.37	31.56	27.18	29.01	78.61	76.81
Set_2	All	MC_N	NP	Dio2/17Sil	22.96	14.99	14.43	18.84	14.08	122.83	106.58
Set_3	All	MC_N	NP	Dio2/17Sil	42.46	24.79	12.06	30.25	29.58	34.21	38.43
Set_1	OR	MC_N	OP	Dio2/17Sil	43.70	30.37	31.56	27.18	29.01	78.61	76.81
Set_2	OR	MC_N	NP	Dio2/17Sil	15.82	10.31	12.08	18.84	14.08	64.06	74.58
Set_3	OR	MC_N	NP	Dio2/17Sil	5.74	18.09	12.06	13.72	15.47	34.21	38.43
Set_2	All	MC_N	OP	Dio2/17Sil	25.10	36.29	49.45	47.17	84.91	132.61	54.90
Set_2	OR	MC_N	OP	Dio2/17Sil	7.81	10.30	23.48	14.97	27.29	51.85	54.90
Set_1	All	MC_O	OP	Dio2/17Sil	69.82	65.44	61.37	69.25	60.66	98.41	254.89
FMS	OR	RF	OP	XLB/200	30.75	95.16	159.59	111.35	67.46	20.85	85.57
W_2	OR	RF	OP	5SilMS/200	42.6	27.88	6.64	50.37	4.9	70.44	52.32
NWU_1	OR	RF	OP	XLB/200	34.91	2.01	240.52	1295.17	128.79	322.43	39.98
NWU_2	All	PAR	OP	XLB/200	14.19	17.52	14.05	12.33	15.06	14.37	14.13

NP	New processing method with manual review of each 2nd dimension peak
MC_O	Conservative linear regression approach to LOD/LOQ
RF	RF used to calculate LOD/LOQ, this approach is similar to conservative linear regression calculations
ALL	All data points included in calculations
Taiwan	Taiwan sample extracts
W1 & W2	Toxic waste sample extracts from Belgium (NMISA and NWU)
FMS	NMISA LOD sample set using clean soil
OP	Old processing method using RF review
MC_N	Routine approach to LOD/LOQ
PAR	Relative peak area percentage used to calculate LOD/LOQ
OR	Outliers removed as determined through y-residuals
SET	NMISA validation sample sets for NIST SRM 1944
NWU_1	South African sample extracts
NWU_2	Large volume South African sample extracts

Table 8.7. *Summary of LOQs obtained using various columns, processing and calculation methods (TCDD/F to HxCDD/F). Calculations are performed using an adequate number of significant figures.*

Parameters affecting LOQ					Limit of Quantitation, LOQ (pg/ g)								
Dataset	All/ outlier	Calc.	Process-ing	Column	2,3,7,8-TCDF	2,3,7,8-TCDD	1,2,3,7,8-PeCDF	2,3,4,7,8-PeCDF	1,2,3,7,8-PeCDD	1,2,3,4,7,8-HxCDF	1,2,3,6,7,8-HxCDF	2,3,4,6,7,8-HxCDF	1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDD
Taiwan	All	MC_O	NP	5SilMS/200	37.56	13.85	423.04	64.18	507.12	109.73	228.62	90.05	128.49	595.43
W_1	All	RF	NP	5SilMS/200	128.75	135.22	376.04	276.65	195.60	33.52	90.69	90.32	282.06	768.32
Set_1	All	RF	NP	Dio2/17Sil	93.83	46.73	257.54	267.23	354.97	300.35	504.94	314.99	393.25	324.66
Set_2	All	RF	NP	Dio2/17Sil	40.86	110.22	189.23	357.54	163.24	185.96	297.80	183.81	205.59	207.99
Set_3	All	RF	NP	Dio2/17Sil	60.79	83.72	407.25	229.31	435.70	197.51	232.70	1046.88	630.52	916.84
Set_1	All	MC_N	OP	Dio2/17Sil	32.63	16.25	89.56	92.93	123.44	104.44	175.59	109.53	136.75	112.90
Set_2	All	MC_N	NP	Dio2/17Sil	11.69	31.54	54.15	102.32	46.72	53.22	85.22	52.60	58.84	59.52
Set_3	All	MC_N	NP	Dio2/17Sil	16.81	23.15	112.61	63.41	120.48	54.62	64.35	289.49	174.35	253.53
Set_1	OR	MC_N	OP	Dio2/17Sil	32.63	16.25	89.56	92.93	69.25	104.44	175.59	109.53	136.75	112.90
Set_2	OR	MC_N	NP	Dio2/17Sil	9.81	31.54	45.09	71.76	40.23	30.13	54.38	52.60	48.40	49.31
Set_3	OR	MC_N	NP	Dio2/17Sil	11.00	17.49	83.88	63.41	53.18	39.98	54.30	162.94	43.69	116.39
Set_2	All	MC_N	OP	Dio2/17Sil	28.67	50.02	193.12	136.14	138.02	136.50	217.63	323.17	63.05	105.99
Set_2	OR	MC_N	OP	Dio2_17Sil	4.93	28.05	62.55	114.91	65.99	31.91	46.34	106.64	63.05	60.79
Set_1	All	MC_O	OP	Dio2/17Sil	37.51	71.59	254.58	205.08	201.34	286.91	287.26	320.63	239.79	386.05
FMS	OR	RF	OP	XLB/200	10.13	3.06	417.83	379.02	67.17	184.49	703.55	210.01	291.06	312.62
W_2	OR	RF	OP	5SilMS/200	12.6	5.91	ND	40.78	158.95	64.83	256.59	89.59	64.83	53.7
NWU_1	OR	RF	OP	XLB/200	10.22	3.71	342.79	40.22	80.94	5544.62	281.34	400.97	48.63	604.13
NWU_2	All	PAR	OP	XLB/200	34.32	38.71	46.22	41.38	45.85	62.70	52.66	48.21	46.33	44.01

NP	New processing method with manual review of each 2nd dimension peak
MC_O	Conservative linear regression approach to LOD/LOQ
RF	RF used to calculate LOD/LOQ, this approach is similar to conservative linear regression calculations
ALL	All data points included in calculations
Taiwan	Taiwan sample extracts
W1 & W2	Toxic waste sample extracts from Belgium (NMISA and NWU)
FMS	NMISA LOD sample set using clean soil

OP	Old processing method using RF review
MC_N	Routine approach to LOD/LOQ
PAR	Relative peak area percentage used to calculate LOD/LOQ
OR	Outliers removed as determined through y-residuals
SET	NMISA validation sample sets for NIST SRM 1944
NWU_1	South African sample extracts
NWU_2	Large volume South African sample extracts

Table 8.8. *Summary of LOQs obtained using various columns, processing and calculation methods (HxCDD/F to OCDD/F). Calculations are performed using an adequate number of significant figures.*

Parameters affecting LOQ					Limit of Quantitation, LOQ (pg/ g)						
Dataset	All/ outlier	Calc.	Process-ing	Column	1,2,3,7,8,9-HxCDD	1,2,3,7,8,9-HxCDF	1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,8-HpCDF	1,2,3,4,7,8,9-HpCDF	OCDD	OCDF
Taiwan	All	MC_O	NP	5SilMS_200	76.62	183.50	89.18	232.54	40.59	208.97	358.03
W_1	All	RF	NP	5SilMS_200	178.36	251.61	580.93	432.97	698.54	483.78	355.67
Set_1	All	RF	NP	Dio2_17Sil	395.03	291.17	302.53	260.54	278.04	753.55	736.27
Set_2	All	RF	NP	Dio2_17Sil	254.15	174.56	168.12	219.39	163.99	1430.72	1241.40
Set_3	All	RF	NP	Dio2_17Sil	471.13	298.87	145.38	364.65	356.52	412.38	463.29
Set_1	All	MC_N	OP	Dio2_17Sil	145.67	101.25	105.20	90.60	96.68	262.04	256.03
Set_2	All	MC_N	NP	Dio2_17Sil	76.52	49.96	48.11	62.78	46.93	409.45	355.27
Set_3	All	MC_N	NP	Dio2_17Sil	141.53	82.64	40.20	100.83	98.59	114.03	128.11
Set_1	OR	MC_N	OP	Dio2_17Sil	145.67	101.25	105.20	90.60	96.68	262.04	256.03
Set_2	OR	MC_N	NP	Dio2_17Sil	52.73	34.35	40.27	62.78	46.93	213.55	248.59
Set_3	OR	MC_N	NP	Dio2_17Sil	19.14	60.30	40.20	45.73	51.56	114.03	128.11
Set_2	All	MC_N	OP	Dio2_17Sil	83.68	120.97	164.85	157.12	283.04	442.05	182.98
Set_2	OR	MC_N	OP	Dio2_17Sil	26.02	34.33	78.25	49.89	90.98	172.84	182.98
Set_1	All	MC_O	OP	Dio2_17Sil	250.63	221.88	197.66	230.01	207.91	330.57	925.06
FMS	OR	RF	OP	XLB_200	92.24	285.48	478.77	334.05	202.39	62.56	265.7
W_2	OR	RF	OP	5SilMS_200	127.8	83.63	240.52	151.12	14.69	211.33	156.95
NWU_1	OR	RF	OP	XLB_200	104.74	6.04	721.55	3885.51	386.37	967.37	119.94
NWU_2	All	PAR	OP	XLB_200	47.3	58.39	46.82	41.11	50.2	47.9	47.09

NP	New processing method with manual review of each 2nd dimension peak
MC_O	Conservative linear regression approach to LOD/LOQ
RF	RF used to calculate LOD/LOQ, this approach is similar to conservative linear regression calculations
ALL	All data points included in calculations
Taiwan	Taiwan sample extracts
W1 & W2	Toxic waste sample extracts from Belgium (NMISA and NWU)
FMS	NMISA LOD sample set using clean soil

OP	Old processing method using RF review
MC_N	Routine approach to LOD/LOQ
PAR	Relative peak area percentage used to calculate LOD/LOQ
OR	Outliers removed as determined through y-residuals
SET	NMISA validation sample sets for NIST SRM 1944
NWU_1	South African sample extracts
NWU_2	Large volume South African sample extracts

Table 8.9. *One-way ANOVA results for the effect of column type on the LOD and LOQ obtained from PCDD/F neat standard. Calculations are performed using an adequate number of significant figures.*

COLUMN	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	Df Residual	MS Residual	F	p
2,3,7,8-TCDF_LOD	0.505832	0.255866	0.149561	431	2	215	1253	14	90	2.406905	0.126345
2,3,7,8-TCDF_LOQ	0.570314	0.325258	0.228866	5605	2	2803	11628	14	831	3.374337	0.063674
2,3,7,8-TCDD_LOD	0.412716	0.170334	0.051811	381	2	191	1857	14	133	1.437133	0.270596
2,3,7,8-TCDD_LOQ	0.437811	0.191678	0.076204	4318	2	2159	18207	14	1301	1.659918	0.225470
1,2,3,7,8-PeCDF_LOD	0.528130	0.278922	0.175911	9006	2	4503	23283	14	1663	2.707683	0.101362
1,2,3,7,8-PeCDF_LOQ	0.601916	0.362303	0.271203	119989	2	59995	211195	14	15085	3.976997	0.042885
2,3,4,7,8-PeCDF_LOD	0.040515	0.001641	-0.140981	36	2	18	22059	14	1576	0.011509	0.988566
2,3,4,7,8-PeCDF_LOQ	0.061044	0.003726	-0.138598	734	2	367	196300	14	14021	0.026182	0.974206
1,2,3,7,8-PeCDD_LOD	0.536732	0.288081	0.186379	10143	2	5072	25066	14	1790	2.832583	0.092686
1,2,3,7,8-PeCDD_LOQ	0.559234	0.312742	0.214563	101465	2	50733	222972	14	15927	3.185407	0.072417
1,2,3,4,7,8-HxCDF_LOD	0.541441	0.293158	0.192181	908689	2	454344	2190967	14	156498	2.903204	0.088157
1,2,3,4,7,8-HxCDF_LOQ	0.540017	0.291618	0.190421	8111458	2	4055729	19703853	14	1407418	2.881680	0.089510
1,2,3,6,7,8-HxCDF_LOD	0.401002	0.160802	0.040917	9105	2	4552	47517	14	3394	1.341300	0.293123
1,2,3,6,7,8-HxCDF_LOQ	0.370572	0.137324	0.014084	69161	2	34581	434477	14	31034	1.114284	0.355578
2,3,4,6,7,8-HxCDF_LOD	0.232546	0.054078	-0.081054	5436	2	2718	95087	14	6792	0.400184	0.677624
2,3,4,6,7,8-HxCDF_LOQ	0.228563	0.052241	-0.083153	47567	2	23784	862965	14	61640	0.385846	0.686886
1,2,3,4,7,8-HxCDD_LOD	0.132395	0.017529	-0.122825	755	2	378	42323	14	3023	0.124889	0.883567
1,2,3,4,7,8-HxCDD_LOQ	0.150966	0.022791	-0.116811	8994	2	4497	385635	14	27545	0.163255	0.850968
1,2,3,6,7,8-HxCDD_LOD	0.463708	0.215025	0.102886	25254	2	12627	92191	14	6585	1.917481	0.183650
1,2,3,6,7,8-HxCDD_LOQ	0.550964	0.303561	0.204070	359081	2	179541	823815	14	58844	3.051130	0.079466
1,2,3,7,8,9-HxCDD_LOD	0.269756	0.072769	-0.059693	2055	2	1027	26179	14	1870	0.549356	0.589274
1,2,3,7,8,9-HxCDD_LOQ	0.284074	0.080698	-0.050631	20829	2	10415	237285	14	16949	0.614473	0.554890
1,2,3,7,8,9-HxCDF_LOD	0.213687	0.045662	-0.090672	776	2	388	16213	14	1158	0.334930	0.720966
1,2,3,7,8,9-HxCDF_LOQ	0.302696	0.091625	-0.038143	14616	2	7308	144903	14	10350	0.706068	0.510336
1,2,3,4,6,7,8-HpCDD_LOD	0.610892	0.373189	0.283644	27148	2	13574	45599	14	3257	4.167632	0.038015
1,2,3,4,6,7,8-HpCDD_LOQ	0.611046	0.373377	0.283859	253394	2	126697	425262	14	30376	4.170985	0.037936
1,2,3,4,6,7,8-HpCDF_LOD	0.546674	0.298852	0.198688	440837	2	220419	1034265	14	73876	2.983628	0.083304
1,2,3,4,6,7,8-HpCDF_LOQ	0.544331	0.296296	0.195767	3913360	2	1956680	9294239	14	663874	2.947366	0.085453
1,2,3,4,7,8,9-HpCDF_LOD	0.389799	0.151943	0.030792	7115	2	3558	39712	14	2837	1.254165	0.315482
1,2,3,4,7,8,9-HpCDF_LOQ	0.417863	0.174609	0.056696	83802	2	41901	396138	14	28296	1.480832	0.260986
OCDD_LOD	0.082432	0.006795	-0.135091	1590	2	795	232404	14	16600	0.047890	0.953394
OCDD_LOQ	0.073073	0.005340	-0.136755	11132	2	5566	2073598	14	148114	0.037579	0.963216
OCDF_LOD	0.329958	0.108872	-0.018431	17981	2	8990	147173	14	10512	0.855217	0.446251
OCDF_LOQ	0.342793	0.117507	-0.008563	189908	2	94954	1426229	14	101873	0.932076	0.416849

Table 8.10. *One-way ANOVA results for the effect of processing on the LOD and LOQ obtained from PCDD/F neat standard. Calculations are performed using an adequate number of significant figures.*

PROCESSING	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
2,3,7,8-TCDF_LOD	0.345531	0.119392	0.060685	201	1	201	1483	15	99	2.033686	0.174327
2,3,7,8-TCDF_LOQ	0.341730	0.116779	0.057898	2012	1	2012	15221	15	1015	1.983295	0.179432
2,3,7,8-TCDD_LOD	0.378422	0.143203	0.086084	320	1	320	1917	15	128	2.507070	0.134189
2,3,7,8-TCDD_LOQ	0.361666	0.130802	0.072856	2946	1	2946	19579	15	1305	2.257297	0.153744
1,2,3,7,8-PeCDF_LOD	0.074289	0.005519	-0.060780	178	1	178	32111	15	2141	0.083242	0.776899
1,2,3,7,8-PeCDF_LOQ	0.105538	0.011138	-0.054786	3689	1	3689	327495	15	21833	0.168957	0.686853
2,3,4,7,8-PeCDF_LOD	0.128277	0.016455	-0.049115	364	1	364	21732	15	1449	0.250953	0.623676
2,3,4,7,8-PeCDF_LOQ	0.131562	0.017309	-0.048204	3410	1	3410	193624	15	12908	0.264201	0.614737
1,2,3,7,8-PeCDD_LOD	0.421066	0.177297	0.122450	6242	1	6242	28967	15	1931	3.232575	0.092341
1,2,3,7,8-PeCDD_LOQ	0.411992	0.169737	0.114386	55069	1	55069	269368	15	17958	3.066564	0.100334
1,2,3,4,7,8-HxCDF_LOD	0.269824	0.072805	0.010992	225670	1	225670	2873985	15	191599	1.177825	0.294934
1,2,3,4,7,8-HxCDF_LOQ	0.271326	0.073618	0.011859	2047708	1	2047708	25767602	15	1717840	1.192025	0.292148
1,2,3,6,7,8-HxCDF_LOD	0.175070	0.030650	-0.033974	1735	1	1735	54887	15	3659	0.474281	0.501538
1,2,3,6,7,8-HxCDF_LOQ	0.183484	0.033666	-0.030756	16956	1	16956	486682	15	32445	0.522591	0.480860
2,3,4,6,7,8-HxCDF_LOD	0.111218	0.012369	-0.053473	1243	1	1243	99280	15	6619	0.187865	0.670868
2,3,4,6,7,8-HxCDF_LOQ	0.108173	0.011701	-0.054185	10654	1	10654	899878	15	59992	0.177599	0.679422
1,2,3,4,7,8-HxCDD_LOD	0.306703	0.094067	0.033671	4052	1	4052	39026	15	2602	1.557507	0.231161
1,2,3,4,7,8-HxCDD_LOQ	0.295429	0.087279	0.026430	34443	1	34443	360186	15	24012	1.434368	0.249636
1,2,3,6,7,8-HxCDD_LOD	0.254952	0.065000	0.002667	7634	1	7634	109811	15	7321	1.042785	0.323363
1,2,3,6,7,8-HxCDD_LOQ	0.280714	0.078800	0.017387	93213	1	93213	1089684	15	72646	1.283117	0.275103
1,2,3,7,8,9-HxCDD_LOD	0.306462	0.093919	0.033513	2652	1	2652	25582	15	1705	1.554809	0.231547
1,2,3,7,8,9-HxCDD_LOQ	0.295822	0.087511	0.026678	22588	1	22588	235527	15	15702	1.438549	0.248978
1,2,3,7,8,9-HxCDF_LOD	0.209723	0.043984	-0.019751	747	1	747	16242	15	1083	0.690111	0.419154
1,2,3,7,8,9-HxCDF_LOQ	0.218229	0.047624	-0.015868	7597	1	7597	151922	15	10128	0.750079	0.400092
1,2,3,4,6,7,8-HpCDD_LOD	0.201035	0.040415	-0.023557	2940	1	2940	69807	15	4654	0.631760	0.439107
1,2,3,4,6,7,8-HpCDD_LOQ	0.188935	0.035697	-0.028590	24226	1	24226	654430	15	43629	0.555269	0.467689
1,2,3,4,6,7,8-HpCDF_LOD	0.236416	0.055892	-0.007048	82447	1	82447	1392655	15	92844	0.888019	0.360945
1,2,3,4,6,7,8-HpCDF_LOQ	0.233214	0.054389	-0.008652	718345	1	718345	12489254	15	832617	0.862756	0.367676
1,2,3,4,7,8,9-HpCDF_LOD	0.061381	0.003768	-0.062648	176	1	176	46651	15	3110	0.056728	0.814967
1,2,3,4,7,8,9-HpCDF_LOQ	0.062930	0.003960	-0.062442	1901	1	1901	478040	15	31869	0.059640	0.810375
OCDD_LOD	0.195815	0.038344	-0.025767	8972	1	8972	225022	15	15001	0.598088	0.451325
OCDD_LOQ	0.201870	0.040752	-0.023198	84956	1	84956	1999774	15	133318	0.637243	0.437168
OCDF_LOD	0.291491	0.084967	0.023964	14033	1	14033	151121	15	10075	1.392846	0.256303
OCDF_LOQ	0.269708	0.072743	0.010925	117562	1	117562	1498574	15	99905	1.176738	0.295149

Table 8.11. *One-way ANOVA results for the effect of calculation used to determine the LOD/ LOQ on the reported LOD/ LOQ. Calculations are performed using an adequate number of significant figures.*

CALCULATION	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
2,3,7,8-TCDF_LOD	0.576701	0.332584	0.178564	560	3	187	1124	13	86	2.15936	0.141929
2,3,7,8-TCDF_LOQ	0.549401	0.301842	0.140728	5202	3	1734	12031	13	925	1.87347	0.183912
2,3,7,8-TCDD_LOD	0.485161	0.235382	0.058931	527	3	176	1711	13	132	1.33398	0.306029
2,3,7,8-TCDD_LOQ	0.457062	0.208906	0.026346	4706	3	1569	17819	13	1371	1.14431	0.367947
1,2,3,7,8-PeCDF_LOD	0.898595	0.807472	0.763043	26073	3	8691	6217	13	478	18.17425	0.000062
1,2,3,7,8-PeCDF_LOQ	0.887327	0.787350	0.738277	260758	3	86919	70426	13	5417	16.04442	0.000117
2,3,4,7,8-PeCDF_LOD	0.755798	0.571231	0.472285	12621	3	4207	9474	13	729	5.77313	0.009795
2,3,4,7,8-PeCDF_LOQ	0.744617	0.554454	0.451636	109247	3	36416	87788	13	6753	5.39256	0.012423
1,2,3,7,8-PeCDD_LOD	0.683795	0.467576	0.344709	16463	3	5488	18746	13	1442	3.80554	0.037014
1,2,3,7,8-PeCDD_LOQ	0.693286	0.480646	0.360795	155939	3	51980	168498	13	12961	4.01036	0.031826
1,2,3,4,7,8-HxCDF_LOD	0.370914	0.137577	-0.061444	426442	3	142147	2673214	13	205632	0.69127	0.573458
1,2,3,4,7,8-HxCDF_LOQ	0.368286	0.135635	-0.063834	3772726	3	1257575	24042584	13	1849430	0.67998	0.579780
1,2,3,6,7,8-HxCDF_LOD	0.684419	0.468429	0.345759	26524	3	8841	30099	13	2315	3.81861	0.036656
1,2,3,6,7,8-HxCDF_LOQ	0.677779	0.459384	0.334627	231363	3	77121	272275	13	20944	3.68222	0.040602
2,3,4,6,7,8-HxCDF_LOD	0.501605	0.251608	0.078902	25292	3	8431	75231	13	5787	1.45686	0.271938
2,3,4,6,7,8-HxCDF_LOQ	0.479905	0.230309	0.052688	209704	3	69901	700828	13	53910	1.29663	0.317282
1,2,3,4,7,8-HxCDD_LOD	0.687275	0.472347	0.350580	20348	3	6783	22730	13	1748	3.87913	0.035046
1,2,3,4,7,8-HxCDD_LOQ	0.676581	0.457762	0.332630	180646	3	60215	213983	13	16460	3.65823	0.041345
1,2,3,6,7,8-HxCDD_LOD	0.790664	0.625150	0.538646	73421	3	24474	44024	13	3386	7.22684	0.004239
1,2,3,6,7,8-HxCDD_LOQ	0.783941	0.614564	0.525617	726966	3	242322	455931	13	35072	6.90934	0.005046
1,2,3,7,8,9-HxCDD_LOD	0.645986	0.417297	0.282828	11782	3	3927	16452	13	1266	3.10328	0.063753
1,2,3,7,8,9-HxCDD_LOQ	0.629964	0.396854	0.257667	102434	3	34145	155681	13	11975	2.85122	0.078305
1,2,3,7,8,9-HxCDF_LOD	0.735599	0.541106	0.435208	9193	3	3064	7796	13	600	5.10967	0.014907
1,2,3,7,8,9-HxCDF_LOQ	0.737318	0.543638	0.438323	86720	3	28907	72798	13	5600	5.16205	0.014407
1,2,3,4,6,7,8-HpCDD_LOD	0.764165	0.583948	0.487937	42481	3	14160	30267	13	2328	6.08204	0.008125
1,2,3,4,6,7,8-HpCDD_LOQ	0.756257	0.571925	0.473138	388140	3	129380	290516	13	22347	5.78949	0.009697
1,2,3,4,6,7,8-HpCDF_LOD	0.442995	0.196245	0.010763	289481	3	96494	1185621	13	91202	1.05803	0.400361
1,2,3,4,6,7,8-HpCDF_LOQ	0.442789	0.196062	0.010538	2589514	3	863171	10618085	13	816776	1.05680	0.400841
1,2,3,4,7,8,9-HpCDF_LOD	0.747849	0.559278	0.457573	26190	3	8730	20638	13	1588	5.49903	0.011614
1,2,3,4,7,8,9-HpCDF_LOQ	0.707139	0.500046	0.384672	239992	3	79997	239948	13	18458	4.33413	0.025231
OCDD_LOD	0.641586	0.411633	0.275856	96320	3	32107	137674	13	10590	3.03168	0.067548
OCDD_LOQ	0.630109	0.397038	0.257893	827717	3	275906	1257013	13	96693	2.85341	0.078163
OCDF_LOD	0.584776	0.341963	0.190108	56476	3	18825	108677	13	8360	2.25191	0.130730
OCDF_LOQ	0.605545	0.366685	0.220535	592613	3	197538	1023524	13	78733	2.50897	0.104493

Table 8.12. *One-way ANOVA results for the effect of calculation used to determine the LOD/ LOQ on the reported LOD/ LOQ (outliers removed). Calculations are performed using an adequate number of significant figures.*

OUTLIER REMOVED	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
2,3,7,8-TCDF_LOD	0.507491	0.257547	0.208051	434	1	434	1250	15	83	5.203312	0.037572
2,3,7,8-TCDF_LOQ	0.517308	0.267608	0.218782	4612	1	4612	12621	15	841	5.480835	0.033450
2,3,7,8-TCDD_LOD	0.518085	0.268413	0.219640	601	1	601	1637	15	109	5.503359	0.033140
2,3,7,8-TCDD_LOQ	0.522130	0.272620	0.224128	6141	1	6141	16384	15	1092	5.621959	0.031557
1,2,3,7,8-PeCDF_LOD	0.101742	0.010351	-0.055625	334	1	334	31955	15	2130	0.156895	0.697608
1,2,3,7,8-PeCDF_LOQ	0.153623	0.023600	-0.041493	7816	1	7816	323368	15	21558	0.362557	0.556083
2,3,4,7,8-PeCDF_LOD	0.151135	0.022842	-0.042302	505	1	505	21591	15	1439	0.350635	0.562574
2,3,4,7,8-PeCDF_LOQ	0.177037	0.031342	-0.033235	6175	1	6175	190859	15	12724	0.485341	0.496668
1,2,3,7,8-PeCDD_LOD	0.498303	0.248305	0.198192	8743	1	8743	26467	15	1764	4.954914	0.041770
1,2,3,7,8-PeCDD_LOQ	0.516290	0.266555	0.217659	86480	1	86480	237957	15	15864	5.451438	0.033861
1,2,3,4,7,8-HxCDF_LOD	0.319955	0.102371	0.042530	317316	1	317316	2782339	15	185489	1.710698	0.210585
1,2,3,4,7,8-HxCDF_LOQ	0.317777	0.100982	0.041048	2808858	1	2808858	25006452	15	1667097	1.684880	0.213882
1,2,3,6,7,8-HxCDF_LOD	0.071513	0.005114	-0.061212	290	1	290	56333	15	3756	0.077106	0.785049
1,2,3,6,7,8-HxCDF_LOQ	0.043996	0.001936	-0.064602	975	1	975	502663	15	33511	0.029091	0.866849
2,3,4,6,7,8-HxCDF_LOD	0.163982	0.026890	-0.037984	2703	1	2703	97820	15	6521	0.414499	0.529416
2,3,4,6,7,8-HxCDF_LOQ	0.179848	0.032345	-0.032165	29451	1	29451	881081	15	58739	0.501397	0.489745
1,2,3,4,7,8-HxCDD_LOD	0.328168	0.107694	0.048207	4639	1	4639	38439	15	2563	1.810383	0.198450
1,2,3,4,7,8-HxCDD_LOQ	0.342491	0.117300	0.058453	46290	1	46290	348339	15	23223	1.993316	0.178402
1,2,3,6,7,8-HxCDD_LOD	0.209816	0.044023	-0.019709	5170	1	5170	112275	15	7485	0.690748	0.418944
1,2,3,6,7,8-HxCDD_LOQ	0.242485	0.058799	-0.003948	69553	1	69553	1113344	15	74223	0.937081	0.348380
1,2,3,7,8,9-HxCDD_LOD	0.432634	0.187172	0.132984	5285	1	5285	22949	15	1530	3.454098	0.082829
1,2,3,7,8,9-HxCDD_LOQ	0.462914	0.214289	0.161909	55311	1	55311	202804	15	13520	4.090998	0.061317
1,2,3,7,8,9-HxCDF_LOD	0.356484	0.127081	0.068886	2159	1	2159	14830	15	989	2.183718	0.160166
1,2,3,7,8,9-HxCDF_LOQ	0.393883	0.155144	0.098820	24748	1	24748	134770	15	8985	2.754504	0.117737
1,2,3,4,6,7,8-HpCDD_LOD	0.196253	0.038515	-0.025584	2802	1	2802	69945	15	4663	0.600873	0.450293
1,2,3,4,6,7,8-HpCDD_LOQ	0.172961	0.029916	-0.034757	20302	1	20302	658354	15	43890	0.462573	0.506786
1,2,3,4,6,7,8-HpCDF_LOD	0.301269	0.090763	0.030147	133885	1	133885	1341217	15	89414	1.497350	0.239954
1,2,3,4,6,7,8-HpCDF_LOQ	0.295721	0.087451	0.026614	1155013	1	1155013	12052586	15	803506	1.437468	0.249148
1,2,3,4,7,8,9-HpCDF_LOD	0.164270	0.026985	-0.037883	1264	1	1264	45564	15	3038	0.415996	0.528684
1,2,3,4,7,8,9-HpCDF_LOQ	0.185391	0.034370	-0.030005	16496	1	16496	463445	15	30896	0.533900	0.476231
OCDD_LOD	0.180976	0.032752	-0.031731	7664	1	7664	226330	15	15089	0.507921	0.486980
OCDD_LOQ	0.199662	0.039865	-0.024144	83107	1	83107	2001623	15	133442	0.622799	0.442305
OCDF_LOD	0.382223	0.146094	0.089167	24128	1	24128	141026	15	9402	2.566340	0.130005
OCDF_LOQ	0.401108	0.160888	0.104947	260016	1	260016	1356120	15	90408	2.876031	0.110557

Table 8.13. *Post-hoc Tukey matrix for the effect of calculation on the LOD/ LOQ; significant variations in the means are indicated in red. Calculations are performed using an adequate number of significant figures.*

1,2,3,7,8-PeCDF_LOD	MC_O	RF	MC_N	PAR	1,2,3,4,7,8-HxCDD_LOD	MC_O	RF	MC_N	PAR	1,2,3,4,6,7,8-HpCDD_LOQ	MC_O	RF	MC_N	PAR
MC_O		0.717698	0.015987	0.062722	MC_O		0.594848	0.802031	0.832670	MC_O		0.204591	0.943491	0.950911
RF	0.717698		0.000245	0.007522	RF	0.594848		0.028414	0.267461	RF	0.204591		0.007536	0.178895
MC_N	0.015987	0.000245		0.935475	MC_N	0.802031	0.028414		0.990295	MC_N	0.943491	0.007536		0.997272
PAR	0.062722	0.007522	0.935475		PAR	0.832670	0.267461	0.990295		PAR	0.950911	0.178895	0.997272	
1,2,3,7,8-PeCDF_LOQ	MC_O	RF	MC_N	PAR	1,2,3,4,7,8-HxCDD_LOD	MC_O	RF	MC_N	PAR	1,2,3,4,7,8,9-HpCDF_LOD	MC_O	RF	MC_N	PAR
MC_O		0.999446	0.004678	0.028665	MC_O		0.645011	0.793776	0.816675	MC_O		0.136021	0.998932	0.976229
RF	0.999446		0.000391	0.015298	RF	0.645011		0.034398	0.278226	RF	0.136021		0.011081	0.163217
MC_N	0.004678	0.000391		0.937111	MC_N	0.793776	0.034398		0.987604	MC_N	0.998932	0.011081		0.982890
PAR	0.028665	0.015298	0.937111		PAR	0.816675	0.278226	0.987604		PAR	0.976229	0.163217	0.982890	
2,3,4,7,8-PeCDF_LOD	MC_O	RF	MC_N	PAR	1,2,3,6,7,8-HxCDD_LOD	MC_O	RF	MC_N	PAR	1,2,3,4,7,8,9-HpCDF_LOQ	MC_O	RF	MC_N	PAR
MC_O		0.196794	0.968322	0.874046	MC_O		0.874523	0.177743	0.369177	MC_O		0.232690	0.996497	0.969505
RF	0.196794		0.009250	0.114468	RF	0.874523		0.004025	0.110472	RF	0.232690		0.023491	0.228159
MC_N	0.968322	0.009250		0.949560	MC_N	0.177743	0.004025		0.988688	MC_N	0.996497	0.023491		0.983971
PAR	0.874046	0.114468	0.949560		PAR	0.369177	0.110472	0.988688		PAR	0.969505	0.228159	0.983971	
2,3,4,7,8-PeCDF_LOQ	MC_O	RF	MC_N	PAR	1,2,3,6,7,8-HxCDD_LOQ	MC_O	RF	MC_N	PAR					
MC_O		0.298018	0.912817	0.791496	MC_O		0.996749	0.093371	0.256646					
RF	0.298018		0.011645	0.117257	RF	0.996749		0.006268	0.133697					
MC_N	0.912817	0.011645		0.935415	MC_N	0.093371	0.006268		0.987452					
PAR	0.791496	0.117257	0.935415		PAR	0.256646	0.133697	0.987452						
1,2,3,7,8-PeCDD_LOQ	MC_O	RF	MC_N	PAR	1,2,3,7,8,9-HxCDF_LOD	MC_O	RF	MC_N	PAR					
MC_O		0.473888	0.042837	0.171367	MC_O		0.882844	0.330070	0.584893					
RF	0.473888		0.179846	0.529158	RF	0.882844		0.012657	0.232160					
MC_N	0.042837	0.179846		0.990175	MC_N	0.330070	0.012657		0.998221					
PAR	0.171367	0.529158	0.990175		PAR	0.584893	0.232160	0.998221						
1,2,3,6,7,8-HxCDF_LOD	MC_O	RF	MC_N	PAR	1,2,3,7,8,9-HxCDF_LOD	MC_O	RF	MC_N	PAR					
MC_O		0.660485	0.755077	0.789823	MC_O		0.994323	0.177360	0.425386					
RF	0.660485		0.030512	0.262685	RF	0.994323		0.015534	0.246866					
MC_N	0.755077	0.030512		0.986841	MC_N	0.177360	0.015534		0.997670					
PAR	0.789823	0.262685	0.986841		PAR	0.425386	0.246866	0.997670						
1,2,3,6,7,8-HxCDF_LOQ	MC_O	RF	MC_N	PAR	1,2,3,4,6,7,8-HpCDD_LOD	MC_O	RF	MC_N	PAR					
MC_O		0.855710	0.578769	0.662096	MC_O		0.168311	0.959961	0.963653					
RF	0.855710		0.036979	0.269591	RF	0.168311		0.006382	0.169817					
MC_N	0.578769	0.036979		0.982266	MC_N	0.959961	0.006382		0.997836					
PAR	0.662096	0.269591	0.982266		PAR	0.963653	0.169817	0.997836						

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METROLOGICAL RESULTS

'*Metrology is the science of measurement and its application. Metrology includes all theoretical and practical aspects of measurement, whatever the measurement uncertainty and field of application*' (JCGM-VIM 2012). It is now accepted that the evaluation of the uncertainty associated with a measurement result is an essential part of any quantitative analysis. Without explicit knowledge of the measurement uncertainty, the statement of an analytical result cannot be considered complete (Ellison & Williams 2012).

Measurement underpins every analysis performed in chemistry laboratories and must be considered in the broadest sense and have very diverse applications. Important decisions (environmental, economic, social, medical, etc.) are based on results of measurements. A very significant part of all measurements are chemical measurements, yet, in spite of their importance, all too often measurement results are of unsatisfactory quality (*i.e.*, incorrect). The concept of metrological traceability underpins the comparability of measurement results and having confidence in that comparability is central to prevent possible technical barriers to trade, especially for South Africa (IPAP 2014).

Quantitative analytical results were addressed in Chapter 7 and were calculated using relative response factors. The preliminary validation for the seventeen PCDD/Fs, including the measurement uncertainty, uncertainty budget and traceability statement, were detailed in Chapter 8. In this Chapter, accurate (metrological) measurement results, with their associated uncertainty of

measurement, for the determination of PCDD/Fs in soil and sediment, will be discussed.

These are the first metrological results for PCDD/Fs using GC×GC-TOFMS methodology. These results are being prepared for publication.

9.1 RESULTS FOR VALIDATION STUDY

The NMISA measurement results were reported using two different approaches and two sets of repeat analyses to verify the results obtained using GC×GC-TOFMS. The first approach used to calculate the LOD/ LOQ and uncertainty followed the general methods applied in routine laboratories (Set 1) (Armbruster & Pry 2008, Miller & Miller 2010, Rocke *et al*. 2003, US EPA 1994a and Van Zoonen *et al.* 1998), and the second more conservative approach based on metrological techniques (Set 2) (Ellison *et al.* 2012, JCGM-GUM 2008 and Meyer 2007). In the routine approach, the LOD was based on the uncertainty of the y-intercept (S_a) whereas the conservative approach considered both the uncertainty of the y-intercept as well as the slope ($S_{y/x}$). In the routine approach, the uncertainty considers the purity of the native and labelled standards, accuracy in the form of bias, repeatability and regression uncertainty. The conservative approach also considers reproducibility as the standard deviation of repeat measurements, and accuracy as the recovery based on repeat analysis of the NIST SRM 1944.

The quality criteria assessed prior to reporting, are based on whether the value reported is above the LOD and LOQ of the method and if the associated uncertainty is fit-for-purpose, using the Rxi®-Dioxin2/ Rxi®-17SilMS column combination and instrument conditions summarized in Table 6.3. The data quality objectives must address whether industry is meeting environmental regulations, then the uncertainty will need to be smaller than if academic pollutant screening is being performed. Any remaining matrix interference after sample clean-up will affect the analytical results as only one ion is used for quantitation. The calculated criteria are summarised in Table 9.1 for the seventeen toxic PCDD/Fs.

Table 9.1. *Summary of NMISA Set 1 and Set 2 data quality criteria (pg/ g)*

Compound	NMISA Set 1			NMISA Set 2		
	Uncertainty U (Rel)%	LOD	LOQ	Uncertainty U (Rel)%	LOD	LOQ
2378-TCDD	18.0	8.42	28.1	37.8	11.2	37.5
2378-TCDF	25.2	1.48	4.93	37.4	20.9	71.6
12378-PeCDD	35.0	19.8	66.0	168	73.2	255
12378-PeCDF	27.6	18.8	62.6	122	60.1	205
23478-PeCDF	34.8	34.5	114	111	58.2	201
123478-HxCDD	41.9	18.9	63.1	211	84.9	287
123678-HxCDD	25.3	18.2	60.8	101	82.4	288
123789-HxCDD	32.3	7.81	26.0	158	95.5	321
123478-HxCDF	36.2	9.57	31.9	129	68.7	240
123678-HxCDF	19.7	13.9	46.3	111	115	386
123789-HxCDF	75.0	10.3	34.3	116	69.8	251
234678-HxCDF	32.8	32.0	106	331	65.4	229
1234678-HpCDD	35.5	15.0	49.9	35.0	61.4	198
1234678-HpCDF	16.5	23.5	78.3	30.3	69.3	230
1234789-HpCDF	27.5	27.3	91.0	190	60.7	208
OCDD	9.41	51.9	172	42.4	98.4	331
OCDF	35.1	54.9	182	8.56	255	925

The results of the participating laboratories (NMISA and MOE) are summarised in Tables 9.2 to 9.4, not taking any quality control criteria into consideration. The two data sets produced during the two different experimental approaches vary significantly from one another. Set 1 results are higher (Table 9.3) than the results from MOE (Table 9.2), whereas Set 2 (Table 9.4) provides results that are lower and can be compared with the results from the MOE, as the NMISA values overlap with the uncertainties reported for the NIST certified values and the MOE values (Figures 9.4 to 9.9). However, the LOD/ LOQs obtained for Set 1 were significantly lower (one-way ANOVA; $p < 0.05$) than for those obtained in Set 2 for 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,7,8,9-HxCDF; 1,2,3,4,6,7,8-HpCDD; 1,2,3,4,6,7,8-HpCDF and 1,2,3,4,7,8,9-HpCDF. The uncertainty calculated for Set 2 (Table 9.4) included the reproducibility

determined over three separate and repeat experiments leading to higher uncertainty for compounds at levels close to the calculated LOD/ LOQs. This is expected as precision decreases close to instrumental limits as discussed in Chapter 8.

Table 9.2. *Summary of results obtained by MOE*

Compound	Concentration (pg/ g)					
	Soil 16	Soil 42	Soil 21	Soil 23	Soil 10	NIST 1944
2,3,7,8-TCDD	0.12	0.13	0.13	0.13	0.14	140
2,3,7,8-TCDF	1.20	0.42	0.99	0.78	3.30	170
1,2,3,7,8-PCDD	0.18	0.12	0.21	0.17	0.32	17
1,2,3,7,8-PCDF	0.37	0.22	0.39	0.43	1.04	44
2,3,4,7,8-PCDF	0.42	0.17	0.44	0.30	1.04	42
1,2,3,4,7,8-HxCDD	0.17	0.09	0.24	0.11	0.38	21
1,2,3,6,7,8-HxCDD	0.39	0.14	1.25	0.35	1.35	48
1,2,3,7,8,9-HxCDD	0.33	0.16	0.54	0.43	0.83	47
1,2,3,4,7,8-HxCDF	0.70	0.28	0.53	0.43	1.50	270
1,2,3,6,7,8-HxCDF	0.47	0.11	0.47	0.26	1.05	97
1,2,3,7,8,9-HxCDF	0.48	0.50	0.76	2.50	0.45	2.2
2,3,4,6,7,8-HxCDF	0.37	0.11	0.43	0.22	0.76	46
1,2,3,4,6,7,8-HPCDD	5.95	0.47	39.00	1.35	57.00	870
1,2,3,4,6,7,8-HPCDF	2.20	0.47	5.05	1.03	6.60	1100
1,2,3,4,7,8,9-HPCDF	0.30	0.13	0.46	0.21	0.60	39
OCDD	41.50	1.75	595.00	5.35	665.00	6500
OCDF	2.10	0.65	9.25	0.96	14.50	1100

Table 9.3. *Summary of results obtained by NMISA using the routine laboratory approach, Set 1*

Compound	Concentration (pg/ g)					
	Soil 16	Soil 42	Soil 21	Soil 23	Soil 10	NIST 1944
2,3,7,8-TCDD	7.82	6.32	7.09	6.80	6.58	132.33
2,3,7,8-TCDF	3.55	1.77	1.37	4.30	2.56	40.50
1,2,3,7,8-PCDD	9.05	8.46	8.53	8.37	8.33	50.24
1,2,3,7,8-PCDF	17.00	53.24	17.53	21.29	18.92	63.98
2,3,4,7,8-PCDF	23.49	23.37	24.57	23.94	23.99	71.89
1,2,3,4,7,8-HxCDD	129.36	14.70	15.26	14.49	14.84	51.72
1,2,3,6,7,8-HxCDD	6.03	6.07	6.19	5.75	7.07	83.94

Compound	Concentration (pg/ g)					
	Soil 16	Soil 42	Soil 21	Soil 23	Soil 10	NIST 1944
1,2,3,7,8,9-HxCDD	3.55	7.12	4.03	6.43	4.51	38.79
1,2,3,4,7,8-HxCDF	2.42	1.94	2.42	2.05	2.85	279.27
1,2,3,6,7,8-HxCDF	10.57	10.43	10.95	10.69	10.47	103.59
1,2,3,7,8,9-HxCDF	8.56	7.99	8.48	7.29	8.21	33.77
2,3,4,6,7,8-HxCDF	25.11	24.41	25.47	24.58	24.69	77.60
1,2,3,4,6,7,8-HPCDD	16.01	9.99	42.69	13.57	57.73	936.99
1,2,3,4,6,7,8-HPCDF	17.11	13.75	19.12	14.68	20.79	1137.50
1,2,3,4,7,8,9-HPCDF	23.18	22.49	22.92	22.48	23.07	71.73
OCDD	50.38	26.06	642.10	30.80	558.23	4905.99
OCDF	39.17	39.01	47.48	40.70	49.33	765.57

Table 9.4. *Summary of results obtained by NMISA using conservative approach, Set 2*

Compound	Concentration (pg/ g)					
	Soil 16	Soil 42	Soil 21	Soil 23	Soil 10	NIST 1944
2,3,7,8-TCDD	5.54	5.54	5.54	5.54	5.54	140.64
2,3,7,8-TCDF	10.43	10.43	10.43	10.43	10.43	34.05
1,2,3,7,8-PCDD	36.60	36.60	36.60	36.60	36.60	29.41
1,2,3,7,8-PCDF	30.04	30.04	30.04	30.04	30.04	39.50
2,3,4,7,8-PCDF	29.10	29.10	29.10	29.10	29.10	44.86
1,2,3,4,7,8-HxCDD	42.45	42.45	42.45	42.45	42.45	23.32
1,2,3,6,7,8-HxCDD	41.18	41.18	41.18	41.18	41.18	55.48
1,2,3,7,8,9-HxCDD	47.74	47.74	47.74	47.74	47.74	30.69
1,2,3,4,7,8-HxCDF	34.33	34.33	34.33	34.33	34.33	176.94
1,2,3,6,7,8-HxCDF	57.87	57.87	57.87	57.87	57.87	78.91
1,2,3,7,8,9-HxCDF	34.91	34.91	34.91	34.91	34.91	51.84
2,3,4,6,7,8-HxCDF	32.72	32.72	32.72	32.72	32.72	15.33
1,2,3,4,6,7,8-HPCDD	30.69	30.69	98.83	30.69	65.42	796.15
1,2,3,4,6,7,8-HPCDF	34.63	34.63	34.63	34.63	34.63	1007.83
1,2,3,4,7,8,9-HPCDF	30.33	30.33	103.95	30.33	30.33	39.67
OCDD	49.20	49.20	524.64	49.20	625.41	6493.68
OCDF	462.53	127.44	462.53	462.53	462.53	1010.50

Based on the LOD and LOQ achieved with the GC×GC-TOFMS, only the NIST SRM 1944 sample could be used for comparison purposes as most of the PCDD/F analytes in the NIST SRM 1944 were above the LOD/ LOQ and could be quantified.

Table 9.5. *Final reported results in pg/ g, using Set 2 and TEQ results in ng WHO-TEQ.kg^{-1} (~pg WHO-TEQ.g^{-1}).*

Compound	I-TEF	MOE		NIST SRM 1944		NMISA	
		Conc (pg/ g)	TEQ	Conc (pg/ g)	TEQ	Conc (pg/ g)	TEQ
2,3,7,8-TCDD	1	140	140	133	133	141	141
2,3,7,8-TCDF	0.1	170	17	39	4	34	3
1,2,3,7,8-PCDD	1	17	17	26	26	29	29
1,2,3,7,8-PCDF	0.3	44	1	45	1	40	1
2,3,4,7,8-PCDF	0.1	42	13	45	14	45	14
1,2,3,4,7,8-HxCDD	0.1	21	2	26	3	23	2
1,2,3,6,7,8-HxCDD	0.1	48	5	56	6	55	6
1,2,3,7,8,9-HxCDD	0.1	47	5	53	5	31	3
1,2,3,4,7,8-HxCDF	0.1	270	27	220	22	177	18
1,2,3,6,7,8-HxCDF	0.1	97	10	90	9	79	8
1,2,3,7,8,9-HxCDF	0.1	2.2	0	19	2	15	2
2,3,4,6,7,8-HxCDF	0.1	46	5	54	5	52	5
1,2,3,4,6,7,8-HPCDD	0.01	870	9	800	8	796	8
1,2,3,4,6,7,8-HPCDF	0.01	1100	11	1000	10	1008	10
1,2,3,4,7,8,9-HPCDF	0.01	39	0	40	0	40	0
OCDD	0.0003	6500	2	5800	2	6494	2
OCDF	0.0003	1100	0	1000	0	1011	0
TOTAL TEQs			**260**		**250**		**250**

Table 9.5 provides a summary of the final reported results for NMISA before considering quality criteria and Table 9.6, after considering quality criteria, which includes the associated uncertainties of the method. Toxic Equivalency Factors (TEF) were calculated for each of the seventeen toxic PCDD/Fs and are generally used to report data to external clients as well as in risk assessment procedures (Van den Berg *et al*. 2006). The Total Toxic Equivalent (TEQ) concentration values were summed for the final results and for the sub-set of five congener results for NIST

SRM 1944 that could be reported. When quality criteria are not factored, then all the NMISA results for Set 2 compare well with the NIST SRM 1944 values. The NIST reference value is a consensus value not confirmed by an independent analytical technique, whereas the MOE value has been confirmed by independent analysis using GC-HRMS.

The NMISA reported uncertainties are generally higher than the NIST SRM 1944 and MOE reported uncertainties. The NMISA uncertainty for OCDF is very low which may be because there is no interference from the internal standard on this compound.

Table 9.6. *Final reported results in pg/ g, using Set 2, with U reported as the associated expanded uncertainty using a coverage factor of K = 2 and TEQ results in ng WHO-TEQ.kg^{-1} (~pg WHO-TEQ.g^{-1}).*

Compound	I-TEF	MOE			NIST SRM 1944			NMISA		
		Conc (pg/ g)	U	TEQ	Conc (pg/ g)	U	TEQ	Conc (pg/ g)	U	TEQ
2,3,7,8-TCDD	1	140	28	140	133	9	133	141	53	141
2,3,7,8-TCDF	0.1	170	34	17	39	15	4	34	13	3
1,2,3,4,6,7,8-HpCDD	0.01	870	174	9	800	7	8	796	279	8
1,2,3,4,6,7,8-HpCDF	0.01	1100	220	11	1000	100	10	1008	305	10
OCDF	0.0003	1100	220	0	1000	100	0	1011	86	0
TOTAL TEQs:				**180**			**160**			**160**

To investigate possible bias associated with the NMISA method, Figures 9.1 to 9.9 compare the two data sets generated by NMISA against the NIST SRM 1944 reference values and the values obtained by the MOE for all seventeen congeners. The TEQs are considered for comparison purposes to assess overall bias using the routine laboratory approach (Set 1) and the more conservative approach (Set 2).

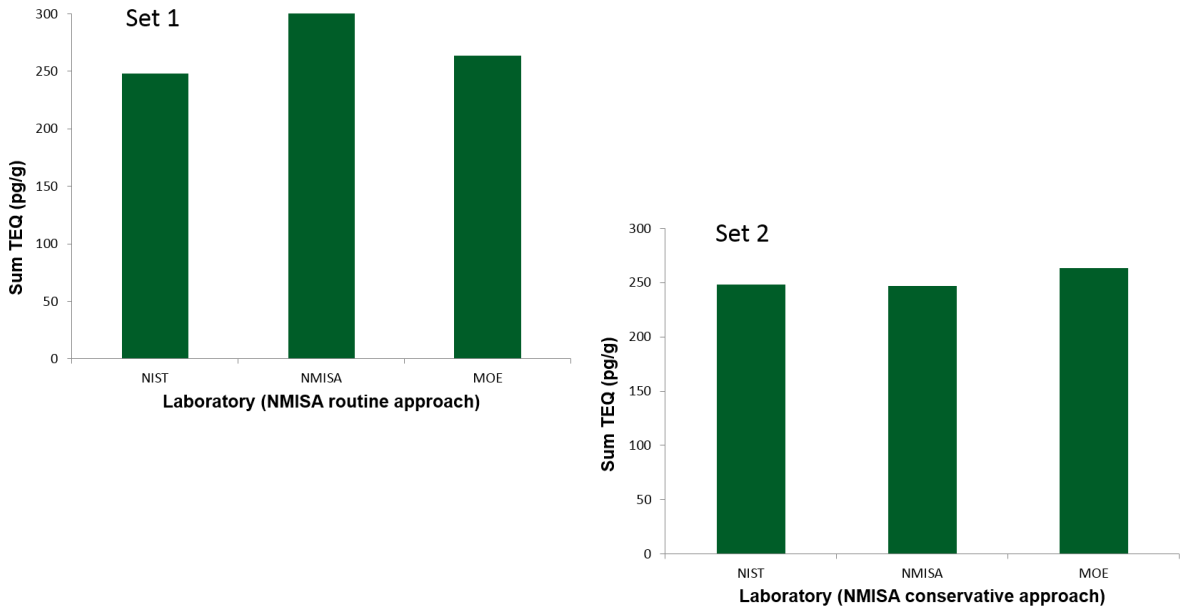

Figure 9.1. *Concentration of ∑PCDD/Fs (pg WHO-TEQ.g^{-1}) for the NMISA values (Set 1 and Set 2) compared with the NIST 1944 reference material and MOE values. The TEQ values were calculated using the updated international toxic equivalence factors (I-TEF) (Van den Berg et al. 2006).*

Figure 9.1 indicates good agreement between the TEQ values from the participating laboratories for all seventeen congeners. The NMISA value for Set 1 (300 pg WHO-TEQ.g^{-1}) is slightly higher than Set 2 (247 pg WHO-TEQ.g^{-1}). The NMISA result obtained for Set 2 agrees very well with the NIST SRM 1944 reference value (certificate value = 248 pg WHO-TEQ.g^{-1}).

Figures 9.2 and 9.3 present congener specific variations between the NMISA TEQ results and the NIST SRM 1944 reference and MOE TEQ results. Figure 9.2 indicates that the largest contributors to the bias in the TEQ results for NMISA Set 1 were the PeCDD/F and HxCDD/F congeners. This is the area in the chromatographic separation that has the most congeners and interferences.

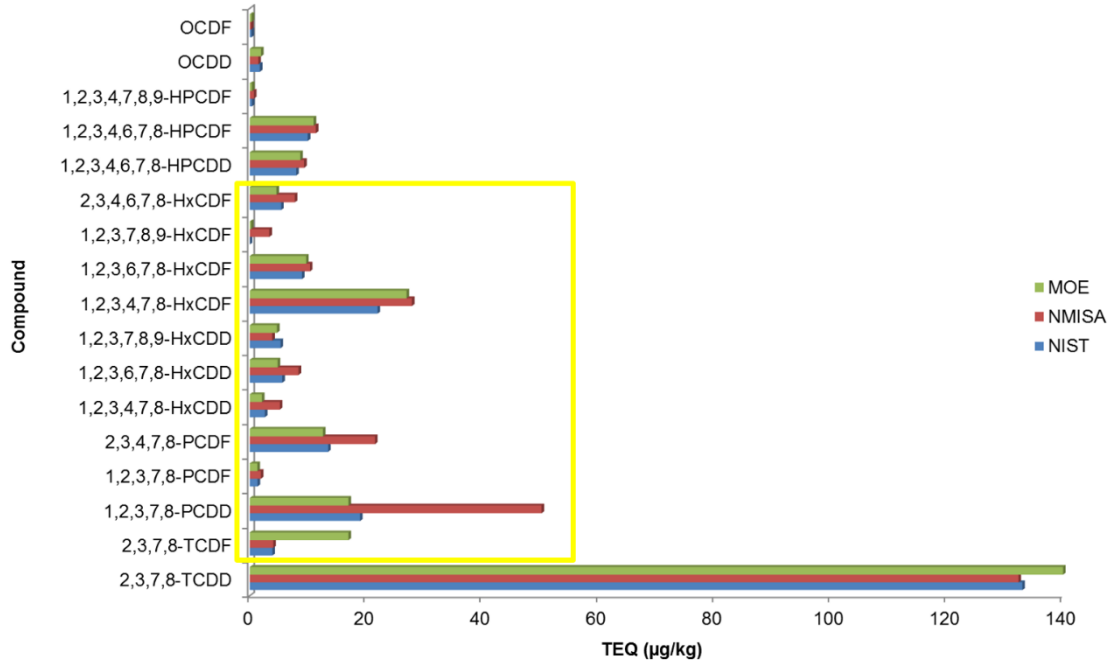

Figure 9.2. *PCDD/F congener TEQ values obtained from the NIST reference value as well as the participating laboratories using data Set 1. The area highlighted in yellow indicates congeners with a positive bias on the calculated TEQ values.*

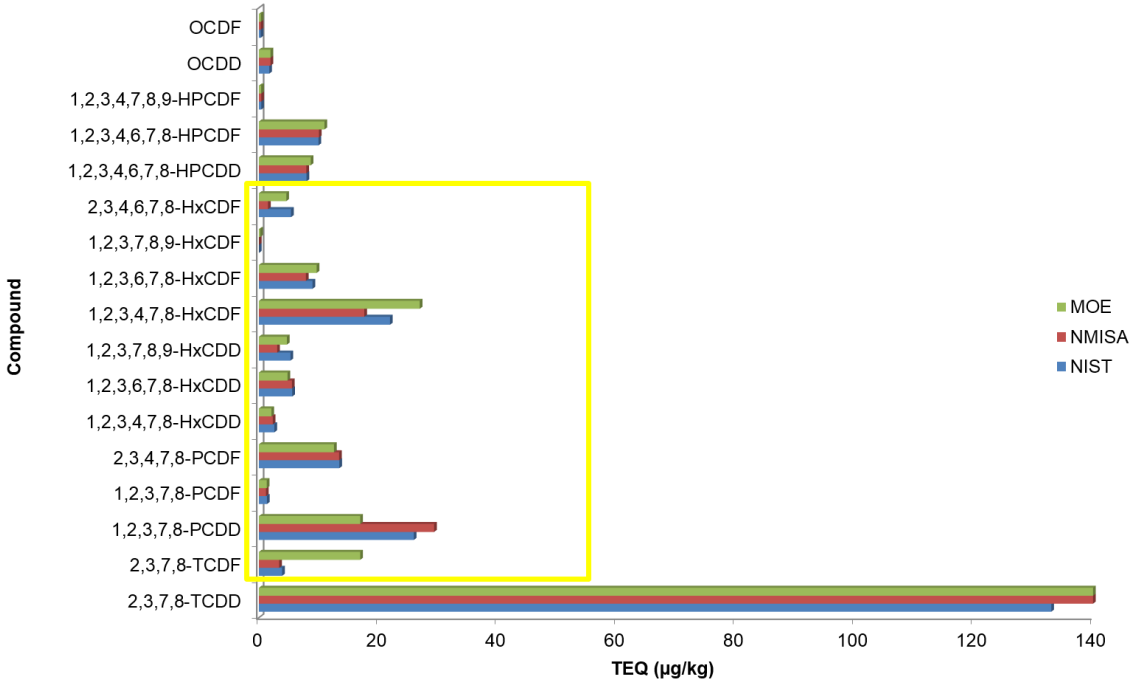

Figure 9.3. *Conservative PCDD/F congener TEQ values obtained from the NIST reference and the participating laboratories using data Set 2. There is only a slight positive bias on 2,3,7,8-TCDD and 1,2,3,7,8-PCDD.*

Due to the observed positive bias obtained for all seventeen congeners, the data sets were inspected more closely based on chlorine substitution and elution order (Figures 9.4 to 9.9).

TCDD/F and PeCDD/F congener values were compared against the NIST reference values and MOE results (Figure 9.4). The analytes indicated in the red blocks for Set 1 could not be reported as values were below the LOQ. Both 2,3,7,8-TCDD and 2,3,7,8-TCDF compare well with the NIST reference value and can be reported. The 1,2,3,7,8-PeCDF is also reported as the uncertainty falls within the uncertainty range for the reference value, although the result obtained (61,8 pg/ g) is higher than the NIST reference value of 45 pg/ g.

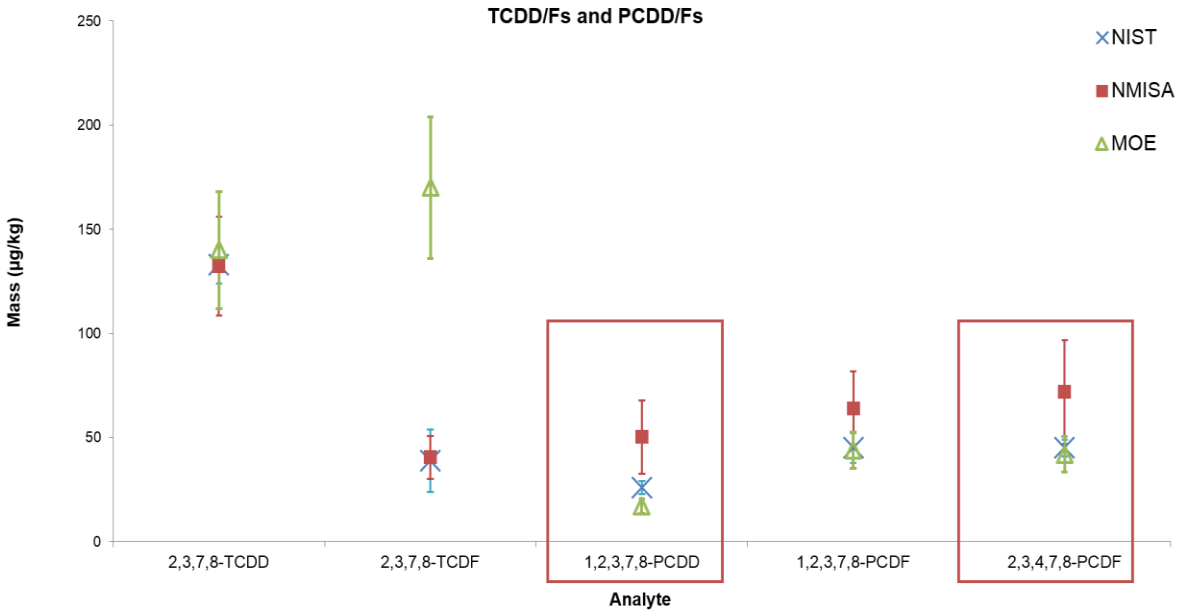

Figure 9.4. *TCDD/F and PeCDD/F congener values for the NIST reference value, MOE and NMISA using data Set 1; the analytes indicated in the red blocks were at levels below the LOQ. Both 2,3,7,8-TCDD and 2,3,7,8-TCDF compare well with the NIST reference value.*

Figure 9.5 compares data obtained for Set 2. The values for 2,3,7,8-TCDD and 2,3,7,8-TCDF compare well to the NIST reference value. There is a slight positive bias on 2,3,7,8-TCDD and 1,2,3,7,8-PCDD for the NMISA results which did not greatly affect the total TEQ value. The analytes indicated in the red blocks (1,2,3,7-

PeCDD, 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF) could not be reported as they were at levels below the LOQ.

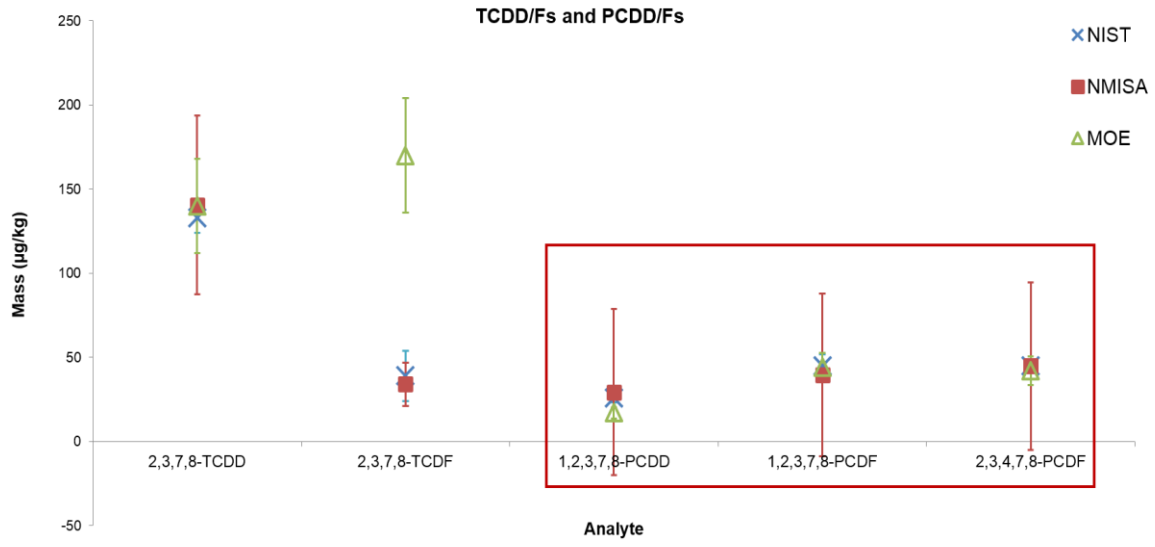

Figure 9.5. *TCDD/F and PeCDD/F congener values for the NIST reference value, MOE and NMISA using data Set 2; the analytes indicated in the red blocks were at levels below the LOQ and their associated uncertainties fall below zero. The values for 2.3,7,8-TCDD and 2,3,7,8-TCDF compare well with the NIST reference value.*

The HxCDD/F congener analytes indicated in the red blocks could not be reported as they were at levels below the LOQ while the analytes in the blue blocks had associated uncertainties greater than 30% (Figure 9.6). In general there is a positive bias associated with NMISA Set 1 results for the HxCDD/F congeners with the exception of 1,2,3,6,7,8-HxCDD and 1,2,3,6,7,8-HxCDF. This could indicate an unidentified co-elution with an interfering compound in this elution area. Mass spectral data corroborates this and can only be addressed with improved clean-up or by adjusting the instrumental parameters.

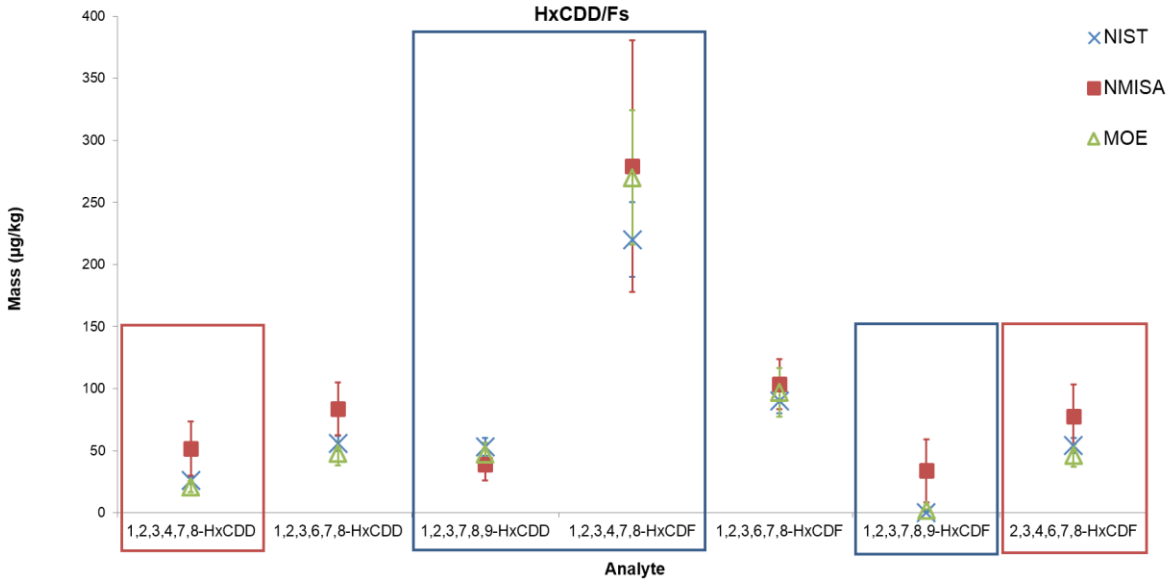

Figure 9.6. *HxCDD/F congener values for the NIST reference value, MOE and NMISA using data Set 1; the analytes indicated in the red blocks were at levels below the LOQ while the analytes in blue blocks had associated uncertainties greater than 30%.*

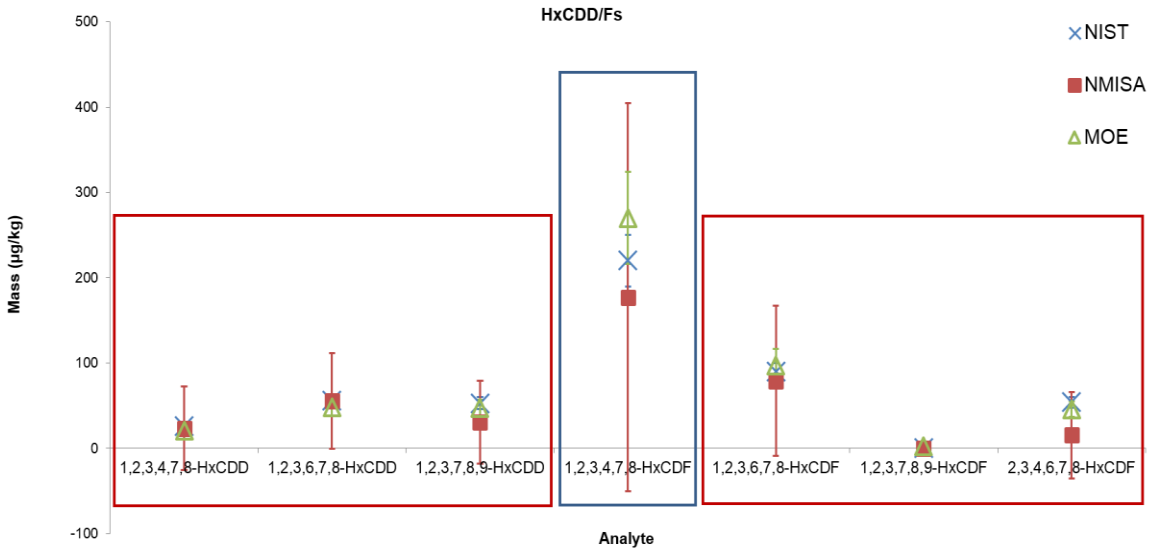

Figure 9.7. *HxCDD/F congener values for the NIST reference value, MOE and NMISA using data Set 2; the analytes indicated in the red blocks were at levels below the LOQ while the analytes in blue blocks had associated uncertainties greater than 30%. With the exception of 1,2,3,4,7,8-HxCDF, there was good agreement between all results obtained.*

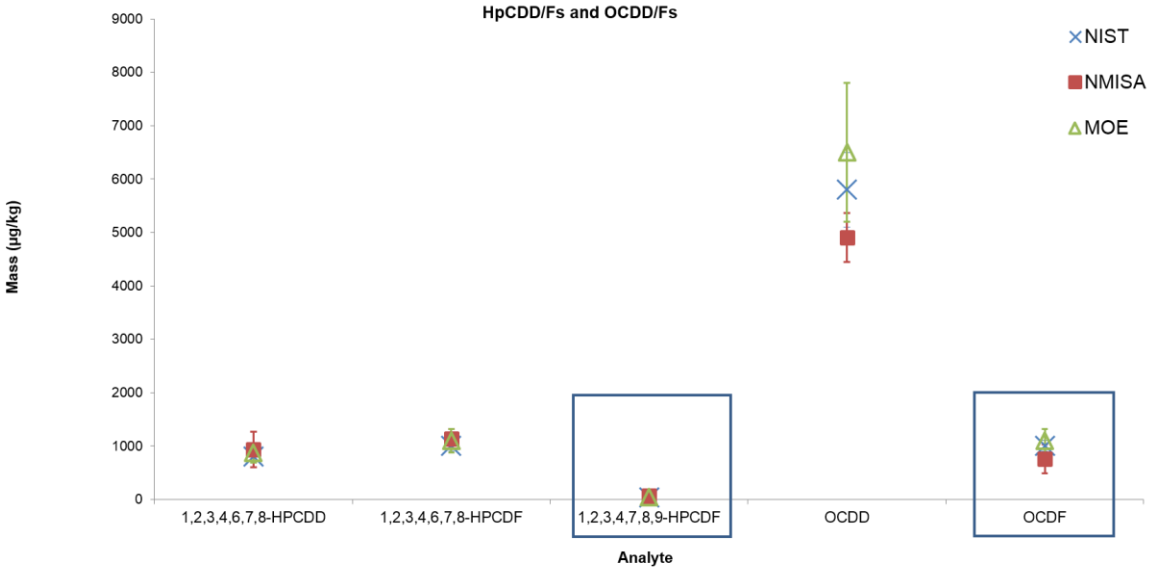

Figure 9.8. *HpCDD/F and OCDD/F congener values for the NIST reference value, MOE and NMISA using data Set 1; the analytes in the blue blocks had associated uncertainties greater than 30%. With the exception of OCDD, there was a good agreement between all results obtained. For OCDD there was a positive bias for the MOE value and a negative bias for NMISA Set 1 results.*

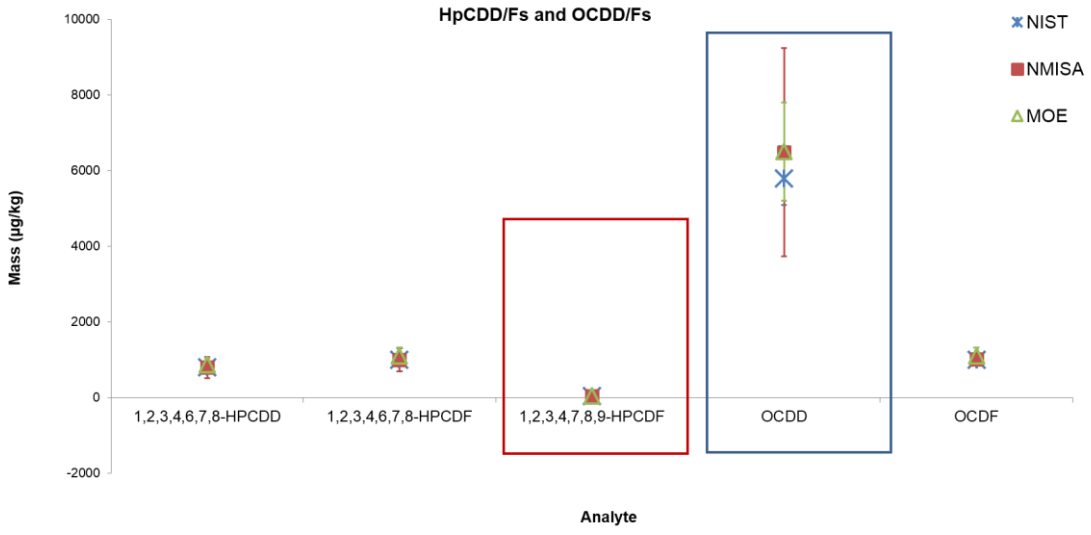

Figure 9.9. *HpCDD/F and OCDD/F congener values for the NIST reference value, MOE and NMISA using data Set 2; the analytes indicated in the red block were at levels below the LOQ while those in the blue block had associated uncertainties greater than 30%. There was good agreement between all results obtained with the exception of OCDD where both the MOE and NMISA Set 2 results had a positive bias.*

Figure 9.7 compares data obtained for Set 2. With the exception of 1,2,3,4,7,8-HxCDF, there was a good agreement between all results obtained. For this compound there was a positive bias for the MOE value and a negative bias for the NMISA result. The HxCDD/F analytes indicated in the red blocks could not be reported as they were at levels below the LOQ, although the results were within the range of the NIST reference value when considering their associated uncertainties. The results for 1,2,3,4,7,8-HxCDF in the blue block, had an associated uncertainty greater than 30% and could also not be reported.

Figure 9.8 compares data obtained for Set 1. The HpCDD/F and OCDD/F analytes indicated in the blue blocks had associated uncertainties greater than 30%. With the exception of OCDD, there was very good agreement between all results obtained. There was a positive bias for the MOE value reported for OCDD and a negative bias for the NMISA result, however both results were within the uncertainty range of the NIST reference value.

Figure 9.9 compares data obtained for Set 2. Soil samples analysed by NMISA fell below the LOD and LOQ with the exception of OCDD in soil sample 10 (625 pg/ g) and soil sample 21 (525 pg/ g) that correlated well with MOE results (sample 10: 665 pg/ g and sample 21: 595 pg/ g). However, although these results compared well, they could not be reported as the uncertainty associated with OCDD is 42%.

Table 9.7 summarises the NMISA results for Set 1 including the quality criteria. Results highlighted in grey are below the LOD/ LOQ and in green have uncertainties > 30%. As reproducibility was not included in the uncertainty calculations, uncertainties above 30% would not be reported. Table 9.8 summarises the results that can be reported (values with an uncertainty less than 30% and that are above the LOQ). Only seven of the seventeen toxic PCDD/F congeners could be reported.

Table 9.9 summarises the NMISA results for Set 2 including the quality criteria. Results highlighted in grey are below the LOD/ LOQ and in green have uncertainties > 40%. As reproducibility was included in the uncertainty calculations, uncertainties above 40% would not be reported. Table 9.10 summarises the results that can be

reported within the set quality criteria of the method. Using this method only five of the seventeen PCDD/F congeners could be reported.

Table 9.7. *Summary of NMISA Set 1 results including quality criteria. Samples highlighted in grey are below the LOD/ LOQ and in green have uncertainties > 30%.*

Compound	Concentration (pg/ g)						Quality criteria		
	Soil 16	Soil 42	Soil 21	Soil 23	Soil 10	NIST 1944	Uncertainty U Rel)%	LOD	LOQ
2378-TCDD	7.82	6.32	7.09	6.80	6.58	132.33	17.99	8.42	28.05
2378-TCDF	3.55	1.77	1.37	4.30	2.56	40.50	25.22	1.48	4.93
12378-PeCDD	9.05	8.46	8.53	8.37	8.33	50.24	34.96	19.80	65.99
12378-PeCDF	17.00	53.24	17.53	21.29	18.92	63.98	27.56	18.77	62.55
23478-PeCDF	23.49	23.37	24.57	23.94	23.99	71.89	34.76	34.47	114.91
123478-HxCDD	129.36	14.70	15.26	14.49	14.84	51.72	41.96	18.91	63.05
123678-HxCDD	6.03	6.07	6.19	5.75	7.07	83.94	25.34	18.24	60.79
123789-HxCDD	3.55	7.12	4.03	6.43	4.51	38.79	32.32	7.81	26.02
123478-HxCDF	2.42	1.94	2.42	2.05	2.85	279.27	36.24	9.57	31.91
123678-HxCDF	10.57	10.43	10.95	10.69	10.47	103.59	19.66	13.90	46.34
123789-HxCDF	8.56	7.99	8.48	7.29	8.21	33.77	75.00	10.30	34.33
234678-HxCDF	25.11	24.41	25.47	24.58	24.69	77.60	32.84	31.99	106.64
1234678-HpCDD	16.01	9.99	42.69	13.57	57.73	936.99	35.52	14.97	49.89
1234678-HpCDF	17.11	13.75	19.12	14.68	20.79	1137.50	16.47	23.48	78.25
1234789-HpCDF	23.18	22.49	22.92	22.48	23.07	71.73	27.49	27.29	90.98
OCDD	50.38	26.06	642.10	30.80	558.23	4905.99	9.41	51.85	172.84
OCDF	39.17	39.01	47.48	40.70	49.33	765.57	35.13	54.90	182.98

Table 9.8. *Summary of results for Set 1 that could be reported considering quality control criteria.*

Compound	NIST 1944 (pg/ g)	Uncertainty U (Rel)%	LOD	LOQ
2378-TCDD	132.33	17.99	8.42	28.05
2378-TCDF	40.50	25.22	1.48	4.93
12378-PeCDF	63.98	27.56	18.77	62.55
123678-HxCDD	83.94	25.34	18.24	60.79
123678-HxCDF	103.59	19.66	13.90	46.34
1234678-HpCDF	1137.50	16.47	23.48	78.25
OCDD	4905.99	9.41	51.85	172.84

Table 9.9. *Summary of NMISA Set 2 results including quality criteria. Samples highlighted in grey are below the LOD/ LOQ and in green have uncertainties > 40%.*

Compound	Concentration (pg/ g)						Quality criteria		
	Soil_16	Soil_42	Soil_21	Soil_23	Soil_10	NIST 1944	Uncertainty U (Rel)%	LOD	LOQ
2378-TCDD	5.54	5.54	5.54	5.54	5.54	140.64	37.8	11.19	37.51
2378-TCDF	10.43	10.43	10.43	10.43	10.43	34.05	37.41	20.86	71.59
12378-PeCDD	36.60	36.60	36.60	36.60	36.60	29.41	168.22	73.20	254.58
12378-PeCDF	30.04	30.04	30.04	30.04	30.04	39.50	122.15	60.09	205.08
23478-PeCDF	29.10	29.10	29.10	29.10	29.10	44.86	111.06	58.19	201.34
123478-HxCDD	42.45	42.45	42.45	42.45	42.45	23.32	210.53	84.90	286.91
123678-HxCDD	41.18	41.18	41.18	41.18	41.18	55.48	101.41	82.36	287.26
123789-HxCDD	47.74	47.74	47.74	47.74	47.74	30.69	157.62	95.47	320.63
123478-HxCDF	34.33	34.33	34.33	34.33	34.33	176.94	128.5	68.66	239.79
123678-HxCDF	57.87	57.87	57.87	57.87	57.87	78.91	111.47	115.74	386.05
123789-HxCDF	34.91	34.91	34.91	34.91	34.91	51.84	115.5	69.82	250.63
234678-HxCDF	32.72	32.72	32.72	32.72	32.72	15.33	330.53	65.44	221.88
1234678-HpCDD	30.69	30.69	98.83	30.69	65.42	796.15	35	61.37	197.66
1234678-HpCDF	34.63	34.63	34.63	34.63	34.63	1007.83	30.27	69.25	230.01
1234789-HpCDF	30.33	30.33	103.95	30.33	30.33	39.67	189.92	60.66	207.91
OCDD	49.20	49.20	524.64	49.20	625.41	6493.68	42.37	98.41	330.57
OCDF	462.53	127.44	462.53	462.53	462.53	1010.50	8.56	254.89	925.06

Table 9.10. *Summary of results for Set 2 that could be reported considering quality criteria*

Compounds	NIST 1944 (pg/ g)	Uncertainty U (Rel)%	LOD	LOQ
2378-TCDD	140.64	37.8	11.07	14.28
2378-TCDF	34.05	37.41	20.86	27.03
1234678-HpCDD	796.15	35	61.37	197.66
1234678-HpCDF	1007.83	30.27	69.25	69.25
OCDF	1010.50	8.56	254.89	925.06

9.2 VALIDATION CONCLUSIONS

The results obtained indicated that a direct comparison between data obtained on the GC×GC-TOFMS and a GC-HRMS, at South African environmentally relevant concentrations, was problematic due to the differences in the obtainable LOD and LOQs. When the LOD/ LOQ were improved using different calculation techniques, problems with positive bias were encountered. When values were calculated by mathematical interpolation and manual peak assignment in blank samples, the obtained results were equivalent to the calculated LOD. The LOD is then equivalent to instrumental noise, thus not meeting the minimum requirements for signal detection as stipulated in US EPA Method 8290A (2007d). It is therefore prudent that the conservative approach, which considered both the uncertainty of the y-intercept as well as the slope ($S_{y/x}$), be used to determine the LOD/ LOQ.

The results obtained for the soil samples and the NIST SRM 1944 had calculated concentrations close to or below the LOD/ LOQ of the GC×GC-TOFMS instrument. When calculating the theoretical concentration of the seventeen toxic PCDD/Fs in the NIST SRM 1944 (considering extraction mass and reconstitution volume), it was observed that the extraction recoveries would need to be greater than 95% to fall above the LOQ. Due to the many steps associated with the extraction and clean-up of PCDD/Fs from soil and sediment, this is a very difficult objective to achieve as most routine laboratories will accept recoveries between 60 – 120%.

The regression analysis has confirmed that the GC×GC-TOFMS results are affected by the sample extraction since matrix effects may influence the obtainable LOD/ LOQ as only one mass is selected for quantitation. This is not a limitation of the GC×GC-TOFMs method; it indicates that many more factors must be considered when performing ultra-trace analysis with nominal mass instrumentation.

Dorman *et al.* (2008) discussed the effect of higher and higher amounts of matrix background on the GC×GC separation. Differences in quantitative accuracy and precision is dependent on whether summed peak areas or calculated peak volumes are used. The area method of calculation was more prone to underestimation (especially at trace levels) than the volume method, but the area method was more repeatable than the volume method. Mass spectrometry might not always provide

enough resolution where the closely eluting PCDD/F peaks are often isomers with essentially the same mass spectra. This may explain the bias associated with the PeCDD/Fs and HxCDD/Fs (Figures 9.2 and 9.3).

The study showed that for concentrations above the LOQ of the GC×GC-TOFMS, results using the GC×GC-TOFMS were comparable with those obtained using GC-HRMS. The bilateral comparison between NMISA and the MOE was a successful benchmarking exercise that has allowed NMISA to establish both an extraction and analytical method for quantitation of PCDD/Fs in sediment. The extraction method provides acceptable results, as the NMISA values did compare with the NIST SRM 1944 values. However, the LOD/ LOQ obtained with the GC×GC-TOFMS in samples containing high levels of matrix interference is a limiting factor. Whether the method is fit-for-purpose for South Africa, where PCDD/F concentrations appear to be very low, will need to be verified.

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CONCLUSIONS

10.1 CONCLUSIONS

South Africa, as a signatory to the Stockholm Convention, has an obligation to undertake appropriate research and cooperation pertaining to POPs, and more particularly, PCDD/Fs. Reliable environmental monitoring is essential to meet consumer demands, to ensure the successful enforcing of international environmental regulations and to overcome technical barriers to trade (TBTs).

With this in mind, NMISA undertook to establish an analysis capability to address PCDD/Fs (dioxins) and other POPs (e.g. dioxin-like PCBs). GC-HRMS remains the gold standard for dioxin detection and analysis as mandated by US EPA Methods 1613B and 8290A for regulatory compliance and litigation. South Africa (and other developing countries) has no established GC-HRMS facility for dioxin analysis and this required the development of an alternative solution that is reasonably priced, and easy to manage and implement.

Since NMISA had invested in the more affordable GC×GC-TOFMS instrumentation, this approach was developed for ultra-trace quantitative analysis of the seventeen toxic PCDD/Fs. The GC×GC-TOFMS analysis was combined with the H4IIE-*luc* bio-assay to provide a useful quantitative and screening tool for PCDD/Fs, reducing the need for expensive GC-HRMS analysis. The bio-assay, which is not PCDD/F specific, gives a good indication of the overall toxicity of the sample and reports the combined effect of all compounds capable of binding to the AhR receptor. It does not identify the causative agent or its associated concentration and instrumental confirmation using GC×GC-TOFMS is required. As with any newly established methodology, the

problems encountered with accurately detecting and quantifying PCDD/Fs at ultra-trace levels amidst contaminant interferences at orders of magnitude higher than the target analytes needed to be resolved.

NMISA, in collaboration with various institutions, initiated a project to optimise the GC×GC-TOFMS screening method. Sample extracts were provided by the collaborators for analysis. The good correlation between results obtained by GC×GC-TOFMS and those obtained by GC-HRMS, establishes the validity of this technique to quantify these compounds at low levels. Although the results were consistently higher than those obtained using GC-HRMS, the methodology using the low resolution GC× GC-TOFMS system is acceptable for quantitative work and is also a valuable tool for overall sample assessment. The sensitivity needed is provided by the focusing effect of the modulator, and the selectivity gain from the two-dimensional chromatographic separation which effectively compensates for that lost in using lower resolution mass spectrometry.

Sample extraction and clean-up are key processes when applying GC×GC-TOFMS analysis with full mass range spectra. Initial sample extraction targeting PCDD/Fs, followed by GC×GC-TOFMS analysis, indicated a need to improve the extraction methodology. If sample matrix interference is still present after clean-up, or if the sample clean-up steps cause analyte losses, then the PCDD/Fs analytes are obscured and accurate quantitation is compromised, resulting in a lack of confidence in the analytical results. Improved sample extraction and adequate clean-up was needed to prevent unacceptable peak overlap and maintain retention of the PCDD/F analytes. Sample extraction and clean-up procedures using the Total Rapid Prep™ system were implemented to improve the dioxin extraction method with the aim of combining automated sample preparation and GC×GC-TOFMS analysis that could be offered as a routine service to industry.

The detection limits and quantitation levels in this method are dependent on the level of interferences, and not only instrumental limitations. The minimum levels as mandated in US EPA Method 1613B are the levels at which the PCDDs/Fs can be determined with no interferences present. Previous work relied on neat standards and the GC×GC-TOFMS was easily able to meet these low levels. The sample extracts

used in the study to compare the results obtained by GC×GC-TOFMS against GC-HRMS, were provided by experienced laboratories and the various analyses considered instrument LOD and not method LOD. When matrix interferences are still present in the extracts, the GC×GC-TOFMS using nominal mass cannot reach the low levels attained by GC-HRMS, where the absence of interference on the accurate mass used for quantitation provides enhanced sensitivity. Higher resolution produces more reliable results when detecting low analyte concentrations in complex matrices. With GC-HRMS, the higher selectivity is related to the improved mass resolution to resolve the analyte mass from matrix-related isobaric interferences. The data processing method using the GC×GC-TOFMS for quantitation, uses a single (nominal mass) extracted ion and any interferences remaining affect the LOD of the PCDD/Fs, with the result that the minimum levels realistically achievable then increase from 1 pg/ g (minimum level as specified for 2,3,7,8-TCDD in US EPA Method 1613B for solids; EPA 1994a) to a higher value. Matrix interference is a hindrance for reaching instrument LOD. This implies a need for improved matrix management, either by improving chromatographic resolution (selectivity) or improving sample clean-up. Reducing such interferences will result in more sensitive detection of analytes in more complex matrices.

The South African soil samples studied showed very high levels of PAHs, aliphatic hydrocarbons and sulphur and the extraction method must include separating the target PCDD/F congeners from any remaining PAHs and other contaminants in the sample. The organic content and matrix interference of South African soil samples (and the NIST SRM 1944 sediment) proved to be very challenging to extract using the method developed for the validation study. Only after assessing the GC×GC-TOFMS data did it become apparent that the extraction method will have to be further optimised to ensure that the target PCDD/Fs are extracted with minimal losses incurred during clean-up.

This study has shown that GC×GC-TOFMS provides a quick, convenient screen for numerous pollutant classes which may be present in environmental samples. The method described is fast, convenient and can achieve detection limits comparable with US EPA Method 1613B requirements if sample extraction and clean-up are optimised to target PCDD/Fs. Retrospective data mining of archived data (extraction dependant)

is possible and has provided key information of other chlorinated and brominated contaminants present in South African waste, soil and sediment samples. NMISA now has a viable GC×GC-TOFMS dioxin analytical method for low level (ultra-trace) quantitative screening of chlorinated compounds that can be offered to South African analytical laboratories for routine dioxin analysis. The method also provides the confirmation needed for the H4IIE-*luc* bio-assay. The work is relevant scientifically in its own right, and is a definitive contribution to the growing compilation of GC×GC methodology, providing highly efficient methods for this demanding environmental application. The developed method was not fully validated as some PCDD/F isomers were below the achievable LOD/ LOQ, or the uncertainty was too large to allow the results to be reported.

10.2 FUTURE WORK

Current EU Legislation requires the confirmation and quantitation of PCDD/Fs and dioxin-like PCBs in foodstuffs and animal feed by isotope dilution GC-HRMS (Commission Regulation 2006a). EU legislation does make provision for the screening of dioxins in foodstuffs and animal feed using alternative mass spectrometric techniques and bio-assays. The regulations have recently been amended (Commission Regulation 2014) allowing the recognition of GC-triple quadrupole mass spectrometry (GC/MS/MS) as a confirmatory tool for checking compliance with maximum levels (Focant 2014, L'Homme *et al*. 2015 and Myers *et al*. 2010). L'Homme *et al*. (2015) have described a fully validated method for PCDD/Fs and DL-PCBs analysis in feed material of plant origin (vegetable oil) using GC/MS/MS following the dedicated EU Regulation 709/2014.

The dti has provided funding to establish an analytical capability and validated method for PCDD/Fs in soil and sediment. In order to meet the stricter EU regulations, NMISA has also procured an Agilent 7010*ci* GC/MS/MS to expand the dioxin capability for South Africa to include foodstuffs and animal feed matrices. Analyses with this instrumentation will be integrated into NMISA work protocols. Further work will be undertaken to provide full method validation, with improved sample preparation. With continued access to the LECO GC-HRT at the University of Pretoria, future confirmatory analysis and accurate quantitation for PCDD/F and dioxin-like PCBs in

complex matrices can be addressed using GC×GC-TOFMS, GC/MS/MS and GC-HRT and South Africa will be in a position to provide information about the accurate levels of hazardous chemicals in environmental and food matrices.

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