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**DEVELOPMENT OF A LABORATORY ANALYTICAL METHOD
BENEFICIAL TO THE POLICIES OF THE CANADA-WIDE STANDARDS
FOR DIOXINS AND FURANS**

By

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B.Sc. (Hons), Queen's University, 2005

A thesis

presented to Ryerson University

in partial fulfillment of the
requirements for the degree of

Master of Applied Science

in the Program of

Environmental Applied Science and Management

Toronto, Ontario, Canada, 2009

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ABSTRACT

Development of a Laboratory Analytical Method Beneficial to the Policies of the Canada-Wide Standards for Dioxins and Furans

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Master of Applied Science

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2009

An enzyme-linked immunosorbent assay (ELISA) was tested for its ability to screen for PCDD/F in soils and sediments at 50, 1000 and 10,000 picograms toxic equivalents per gram of soil (pgTEQ g⁻¹). The correlation between ELISA and GC-HRMS was strong for samples between 11 and 27,000 pgTEQ g⁻¹ (n=48, r²=0.994, slope=0.94). These results relied on two concepts developed in this thesis. The first, a congener correction factor, corrects ELISA results for differences in how ELISA and GC-HRMS calculate the dioxin content of a sample. The congener correction factor increased the correlation between ELISA and GC-HRMS TEQ values calculated using World Health Organization (WHO) toxic equivalency factors (TEF) from 83% to 94%. The correlation between ELISA and GC-HRMS TEQ values calculated using North Atlantic Treaty Organization (NATO) TEF remained strong when the correction factor was applied, falling from 102% to 94%. The second concept, a sample algorithm allows ELISA to efficiently measure unknown PCDD/F concentrations between 30 and 10,500 pgTEQ g⁻¹. The algorithm successfully placed 24 of 28 samples into their correct concentration ranges in a maximum of two ELISA each. A cost analysis of using the algorithm predicted that ELISA can screen samples three times faster than GC-HRMS while at a 60% reduction in operating cost. The success of ELISA in conjunction with its time and cost savings indicate that it can replace GC-HRMS in situations where the high precision of GC-HRMS is not required.

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1. DIOXINS AND FURANS: PROPERTIES, REDUCTION INITIATIVES AND MEASUREMENT TECHNIQUES

1.1. Overview

Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are a group of persistent, bioaccumulative chemicals that are toxic to both humans and wildlife (Mandal, 2005; Pesatori et al., 2003; Pelclova et al., 2002; Environment Canada, 1990). They are largely man-made (Srogi, 2008; Mandal, 2005) with many different industries contributing to their release into the environment (CCME, 2004). Toxic effects of exposure to dioxins and furans (referred to herein as dioxins) include wasting syndrome, changes to the immune and reproductive systems, endocrine disruption and chloracne (Schechter et al., 2006; Mandal, 2005; Birnbaum and Tuomisto, 2000). Many of these effects have been observed in fish, bird and mammal studies at body burdens that approach levels present in the background human population (Martinez et al., 2003; Birnbaum and Tuomisto, 2000). Due to the toxicity of dioxins, their creation and emission into the environment are controlled and monitored with international and national initiatives. Internationally, the Stockholm Convention on persistent organic pollutants has targeted them for reduction or elimination (Stockholm Convention, 2008). In Canada, the major dioxin reduction initiative is the *Canada Wide Standards (CWS) for Dioxins and Furans* (CCME, 2001). Although this initiative has achieved some interim successes, the CWS will have difficulty in reaching its goal of virtually eliminating all Canadian dioxin emissions. In order to reach this goal, increased testing of known and suspected dioxin emitting sources as well as validation of new pollution prevention technologies is recommended (CCME, 2004). An increase in source testing and technology validation will likely rely on gas

chromatography-high resolution mass spectroscopy (GC-HRMS) as it has the necessary sensitivity and accuracy.

The problem with an increased use of GC-HRMS for dioxin analysis is that it is expensive and time-consuming. As such, there is interest in finding new technologies that can replace the use of GC-HRMS in instances where its high precision and accuracy are not necessarily required. One such instance is the routine testing of sites for potential dioxin soil contamination. The aim of this thesis is to show that an enzyme-linked immunosorbent assay (ELISA) developed by Cape Technologies is capable of becoming a low cost and rapid screening tool for dioxin contaminated soil. This will be accomplished by introducing and developing two new concepts, a congener correction factor and a sample algorithm, and examining their effects on the efficiency of ELISA and its correlation to GC-HRMS. A discussion on the applicability of using ELISA as a screening tool at existing and future dioxin contaminated sites will follow. If the improved ELISA can relieve some of the burden placed on GC-HRMS, GC-HRMS can become more available to help improve the successes of Canadian dioxin initiatives and thus improve the protection of human health and the environment.

1.2. Properties and Toxicity

1.2.1. Structure and toxicity mechanism

The general structures of dioxins and furans are shown in Figure 1.

Figure 1. General structure for dioxins (top) and for furans (bottom)
Source: CCME, 2002

As this group of molecules can contain any amount of chlorination on their benzene rings, 75 PCDD and 135 PCDF congeners exist. Of these 210 congeners, 7 PCDD congeners and 10 PCDF congeners display “dioxin-like” toxicity, but to differing degrees (CCME, 2002). The most toxic of these congeners is 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (2,3,7,8-TCDD). Concentrations of each toxic congener are reported in Toxicity Equivalents (TEQ) by converting the concentration of each congener to the concentration of 2,3,7,8-TCDD that would have the equivalent level of toxicity. This is calculated by multiplying the Toxic Equivalency Factor (TEF) of a specific congener with either its mass or concentration (CCME, 2002) to get values typically reported in pgTEQ g⁻¹ for solid samples or pgTEQ L⁻¹ for liquids. When dealing with mixtures of dioxin congeners, TEQ values for each congener are summed up to get the total TEQ. Typically, the TEQ of a mixture is reported in order to avoid having to report values for

each individual congener concentration. This simplifies the reporting of dioxin concentrations and allows for a simpler comparison between different mixtures of dioxin congeners.

The toxicity of the dioxin congeners is thought to be triggered once the dioxin molecule binds to an intracellular protein known as the aryl hydrocarbon (Ah) receptor (Cuthill et al., 1988). The dioxin molecule binds stereospecifically to a ligand molecule on the Ah receptor and activates it. Once it is bound, the receptor-ligand complex interacts with dioxin-response elements (DRE) that transcribe various genes whose products are thought to bring about the toxicity within the organism (Whitlock, 1990). Depending on the organism and the extent of exposure to dioxin, many toxicological effects can occur.

1.2.2. Toxicological effects of dioxins and furans

Toxicological studies using 2,3,7,8-TCDD have been performed on many small mammals. Kransler et al., 2007 reports that guinea pigs and rhesus monkeys seem to be the most susceptible to dioxin poisoning whereas hamsters are far less harmed from dioxin exposure. The LD₅₀ for male guinea pigs has been estimated to be as little as 0.6 µgTEQ kg⁻¹ (Schwetz, et al., 1973) whereas the LD₅₀ for hamsters was estimated to be upwards of 5000 µgTEQ kg⁻¹ (Henck et al., 1981). Despite the large difference in LD₅₀ values, symptoms of acute toxicity tend to include a loss in fat, shrinkage of the spleen, changes in blood cell count and the thickening of the gastrointestinal tract (US EPA, 2003 draft). Symptoms of chronic exposure tend to include immunotoxic, reproductive and developmental effects. Dioxin exposure is also thought to cause cancers in small mammals but not directly. It does so by altering chemicals found in the body making them more damaging to DNA or by affecting cell growth pathways that in turn can

promote cancer-causing mechanisms (US EPA, 2003 draft). A list of some symptoms of chronic exposure reported for small mammals is given in Table 1.

Table 1. Reported symptoms of dioxin exposure in small mammals

Type of Effect	Symptom	Reference
Immunotoxic	Endocrine disruption	Birnbaum and Tuomisto, 2000
	Increased sensitivity to bacteria such as <i>Salmonella</i>	Thigpen et al., 1975
	Increased sensitivity to viruses such as influenza	Burleson et al., 1996
Reproductive	Reduced fertility, smaller litter sizes and changes to male reproductive organs	US EPA, 2003 draft
	Mutation of spermatozoa	McConnell and Moore, 1978
In vivo exposure	Decreased thymic weight, irregular T-cell counts, heightened susceptibility to autoimmune diseases.	Mustafa et al., 2008
Carcinogenic	Causes hepatocellular carcinomas and squamous cell carcinomas in the lungs	Kociba et al., 1978

Humans are also susceptible to the toxic effects of dioxins. However, it is difficult to determine the exact health effects on humans since incidents that expose humans to dioxins often expose them to other contaminants as well (Environment Canada, 1990). Incidents of dioxin exposure include the use of Agent Orange as a chemical defoliant in Vietnam, which contained 2,3,7,8-TCDD as a by-product (Schecter et al., 2006; Dow, no date), a 1976 explosion in Seveso, Italy where dioxins were released from a 2,4,5-trichlorophenol plant (Bertazzi et al., 1997) and a 1968 incident where furan had contaminated Yusho rice oil in Fukuoka, Japan (Schecter et al., 1994).

Incidents of human exposure to dioxins are not just relegated to events in the past. In recent years, it was discovered that residents of Midland, Michigan were being exposed to dioxin contaminated soil as a result of industrial activities found upstream of the Tittabawassee River. These activities have contaminated soils that are 10 to 20 times the levels found in nearby

unaffected areas (Hilscherova et al., 2003). An incident in 2008 involved the consumption of dioxin contaminated pig meat in Ireland. An estimated €125m of pig meat was destroyed after dioxin concentrations reaching 80 to 200 times safe levels were detected in routine sampling of pig feed being supplied to Irish farms (MacConnell and Healy, 2008).

Human exposure to dioxins can cause weakness, chloracne (a skin rash) and other types of lesions and cysts (Srogi, 2008; Pesatori et al., 2003; Environment Canada, 1990; WHO, 1989). Other effects specifically linked to furan exposure include neurological and psychological effects, birth defects, and foetal deaths (Environment Canada, 1990). The possibility of dioxin being a human carcinogen has been debated over the past 20 years and now the US Environmental Protection Agency has classified dioxins as “likely to be carcinogenic to humans” (The National Academies, 2006).

1.2.3. Releases and bioavailability of dioxins and furans

Dioxins can only cause the health effects discussed above if organisms are exposed to the toxin. Consequently, a link must be made that connects the emission of dioxin into the environment to a sink that makes dioxin bioavailable to organisms. Dioxins are predominantly emitted into the environment as a by-product of incomplete combustion and chlorination processes (Environment Canada, 1990). The National Pollution Release Inventory (NPRI) considers the smelting of aluminum and lead, the operation of electric arc furnaces, the combustion of fuel in the pulp and paper industry and the manufacturing of iron as some specific industries that produce dioxin as a by-product (NPRI, 2003). It also lists the combustion of non-hazardous solid waste, biomedical waste and municipal waste as sources. While many of these anthropogenic activities emit dioxin

into the atmosphere through air emissions, the main sinks for dioxins are soils and sediments (Environment Canada, 1990).

Dioxin enters the soil through aerial deposition where it can accumulate to levels that can affect the surrounding environment. Srogi, 2008 notes that the majority of emitted dioxin gets adsorbed onto dusts and soot where it then gets deposited into soils. Aerial deposition rates into soils around a Turkish industrial area with dioxin levels similar to many sites around the world were estimated to be between 7.6 and 66.3 ngTEQ m⁻² year⁻¹ (Bakoglu and Karademir, 2005). These values can be considered problematic since they are greater than the depositions of between 3.4 and 13.6 pgTEQ m⁻² day⁻¹ which are the values that are suggested to keep the tolerable daily intake (TDI) of dioxins below the 1-4 pgTEQ kg⁻¹ day⁻¹ level proposed by the WHO (De Fre et al., 2000).

Since the majority of dioxin pollution in Canada enters the environment by way of air emissions (CCME, 2002), not only must soil deposition occur to expose organisms to heightened levels of dioxins but the dioxins must persist in the soil in order to accumulate to levels that may be considered harmful. A study performed by Fattore et al., 2003 concludes that existing soil contamination from the Seveso incident of 26 years ago is not re-entering the atmosphere and is staying relatively stable in the soil. In Vietnam, levels of dioxin exceeding 1,000,000 ppt have been measured in soils three to four decades after Agent Orange was used (Schecter et al., 2006). A study by Hilscherova and Kannan, 2003 shows that dioxins from a contaminated water source can accumulate and persist in the surrounding soil. The accumulation and persistence of dioxins in soil can explain how some areas have elevated levels of soil contamination when there are no

releases to soils nearby. For instance, soils around the pulp and paper industry in British Columbia have reported dioxin concentrations of up to 255 ngTEQ kg⁻¹ (Environment Canada, 2005) when natural background levels should be only 4 ngTEQ kg⁻¹ (CCME, 2002).

While the accumulation and persistence in soil are responsible for elevating the levels of dioxin in the environment, its soil bioavailability makes it especially harmful. One study estimates that roughly 25% of the dioxin contamination in soil found in Midland Michigan can be directly absorbed by humans through oral ingestion (Ruby et al., 2002). A 2005 study by Watanabe et al. links the bioconcentration of dioxins in crows around a waste dumping site specifically to their ingestion of soil rather than their exposure to other sources of dioxin in the area. A food chain transfer study for dioxin originating from the spraying of Agent Orange in Vietnam has confirmed that bioaccumulation and bioconcentration of dioxin does occur, travelling from soils to pond sediments then into fish and ducks and finally into the local residents who consume these animals (Dwernychuk et al., 2002). Another bioaccumulation link between contaminated soils and humans was noted in residents residing near a contaminated dump site in India. Links were made between the accumulation of dioxin from cows that graze on dump wastes into mothers who ingest milk and meat from these cows; dioxin is then passed into their children through breastfeeding (Kunisue et al., 2004). These studies tend to confirm that while the majority of dioxin enters into the bottom of the food chain through the ingestion of contaminated soil by animals and small organisms, 98.8% of human exposure to dioxins occurs through ingestion of meats and dairy products (Travis and Hattemer-Frey, 1991).

1.3. International and Canadian Dioxin and Furan Initiatives

Since dioxin is known to be toxic, persistent and bioaccumulative, many dioxin initiatives have been developed on both the international and national level. On the international level, the World Health Organization (WHO) and the North Atlantic Treaty Organization (NATO) both have dioxin programs in place that act as reference points for dioxin programs found in countries around the world. On a national level, many countries have regulations that attempt to monitor, control and prevent the release of dioxin into the environment. This section will examine the WHO and NATO programs as well as the Canada-Wide Standards for Dioxins and Furans which is the leading Canadian dioxin reduction plan.

1.3.1. NATO dioxin initiatives

NATO's major contribution to the regulation of dioxins on an international scale was the introduction of a TEF scheme that unified several previously existing schemes that had slight differences (Kutz et al., 1990). The international toxicity equivalency factors (I-TEF) combined the better studied toxicity data of 2,3,7,8-TCDD with the very limited toxicity data on other 2,3,7,8-substituted dioxins and furans to develop individual I-TEF values. Specifically, long-term whole animal studies were used and when not available, short-term studies, subchronic effects data, acute toxicity studies, and *in vivo* and *in vitro* enzyme induction data were used (Kutz et al., 1990). The I-TEF values developed by NATO are given in the Table 2.

Table 2. NATO derived I-TEF values

Dioxin congener	I-TEF value	Furan congener	I-TEF value
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDD	0.5	2,3,4,7,8-PeCDF	0.5
		1,2,3,7,8-PeCDF	0.05
1,2,3,4,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDD	0.1	1,2,3,7,8,9-HxCDF	0.1
1,2,3,6,7,8-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1
		2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,4,6,7,8-HpCDF	0.01
		1,2,3,4,7,8,9-HpCDF	0.01
OCDD	0.001	OCDF	0.001

Source: Kutz et al., 1990

Interestingly, Kutz et al., 1990 states that the I-TEF scheme was meant only as an interim measure to be used until congener specific toxicities or toxicities of dioxin mixtures could be better characterized. The I-TEF values were to be periodically updated when the science behind dioxin toxicity improves. Despite these recommendations, the I-TEF values have never been updated and this scheme continues to be the method used by some agencies to report dioxin congener concentrations including the US EPA for calculating emission concentrations, the European Union Directive on Incineration and the Ontario Ministry of the Environment's *Guidance on Site Specific Risk Assessment for Use at Contaminated Sites in Ontario* (A.J. Chandler, 2006; OMOE, 1996). The Canada-Wide Standards for Dioxins and Furans also use the NATO scheme for at least its Waste Incineration guidelines (CCME, 2001).

The alternative to using the NATO I-TEF values is the use of TEF values developed by the WHO. Switching from NATO I-TEF to WHO TEF values has been shown to increase the TEQ of a sample by approximately 10% (Van Leeuwen, 2000; Sinclair Knight, 2001; US EPA, 2005b draft). This may be one reason why the WHO TEF values have not been adopted by initiatives

aimed at reducing dioxin levels; doing so will immediately make a source seem like a greater dioxin emitter even though the actual quality of its emissions has not changed. Despite the lack of use of the WHO scheme for dioxin reducing initiatives, the scheme is often used for assessing health risks. Environment Canada has officially adopted the WHO scheme for managing human health risks for dioxins in food, water, air and consumer products; Canadian soil quality guidelines also use the WHO scheme (CCME, 2002). The WHO scheme for the quantification of dioxin congeners remains one of the WHO's main international dioxin-based initiatives.

1.3.2. WHO dioxin initiatives

The first WHO TEF values introduced in 1994 were identical to I-TEF values but they also included TEF values for dioxin-like PCBs after a need was identified to characterize them (A.J. Chandler, 2006). They were re-evaluated twice – once in 1998 (WHO98 values) and again in 2005 (WHO05 values). The 1998 re-evaluation removed the di-*ortho* PCB TEF values, readjusted some mammalian TEF values and introduced TEF values specifically for birds and fish (Van den Berg et al., 2005). The 2005 re-evaluation altered some TEF values based on further scientific studies and switched the TEFs to a logarithmic scale (Van den Berg et al., 2005). WHO94, WHO98 and WHO05 TEF values are given in Table 3.

Table 3. WHO94, WHO98 and WHO05 TEF values

Congener	WHO94 TEF	WHO98 TEF	WHO05 TEF
Dioxins			
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	0.5	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01	0.01
OCDD	0.001	0.0001	0.0003
Furans			
2,3,7,8-TCDF	0.1	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01
OCDF	0.001	0.0001	0.0003
Dioxin-like PCBs			
3,3',4,4'-TeCB	0.0005	0.0001	0.0001
3,4,4',5-TCB		0.0001	0.0003
2,3,3',4,4'-PeCB	0.0001	0.0001	0.00003
2,3,4,4',5-PeCB	0.0005	0.0005	0.00003
2,3',4,4',5-PeCB	0.0001	0.0001	0.00003
2',3,4,4',5-PeCB	0.0001	0.0001	0.00003
3,3',4,4',5-PeCB	0.1	0.1	0.1
2,3,3',4,4',5-HxCB	0.0005	0.0005	0.00003
2,3,3',4,4',5'-HxCB	0.0005	0.0005	0.00003
2,3',4,4',5,5'-HxCB	0.00001	0.00001	0.00003
3,3',4,4',5,5'-HxCB	0.01	0.01	0.03
2,2',3,3',4,4',5-HpCB	0.0001		
2,2',3,4,4',5,5'-HpCB	0.00001		
2,3,3',4,4',5,5'-HpCB	0.0001	0.0001	0.00003

Source: A.J. Chandler, 2006; Van den Berg et al., 2005

The development of these new TEF values was done through the International Program on Chemical Safety (IPCS). In addition to the IPCS, the WHO has other international dioxin programs. Several are listed on their website (WHO, 2007). These include a comprehensive risk

assessment on PCDD, PCDF and dioxin-like PCBs completed in 2001 (JECFA, 2001), the establishment of the *Code of Practice for the Prevention and Reduction of Dioxin and Dioxin-like PCB Contamination in Foods and Feed* (FAO/WHO, 2006) and an estimate of the tolerable daily intake (TDI) for humans (WHO, 1998). The WHO has also collaborated with the United Nations Environmental Program (UNEP) on implementation of the Stockholm Convention, which is dedicated to reducing emissions of persistent organic pollutants and completing estimates of dioxin releases from every region across the globe (UNEP, 2003). The global report is based on emission estimates and environmental guidelines of each specific country. Since many countries lack the technology and resources needed to estimate and regulate dioxin emissions, their emissions are not suitably quantified in the UNEP report. For other countries, including Canada, dioxin emissions are well documented. Canadian estimates are made through annual NPRI reporting and the Canada-Wide Standards for Dioxins and Furans (UNEP, 2002).

1.3.3. The Canada-Wide Standards for Dioxins and Furans

1.3.3.1. Scope and development

The development of the CWS for dioxins and furans stem from the findings of the 1990 Priority Substances List (PSL1) – a list made collaboratively by Environment Canada and Health Canada (Environment Canada, 1990). The PSL1 report on dioxins presents findings on the properties of dioxins, their sources, environmental fate, toxicological effects, ecosystem impact and environmental and human health risks. Their findings emphasize four crucial properties of dioxins; they are persistent in the environment, they bioaccumulate in the food chain, they are largely anthropogenic and they are toxic as defined by the *Canadian Environmental Protection Act* (CEPA, 1999). These four characteristics of dioxins make them eligible for the *Toxic*

Substances Management Policy (Environment Canada, 1995), which is a policy instrument dedicated to following the principles and goals of CEPA.

The *Toxic Substances Management Policy* (TSMP) has designated dioxins as Track 1 Substances, which means that they should be ‘virtually eliminated’ in Canada. The term ‘Virtual Elimination’ is used by CEPA to signify that the use and emission of a pollutant should be reduced down to the lowest level of quantitation (LLOQ) – the lowest level measurable by routine analysis. The LLOQ for dioxins found in air is 32 pgTEQ m⁻³ and in soil, it is 9 pgTEQ g⁻¹ (CCME, 2004). Methods on how to reach a virtual elimination of dioxin emissions is quantified by the 2001 *Canadian Wide Standards (CWS) for Dioxins and Furans* (CCME, 2001). The CWS has set reduction goals for certain targeted dioxin sources in the hopes that these targets will be met within the given timeframe and be made more stringent until Virtual Elimination is achieved. These sources are waste incineration (municipal solid waste, hazardous waste, sewage sludge and medical waste); burning salt laden wood in coastal pulp and paper boilers in British Columbia; residential wood combustion; iron sintering; electric arc furnace steel manufacturing; and conical municipal waste combustion in Newfoundland (CCME, 2001). Initial estimates predicted that eliminating dioxin releases from these sources would reduce dioxin emissions by 80% (CCME, 2001). This would mark substantial progress towards achieving Virtual Elimination.

Achieving a virtual elimination of dioxin emissions is not just important in terms of following the precautionary principles outlined in CEPA but it is also important in order to meet the recommendations made in the Canadian soil quality guidelines for dioxins (CCME, 2002).

These guidelines state that since the most sensitive receptors (toddlers and infants) currently have an estimated daily intake of dioxin (EDI) that exceeds their tolerable daily intake (TDI), it is recommended that dioxin levels be reduced down to 4 pgTEQ g^{-1} – the natural Canadian dioxin background level. In other words, it is necessary to virtually eliminate dioxins from the environment. While the CWS have made progress in reaching Virtual Elimination, several deficiencies will prevent them from fully eliminating dioxin releases.

1.3.3.2. Deficiencies in the CWS and a solution

Two major deficiencies that exist within the CWS prevent them from reaching Virtual Elimination; they do not address the 80% of Canadian dioxin emissions as initially claimed and the sources that are addressed have stalled in their attempts to reach Virtual Elimination. While the six targeted dioxin sources were initially estimated to release 80% of all Canadian dioxin emissions, in reality they only account for approximately 56% of 1999 atmospheric releases (CCME, 2004). The discrepancy is due to the quantification of additional sources of dioxin emissions. Of the remaining 44%, the 2004 CCME report predicts that 15% are currently controlled through other initiatives and the remainder, which is released through combustion of fuel and the burning of residential and municipal waste, goes completely unregulated. What is perhaps more troubling is the lack of testing and research for many sectors that have the potential of being significant dioxin emitters. These potential sources include but are not limited to asphalt plants, magnesium production, petroleum refineries, crematoria and secondary copper smelting (CCME, 2004). The 2004 report by the CCME recommends that:

Industry, governments and other stakeholders should undertake cooperative scientific research programs to characterize smelter releases, validate predictive models, investigate

environmental behaviours and effects, and identify and develop pollution prevention opportunities and technologies.

The creation of scientific research programs and the development of pollution prevention technologies will not only help identify other dioxin sources but will also help the sources currently targeted by the CWS reach their goal of Virtual Elimination.

The interim targets and the progresses of the sources currently targeted by the CWS are summarized in Table 4.

Table 4. Interim progress for the six industries targeted by the CWS

Dioxin Source	Interim Targets	Targets met?	Comments	Reference
Electric Arc Furnaces	By 2006: <150 pgTEQ m ⁻³ By 2010: <100 pgTEQ m ⁻³	Yes, as well as 2010 targets	This source has been very successful in eliminating its dioxin emissions. Some sources have even achieved emissions below the LLOQ. Facilities that have not reached the LLOQ now have information on how to do so.	William Lemmon and Associates, 2004a
Iron Sintering Plants	By 2005: <500 pgTEQ m ⁻³ By 2010: <200 pgTEQ m ⁻³	2005 target was met	The final remaining iron sintering plant in Canada (Stelco Hamilton) reached its 2005 interim goal and has since been shut down thus removing its contribution to the release of dioxin emissions.	William Lemmon and Associates, 2004b; McCarthy, personal communication
Conical Waste Combustion	By 2005: Close 40% of facilities. By 2008: Close all facilities.	2005 target has been met	As of 2005, 24 of 58 burners have been shut down, reducing dioxin emissions by 52%. No information was found on closure of the remaining facilities.	CCME, 2005
Waste Incineration	All incinerators: <80 pgTEQ m ⁻³	Most facilities have met this target	An 85% reduction in emissions has been reported but this is based on largely estimated amounts of combusted waste. Additionally, there is a lack of testing in older medical incinerators and little data on the dioxin content of combustion residue such as fly ash.	CCME, 2007
Coastal Pulp and Paper Boilers	New boilers <100 pgTEQ m ⁻³ Existing Boilers: <500pgTEQm ⁻³	Not consistently	Facilities are struggling to consistently meet the targets that are 3 and 15 times the LLOQ. Research on new pollution prevention technology is lacking. This industry will not reach Virtual Elimination in the near future.	CCME, 2006
Residential/ Municipal Waste Combustion	This source has been addressed by the CWS but no targets have been set.	N/A	This is a non-point source that is estimated to release 15% of Canada's dioxin releases. Educational programs have been suggested to attempt to curb residential waste burning.	CCME, 2004

The majority of the six targeted dioxin sources have been at least partially successful in reaching the interim goals set up by the CWS. However, with the exception of Electric Arc Furnaces, which have been very successful in eliminating dioxin releases and Conical Waste Combustors, which will reduce dioxins through a phase-out, all other existing sources would benefit from additional source testing to better quantify dioxin releases. They would also benefit from an increase in the validation of new pollution prevention technologies. New pollution prevention technologies would be especially useful because they can not only allow sources to meet interim targets but can contribute to the advancement of interim targets towards the eventual goal of Virtual Elimination.

The major obstacle in increasing source testing and validating new pollution prevention technologies is the limited capacity of dioxin testing facilities. For instance, an external review of the successes of the CWS for Canadian incinerators has suggested that many small incinerators in fact meet the specifications that require them to be tested for dioxin emissions but a lack of testing capacity and the high cost of doing so makes it unlikely to ever happen (A.J. Chandler, 2006). It is also reasonable to conclude that if this is the case, then the extensive testing that would be required to validate new pollution prevention technologies would also be difficult to complete.

Currently, dioxin testing is done through use of gas chromatography-high resolution mass spectroscopy (GC-HRMS) as it is highly accurate and can provide concentrations for each of the 17 most toxic dioxin and furan congeners in only one analysis. The basic concept of GC-HRMS is described in Reiner et al., 2006. Dioxin and other organics are extracted three times from the

sample matrix. The extract is then run through a series of cleanup columns, which can include silica, alumina and reversed-phased HPLC; each targets specific contaminants for removal (Chen, 2008). Using gas chromatographic columns, the dioxin congeners are separated from the remaining co-extractants and are eluted sequentially starting with the tetrachlorinated followed by the penta-, hexa-, hepta- and octachlorinated congeners (Reiner et al., 2006). The elutes are then fed through a mass spectrometer that records the molecular fragments of the congeners. The mass spectrometer gives output on quantities of all dioxin and furan congeners within the sample and using mathematical software, the amounts of the 17 most toxic dioxin and furan congeners can be inferred.

The problem with using GC-HRMS is that it is cost-prohibitive, very time-consuming and requires highly trained personnel. If GC-HRMS is to be used for increased source testing and more technology validation, some of the testing currently being handled by GC-HRMS should be offset to newer technologies that prove to be either less expensive, faster, or both. One new technology that has been shown to have distinct advantages over GC-HRMS in these respects is an enzyme-linked immunosorbent assay (ELISA).

1.4. ELISA

1.4.1. Comparisons to GC-HRMS

ELISA is a technology that has numerous uses including testing for HIV, pregnancy, anabolic steroids, illicit drugs and other toxins in food, blood and tissue (Kimball, 2008). The use of ELISA for dioxin screening is relatively new and ELISA would have both advantages and disadvantages in comparison to GC-HRMS. The use of ELISA is advantageous since it has the

capability of being a faster and less expensive alternative to GC-HRMS. The US Environmental Protection Agency (US EPA), under its Superfund Innovative Technology Evaluation Program, has examined the use of an ELISA produced by Cape Technologies (cape-tech.com) to detect dioxins in soils and sediments. By testing the ability of ELISA to screen for dioxins in 209 soil and sediment samples, the US EPA concluded that Cape Technologies ELISA kits would have the time and cost advantages over US EPA Methods 1613B and 1668A (which use GC-HRMS) shown in Table 5.

Table 5. Comparisons for 209 soil samples analyzed by GC-HRMS and ELISA

	GC-HRMS	ELISA	Savings
Analysis Time	Eight Months	Three Weeks	91% reduction
Total Cost	\$398,029	\$59,234	85% reduction

Source: US EPA, 2005a

The cost and time saving advantages of ELISA shown here may be slightly exaggerated (as demonstrated in Section 2.2 of this thesis) but the fact remains that the technology is much less expensive and faster than GC-HRMS analysis. ELISA is also much simpler to operate and does not need PhD level personnel. However, due to the nature of the technology, an ELISA analysis is capable of only reporting the total TEQ of the sample whereas a GC-HRMS analysis can report the concentrations of the 17 most toxic dioxin and furan congeners. With these concentrations, the total TEQ of the sample is calculated.

The US EPA report examined other variables including accuracy, precision, comparability, detection limits, false positive (FP) and false negative (FN) rates and sample throughput in order to compare the performance of both analytical techniques. ELISA results for performance evaluation samples (n=58), which have known quantities of dioxin, and for environmental

samples collected from 10 superfund sites (n=128) were compared in the US EPA study to GC-HRMS results for the same samples. A series of sample extracts (n=23) were also compared through both techniques to remove any effects that the soil matrix may have had on results. Table 6 on the following page presents the ELISA performance results obtained by the US EPA for these 209 samples, the ELISA product specifications reported by Cape Technologies and what the US EPA states the ideal results should be. False positive values are presented as the percentage of samples within range of a threshold value that ELISA detects as being above the threshold but GC-HRMS detects as being below. False negative values are presented as the percentage of samples within range of a threshold value that ELISA detects as being below the threshold but GC-HRMS detects as being above.

Table 6. Comparisons of US EPA and Cape Tech ELISA performance

Testing Criteria	Ideal Value	US EPA, 2005a Report Findings	Cape-Tech Specifications
Accuracy – presented as percent recovery of the GC-HRMS determined dioxin concentration	100%	236% mean 182% median 30% minimum 595% maximum (n=58)	95% (n=23) ¹
Precision – presented as %CV ²	<20%	71% mean 63% median 0% minimum 187% maximum (n=4, 7 or 8)	13±14% (n=23) ¹
Comparability – presented by dividing the difference between ELISA and GC-HRMS results by the average of these two numbers and multiplying by 100.	>-25% and <25%	-26% median -199% minimum 198% maximum (n=209)	24% median 47% max ¹ (n=23)
Detection limit	As low as possible	Between 12 and 35 pgTEQ g ⁻¹ (n=81)	4 pgTEQ g ⁻¹ ³
False positive (FP) and False negative results (FN)	0%	20 pgTEQ g ⁻¹ : FP 11%, FN 6% 50 pgTEQ g ⁻¹ : FP, FN 4-10% (n=209) ⁴	500pgTEQg ⁻¹ : FP 9% FN 1% (n=74) ^{5, 6}
Sample throughput		19 samples per day	10 samples per day ⁷

1 – Cape-Tech, 2003a.

2 – The %CV, or interbatch imprecision for duplicates of one sample is calculated as follows

$$(\text{Zady, 1999}): \%CV = \frac{\text{Standard Deviation}}{\text{Geometric Mean}} \times 100$$

3 – Cape-Tech, no date.

4 – False positive/negative rates were determined around thresholds of 20 and 50 pgTEQ g⁻¹.

5 – These results were a combination of two different runs (n=56 and n=18).

6 – Cape-Tech, 2002.

7 – US EPA, 2005a.

The findings of the US EPA Superfund Innovative Technology Evaluation report conclude that while there are both time and cost advantages in using ELISA, the technique does have its drawbacks. The inability of the US EPA to reach the ideal values listed in Table 6 indicates that

it is less accurate and less sensitive than its GC-HRMS counterpart. However, the accuracy of ELISA demonstrated by both Cape Technologies and the US EPA could potentially be improved by using a correction factor that is discussed in Section 2.1 of this thesis. The discrepancy between the ELISA results obtained by the US EPA and the Cape Technologies product specifications highlights the fact that running a successful ELISA is dependant on many variables; changes in factors such as the technique of the operator and the conditions of the laboratory set-up can have large effects on the results obtained. Finally, it should be emphasized again that ELISA is only capable of reporting the total TEQ of a sample whereas GC-HRMS is capable of reporting congener specific concentrations.

It is clear from characteristics of ELISA that it cannot serve as a complete replacement to GC-HRMS. This is especially true for instances such as the routine testing of stack emissions, where ELISA would lack the necessary sensitivity or in the validation of new dioxin prevention technologies, where ELISA would not have adequate accuracy. However, ELISA can excel in one very important area; it can act as an initial screening tool that can offset much of the burden on GC-HRMS. In fact, the 2005a US EPA report notes that ELISA has the potential of screening for dioxins at 20 pgTEQ g^{-1} and 50 pgTEQ g^{-1} – levels that are in range for the testing of dioxins in soil. ELISA can therefore complement GC-HRMS as a site assessment tool. The efficiency of ELISA analysis can be exploited to quickly and extensively map a site in terms of what areas are clean and what areas are most contaminated. Clean-up efforts can be better focused, the remediation of the site can be hastened and GC-HRMS can be made more available for high priority soil sampling, more routine dioxin source testing and the validation of new dioxin reducing technologies.

1.4.2. Description of ELISA technology

The principles behind ELISA are described in an ELISA kit insert provided by Cape Technologies (Cape-Tech, 2003b). ELISA works by binding dioxins dissolved in methanol to anti-dioxin antibodies that are coated on an immunoassay tube. The anti-dioxin antibodies specifically target 2,3,7,8-TCDD and they do so by shape recognition. The other congeners, which all have a similar shape to 2,3,7,8-TCDD are also targeted by the antibodies but to differing degrees. The degree of reaction between a particular congener and the anti-dioxin antibodies is known as its cross reactivity. The concept of cross reactivity is discussed in further detail in Section 2.1. Once the dioxins are bound to the coated surface of the immunoassay tube, the unbound material is washed away and a competitor-HorseRadish Peroxidase (HRP) conjugate is added to the tube. The competitor is a dioxin molecule chemically conjugated to the HRP enzyme. The competitor occupies the remaining antibody sites that are not taken up by dioxin molecules. Hence, the amount of bound competitor-HRP is inversely proportional to the amount of bound dioxin. The tube is allowed to incubate after which the unbound excess competitor is washed away. A solution containing a peroxide and a chromogen is then added. The peroxidase enzyme cleaves the peroxide substrate to liberate oxygen radical which oxidizes the colourless chromogen to its coloured form. The colour intensity is dependant on the amount of bound competitor-HRP and therefore is inversely proportional to the amount of bound dioxin. Finally, a stop solution is added to halt and stabilize the colour change. The colour intensity, or optical density (OD), is measured spectrophotometrically as absorbance. The ELISA process is depicted in Figure 2.

Figure 2. Principles of the ELISA technology
Source: Cape-tech.com

Once the OD reading for each sample is measured from the spectrophotometer, they are converted into a TEQ value. The conversion requires the development of a relationship curve

between OD readings and TEQ values. This curve is made by fitting a series of standard and negative control (NC) OD readings to a four-parameter equation using Microsoft Excel's solver function in a spreadsheet provided by Cape Technologies (Cape-Tech, 2004). The four parameter equation is as follows:

$$Y = \frac{A - D}{1 + (X/C)^B} + D \quad (\text{Eq. 1})$$

Where

- X = pg per ELISA tube (2378-TCDD for standards, TEQ for samples)
- Y = normalized ELISA response (OD as % of NC OD)
- A = Y value of upper asymptote
- B = degree of curvature (relative slope of middle region)
- C = X value at 50% point of curve
- D = Y value of lower asymptote

Values for A, B, C and D are found automatically through the Microsoft Excel solver function in the spreadsheet provided by Cape Technologies.

1.4.3. Current state of ELISA technology

As mentioned in Section 1.4.1, the US EPA has shown that ELISA has the potential of screening for dioxins in soil. In fact, these findings have translated into the US EPA Method 4025 – an officially approved method that uses Cape Technology ELISA kits to screen for dioxins at 500 pgTEQ g⁻¹ (US EPA, 2002). Generally speaking, validation of this method in Canada would be required before ELISA is widely accepted by Canadian institutions. The validation would involve the formal licensing and accreditation of ELISA in established laboratories. This thesis takes the first steps towards validation. Other academic papers have also tested the use of ELISA to screen for dioxins in soil. Nording et al., 2007; Nording et al., 2006; Nickkova, 2004 and Shan et al., 2001 all have studied this use of ELISA; however, their sample sizes were small

($n=15$, $n=10$, $n=12$ and $n=6$, respectively) and their findings were very preliminary. Van Emon et al., 2008 offers a more complete study of ELISA as a screen for dioxins in soil. This paper analyzed in triplicate 75 soil samples from a Superfund site in Rhode Island, USA by both ELISA and GC-HRMS. The comparison of both techniques gave very favourable results; the standard deviation of the triplicate ELISA results for each sample was $\pm 20\%$, ELISA results were on average only 12% higher than those of GC-HRMS, FP and FN rates were 6.7% and 8.0% respectively at $1,000 \text{ pgTEQ g}^{-1}$ and the correlation between both methods was strong ($r = 0.79$). Their study concluded that ELISA could be used as a complementary screening process at $1,000 \text{ pgTEQ g}^{-1}$.

While these studies confirm that ELISA can be used as a screening tool, several aspects of the ELISA method can still be improved. The van Emon et al., 2008 study, the Nording et al., 2007 study and the Nording et al., 2006 study all acknowledge that differences in how ELISA and GC-HRMS calculate TEQ mean that both methods produce results that are only approximately equal to the other. The van Emon et al., 2008 study emphasizes this fact by reporting ELISA TEQ values in terms of an 'ELISA-EQ'. Furthermore, all aforementioned studies have depended on prior analysis by GC-HRMS to provide the samples' dioxin concentrations and congener profiles; no studies were found that discussed the use of ELISA to analyze soil samples that have not been previously analyzed by GC-HRMS. This is an issue because dioxin levels in soil can range anywhere from the Canadian background level of 4 pgTEQ g^{-1} (CCME, 2002) to over $10,000 \text{ pgTEQ g}^{-1}$ – the maximum dioxin concentration level that can be reached in Ontario before remediation is required (OMOE, 1996). No literature was found that discussed how to

overcome the fact that one ELISA test would be inadequate to determine a dioxin concentration that could fall anywhere across this range.

The following Method Development Section will introduce and develop two new concepts that overcome these problems. A correction factor that accounts for the differences in how ELISA and GC-HRMS convert values to TEQ and a method of determining how the correction factor can be calculated for a site are discussed in Section 2.1. Section 2.2 describes a sample algorithm that details how to efficiently introduce a sufficient amount of dioxin into an ELISA tube from a sample with an unknown dioxin concentration. Section 2.3 addresses the need to account for background dioxin concentration when analysing and reporting ELISA results. These improvements to the ELISA procedure will be tested in Section 3 by using ELISA to analyze soil and sediment samples from a dioxin contaminated site and comparing those results to GC-HRMS. If these new concepts are found to be sound then they will contribute to the advancement of ELISA technology and allow it to replace GC-HRMS for the routine screening of soil and sediment samples.

It should be noted that Nording et al., 2007 also offers a method to correct for the differences in how ELISA and GC-HRMS convert values to TEQ. However, their method is simply to multiply the correlation between ELISA and GC-HRMS for a group of samples from the same site by the inverse slope to get a correlation for that dataset that is closer to 1. This is not an ideal correction method since it is entirely empirical and depends on having a strong enough dataset to generate a reasonable slope. It cannot be used for individual samples or small datasets.

The correction factor introduced in Section 2.1 is based more on theory and can be applied even to individual samples.

One of the most common methods for determining the concentration of a substance in a sample is by comparing the intensity of a characteristic spectral line to that of a standard. This method is based on the assumption that the intensity of the spectral line is proportional to the concentration of the substance. However, this assumption is only valid for a limited range of concentrations. At higher concentrations, the intensity of the spectral line increases more slowly than at lower concentrations, leading to a non-linear relationship between intensity and concentration. This non-linearity can be corrected by using a correction factor, which is a function of the concentration. The correction factor is determined by comparing the intensity of the spectral line to that of a standard at a known concentration. The correction factor is then used to adjust the intensity of the spectral line in the sample, so that it can be compared to the intensity of the standard at the same concentration. This method is known as the standard addition method, and it is a widely used technique for determining the concentration of a substance in a sample.

Concentration (mg/L)	Intensity (a.u.)	Corrected Intensity (a.u.)
0.0	0.0	0.0
0.5	1.2	1.2
1.0	2.4	2.4
1.5	3.6	3.6
2.0	4.8	4.8
2.5	5.8	5.8
3.0	6.8	6.8
3.5	7.8	7.8
4.0	8.8	8.8
4.5	9.8	9.8
5.0	10.8	10.8
5.5	11.8	11.8
6.0	12.8	12.8
6.5	13.8	13.8
7.0	14.8	14.8
7.5	15.8	15.8
8.0	16.8	16.8
8.5	17.8	17.8
9.0	18.8	18.8
9.5	19.8	19.8
10.0	20.8	20.8

2. METHOD DEVELOPMENT

2.1. The Congener Correction Factor

ELISA works by capturing analytes using antibodies, which are already bound to the surface of an ELISA tube. For this particular ELISA, the target analyte is 2,3,7,8-TCDD. Molecules with a similar shape (the other dioxin and furan congeners) will also bind to the antibodies but to differing degrees. The degree of binding for a particular congener is known as its cross reactivity. The percent cross reactivity values for various dioxin and furan congeners (and some PCBs) for the ELISA developed by Cape Technologies are provided in Table 7. Cross reactivity values for these congeners and PCBs were determined experimentally by Cape Technologies (Cape-Tech, 2003b) by dividing the result of an ELISA analysis of a pure sample of 2,3,7,8-TCDD by the result of an ELISA analysis of a pure sample of each congener at the same concentration. This was done at four different concentrations across two to four independent trials.

Table 7. Cross reactivity values for 27 dioxin and furan congeners and 5 dioxin-like PCBs

<u>Compound</u>	<u>Percent Crossreactivity</u>	<u>Compound</u>	<u>Percent Crossreactivity</u>
<u>Toxic Dioxin Congeners</u>		<u>Other PCDD/F Congeners</u>	
2,3,7,8-TCDD	100	2,3-dichlorodibenzo- <i>p</i> -dioxin	0.13
1,2,3,7,8-PeCDD	105	2,7-dichlorodibenzo- <i>p</i> -dioxin	0.003
1,2,3,4,7,8-HxCDD	1.6	2,3-dichlorodibenzofuran	0.02
1,2,3,6,7,8-HxCDD	7.9	2,7-dichlorodibenzofuran	<0.002
1,2,3,7,8,9-HxCDD	39	2,3,7-trichlorodibenzo- <i>p</i> -dioxin	24
1,2,3,4,6,7,8-HpCDD	0.7	2,3,8-trichlorodibenzofuran	0.26
OCDD	<0.001	1,2,3,4-TCDD	<0.001
		1,2,3,4-TCDF	<0.001
		1,3,6,8-TCDD	0.05
		1,3,6,8-TCDF	0.007
<u>Toxic Furan Congeners</u>		<u>PolyChlorinated Biphenyls</u>	
2,3,7,8-TCDF	20	3,3',4,4' (PCB 77)	0.4
1,2,3,7,8-PeCDF	4.6	3,3',4,4',5 (PCB 126)	0.5
2,3,4,7,8-PeCDF	17	2,2',4,4',5 (PCB 153)	<0.1
1,2,3,4,7,8-HxCDF	0.4	3,3',4,4',5,5' (PCB 169)	<0.1
1,2,3,6,7,8-HxCDF	1.0	Aroclor 1254	<0.1
1,2,3,7,8,9-HxCDF	3.3		
2,3,4,6,7,8-HxCDF	4.9		
1,2,3,4,6,7,8-HpCDF	0.02		
1,2,3,4,7,8,9-HpCDF	0.9		
OCDF	<0.001		

Source: Cape-Tech, 2003b

Since GC-HRMS dioxin results are always reported in TEQ, ELISA results are also reported in equivalent amounts of 2,3,7,8-TCDD and cross reactivity values are set relative to the cross reactivity of 2,3,7,8-TCDD. One of the fundamental differences between GC-HRMS and ELISA is that GC-HRMS results report congener concentrations which must be each multiplied by the corresponding TEF value to get TEQ whereas ELISA results (which are reported as 2,3,7,8-TCDD equivalents) already account for the cross reactivity of the congeners; the congener concentrations themselves cannot be determined.

The polyclonal antibody used for the Cape Technologies ELISA was selected since it had congener cross reactivity values that approximate their respective TEF values. Therefore, cross reactivity values roughly represent the relative toxicity of each congener. Since cross reactivity values are only roughly equal to both NATO and WHO derived TEF values, GC-HRMS TEQ and ELISA results are not entirely comparable. For some congeners, the difference between cross reactivity and TEF is negligible but for others, the difference is distinct. For instance, 2,3,4,7,8-pentachloro dibenzofuran (PeCDF) has a cross reactivity of 0.17 but its NATO derived TEF is 0.5. If a sample of pure 2,3,4,7,8-PeCDF was analyzed by both ELISA and GC-HRMS, the ELISA result would underestimate the GC-HRMS TEQ by 66%. The congener correction factor proposed below will correct for this discrepancy.

The correction factor will allow for a direct comparison between ELISA results and GC-HRMS TEQ. Since GC-HRMS and ELISA use different methods to convert dioxin and furan congeners into equivalent amounts of 2,3,7,8-TCDD, only the concentrations of the congeners themselves

are directly comparable. Assuming a sample is composed of pure 'Congener A', its concentration can be determined from an ELISA result as follows:

$$[\text{Congener A}] = \frac{\text{ELISA}_{\text{TCDD Equivalents}}}{\text{Cross reactivity}_A} \quad (\text{Eq. 2a})$$

With GC-HRMS, the concentration of Congener A is measured directly and can be equated to the TEQ of the sample as follows:

$$[\text{Congener A}] = \frac{\text{GC - HRMS}_{\text{TEQ}}}{\text{TEF}_A} \quad (\text{Eq. 2b})$$

Setting Eq. 2a equal to Eq. 2b,

$$\frac{\text{GC - HRMS}_{\text{TEQ}}}{\text{TEF}_A} = \frac{\text{ELISA}_{\text{TCDD Equivalents}}}{\text{cross reactivity}_A} \quad (\text{Eq. 2c})$$

$$\text{GC - HRMS}_{\text{TEQ}} = \frac{\text{ELISA}_{\text{TCDD Equivalents}}}{\text{cross reactivity}_A} \times \text{TEF}_A$$

An ELISA result for a sample composed of a single congener is thus corrected by multiplying the uncorrected ELISA result by the congener's TEF and dividing by its cross reactivity. This corrected ELISA result is now directly comparable to GC-HRMS TEQ.

$$\text{ELISA}_{\text{Congener Corrected}} = \text{ELISA}_{\text{Uncorrected}} \times \frac{\text{TEF}}{\text{cross reactivity}} \quad (\text{Eq. 2d})$$

The term 'correction factor' will herein refer to $\text{TEF} \div (\text{cross reactivity})$. NATO TEF values for each congener are given in Table 2, WHO TEF are given in Table 3 and cross reactivity values

are given in Table 7. The congener corrected ELISA will not only be directly comparable to GC-HRMS TEQ results but it will also be more comparable to all dioxin environmental regulations since regulations use TEF values to convert dioxin concentrations into TEQ.

On a side-note, this congener correction factor shows promise for other similar ELISA applications. For instance, Abraxis LLC (abraxiskits.com) produces an ELISA kit that semi-quantitatively screens for PCBs in soil, sediment, fish tissue and other matrices. Abraxis LLC also identifies that their kit only approximates the GC-MS derived PCB TEQ of a sample due to differences between cross reactivity and PCB TEF values (US EPA, 2005c). The concept of the correction factor can be equally applied to solve the problem of discrepancy between PCB ELISA and GC-MS.

Applying the correction factor to real samples, such as soils and sediments, poses a challenge since they are composed of numerous congeners, each with their own cross reactivity and TEF value. This problem has been resolved by introducing the concept of 'composite cross reactivity' to complement the existing concept of 'composite TEF'. Composite cross reactivity and TEF values can be calculated by multiplying the individual cross reactivity and TEF values for each congener in a sample by its percent composition and summing the results. By using the resulting composite TEF and composite cross reactivity values, the correction factor in Equation 2d can be used for samples with congener mixtures. The calculations (shown in Table 8) of composite cross reactivity values, composite TEF values, and the resulting correction factor require only the percent composition of the sample in question. The sample calculations in Table 8 are for one of twenty-seven soil samples provided by the MOE that are used in Section 3 of

this thesis. The method shown in Table 8 is the one used to calculate all correction factors found in this thesis. For example, it has been used to generate the correction factors for the 30 emission sources found in Tables 10 and 11.

Table 8. Calculating composite correction factors and composite TEF values

Congener	Percent Composition	WHO 1998 TEF	ELISA cross reactivity	Contribution toward composite TEF	Contribution toward composite cross reactivity
1234678-heptachlorodioxin	8.53%	0.01	0.007	8.53E-04	5.97E-04
1234678-heptachlorofuran	1.07%	0.01	0.0002	1.07E-04	2.15E-06
1234789-heptachlorofuran	0.17%	0.01	0.0002	1.65E-05	3.30E-07
123478-hexachlorodioxin	0.44%	0.1	0.016	4.40E-04	7.05E-05
123478-hexachlorofuran	0.61%	0.1	0.004	6.06E-04	2.42E-05
123678-hexachlorodioxin	1.65%	0.1	0.079	1.65E-03	1.30E-03
123678-hexachlorofuran	0.30%	0.1	0.01	3.03E-04	3.03E-05
123789-hexachlorodioxin	0.85%	0.1	0.39	8.53E-04	3.33E-03
123789-hexachlorofuran	0.02%	0.1	0.033	2.26E-05	7.45E-06
12378-pentachlorodioxin	0.66%	1	1.05	6.61E-03	6.94E-03
12378-pentachlorofuran	0.08%	0.05	0.046	4.13E-05	3.80E-05
234678-hexachlorofuran	0.36%	0.1	0.049	3.58E-04	1.75E-04
23478-pentachlorofuran	0.27%	0.5	0.17	1.36E-03	4.63E-04
2378-tetrachlorodioxin	0.09%	1	1	8.53E-04	8.53E-04
2378-tetrachlorofuran	0.19%	0.1	0.2	1.87E-04	3.74E-04
Octachlorodioxin	82.59%	0.0001	0.00001	8.26E-05	8.26E-06
Octachlorofuran	2.12%	0.0001	0.00001	2.12E-06	2.12E-07
Composite TEF				0.0143	-
Composite cross reactivity				-	0.0142
Correction Factor				1.01	

In the example shown in Table 8, the correction factor is quite close to unity. What this implies is that when all congeners are considered simultaneously, discrepancies between TEF and cross reactivity values (such as the one mentioned previously for 2,3,4,7,8-PeCDF) may be effectively evened out or found inconsequential. ELISA results would not have to be corrected and could be compared directly to GC-HRMS TEQ. However, the question remains as to whether or not

some congener profiles will produce correction factors that differ significantly from unity and thus would require their usage when comparing ELISA results to GC-HRMS. To answer this question, congener profiles of several different dioxin sources were examined.

2.1.1. *Source specific congener profiles*

Average congener profiles of several dioxin sources were obtained through the *Database of Sources of Environmental Releases of Dioxin-like Compounds in the United States* (NCEA, 2001). Database information for each source type was collected from various facilities across the United States as of 1995 – the latest year that could be consistently evaluated for the given facilities. This data, which is composed of emission testing results on the quantity of the 17 toxic dioxin and furan congeners that have measured TEF values, were used to develop source specific emission factors and thus estimates on the quantity of dioxin emitted from each source. While the database states that the quantity of emissions has been significantly reduced since 1995, the information found in the database will only be used to obtain the congener profile of each source (i.e. the congener percent composition of the dioxin being released) and not the quantity of dioxin released. Table 9 lists the sources that were included in the NCEA database.

Table 9. Sources included in the NCEA, 2001 database

Industry	Source
Metal Smelting and Refining	Ferrous Foundry
	Primary Copper Smelting
	Primary Ferrous Metal Smelting
	Secondary Aluminum Smelters
	Secondary Copper Refinery
	Secondary Lead Smelters
Other High Temperature Sources	Cement Kiln Burning Hazardous Waste with the Inlet Temperature of the Air Pollutant Control Device greater than 450F
	Cement Kiln Burning Hazardous Waste with the Inlet Temperature of the Air Pollutant Control Device less than 450F
	Cement Kilns not Burning Hazardous Waste
	Kraft Black Liquor Recovery Boilers
	Lightweight Aggregate Kilns
	Petroleum Refining Catalyst Regeneration
Power Energy Generation	Coal Fired Plants
	Diesel Fuelled Trucks
	Industrial Wood Combustion
	Leaded Gas-Fuelled Vehicles
	Oil Fired Plants
	Residential Oil Furnace
	Unleaded Gas-Fuelled Vehicles
Uncontrolled Combustion	Barrel Burning for House Waste
	Combustion of Landfill Gas
Waste Incineration	Bleached Chemical Pulp Mills
	Boilers- Hazardous Wastes
	Cement Kilns Burning Hazardous Waste
	Crematoria
	Incinerator- Hazardous Wastes
	Medical Waste Incineration
	Municipal Solid Waste Incinerators
	Sewage Sludge Incinerator
	Tire Combustion

Table 10 and Table 11 list the congener correction factors that were calculated based upon the congener profiles of each source. The sources were grouped by whether or not an uncorrected ELISA would underreport (Table 10) or over-report (Table 11) the dioxin content of a sample from that particular source in comparison to GC-HRMS TEQ values.

Table 10. Sources that an uncorrected ELISA will underreport

Source	Composite WHO98 TEF	Composite cross reactivity	Source-specific Correction Factor	Uncorrected ELISA will underreport by:
Secondary Copper Refinery	0.051	0.019	2.68	63%
Petroleum Refining Catalyst Regeneration	0.072	0.033	2.19	54%
Incinerator- Hazardous Wastes	0.066	0.034	1.93	48%
Medical Waste Incineration	0.051	0.028	1.84	46%
Leaded Gas-Fuelled Vehicles	0.054	0.030	1.78	44%
Barrel Burning for House Waste	0.129	0.075	1.71	41%
Secondary Aluminum Smelters	0.106	0.062	1.70	41%
Crematoria	0.061	0.040	1.50	33%
Cement Kilns Burning Hazardous Waste	0.187	0.125	1.49	33%
Coal Fired Plants	0.058	0.043	1.35	26%
Ferrous Foundry	0.041	0.031	1.33	25%
Boilers- Hazardous Wastes	0.066	0.050	1.32	24%
Municipal Solid Waste Incinerators	0.049	0.037	1.31	24%
Cement Kiln Burning Hazardous Waste with the Inlet Temperature of the Air Pollutant Control Device Greater than 450F	0.148	0.118	1.26	20%
Secondary Lead Smelters	0.184	0.152	1.21	17%
Kraft Black Liquor Recovery Boilers	0.044	0.037	1.17	14%
Unleaded Gas-Fuelled Vehicles	0.030	0.026	1.16	14%
Industrial Wood Combustion	0.065	0.056	1.16	14%
Oil Fired Plants	0.042	0.038	1.12	11%
Bleach Chemical Pulp Mills	0.034	0.032	1.09	8%
Cement Kilns not Burning Hazardous Waste	0.099	0.092	1.07	7%
Tire Combustion	0.047	0.043	1.07	7%
Diesel Fuelled Trucks	0.027	0.025	1.05	5%
Primary Ferrous Metal Smelting	0.177	0.173	1.02	2%

Footnote:

The extent of underreporting is calculated as $[1 - 1/(\text{cross reactivity})] \times 100$.

Table 11. Sources that an uncorrected ELISA will over-report

Source	Composite WHO98 TEF	Composite CR	Source- specific Correction Factor	Uncorrected ELISA will over-report by:
Combustion of Landfill Gas	0.094	0.128	0.74	36%
Cement Kiln Burning Hazardous Waste with the Inlet Temperature of the Air Pollutant Control Device Less than 450F	0.102	0.108	0.94	6%
Sewage Sludge Incinerator	0.119	0.120	0.99	1%

Footnote:

The extent of over-reporting is calculated as $[1/(\text{cross reactivity})-1] \times 100$.

Based on the congener profiles provided by the dioxin database, applying a correction factor will be necessary when using ELISA on samples originating from the majority of the listed sources. Uncorrected ELISA results can range anywhere from underreporting a sample's TEQ by 63% (Secondary Copper Refinery) to over-reporting TEQ by 36% (Combustion of Landfill Gas). Use of the correction factor will correct for these discrepancies. Interestingly, it seems that an uncorrected ELISA will generally underreport dioxin concentrations as 'Combustion of Landfill Gas' is the only source with a congener profile that will lead to a significant over-reporting. However, it must be emphasized that this finding is only true for the Cape Technologies ELISA. The ELISA used by other laboratories may use other polyclonal antibodies and thus would have different cross reactivity values. Therefore, new correction factors must be determined for different ELISA in order to compare those ELISA to GC-HRMS.

It is important to point out that the listed source-specific correction factors are not intended to be used quantitatively. Each site will have its own unique congener profile and this profile can be used to calculate a site-specific correction factor. The term 'site-specific correction factor' was

previously coined by Nording et al., 2007 however as discussed previously their correction factor is rather different.

Certain assumptions are required to form the basis for the use of a site-specific correction factor. The congener profile of the contaminated site is assumed to remain roughly consistent across the site. That is to say, the congener profiles of each sample would be roughly but not exactly equal. This assumption is known to hold true when the site has a single source of dioxin emission (OMOE, 2007). This assumption may also hold true for sites with multiple sources of dioxin emissions if the combined emissions produce a congener profile that has little variability. To state this assumption in other words, it is assumed that a site can have a wide range of different dioxin concentrations but that the congener profile (or percent composition) of the site's samples will remain roughly consistent. This assumption can be verified by running GC-HRMS on samples from a particular site to ensure that there are no differences in congener profiles.

The site-specific congener profile is an average of the congener profiles of every sample taken from the site. However, this approach would require GC-HRMS analysis on each sample from the site as it is the only technique that gives congener concentrations. ELISA would become dependent on GC-HRMS analysis and the benefits of using ELISA as a way to reduce the burden on GC-HRMS would effectively be lost. A practical solution to this problem would be to calculate a site-wide average congener profile based on the fewest number of GC-HRMS analyses. It therefore becomes a question of knowing how many GC-HRMS analyses are necessary to calculate an average congener profile that reasonably resembles the actual site-wide average congener profile.

In order to calculate an average site-wide congener profile, the average percent composition of each congener must be known. With the assumption that a site has a single, average congener profile and that each sample taken from that site has a profile that is similar to this average value, it follows that each congener in a sample should have a statistical distribution about the site's average value for that same congener. The primary objective becomes how to determine the fewest number of GC-HRMS analyses needed to calculate an average site-wide congener profile that is reasonably close to its true value. 'Reasonably close' will be defined as $\pm 5\%$ of its true value. To accomplish this objective, a two-stage process is required. The first stage, expanded upon in Section 2.1.2 is to determine which type of statistical distribution best represents the congener percent compositions of samples collected from a particular site. This first stage is necessary for the correct determination of the fewest number of GC-HRMS analyses needed, which is the second stage (Section 2.1.3).

2.1.2. *Method of determining the distribution type that best represents the congener percent compositions of samples collected from a particular site*

The reasoning behind needing to determine the distribution type is as follows. The formula used to calculate an average percent composition for each congener will be different depending on which distribution type best fits the data. For instance, the average value, or mean, of a normal distribution is calculated as

$$\bar{x} = \sum_{i=1}^n \frac{x_i}{n} \quad (\text{Eq. 3})$$

whereas for a lognormal distribution, the mean value is calculated as

$$\bar{x} = \exp\left(\mu + \frac{\sigma^2}{2}\right) \quad (\text{Eq. 4})$$

where μ and σ are the mean and standard deviation of normally distributed $\ln(x)$ (NIST/SEMATECH, 2008e).

Distributions of the percent compositions for each of the 17 reported dioxin and furan congeners were examined for 25 soil samples provided by the Ontario Ministry of the Environment (MOE) from a site with an unknown source of dioxin contamination. These samples had a wide range of dioxin concentrations ($11 - 27,000 \text{ pgTEQ g}^{-1}$) and were sampled at several sampling points about the site, making them an ideal dataset to test whether or not the congener percent compositions of samples taken at a site can be fitted to a particular distribution type. Methodology to determine the ideal distribution type of this dataset was adapted from several sections of the NIST/SEMATECH e-handbook of Statistical Methods (NIST/SEMATECH, 2008a). The first step was to run a Tukey-Lambda analysis.

2.1.2.1. *Tukey-Lambda analysis*

The Tukey-Lambda plot gives an initial estimate of the distribution type that best fits a dataset. It plots Tukey-Lambda probability plot correlation coefficients on the y-axis against the different shape parameters that are used to make up these probability plots on the x-axis (NIST/SEMATECH, 2008b). The optimal shape parameter will be the one that produces the highest probability plot correlation coefficient and as such will indicate which type of distribution best fits the data. Optimal shape parameters (λ) of a Tukey-Lambda plot relate to distribution types as shown in Table 12.

Table 12. Relation between Tukey-Lambda shape parameters and distribution type

Optimal Tukey-Lambda shape parameter	Distribution type
$\lambda = -1$	Approximately Cauchy
$\lambda = 0$	Exactly logistic
$\lambda = 0.14$	Approximately normal
$\lambda = 0.5$	U-shaped
$\lambda = 1$	Exactly uniform

A web-based statistics program was used to run the Tukey-Lambda analysis (Wessa, 2008). Sample output from the Tukey-Lambda program for the $n = 25$ dataset of 1,2,3,7,8-PeCDF percent compositions is shown in Figure 3.

Figure 3. Sample Tukey-Lambda output for 1,2,3,7,8-PeCDF

Tukey-Lambda correlation coefficients for different shape parameters for 1,2,3,7,8-PeCDF are shown in Table 13.

Table 13. Tukey-Lambda correlation coefficient values for 1,2,3,7,8-PeCDF

Distribution	Correlation
Approx. Cauchy (lambda=-1)	0.8373
Exact Logistic (lambda=0)	0.9712
Approx. Normal (lambda=0.14)	0.9722
U-shaped (lambda=0.5)	0.9672
Exactly Uniform (lambda=1)	0.9595

Figure 3 and Table 13 indicate that the percent compositions of 1,2,3,7,8-PeCDF are best correlated to a shape parameter of 0.14, which indicates that the data best fits a near-normal distribution. The same analysis was completed for all 17 congeners. The results are listed in Table 14.

Table 14. Tukey-Lambda correlation coefficient values for 17 dioxin and furan congeners

Congener	Site-average sample composition	Approx. Cauchy	Exactly Logistic	Approx. Normal	U-shaped	Exactly Uniform	Decision
Octachlorodioxin	30.91%	0.7525	0.9498	0.9583	0.9673	0.9686	Uniform
1234678-heptachlorodioxin	17.74%	0.7761	0.9757	0.9827	0.9878	0.9853	Normal
123678-hexachlorodioxin	8.97%	0.7081	0.9508	0.9637	0.9803	0.9858	Uniform
1234678-heptachlorofuran	6.82%	0.8051	0.9753	0.9794	0.9793	0.9739	Normal
123678-hexachlorofuran	5.37%	0.7505	0.9597	0.9679	0.9751	0.9737	Normal
123789-hexachlorodioxin	5.15%	0.7107	0.9495	0.9620	0.9775	0.9823	Uniform
234678-hexachlorofuran	4.81%	0.7897	0.9610	0.9640	0.9608	0.9521	Normal
12378-pentachlorodioxin	3.96%	0.7200	0.9535	0.9658	0.9815	0.9868	Uniform
23478-pentachlorofuran	3.80%	0.7605	0.9535	0.9601	0.9643	0.9612	Normal
Octachlorofuran	3.22%	0.6581	0.8552	0.8624	0.8672	0.8638	Normal
123478-hexachlorofuran	3.15%	0.7847	0.9495	0.9539	0.9550	0.9510	Normal
2378-tetrachlorofuran	2.20%	0.8161	0.9855	0.9878	0.9831	0.9735	Normal
123478-hexachlorodioxin	1.69%	0.6809	0.9258	0.9399	0.9591	0.9668	Uniform
12378-pentachlorofuran	1.02%	0.8377	0.9713	0.9722	0.9672	0.9595	Normal
1234789-heptachlorofuran	0.61%	0.9048	0.9853	0.9783	0.9571	0.9375	Logistic
2378-tetrachlorodioxin	0.42%	0.8819	0.8383	0.8216	0.7855	0.7591	Cauchy
123789-hexachlorofuran	0.14%	0.8230	0.7444	0.7230	0.6775	0.6442	Cauchy

Footnotes:

1. To calculate a site-averaged composition, it was assumed for the time being that the congeners are normally distributed.
2. The bolded entries indicate where the highest correlation coefficient value falls. Where two values are bolded, the maximum value falls between the two distribution types.

The results show that 14 of the 17 congeners (98.8% of the average sample composition) are best fit to either an approximately normal or a uniform distribution. Both of these distributions are considered equivalent for the purposes of this analysis since they would both use the same formula for determining average congener profile values. While the percent composition distributions of three congeners do not fit a normal distribution very well, they make up less than 2% of the average sample composition for the MOE site.

Although Tukey-Lambda has determined that the data is near normal, it does not distinguish between a normal distribution and a lognormal distribution. While the difference between the two may seem trivial, many environmental samples show lognormal behaviour (Limpert et al., 2001) and even dioxin concentrations in soil have been suggested to be distributed lognormally (van Emon et. al, 2008). Whether or not the distribution of a congener's percent composition is better fit to a normal or a lognormal distribution can be determined by using a lognormal probability plot correlation coefficient (PPCC) plot to determine the ideal lognormal shape parameter.

2.1.2.2. Determining the ideal lognormal shape parameter

In a lognormal PPCC plot, the "lognormal probability plot correlation coefficients" are plotted on the y-axis and the lognormal shape parameters that are used to create them are plotted on the x-axis (NIST/SEMATECH, 2008c). The shape parameter with the highest correlation coefficient will be the ideal lognormal shape parameter for that particular dataset. If the optimum correlation coefficient of the lognormal PPCC plot is at zero then the data is best fit to a normal distribution. Figure 4 shows the lognormal PPCC plots for 1,2,3,4,6,7,8-HpCDD on the left and 1,2,3,7,8,9-HxCDF on the right. The left figure indicates that 1,2,3,4,6,7,8-HpCDD is

best fit to a normal distribution since the optimal probability correlation coefficient is at zero. The right figure indicates that 1,2,3,7,8,9-HxCDF is best fit to a lognormal distribution with a shape parameter of 2.9. The lognormal PPCC analyses were completed manually using Microsoft Excel.

Figure 4a. Lognormal PPCC plots for 1,2,3,4,6,7,8-HpCDD (left) and Figure 4b. 1,2,3,7,8,9-HxCDF (right)

As mentioned above, the “lognormal probability plot correlation coefficients” are plotted on the y-axis of the lognormal PPCC plot. Values for the “lognormal probability plot correlation coefficients” are generated as follows. A lognormal probability plot is generated for each shape parameter. For example, a series of shape parameter values ranging from 0.1 to 5 with increments of 0.1 will generate a total number of 50 lognormal probability plots. On each plot, the x-axis is the inverse cumulative distribution function of the lognormal distribution evaluated at the “normalized ordered statistic medians” (defined below). The y-axis is the congener percent composition dataset ranked in increasing order. Each plot generates a correlation coefficient for that particular shape parameter. The distribution is considered to be a good fit for the dataset when the lognormal probability plot shows a linear line with a strong correlation coefficient.

A probability plot for 1,2,3,4,6,7,8-HpCDD evaluated at its optimal lognormal shape parameter of 0 (i.e. a normal distribution) is shown in Figure 5. The resulting r-squared value of 0.97308 provides the first data point on Figure 4a. The remaining datapoints in Figure 4a were provided by similar procedures with different shape parameters. Datapoints in Figure 4b are obtained by applying the same procedure to the 1,2,3,7,8,9-HxCDF percent composition dataset.

Figure 5. Lognormal probability plot for 1,2,3,4,6,7,8-HpCDD evaluated at $\lambda = 0$

The “normalized ordered statistic medians” (or m_i) needed to generate a lognormal probability plot are the medians between the i th-ordered datapoint and the location of where the i th datapoint should be on a normal probability plot (Filliben, 1975). In general, probability plots can be created for any distribution where the inverse cumulative distribution function is known (NIST/SEMATECH, 2008d). For a lognormal distribution, the inverse cumulative distribution G is:

$$G(m_i) = \exp(\lambda \Phi^{-1}(m_i)) \quad m_i \in (0,1) \quad (\text{Eq. 5})$$

Where m_i is the i th ordered statistical median, λ is the lognormal shape parameter and Φ^{-1} is the inverse cumulative distribution function of the normal distribution evaluated at m_i (NIST/SEMATECH, 2008e).

The inverse cumulative distribution function of the normal distribution is (Weisstein, E., no date B):

$$\Phi^{-1}(m_i) = \sqrt{2} \operatorname{erf}^{-1}(2m_i - 1) \quad (\text{Eq. 6})$$

Where erf^{-1} is the inverse error function which is estimated as (Weisstein, E., no date A):

$$\operatorname{erf}^{-1}(x) = \frac{1}{2} \sqrt{\pi} \left(x + \frac{\pi}{12} x^3 + \frac{7\pi^2}{480} x^5 + \frac{127\pi^3}{40320} x^7 + \frac{4369\pi^4}{5806080} x^9 + \dots \right) \quad (\text{Eq. 7})$$

The statistical ordered medians are approximated as follows (Filliben, 1975):

$$m_i = \begin{cases} 1 - m_n & i = 1 \\ (i - 0.3175)/(n + 0.365) & i = 2, 3, \dots, n-1 \\ 0.5^{(i/n)} & i = n \end{cases} \quad (\text{Eq. 8})$$

The final step of stage one – the determination of the type of statistical distribution for the 17 congeners – is as follows. Lognormal PPCC plots (as illustrated by two congeners in Figure 4a and 4b, respectively) were generated for the distribution of the remaining 15 toxic congeners' percent compositions. Optimal lognormal shape parameters were calculated and then summarized in Table 15.

Table 15. Optimal lognormal shape parameters for 17 dioxin and furan congeners

Congener	Optimal Shape Parameter
Octachlorodioxin	0.315
1234678-heptachlorodioxin	Normal
123678-hexachlorodioxin	Normal
1234678-heptachlorofuran	Normal
123678-hexachlorofuran	0.168
123789-hexachlorodioxin	Normal
234678-hexachlorofuran	0.468
12378-pentachlorodioxin	Normal
23478-pentachlorofuran	0.340
Octachlorofuran	0.769
123478-hexachlorofuran	Normal
2378-tetrachlorofuran	0.056
123478-hexachlorodioxin	Normal
12378-pentachlorofuran	0.330
1234789-heptachlorofuran	0.014
2378-tetrachlorodioxin	2.379
123789-hexachlorofuran	2.930

Footnote:

When the optimal shape parameter is 0, the congener's percent composition is best fit to a normal distribution.

Table 15 shows that seven congeners are best fit to a normal distribution and ten are best fit to lognormal distributions with varying shape parameters. The ideal situation would be if each congener assumed a normal distribution because in this case, the optimal shape parameter would not have to be determined. The entire objective of examining which distribution best describes the site-wide spread of a congener's percent composition is to determine how to calculate the congener's site-wide average percent composition. Therefore, the average composition for the least normally distributed (i.e. the most lognormally distributed) congener, 1,2,3,7,8,9-HxCDF was calculated by using both the normal and lognormal methods of calculating an average value. This is shown in Table 16. If the estimation of the average value calculated both ways is

sufficiently similar then this would resemble the ideal situation since all the congeners could suitably be considered normally distributed.

Table 16. Normal and lognormal averages for the most lognormally distributed congener

Method of calculating an average	Equation	Value for the site's average composition of 1,2,3,7,8,9-HxCDF
Normal Distribution	$\bar{x} = \sum_{i=1}^n \frac{x_i}{n}$	1.43%
Lognormal Distribution	$\bar{x} = \exp\left(\mu + \frac{\sigma^2}{2}\right)$	1.38%

The results in Table 16 demonstrate that the two methods both give similar estimates for the average composition of the most lognormally distributed congener. Therefore, the conclusion of stage one is that all congeners can be treated as normally distributed. This conclusion is necessary for progression into stage two (Section 2.1.3).

2.1.3. Determining the fewest required number of GC-HRMS analyses

This section will determine the fewest number of GC-HRMS analyses necessary to calculate a site-wide average congener profile that would be sufficiently close to the best estimate of the site's actual average congener profile. With this average congener profile, an average site-wide correction factor can be calculated that can be applied to all ELISA results from that site. Before calculating an average congener profile, the distribution of the percent compositions of each congener had to be determined. From the statistics provided in the previous sections, it was found that the distribution of the congener percent compositions of the 25 provided samples from the MOE site could suitably be treated as normal. As such the average value for each congener

percent composition is calculated as $\bar{x} = \sum_{i=1}^n \frac{x_i}{n}$.

The best estimate of the average site-wide congener profile for the MOE site was considered to be the average congener profile calculated using all 25 provided samples. This profile and its associated correction factor are given in Table 17.

Table 17. The average congener profile for the MOE soil samples

Congener	Percent Composition
Octachlorodioxin	30.91%
1234678-heptachlorodioxin	17.74%
123678-hexachlorodioxin	8.97%
1234678-heptachlorofuran	6.82%
123678-hexachlorofuran	5.37%
123789-hexachlorodioxin	5.15%
234678-hexachlorofuran	4.81%
12378-pentachlorodioxin	3.96%
23478-pentachlorofuran	3.80%
Octachlorofuran	3.22%
123478-hexachlorofuran	3.15%
2378-tetrachlorofuran	2.20%
123478-hexachlorodioxin	1.69%
12378-pentachlorofuran	1.02%
1234789-heptachlorofuran	0.61%
2378-tetrachlorodioxin	0.42%
123789-hexachlorofuran	0.14%
Best estimate of the actual site-wide correction factor for soil samples	1.0952

Therefore, the best estimate of the actual site-wide correction factor is 1.0952. In order to determine the fewest number of samples needed to be analyzed by GC-HRMS in order to obtain a site-wide correction factor that is sufficiently similar to 1.0952, average congener profiles were calculated for randomly generated sample sets of $n=1,2,3,4,\dots,25$ samples taken from the 25 available samples. Each of these sample sets was randomly generated 200 times from the 25 available samples and was used to calculate an estimate of the site-wide correction factor. The methodology on how this is accomplished is explained in Appendix A. The 200 correction

factors generated for each sample size of $n=1,2,3,4,\dots,25$ are presented in the Figure 6 as a percentage of the best estimate of the actual site-wide correction factor 1.0952.

Figure 6. Correction factors for the 200 datasets at samples sizes $n=1,2,3,\dots,25$

From the spearhead shape of Figure 6, it can be concluded that running too few GC-HRMS analyses to create a site-side correction factor can lead to a correction factor that varies too much from the best estimate of its actual value. Running too many GC-HRMS analyses is also not beneficial because exceeding a certain sample size will no longer appreciably change the average site-wide correction factor. To quantify this observation, the site-wide correction factor was considered acceptably close to the best estimate of its actual value once it was $\pm 5\%$ of 1.0952. In order to determine how many samples will be needed to meet this criterion, the standard deviation of the spread of data for each sample size was calculated. Since the data spreads at each sample size were found to be normally distributed (by running a Tukey-Lambda analysis)

and based on the fact that 95% of the data from a normal distribution falls within two standard deviations (Moore and McCabe, 1999), Figure 7 was generated.

Figure 7. Two standard deviations of the spread of correction factors at each sample size

The $\pm 5\%$ cut-off line intercepts two standard deviations at a sample size of 7. Therefore by running GC-HRMS on 7 samples and using their averaged congener profile to create a site-wide correction factor, one can be 95% confident that the resulting correction factor will be within 5% of the best estimate of its actual value. While the requirement of 7 samples is ultimately a function of the size of the dataset (the 25 soil samples) from which they would be selected, it is likely that after a certain dataset size is reached, the number of required samples would not change.

It should be emphasized that the requirement of 7 GC-HRMS samples to produce a site-wide correction factor is only valid for soils from this particular site. Factors such as different soil types, different congener profiles and different distributions types for the percent compositions of each congener will alter the number of required samples. The method of estimating the number of samples required to calculate a site-wide correction factor is therefore meant only as a template. Comparisons between a number of sites, their specific characteristics and how they contribute to different estimations of the number of GC-HRMS samples needed for a site-wide correction factor is beyond the scope of this thesis. However, for the purposes of method validation, the number of GC-HRMS samples needed for a site-wide correction factor was again determined for another sample set comprising 30 dioxin contaminated sediments from the same site. Although the 25 soil samples and 30 sediment samples were from the same site, they were not combined to estimate the number of samples needed for a site-wide correction factor since the different characteristics between soils and sediments could potentially lead to them having widely different dioxin congener profiles.

2.1.4. Calculating a site-wide correction factor using a different sample set

The methodology detailed in Sections 2.1.2 and 2.1.3 is briefly summarized in Table 18 on the next page. It will be repeated for the 30 sediment sample set. Table 19 gives the results of the Tukey-Lambda analysis for the congener profiles of the 17 toxic congeners found in the sediment samples.

Table 18. Summary of the two-stage process used to calculate a site-wide correction factor

Section	Stage	Description	Subsection	Step	Purpose	Related Tables and Figures
Section 2.1.2	Stage 1	Determines the best statistical distribution type for the percent compositions of 17 congeners at a contaminated site	2.1.2.1	Tukey-Lambda analysis	Gives an initial estimate of the distribution type that best fits a dataset	Table 12 Table 13 Table 14 Figure 3
			2.1.2.2	Lognormal PPCC plot analysis	Determines whether a normal or a lognormal distribution best fits the dataset	Table 15 Table 16 Figure 4a Figure 4b Figure 5
Section 2.1.3	Stage 2	Determines the fewest number of GC-HRMS samples needed to produce a suitable site-wide correction factor	2.1.3	Calculate the best estimate of the site-wide congener profile using the distribution type found in Stage 1	Produces the best estimate of the site-wide correction factor	Table 17
				Produce and analyze a "spearhead" graph	Determines the fewest amount of samples needed to produce a site-wide correction factor that 95% of the time is within 5% of the best estimate of the site-wide correction factor	Figure 6 Figure 7

Table 19. Tukey-Lambda correlation coefficient values for 17 dioxin and furan congeners

Congener	Site-average sample composition	Approx. Cauchy	Exactly Logistic	Approx. Normal	U-shaped	Exactly Uniform	Decision
1234678-heptachlorodioxin	24.26%	0.7492	0.9777	0.9851	0.9896	0.9862	U-shaped
Octachlorodioxin	18.72%	0.7697	0.9509	0.9510	0.9407	0.9264	Normal
123678-hexachlorodioxin	12.91%	0.7712	0.9875	0.9925	0.9917	0.9841	Normal
123789-hexachlorodioxin	6.86%	0.8054	0.9892	0.9898	0.9807	0.9676	Normal
1234678-heptachlorofuran	6.26%	0.8673	0.9043	0.8926	0.8639	0.8409	Logistic
123478-hexachlorofuran	5.14%	0.7879	0.9682	0.9680	0.9569	0.9418	Normal
123678-hexachlorofuran	5.03%	0.7742	0.9695	0.9736	0.9721	0.9649	Normal
12378-pentachlorodioxin	4.77%	0.6991	0.9625	0.9742	0.9863	0.9875	Uniform
234678-hexachlorofuran	3.95%	0.8800	0.9533	0.9427	0.9141	0.8896	Logistic
23478-pentachlorofuran	2.63%	0.7632	0.9716	0.9754	0.9722	0.9627	Normal
123478-hexachlorodioxin	2.47%	0.7639	0.9824	0.9875	0.9870	0.9794	Normal
Octachlorofuran	1.99%	0.8164	0.8516	0.8385	0.8066	0.7812	Logistic
2378-tetrachlorofuran	1.69%	0.8764	0.9414	0.9305	0.9022	0.8785	Logistic
1234789-heptachlorofuran	1.10%	0.7781	0.9777	0.9803	0.9748	0.9636	Normal
12378-pentachlorofuran	1.06%	0.7285	0.9764	0.9864	0.9955	0.9950	U-shaped
2378-tetrachlorodioxin	0.86%	0.7587	0.9622	0.9674	0.9679	0.9621	U-shaped
123789-hexachlorofuran	0.30%	0.7772	0.6677	0.6452	0.6001	0.5696	Cauchy

Footnotes:

1. To calculate a site-average composition, it was assumed for the time being that the congeners are normally distributed.
2. The bolded entries indicate where the highest correlation coefficient value falls. Where two values are bolded, the maximum value falls between the two distribution types.

From the Tukey-Lambda analysis, roughly 55% of the average congener profile of the sediment samples is best fit to a near-normal distribution. However, 91% of the data has a high correlation coefficient value of at least 0.93 suggesting that most of the congeners can fit a near-normal distribution.

PPCC plots were then generated for each congener to determine if the data is better suited to a normal distribution or a lognormal one. The resulting shape parameters are listed in Table 20.

Table 20. Optimal lognormal shape parameters for the 17 dioxin and furan congeners

Congener	Optimal Shape Parameter
1234678-heptachlorodioxin	0.154
Octachlorodioxin	0.467
123678-hexachlorodioxin	Normal
123789-hexachlorodioxin	Normal
1234678-heptachlorofuran	0.636
123478-hexachlorofuran	0.246
123678-hexachlorofuran	Normal
12378-pentachlorodioxin	0.215
234678-hexachlorofuran	0.688
23478-pentachlorofuran	0.227
123478-hexachlorodioxin	Normal
Octachlorofuran	1.677
2378-tetrachlorofuran	0.791
1234789-heptachlorofuran	0.195
12378-pentachlorofuran	0.084
2378-tetrachlorodioxin	0.344
123789-hexachlorofuran	5.376

Footnote:

When the optimal shape parameter is 0, the congener's percent composition is best fit to a normal distribution.

All congeners have optimal shape parameters of less than 1 (except for octachlorofuran and 1,2,3,7,8,9-HxCDF which make up less than 2.5% of the average sample percent composition). Therefore, all of the congeners will be considered normally distributed about their respective

average site-wide value. At this point, the determination of statistical distribution type (stage one) is complete.

The average site-wide congener profile for the sediment samples (calculated using the average formula of a normal distribution) and its associated correction factor are given in Table 21.

Table 21. Average congener profile for the MOE sediment samples

Congener	Percent Composition
1234678-heptachlorodioxin	24.26%
Octachlorodioxin	18.72%
123678-hexachlorodioxin	12.91%
123789-hexachlorodioxin	6.86%
1234678-heptachlorofuran	6.26%
123478-hexachlorofuran	5.14%
123678-hexachlorofuran	5.03%
12378-pentachlorodioxin	4.77%
234678-hexachlorofuran	3.95%
23478-pentachlorofuran	2.63%
123478-hexachlorodioxin	2.47%
Octachlorofuran	1.99%
2378-tetrachlorofuran	1.69%
1234789-heptachlorofuran	1.10%
12378-pentachlorofuran	1.06%
2378-tetrachlorodioxin	0.86%
123789-hexachlorofuran	0.30%
Best estimate of the site-wide correction factor for the sediment samples	1.0245

Stage Two involves determining the fewest number of samples needed to be analyzed by GC-HRMS in order to produce a site-wide correction factor with a value approximating 1.0245. Average congener profiles were calculated for 200 randomly generated sample sets of $n=1,2,3,4,\dots,30$ samples taken from the 30 available samples. Each of these sample sets was used to calculate an estimate of the site-wide correction factor. The 200 correction factors

generated for each sample size of $n=1,2,3,4,\dots,30$ are presented in Figure 8 as a percentage of the best estimate of the actual site-wide sediment correction factor 1.0245.

Figure 8. Correction factors for the 200 datasets at samples sizes $n=1,2,3,\dots,30$

As was done for the sample set of 25 soil samples, the fewest number of GC-HRMS samples needed was determined by looking at the intercept between the lines of ± 2 standard deviations of the distribution at each sample size and $\pm 5\%$ of the actual site-wide correction factor. Figure 9 shows that the intercept between the ± 2 standard deviation line and the $\pm 5\%$ line intersect between sample sizes of one and two. Therefore, only two sediment samples are needed to be 95% confident that the resulting correction factor will be within 5% of the best estimate of the actual site-wide sediment correction factor.

Figure 9. Two standard deviations of the spread of correction factors at each sample size

The need of only two GC-HRMS sediment samples to calculate a site-wide correction factor for sediments taken from this site indicates that all of these sediments share a more uniform or homogeneous congener profile. In contrast, the soils samples exhibited a more heterogeneous congener profile. Three processes can contribute to this difference. The first process is photodegradation. Air emissions of dioxin are deposited on soils. Photodegradation can cause the breakdown of congeners in the soil, exposed to sunlight (Isosaari et al., 2004). Sunlight is less accessible to sediments. The second process is mixing. When soils eventually get washed into local waters and become sediments, mixing can occur which can increase homogeneity. The third process involves the selective solubility of various congeners in water (hydrophilicity). Higher chlorinated congeners will have slightly more ionic features (due to the greater degree of substitution around the dibenzo core) and thus may be more likely to dissolve and be washed

away by water, which is polar. One could hypothesize that sediments would be richer in concentrations of the lower chlorinated congeners.

The dichotomy of congener abundance between soils and sediments is examined in light of the above mixing and hydrophilicity hypotheses. The average percent compositions of each dioxin/furan homologue (i.e. group of congeners with the same level of chlorination) are plotted in Figure 10 to test the hydrophilicity hypothesis. If the hydrophilicity hypothesis is true, the sediments should exhibit a shift in congener abundance towards the lesser chlorinated homologues since the higher chlorinated homologues are dissolved away. The mixing hypothesis is tested by comparing the magnitude of error bars (± 1 standard deviation), which is a measure of homogeneity. If the mixing hypothesis is true, the sediments must have smaller error bars for each and every single homologue.

Figure 10 Average composition of each dioxin/furan homologue for soils (n=25) and sediments (n=30)

Figure 10 provides evidence that both hypotheses are true. The mixing hypothesis is confirmed since the congener profiles of sediments are indeed more homogeneous than soils; for each and every homologue the standard deviation of the sediments is always smaller than that of the soils. The hydrophilicity hypothesis is confirmed since the sediments are, to a certain extent, more abundant in lower chlorinated homologues. That is, sediments are composed of fewer octachlorinated congeners in comparison to soils and they are most abundant in hexachlorinated congeners. Conversely, soils are most abundant in octachlorinated congeners. The least abundant of the 5 dioxin homologues are the penta- and tetrachlorinated congeners. No difference is observed in their relative abundance, perhaps because they have the lowest level of chlorination and thus would be least likely to dissolve in water.

The above findings have the following practical implications. Since sediments have a smaller distribution of congeners and a greater homogeneity, ELISA may be more amenable to screening for dioxins in sediment samples. More homogeneity in sample composition implies that fewer initial GC-HRMS analyses are required to develop a site-specific correction factor and thus, fewer resources are expended before ELISA can be used. This observation is confirmed by determining that only two sediments are required for developing a sediment site-specific correction factor whereas the soils require seven samples.

The difference between the findings of the soil and sediment sets reaffirms that the sample matrix and other site specific characteristics can have an effect on the number of GC-HRMS samples needed to produce a site-wide correction factor. Future research would require studying

the effects that specific site characteristics would have on the number of samples needed to calculate site-wide correction factors.

The ability of the site-wide congener correction factors to improve ELISA results for both the soil and the sediment samples from the MOE site is examined in Section 3

2.2. The Sample Algorithm

2.2.1. Sample algorithm development

Contaminated soils can have a wide range of possible dioxin concentrations and it is important for ELISA to be able to screen across this entire range. Ideally, ELISA should be able to screen at low, medium and high levels of about 50, 1000 and 10,000 pgTEQ g⁻¹, respectively. The reason for these three screening levels is that they are relevant to North American dioxin testing, as described in Table 22 on the following page.

Table 22. Soil criteria from the United States and Ontario

Country	Soil Criteria ¹	Comments
United States (De Rosa et al., 1997)	Screening Level: 50 pgTEQ g ⁻¹	<i>These criteria are developed by the US Agency for Toxic Substances and Disease Registry (ATSDR).</i> Soils are initially screened at 50 pgTEQ g ⁻¹ . However, soils with dioxin concentrations below the Screening Level may still require further evaluation.
	Evaluation Level: Between 50 and 1000 pgTEQ g ⁻¹	Soils with dioxin concentrations within the Evaluation Level require site specific investigations into bioavailability, exposure pathways and surrounding dioxin background levels.
	Actionable Level: 1000 pgTEQ g ⁻¹	Soils with dioxin concentrations above the Actionable Level require a response action to address the contamination.
Ontario, Canada (OMOE, 1996)	Upper Concentration Limit for Soil: 10,000pgTEQ g ⁻¹	Soil concentrations above this value must undergo some form of Ontario Site Specific Risk Assessment Level II remediation. This can include engineering solutions or the blocking of exposure pathways. This value is an absolute maximum and soils with concentrations below this value should still be examined for remediation options.

Footnote:

TEQ values are calculated using the NATO toxic equivalency factors.

One challenge to using any analytical method for measuring a wide range of concentrations is that often the method has a limited dynamic range of dose-response. ELISA is no exception. For example, if a soil sample with a low dioxin concentration, such as 50 pgTEQ g⁻¹, was processed by an ELISA designed for screening at levels nearing 10,000 pgTEQ g⁻¹, the ELISA results would not be accurate. Similarly, analyzing a sample with a high concentration would not yield accurate results if screened at 50 pgTEQ g⁻¹. This dilemma was resolved by the

development of a sample algorithm. This algorithm ensures an efficient introduction of a sufficient amount of dioxin into an ELISA tube from a soil sample with unknown dioxin concentration.

The algorithm must meet four key requirements in order to be effective. The four requirements are:

- 1) Enable ELISA to screen between 50 and 10,000 pgTEQ g⁻¹;
- 2) Introduce between 5 and 35 pgTEQ of dioxin into an ELISA tube;
- 3) Introduce a minimal but constant aliquot of sample in methanol into the ELISA tube and
- 4) Prepare a sufficient sample volume to be able to run ELISA in duplicate or triplicate.

These requirements can be met by altering how the sample is prepared and subsequently introduced into the ELISA tube. Between 5 and 35 pg of dioxin must always be introduced into an ELISA tube since the ELISA analysis is most sensitive at quantities of 20±15 pgTEQ per tube (Cape Technologies, 2003b). The amount of methanol being introduced into the ELISA tube must be minimized since methanol has been found to denature the antibodies (Zhang, 2008). While this is true, methanol is used since dioxin must be dissolved in an organic solvent that is miscible with the aqueous buffer (Zhang, 2008). For the purposes of reproducibility and contingency, the sample volume to be prepared for ELISA should be sufficient for duplicate or triplicate runs. Three ELISA tests have been designed (Table 23) in order to meet all four of these requirements – tests whose midpoints are centered around 6000, 900 and 120 pgTEQ g⁻¹.

Table 23. Algorithm test level ranges in pgTEQ g⁻¹

Test level (the midpoint)	Maximum detection level	Minimum detection level
6,000	10,500	1,500
900	1,500	225
120	225	30

Although three ELISA tests have been designed to cover between 30 and 10,500 pgTEQ g⁻¹, in reality only two of these tests are required for any given sample. The 900 pgTEQ g⁻¹ test will always be run first. If the sample dioxin concentration falls within range, the analysis is complete. If the sample proves to have a dioxin concentration above the range of the 900 pgTEQ g⁻¹ test, the 6,000 pgTEQ g⁻¹ test would be run next. If the dioxin concentration is below the range of the 900 pgTEQ g⁻¹ test, the 120 pgTEQ g⁻¹ test would follow. The algorithm and how it directs subsequent sample preparation is illustrated in Figure 11.

Figure 11. The sample algorithm

The ability of the sample algorithm to effectively screen samples into their correct test level is validated in Section 3.

2.2.2. Cost analysis of using the sample algorithm

With the concept of the sample algorithm in place, it is no longer correct to state that each sample will require only one ELISA analysis. As such, when comparing the costs of ELISA and GC-HRMS, the possibility of having to run more than one ELISA for each sample must be considered. To estimate the total cost of running samples with ELISA, a cost analysis was

performed on 36 soil samples provided by the MOE with an additional four samples. The reason for including the four extra samples was to have four equal ELISA runs of ten samples each. These 40 samples, listed in Table 24, have concentrations that fall into all three test levels of the algorithm.

Table 24. Forty samples used in the cost analysis and their appropriate algorithm test level

Sample Order	pgTEQ/g	Test Level	Sample Order	pgTEQ/g	Test Level
1	0.90	120	21	5.0	120
2	43	120	22	0.37	120
3	1.5	120	23	3700	6000
4	180	120	24	4.0	120
5	18	120	25	1.7	120
6	47	120	26	0.7	120
7	4.0	120	27	0.77	120
8	3.2	120	28	225	120
9	1300	900	29	15	120
10	410	900	30	67	120
11	27	120	31	12	120
12	79	120	32	11	120
13	6.5	120	33	1.4	120
14	5.3	120	34	6	120
15	25	120	35	18000	6000
16	23	120	36	27000	6000
17	8.4	120	37	120	120
18	4.4	120	38	120	120
19	430	900	39	120	120
20	60	120	40	900	900

The completion time for each step of an ELISA run consisting of ten samples and ten standards per run and the manpower needed are shown in Table 25.

Table 25. Completion times and employees needed for each ELISA step

ELISA Step	Completion Time	Employees Needed
Glassware Cleaning and ELISA ¹	5 days	1
Sample Preparation and Weighing	0.5 days	1
First Extraction	1 day	1
Evaporation	0.5 days	1
ELISA	1 day	2
Second Extraction ²	0.5 days	1

Footnotes:

1. The 5 days required for glassware cleaning and ELISA proofing will be sufficient for all of the ELISA runs necessary to complete the 40 samples.
2. The second extraction is needed for samples that require a second ELISA analysis at either 120 pgTEQ g⁻¹ or 6,000 pgTEQ g⁻¹. This step involves only solvent exchange and so it takes half the time of the first extraction.

Labour and material costs are shown in Table 26.

Table 26. Labour and material costs for an ELISA run

Item	Cost or Rate
ELISA materials per run (20 tubes plus reagents)	\$400
Additional materials per ELISA run	\$50
Labour @ \$32 per hour	\$256 per day

Footnote:

The cost of the ELISA materials is based on the Cape Technologies price of \$2,000 for 100 tubes and their reagents. Additional material and labour costs are approximations.

Figure 12 on the following page details the timeline and resources needed to run the 40 samples listed in Table 24 either once for samples within the 900 pgTEQ g⁻¹ range, or twice for samples requiring an additional test at either the 120 or the 6,000 pgTEQ g⁻¹ level.

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Runs	Day 1 - 5	Day 6 5.5 6	Day 7 6.5 7	Day 8 7.5 8	Day 9 8.5 9	Day 10 9.5 10	Day 11 10.5 11	Day 12 11.5 12	Day 13 12.5 13	Day 14 13.5 14	Day 15 14.5 15	Day 16 15.5 16
1		Weigh	Extract	Evap	ELISA		Extract 2		ELISA 2			
2	Glassware cleaning and ELISA	Weigh		Extract	Evap	ELISA		Extract 2		ELISA 2		
3			Weigh		Extract	Evap	ELISA		Extract 2		ELISA 2	
4			Weigh			Extract	Evap	ELISA		Extract 2		ELISA 2
First Round ELISA Results					120	120	120	120				
					120	120	120	120				
					120	120	6000	120				
					120	120	120	120				
					120	120	120	6000				
					120	120	120	6000				
					120	120	120	120				
					900	120	120	120				
Second Round ELISA Results					900	900	120	900	120	120	120	120
									120	120	120	6000
									120	120	120	6000
									120	120	120	120
									120	120	120	120
									120	120	120	120
									120	120	120	empty
									120	120	120	empty
Employees needed	1	1	2	2	4	4	4	3	3	3	2	2
	Day 1 - 5	Day 6 5.5 6	Day 7 6.5 7	Day 8 7.5 8	Day 9 8.5 9	Day 10 9.5 10	Day 11 10.5 11	Day 12 11.5 12	Day 13 12.5 13	Day 14 13.5 14	Day 15 14.5 15	Day 16 15.5 16

Figure 12. Timeline and employees needed to analyze the samples provided by the MOE

Footnotes:

1. Each number (120, 900 or 6000) indicates the correct test level for each of the 40 samples being tested.
2. The text with the coloured font indicates a sample that has been tested at the correct test level. The filled text indicates a sample that must be tested a second time at its correct test level.

Figure 12 indicates that nine ELISA tests are needed as well as 35 employee days with a sample turnaround time of 16 working days. The estimated cost is shown in Table 27.

Table 27. Cost per sample when using the sample algorithm

Item	Cost
9 ELISA runs @ \$450	\$4050
35 employee days @ \$256 per day	\$8960
Total for 40 samples	\$13,010
Cost per sample	\$325

This estimate compares favourably to a previously reported estimate of \$300 per sample (Chen, 2008).

Four labs (Maxxam Analytics Inc., Wellington Laboratories Inc., AGAT Laboratories and ALS Laboratory Group) were contacted to obtain information on their costs of running GC-HRMS dioxin analysis for soil samples. They gave an average price of \$800 per sample with an average turnaround time of 45 days for 40 samples. Therefore, in comparison, running ELISA with the algorithm in place generates soil sample results three times faster and 60% less expensive.

2.3. The Background Contamination Correction Method

The third and final improvement to ELISA is a method that accurately accounts for possible sources of dioxin that come into contact with our sample during sample preparation and ELISA analysis. Background dioxin contamination that is introduced into an ELISA tube will originate from glassware and reagents that are used throughout the entire ELISA procedure. Glassware may contain trace amounts of dioxin even after the cleaning procedures described by Zhang 2008 are followed and even the purest reagents may contain dioxin contamination at the picogram level. As such, the reagents and cleaned glassware must be tested for their background dioxin

levels and the background levels of the materials used during sample preparation must be subtracted from the results given by ELISA. To do so, each piece of glassware and reagent that is used in an ELISA run is first tested with ELISA for its background dioxin level.

Since background levels are reported on a per unit basis (for example 'x' picograms per tube or 'y' picograms per mL) and different solution volumes are being transferred during different points of the procedure, it becomes a question of determining how to correctly address the amount of background dioxin that actually enters an ELISA tube. Table 28 on the following page identifies each source of background contamination and how to calculate what amount of contamination gets passed onto the next step. The step-by-step procedure for running a soil ELISA and the listed step numbers that are referred to in Table 28 are provided in Appendix B. The background levels provided in Table 28 represent typical values. An empirical assumption was made that the entire amount of dioxin that was measured for a piece of glassware enters the total amount of organic solvent that comes in contact with that glassware.

Table 28. Background dioxin calculation methodology

Section	Steps in Appendix B	Background Source		Value	Section Totals
Background for the total extract in Extraction Tube	Step 3.1.1	Na ₂ SO ₄ background	pg	0.10	
	Step 3.1.4	Extraction Tube background	pg	0.98	
	Step 3.2.2	DMF vial background	pg	0.28	
	Step 3.2.2	DMF solution background	pg	0.15	
	Step 3.2.3	Mixing beads background	pg	0.30	
		Extraction Tube total	pg		= 1.81
Background of extract transferred to Oxidation Tube	Step 3.2.2	Extraction Tube total volume	ml	15	
	Step 3.2.9	Volume transferred from Extraction tube to Oxidation tube	ml	1.8	
		Background transferred from Extraction tube to Oxidation tube	pg	$1.81 \times \frac{1.8 \text{ ml}}{15 \text{ ml}}$	= 0.22
Background for the solution in Oxidation Tube		Extract background	pg	0.22	
	Step 3.2.8	Oxidation tube background	pg	0.54	
	Step 3.2.8	Hexane solution background	pg	0.45	
	Step 3.2.8	Hexane transfer vial background	pg	0.32	
	Step 3.2.11	Acid background	pg	0.65	
		Oxidation Tube total	pg		= 2.18
Background for the solution in the Evaporation Vial		Oxidation Tube background	pg	2.18	
	Step 3.2.15	Evaporation Vial	pg	0.17	
	Step 3.2.16	Pasteur pipette for hexane transfer background	pg	0.23	
		Evaporation Vial total	pg		= 2.58
Final Sample Background		$2.58 \times \frac{\text{Volume Transferred into ELISA Tube (mL)}}{\text{Total Reconstituted Volume (mL)}}$			

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The final background of the sample being introduced into the ELISA tube will either be 5% of the total Evaporation Vial dioxin background if running the 6,000 pgTEQ g⁻¹ test, 33% if running the 900 pgTEQ g⁻¹ test or 50% if running the 120 pgTEQ g⁻¹ test. This method of accounting for all glassware and for the volumes of sample being transferred throughout the entire ELISA procedure will allow for a more precise way of calculating background contamination and thus will improve upon the method previously used in our lab to subtract background levels from ELISA results. This background correction method was used for all samples run in this thesis.

3. EXPERIMENTAL

3.1. Sample Description

Thirty-six soil samples and twenty-eight sediment samples were provided by the Ontario Ministry of the Environment from a site contaminated by an unknown dioxin source. The samples had dioxin concentrations ranging from 11 to 27,000 pgTEQ g⁻¹. These concentrations were determined by GC-HRMS analysis by laboratories at the MOE. A certified reference material (CRM) sample was also provided by the MOE. The CRM sample was used to prove that ELISA is able to report an accurate estimate of dioxin concentration in a sample without prior knowledge of the GC-HRMS determined dioxin concentration. It was also used to examine the effects of spatial variability of dioxin within a container of sample. The soil and sediment samples were used to test the ability of our lab to correlate ELISA to GC-HRMS results, then to test the ability of ELISA to function as a screen at 50, 1000 and 10,000 pgTEQ g⁻¹. Next, the benefits of applying congener correction factors were examined followed by a test of the sample algorithm. All ELISA runs incorporated the method described in Section 2.3 to subtract background contamination from ELISA results. All ELISA results also incorporated the congener correction factor except for the CRM sample (since the congener profile was not provided beforehand) and for the uncorrected soil and sediment results used to examine the benefits of applying the correction factor.

Sample preparation and analysis were modified from the methodology provided by Cape Technologies (Cape-tech, 2002) as described in Zhang, 2008. A detailed standard operating procedure, including a list of equipment, materials and chemicals needed for sample preparation,

extraction, oxidation and evaporation is provided as Appendix B. Sections 3.2 and 3.3 offer a brief outline of the sample preparation and ELISA steps.

3.2. Sample Preparation

Each soil and sediment sample was homogeneously mixed using a scoopula and a 5 g portion containing particles visibly smaller than approximately 2 mm was weighed out. The 5 g portion was dried with 4 g anhydrous sodium sulphate and the dioxin was extracted on a rugged rotator for 2 hours into 15 mL dimethylformamide (DMF). Approximately 10 g of CRM material (ID# PB2) were available (from the MOE Quality and Reference Section) from which eight replicates were prepared. The first contained 2 g of soil and was extracted into 6 mL DMF; the last seven contained 1 g of soil and were extracted into 3 mL DMF. CRM replicates only contained 1 or 2 g in order to maximize usage of the CRM supply. After extraction of soils, sediments and CRM, an aliquot of each sample in DMF (either 200 μ L or 1 mL, depending on the test level being run) was then solvent-exchanged into 6 mL hexane. The remaining amount of sample in DMF was stored for further experiments. The solution of DMF and hexane was oxidised on a rugged rotator for 15 minutes using 10 times the aliquot amount of fuming sulphuric acid (15% sulphur trioxide in concentrated sulphuric acid). The solvent mixture was centrifuged for five minutes at 2000 $\times$ g in order to achieve a good phase separation. The hexane supernatant was transferred into an evaporation tube containing 150 μ L keeper (80:20 methanol:polyethylene glycol [PEG] + 100 ppm Triton X-100). The hexane was completely evaporated at 55°C. The remaining keeper residue was reconstituted in either 70 or 120 μ L methanol, depending on the test level being run. Cape Technologies recommends additional cleanup for samples that exhibit cloudiness during reconstitution (Cape-tech, 2002). Since no cloudiness was seen, samples were tested without any further cleanup.

3.3. ELISA Analysis

The ELISA tubes and all ELISA reagents were purchased from Cape Technologies. The optimised ELISA procedure is described fully in Zhang, 2008 but is summarized here. All ELISA analyses were performed by Wei Zhang, who has been responsible for ELISA analysis in previous fish studies, in order to minimise variability in results due to switches in the operator. Ten samples were tested alongside two negative controls and eight standards. Standards were also prepared by Wei Zhang. An aliquot of the sample in methanol (either 50 μ L, or 5 μ L diluted with 45 μ L clean methanol, depending on the test level) was added to a washed ELISA tube containing 500 μ L sample diluent. The solution was allowed to incubate for two hours. The liquid was discarded and the tube was washed with 1 mL of Wash Solution 1, which is provided in the Cape Technologies kit. Once the wash is discarded, 500 μ L of competitor-horseradish peroxidase (HRP) conjugate was added in order to bind to the remaining free antibodies in the tube. After incubation in the dark, the liquid in the tubes was discarded and the tube was washed again four times using deionized distilled water (DDW). Five hundred microlitres of a HRP substrate solution was added into the ELISA tube. A blue colour then begins to develop. This solution was allowed to react in the dark after which 500 μ L of a stop solution was added to halt the colour production and turn the solution from blue to yellow. Colour production is inversely proportional to the amount of dioxin in the sample tube and the solution's absorbance was measured using a spectrophotometer (Abraxis LCC model no. 108921-500). The final raw ELISA result given in equivalents of 2,3,7,8-TCDD was obtained by converting the absorbance reading through use of a standard curve based on a four parameter equation described in Section 1.4.2.

3.4. Results

Soil Samples

From the 36 soil samples provided by the MOE, 10 were run in duplicate to make a total of 46 soil samples. Of these 46 samples, 7 were lost during sample preparation and 12 samples fell out of the optimal sensitivity range of the ELISA: between 5 and 35 pgTEQ per ELISA tube. When these 46 soil samples were analyzed, the sample algorithm had not yet been developed so the 12 samples that fell out of range were not re-run in their correct range; their results were omitted. The remaining 27 samples consisted of 7 samples run in duplicate and 13 unique samples. These samples were used to examine: (1) the correlation between ELISA and GC-HRMS, (2) the effects of using a correction factor and (3) the ability of ELISA to screen at 50, 1000 and 10,000 pgTEQ g⁻¹.

Sediment Samples

Twenty-one of the twenty-eight sediment samples provided valid results. The other seven sediments were not used since they provided inaccurate recoveries (1%, 16%, 22%, 27%, 29%, 31% and 1087%). Deletion of these experimental outliers was based on the following two rationales. Firstly, while samples should not be excluded solely on the basis of low recovery, the six samples with low recoveries mentioned here were all from the same experiment. This particular experiment used HRP conjugate that was suspected of being deteriorated and so it is possible that this ELISA lacked the usual sensitivity of the other experiments. Secondly, the sample with a recovery of 1087% is an obvious outlier. A spatial variability of dioxin content within the sample container itself could be one explanation. The samples used for ELISA and

GC-HRMS analyses were all taken from a larger sample container. It is possible that the dioxin content of the two sample portions were significantly different.

The 21 remaining sediment results were used to study: (1) the correlation between ELISA and GC-HRMS, (2) the effects of the correction factor, (3) the ability of ELISA to screen at 50, 1000 and 10,000 pgTEQ g⁻¹ and (4) the ability of the sample algorithm to place samples within the correct ELISA testing range (120, 900 or 6,000 pgTEQ g⁻¹).

3.4.1. CRM sample results

The eight CRM samples were run for two purposes. The first was to establish that our laboratory team is capable of using ELISA to accurately report a sample's dioxin concentration without prior knowledge of its value. The second purpose was to verify the assumption that although each soil and sediment sample portion was taken from a larger sample container, the dioxin content is evenly distributed within the container thus producing low spatial variability. This is a rather important assumption since GC-HRMS and ELISA analyses used different sample portions collected from the same container. Any spatial variability would reduce the comparability between the two techniques. The verification of this assumption was done by analyzing 8 separate portions of the CRM material taken from the larger sample container and comparing their predictions of dioxin content.

Figure 13 below shows the concentration of the CRM sample as determined from the eight sample portions taken from the same CRM sample container. Included is the average of the eight results and the variability, or uncertainty, in dioxin concentration calculated as ± 1 standard deviation.

Figure 13. ELISA results for the eight CRM samples and the $n=8$ average with error bars

The average CRM dioxin concentration was determined by ELISA to be 79 ± 19 pgTEQ g⁻¹ ($\pm$ SD). The uncertainty, calculated as ± 1 standard deviation (Fowle, 2003), is therefore 24%. The dioxin concentration of this CRM was previously determined by several GC-HRMS facilities as being 61 ± 7.4 pgTEQ g⁻¹ (with NATO TEF values) or 62 ± 7.7 pgTEQ g⁻¹ (with WHO98 TEF values). The ELISA CRM result is therefore roughly 30% above this value. However, the discrepancy can be attributed to an evaporation effect in the ELISA tube. In these experiments, the sample was reconstituted into 70 μ L of methanol but only 50 μ L were transferred into the ELISA tube. With such low volumes of methanol, any solvent evaporation will increase the concentration of the aliquot being transferred and thus will increase the amount of dioxin being detected by ELISA. The reason for transferring only a portion of the 70 μ L is not straightforward and is explained in Appendix C.

An uncertainty of 24% in trace level chemistry is not unusual. For the GC-HRMS labs that reported the dioxin values used in this thesis, uncertainty on 12 spiked soil samples over months

of multiple runs was found to be between 15 and 20% (Brunato and Macpherson, 2006). Since the uncertainty is based on artificial spikes and not real soil replicates from the same sample container, it may not be indicative of the uncertainty created in comparing two soil sample portions from the same sample container. However, for the purpose of this study, it is reasonable to assume that the GC-HRMS uncertainty on the dioxin content of soil and sediment samples is 20%.

The same GC-HRMS lab also has found that uncertainty on their CRM sampling is significantly lower (Dr Eric Reiner, personal communication). CRM samples are rigorously grinded and thus more uniformly mixed than regular soil and sediment samples. It is likely that the uncertainty of the soil and sediment samples being analyzed by ELISA is higher than 24%; however this value is a reasonable assumption to apply.

The uncertainties of 20% for GC-HRMS results and 24% for ELISA results will be added as theoretical error bars in Figures 14, 15, 18, 19 and 20.

The findings of the CRM replicates indicate that our laboratory team is capable of using ELISA to accurately report dioxin concentrations without prior knowledge of their values. The findings also show that there is relatively low variability among different sample portions originating from the same sample container. Therefore, a comparison between ELISA and GC-HRMS results is indeed possible.

3.4.2. Correlation between ELISA and GC-HRMS

The overall correlation between GC-HRMS results obtained using NATO TEF values and congener corrected ELISA results for soil and sediment samples with dioxin concentrations between 11 and 27,000 pgTEQ g⁻¹ is plotted in Figure 14 ($n=48$, $r^2=0.994$, slope=0.94). Included are the 90% confidence interval and the theoretical $\pm 20\%$ and $\pm 24\%$ x- and y-uncertainty bars.

Figure 14. Correlation between ELISA and GC-HRMS results for the $n=48$ sample set

A magnification of the low-lying data points is plotted in Figure 15.

Figure 15. Magnification of the low-lying datapoints found in Figure 14

When the uncertainty is accounted for, the majority of data points fall within the 90% confidence interval. Significant exceptions are the three rightmost data points in Figure 15; One of these samples introduced 34 pg of dioxin into an ELISA tube. This approaches the maximum allowable amount of 35 pg and may have led in this instance to a recovery that was slightly low. This sample could have been re-run at a higher testing level; however, it was run before the development of the sample algorithm. The other two are results from samples taken from the bottom of a harbour. These two samples will be discussed in greater detail below.

The r-squared value of the $n=48$ regression line is 0.994 with a slope of 0.94. This r-squared value is an improvement over what was reported by the manufacturers of the ELISA kit for a smaller set of 23 soil samples ($r^2=0.90$, Cape-tech, 2003a). Furthermore, the sample set in this

study spans a greater range of dioxin concentrations (11–27,000 pgTEQ g⁻¹) compared to the span of the manufacturer's sample set (50–1000 pgTEQ g⁻¹). It is believed that this improvement can be attributed to the optimized ELISA procedure developed in our laboratory.

Two aerial maps were produced using Google Earth (earth.google.com) of the dioxin contaminated site. The first displays the soil samples and the second displays the sediments. Each map superimposes the ELISA results as blue bars, the GC-HRMS results as yellow bars and the sampling depths as red numbers. The bars are all to scale except for values that exceed 430 pgTEQ g⁻¹ since they would not fit into the figures. These bars have a triangle icon on their peaks. A link between sampling depth and dioxin concentration was examined but no significant relationship was found. The aerial maps can be found as Figure 16 and Figure 17 on the following pages.

The major conclusion that can be drawn from the aerial maps is that in general ELISA has the ability to map a site just as well as GC-HRMS. For the majority of the samples, areas with high concentrations can be located with either ELISA or GC-HRMS results; conversely areas of low concentrations can equally be identified. However, ELISA does underestimate the TEQ of some samples. For two of these samples (the two leftmost points in Figure 17 with low underestimates) the fact that they were collected from deep water in the middle of the harbour may have influenced the ELISA result. Sediments at the bottom of the harbour may have other contaminants that affect the ability of ELISA to function properly. These samples may have benefitted from a sample cleanup step before analysis whereas samples collected in shallow water or on land did not seem to require cleanup.

When considering the other samples where ELISA underestimated TEQ values, it should be reemphasized that ELISA is intended only as a screening tool. Some of the samples whose dioxin levels have been underestimated by ELISA in fact fall within the same screening level (either below 50pgTEQ g⁻¹, between 50 and 1,000 pgTEQ g⁻¹ or above 10,000 pgTEQ g⁻¹) whether screened by ELISA or GC-HRMS. If this is the case then ELISA has been successful in its capacity as a screening tool despite dioxin underestimation. The overall ability of ELISA to act as a screening tool at the three screening levels will be discussed next.

Figure 16. Upstream aerial map of the studied site with an overlay of the soil depths in cm (red), ELISA results (blue) and GC-HRMS results (yellow)

Figure 17. Downstream aerial map of the studied site with an overlay of the sediment depths in m (red), ELISA results (blue) and GC-HRMS results (yellow)

3.4.3. Evaluating ELISA as a screening tool

Screening at 10,000 pgTEQ g⁻¹

Four soil samples had dioxin values that approached 10,000 pgTEQ g⁻¹ and thus were used to assess ELISA as a screening tool at the 10,000 pgTEQ g⁻¹ decision level. No sediments had dioxin concentrations that approached this screening level. A strong correlation was shown (Figure 18) between congener corrected ELISA results and NATO-based GC-HRMS results ($n=4$, $r^2=0.990$, slope=0.92).

Figure 18. Correlation between ELISA and GC-HRMS determined dioxin concentrations for samples near the 10,000 pgTEQ g⁻¹ screening level.

Screening at 10,000 pgTEQ g⁻¹ produced no false positives and no false negatives. Although conclusions drawn from only 4 samples may be limited, results seem to indicate that ELISA would be capable of screening at 10,000 pgTEQ g⁻¹.

Screening at 1,000 pgTEQ g⁻¹

Fourteen sediment samples and four soil samples with dioxin concentrations between 220 and 1600 pgTEQ g⁻¹ were used to assess the ability of ELISA to screen for dioxins at 1,000 pgTEQ g⁻¹ (Figure 19).

Figure 19. Correlation between ELISA and GC-HRMS determined dioxin concentrations for samples near the 1,000 pgTEQ g⁻¹ screening level.

Screening at 1,000 pgTEQ g⁻¹ produced no false positives and 2 false negatives. One point is considered a borderline false positive and another is considered a borderline false negative. A borderline false positive/negative is a false positive/negative sample with an uncertainty that crosses either of the screening lines. Since their uncertainties overlap a screening line, they will not be considered true false positives/negatives. The false negative rate at this screening level is therefore 11% and the false positive rate is 0%. Despite the false negative result for these two

samples, ELISA still predicts them to have dioxin levels significantly higher than background. In practice, ELISA has still successfully flagged those soils as warranting further analysis by GC-HRMS.

Screening at 50 pgTEQ g⁻¹

Five sediment and eighteen soil samples were used to assess the ability of ELISA to screen at 50 pgTEQ g⁻¹. The correlation between ELISA and GC-HRMS was much weaker at the 50 pgTEQ g⁻¹ decision level – likely because 50 pgTEQ g⁻¹ begins to approach the detection limit for ELISA. However, this is the lowest threshold for dioxin screening, so it is more important to note the rate of false negatives. Of the 23 samples in the range of 50 pgTEQ g⁻¹, one sample run in duplicate (circled on Figure 20) produced false negatives and there were no false positives. Three samples are considered borderline false positives and four samples are considered borderline false negatives but their uncertainties overlap the 50 pgTEQ g⁻¹ screening lines (dotted circles on Figure 20).

Figure 20. Correlation between ELISA and GC-HRMS determined dioxin concentrations for samples near the 50 pgTEQ g⁻¹ screening level

The false negative rate at this screening level is 9% and the false positive rate is 0%. The false negative result for the one duplicate sample is somewhat inconsequential since the dioxin concentration of the sample as determined by GC-HRMS is low (67 pgTEQ g⁻¹) and likely not dissimilar enough from its ELISA values (26 and 36 pgTEQ g⁻¹) to affect any decision about the possible health impacts of being exposed to this soil.

3.4.4. The benefits of the congener correction factor

By comparing the correlation between GC-HRMS TEQ values and ELISA results with and without application of the correction factor, it can be determined if the correction factor can successfully account for the differences between TEF and cross reactivity results and improve

the correlation between ELISA and GC-HRMS. This comparison was done for soils and sediments by looking at the correlation between ELISA and GC-HRMS results calculated with both NATO and WHO98 TEF values. The WHO98 TEF values were used instead of the WHO05 values since the GC-HRMS results were reported using the 1998 values.

Since congener profiles were available for each sample, the effects of applying sample-specific congener correction factors were examined in addition to examining the effects of applying site-wide correction factors. The soil and sediment site-wide correction factors used average congener profiles calculated with seven soil samples and two sediment samples, respectively. Recall from Section 2.1 that seven soils and two sediments were required to produce correction factors that are 95% of the time within 5% of the best estimate of the actual soil and sediment site-wide correction factors. Microsoft Excel was used to randomly select seven soil samples and two sediment samples. Their resulting correction factors are found in Table 29.

Table 29. Average site-wide correction factors for soil samples and sediment samples from the study site

	Soils (n=7)	Sediments (n=2)
Using NATO TEF values	0.878	0.795
Using WHO98 TEF values	1.11	1.01

The calculation for how the correction factors were calculated for the seven soil samples is shown in Appendix D.

When the sample-specific congener correction factors were applied, a minor decrease in the agreement between ELISA and GC-HRMS values calculated with NATO TEF was observed. However, the agreement between ELISA and GC-HRMS values calculated using WHO98 TEF improved by 11% (Table 30). Applying the site-wide correction factors gave similar results

however it decreased the agreement between ELISA and GC-HRMS values calculated with NATO TEF slightly more and improved the agreement between ELISA and GC-HRMS values calculated with WHO98 TEF slightly less.

Table 30. Effect of the correction factor on the correlation between ELISA and GC-HRMS

n = 48 (27 soils and 21 sediments)	Uncorrected ELISA	Applying Sample-specific correction factor	Applying site-specific soil and sediment correction factors
Correlation to GC-HRMS using NATO TEF values	Slope = 1.02 $R^2 = 0.992$	Slope = 0.94 $R^2 = 0.995$	Slope = 0.89 $R^2 = 0.991$
Correlation to GC-HRMS using WHO98 values	Slope = 0.83 $R^2 = 0.992$	Slope = 0.94 $R^2 = 0.994$	Slope = 0.92 $R^2 = 0.991$

Footnote:

While the soil and the sediment samples both had their individual correction factors, their results were combined to examine the correlation between ELISA and GC-HRMS.

These results seem to confirm that each sample would benefit from being corrected using its unique congener profile but site-wide correction factors are still capable of adequately accounting for the differences in how ELISA and GC-HRMS calculate TEQ values.

The findings of these results are two-fold. Firstly, it appears that congener corrected ELISA results have the same correlation to GC-HRMS TEQ values regardless of whether GC-HRMS uses WHO or NATO TEF values – in this case it is a correlation of 94%. An uncorrected ELISA will always correlate differently to GC-HRMS values depending on which TEF scheme GC-HRMS used to calculate TEQ. This implies that with the correction factor it no longer becomes necessary to state which TEF scheme you are comparing ELISA results to. Secondly, applying congener correction factors to ELISA results has the potential of improving the correlation between ELISA and GC-HRMS even when the factors are close to unity. In future work, it

would be valuable to confirm the beneficial effect of the correction factor for a site that produces a correction factor with a value that deviates significantly from unity.

3.4.5. Using the sample algorithm

Twenty-eight sediment samples were analyzed using the sample algorithm to determine how well it can place samples into their correct testing range. All samples were first run at the 900 pgTEQ g⁻¹ testing level then, if necessary, were run a second time at either the 120 or the 6,000 pgTEQ g⁻¹ level. Recall that a sample is considered to be within the correct range of the testing level being run when between 5 and 35pg are measured per ELISA tube. If less than 5pg are measured during the 120 pgTEQ g⁻¹ test then the results are reported as “below 30 pgTEQ g⁻¹”. Conversely, if greater than 35pg are recorded during the 6,000 pgTEQ g⁻¹ test then the results are reported as “above 10,500 pgTEQ g⁻¹”. The results, shown in Table 31 on the following page, indicate that of the 28 sediment samples, 24 were placed within their correct test range and 4 were not. This gives the sample algorithm an accuracy of 86%. Of the four incorrectly placed samples, Sed07 (which is the sample that had a recovery of 1%) was predicted by both the 1st and 2nd ELISA runs to be placed within the 120 pgTEQ g⁻¹ testing level. This implies perhaps that ELISA did not incorrectly underestimate the dioxin content of this sample but that the sample indeed had low levels of dioxin.

Table 31. Results of running the 28 sediment samples through the sample algorithm

Sample	1st ELISA results (pg/tube)	Is the 1 st ELISA result within the 900 pgTEQ g ⁻¹ range?	Is this decision correct?	2nd ELISA result (pg/tube)	Is the 2 nd ELISA result in or below the 120 pgTEQ g ⁻¹ test range?	Is this decision correct?	NATO TEQ	Correct Test Level
Sed 01	6.06	Yes	Yes				220	900
Sed 02	9.81	Yes	Yes				230	900
Sed 03	7.79	Yes	Yes				470	900
Sed 06	26.10	Yes	Yes				1600	900
Sed 08	18.22	Yes	Yes				1400	900
Sed 12	7.90	Yes	Yes				350	900
Sed 13	22.30	Yes	Yes				840	900
Sed 14	31.53	Yes	Yes				940	900
Sed 15	10.11	Yes	Yes				310	900
Sed 16	6.13	Yes	Yes				220	900
Sed 18	18.25	Yes	Yes				720	900
Sed 22	6.45	Yes	Yes				320	900
Sed 28	6.02	Yes	Yes				230	900
Sed 04	0.73	No, run at 120 pgTEQ g ⁻¹	Yes	4.38	Yes	Yes	77	120
Sed 05	1.06	No, run at 120 pgTEQ g ⁻¹	Yes	3.87	Yes	Yes	130	120
Sed 10	3.78	No, run at 120 pgTEQ g ⁻¹	Yes	5.03	Yes	Yes	73	120
Sed 11	3.62	No, run at 120 pgTEQ g ⁻¹	Yes	3.79	Yes	Yes	93	120
Sed 19	1.22	No, run at 120 pgTEQ g ⁻¹	Yes	0.31	Yes	Yes	55	120
Sed 20	0.05	No, run at 120 pgTEQ g ⁻¹	Yes	9.79	Yes	Yes	38	120
Sed 24	2.71	No, run at 120 pgTEQ g ⁻¹	Yes	2.91	Yes	Yes	65	120
Sed 25	1.18	No, run at 120 pgTEQ g ⁻¹	Yes	12.26	Yes	Yes	51	120
Sed 26	0.50	No, run at 120 pgTEQ g ⁻¹	Yes	12.76	Yes	Yes	49	120
Sed 23	4.27	No, run at 120 pgTEQ g ⁻¹	Yes	Sample was lost during preparation			220	120
Sed 27	1.40	No, run at 120 pgTEQ g ⁻¹	Yes	Sample was lost during preparation			62	120
Sed 09	21.06	Yes	No				70	120
Sed 17	6.05	Yes	No				170	120
Sed 21	8.19	Yes	No				150	120
Sed 07	1.09	No. Run at 120 pgTEQ g ⁻¹	No	4.44	Yes	No	1400	900

4. APPLICATION OF ELISA AS A SCREENING TOOL

Since the optimizations suggested in the previous sections have been shown to improve the accuracy and efficiency of an ELISA for dioxins in soil, the remaining task is to identify existing and future dioxin contaminated sites where ELISA can be applied. To do so, contaminated sites that currently exist within Canada are identified and the likelihood of an emergence of future dioxin contaminated sites in Canada is discussed..

4.1. New or Future Contaminated Sites in Canada

Dioxins are largely created and released as combustion by-products or industrial effluents. New contaminated sites would have to arise from large aerial depositions of dioxin air emissions or from industrial spills directly to soil. A spill database compiled by Ryerson University of all environmental spills in Ontario to land, air and water between 1987 and 2007 was examined for instances where dioxin may have been released. Only two incidents were found. The first was a 100 L spill into the St. Clair River of effluent with traces of dioxin at a concentration of only 0.2 parts per quadrillion. The second was a spill in 2005 of dioxin impacted groundwater into Snyder Creek in Waterloo that was considered to have only a potential of contaminating surface water. Both of these releases are into water and at low concentrations. As such, an ELISA for soil could not be used.

The National Pollution Release Inventory (NPRI) was also examined for instances across Canada where dioxin may have been released, disposed of or spilled into the air, water and soil since 2000 – the first year that dioxins were reported. No large releases were reported and the largest

emitters still only released trace quantities of dioxin very gradually throughout the course of a particular year.

The lack of spills in Ontario over the past 20 years and in the NPRI database is consistent with the findings of Environment Canada's *Risk Evaluation Determining Whether Environmental Emergency (E2) Planning is Required Under the Environmental Emergency Regulations set under the Canadian Environmental Protection Act* (Environment Canada, 2006). Dioxins are considered under section 199 of CEPA, 1999 and as such, it must be determined if they require an E2 plan. Environment Canada concludes that while dioxin is considered toxic under CEPA, 1999, no industries are known to distribute, store or use the chemical. Dioxins are produced only in trace amounts and do not have a realistic emergency pathway for large scale release. Since no spills are actually anticipated in Canada, Environment Canada does not require an E2 plan for dioxins.

The findings of the spill database and NPRI reporting and the lack of a dioxin E2 plan indicates that the occurrence of future dioxin contaminated sites in Canada is unlikely. An ELISA for dioxins in soil would be best used at existing Canadian dioxin contaminated sites.

4.2. Existing Contaminated Sites

Within Canada, ELISA would be very beneficial in screening for dioxins in many of the federal sites that have dioxin contaminated soils. The Federal Contaminated Sites Inventory (FCSI, 2005) lists all of the federally controlled contaminated sites across Canada. It includes details such as the types of contaminants, the volume of contaminated material and most importantly, the current status of the site. The current status of the site is important since it indicates which

sites would benefit from ELISA and which sites are not at a stage where ELISA could be used.

Table 32 below lists the Federal contaminated 10-step process and in which of those steps ELISA could be used.

Table 32. The contaminated site inventory steps in which ELISA could be used

Step	Number of Dioxin Contaminated Sites Currently at this Step	Can ELISA be used in this step?
1. Identify Suspect Sites	2	No
2. Historical Review	18	No
3. Initial Testing Program	4	Yes
4. Classify Contaminated Site Using the CCME National Classification System	32	No
5. Detailed Testing Program	6	Yes
6. Reclassify the Site Using CCME National Classification System	11	No
7. Develop Remediation/Risk Management Strategy	34	No
8. Implement Remediation/Risk Management Strategy	5	Yes
9. Confirmatory Sampling and Final Reporting	19	Yes
10. Long-Term Monitoring (Optional)	3	Yes

Currently, 37 sites exist where ELISA could be of some benefit. The remaining 97 sites, while not currently at a stage where ELISA could be of use, will at some point reach one of these stages. One caveat to using ELISA would be that some sites with contaminants such as hydrocarbons or large quantities of PCBs may have to be omitted since these contaminants could potentially interfere with the ELISA process. Nevertheless, matrix interference by PCBs and other hydrocarbons could be eliminated by sample cleanup before ELISA. Upon examination of the list of sites (which is too extensive to include in this thesis), it seems that for many, ELISA could prove to be an important asset to the remediation process. ELISA can increase the total

quantity of samples from a site, improve the mapping of contaminated areas, help re-evaluate the remediation strategy and decrease the cost of future site monitoring.

In addition to the 114 federally-controlled contaminated sites, many privately owned sites could also benefit from an ELISA for dioxins in soil. The major obstacle in dioxin testing of privately owned sites is that often the land owners would prefer not knowing if their property has dioxin contamination. A conversation in Fall 2007 with Dr. Jagani, president of AiMS Environmental revealed that dioxin testing at a site is only required when dioxin contamination is suspected. Therefore, it is unlikely that a land owner will voluntarily determine if the site has dioxin contaminated soil since this would introduce additional remediation costs. While this may be the case for privately owned lands, discussions with four analytical labs (summarized in Table 33) indicate that GC-HRMS testing for dioxins in soil is still frequently performed.

Table 33. The frequency of dioxin testing in soil at four analytical labs

Laboratory	Price per Sample	Sample Turnaround Time	Comments given towards the frequency of dioxin analysis.
Maxxam Analytics Inc.	\$900	20 samples in 15 days	They run dioxin soil testing everyday.
ALS Laboratory Group	\$700-1000	20 samples in 28 days	Testing for dioxins in soils is frequent.
AGAT Laboratories	\$750-800	15 samples in 14 days	They run dioxin samples all the time.
Wellington Laboratories Inc.	\$650	15 samples in 21 days	Busy but testing frequency is declining.

Dioxin testing through GC-HRMS is still frequently done. Therefore, developing ELISA as a screening tool can indeed prove to be beneficial as the market for dioxin testing is still strong.

5. CONCLUSIONS

The findings of this thesis have confirmed that ELISA is fully capable of functioning as a screening tool for dioxin contaminated soils and sediments. When the congener correction factor is applied, the correlation between ELISA and GC-HRMS was strong ($n=48$, $r^2=0.994$, slope=0.94). Inspection of two aerial maps has shown that ELISA has successfully identified areas of high and low dioxin concentrations from a site with contaminated soils and sediments. Finally, ELISA produced no false positives and low false negative rates of 0%, 11% and 9% for the three screening levels important to North American dioxin testing – 10,000 pgTEQ g⁻¹, 1,000 pgTEQ g⁻¹ and 50 pgTEQ g⁻¹. To add to these successes, a costing analysis of ELISA has shown that it can produce results three times faster than GC-HRMS and concomitantly, a 60% reduction in operating cost with the sample algorithm in place.

The congener correction factor resolves the discrepancy between how ELISA and GC-HRMS report dioxin concentrations to a degree that has not been previously published. This discrepancy has previously been addressed to some extent by either using an ELISA equivalent of TEQ (ELISA-EQ) when reporting values or by correcting site-specific datasets using empirical methods. Both of these methods are irrelevant to any practical use of ELISA as a screening tool for dioxins in soil. The concept of ELISA-EQ is not acceptable since it does nothing to account for the differences between GC-HRMS TEQ and ELISA results. ELISA would remain a technology that can only roughly approximate dioxin concentrations and thus fails to provide results that can be compared to policies and environmental guidelines, which always utilize GC-HRMS derived TEQ values. The use of empirical methods quoted in other

research (Nording et al., 2007) to correct ELISA results is unrealistic. The empirical correction factor involves applying the inverse slope of the correlation between ELISA and GC-HRMS to subsequent ELISA samples from that site. To produce an empirical factor, a restrictively large number of samples need to be analyzed by GC-HRMS. Without a large number of samples, an accurate determination of the correlation between ELISA and GC-HRMS (and hence an accurate empirical correction factor) could not be established. In sharp contrast, the correction factor developed in this study site requires only seven GC-HRMS determination for soil samples and only two GC-HRMS determinations for sediment samples. Furthermore, since the correction factor is essentially a method to calibrate ELISA results to GC-HRMS TEQ, it is constrained by the restrictions placed on calibration curves. That is, the calibration value is only valid for the range that was used to generate it. Therefore, the samples used to generate the empirical factor must cover the anticipated range of dioxin concentration values for that particular site. If a sample was analyzed by subsequent ELISA and found to be above the concentration range used to generate the empirical factor, the factor could not be used. This is again in sharp contrast to the correction factor developed in this work as it can be applied to ELISA results for any sample taken from a site, regardless of the sample's concentration. As a result of the flaws of empirical correction factors, useful, accurate ones would be very difficult to generate. They would depend on a great deal of initial GC-HRMS sampling, would potentially require additional GC-HRMS analysis during the course of ELISA site surveying and thus would command unacceptably large investments of time, money and resources,

Unlike the empirical approach, the congener correction factor proposed in this work is mathematically derived from first principles of how ELISA and GC-HRMS convert dioxin

concentrations into 2,3,7,8-TCDD equivalents. Therefore, it circumvents the problems inherent in ELISA-EQ and empirical correction factors thereby rendering them obsolete. In practice, the congener correction factor is the only viable method of correcting samples in real situations. In addition to being practical in ELISA site surveying, the correction factor is fully capable of accounting for the likelihood that ELISA will either incorrectly over- or under-report dioxin releases from many different dioxin emitting sources including smelters, incinerators and other combustion processes.

When ELISA results were corrected using sample-specific correction factors and were correlated to GC-HRMS TEQ, it seems that they correlated equally well to GC-HRMS regardless of which TEF scheme (WHO or NATO) GC-HRMS used. The impact of this finding is that unlike GC-HRMS, which could report in either NATO-TEQ or WHO-TEQ, a congener corrected ELISA simply reports its values and can be compared directly to both schemes. A fundamental impact is that major disagreements between NATO and WHO and between ELISA and GC-HRMS are removed.

For sites with dioxin contaminated soils, sample-specific correction factors cannot be used as they would rely on a GC-HRMS analysis for each ELISA analysis. Consequently, the use of site-specific correction factors is required for ELISA to be accurate. To generate site-specific correction factors, spearhead plots were generated for both the soil and sediment datasets. Examination of these spearhead plots determined that for this site, site-specific correction factors required averaged congener profiles of seven samples for soils and two samples for sediments. The use of averaged site-specific correction factors proved to have the same benefits of sample-

specific correction factors. Correlations between ELISA and WHO98-derived TEQ improved by 9% to a correlation of 92% and correlations between ELISA and NATO-derived TEQ remained strong falling from a correlation of 102% to a correlation of 89%. Similar to use of sample-specific correction factors, average site-specific correction factors can improve the correlation between ELISA results and GC-HRMS TEQ regardless of which TEF scheme is used.

The sample algorithm offers a simple solution to the problem of efficiently screening soils that may have widely different dioxin concentrations. US EPA Method 4025 (US EPA, 2002) and Cape Technologies (Cape-Tech, 2003a; Cape-Tech, 2003b; Cape-Tech, 2002) offer only methods to screen at one particular screening level and do not discuss screening across a continual range of values. A test of the sample algorithm successfully placed 24 of 28 samples into their correct testing level: either at 120, 900 or 6,000 pgTEQ g⁻¹. One other sample was likely placed in the correct testing level however these results disagreed with the GC-HRMS prediction for the dioxin content of that sample. Since the high success rate of the sample algorithm was accomplished in a maximum of two ELISA runs per sample, the sample algorithm improves the efficiency of ELISA as a monitoring tool for any given soil sample.

6. RECOMMENDATIONS

Based on the findings of this thesis, it is recommended that the sample algorithm and congener correction factor should be employed when using ELISA as a screening tool for soils and sediments. The two new concepts mark advancements in areas that have previously impeded the practicality of ELISA – the discrepancy between the methods used by ELISA and GC-HRMS to report total dioxin concentrations of a sample and the lack of a way to efficiently screen soils across a wide range of dioxin concentrations. Adoption of these concepts has finally transformed ELISA from a potentially useful into a fully functional screening tool. This reality was confirmed by an accurate mapping of soil and sediments at a dioxin contaminated site.

A second recommendation is that the congener correction factor be applied to other ELISA technologies. For example, the current PCB ELISA in the market suffers the same limitation of discrepancy to GC-MS. Adaptation of the congener correction factor would allow ELISA to better analyse for PCBs in soil, sediment, fish tissue and other matrices. The implication of this is that the correction factor can impact other markets beyond dioxin testing and can lead to the factor becoming a vital part of many different ELISA technologies.

It is proposed that ELISA be used as a screening tool at the 114 federally controlled sites with dioxin contamination. With a need for a minimal amount of GC-HRMS soil analyses at each site, ELISA can fulfil the initial testing, detailed testing, confirmatory sampling and long term monitoring steps detailed in the Federal Contaminated Sites Inventory 10-step process.

It is further recommended that sites with dioxin contaminated sediments should be targeted first since sediments will likely require fewer initial GC-HRMS analyses to produce a site-wide correction factor that will correct subsequent ELISA results. The implication is that contaminated water bodies and drinking water sources can be quickly and efficiently dealt with by ELISA.

ELISA should also be used to enable the Canada-Wide Standards for Dioxins and Furans to achieve their objective of a Virtual Elimination of dioxin emissions. Despite the ambitious aim of the CWS, the program targets only five industries that emit just 56% of 1999 Canadian atmospheric emissions (CCME, 2004) and two of these industries are struggling to meet even interim reduction goals. Additionally, backyard burning (which is the largest Canadian emitter of dioxins) is completely unregulated, small facilities that fall within the five targeted industries go unmonitored and dioxin emissions from many industries that are potential dioxin emitters are completely unquantified (CCME, 2004).

The Canadian Council of Ministers of the Environment has expressed a desire to increase dioxin monitoring and validate new pollution prevention technologies in order improve upon the performance of the CWS. The dioxin monitoring and pollution prevention technologies would rely on GC-HRMS; however, GC-HRMS testing is at capacity and would be unable to accommodate any additional testing. Therefore, ELISA, in conjunction with the correction factor and sample algorithm, should be used as a screening tool to offset some of the burden placed on GC-HRMS and allow it to become more available for the high-precision, high-accuracy testing that would be beneficial to the policies of the CWS.

Before ELISA can be used as a screening tool at federal contaminated sites, future in-house method development is required. ELISA must become an accredited method at the MOE by a certified Canadian environmental analytical laboratory auditor. This would be a key step in establishing a Canadian center of excellence in dioxin monitoring and cleanup.

Academic research is desirable to fully understand how sediment transport, dioxin source type and soil chemistry can affect the resulting correction factors. This knowledge could potentially promote use of correction factors specific to the dioxin source. The advantage of using source-specific correction factors is that no initial GC-HRMS testing at contaminated sites would be required before use of ELISA.

Finally, the benefits of a correction factor should be extended into use with air, water and food samples. ELISA can then become an integral component of all dioxin monitoring and testing, can contribute directly to an improvement of the Canada-Wide Standards for Dioxins and Furans, and can further mitigate the impact of dioxins on humans, wildlife and the environment.

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Appendix A – Methodology for the generation of the 200 randomly ordered datasets used to create the spearhead figures.

Appendix A presents the methodology applied to generate the 200 datasets that were used to create the spearhead figure found in the main text as Figure 6. The first step in the process is to sort the percent compositions of the 25 soil samples (S1—S25) using a random number generator. This produces a unique order of the 25 sample dataset (Table A1). From the order in Table A1, average percent compositions were calculated for sample sizes of $n=1,2,3,\dots,25$ (Table A2). For instance, the sample order in Table A1 indicates that the average percent composition for $n=4$ was calculated using the percent compositions of samples S7, S9, S10 and S2. Composite cross reactivity and TEF values (Tables A3 and A4, respectively) were then calculated by multiplying the individual cross reactivity and TEF values for each congener by its average percent composition at each sample size and summing the results. Finally, the correction factors for each sample size were calculated by dividing the composite TEF value by the composite cross reactivity value. Table A5 shows the correction factors calculated for each sample size for this particular sample order (presented in Table A5 as 'Dataset Order 1'). This entire process was repeated 200 times using a Microsoft Excel macro and the resulting correction factors for five other random dataset orders are also shown in Table A5.

Table A 1 Example of one random ordering of the percent compositions of the 25 soil samples

Random #	Sample #	1234678-heptachlorodioxin	1234678-heptachlorofuran	1234789-heptachlorofuran	123478-hexachlorodioxin	123478-hexachlorofuran	123678-hexachlorodioxin	123678-hexachlorofuran	123789-hexachlorodioxin	123789-hexachlorofuran	12378-pentachlorodioxin	12378-pentachlorofuran	234678-hexachlorofuran	23478-pentachlorofuran	2378-tetrachlorodioxin	2378-tetrachlorofuran	Octachlorodioxin
0.0093	S7	0.12	0.07	0.00	0.01	0.02	0.03	0.05	0.02	0.00	0.02	0.01	0.04	0.03	0.00	0.02	0.51
0.0280	S9	0.12	0.08	0.00	0.01	0.02	0.04	0.05	0.02	0.00	0.02	0.01	0.04	0.03	0.00	0.02	0.50
0.0704	S10	0.20	0.05	0.01	0.01	0.03	0.09	0.03	0.04	0.00	0.03	0.00	0.02	0.02	0.00	0.01	0.43
0.0724	S2	0.12	0.05	0.00	0.01	0.02	0.02	0.02	0.02	0.00	0.01	0.01	0.01	0.02	0.00	0.02	0.59
0.0990	S5	0.15	0.10	0.01	0.02	0.04	0.07	0.05	0.05	0.01	0.04	0.02	0.05	0.05	0.01	0.03	0.24
0.1002	S14	0.22	0.08	0.01	0.02	0.04	0.13	0.07	0.07	0.00	0.06	0.01	0.06	0.05	0.00	0.03	0.14
0.1580	S3	0.20	0.04	0.01	0.02	0.03	0.08	0.03	0.06	0.00	0.03	0.01	0.02	0.02	0.00	0.01	0.43
0.1696	S12	0.25	0.06	0.01	0.02	0.04	0.15	0.05	0.08	0.00	0.06	0.01	0.04	0.03	0.00	0.02	0.15
0.2133	S23	0.17	0.10	0.01	0.02	0.04	0.10	0.09	0.07	0.00	0.05	0.01	0.11	0.07	0.01	0.03	0.11
0.2569	S25	0.26	0.06	0.01	0.02	0.04	0.15	0.05	0.07	0.00	0.06	0.01	0.04	0.03	0.00	0.02	0.16
0.3092	S8	0.13	0.06	0.00	0.01	0.02	0.04	0.03	0.02	0.00	0.02	0.01	0.03	0.02	0.00	0.02	0.52
0.3505	S19	0.19	0.09	0.01	0.02	0.05	0.13	0.10	0.08	0.00	0.05	0.02	0.08	0.07	0.00	0.04	0.05
0.3710	S21	0.22	0.06	0.01	0.02	0.04	0.12	0.05	0.07	0.00	0.04	0.01	0.04	0.03	0.00	0.02	0.25
0.4408	S18	0.22	0.05	0.01	0.02	0.04	0.10	0.04	0.06	0.00	0.03	0.01	0.03	0.02	0.00	0.01	0.34
0.6495	S24	0.26	0.06	0.01	0.02	0.04	0.14	0.05	0.08	0.00	0.06	0.01	0.04	0.03	0.01	0.02	0.15
0.6654	S16	0.20	0.06	0.01	0.02	0.03	0.09	0.04	0.05	0.00	0.04	0.01	0.03	0.03	0.00	0.02	0.34
0.6873	S4	0.14	0.08	0.01	0.01	0.02	0.05	0.04	0.03	0.00	0.02	0.01	0.03	0.03	0.01	0.02	0.45
0.7103	S22	0.14	0.10	0.01	0.02	0.04	0.09	0.10	0.06	0.00	0.05	0.01	0.11	0.08	0.01	0.03	0.12
0.7138	S1	0.12	0.05	0.00	0.01	0.02	0.03	0.02	0.02	0.00	0.02	0.01	0.02	0.02	0.00	0.02	0.56
0.7454	S11	0.19	0.10	0.01	0.03	0.04	0.13	0.10	0.06	0.00	0.07	0.02	0.08	0.07	0.00	0.04	0.05
0.8346	S20	0.20	0.08	0.01	0.02	0.04	0.12	0.08	0.07	0.00	0.06	0.01	0.07	0.05	0.00	0.02	0.14
0.8527	S15	0.09	0.01	0.00	0.00	0.01	0.02	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.83
0.9118	S6	0.20	0.09	0.01	0.02	0.05	0.13	0.09	0.08	0.00	0.06	0.01	0.08	0.06	0.00	0.04	0.06
0.9316	S17	0.18	0.10	0.01	0.03	0.04	0.12	0.10	0.08	0.00	0.06	0.02	0.10	0.07	0.00	0.03	0.05
0.9920	S13	0.15	0.03	0.00	0.01	0.02	0.05	0.02	0.03	0.00	0.02	0.00	0.02	0.01	0.00	0.01	0.54

Table A 2 Average percent compositions of each congener for sample sizes of n=1,2,3,...,25

Congener	n=1	n=2	n=3	n=4	n=5	n+1	n=23	n=24	n=25
1234678-heptachlorodioxin	0.12	0.12	0.15	0.14	0.14	...	0.18	0.18	0.18
1234678-heptachlorofuran	0.07	0.08	0.07	0.06	0.07	...	0.07	0.07	0.07
1234789-heptachlorofuran	0.00	0.00	0.00	0.00	0.01	...	0.01	0.01	0.01
123478-hexachlorodioxin	0.01	0.01	0.01	0.01	0.01	...	0.02	0.02	0.02
123478-hexachlorofuran	0.02	0.02	0.02	0.02	0.02	...	0.03	0.03	0.03
123678-hexachlorodioxin	0.03	0.03	0.05	0.05	0.05	...	0.09	0.09	0.09
123678-hexachlorofuran	0.05	0.05	0.04	0.04	0.04	...	0.05	0.06	0.05
123789-hexachlorodioxin	0.02	0.02	0.03	0.03	0.03	...	0.05	0.05	0.05
123789-hexachlorofuran	0.00	0.00	0.00	0.00	0.00	...	0.00	0.00	0.00
12378-pentachlorodioxin	0.02	0.02	0.02	0.02	0.03	...	0.04	0.04	0.04
12378-pentachlorofuran	0.01	0.01	0.01	0.01	0.01	...	0.01	0.01	0.01
234678-hexachlorofuran	0.04	0.04	0.04	0.03	0.04	...	0.05	0.05	0.05
23478-pentachlorofuran	0.03	0.03	0.03	0.03	0.03	...	0.04	0.04	0.04
2378-tetrachlorodioxin	0.00	0.00	0.00	0.00	0.00	...	0.00	0.00	0.00
2378-tetrachlorofuran	0.02	0.02	0.02	0.02	0.02	...	0.02	0.02	0.02
Octachlorodioxin	0.51	0.50	0.48	0.51	0.45	...	0.31	0.30	0.31
Octachlorofuran	0.04	0.04	0.04	0.05	0.04	...	0.03	0.03	0.03

Table A 3 Composite WHO98 TEF values for sample sizes of n=1,2,3,...,25

Congener	WHO98 TEF	n=1	n=2	n=3	n=4	n=5	n+1	n=23	n=24	n=25
1234678-heptachlorodioxin	0.01	0.0012	0.0012	0.0015	0.0014	0.0014	...	0.0018	0.0018	0.0018
1234678-heptachlorofuran	0.01	0.0007	0.0008	0.0007	0.0006	0.0007	...	0.0007	0.0007	0.0007
1234789-heptachlorofuran	0.01	0.0000	0.0000	0.0000	0.0000	0.0001	...	0.0001	0.0001	0.0001
123478-hexachlorodioxin	0.1	0.0007	0.0007	0.0010	0.0009	0.0011	...	0.0017	0.0017	0.0017
123478-hexachlorofuran	0.1	0.0018	0.0019	0.0023	0.0022	0.0025	...	0.0032	0.0032	0.0032
123678-hexachlorodioxin	0.1	0.0034	0.0035	0.0052	0.0045	0.0050	...	0.0090	0.0091	0.0090
123678-hexachlorofuran	0.1	0.0046	0.0046	0.0041	0.0035	0.0039	...	0.0053	0.0055	0.0054
123789-hexachlorodioxin	0.1	0.0023	0.0024	0.0030	0.0027	0.0031	...	0.0051	0.0053	0.0052
123789-hexachlorofuran	0.1	0.0000	0.0001	0.0001	0.0001	0.0002	...	0.0001	0.0001	0.0001
12378-pentachlorodioxin	1	0.0206	0.0207	0.0247	0.0216	0.0259	...	0.0394	0.0404	0.0396
12378-pentachlorofuran	0.05	0.0003	0.0003	0.0003	0.0003	0.0005	...	0.0005	0.0005	0.0005
234678-hexachlorofuran	0.1	0.0044	0.0044	0.0037	0.0032	0.0035	...	0.0047	0.0049	0.0048
23478-pentachlorofuran	0.5	0.0158	0.0162	0.0139	0.0126	0.0151	...	0.0188	0.0196	0.0190
2378-tetrachlorodioxin	1	0.0020	0.0020	0.0024	0.0024	0.0049	...	0.0043	0.0043	0.0042
2378-tetrachlorofuran	0.1	0.0018	0.0019	0.0016	0.0017	0.0020	...	0.0022	0.0023	0.0022
Octachlorodioxin	0.0001	0.0001	0.0001	0.0000	0.0001	0.0000	...	0.0000	0.0000	0.0000
Octachlorofuran	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	...	0.0000	0.0000	0.0000
Composite TEF value		0.0597	0.0605	0.0645	0.0577	0.0699	...	0.0970	0.0995	0.0974

Table A 4 Composite cross reactivity values for sample sizes of n=1,2,3,...,25

Congener	ELISA cross reactivity	n=1	n=2	n=3	n=4	n=5	n+1	n=23	n=24	n=25
1234678-HpCDD	0.007	0.0009	0.0008	0.0010	0.0010	0.0010	...	0.0012	0.0013	0.0012
1234678-HpCDF	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	...	0.0000	0.0000	0.0000
1234789-HpCDF	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	...	0.0000	0.0000	0.0000
123478-HxCDD	0.016	0.0001	0.0001	0.0002	0.0001	0.0002	...	0.0003	0.0003	0.0003
123478-HxCDF	0.004	0.0001	0.0001	0.0001	0.0001	0.0001	...	0.0001	0.0001	0.0001
123678-HxCDD	0.079	0.0027	0.0027	0.0041	0.0036	0.0040	...	0.0071	0.0072	0.0071
123678-HxCDF	0.01	0.0005	0.0005	0.0004	0.0004	0.0004	...	0.0005	0.0006	0.0005
123789-HxCDD	0.39	0.0088	0.0092	0.0119	0.0106	0.0122	...	0.0200	0.0205	0.0201
123789-HxCDF	0.033	0.0000	0.0000	0.0000	0.0000	0.0001	...	0.0000	0.0000	0.0000
12378-PeCDD	1.05	0.0216	0.0217	0.0260	0.0227	0.0271	...	0.0413	0.0424	0.0416
12378-PeCDF	0.046	0.0002	0.0003	0.0002	0.0003	0.0004	...	0.0005	0.0005	0.0005
234678-HxCDF	0.049	0.0022	0.0021	0.0018	0.0016	0.0017	...	0.0023	0.0024	0.0024
23478-PeCDF	0.17	0.0054	0.0055	0.0047	0.0043	0.0051	...	0.0064	0.0066	0.0065
2378-TCDD	1	0.0020	0.0020	0.0024	0.0024	0.0049	...	0.0043	0.0043	0.0042
2378-TCDF	0.2	0.0036	0.0037	0.0032	0.0033	0.0040	...	0.0044	0.0045	0.0044
OCDD	0.00001	0.0000	0.0000	0.0000	0.0000	0.0000	...	0.0000	0.0000	0.0000
OCDF	0.00001	0.0000	0.0000	0.0000	0.0000	0.0000	...	0.0000	0.0000	0.0000
Composite cross reactivity value		0.0480	0.0489	0.0561	0.0502	0.0612	...	0.0886	0.0908	0.0889

Table A 5 Correction factors for six of the randomly ordered datasets for sample sizes of n=1,2,3,...,25

Dataset Order	n=1	n=2	n=3	n=4	n=5	n+1	n=23	n=24	n=25
1	1.2434747	1.238028	1.150067	1.149022	1.143142	...	1.094665	1.096141	1.095236
2	1.1508664	1.114487	1.115505	1.095876	1.074133	...	1.103843	1.099435	1.095236
3	1.143615	1.096656	1.047276	1.047617	1.072481	...	1.096042	1.09342	1.095236
4	1.1918521	1.219093	1.158994	1.15664	1.121179	...	1.091236	1.094585	1.095236
5	1.1173548	1.068991	1.132826	1.126969	1.113866	...	1.092519	1.091962	1.095236
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
200	1.1652523	1.09718	1.076373	1.114641	1.106812	...	1.097173	1.097802	1.095236

Footnote: The data presented in Tables B1 to B4 were used to calculate the correction factors listed as Dataset 1.

Appendix B – Draft of a standard operating procedure for soil and sediment samples at the MOE

This draft Standard Operating Procedure (SOP) describes the general methodology that has been developed to prepare samples in a soil matrix using Enzyme-Linked Immunosorbent Assay (ELISA). It has been written to describe the changes needed for soil analysis to the MOE Draft Method 3486 (Lo and Clarke, no date) and so it only includes sample weighing, extraction, oxidation and evaporation. These four steps are a modified version of the methodology presented by Cape Technologies (Cape-Tech, 2002).

1. APPARATUS**1.1. Equipment****1.1.1. Sample Weighing**

Digital scale - Explorer Pro Ohaus EP2102C
Calibration weights
Fume hood

1.1.2. Extraction and Oxidation

Rugged rotator
Centrifuge
Vortex mixer
Fume hood

1.1.3. Evaporation

Parker Balston Nitrovap Generator
Accublock Digital Dry Bath
Bucket for nitrogen purge
Nitrogen drying apparatus
Fume hood
Vortex mixer

1.2. Labware

1.2.1. Sample Weighing

Blue capped centrifuge tube – 1 for each sample
 Gloves
 Kimwipes
 Labelling tape
 Metal scoopula
 Protective breathing mask
 Small 50 mL beaker
 Teflon-capped glass vial – 1 for each sample

1.2.2. Extraction and Oxidation

16mm borosilicate glass vial – 1 per sample plus 2 additional
 16mm borosilicate glass vial with 1 mL marking
 16mm borosilicate glass vial with 2 mL marking
 16mm borosilicate glass vial with 6 mL marking
 16mm borosilicate glass vial with 10 mL marking
 16mm borosilicate glass vials M1, M2 and M3 for pipette cleaning
 Aluminum foil
 Clean 100 mL beaker for DMF
 Glass beads – 6 per sample
 Gloves
 Kimwipes
 Metal scoopula
 Parafilm
 Pasteur pipettes– 2 per sample plus one additional
 Positive displacement pipettor 100 μ L range
 Positive displacement pipettor replacement bores – 4 per sample
 Protective Eyewear
 Teflon-capped glass vial – 2 per sample plus one additional
 Teflon-capped vial with 15 mL marking

1.2.3. Evaporation

Aluminum foil
 Gloves
 Kimwipes
 Parafilm
 Pasteur pipettes to connect to the drying apparatus – one for each sample
 Protective Eyewear

1.3. Reagents

1.3.1. Sample Weighing

Anhydrous Sodium Sulphate

1.3.2. Extraction and Oxidation

Dimethylformamide (DMF)

Fuming sulphuric acid

Hexane

Keeper solution

Methanol

1.3.3. Evaporation

None

2. GENERAL CONSIDERATIONS

The sample weighing, extraction, oxidation and evaporation steps are written for the preparation of one soil sample at a time. However, 30 samples can be extracted and oxidized at once and 24 can be evaporated at once. Samples can be sealed with parafilm and stored in the fridge at any point in the procedure listed here. This SOP was written for soil samples that do not require clean up before ELISA analysis. It was also written to take the sample algorithm into consideration.

3. PROCEDURE

Note: as samples may contain high levels of dioxins and other contaminants, be sure to always wear gloves and a protective breathing mask and always work in a fume hood.

3.1. Sample Weighing

3.1.1. Tare a blue-capped centrifuge tube being held upright in a small 50 mL beaker on a digital scale. Use a metal scoopula to measure 4 g of anhydrous sodium

sulphate into the tube. Record the weight. Be sure to wipe the scoopula clean before proceeding to the next step.

- 3.1.2. Tare a Teflon-capped glass vial being held upright in a small 50 mL beaker on a digital scale.
- 3.1.3. Retrieve a soil sample and using a metal scoopula, stir the sample to ensure good homogeneity. If there are any large soil chunks, use the scoopula to break them apart.
- 3.1.4. Weigh out 5 g of soil into the tared glass vial and record the exact weight. The sample should contain only finely broken up soil with no vegetation, large chunks, rocks or any other debris.
- 3.1.5. Wipe off the scoopula with a clean Kimwipe or wash if necessary.

3.2. Extraction and Oxidation

- 3.2.1. Empty the anhydrous sodium sulphate from the blue-capped centrifuge tube into the glass vial containing the soil sample. Stir the mixture with a metal scoopula until the sample is free flowing.
- 3.2.2. In a fume hood, carefully decant DMF into a clean 100 mL beaker. Pour 15 mL of DMF into an empty Teflon-capped glass vial using the Teflon-capped vial with the 15 mL marking as a guide. Caution: DMF is a hazardous substance. Wear gloves and safety glasses and proceed with care.
- 3.2.3. Add the DMF and six glass mixing beads into the soil mixture.
- 3.2.4. After ensuring that the cap is tightened, attach the vial to the rugged rotator. To keep the wheel balanced, ensure that vials are attached in pairs across the wheel from each other.
- 3.2.5. Select a rotation speed that allows the solid mixture inside to tumble. To allow for complete extraction, the sample should remain on the wheel for 2 hours.
- 3.2.6. Check periodically to make sure that the solid mixture continues to tumble inside the vial and does not start to stick to the bottom.

Note: Be sure to also check that the tubes do not leak during the entire 2 hour extraction.

- 3.2.7. Centrifuge the sample for 15 minutes at $1000\times g$ to fully separate the different fractions. To keep the centrifuge balanced, ensure that vials are placed in pairs across the centrifuge from each other.
- 3.2.8. In a fume hood, carefully pour 6 mL hexane into a new borosilicate glass tube using the borosilicate glass tube with the 6 mL marking as a guide. Transfer this into a new Teflon-capped vial.
- 3.2.9. Using a positive displacement pipettor, transfer either 200 μ L or 1 mL of DMF extract to a Teflon-capped vial containing 6 mL hexane. Inject the DMF below the surface of the hexane. The amount of extract to be transferred is dependant on which test level is being followed. See the algorithm in Section 2.2 of the main thesis body for details.
- 3.2.10. Using a Pasteur pipette, transfer the remaining DMF into another Teflon-capped vial for storage. Extracts are stable for one month at room temperature in a tightly sealed vial. The soil mixture can now be discarded in the proper waste container.
- 3.2.11. In a fume hood, carefully pour either 2 mL fuming sulphuric acid into a new borosilicate glass tube using the borosilicate glass tube with the 2 mL marking as a guide or 10 mL fuming sulphuric acid into a Teflon-capped vial using the Teflon-capped vial with the 10 mL marking as a guide. The amount of acid to be poured is dependant on which dioxin decision level test is selected. See the algorithm in Section 2.2 for details. **Caution: fuming sulphuric acid is corrosive and hygroscopic. Wear gloves and safety glasses and proceed with care.**
- 3.2.12. Using a Pasteur pipette, slowly transfer the acid into the Teflon-capped vial containing hexane and DMF extract. The acid should not be exposed in air for too long as this may lead to adsorption of water which will decrease the SO_3 concentration in solution and cause incomplete oxidation of the sample.
- 3.2.13. Tightly cap the vial and reattach the sample to the rugged rotator. To keep the wheel balanced, ensure that vials are attached in pairs across the wheel from each other. The sample should be spun for 15 minutes to allow for complete oxidation. At the end of the oxidation, the vial should be warm but not hot.
- 3.2.14. Centrifuge the sample at $2000\times g$ for 5 minutes to achieve full separation of phases.

Note: At this point, the acid phase should be dark reddish brown and should have a clean separation from the hexane layer. If the DMF extract was originally dark then acid layer may also be black or dark grey. If the interface between the hexane and the acid is not sharp and shows signs of cloudiness or flocculation, oxidation was incomplete and **steps 3.2.11 to 3.2.14 should be repeated.**

- 3.2.15. Using a positive displacement pipettor, dispense 150 μ L PEG-Triton Keeper solution into the bottom of a fresh borosilicate tube.
- 3.2.16. Using a Pasteur pipette, transfer as much of the sample's oxidized upper hexane layer as possible into the tube with the Keeper solution. Be sure not to transfer any of the lower layer as this contamination will carry through into the ELISA analysis.
- 3.2.17. Cover the top of the tube in aluminum foil and wrap in parafilm. Store at room temperature until sample is to be evaporated. Typically the evaporation is performed one day before ELISA is run.

3.3. Evaporation

- 3.3.1. Turn on the waste air stream with tubing that leads to the white waste bucket. Leave the air running for 10 minutes to purge the system of any dust.
- 3.3.2. Attach a new Pasteur pipette for each sample that is to be evaporated to one of 24 hose endings coming from the drying apparatus connected to the dry bath. Make sure that all unused hose endings also have Pasteur pipettes attached as this prevents nitrogen from leaking from an open ending.
- 3.3.3. After running the waste air stream for 10 minutes, close this air valve and open the adjacent valve that is connected to the Nitrovap system. Turn the lever on the Nitrovap system from 'Standby' to 'Nitrogen On'. Nitrogen should now be flowing to the drying apparatus.
- 3.3.4. Adjust the nitrogen flowrate to 0.1 psi using the pressure gauge next to the drying apparatus.
- 3.3.5. Turn on the dry bath and adjust the temperature to 55°C.
- 3.3.6. Place the sample tube in a dry bath slot and slip the clean Pasteur pipette connected to the hose ending through the slotted scaffolding supports so that the pipette tip is blowing nitrogen onto the top of the sample tube.
- 3.3.7. Readjust the pressure again so that the nitrogen is swirling the liquid in the tube but the surface seems undisturbed. Blowing too much nitrogen will evaporate the sample too quickly and will lead to an excess loss of analyte.
- 3.3.8. Wrap the dry bath with a layer of tin foil to conserve the heat and protect the sample from any dust.
- 3.3.9. Periodically check the sample so that it can be removed as soon as it is finished evaporating. It is finished when there is only a small portion of Keeper remaining

on the bottom of the tube and there is no detectable hexane odour from the tube. Do not overdry the sample.

3.3.10. Once the sample has evaporated, remove it from the bath, seal it with an aluminum foil cover and parafilm it closed.

3.3.11. Turn off the dry bath, close the air valve feeding into the Nitrovap system and turn the lever on the Nitrovap system from 'Nitrogen On' to 'Standby'.

The remaining ELISA procedure is essentially unchanged from what is reported in (Lo and Clarke, no date) and so it is not included here.

Amount of sample in to the ELISA tube (µl)	Amount of methanol in the evaporated sample (µl)	Sample algorithm testing to methanol (µg)
20	120	200

Appendix C – The trade-off between ELISA accuracy and the elimination of detection gaps in the sample algorithm

When a sample is being analyzed for dioxin content by ELISA, the intent of the analysis must be clarified. There are two possible intents. One intent would be to screen samples over a wide concentration range in which case absolute accuracy is not essential. The other intent would be to determine the dioxin content as accurately as possible within a narrow concentration range. This intent would be relevant for applications such as food analysis. Depending on the desired intent, different sample preparation procedures should be followed.

For the first intent, the procedure outlined in the sample algorithm should be followed. The three test levels of the algorithm (the 120, 900 and 6,000 pgTEQ g⁻¹ tests) are capable of measuring for dioxin concentrations that range from 30 to 10,500 pgTEQ g⁻¹. However, in order to do so, a certain level of accuracy within the ELISA is lost. The reason for this is as follows. The final step in the ELISA process is to reconstitute the evaporated sample into a volume of methanol and transfer sufficient methanol into the ELISA tube for analysis. The methanol and transfer volumes for the three testing levels of the sample algorithm are shown in Table C1.

Table C 1. Reconstitution and transfer volumes for the testing levels of the sample algorithm

Sample algorithm testing level (pgTEQ g ⁻¹)	Amount of methanol to be added to reconstitute the evaporated sample (μL)	Amount of sample in methanol to be transferred to the ELISA tube (μL)
120	70	50
900	120	50
6,000	70	5

As shown in Table C1, the transferred amount of methanol is less than the amount of methanol needed for reconstitution. As such, any evaporation that occurs with the reconstituted methanol

will concentrate the amount of methanol being transferred into the ELISA tube and will cause higher than expected ELISA results.

For the three test levels of the algorithm, only a portion of the methanol is being transferred to ensure that the correct amount of dioxin is entering the ELISA tube. Adding the full amount of reconstituted methanol will alter the sensitivity range of the ELISA analysis thus causing detection gaps within the sample algorithm. For instance, if the full methanol volume was transferred for the 900 pgTEQ g⁻¹ test, then the ELISA would be sensitive only to dioxin values between 75 and 525 pgTEQ g⁻¹. Since the 6,000 pgTEQ g⁻¹ test is sensitive between 1500 and 10,500 pgTEQ g⁻¹, there would be a detection gap between 525 and 1500 pgTEQ g⁻¹. Due to various constraining variables, no mathematical solution was found that has three testing levels covering between 30 and 10,000 pgTEQ g⁻¹ while simultaneously introducing the full amount of reconstituted methanol. Therefore if the intent of analysis is to determine the actual dioxin content of a sample, there is a necessary trade-off between ELISA accuracy and the necessity of eliminating detection gaps within the algorithm.

For the second intent which focuses on a higher accuracy, it is not necessary to have a sample procedure that covers a wide range of dioxin concentrations. In other words, the problem of eliminating detection gaps does not exist. Therefore, sample preparation can be modified accordingly so that the full amount of reconstituted methanol is added into the ELISA tube. The effects of methanol evaporation are eliminated and a higher level of ELISA accuracy is maintained.

Appendix D – Calculation of the n=7 congener correction factor for soil samples

Appendix D presents the calculations required to generate the WHO98 and NATO n=7 site-wide soil correction factors. The first step in the process is to sort the congener percent compositions of the 25 soil samples (S1—S25) using a random number generator. This produces a unique order of the 25 sample dataset (Table D1). The first seven samples in Table D1 (S11, S14, S17, S21, S25, S4 and S8) were used to generate an average congener profile. This profile was used to calculate correction factors using WHO98 and NATO TEF values (Tables D2 and D3 respectively). As predicted, these n=7 site-wide correction factors were within 5% of the WHO98 and NATO n=25 site-wide correction factors, respectively.

Table D 1. Ordering of the 25 soil samples used to generate the n=7 site-wide correction factors

Random #	Sample #	1234678-heptachlorodioxin	1234678-heptachlorofuran	1234789-heptachlorofuran	123478-hexachlorodioxin	123478-hexachlorofuran	123678-hexachlorodioxin	123678-hexachlorofuran	123789-hexachlorodioxin	123789-hexachlorofuran	12378-pentachlorodioxin	12378-pentachlorofuran	234678-hexachlorofuran	23478-pentachlorofuran	2378-tetrachlorodioxin	2378-tetrachlorofuran	Octachlorodioxin
0.0991	S11	0.19	0.10	0.01	0.03	0.04	0.13	0.10	0.06	0.00	0.07	0.02	0.08	0.07	0.00	0.04	0.05
0.1305	S14	0.22	0.08	0.01	0.02	0.04	0.13	0.07	0.07	0.00	0.06	0.01	0.06	0.05	0.00	0.03	0.14
0.1452	S17	0.18	0.10	0.01	0.03	0.04	0.12	0.10	0.08	0.00	0.06	0.02	0.10	0.07	0.00	0.03	0.05
0.1631	S21	0.22	0.06	0.01	0.02	0.04	0.12	0.05	0.07	0.00	0.04	0.01	0.04	0.03	0.00	0.02	0.25
0.1869	S25	0.26	0.06	0.01	0.02	0.04	0.14	0.05	0.08	0.00	0.06	0.01	0.04	0.03	0.01	0.02	0.15
0.2013	S4	0.14	0.10	0.01	0.02	0.04	0.09	0.10	0.06	0.00	0.05	0.01	0.11	0.08	0.01	0.03	0.12
0.2077	S8	0.13	0.06	0.00	0.01	0.02	0.04	0.03	0.02	0.00	0.02	0.01	0.03	0.02	0.00	0.02	0.52
0.2098	S24	0.26	0.06	0.01	0.02	0.04	0.15	0.05	0.07	0.00	0.06	0.01	0.04	0.03	0.00	0.02	0.16
0.2180	S2	0.12	0.05	0.00	0.01	0.02	0.03	0.02	0.02	0.00	0.02	0.01	0.02	0.02	0.00	0.02	0.56
0.2212	S15	0.09	0.01	0.00	0.00	0.01	0.02	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.83
0.2267	S9	0.12	0.08	0.00	0.01	0.02	0.04	0.05	0.02	0.00	0.02	0.01	0.04	0.03	0.00	0.02	0.50
0.2570	S5	0.15	0.03	0.00	0.01	0.02	0.05	0.02	0.03	0.00	0.02	0.00	0.02	0.01	0.00	0.01	0.54
0.2792	S16	0.20	0.09	0.01	0.02	0.05	0.13	0.09	0.08	0.00	0.06	0.01	0.08	0.06	0.00	0.04	0.06
0.3163	S1	0.12	0.07	0.00	0.01	0.02	0.03	0.05	0.02	0.00	0.02	0.01	0.04	0.03	0.00	0.02	0.51
0.3264	S18	0.22	0.05	0.01	0.02	0.04	0.10	0.04	0.06	0.00	0.03	0.01	0.03	0.02	0.00	0.01	0.34
0.4717	S22	0.14	0.08	0.01	0.01	0.02	0.05	0.04	0.03	0.00	0.02	0.01	0.03	0.03	0.01	0.02	0.45
0.4733	S3	0.20	0.04	0.01	0.02	0.03	0.08	0.03	0.06	0.00	0.03	0.01	0.02	0.02	0.00	0.01	0.43
0.4924	S6	0.20	0.08	0.01	0.02	0.04	0.12	0.08	0.07	0.00	0.06	0.01	0.07	0.05	0.00	0.02	0.14
0.5319	S7	0.12	0.05	0.00	0.01	0.02	0.02	0.02	0.02	0.00	0.01	0.01	0.01	0.02	0.00	0.02	0.59
0.6216	S19	0.19	0.09	0.01	0.02	0.05	0.13	0.10	0.08	0.00	0.05	0.02	0.08	0.07	0.00	0.04	0.05
0.6392	S20	0.20	0.06	0.01	0.02	0.03	0.09	0.04	0.05	0.00	0.04	0.01	0.03	0.03	0.00	0.02	0.34
0.7023	S23	0.17	0.10	0.01	0.02	0.04	0.10	0.09	0.07	0.00	0.05	0.01	0.11	0.07	0.01	0.03	0.11
0.7357	S12	0.25	0.06	0.01	0.02	0.04	0.15	0.05	0.08	0.00	0.06	0.01	0.04	0.03	0.00	0.02	0.15
0.8612	S25	0.15	0.10	0.01	0.02	0.04	0.07	0.05	0.05	0.01	0.04	0.02	0.05	0.05	0.01	0.03	0.24
0.9006	S10	0.20	0.05	0.01	0.01	0.03	0.09	0.03	0.04	0.00	0.03	0.00	0.02	0.02	0.00	0.01	0.43

Table D 2. Calculation of the n=7 site-wide correction factor using WHO98 TEF values

Congener	n=7 average percent composition	WHO 1998 TEF	ELISA cross reactivity	Congener's contribution toward composite TEF	Congener's contribution toward composite cross reactivity
1234678-HpCDD	19.12%	0.01	0.007	1.91E-03	1.34E-03
1234678-HpCDF	7.91%	0.01	0.0002	7.91E-04	1.58E-05
1234789-HpCDF	0.64%	0.01	0.0002	6.42E-05	1.28E-06
123478-HxCDD	2.06%	0.1	0.016	2.06E-03	3.30E-04
123478-HxCDF	3.56%	0.1	0.004	3.56E-03	1.42E-04
123678-HxCDD	11.13%	0.1	0.079	1.11E-02	8.79E-03
123678-HxCDF	7.07%	0.1	0.01	7.07E-03	7.07E-04
123789-HxCDD	6.34%	0.1	0.39	6.34E-03	2.47E-02
123789-HxCDF	0.10%	0.1	0.033	9.96E-05	3.29E-05
12378-PeCDD	5.16%	1	1.05	5.16E-02	5.41E-02
12378-PeCDF	1.23%	0.05	0.046	6.15E-04	5.66E-04
234678-HxCDF	6.55%	0.1	0.049	6.55E-03	3.21E-03
23478-PeCDF	5.07%	0.5	0.17	2.53E-02	8.62E-03
2378-TCDD	0.45%	1	1	4.49E-03	4.49E-03
2378-TCDF	2.69%	0.1	0.2	2.69E-03	5.38E-03
OCDD	18.37%	0.0001	0.00001	1.84E-05	1.84E-06
OCDF	2.57%	0.0001	0.00001	2.57E-06	2.57E-07
	Composite TEF			0.124	—
	Composite cross reactivity			—	0.112
	WHO98 Correction Factor			1.11	

Table D 3. Calculation of the n=7 site-wide correction factor using NATO TEF values

Congener	n=7 average percent composition	WHO 1998 TEF	ELISA cross reactivity	Congener's contribution toward composite TEF	Congener's contribution toward composite cross reactivity
1234678-HpCDD	19.12%	0.01	0.007	1.91E-03	1.34E-03
1234678-HpCDF	7.91%	0.01	0.0002	7.91E-04	1.58E-05
1234789-HpCDF	0.64%	0.01	0.0002	6.42E-05	1.28E-06
123478-HxCDD	2.06%	0.1	0.016	2.06E-03	3.30E-04
123478-HxCDF	3.56%	0.1	0.004	3.56E-03	1.42E-04
123678-HxCDD	11.13%	0.1	0.079	1.11E-02	8.79E-03
123678-HxCDF	7.07%	0.1	0.01	7.07E-03	7.07E-04
123789-HxCDD	6.34%	0.1	0.39	6.34E-03	2.47E-02
123789-HxCDF	0.10%	0.1	0.033	9.96E-05	3.29E-05
12378-PeCDD	5.16%	0.5	1.05	2.58E-02	5.41E-02
12378-PeCDF	1.23%	0.05	0.046	6.15E-04	5.66E-04
234678-HxCDF	6.55%	0.1	0.049	6.55E-03	3.21E-03
23478-PeCDF	5.07%	0.5	0.17	2.53E-02	8.62E-03
2378-TCDD	0.45%	1	1	4.49E-03	4.49E-03
2378-TCDF	2.69%	0.1	0.2	2.69E-03	5.38E-03
OCDD	18.37%	0.001	0.00001	1.84E-04	1.84E-06
OCDF	2.57%	0.001	0.00001	2.57E-05	2.57E-07
	Composite TEF			0.099	—
	Composite cross reactivity			—	0.112
	NATO Correction Factor			0.878	