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Persistent organic pollutants (POPs) in soil associated with an active incinerator in Potchefstroom, South Africa

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Dissertation submitted for the degree Magister Environmental Sciences at the North-West University, Potchefstroom Campus.

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Abstract

POPs are a group of chemicals that have been extensively studied over the last few years. The main reason that these chemicals have received so much scientific attention is the myriad of negative effects they have on the environment and human health. The properties that cause the deleterious effects include a high molecular stability, rendering them highly persistent. Added to this is the lipophilic and hydrophobic nature of the compounds. POPs will thus tend to bio-accumulate and bio-magnify in the environment, causing a direct threat to humans and wildlife. To address this threat, the Stockholm Convention on Persistent Organic Pollutants, under the supervision of United Nations Environment programme (UNEP), was initiated and became legally binding on 17 May 2004. All countries, including South Africa, which ratified this agreement, will be expected to monitor and regulate the formation of POPs.

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are all members of the dioxin-like family of POPs. This family of chemicals pose serious health threats such as carcinogenic effects and negative effects on reproduction. These substances, with the exception of PCBs, are formed unintentionally as by-products of industrial and thermal processes. One of the main sources of dioxin-like chemicals is medical waste incinerators.

In this project the area surrounding a medical waste incinerator was monitored using a bio-assay technique. The determination of dioxin concentrations is usually preformed by chemical analysis, however, bio-assays have proven themselves to be a cheaper and time-saving screening method. The Toxic Equivalency Quotient (TEQs) determined through bio-assays can support chemical analysis in determining

biologically-relevant risk assessments since bio-assay data has ecotoxicological relevance. These assays represent an integrated biological response to chemical pollutants, where biological effects are accounted for which is not possible in chemical analyses. One of the bio-assays used in the determination of the dioxin-like chemical TEQ is the H411E reporter gene bio-assay. This assay is based on the Ah-receptor mediated toxicity of dioxin-like chemicals. Using this technique the TEQs for areas surrounding an active incinerator were determined, to indicate the distribution of these substances. The TEQs for the soil samples collected ranged between non-detectable and 154 ngTEQ/kg. There was no clear distributional pattern and the total organic carbon content in the soil did not seem to play a crucial role in the distribution of dioxin-like chemicals. Although a decrease in soil tillage showed a corresponding increase in TEQ. The predominant wind direction was taken into account but no correlation could be seen. However, meteorological parameters such as the ambient temperature and low precipitation in the area may have contributed to lower TEQ values. Cytotoxicity excluded data points and the phenomenon has to be addressed.

High TEQ values in a residential area where free-range chickens are raised pose a serious concern to the level of dietary dioxin-like chemical intake. Eggs in the area could theoretically contain between 2.75 and 28.75 pgTEQ/g egg fat. Further studies are needed to determine how much dioxin-like chemicals are being transferred to humans through the consumption of free-range eggs.

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Keywords: PCDD, PCDF, PCB, H411E reporter gene bio-assay, TEQ, medical waste incinerator, soil, distribution.

Die voorkoms van persisterende organiese besoedelstowwe in grond rondom 'n aktiewe verbrandingsoond te Potchefstroom, Suid-Africa.

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Opsomming

Persisterende organiese besoedelstowwe is 'n groep verbindings wat oor die afgelope paar jaar baie aandag onder die wetenskaplike gemeenskap geniet het. Die hoofrede hiervoor, is die talle negatiewe effekte wat die verbindings op die natuur en menslike gesondheid uitoefen. Die eienskappe verantwoordelik vir die skadelike effekte is onder andere die verbindings se hoë molekulêre stabiliteit sowel as die lipofiliese en hidrofobiese aard van die stowwe. Persisterende organiese besoedelstowwe het dus die geneigdheid om in biologiese materiaal te versamel en die konsentrasie van die stowwe verhoog soos wat hoër op in die hiërargie van voedselkettings beweeg word. Die versterkte effek van die besoedelstowwe bedreig beide die gesondheid van die omgewing sowel as dié van die mens. Om hierdie bedreiging aan te spreek is die Stockholmkonvensie onder leiding van die Verenigde Nasies tot stand gebring en op 17 Mei 2004 het dit internasionale wetgewing geword. Alle lande wat ondertekenaars is, Suid-Afrika ingesluit, onderneem om daarvolgens die produksie van die gevaarlike stowwe, soos in die Konvensie gelys, te moniteer en te reguleer.

Poligechloreerde dibenso-*p*-dioksiene (PCDDs), poligechloreerde dibensofurane (PCDFs) en poligechloreerde bifeniele (PCBs) behoort aan die dioksienagtige POPs groep. Die groep chemiese verbindings hou ernstige gesondheidsgevolge in, soos 'n verhoogde geneigdheid tot kanker en 'n negatiewe impak op die voortplantingstelsel. Hierdie stowwe, met die uitsondering van PCBs, word nie doelbewus geproduseer nie en word as byprodukte in industriële en termiese prosesse vervaardig. Een van die hoofbronne is verbrandingsoonde vir mediese afval.

Tydens hierdie studie is die omgewing om 'n mediese verbrandingsoond gemoniteer deur gebruik te maak van 'n weefselkultuursellyn tegniek. Die meting van dioksien-agtige stowwe se konsentrasies word normaalweg gedoen met behulp van chemiese analises, maar daar is bewys dat die weefselkultuursellyn-toetsmetode goedkoper en tydsbespared is. Die Toksiese Ekwivalensie Kwosiënt (TEKs) bepaal deur die weefselkultuursellyn-toets kan chemiese analises ondersteun in die bepaling van biologies-toepaslike risiko beramings. Hierdie metode stel 'n volledige biologiese reaksie voor op die chemiese besoedelstowwe, waar die biologiese effekte in ag geneem word. Dit is nie moontlik met chemiese analises nie.

Een van die weefselkultuursellyne wat gebruik kan word is die H4IIE-weefselkultuursellyn-toets. Hierdie toets is gegrond op die Ah-reseptor bemiddelde toksisiteit van dioksiene. Deur hierdie tegniek is die TEK vir die gebied om 'n aktiewe verbrandingsoond bepaal om die verspreiding van hierdie stowwe aan te toon. Die TEK-waardes vir die grondmonsters varieer tussen die deteksie drumpelwaarde en 154 ngTEK/kg. Daar was geen duidelike verspreidingspatroon waarneembaar nie en die totale organiese inhoud van die grond het geen beduidende invloed op die verspreiding van dioksiene gehad nie. Die mate van grondbewerking het egter wel 'n verwantskap met TEK waardes getoon. Hoe laer die mate van bewerking hoe hoër die ooreenstemmende TEK waarde. Die oorheersende windrigting is in ag geneem, maar geen duidelike verwantskap is waarneembaar nie. Meteorologiese parameters, soos die temperatuur en lae reënval in die area, kon moontlik 'n bydra gelewer het tot die lae TEK-waardes. Sitotoksisiteit het veroorsaak dat datapunte uitgesluit moes word en hierdie verskynsel moet aangespreek word.

Hoë TEK waardes in woongebiede waar vryloop hoenders aangehou word, kan ernstige gevolge inhou vir die hoeveelheid dioksien-agtige stowwe wat deur die mense se dieet ingeneem word. Eiers kan teoreties tussen 2.75 en 28.75 pgTEK/g eiervet bevat. Verdere studies is nodig om vas te stel hoeveel dioksien-agtige stowwe deur die inname van vryloop hoendereiers na mense verplaas word.

Erkenning vir finansiële ondersteuning deur die Nationale Navorsingstigting (NRF) word hiermee verleen en gevolgtrekkings is diè van die outeurs alleen.

Sleutelwoorde: PCDD, PCDF, H4IIE-weefselselkultuurlyn, TEK, verbrandingsoond, grond, dioksienverspreiding.

Contents

Abbreviations

AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of variance
APCS	Air pollution control system
Arnt	Aryl hydrocarbon nuclear translocator
ARC	Agricultural Research Council
CV	Coefficient of variation
CP	Chlorinated phenols
DMEM	Dulbecco's Modified Eagle's Medium
DRE	Dioxin responsive element
DWAF	Department of Water Affairs and Forestry
E	East
EC	Effective concentration
EDTA	Ethylene-diamine-tetra-acetic-acid
ELISA	Enzyme-linked immunosorbent assay
ENE	East-North-East
ESE	East-South-East
EROD	Ethoxyresorufin-*o*-deethylase
EU	European Union
FBS	Foetal bovine serum
$Fe(NH_4)_2(SO_4)_2$	Iron (II) ammonium sulphate
GC	Gas chromatography
GPS	Global positioning system
HpCB	Heptachlorinated biphenyl
HpCDD	Heptachlorodibenzo-*p*-dioxin
HpCDF	Heptachlorodibenzofuran
HPLC	High performance liquid chromatography
HSP90	Heat shock protein
HxCB	Hexachlorinated biphenyl
HxCDD	Hexachlorodibenzo-*p*-dioxin
HxCDF	Hexachlorodibenzofuran
I-TEF	International toxicity equivalency factors
I-TEQ	International toxic equivalency quotient
$K_2Cr_2O_7$	Potassium dichromate
Max	Maximum
Min	Minimum
MTT	3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
N	North
NE	North-East
NIEHS	National institute of environmental health sciences.
NNE	North-North-East
NW	North-West
NWW	North-West-West
OCDD	Octachlorodibenzo-*p*-dioxin
OCDF	Octachlorodibenzofuran
PBS	Phosphate buffered saline
PCPs	Polychlorophenols
PCB	Polychlorinated biphenyl
PCBzs	Polychlorobenzenes
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	Polychlorinated dibenzofuran
PeCB	Pentachlorinated biphenyl

PeCDD	Pentachlorodibenzo-*p*-dioxin
PeDDF	Pentachlorodibenzofuran
PHAH	Polyhalogenated aromatic hydrocarbons
POPs	Persistent organic pollutants
RLU	Relative light units
REP	Relative potencies
R^2	Correlation coefficient
S	South
SE	South-East
SSE	South-South-East
SSW	South-South-West
Stdev	Standard deviation
SW	South-West
TCB	Tetrachlorinated biphenyl
TCDD	2,3,7,8 – Tetrachlorodibenzo-*p*-dioxin
TCDF	Tetrachlorodibenzofuran
TDI	Total daily intake
TE	Toxicity equivalents
TEF	Toxicity equivalency factors
TEQ	Toxic equivalency quotient
TOC	Total organic carbon
UK	United Kingdom
UN	United Nations
UN-ECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
U.S. EPA	United States Environmental Protection Agency
W	West
WHO	World Health Organisation
WNW	West-North-West
WSW	West-South-West

Chapter 1: Introduction and Literature review

1.1. Introduction

Persistent organic pollutants (POPs) are a group of industrial and agricultural chemicals that exhibit several common properties (Corsolini, Kannan, Imagawa, Focardi & Giesy, 2002). These physical properties, which include a high molecular stability, resistance to chemical, photochemical and biological breakdown and miscibility with organic solvents (Safe, 1995), enhanced the usefulness of substances such as polychlorinated biphenyls (PCBs) and certain insecticides leading to their wide-spread use.

Before the environmental consequences became clear, these characteristics appeared to make these substances ideal industrial chemicals, insecticides and pesticides. These properties also increased the persistence of POPs in the environment (Godduhn & Duffy, 2003). Persistence means that neither transformation nor bio-degradation processes play an important role in the environmental cycling of these chemicals (Fiedler, 1996). Because the structure of POPs are not easily or readily changed, new releases into the environment will lead to an increase in their concentration (Fiedler, 1996). In conjunction with the above-mentioned characteristics, these compounds are normally hydrophobic, lipophilic and semi-volatile, increasing the likelihood of bio-accumulation (Godduhn & Duffy, 2003). Bio-accumulation is the process by which a chemical's concentration in an organism exceeds that in the environment (Webster, Cowan-Ellsberry & MacCarty, 2004). This characteristic is linked to the ability of POPs to cause a variety of short and long-term toxic responses in humans and wildlife (Corsolini *et al.*, 2002). These chemicals pose a serious risk to environmental and human health.

The most alarming characteristic POPs possess, is their tendency to become geographically widely distributed (Anon, 2004a). Certain POPs have the ability to undergo long-range atmospheric transport (Prevedouros, MacLeod, Jones, & Sweetman, 2004). Long-range transport leads to relatively-high concentrations in remote areas with little human activity. There are two main proposed forms of long-range transport of POPs in the atmosphere: chemicals that are transported through the one-hop process and chemicals that are transported through the multi-hop process (Breivik & Heimstad, 2005). The one-hop process occurs when pollutants

are transported by winds and deposited without having the capability to re-enter the atmosphere to the same extent as the multi-hop compounds. Multi-hop compounds are thought to represent the greatest portion of POPs. These chemicals have the capability to re-enter the atmosphere after initial deposition (Figure 1.1). Multi-hop chemicals evaporate, travel and condensate a number of times before being trapped, generally in colder areas (Breivik & Heimstad, 2005). These substances are not only transported through air but also through water and land transport of product and waste as illustrated in Figure 1.2. Combined with this, POPs are long-lived in the environment, and the global journey of a POP molecule, in theory, may take decades from its initial point of release until it is permanently trapped in an environment (Breivik & Alcock, 2002).

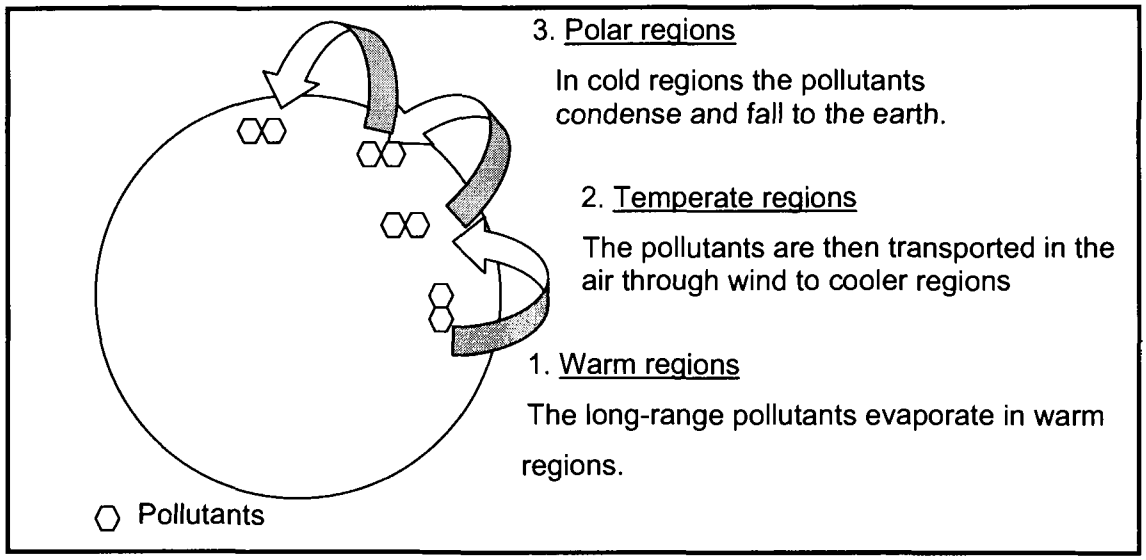

Figure 1.1: The multi-hop (grasshopper) movement of organic chemicals from a warm region to a polar region (adapted from Anon, 2005a).

Due to their long-range transport potential and harmful effects on man and the environment, an international agreement, the Stockholm Convention on POPs, was initiated to reduce future environmental burdens (Breivik, Alcock, Li, Bailey, Fiedler & Pacyna, 2004). The Stockholm Convention is a global treaty with the main objective to protect human health and the environment from the effects of POPs (Anon, 2004a). Contaminants listed in the Stockholm Convention are persistent, bio-accumulative and toxic, with the capacity to travel long distances by various pathways. The level and mechanism of toxicity, however, do not have to be understood for a chemical to be listed in the Stockholm Convention (Goddhunn & Duffy, 2003). South Africa ratified this agreement on 4 September 2001 (Stockholm

Convention, 2005) and like all other nations that ratified the Convention, agreed to lower emissions and ultimately eliminate the intentional and unintentional release of POPs into the environment (Bouwman, 2004). To this end, the sources of POPs must be quantified with a standardised and consistent methodology, in order to allow monitoring between countries (United Nations Environment Programme (UNEP), 2003). This Convention came into force on 17 May 2004.

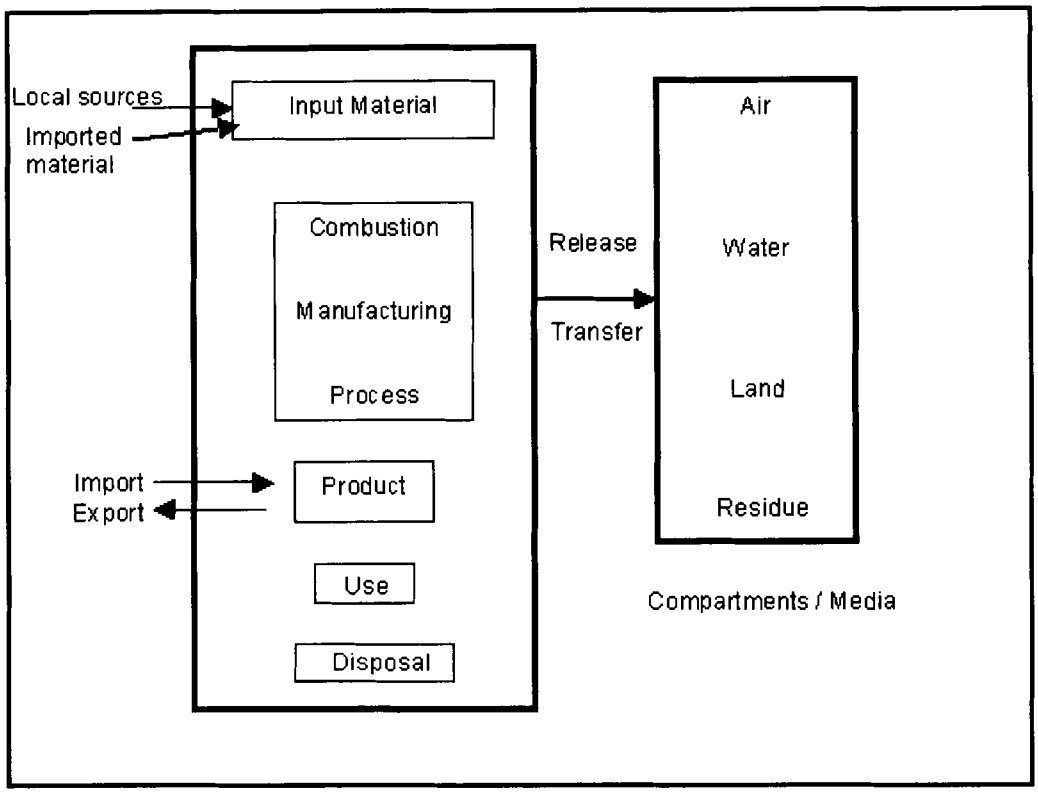

Figure 1.2: The life cycle and distribution of the dioxin group of persistent organic pollutants (UNEP, 2003).

During this study, three groups of POPs: polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like PCBs, collectively known as dioxin-like chemicals were studied. Dioxin-like compounds belong to a class of compounds known as polyhalogenated aromatic hydrocarbons (PHAHs) (Hurst, Balaam, Chon-Man, Thain, & Thomas, 2004), and are of the most toxic chemicals known to man. Furthermore, PCDDs, PCDFs and PCBs are listed in Annex C of the Stockholm Convention. These chemicals are unintentionally produced (in this context PCBs are mentioned as by-products formed during industrial processes) from anthropogenic sources. PCBs industrially produced are additionally included in Annex A, chemicals listed for elimination. The United States Environmental Protection Agency (U.S. EPA) recognises this group of chemicals as a threat to

public health (McKay, 2002) making them an important research focus point. Furthermore, the lack of accurate and complete data for POPs is considered one of the greatest shortcomings in understanding the distribution and fate of POPs in the environment (Breivik, Sweetman, Pacyna & Jones, 2002), making research in this area a necessity.

One of the main source categories of dioxin-like chemicals is combustion (Fiedler, 1996) and the largest single contributor to the release of dioxin-like chemicals being medical waste incineration (Tuppurainen, Halonen, Ruokojärvi, Tarhanen & Ruuskanen, 1998). For this reason, this project focused on the distribution of dioxin-like chemicals possibly released from an active incinerator burning a mixture of medical waste and animal carcasses.

The emission of dioxin-like chemicals from a medical waste incinerator tend to have a large portion of the total dioxin release deposited locally due to greater fraction of the dioxins being associated with larger particles and shorter stacks (Lohman & Seigneur, 2001). Accordingly the sampling area was in a 2.5 km^2 surrounding the incinerator based on a similar study by Dominigo, Schumacher, Llobet, Muller & Rivera (2001). As dioxins are lipophilic they tend to accumulate in the organic material of soil. Soil also tends to retard the movement of POPs once adsorbed due dioxin-like chemicals immobility and long half-life in the matrix (Nouwen, Cornelis, De Fré, Wevers, Viaene, Mensink, Patyn, Verschaeve, Hooghe, Maes, Collier, Schoeters, Van Cleuvenbergen & Geuzens, 2001). Soil is thus an ideal material for sampling.

Seeing as there were no dioxin analysis facilities in South Africa at the time of the study and that the analysis of these compounds internationally proved to be very expensive a cheaper alternative technique had to be implemented. Biological analyses are cost and time effective. One of these analysis techniques that can be used to determine the amount of dioxins in the soil is the H4IIE reporter gene assay (Hilscherova, Machala, Kannan, Blackenship, Giesy, 2000). This assay was implemented during the soil analyses for dioxins in this study

It is important to study the characteristics of dioxin-like chemicals in South Africa due to the fact that very little research has been done on these substances in South Africa. The main body of research into dioxin-like chemicals has been done in the Northern hemisphere in well-developed counties. The climatic and technological

differences that occur in South Africa to these previously researches areas make it necessary to seek new information on the current level and where possible characteristics of dioxins in the environment.

The aim of this project was to determine the possible environmental contamination and risks of dioxin-like substances in soils associated with an active incinerator in Potchefstroom. In order to achieve this aim, the following objectives were set:

- Collection and extraction of soil samples, as well as incinerator ash from all incinerators present in the Potchefstroom area.
- Investigate the usability of the H4IIE bio-assay in the assessment of soil samples.
- Determine the total organic content of the soil samples collected.
- Plot the results geographically, and investigate the possible influence of climatic factors on the distribution of dioxin-like chemicals.
- Assess the implications and potential risks of the contamination.
- Formulate recommendations.

This study, as far as I am aware of, constitutes the first investigation of its kind in South Africa, and probably Africa as well.

To assist in the planning, execution and interpretation of this project, an in-depth understanding of dioxin-like chemicals is required. This will be presented in sections 1.2 to 1.6.

1.2 Background to dioxins, dibenzofurans and dioxin-like PCBs

1.2.1. PCBs

PCBs were used in great quantities because of favourable chemical characteristics such as high chemical stability, low flammability, good heat conduction, a high dielectric constant and low electrical conductivity (Mason, 1991). These characteristics made PCBs ideal for use in a variety of open, nominally-closed and closed systems (Breivik *et al.*, 2004). Open uses included plasticizers, surface coatings, inks, laminating and impregnating agents and paints. Nominally-closed and closed systems included hydraulic and heat transfer liquids, transformers, capacitors, generators, and a number of other industrial applications (Breivik *et al.*, 2004, National Institute of Environmental Health Sciences (NIEHS), 2005).

Much of the environmental behaviour of PCBs can be related to their physical characteristics (McKay, 2002; NIEHS, 2005). The non-polar nature of PCBs indicates that these compounds are hydrophobic and strongly lipophilic. PCBs also exhibit a high predilection for smooth surfaces. Combined with the above-mentioned characteristics, it explains why these chemicals are so easily adsorbed onto soil and sediment particles (McKay, 2002). Furthermore, PCBs are also stabilised onto the surface of water bodies due to their physical and chemical properties (McKay, 2002). The distribution of PCBs throughout the world suggests that PCBs are transported mainly through air. The ability of PCBs to volatilise from landfills into the atmosphere and to resist degradation at low incinerating temperatures, makes atmospheric transport the primary mode of global distribution (World Health Organisation (WHO), 2000).

After PCBs were shown to effect mammalian reproduction and cause liver damage, the production of this chemical group was restricted. Although these chemicals are now no longer produced, thousands of tons still remain in equipment, storage and waste dumps (UNEP, 2004). These chemicals are also still found in the environment due to their accumulation in biological matrices (Mason, 1991; Axelman & Browman, 1999). In addition to industrial sources, PCBs can also be formed as unwanted by-products during large-scale industrial production and from biochemical processes in sewage and compost or chemical reactions (Langer, 1998).

Certain PCB isomers exhibit toxic effects similar to PCDDs and PCDFs. PCBs substituted with zero or one chlorine atom in the 2'2 or 6'6 (*ortho*) positions (Figure 1.3) on the phenol ring and one or more *meta* or *para* chlorines on each ring can assume a planar configuration. This leads to a molecule similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These coplanar PCBs are termed dioxin-like PCBs (Lemieux, Lee, Ryan & Lutes, 2001).

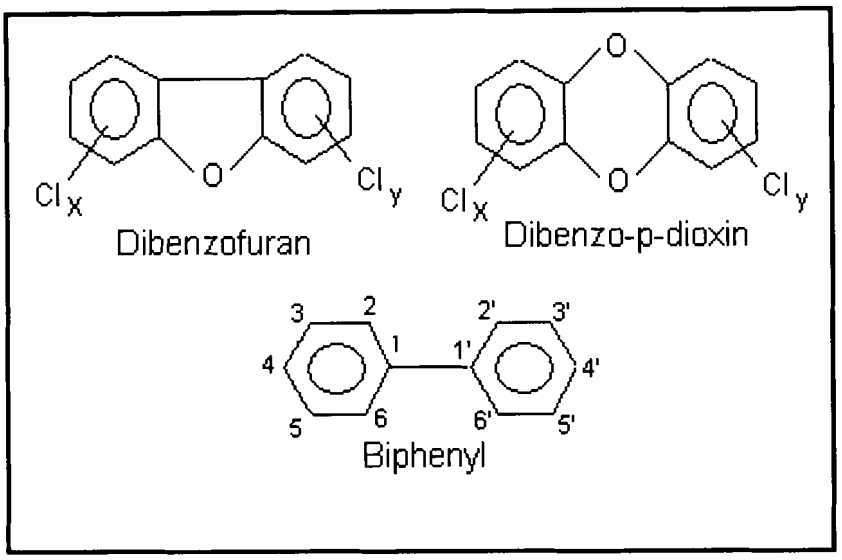

Figure 1.3: The chemical structure of dibenzo-*p*-dioxins, dibenzofurans and dioxin like-PCBs (adapted from Anon, 2004b).

1.2.2. PCDD/Fs

PCDDs and PCDFs (Figure 1.3) on the other hand, are not intentionally produced and have no industrial value (Fiedler, 1996) other than a small amount produced for scientific purposes. These chemicals are by-products of a number of industrial and thermal processes, especially those involving chlorinated chemicals (Fiedler, 1996). Furthermore, these chemicals can enter the environment through secondary sources such as landfills and compost, especially when compost and liquid manure are used in agricultural applications (Fiedler, 1996). It is suspected that these chemicals can also be formed through natural formation processes (Hoekstra, De Weerd, De Leer & Brinkman, 1999).

1.3. Dioxin formation and sources

PCDDs and PCDFs can be formed in a variety of industrial and thermal processes. Combustion sources especially contribute to ambient air levels (Fiedler, 1996). It has been shown that there are only four main components needed for dioxin formation: carbon, chlorine, oxygen and the presence of a metal catalyst (Ruokojärvi, Asikainen, Tuppurainen, Ruuskanen, 2004). Dioxin-like chemicals are especially formed during incomplete combustion where chlorine is available in the feedstock or in the air supply (Hays & Aylward, 2003).

1.3.1. Natural formation of PCDDs & PCDFs

Dioxin-like chemicals can be formed not only through anthropogenic, but also through natural processes. The presence of these substances in earth cores dated to periods before large-scale manufacturing and use of chlorinated chemicals have been confirmed (McKay, 2002). Additionally, residues in marine sediment cores suggest the natural formation of these chemicals on the surface of the ocean (Hashimoto, Wakimoto & Tatsukawa, 1995). However, the influence that long-range transport from land emissions could have had on the concentration of dioxin-like chemicals in these marine sediment cores, had, by then, not yet been elucidated (Hashimoto *et al.*, 1995).

These substances can also be formed biologically, especially in forest soils and sediments (McKay, 2002). Experiments done in the soil of a Douglas fir forest (Hoekstra *et al.*, 1999) have led to the development of a possible mechanism of formation. The first step in this mechanism depends on the natural formation of chlorinated phenols (CP) from organic matter and inorganic chloride through *de novo* synthesis or chloroperoxidase catalysed chlorination (Hoekstra *et al.*, 1999). This reaction then proceeds via an anion or radical reaction that would lead to the production of both PCDDs and PCDFs as indicated in Figure 1.4 (Hoekstra *et al.*, 1999). Formation has also been noted in sewage sludge and compost under normal environmental conditions. PCDDs and PCDFs are then formed by peroxidates from chlorinated organic reservoirs (McKay, 2002). Even though these substances are likely to be formed through biochemical and geochemical processes as well as natural combustion processes (forest fires, volcanoes), there has been a meaningful increase in environmental levels coinciding with the large-scale production and use of chlorinated chemicals (Hays & Aylward, 1993: McKay, 2002).

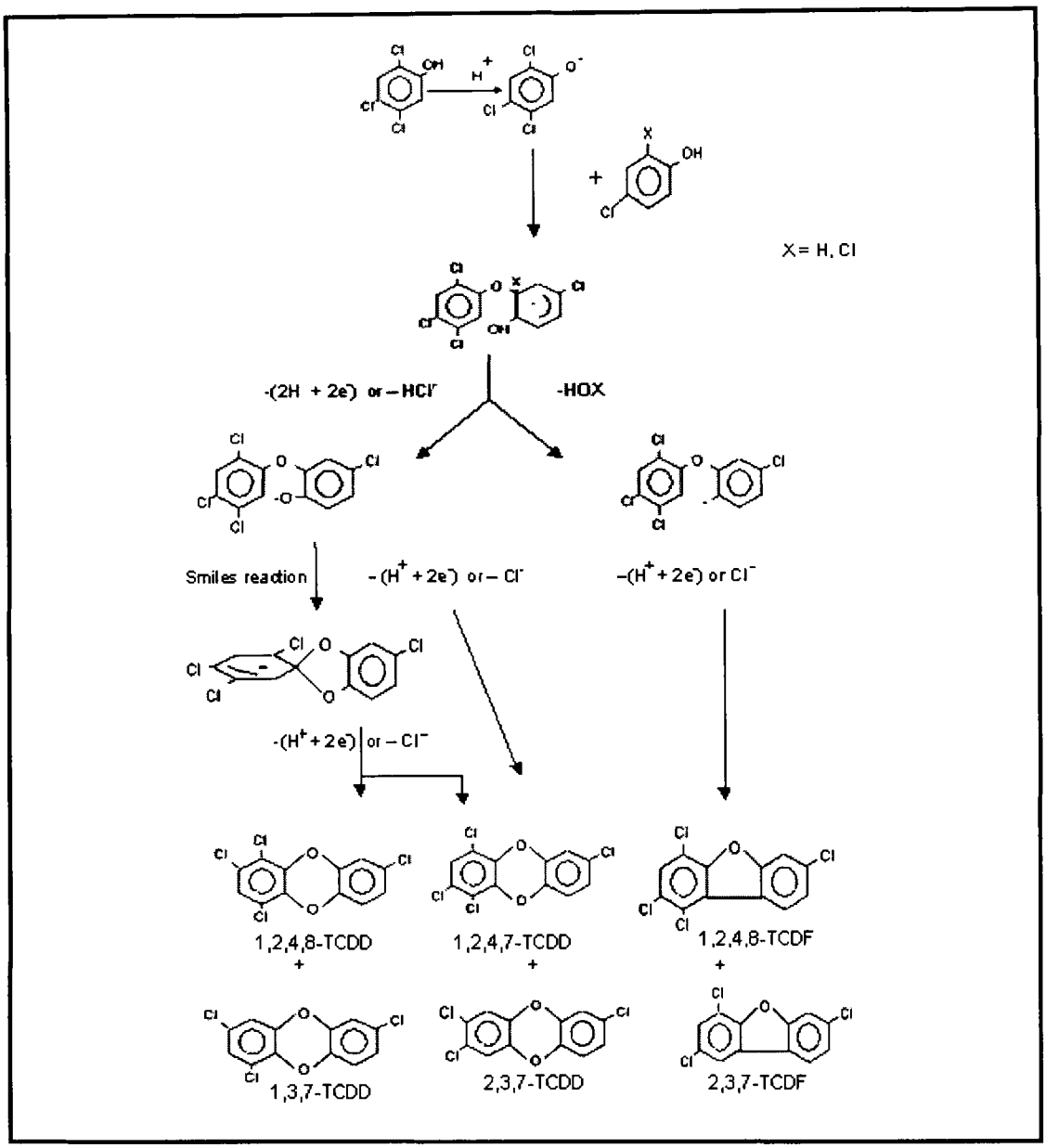

Figure 1.4: Proposed natural formation mechanism of PCDD and PCDF congeners mediated by peroxidase (Hoekstra *et al.*, 1999).

1.3.2. Major source categories of PCDDs and PCDFs

There are three main categories of dioxin sources: chemical-industrial sources, thermal, or combustion sources and reservoirs (Fiedler, 1996). According to Anderson & Fisher (2002) and UNEP (2003) there are four processes or sources from which dioxin-like chemicals can be released:

- chemical production processes (chloro-chemical industries and paper and pulp industry);

- thermal and combustion processes (waste incinerators, power generation and metal production);
- biogenic processes (formation of dioxins from precursors such as penta-chlorophenol); and
- reservoir sources (historical pesticide stores, dumps and contaminated sites).

The difference between the two groups of categories is that the second group includes a separate category for biogenic processes that could lead to the formation of dioxin-like chemicals.

The U.S. EPA has estimated that 70 % of all quantifiable environmental emissions were contributed by air emissions from three source categories: municipal waste incineration, backyard burning and medical waste incinerators (Van Overmeire, Clark, Brown, Chu, Cooke, Denison, Baeyens, Srebrnik & Goeyens, 2001). Medical waste incinerators are probably the largest contributors to the formations of PCDDs and PCDFs, followed by municipal waste incinerators and landfill fires (Tuppurainen *et al.*, 1998). Medical waste can be defined as solid waste generated during the treatment, diagnoses or immunisation of humans and animals (Lee, Liow, Tsai, & Hsieh, 2002). The incineration of various wastes or the combustion of various materials containing chlorine, lead to the formation and emission of polychlorobenzenes (PCBzs), polychlorophenols (PCPs), PCBs, PHAHs, PCDDs and PCDFs (Lavric, Konnov & De Ruyck, 2005).

1.3.3. Formation of PCDDs and PCDFs

Flame chemistry in incineration systems involves the formation of many organic products of incomplete combustion, including dioxin-like chemicals (Figure 1.5). There are two temperature windows in which dioxins and furans can be formed. The homogenous route describes the pathway where these substances are formed at temperatures between 500 and 800 °C and the heterogeneous route where the temperature window of formation is between 200 and 400 °C (Stanmore, 2004). Trace quantities of PCDDs and PCDFs can be formed under appropriate conditions when carbon, hydrogen and chloride are present. Formation may be in the vapour phase or on solid surfaces such as soot or ash particles (Stanmore, 2004).

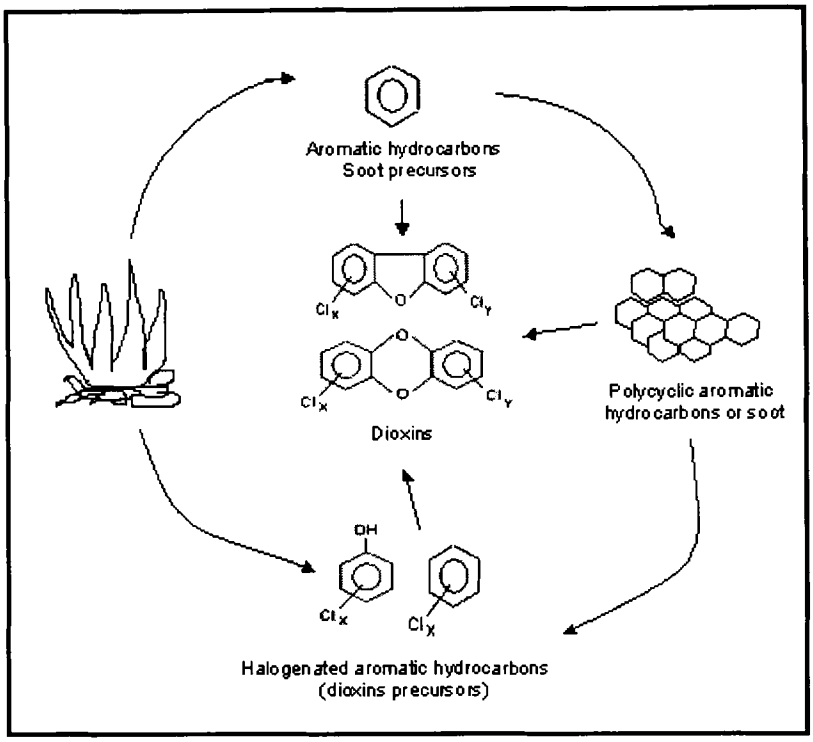

Figure 1.5: The formation pathway of dioxins during combustion processes (adapted from Anon, 2004c).

These substances are mainly formed through pyrosynthesis of small hydrocarbons or from the decomposition of aromatic macromolecules (Fullana & Sidhu, 2005) through three pathways. (1) The homogeneous route is the result of pyrolitic rearrangement of chlorinated precursors (small organic molecules), such as chlorophenols and chlorobenzens in the gas phase (Figure 1.6). This route occurs at high temperatures. (2) The heterogeneous formation (Figure1.7) is a catalysed reaction, which takes place on the ash or soot particles present in combustion systems (Stanmore, 2004). The formation of dioxins through the heterogeneous route can be divided into four primary stages (Tuppurainen *et al.*, 1998):

- Formation of ashes, products of incomplete combustion, carbon monoxide, volatile compounds and organic radicals.
- Formation of surface-active compounds with absorbed dioxin precursors, transitional metal salts, and oxides.
- Occurrence of complex organic reactions.
- Partial de-sorption of products from the surface.

(3) The third route, the *de novo* formation, occurs at lower temperatures between 250-350 °C and involves the oxidisation and chlorination of any unburned carbon in the particles present. The reaction pathway is based on the presence of pre-existing

macro-molecular structures such as 3-ring carbon skeletons (Tuppurainen, Halonen, & Ruuskanen, 1996; Stanmore, 2004). For the *de novo* synthesis to occur, oxygen is essential. The formation rate increases with the oxygen concentration by a reaction order of approximately 0.5 (Huang & Buekens, 1996). Precursor routes are classified into further subcategories (Altwicker, 1996; Lavric *et al.*, 2005):

- Formation from chemically similar compounds.
- Rapid formation and combustion of intermediates.
- Pathways to PCDFs (the mechanism of *de novo* reactions does not explain PCDF formation well and it is necessary to look at other sources).
- Formation from carbonaceous matrices within fly ash (*de novo* synthesis).
- Other *de novo* synthesis mechanisms that include C, H, O and Cl.

Various classes of precursors are capable of dioxin formation, with the possession of an aromatic ring or chloride and oxygen atom not being a prerequisite. For this reason, there is a large number of different compounds in flue gas, which can contribute to dioxin formation (Addink & Olie, 1995). The most important of these routes seems to be the homogeneous pathway (Tuppurainen *et al.*, 1996).

In a thermal system, the final dioxin emission will result from the difference between the rates of formation and thermal degradation. For this reason, the degradation of dioxins is an important consideration in the total formation of dioxins. The degradation temperatures of dioxin-like chemicals are higher than those for formation, illustrating the importance of a sufficiently high operating temperature (Stanmore, 2004). Dioxins are also formed in the post-combustion zone as illustrated by the increase of dioxin concentrations as the flue gas leaves the combustion chamber (Addink & Olie, 1995). In this area the temperatures are lower and conditions ideal for dioxin formation with the fly ash acting as a catalyst (Addink & Olie, 1995). Finally, dioxin emissions from combustion sources can also occur due to dioxin contamination of the raw fuel (Huang & Beukens, 1996).

The formation of dioxins in these systems can, however, be controlled through upgrading the plants and the addition of systems to reduce pollutant emissions. In modern facilities, with the proper processing, the problem of dioxin formation can be controlled to a major extent (Ruokojärvi *et al.*, 2004). Incinerators with high quality air pollution control systems (Addink & Altwicker, 2004), reduced emissions of PCDD and PCDFs through end-of-pipe removal techniques, the use of chemical inhibition, control of waste composition, improvement of combustion conditions and prevention

of formation in the post-combustion zone can all lower the PCDD and PCDF emissions (Ruokojärvi *et al.*, 2004).

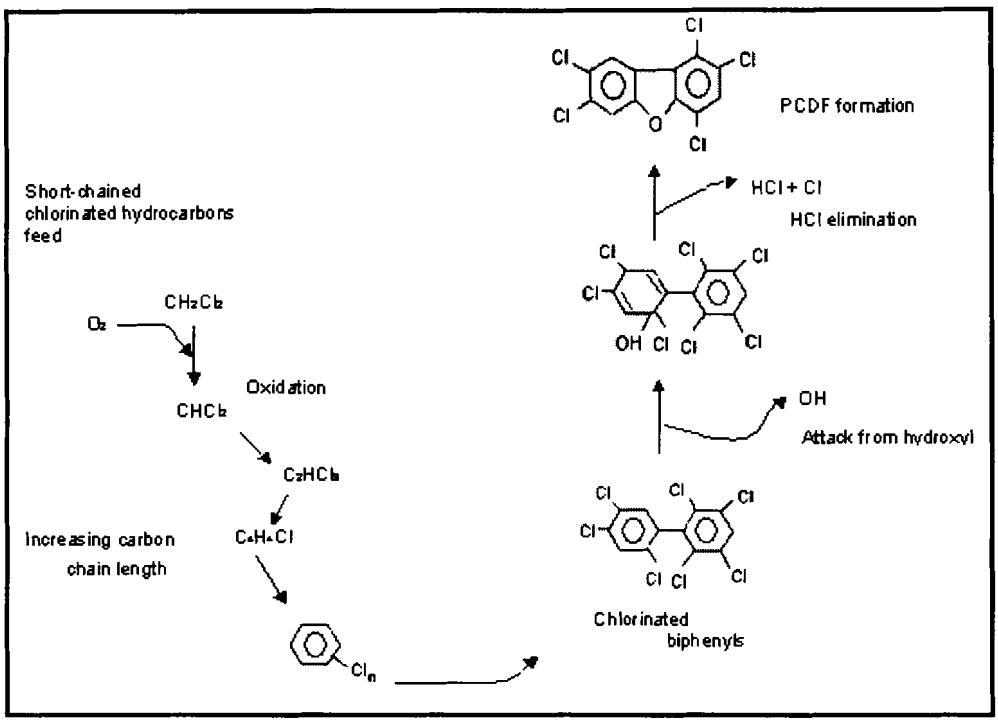

Figure 1.6: Mechanism of the homogenous pathways for PCDD/F formation (Environment Australia, 1999).

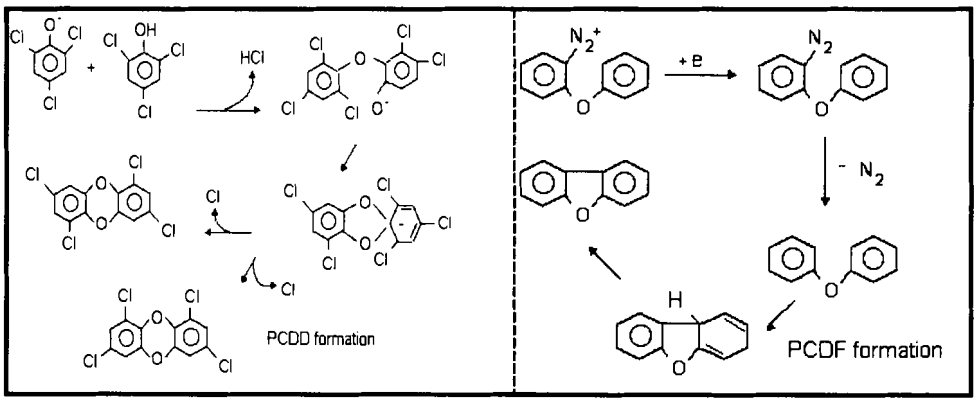

Figure 1.7: Mechanistic view of the formation of PCDD and PCDF in the fly-ash catalysed precursor pathway (Environment Australia, 1999).

1.4. PCB formation

Sources of PCBs are discussed in section 1.2.1. The production of industrial PCBs involves the chlorination of biphenyl in the presence of a catalyst, and depending on reaction conditions, the chlorination can vary between 21 and 68% (Breivik *et al.*, 2004). It is generally believed that emission from combustion sources results from the incomplete destruction of these industrial PCBs. However, PCBs can also be synthesised during combustion processes (Lemieux *et al.*, 2001). With the implementation of more stringent laws concerning the intentional production of PCBs, the significance of unintentionally produced PCBs becomes increasingly important.

Although there has been relatively little research done on the specific formation of PCBs, the following mechanism has been postulated corresponding to the formation of PCDDs and PCDFs (Lemieux *et al.*, 2001; Dyke, 2005):

- PCBs present in the fuel can pass through the combustion process un-destroyed or partially destroyed leading to emissions of this substance.
- PCBs may be formed in the gas phase during combustion.
- PCBs may also be formed by heterogeneous reactions involving precursor chemicals or *de novo* synthesis from carbon in the presence of particulate ash.

It has also been found that PCBzs can be formed directly through radical mechanisms or through the combustion of chlorine and chlorophenyl radicals. The combustion of two chlorophenyl radicals then gives rise to PCBs (Tuppurainen, *et al.*, 1998). For the formation of PCBs, as for the formation of dioxins, the two most important parameters are the residence time of the gases in the post-combustion zone, and the small-size fraction of the particulate matter in the system (Dyke, 2005).

As with dioxins, the optimum temperature for the formation of PCBs in the *de novo* synthesis is 300 °C, with an optimum formation at 350 °C. As the oxygen concentration decreases, there is a corresponding shift towards lower chlorinated congeners, suggesting that an electrophilic aromatic substitution occurs (Schoonenboom, Tromp & Olie, 1995). The formation then proceeds through a two-stage mechanism:

- First the surface of a carbon is chlorinated through an electrophilic aromatic substitution.

- Then oxidative decomposition of the chlorinated carbon occurs, yielding side-by-side chlorinated PCBs, PCDDs and PCDFs (Schoonenboom *et al.*, 1995).

1.5. Transport and environmental fate of dioxin-like chemicals after formation

1.5.1. Chemical structure and properties of PCDDs, PCDFs and PCBs

As previously discussed PCDDs, PCDFs and dioxin-like PCBs have similar chemical structures, as is illustrated in Figure 1.3. These chemicals therefore share common chemical and physical properties. Each of these chemical groups is comprised of two benzene rings connected by oxygen or carbon bonds. In the structure of PCDDs, two oxygen atoms on either side of the molecule connect the benzene rings. In PCDFs the benzene rings are connected by an oxygen bond on one side of the molecule and a carbon bond on the other (McKay, 2002). Presently there are 75 dioxin and 135 dibenzofuran congeners known to man (Stanmore, 2004). Only 17 congeners have been shown to have potential health risks, while the rest of the congeners are thought to pose no risk to human health (Seys, 1997). There are 209 possible PCB congeners, however, only 130 of these have been identified in commercial products (WHO, 2000). Environmental PCB residues normally contain complex mixtures of congeners and bring about a broad spectrum of biological responses (Langer, 1998).

PCDDs, PCDFs and dioxin-like PCBs have a number of characteristics that make them an important environmental concern including the following (Van Overmeire, *et al.*, 2001; McKay, 2002; Breivik & Alcock, 2002):

- high melting point;
- low vapour pressure;
- good stability and affinity for non-polar conditions;
- accumulation and bio-magnification in the food chain due to fat solubility;
- pronounced resistance to metabolic degradation;
- tendency to be strongly absorbed on surfaces of particulate matter; and
- semi-volatility.

These properties import the ability to cause deleterious effects on cells and tissue (Hilscherova *et al.*, 2000). Even though these chemicals are highly persistent, and degradation takes an extended period to occur, the levels and environmental fate of

these chemical groups has never specifically been studied under South African conditions. South Africa's climate is different to that of the northern hemisphere where most studies concerning these chemicals have been done. This poses a question as to how these chemicals will react in the South African climate, especially when it is taken into consideration that these chemicals have a shorter half-life in summer than in winter because of elevated temperature and light intensity (Stanmore, 2004). South Africa has extended summers with high temperatures while northern hemisphere countries have extended winters with precipitation.

1.5.2. Deposition of dioxin-like chemicals

Since dioxin-like chemicals are stable and tend to accumulate in carbon-rich matrices such as soil and sediments, they have spread into almost all environmental compartments (Ruokojärvi *et al.*, 2004). After being released from the sources (Section 1.4.2), the compounds can be deposited, *inter alia*, on soil and plants. They then remain in these matrices due to low mobility and persistence (Pereira, 2004). Once deposited, dioxin-like chemicals tend to remain in the upper surfaces. The main method of plant contamination is through wet and dry deposition (Pereira, 2004). Dry particle deposition is dominated by coarse particles, while wet composition is predominantly fine particles. Fine particles are associated with the higher chlorinated congeners (Moon, Lee, Choi & Ok, 2005). During studies in South Korea, it was found that seasons also play a role on the amount of dioxin-like chemicals that are deposited in an area. Deposition fluxes show high levels in winter, moderate levels in spring and autumn and low levels in summer (Moon *et al.*, 2005). This can be due to a greater amount of combustion in winter (Moon *et al.*, 2005, Lohmann & Jones, 1998), as well as the tendency of these pollutants to have an increased magnitude of deposition and reduced revolatilisation at low temperatures (Backe, Cousins & Larsson, 2004). Lower deposition in summer can be attributed to higher levels of photodegradation, scavenging by plants, and reactions with OH^- radicals that lead to the decomposition of dioxin-like chemicals (Lohmann & Jones, 1998; Moon *et al.*, 2005).

1.5.3. The transport of dioxin-like chemicals

The majority of dioxin-like chemical emissions tend to be transported beyond 100 km of their formation site (Lohman & Seigneur, 2001). Thus, most of the dioxin-like chemicals are not deposited locally. The exceptions are emissions from waste

incinerators, medical waste incinerators and vehicles. These sources tend to have a greater fraction of their total dioxin releases deposited locally. One of the reasons for this is that these sources have a large portion of dioxins associated with larger particles that will settle near to the point of origin (Lohman & Seigneur, 2001). Dominigo, Schumacher, Llobet, Muller & Rivera (2001) studied the concentration PCDDs and PCDFs in the vicinity of a municipal waste incinerator. Their sampling sites started at a distance of 250 m to 1500 m from the incinerator stack. It must be mentioned that the fraction of dioxins deposited in an area will depend upon the particle size, distribution, congener profile, source characteristics, meteorological conditions and the land-use of the area (Lohman & Seigneur, 2001). The land-use can be an important factor in the concentration of dioxins in soil. Forrest areas can produce dioxins through natural pathways (Hoekstra, *et al.*, 1999) and agricultural areas tend to have fewer sources of pollutants when compared with urban and industrial areas. There is also speculation that the tillage and erosion of agricultural soil can play a role in the destruction or dilution of dioxin-like chemicals in soil (Rogowski & Yake, 2005). All these factors have to be considered when studying the distribution and transport of dioxin-like chemicals from a point source.

Since dioxin-like chemicals are poorly water-soluble and possess a high octanol-water coefficient, they tend to associate strongly with soils and sediments (Lohmann & Jones, 1998). The greatest deposition to soil occurs through wet deposition, however, dry deposition does increase at cooler temperatures (Lohmann & Jones, 1998). The deposition to soil also depends on the variable characteristics of the soil such as organic carbon content, moisture content, texture, structure and porosity (Backe *et al.*, 2004). The better a soil can retard the movement of small particles, the better that soil will be able to retain dioxin-like chemicals (Brzuzy & Hites, 1995). Characteristics such as pH play a negligible role when looking at dioxin-like chemicals and since these chemicals are non-polar and non-ionic their abundance will not be strongly affected by this characteristic (Brzuzy & Hites, 1995).

1.6. Legislation concerning incineration and air quality.

Seeing that PCDDs, PCDFs and PCBs disperse in the environment (section 1.5), it is becoming increasingly important to limit their releases and to measure their occurrence. A country's legislation and policies can increase awareness of these substances and their effect on the environment. According to Pereira (2004) the

control of dioxin sources and the revision of legislation are the main strategies to control human exposure to these substances.

According to Gochfeld (1995), incineration is considered one of the four primary ways to manage solid wastes. The other primary ways are source reduction and re-use, recycling, composting and land filling. Incineration is currently used to destroy waste by reducing volume and destroying harmful constituents (Gochfeld, 1995). Waste volume can be reduced by up to 90% when incinerated and the reactivity of waste is reduced through the destruction of organic compounds (Dominigo, *et al.*, 2001). The incineration of waste is the process where waste is burned to ash, using very high temperatures (U.K. (United Kingdom) Environment Agency, 2004).

The use of these systems has to be continued since there are few alternatives that are practically and financially possible. In European countries where stringent controls are placed on the incineration processes, evidence suggests that waste management has a relatively small impact on health. In the United Kingdom (UK) well-controlled municipal solid waste incineration contributes less than 1% of the total dioxin emissions (U.K. Environmental Agency, 2004).

1.6.1. International legislation

According to the Directive 2000/76/ec (2000) of the European Parliament and Council, dating from 4 December 2000 on the Incineration of Waste, the following applies to legislation governing dioxin formation in incineration processes:

- *"The fifth environment action program sets as an objective a 90% reduction of dioxin emissions of identified sources by 2005."*
- *"The protocol on POPs signed by the community within the framework of the United Nations (UN) Economic Commission for Europe (UN-ECE) Convention on long-range trans-boundary air pollution sets legally binding limit values for the emission of dioxins and furans of 0.1 ng/m^3; Toxicity equivalents (TE) for installations burning more that 3 t/h of municipal solid waste, 0.5 ng/m^3 TE for installations burning more than 1 t/h of medical waste, and 0.2 ng/m^3 TE for installations burning more than 1 t/h of hazardous waste."*
- *"The incineration of hazardous waste with a content of more than 1% of halogenated organic substances, expressed as chlorine has to comply with*

certain operational conditions in order to destroy as many organic pollutants such as dioxins as possible."

- *"The incineration of waste which contains chlorine generates flue gas residues should be managed in a way that minimises their amount and harmfulness."*
- *"Article 4 of Council Directive 75/442/EEC of 15 July 1975 on waste requires member states to take the necessary measures to ensure that waste is recovered or disposed of without endangering human health and the environment "*
- Lastly according to Article 11*: "Measurement requirements as listed in Directive 2000/76/EC, at least two measurements per year of heavy metals, dioxins and furans; one measurement at least every three months shall however be carried out for the first 12 months of operation. Member states may fix measurement periods where they have set emission values for polycyclic aromatic hydrocarbons or other pollutants."*

In Ontario, Canada, dioxins and furans are being reduced through a comprehensive programme of regulatory, monitory, abatement, research, and educational development (Canadian Ministry of the Environment, 1997). This Canadian province has developed guidelines that integrate limits for the intake of dioxins and furans from all into a single, overall standard, the Tolerable Daily Intake (TDI). For humans the TDI is 10 pg TCDD per kilogram body weight per day. Furthermore, Ontario has specific standards concerning dioxins as indicated in Table 1.1 (Canadian Ministry of the Environment, 1997).

Table 1.1: Ontario's standards for dioxins, reported in Toxic Equivalency Quotient (TEQ) (Canadian Ministry of the Environment, 1997).

Matrix	Matrix specification	Dioxin standard
Air	Ambient air quality criterion (24 hours)	5 pgTEQ/m^3
Drinking water	Interim maximum allowable concentration	15 pgTEQ/ ℓ
Surface water	Water quality guideline in preparation	
Surface soil	Residential soil remediation criterion	1000 ngTEQ/kg
Surface soil	Agricultural soil remediation criterion	10 ngTEQ/kg

1.6.2. South African legislation

In strong contrast to international tendencies to apply strict legislation upon the release of dioxins, South Africa currently has limited legislation concerning this group of chemicals. The only policy mentioning dioxins occurs under the Waste Management Policy, in process 39: Waste Incineration Processes. This policy states that the average dioxin and furan concentration in the gas emissions of Class 1 incinerators (incinerators in which the waste serves as fuel or supplementary fuel in industrial processes) and Class 2A incinerators (incinerators for hazardous and potentially hazardous wastes) should not exceed 80 ng/m^3 total dioxins and furans if measured for a period of 6 to 16 hours, or 0.2 ng International Toxic Equivalent Quotient per cubic meter (I-TEQ/m^3), or result in an excess cancer risk of 1:100000 on the basis of annual average exposure. For class 2B-1 incinerators (medical waste incinerators at more than 10kg/day), the gas temperature, measured against the inside wall in the secondary chamber and not in the flame zone, should not be less than 1100 °C if materials containing 1% or more halogenated hydrocarbons are combusted (Department of Water Affairs and Forestry (DWAF), 2005).

The National Environmental Management Air Quality Bill makes no mention of dioxin-like chemicals, and the only chemicals listed in the ambient air quality standards are ozone, nitrogen oxides, nitrogen dioxide, sulphur dioxide, lead and particulate matter with a particular size less than 10 microns. Furthermore, the act also addresses the total suspended solids released into the air (National Environmental Management: Air Quality Bill, 2004).

Compared to international standards, there is therefore very little legislation concerning dioxins in South Africa. Until more severe measures are applied, the formation of these substances remains a potentially, although, not yet quantified, serious health and environmental risk. One of the greatest challenges facing South Africa is that currently there are no dioxin analysis facilities in South Africa, making dioxin analyses very expensive since samples have to be analysed abroad (Baldwin, 2004). This makes the implementation of inexpensive techniques essential. Legislation on dioxin emissions is imperative due to the serious threat dioxin-like chemicals hold for human and environmental health, not only in the country of origin but in all areas to where these chemicals may travel to.

1.7. Health impacts.

Chemicals that cause health effects similar to TCDD, the most toxic congener of the dioxin group of chemicals, are of great concern to human health. The effects these chemicals can have, are: hepatoxicity, immunotoxicity, tumour promotion, carcinogenesis, embryo toxicity, dermal toxicity, wasting syndrome, teratogenicity, lethality, disturbance of hormone steroid action, endocrine disruption and profound alteration in neural development. (Poland & Knutson, 1982; Schmitz, Hagenmaier, Hagenmaier, Bock, & Schrenk, 1994; Schwirzer, Hofmaier, Kettrup, Nerdinger, Schramm, Thoma, Wegenke & Wiebel, 1998; Hilscherova *et al.*, 2000; Jin, Jung, Lee, & Kim, 2004).

1.7.1. Toxicity of dioxin-like chemicals

TCDD in humans causes a variety of toxic responses including chloracne, tumour promotion, thymic involution, hydronephrosis, cleft palate and wasting syndrome. After TCDD has been deposited into the adipose tissue (specialised connective tissue that functions as the major storage site for triglycerides) where this chemical accumulates, TCDD inhibits glucose transport, lipoprotein lipase activity and fatty acid synthesis. The expression of adipose differentiation-specific transcription factors is also inhibited in the presence of TCDD (Shimba, Todoroki, Aoyagi & Tezuka, 1998). Furthermore the U.S. EPA (among others) has confirmed that dioxins are a cancer hazard and exposure can also cause severe reproductive and developmental problems (McKay, 2002). One of the reproductive influences these chemicals has, is to lower the male/female sex ratio of birth in the offspring of people exposed to high levels of TCDD (Mocarelli, Gerthoux, Ferrari, Patterson, Kieszak, Brombilla, Vincoli, Signorini, Tramacere, Carreri, Sampson, Turner, & Needham, 2000). PCBs have been reported to cause changes in the immune system, behavioural alterations, impaired reproduction, anaemia, as well as liver, stomach and thyroid gland injuries in animals (Wikipedia, 2005). Acute PCB effects include chloracne, and changes in the pigmentation of the skin and nails (Pereira, 2004). Dioxin-like chemicals also have the potential to disrupt multiple endocrine pathways (Mandal, 2005). This can result in reproductive problems, cancers, and other toxic responses that are related to growth, development and differentiation (Sanderson & Van den Berg, 2003).

The toxicity of these PCDDs, PCDFs and PCBs are usually restricted to those congeners with four chlorine atoms or more in the molecule (Table 1.2), with all having the 2,3,7,8 positions occupied (Stanmore, 2004). Even though acute toxicity to higher animals is limited, these chemicals have been shown to cause chronic damage (Bernes, 1995). There are marked species differences in the sensitivity to dioxin-like chemicals. The resulting pathological expression caused by exposure also varies among tissues and organs (Matsumura, 1983).

Table1.2: The toxic congeners of dioxin-like chemicals (Seys, 1997; Fiedler, 2003; U.S. EPA, 2005).

Dioxins	Dibenzofurans	Dioxin-like PCBs
2,3,7,8-TCDD	2,3,7,8-TCDF	3,3',44'-TCB
		3,4,4',5-TCB
		2,3,3',4,4'-PeCB
	2,3,4,7,8-PeCDF	2,3,4,4',5-PeCB
1,2,3,7,8-PeCDD	1,2,3,7,8-PeCDF	2,3',4,4',5-PeCB
		2,3',4,4'5'-PeCB
		3,3',4,4',5-PeCB
1,2,3,4,7,8-HxCDD	1,2,3,4,7,8-HxCDF	2,3,3',4,4',5-HxCB
1,2,3,6,7,8-HxCDD	1,2,3,7,8,9-HxCDF	2,3,3',4,4',5'-HxCB
1,2,3,7,8,9-HxCDD	1,2,3,6,7,8-HxCDF	2,3',4,4',5,5'-HxCB
	2,3,4,6,7,8-HxCDF	3,3',4,4',5,5'-HxCB
1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,8-HpCDF	2,3,3',4,4',5,5'-HpCB
OCDD	1,2,3,4,7,8,9-HpCDF	
	OCDF	

HpCB	Heptachlorinated biphenyl	OCDF	Octachlorodibenzofuran
HpCDD	Heptachlorodibenzo-*p*-dioxin	PeCB	Pentachlorinated biphenyl
HpCDF	Heptachlorodibenzofuran	PeCDD	Pentachlorodibenzo-*p*-dioxin
HxCB	Hexachlorinated biphenyl	PeDDF	Pentachlorodibenzofuran
HxCDD	Hexachlorodibenzo-*p*-dioxin	TCB	Tetrachlorinated biphenyl
HxCDF	Hexachlorodibenzofuran	TCDF	Tetrachlorodibenzofuran
OCDD	Octachlorodibenzo-*p*-dioxin		

Additionally these chemicals are classified as a severe environmental threat because they are widely distributed throughout the environment as a result of atmospheric transport and deposition (section 1.6). Since, and as early as, the 1960s, organohalogen compounds have been identified in almost every component of the global ecosystem, including air, water, aquatic sediments, fish, wildlife and human tissue (Safe, 1995). Eventually dioxin-like chemicals enter the food chain through

particles and dust that adsorb to plants and soil (Figure 1.8). The primary pathways for dioxin-like chemicals to enter the food chain is air-to-plant-to-animal, and from water/sediment-to-fish (Van Overmeire *et al.*, 2001). Significant dioxin-like activity has been observed in eggs of birds as well as birds at different stages of development (Giesy, Hilscherova, Jones, Kannan, & Machala, 2002), showing that these chemicals have found their way into the higher levels of the food chain.

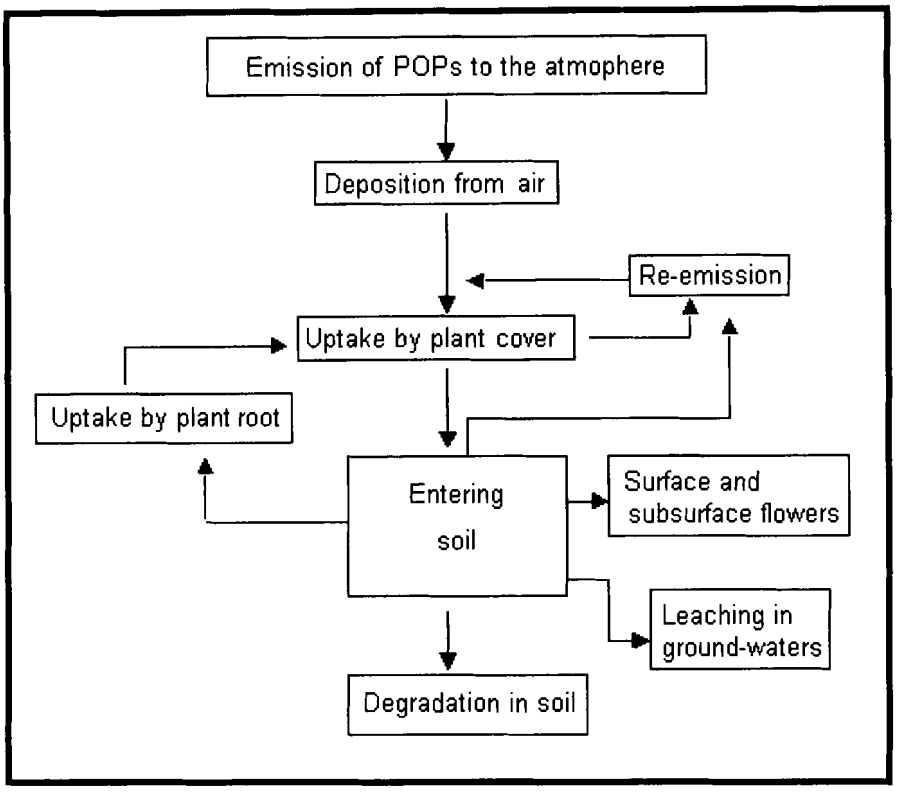

Figure 1.8: Conceptual model of the behaviour of POPs in the air-plant-soil system (Galiulin, Bashkin & Galiulina, 2002).

1.7.2. The movement of dioxin-like chemicals through the food chain.

The amount of PCDDs, PCDFs and PCBs that are capable of entering the food chain depends on the bio-availability of these substances. Bio-availability is the accessibility of a pollutant to an organism. Bio-availability is influenced by the process of aging, as well as the chemical and biological characteristics of a substance (Reid, Jones, & Semple, 2000). Aging is a term used to describe the reduction in availability of certain POPs when they have resided in soil for an extended period. Slow processes such as diffusion and chemical degradation can cause this decrease in the impact of toxic compounds over extended time periods (Alexander, 1995).

Once a pollutant has entered the food chain, direct human exposure to contamination is bound to occur as humans are exposed to toxic dioxin congeners daily through their diet (Hays & Aylward, 2003: Kitamura, Takazawa, Hashimoto, Choi, Ito, & Morita, 2004). Since the gastrointestinal permeability and diffusion capability across membranes correlate with the lipophilicity of a substance (Dybing, Doe, Grotenh, Kleiner, O'Brien, Renwick, Schlatter, Steinberg, Tritscher, Walker & Younes, 2002), the uptake of dioxin-like chemicals through the food chain is a serious concern. Other pathways through which people can be potentially exposed to dioxin-like chemicals include (Meneses, Schumacher, & Domingo, 2004.):

- The intake of contaminated soil
- Inhalation of re-suspended particles
- Dermal absorption.

However, the exposure to PCDDs and PCDFs experienced by an individual is dominated by the food chain pathway, which accounts for over 98% of the total uptake (Eduljuee & Gair, 1997).

1.7.3. Dioxin-like chemicals in the human diet.

Available information from industrialised countries indicates that the daily intake of dioxin-like chemicals during the last decades varied roughly between 2-10 pgTEQ/kg bw (body weight) per day for a 60 kg adult. However, with stringent laws concerning formation and release in European countries, a significant decrease in intake has been reported (Baeyens, Verstraete & Goeyens, 2004). A foetus, before birth and a baby when breast feeding, are the subjects in the food chain consuming the highest concentration PCDDs, PCDFs and PCBs in its daily fat intake (Koppe, 1995). Due to an infant's high risk of exposure and probable sensitivity, a breast-fed baby is regarded as the primary risk group for these toxins (Hanberg, 1996). Since dioxin-like chemicals have a long half-life in the human body (greater than seven years in adults for certain congeners), body burdens do not change rapidly in response to changes in intake exposure levels (Hays & Aylward, 2003). Dioxin-like chemicals have been identified in almost all species, including humans (Mocarelli *et al.*, 2000). This raises serious questions about the effect of PCDDs, PCDFs and PCBs.

The fate of potentially toxic chemicals in the body describes the processes of absorption, distribution, bio-transformation and excretion of these chemicals. The processes can be described as follows (Dybing *et al.*, 2002):

- Absorption is the process by which a substance enters the body. The chemical characteristics of the substances determine the rate and extent of its absorption.
- Distribution is the process by which a chemical circulates and partitions through the body. This process is crucial for substances' toxicity. For a substance to reach the site of action, it first has to be transported to this site. Often this means the substance has to transverse cellular membranes and other physical barriers.
- Bio-transformation is the process by which a chemical is structurally changed in the body through enzymatic or non-enzymatic reactions. Metabolic reactions can lead to a decrease in a chemical's toxicity. However, many times the metabolites formed, are themselves toxic and reactive in an organism.
- Excretion describes the process by which a chemical is removed from the body.

The susceptibility of a chemical to these processes will determine the toxicity to, and half-life in the human body. When the body absorbs PCDDs, PCDFs and PCBs, they accumulate in lipoproteins, especially in blood, liver and fat tissue. The metabolism of dioxin-like chemicals is only possible through transformation processes. During these processes, these chemicals are transformed into polar metabolites through the epoxidation (a chemical reaction in which an oxygen atom is joined to an olefinically unsaturated molecule to form a cyclic, three-membered ether) of the molecules with the corresponding formation of hydroxyl-derivatives and glucuronidation of the dioxins (Pereira, 2004; Anon, 2005b). These metabolites are less toxic, and un-metabolised dioxin-like chemicals are partially excreted (Pereira, 2004). In women the main route of dioxin excretion is through lactation (Hanberg, 1996), increasing the threat to infants. PCBs on the other hand form reactive metabolites that are persistent, including hydroxylated and methylsulfonyl metabolites (Hanberg, 1996).

Values set for the regulation of these substances are often based on TEQs, including emission limits (Dyke & Stratford, 2002) that will eventually determine the amount of

dioxin-like chemicals populations are exposed to. The concept of TEQ is described next.

1.8. Toxic equivalency quotient.

In this approach, the biological or toxic potencies of a mixture of dioxins and dioxin-like chemicals are expressed relative to a benchmark dioxin, usually 2,3,7,8-TCDD, since it is the most potent congener (Hahn, 2002). The TEQ approach is an attempt to provide an integrated assessment of the toxic potential of an environmental mixture and thus represents the total 2,3,7,8–TCDD-toxic potency of the mixture of dioxin-like components (Schwirzer *et al.*, 1998; Van Overmeire *et al.*, 2001; Hahn, 2002).

TEQs are calculated by multiplying the Relative Potency (REP) for the specific assay or the International Toxic Equivalency Factor (I-TEF) by the concentration of the specific congener, giving the total sum TEQ per mass unit (Hilscherova *et al.*, 2000). The REP of samples are usually calculated as the amount of standard (TCDD) giving the same response as the sample, based on the amount needed to produce 50% of the maximal response (Giesy *et al.*, 2002). Toxic Equivalency Factor (TEF) values are consensus values based on different assays and analyses; these values are suitable for risk assessment. Currently there are two sets of TEF values, the I-TEF and the WHO-TEF. The WHO-TEF values are more recent and include TEF values for the dioxin-like PCBs. Furthermore, the WHO-TEF distinguishes between species, having different values for humans/mammals, fish and birds (Fiedler, 2003). The different TEF values are shown in Table 1.3. The TEQ concentration can also be determined by summing the products of multiplying the concentrations of various molecules for which a TEF has been assigned by its respective TEF (TEQ = (TEF x [PCDDs]) + (TEF x [PCDFs])+ ...) (Lemieux *et al.*, 2001; Cooke, Clark, Goeyens, & Baeyens, 2000).

The TEQ approach is very important when dealing with dioxin-like chemicals. Humans that are exposed to PCDDs, PCDFs and PCBs are usually exposed to a mixture of these chemicals (Maruyama, Yoshida, Tanaka, & Nakanishi, 2003). To truly assess the possible risk the population is exposed to, all possible toxic congeners have to be taken into account. To include a compound in a TEF-scheme the following criteria have to be met (WHO, 2000):

- The compound should show a structural relationship to PCDDs and PCDFs.

- It should bind to the Aryl hydrocarbon receptor (AhR-receptor).
- It should elicit dioxin-specific biochemical and toxic responses.
- It should be persistent and accumulate in the food chain.

Table 1.3 TEF values for the toxic congeners of the dioxin-like chemicals *.

Congener	I-TEF	WHO-TEF		
		Humans/Mammals	Fish	Birds
2,3,7,8-TCDD	1	1	1	1
1,2,3,7,8-PeCDD	0.5	1	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1	0.5	0.05
1,2,3,7,8,9-HxCDD	0.1	0.1	0.01	0.01
1,2,3,6,7,8-HxCDD	0.1	0.1	0.01	0.1
1,2,3,4,6,7,8-HeCDD	0.01	0.01	0.001	<0.001
OCDD	0.001	0.0001	-	-
2,3,7,8-TCDF	0.1	0.1	0.05	1
1,2,3,7,8-PeCDF	0.05	0.05	0.05	0.1
2,3,4,7,8-PeCDF	0.5	0.5	0.5	1
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1	0.1
1,2,3,4,6,7,8-HeCDF	0.01	0.01	0.01	0.01
1,2,3,4,7,8,9-HeCDF	0.01	0.01	0.01	0.01
OCDF	0.001	0.0001	0.0001	0.0001
3,4,4'5-TCB		0.0001	0.0005	0.1
3,3',4,4'-TCB		0.0001	0.0001	0.05
3,3',4,4'5-PeCB		0.1	0.005	0.1
3,3',4,4',5,5-HxCB		0.01	0.00005	0.001
2,3,3',4,4'-PeCB		0.0001	<0.000005	0.0001
2,3,4,4',5-PeCB		0.0005	<0.000005	0.0001
2,3',4,4',5-PeCB		0.0001	<0.000005	0.00001
2',3,4,4',5-PeCB		0.0001	<0.000005	0.00001
2,3,3',4,4',5-HxCB		0.0005	<0.000005	0.0001
2,3',4,4',5'-HxCB		0.0005	<0.000005	0.0001
2,3,3',4,4',5,5'-HxCB		0.00001	<0.000005	0.00001
2,3,3',4,4'5,5'-HpCB		0.0001	<0.000005	0.00001

* According to Van den Berg, Birnbaum, Bosveld, Brunström, Cook, Feeley, Giesy, Hanberg, Hasegawa, Kennedy, Kubiak, Larsen, Rolaf van Leeuwen, Liem, Nolt, Peterson, Poellinger, Safe, Schrenk, Tillitt, Tysklind, Younes, Wærn, Zacharewski, 1998; Fiedler, 2003.

For the above reasons, dioxin-like PCBs can be included since there is general agreement that PCDDs, PCDFs and dioxin-like compounds proceed through the action of the Ah-receptor (Baeyens *et al.*, 2004).

There are two main methods that can be employed to measure TEQs:

1. Chemical-analysis, including capillary gas chromatography coupled with mass spectrometry, two-dimensional gas spectrometry and fast gas spectrometry can be used in conjunction with TEFs to address and facilitate risk assessment (Pereira, 2004).
2. Bio-analytical methods that directly provide a total overall TEQ value (Overmeire *et al.*, 2001).

1.8.1 Chemical analysis

Chemical analysis is the standard method for determining TEQs. There is also a widespread acceptance that TEQs derived from TEFs can be an effective tool to assess and regulate complex mixtures of dioxins (Dyke & Stratford, 2002). However, there are a number of limitations to this method (Hanberg, 1996; Schwizer *et al.*, 1998; Giesy *et al.*, 2002; Fernàndez, Cagigal, Vega, Urzelai, Babín, Pro & Tarazona, 2005):

- This type of analysis is laborious and time consuming.
- Chemical analysis does not take chemical interactions such as synergistic or antagonistic effects into account.
- TEFs are available for only a limited number of congeners.
- TEFs arrived at biologically, may not be suitable due to interspecies differences in specificity.
- TEF-concept assumes additive effects for all chemicals present.
- Samples may contain compounds that are not routinely detected, whose contribution to toxicity may be overlooked.
- Detailed analysis of trace contaminants needs specialised equipment, which is not always available and might be prohibitively expensive.
- Total concentrations can overestimate the real risk, as ageing processes can strongly reduce the bio-availability and subsequently the toxicity of pollutants.

1.8.2. Bio-analytical techniques

Biological analysis on the other hand is cost-and-time-effective, especially when screening complex matrices (Schwirzer *et al.*, 1998), and allows a higher sample throughput (Overmeire *et al.*, 2001). Bio-assays also lead to higher TEQ values, as all congeners and their interactions are taken into account (Schwirzer *et al.*, 1998). Bio-assays measure biological responses e.g. enzyme activity, transcription and expression of reporter genes, ligand-receptor binding or an antigen-antibody reaction (Overmeire *et al.*, 2001; Giesy *et al*, 2002). Bio-analytical methods give an overall TEQ value and thus an overall assessment of dioxin-like toxicity, without providing information on individual congeners (Overmeire *et al.*, 2001). There are a number of bio-assays that can be used to determine dioxin and dioxin-like compound potencies including, H411E reporter gene bio-assay, Enzyme-linked immunosorbent assay (ELISA), Ethoxyresorufin-O-deethylase (EROD) bio-assay and immuno-assays (Behnish, Hosoe, & Sakai, 2001). Since the H4IIE system was employed during this study, a more detailed description is relevant.

1.9. H411E reporter gene bio-assay

There is a general consensus that a soluble intracellular protein, the AhR-receptor, mediates the biological effects including toxic and biochemical effects of TCDD and other dioxin-like chemicals (Denison & Deal, 1990; Hanberg, 1996; Giesy *et al.*, 2002). Toxicity is produced as a result of changes in gene expression mediated through the Aryl Hydrocarbon Receptor (AhR), or interference with other signalling pathways (Hurst *et al*, 2004). The structure-activity relationship of dioxin-like chemicals shows that the toxicity of each congener correlates to the binding affinity of the congener to the AhR receptor (Hanberg, 1996). Variability in the sensitivity to AhR-active substances by different species has led to different TEFs for humans, fish and birds (Janošek, Hilscherová, Bláha & Holoubek, 2005)

1.9.1. Biochemical background

The AhR is a type II nuclear receptor (Janošek *et al.*, 2005), belongs to the helix-loop-helix group of proteins, and functions as a transcription factor (Shimba *et al.*, 1998; Giesy *et al.*, 2002) that modulates the responses to halogenated aromatic

hydrocarbons, polynucleur aromatic hydrocarbons, combustion products and phytochemicals such as flavinoids (Mandal, 2005). Unliganded AhR exists in the cytoplasm in a complex with 90-kDa heat shock protein (Hsp90) (Denison & Deal, 1990; Shimba *et al.*, 1998), a p23 protein and an immunophilin-like protein (Mandal, 2005). This configuration is un-reactive and the hsp90 keeps the ligand in a configuration that is suitable for binding dioxin-like chemicals and additionally prevents binding to DNA (Hanberg, 1996). Under the normal physiological conditions, the AhR is involved in the normal development of the liver, heart, vascular system and spleen, thymus, kidney, and may also play an important role in the regulation of xenobiotic metabolism as well as the maintenance of homeostatic functions (Shimba *et al.*, 1998; Wong, Wang, Wen, Buhle, & Hu, 2004). Furthermore, in the absence of exogenous ligands, the AhR affects metabolism of endobiotics (natural occurring substances) and plays a role in cell cycle regulation (Bock & Köhle, 2005). The binding of dioxin-like chemicals to the AhR-receptor induces phase I and phase II enzymes, release of alanine aminotransferase from the liver into the plasma, severe neurological toxicity, and receptor mediated tumour promotion (Parzefall, 2002).

Since it is thought that most of the toxic effects of dioxin-like chemicals are caused through the AhR-mechanism, most bio-assays are based on the following assumptions (Behnisch *et al.*, 2001):

- The compounds share structural relationships.
- The compounds bind to AhR.
- The compounds cause AhR-mediated biochemical effects.
- These compounds are persistent and accumulate in the food chain.

The current understanding of the AhR-mechanism is illustrated in Figure 1.9. After the dioxin-like chemical diffuses into the cell, it binds with high affinity to the cytosolic AhR, and the Hsp dissociate from the complex. The AhR subunit ligand complex is activated and translocated to the nucleus of the cell. The binding of the ligand increases the rate of nucleus import of the AhR, but does not eliminate nuclear export of the AhR since the AhR shuttles between the cytosol and the nucleus in the absence of the exogenous ligand (Richter, Tillitt & Hannink, 2001).

In the nucleus, the AhR ligand-complex forms a heterodimer with Arnt (Aryl hydrocarbon nuclear translocator) protein. The heterodimeric ligand complex then binds with high affinity to the specific DNA sequence (Safe, 1995; Mandal, 2005)

5'-TGCGTG-3',
3'-ACGCAC-5'

the Dioxin Responsive Element (DRE) located in the promoter region of CYP1A1 gene that is directly controlled by the AhR mechanism (Mandal, 2005). This causes DNA bending, disruption of chromatin and nucleosome as well as increased promoter accessibility and transcriptional activation of adjacent responsive genes. This induces the transcription of a number of genes, and subsequently the production of proteins such as P-450 cytochrome (Safe, 1995; Hilscherova *et al.*, 2000; Nie, Blakenship, & Giesy, 2001; Behnish *et al.*, 2001).

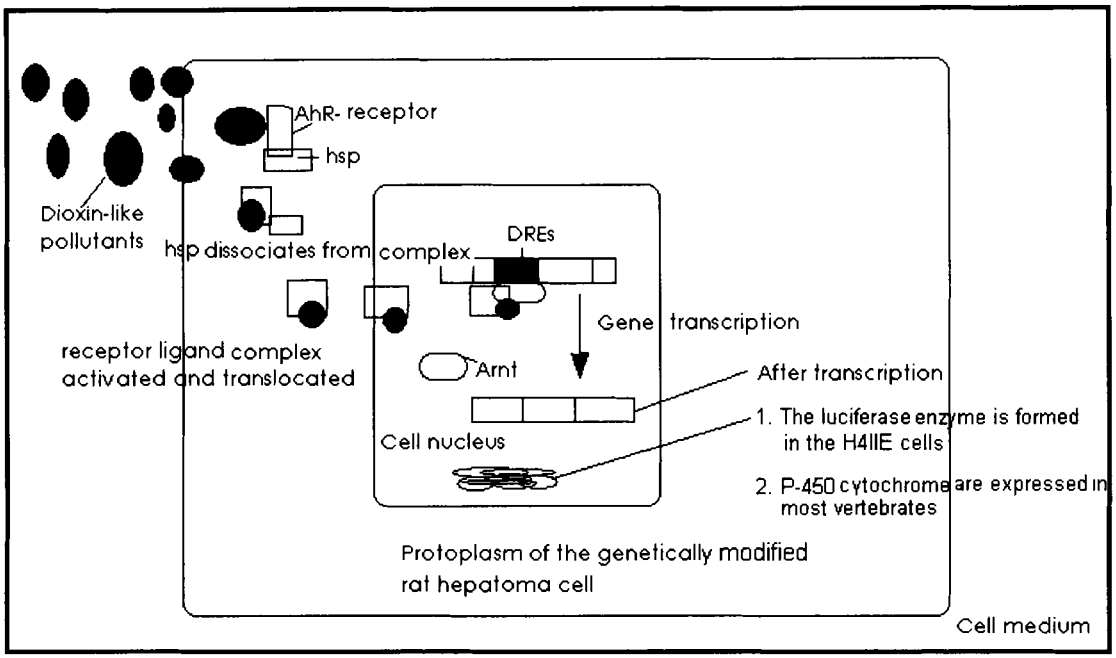

Figure 1.9: The AhR-mechanism of dioxin-like chemical activity.

One of the bio-assays based on this AhR mechanism is the H411E reporter gene bio-assay. The H411E-*luc* cells are rat hepatoma cells, which have been stably transfected with a firefly luciferase reporter gene under control of the DRE (Nie *et al.*, 2001) and thus the AhR-mechanism. In the stable transfection, the gene of interest becomes a permanent part of the cell genome (Figure 1.9). These cells are thus suitable for long-term experiments and their results are more reproducible than those found in assays using transient transfection (Hilscherova *et al.*, 2000). In addition, permanent cell lines avoid the use of primary cultures and no live animals are needed. The resulting recombinant cell lines then respond to PCDDs, PCDFs and PCBs exposure with the induction of luciferase, after the DRE activates adjacent responsive genes, which, in the presence of the luciferase-substrate, produces a

luminescent signal proportional to the cell's response to the dioxin-like chemicals in the sample.

The H411E bio-assay has advantages over traditional analytical chemistry techniques (Whyte & Tillitt, 2004):

- It reveals the cumulative biological activity of structurally similar contaminants.
- It reveals the potential interactions that occur between contaminants present in complex mixtures in environmental samples.
- It is a valuable monitoring tool as it enables the assessment and ranking of the potential toxicity of samples.
- H411E-derived potency estimates can be used to assess the risk to the environment.
- This assay estimates the contaminant burden that organisms could contribute to higher trophic levels or its progeny.
- This bio-assay has a high degree of sensitivity and can be rapidly preformed.

Furthermore, the production of luciferase in itself has a number of advantages (Behnish *et al.*, 2001):

- production of more copies of the vectors in the cell than natural P-450 enzyme;
- stability of the *luc*-protein is greater than cytochrome P-450; and
- cellular response can be measured easily.

Together, the above-mentioned characteristics make the H411E bio-assay an ideal tool in the environmental monitoring of dioxin-like chemicals. However, this bio-assay cannot replace the more conventional chemical analyses, since there is a major drawback to the specificity of this method. The bio-assay measures the total dioxin-like activity and supplies no information on the specific congeners or chemicals that are present in the sample (Environmental Health Perspectives, 1997). The data generated is also only a relative measure of the concentration of compounds in the sample that activate the AhR-receptor and not an absolute predictor of the actual toxicity of the extract (Sanderson, Aarts, Brouwer, Froese, Denison & Giesy, 1996). Chemical analysis is thus crucial if information is needed concerning specific congeners and their concentration in the sample, especially when the source of contamination has to be elucidated (Vanderperren, Van Wouwe, Behets, Windal, Van Overmeire & Fontaine, 2004).

Chapter 2 Materials and Methods

2.1. Determining the area of sampling and site description.

As discussed earlier, the incineration process is ideal for the formation of dioxin-like chemicals. For this reason the project focused on incinerators. It was determined that the Potchefstroom area has four sites that at one time housed, or still houses, incinerators. These four sites are:

- Veterinary Diagnostic Services, Department of Agriculture, Conservation and Environment, North West Province, located on the grounds of the Agricultural Research Council (ARC);
- Witrand Hospital;
- The Provincial hospital of Potchefstroom (Kallie de Haas); and
- Municipal Waste Water Treatment Plant.

After investigating each of these sites, it was found that only the incinerator at the Veterinary Diagnostic Services was operational at the time of sampling. Ash samples were also taken at each of the above-mentioned sites even though the incinerators were no longer active. The exact TEQ contribution from each of the possible sources that were not currently active at the time of sampling was considered confidential, therefore the sites were allocated codes. Notably, there were two incinerators at one of these locations, and an ash sample was taken from each. Since the incinerator located at the Veterinary Diagnostic Services was the only operating incinerator during the study period, it was the focus of this study.

The Veterinary Diagnostic Services incinerator was used to dispose of animal carcasses (Figure 2.1) as well as any medical waste that the veterinary research and testing generated. The incinerator had no Air Pollution Control System (APCS) at the time of sampling. On average, one 25 ℓ autoclave bag of medical waste was burned per day and an average of 150 animal carcasses (weighing approximately 450 kg per carcass) were burned in a year. The ash generated by the incinerator was collected for use in agricultural applications (Dr. J. Kangumba, personal communication).

It has been observed that medical waste incinerators deposit dioxins in a radius relatively close to the incinerator, because these incinerators tend to have relatively short stacks (Lohman & Seigneur, 2001). Further studies have shown that under South African conditions, the temperature range (150 °C – 450 °C) where dioxins

could be formed, is in the transition area between the stack outlet and the turbulent mixing region after the gases have left the stack tip (Brent & Rogers, 2002). Therefore, sampling was done in an evenly spaced grid surrounding the incinerator, with each point being 500 m apart from the previous point, as illustrated in Figure 2.2. The sampling distribution was similar to the layout used by Domingo *et al* (2001). The total area of sampling was 2.5 km^2. The co-ordinates shown in Figure 2.2 were the pre- determined co-ordinates for site sampling.

Figure 2.1: This figure shows a carcass (left) that was later disposed of in the incinerator (right).

2.2. Sample collection

The location of each sample was determined through the use of an E-Map: Garmen Global Positioning System (GPS) (Figure 2.3). The samples were taken in April 2004, as close to the pre-determined co-ordinates as possible. Table 2 lists both the pre-determined and actual co-ordinates of the sampling sites as well as their physical address where possible. The samples were not always collected on the pre-determined position since it was not always practically feasible. Some of the sites were on private property or developed areas as illustrated in Figure 2.4.

The samples were collected using only stainless steel or glass equipment to prevent any accidental contamination of the samples (Figure 2.3). All equipment was washed with phosphate free soap, rinsed with acetone to remove all polar organic compounds and then hexane to remove any non-polar organic compounds. In the field, equipment was rinsed with distilled water, acetone and hexane after each sampling (U.S. EPA, 1994a; U.S. EPA, 1994b).

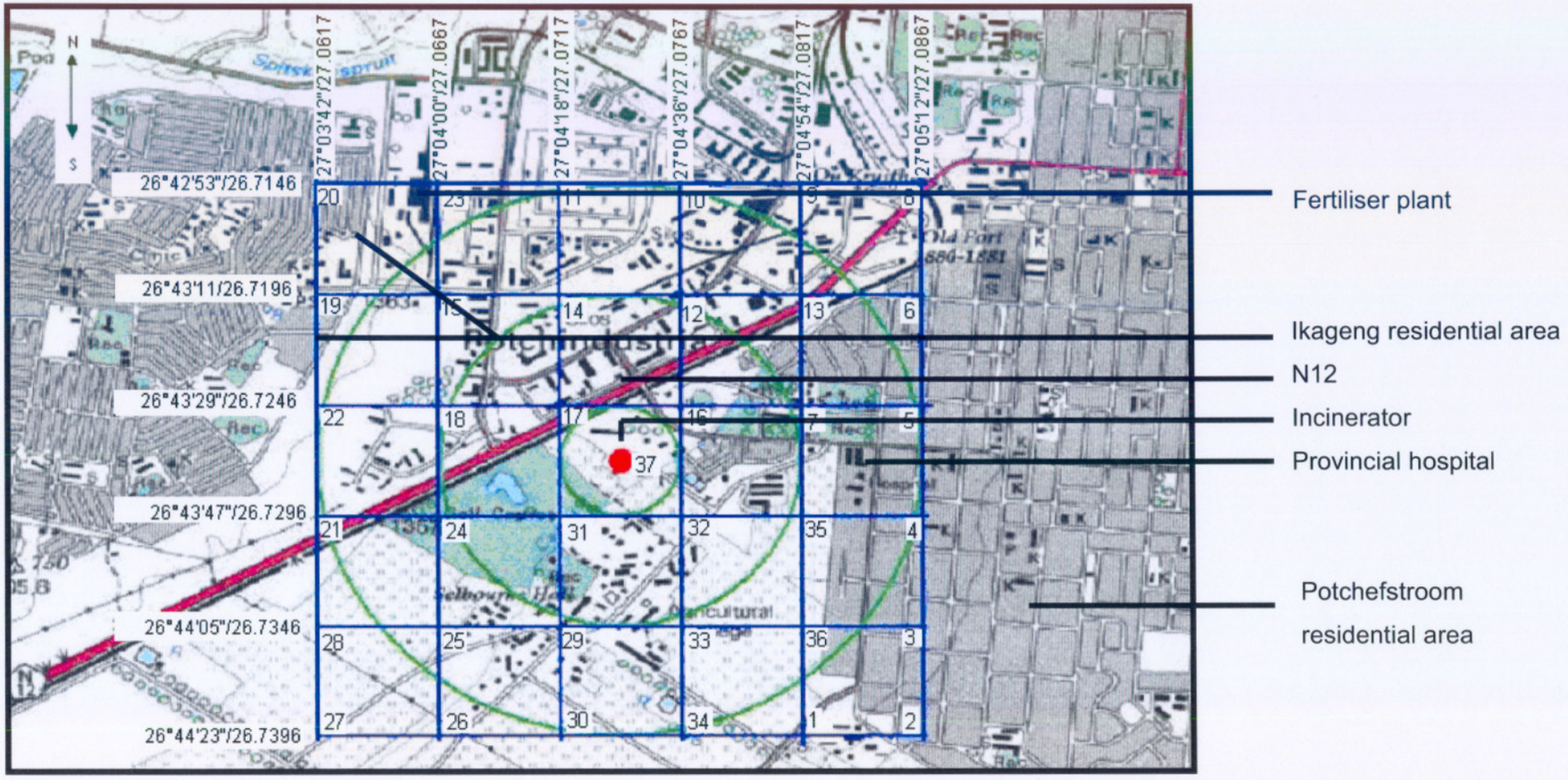

Figure 2.2: A map (scale 1cm: 263.15 m) showing the sampling grid, and co-ordinates in the area surrounding the incinerator of the Veterinary Diagnostic Services. The sampling points were located close to the intersections of the grid, and the edges of the grid are 2.5 km in length.

Table 2.1: Co-ordinates of the specific grid denoting the sampling sites in Potchefstroom and the accuracy of the GPS as recorded at the time of sampling.

Grid	Predetermined co-ordinates	Actual sampling co-ordinates	GPS Accuracy (m^2)	Address/ description of site
1	S 26°44.383' E 27°05.200'	S 26°44.389' E 27°05.208'	5.8	201 Kock Street.
2	26°44.383' 27°04.900'	26°44.379' 27°04.934'	6.9	Abraham Kriel Children's Haven.
3	26°44.083' 27°05.200'	26°44.078' 27°05.182'	5.4	146 Kamp Street.
4	26°43.783' 27°05.200'	26°43.794' 27°05.204'	5.4	104 Kamp Street.
5	26°43.483' 27°05.200'	26°43.459' 27°05.210'	9.9	60 Kamp Street.
6	26°43.183' 27°05.200'	26°43.184' 27°04.910'	6.9	9 Kamp Street.
7	26°43.483' 27°04.900'	26°42.837' 27°05.183'	5.8	In the grounds of Volkskool.
8	26°42.833' 27°05.200'	26°42.837' 27°05.183'	5.1	Open patch of ground near the old powder magazine.
9	26°42.833' 27°04.900'	26°42.833' 27°04.909'	6.5	Industrial area on Curlewis Street.
10	26°42.833' 27°04.600'	26°47.902' 27°04.579'	7.0	Industrial area on Curlewis Street.
11	26°42.833' 27°04.300'	26°42.852' 27°04.227'	3.5	Samples taken from the graveyard currently in use. The samples were taken in an area that has not been utilised.
12	26°43.183' 27°04.600'	26°43.207' 27°04.616'	9.0	Sample collected from soil in front of an air conditioner outlet.
13	26°43.183' 27°04.900'	26°43.193' 27°04.900'	5.2	In an open field across from Champion Tyres in

				the industrial area.
14	26°43.183' 27°04.300'	26°43.182' 27°04.271'	5.3	Near the Independent Electoral Commission offices
15	26°43.183' 27°04.000'	26°43.185' 27°05.182	7.0	An open area south of a fertiliser plant.
16	26°43.483' 27°04.600	26°43.517' 27°04.604'	3.9	In an unused old rugby field of a school. The sample was taken from an ant mound.
17	26°43.483 27°04.300	26°43.484' 27°04.374'	14.9	On the main road to Klerksdorp across from an engineering company.
18	26°43.483' 27°04.000'	26°43.516' 27°04.008'	7.0	An old graveyard in the industrial part of Potchefstroom.
19	26°43.183' 27°03.700'	26°43.198' 27°03.741'	4.8	An open field next to the police station, Ikageng.
20	26°42.833 27°03.700'	26°42.882' 27°03.706'	5.5	76 Ramatsani Street, Ikageng.
21	26°43.783' 27°03.700'	26°43.802' 27°03.467'	4.6	Open field, part of ARC rehabilitation programme near Klerksdorp main road.
22	26°43.483' 27°03.700'	26°43.447' 27°03.642'	8.7	Open field in Katelego.
23	26°42.833 27°04.000'	26°42.967' 27°04.063'	6.8	Area next to fertiliser plant
24	26°43.783' 27°04.000'	26°43.889' 27°03.931'	5.1	ARC golf course.
25	26°44.083' 27°04.000'	26°44.086' 27°03.988'	4.0	ARC agricultural experiment.
26	26°44.383' 27°03.000'	26°44.273' 27°04.054'	4.7	ARC grazing area.
27	26°44.838' 27°03.700'	26°44.350' 27°03.701'	5.8	ARC agricultural experimental area.

28	26°44.083' 27°03.700'	26°44.056' 27°03.532'	3.8	ARC grazing area.
29	26°44.083' 27°04.300'	26°44.110' 27°04.325'	9.5	Near a building on the ARC.
30	26°44.383' 27°04.300'	26°44.383' 27°04.307'	3.5	ARC, next to an internal road.
31	26°43.783' 27°04.300'	26°43.815' 27°04.296'	3.7	ARC golf course.
32	26°43.783' 27°04.600'	26°43.788' 27°04.555'	4.2	ARC grazing area.
33	26°44.083' 27°04.600'	26°44.045' 27°04.688'	3.9	ARC field not used.
34	26°44.383' 27°04.600	26°44.320' 27°04.605'	4.5	ARC field not used.
35	26°43.783' 27°04.900'	26°43.761' 27°04.857'	6.8	ARC agricultural experimental area.
36	26°44.083' 27°04.900'	26°44.087' 27°04.923'	6.7	Banister Road area next to Public road works premises.
37	26°43.699' 27°04.408'	26°43.699' 27°04.408'	5.1	The Veterinary Diagnostic Services Incinerator located at the ARC.

At each site, soil was collected from five separate locations within a radius of 5 m, and a composite sample was prepared. Three glass containers with screw lids were filled with the composite sample and immediately placed into a brown paper bag and stored at 4 °C (U.S. EPA, 1999). These precautions prevented the possible degradation of the target compounds in the sample due to Ultra Violet (UV) rays, bio-degradation through micro-organisms and heat. Directly placing the samples into a temperature lower than 4 °C also retained as much of the organic carbon present in the sample as possible (Schumacher, 2002).

Figure 2.3: Some of the equipment used during sampling in the field. This photo shows the GPS (indicated by an arrow) used to locate the sampling positions and the stainless steel containers used to collect the composite samples.

Ash samples were collected from the incinerator and directly placed into glass containers. Composite samples were not truly viable since all the ash was collected from a single, small area. In one case, the incinerator was no longer present on the grounds. A mixture of ash and soil was taken from the site where the incinerator had once stood.

Figure 2.4: One of the sample sites off the main road to Klerksdorp.

The analysis process is represented in Figure 2.5 and shows the steps taken to determine the TEQ of each sample. After sample collection, the samples were transported to the laboratory and stored at –5 °C until the extraction process was initiated.

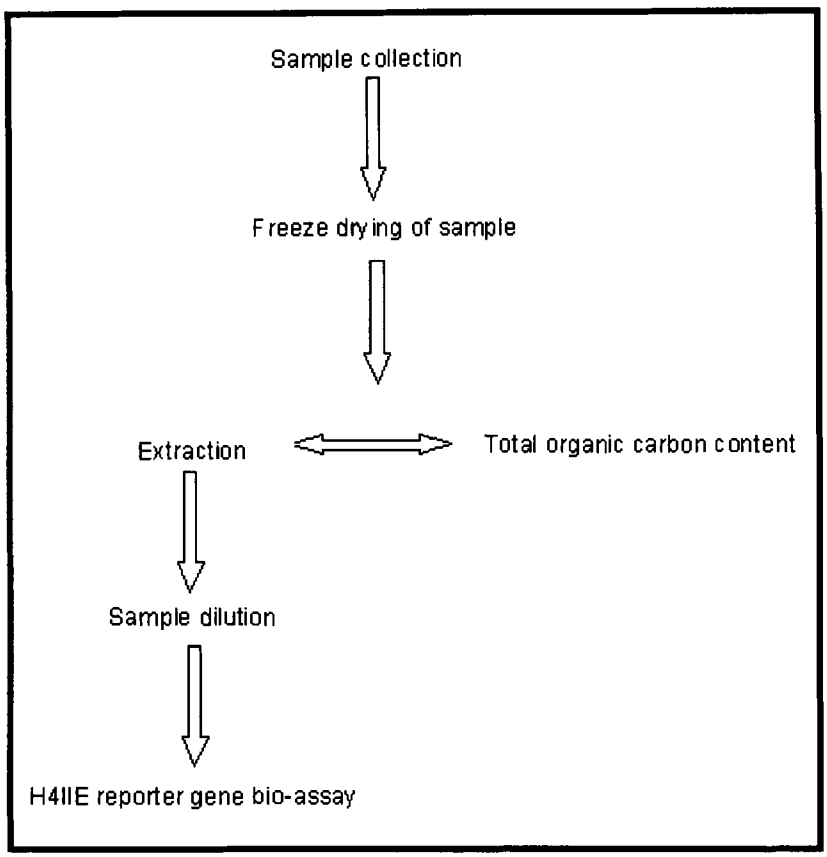

Figure 2.5: The process followed in sample analysis.

2.3. Extraction of the soil samples

The extraction process was divided into five phases:

- Freeze drying of samples (Section 2.3.1)
- Soxhlet extraction (Section 2.3.2)
- Rotary evaporation (Section 2.3.3)
- Acid wash (Section 2.3.4)
- Nitrogen gas evaporation (Section 2.3.5)

Each of these phases will be discussed separately.

2.3.1. Freeze drying and homogenising of samples

The samples that were collected contained varying amounts of moisture. To remove this moisture, the samples were freeze-dried to prevent degradation of the target compounds. Conventional heat drying was not an option as the heat could degrade the target pollutants in the samples. The process took approximately 48 hours,

depending on the water content of the sample. The removal of water was necessary to ensure that the weight determination of the samples extracted was accurate. After freeze-drying, samples were ground in a mortar and pestle (Koh, Khim, Villeneuve, Choi, Ito & Morita 2005), and then sieved (0.5 mm) to ensure that the soil particles were all of the same size.

2.3.2 Soxhlet extraction

Extraction equipment (Figure 2.6) was cleaned as previously described in section 2.2 covering sample collection. 400 ml hexane was used as solvent and boiling chips were not used since the chips themselves could contaminate the sample. All the extraction chemicals were HPLC (high performance liquid chromatography) grade from Burdick & Jackson (U.S. EPA, 1994a; U.S. EPA, 1996a). A 40 g mixture containing equal parts of soil and anhydrous sodium sulphate was placed on top of a glass wool layer in the glass tube of the Soxhlet apparatus (U.S. EPA, 1996a). The glass wool stopped the soil from filtering into the round-bottomed flask (Figure 2.6). Before use, the glass wool was extracted in a mixture of hexane and dichloromethane (1:3) to ensure that all dioxin-like chemicals possibly present had been removed. The sodium sulphate was added to ensure no moisture was present in the sample.

The ash samples were too fine for the use of glass wool and were extracted using Whatman® single thickness (external diameter 45 mm x external length 123 mm) cellulose extraction thimbles (U.S. EPA, 1996a). The extractions were left to run for no less than 16 hours. The rate of percolation had to be maintained at three percolations per hour to ensure that all possible PHAHs were removed from the sample. After the 16-hour period of Soxhlet extraction, the extract was cooled and concentrated.

2.3.3. Rotary evaporation

During this phase the hexane was evaporated to concentrate the sample. The sample was concentrated to the barest minimum so that after transferring it into a prepared test tube and rinsing with hexane the end volume was adjusted to 10 ml. The rotary evaporator was fitted with a splashguard to minimise sample loss.

Precaution was taken not to evaporate the sample completely; the sample would then be lost (U.S. EPA, 1994a; U.S. EPA, 1999; Yang, Lee, Park, & Lee, 1999).

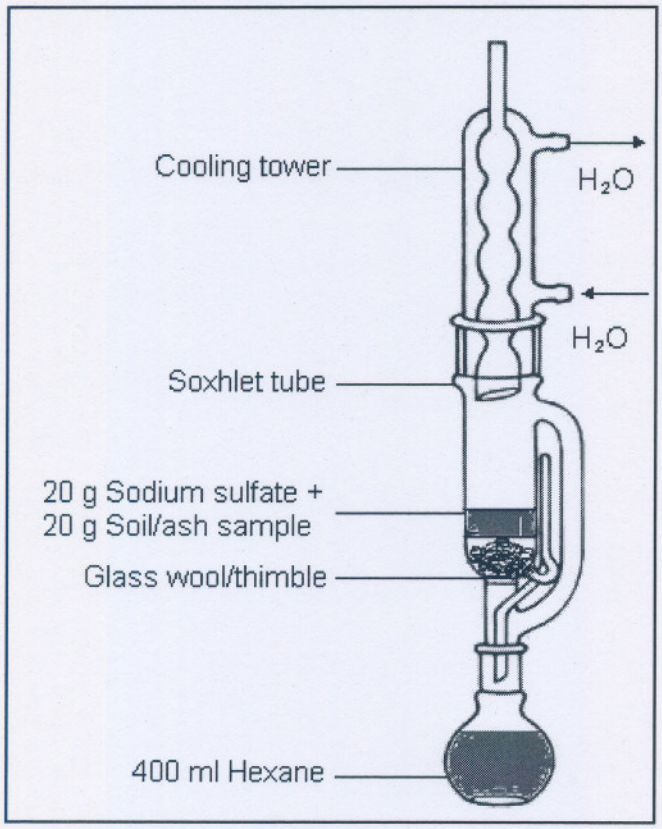

Figure 2.6: A drawing of the Soxhlet set-up used, from Anon (2004d).

2.3.4. Acid wash

The 10 ml concentrate was washed three times with an equal volume of 95% sulphuric acid (Merck) (Masunaga, Morinaka & Nakanishi, 2002). The acid and hexane were mixed by gently shaking the separation funnel, with frequently venting the mixture. Care was taken to allow the phases to separate properly, before the acid phase was collected and discarded. This was repeated until the hexane phase was completely clear. The process was repeated once with double ionised water (18 MΩ) to remove any traces of the sulphuric acid. To remove any traces of water from the hexane portion, it was funnelled through glass wool topped with anhydrous sodium sulphate.

2.3.5. Nitrogen evaporation

The 10 ml hexane portion had to be concentrated further for use in the bio-assay. Since the volume was now relatively small, evaporation had to proceed at a controlled rate. Nitrogen gas evaporation was an ideal means to achieve concentration since the gas is inert. The needles were pre-cleaned with acetone and hexane and the nitrogen flow was weak to prevent the loss of samples through displacement, but still facilitating evaporation. The final volume was 0.5 ml. (U.S. EPA, 1994a).

2.4. Storage of extracted samples

The 0.5 ml sample was transferred to an amber GC (gas chromatography) vial (U.S. EPA, 1994b). The volume was increased to 1 ml with the addition of hexane. The samples were stored at 4 °C until used in the H4IIE-*luc* bio-assay.

2.5. Carbon content determination

After freeze-drying of the sample (Section 2.3.1), samples were sieved to remove larger particles since the removal of larger particles will result in a more accurate measurement of the Total Organic Carbon (TOC) (Schumacher, 2002). The standard wet chemistry technique used for determining the TOC, the rapid dichromate oxidation method, better known as the Walkley-Black method, was used (Schumacher, 2002; University of Exeter, 2005; Schulte, 2005), as follows:

Potassium Dichromate ($K_2Cr_2O_7$), with a concentration of 0.167 mol/dm^3, was added to an accurately weighed soil sample between 0.5 and 1 g. The carbon reduces Cr^{6+} to Cr^{3+} (equation 1). After mixing well, 95% sulphuric acid was quickly added while working in a fume cupboard. This mixture was then left to cool for at least 30 minutes. After the reaction was completed and the mixture cooled, distilled water and chemically-pure orthophosphate acid (Merck) was added. This provided a clearer suspension for viewing the endpoint of titration and the orthophosphate complexes with Fe^{3+} that would otherwise interfere with the endpoint. The solution was then titrated with Iron (II) Ammonium Sulphate ($(Fe(NH_4)_2(SO_4)_2)\cdot 6H_2O$) with a 0.5 mol/dm^3 concentration to reduce the remaining Cr^{6+} (equation 2) using Barium Diphenylamine p-sulphonate as an indicator.

1. $K_2Cr_2O_7 + 3\ C + 16\ H^+ \rightarrow 4\ Cr^{3+} + 3\ CO_2 + 2\ H_2O$

2. $6\ Fe^{2+} + Cr_2O_7^{2-} + 14\ H^+ \rightarrow 2\ Cr^{3+} + 6\ Fe^{3+} + 7\ H_2O$

Together with the sample titration, a blank titration was run to determine the endpoint without any organic carbon present. When calculating the TOC the following equation was used:

$$\%\ TOC = \frac{([ml\ Fe^{2+}\ Blank - ml\ Fe^{2+}\ sample] \times concentration\ Fe^{2+}\ in\ mol/dm^3 \times 0.3 \times 1.4)}{Mass\ soil\ sample\ (g)}$$

A correction factor of 1.4 was used instead of the traditional 1.3 as suggested in Schumacher (2002), making this technique more comparable with other TOC determination techniques. This correction factor compensates for the fact that only approximately 71.4% of the organic carbon present participates in the redox reaction.

2.6. H4IIE Bio-assay

The H4IIE cells were grown in a sterile environment with 5% carbon dioxide at 37 °C in cell culture dishes (Greiner Bio-one; 100 x 20 mm, sterile) with a working volume of 12 ml. The cells were grown on Dulbecco's Modified Eagle's Medium (DMEM) containing L-glutamine, 1000 mg/l glucose without phenol red and sodium bicarbonate. It is important that the medium used should not contain phenol red since the presence of this chemical might influence the response of the cells. All chemicals and mediums used in cell culture were obtained from Sigma Aldrich with the exception of trypsin/versene (in Ca^{2+} and Mg^{2+} free Phosphate Buffered Saline (PBS) with 0.25% trypsin and 0.1% versene Ethylene-Diamine-Tetra-Acetic-Acid (EDTA)); (Highveld Biological) and Foetal Bovine Serum, (FBS) (Gibco). When the DMEM was prepared, sodium bicarbonate was added to the stock solution and the pH of the medium was adjusted to 7.4 to compensate for changes caused by sterile filtration. Before use in cell culture the DMEM stock solution was supplemented with European Union (EU) approved, 10% FBS (Davis, 2002).

Before a bio-assay could commence, the sample had to be diluted to different concentrations. Initially a three-times solution series was prepared to create a dose-response curve. The dilution factor was modified depending on the outcome of the

initial bio-assay. Standard curves were run concurrently on alternating plates using the most toxic dioxin congener 2,3,7,8-TCDD (Ultra Scientific). The concentration of the TCDD had to be known to allow data to be converted to usable TEQ values.

The completion of a bio-assay took five days. On the first day, the 96-microwell plates were seeded with approximately 50 000 cells/ml cell suspension (adapted from Whyte & Tillitt, 2004). The cells were trypsinised and a suspension made containing DMEM supplemented with hormone-stripped FBS since the presence of hormones could influence the response of the cells. The 60 interior wells of a 96–microwell plate were seeded with 250-µl cell suspension and the 36 exterior wells were filled with 250 µl PBS without Ca^{2+} and Mg^{2+} (Schmitz *et al.*, 1994; Masunaga *et al.*, 2002) as illustrated in Figure 2.7.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		TCDD 4.1	TCDD 4.1	TCDD 4.1	Hexane	Sample 1	Sample 1	Sample 1	Sample 2	Sample 2	Sample 2	
C		TCDD 4.2	TCDD 4.2	TCDD 4.2	Hexane	Sample 1:3	Sample 1:3	Sample 1:3	Sample 1:3	Sample 1:3	Sample 1:3	
D		TCDD 4.3	TCDD 4.3	TCDD 4.3	Hexane	Sample 1:9	Sample 1:9	Sample 1:9	Sample 1:9	Sample 1:9	Sample 1:9	
E		TCDD 4.4	TCDD 4.4	TCDD 4.4	Blank control	Sample 1:27	Sample 1:27	Sample 1:27	Sample 1:27	Sample 1:27	Sample 1:27	
F		TCDD 4.5	TCDD 4.5	TCDD 4.5	Blank control	Sample 1:81	Sample 1:81	Sample 1:81	Sample 1:81	Sample 1:81	Sample 1:81	
G		TCDD 4.6	TCDD 4.6	TCDD 4.6	Blank control	Sample 1:243	Sample 1:243	Sample 1:243	Sample 1:243	Sample 1:243	Sample 1	
H												

TCDD 4.1 = 7.8×10^{-2} mg/µl

TCDD 4.2 = 1.2×10^{-2} mg/µl

TCDD 4.3 = 3×10^{-3} mg/µl

TCDD 4.4 = 7.5×10^{-4} mg/µl

TCDD 4.5 = 1.88×10^{-4} mg/µl

TCDD 4.6 = 4.69×10^{-5} mg/µl

Figure 2.7: The basic layout of a 96-well plate during an H4IIE bio-assay indicating the contents of the different wells. The positions of the different components can be varied on each plate.

The cells were incubated overnight and dosed with 2.5 µl of the extract on day two of the assay (Masunaga *et al.*, 2002). Three repetitions of each extract concentration were dosed for statistical accuracy. Every second plate dosed also contained the TCDD standards and each plate contained a blank and solvent control (Schmitz *et al.*, 1994; Masunaga *et al.*, 2002). The cells were then incubated for 72 hr. After incubation, the plates were rinsed three times with PBS containing Mg^{2+} and Ca^{2+}. The addition of the Mg^{2+} and Ca^{2+} insures that there is an excess of these ions to

preclude them as a possible rate limiting factor during the light producing chemical reaction. Luclite® reagent (PerkinElmer) was then added to the wells. After an incubation of 10 min., the light emission of the cells was read using a luminometer (Microplate Fluorescence Reader Flx 800, Bio-Tek Instruments).

The results were then statistically analysed, using standard deviation and standard error calculations and the TEQ of each sample was determined using the TCDD standard graphs. The coefficient of variation was kept as close to or under 20 as possible to improve the accuracy of the statistical data analysis.

2.7. Data analysis

During the H4IIE bio-assay, TCDD standards were run concurrently with the samples. The standard data were analysed as illustrated in Table 2.2, Table 2.3 and Figures 2.8 and 2.9. A prepared TCDD dilution series was used with known concentrations in a 4:1 serial dilution (Nicks & Tillitt, 2003). Solvent corrections were done on all samples and standard readings to compensate for the cell's reaction to the hexane. Included on each plate, three wells were solely dosed with hexane (Masunaga, *et al.*, 2002), to determine the effect of the solvent. When the high concentrations of TCDD were run next to the solvent control wells, the results were of no value since the neighbouring TCDD then had an effect on the reading. This phenomenon of interfering signals emanating from adjacent wells is referred to as cross-talk (Anon, 2005c). For the same reason, samples were never dosed directly next to a standard since this could lead to a false positive response or an inflated luminometer reading. To compensate for the solvent's influence on the cells, the hexane readings were subtracted from the standard (Table 2.3) as well as the sample values.

The log values of the TCDD concentration were plotted against the average of the Relative Light Units (RLU) generated in the H4IIE bio-assay as shown in Figure 2.8. Sample values were then related to the maximum value for TCDD as well as the Effective Concentration (EC) values generated from the TCDD dose response curves. EC values were calculated as the concentration of a substance relative to the maximal activity induced by the TCDD standard. The Coefficient of Variation (CV) values were kept under or as close as possible to the 20% value as the CV should not exceed 20%. A CV value higher than this would indicate unreliable data

(Grantley, 2002). Figure 2.9 shows the log concentration of the TCDD standards against the mean RLU and includes the Standard Deviation (Stdev). Only values greater or equal to 20% of the maximum TCDD response were used in calculations, because it is necessary to use a standard range of response when evaluating environmental samples (Whyte, Schmitt & Tillitt, 2004).

Table 2.2: Data analyses of the TCDD standard values generated during an H4IIE bio-assay.

RLU Hexane	log TCDD	RLU TCDD	RLU TCDD	RLU TCDD	Mean RLU value	Stdev	CV	%TCDDmax
55.386	2.08	353.51*	397.86*	300.91*	350.76	48.53	13.84	77.03
56.713	1.48	473.67	489.55	402.83	455.35	46.17	10.14	100.00
49.229	0.88	395.74	446.16	339.6	393.83	53.31	13.54	86.49
Mean	0.27	273.94	366.45	331.39	323.93	46.70	14.42	71.14
53.776	-0.33	163.13	191.65	175.67	176.82	14.29	8.08	38.83
	-0.93	112.89	79.166	125.17	105.74	23.82	22.53	23.22

*Cells showing a cytotoxic response

Table 2.3: RLU values after the solvent correction has been implemented

RLU TCDD	RLU TCDD	RLU TCDD	Mean RLU value	Stdev	CV	%TCDDmax
299.73	344.08	247.13	296.98	48.53	16.34	73.95
419.89	435.77	349.05	401.57	46.17	11.50	100
341.96	392.38	285.82	340.06	53.31	15.68	84.68
220.16	312.67	277.61	270.15	46.70	17.29	67.27
109.35	137.87	121.89	123.04	14.29	11.62	30.64
59.11	25.39	71.39	51.97	23.82	45.83	12.94

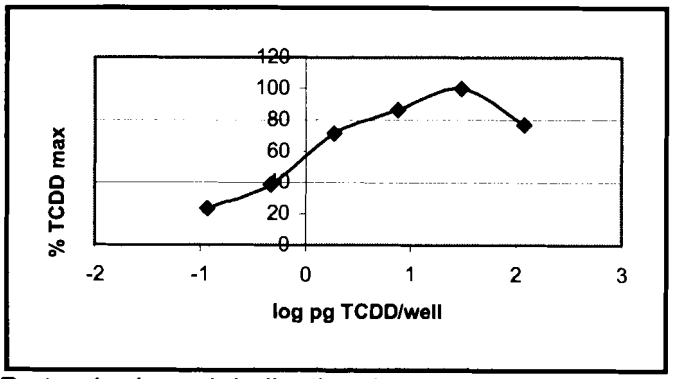

Figure 2.8: TCDD standard graph indicating the response as a percentage of the maximum response to the standard.

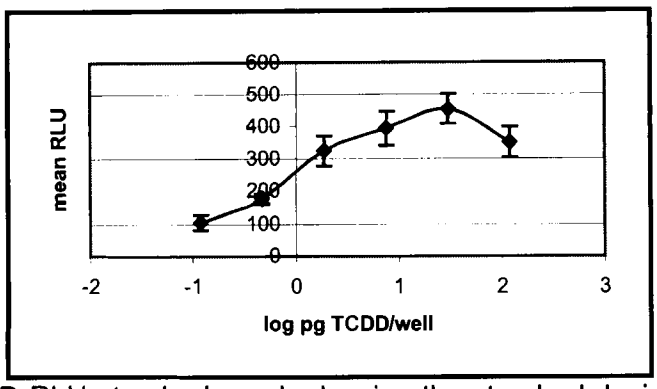

Figure 2.9: TCDD RLU standard graph showing the standard deviation of the mean relative light units generated during the H4IIE bio-assay.

From the straight line section of the dose response curve shown in Figure 2.8, the following parameters were calculated: slope, intercept, Correlation Coefficient (R^2) and the EC values.

Slope	33.42	EC20	0.09	pg/well TCDD
Intercept	54.81	EC50	0.72	pg/well TCDD
R^2	0.98	EC80	5.67	pg/well TCDD

TEQ values were calculated by comparing the luciferase activity in the cells caused by the pollutant to that of the TCDD standard (Schramm, Klimm, Hofmaier & Kettrup, 2001). The linear portion of the slope derived from the samples were normalised to the linear portion of the slope derived from the TCDD, resulting in a measure of the REP values (Nicks & Tillitt, 2003). Thus, the EC values generated in the sample data analyses were expressed in terms of the TCDD standard EC values, generating the REP values. The same calculations used in the TCDD standard dose responses were followed in the data analyses for the samples as shown in Table 2.4 and Figures 2.10 and 2.11. The greatest difference being that the mean relative light units were then expressed as a percentage response of the maximum TCDD response. The end results of the H4IIE bio-assay for sample 5 are summarised in Table 2.5.

Table 2.4: Representative illustration of sample data analyses.

Sample dilution	log µl/well	RLU Sample 5	RLU Sample 5	RLU Sample 5	Mean RLU Sample 5	Stdev	CV	%TCDDmax
2.5	0.40	408.16	392.40	317.13	372.56	48.65	13.06	81.82
0.83	-0.08	250.02	293.45	389.69	311.05	71.48	22.98	68.31
0.28	-0.56	157.28	177.72	187.24	174.08	15.31	8.79	38.23
0.09	-1.03	67.56	124.67	100.56	112.62	17.05	15.14	24.73
0.03	-1.51	75.58	70.13	64.20	69.97	5.69	8.14	15.37
0.01	-1.99	55.89	52.05	52.69	53.54	2.05	3.84	11.76

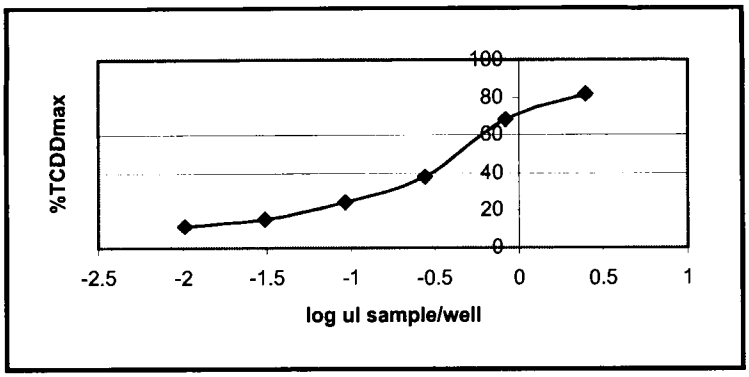

Figure 2.10: Standard dose response graph for sample 5 indicating the response as a percentage of the maximum response of the TCDD standard.

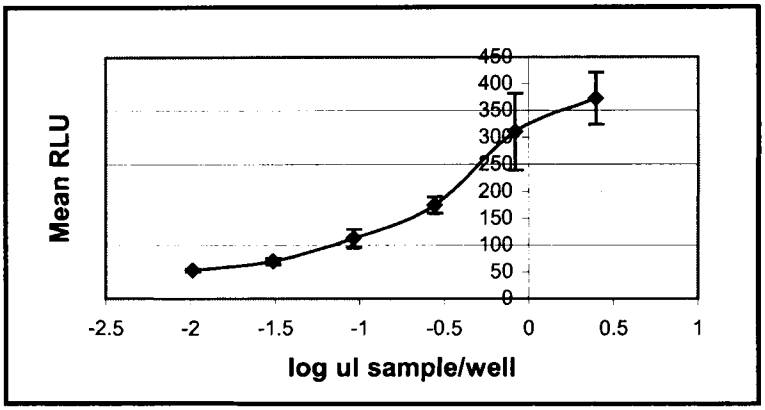

Figure 2.11: The dose response graph of sample 5 showing the standard deviation of the mean relative light units generated during the H4IIE bio-assay.

Table 2.5: Results for sample 5 as calculated from Figure 2.8 to Figure 2.11.

Description of straight line portion of graph		EC (µl/well)		REP values		
Slope	42.20	EC20	0.078	REP20	1.16	pg TEQ/ul
Intercept	66.68	EC50	0.40	REP50	1.78	pg TEQ/ul
R^2	0.98	EC80	2.07	REP80	2.74	pg TEQ/ul
				REP20	0.06	ng TEQ/g sediment
				REP50	0.09	ng TEQ/g sediment
				REP80	0.14	ng TEQ/g sediment

2.8. Geographical representation of data.

After the TEQs were determined for all the data points in the grid surrounding an active incinerator, the data were plotted geographically using GS+ (Version 7.7). Before the data could be read into this programme the co-ordinates had to be

translated into a compatible format with the DIDGER program. All data points for which values could not be determined, were given default values of zero. After the distribution maps were plotted, MapViewer (Version 6) was used to include reference points on the maps to facilitate the interpretation of these geographical maps. Maps were plotted for the %TOC, REP 50 values as well as the normalised TEQs for the entire grid.

2.9 Statistical analysis

The data was analyses statistically using the statistical programme Statistica (Version 7). The first step in the statistical analysis was to determine the normality of data. To achieve this the Shapiro-Wilks test was used. The effect of different parameters on the amount of dioxin-like chemicals in the soil was then statistically analysed using both parametric and non-parametric statistics. To determine the effect of tilling on the concentration of dioxin-like chemicals Analysis of Variance (ANOVA) was done. The relationship between TOC and the TEQ was determined using both regression analysis and the Spearman rank order correlation. Combined effects were determined using multiple regression analyses. Zero values were either excluded from the data set or log transformed (log (TEQ + 1)) depending on the nature of the analysis implemented.

Chapter 3: Results

3.1. Summarised H4IIE data

The results of the soil samples were calculated as explained in Chapter 2. The results of the soil samples collected in a grid surrounding an active incinerator are summarised in Table 3.1. The EC and REP values are reported with the percentage TOC for all of the soil samples taken as well as the normalised data for REP50. Of the REP20-80 data generated, the REP50 values were chosen to report normalised data, as these values could be directly measured for the majority of the soil samples without extrapolating the dose response curve. The TEQ reported throughout the rest of the results and discussion will refer to the TEQ values for REP50. Normalised data was calculated by dividing the REP values by the percentage carbon present in that specific sample and converting to 100% carbon content. Through normalising the TEQ values, the effect of carbon variation on the amount of dioxin-like chemicals present in the samples was eliminated.

The concentration of dioxin-like chemicals in the samples with a zero reading was below the detection limit of the H4IIE bio-assay and the data was entered as 0, meaning "not detected" (Baccarelli, Pfeiffer, Consonni, Pesatori, Bonzini, Patterson, Bertazzi & Landi, 2005). The lowest limit of detection in this study was 0.181 pg TCDD/well. Previously reported detection limits were between 0.064 and 0.448 pg TCDD/well (Sanderson *et al.*, 1996) showing a good association with the measured lowest limit. When calculating the TEQs of the samples, a relative potency range has to be standardised. For this reason points falling below 20% were not used since the standard range of response used by Whyte *et al.* (2004) was between 20 and 80% of the TCDD maximum response.

Sample 10 was cytotoxic to the H4IIE cell line at all concentrations and further analysis of the sample was not possible, even though a response was detected. To create the distribution map (section 3.2), this sample was also allocated a value of 0. The table also shows that there was a great amount of variation in the TEQ values from the different sampling locations. The TEQ varied between 122.00 ngTEQ/kg and the detection limit of the method and the normalised TEQ values varied between the detection limit and 5731 ngTEQ/kg. Samples 6 and 22 had low R^2 values with a R^2 value of 0.63 and 0.46 respectively. The assays were repeated numerous times with different series of dilutions, but better results could not be obtained. Samples 6

and 22 never responded in a completely linear manner. The REP50 TEQ varied considerably from the normalised values: the REP50 TEQs ranged between 0 and 154 ngTEQ/kg compared to the normalised data that ranged between 0 and 5731 ngTEQ/kg. Even though the values were different the basic trends in distribution were similar as can be seen in Figures 3.2 and 3.3.

Table 3.1: TEQ values for the samples collected in the soil surrounding an active incinerator in the Potchefstroom area.

Sample	R^2	EC20	EC50	EC80	REP20	REP50	REP80	%TOC	Normalised data REP50
			ul/well			ng TEQ/kg			ngTEQ/Kg
1	0.99	0.15	0.51	1.74	58.00	122.00	257.00	3.19	3821
2	0.82	0.19	3.26	55.41	20.00	4.00	1.00	0.96	418
3	0.95	0.18	0.79	3.52	44.00	36.00	30.00	3.89	927
4	0.95	0.56	2.89	14.99	11.00	28.00	67.00	2.91	964
5	0.97	0.13	0.56	2.35	67.00	97.00	142.00	5.27	1840
6	0.63	0.04	0.76	13.74	355.00	65.00	12.00	2.96	2197
7	0.93	0.23	1.39	8.29	27.00	58.00	121.00	3.17	1829
8	0.82	0.41	3.97	38.53	7.00	5.00	3.00	0.99	507
9	0.91	0.21	1.45	10.12	4.00	10.00	29.00	1.37	733
10	-	0+	0+	0+	0+	0+	0+	3.72	0+
11	-	0	0	0	0	0	0	4.85	0
12	0.91	0.37	1.64	7.38	11.00	7.00	4.00	1.59	440
13	0.97	0.40	0.92	2.11	10.00	13.00	15.00	3.88	335
14	0.91	0.66	1.64	4.09	13.00	14.00	16.00	0.90	1550
15	0.93	0.24	1.32	7.14	58.00	36.00	23.00	4.09	881
16	-	0	0	0	0	0	0	2.14	0
17	0.85	0.32	0.90	2.53	26.00	26.00	25.00	1.93	1346
18	-	0	0	0	0	0	0	5.38	0
19	0.96	0.07	0.34	1.57	38.00	33.00	28.00	4.83	683
20	0.95	0.16	0.62	2.46	47.00	69.00	101.00	1.26	5476
21	0.85	0.11	1.34	16.79	27.00	8.00	3.00	2.44	328
22	0.46	0.31	14.13	644.41	9.00	1.00	0.07	2.45	041
23	0.96	0.96	5.48	31.35	2.00	3.00	6.00	1.37	220
24	-	0	0	0	0	0	0	3.09	0
25	-	0	0	0	0	0	0	0.74	0
26	-	0	0	0	0	0	0	5.08	0
27	-	0	0	0	0	0	0	0.88	0
28	0.94	0.21	0.54	1.34	69.00	154.00	342.00	2.69	5731
29	0.97	0.54	2.05	7.80	8.00	11.00	14.00	2.42	456
30	0.95	1.79	211.67	25092.36	2.50	0.10	0	2.35	4
31	-	0	0	0	0	0	0	2.12	0
32	-	0	0	0	0	0	0	3.52	0
33	-	0	0	0	0	0	0	1.20	0
34	-	0	0	0	0	0	0	1.48	0
35	-	0	0	0	0	0	0	0.99	0
36	-	0	0	0	0	0	0	1.95	0
37	0.99	0.32	1.23	4.70	25.00	25.00	25.00	3.17	788

+ Indicates that there was a response in the sample and that the value of the response could not be determined.

0 Value below the detection limit

The ash samples collected at the various incinerators are summarised in Table 3.2 With the exception of the ash collected at the old incinerator C3, no values could be calculated due to a high occurrence of cytotoxicity in the bio-assay. This was also seen in all the soil samples collected in the direct vicinity of the incinerators, except for Grid 37. This sample was collected at the active incinerator on the premises of the ARC. The incinerator at C2 had been demolished and the sample was taken from the area where the incinerator once stood. This sample could not be classified as ash, since soil was also present in the sample.

Table 3.2: TEQ values for ash collected from incinerators in the Potchefstroom area and soil directly surrounding the incinerators.

Sample	R^2	EC20	EC50	EC80	REP20	REP50	REP80	%TOC	Normalised data REP50
		ul/well			ng TEQ/g				ngTEQ/Kg
Grid 37 Ash	-	0+	0+	0+	0+	0+	0+	13.555	0+
C1 Ash*	-	0+	0+	0+	0+	0+	0+	8.847	0+
C2 Ash*#	-	0+	0+	0+	0+	0+	0+	11.325	0+
C4 Ash new incinerator*	-	0+	0+	0+	0+	0+	0+	4.200	0+
C4 Ash old incinerator*	0.91	0.373	1.138	3.471	0.008	0.018	0.041	4.852	370.98
C1 soil	-	0+	0+	0+	0+	0+	0+	0.420	0+
C2 soil	-	0+	0+	0+	0+	0+	0+	4.977	0+
C4 soil	-	0+	0+	0+	0+	0+	0+	1.197	0+
Grid 37 Ground	0.99	0.321	1.227	4.697	0.025	0.025	0.025	3.171	788.40

\+ Indicates that there was a response in the sample and that the value of the response could not be determined.
0 Values below the detection limit.
\- Further calculations not possible.
* Incinerators were not active at the time of sampling.
\# Mixture of ash and soil.

3.2 Geographical distribution

The %TOC as summarised in Table 3.1 is graphically presented in Figure 3.1. This figure shows the distribution of the TOC as interpolated from the 37 points on the grid surrounding the incinerator. The distribution map was compiled using GS+ (Version 7.22) and Map Viewer (Version 6). The distribution map also contains reference markers (such as roads and facilities) to allow easier interpretation of the visual data. The same programmes were used for Figure 3.2 and Figure 3.3. Figure 3.2 shows the interpolated REP50 (ngTEQ/kg) data for each point on the sampling grid before the TOC was taken into account. Figure 3.3 shows the soil organic content normalised TEQ distribution.

Table 3.3: Soil tillage category allocation and associated TEQ (ngTEQ/ kg) of the 37 sampling sites around an active incinerator.

Sampling site	Soil tillage category	TEQ (ngTEQ/kg)	Sampling site	Soil tillage category	TEQ (ngTEQ/kg)
21	1	8	2	3	4
25	1	0	3	3	36
27	1	0	4	3	28
35	1	0	5	3	97
13	2	13	6	3	65
15	2	36	7	3	58
16	2	0	8	3	5
18	2	0	9	3	10
19	2	33	10	3	0
22	2	1	11	3	0
26	2	0	12	3	7
28	2	154	14	3	14
29	2	11	17	3	26
30	2	0.1	20	3	69
32	2	0	23	3	3
33	2	0	21	3	0
34	2	0	31	3	0
36	2	0	37	3	25
1	3	122			

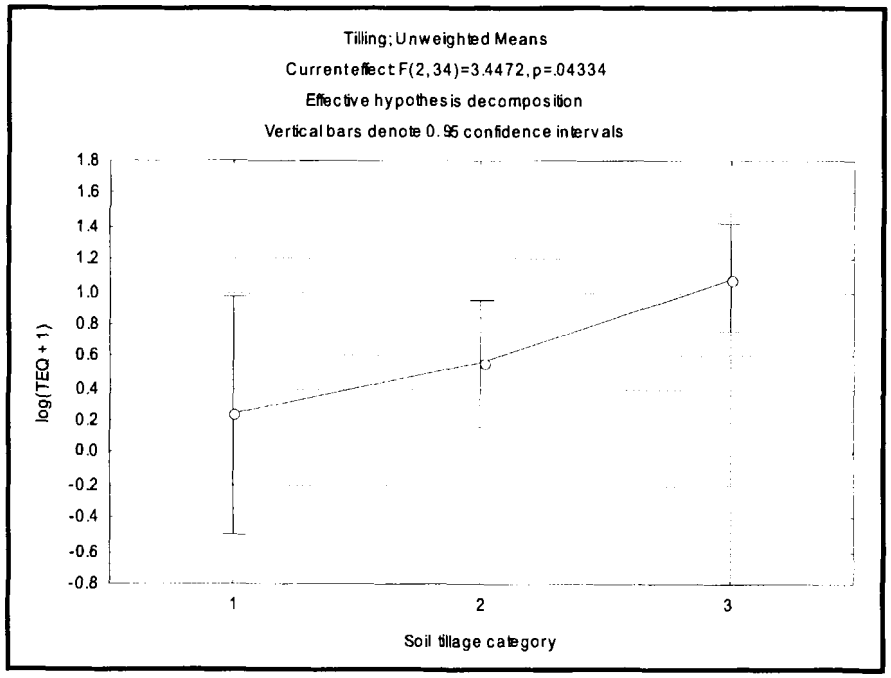

Figure 3.4: The association between tillage and the TEQ concentration in soil.

3.4. Relationship between TOC and TEQ

Since soil rich organic substances have a higher capacity for the sorption of pollutants from the atmosphere (Prevedouros *et al.*, 2004), a relationship should exist between the TOC in soil and the TEQ of the area. To determine the relationship between the TOC content of the soil and the measured TEQ values before normalisation, regression analysis was done on the log transformed REP 50 TEQs and the corresponding %TOC values (Hassanin, Lee, Steinnes & Jones, 2005). A computer programme, Statistica (version 7), was used in this analysis. Samples that fell below the detection limit of the method were assigned a zero value, and not used in calculations. REP50 values were chosen because these values were actually measured for most of the samples, thus they are not extrapolated values from the dose response curves.

The Shapiro-Wilks test was performed to determine the normality of the data. Normality was confirmed with a p-value of 0.056 and a normal regression analysis was executed (Figure 3.5; $R^2 = 0.173$; $p = 0.048$). This shows that approximately 17.3% of the variance in the TEQ data can be explained or is correlated to the %TOC in the soil samples. The null hypothesis in straight-line regressions assumes that the variables are not correlated and that the slope = 0 (Linear regression, 2005). A p-value < 0.05 indicates that a significant correlation exists and that the slope is greater than 0.

The linear relationship between the TEQ and the TOC changed when the zero values were included in the data set (Figure 3.6). When 0 values were included no statistically significant linear relationship was found and the slope was equivalent to 0 ($p > 0.05$). In this analysis only 0.7% of the variance of the TEQ values could be explained by the TOC in comparison with the 17.3 % if 0 values were excluded.

Statistical analysis was also done for the residential area because the area could show a different tendency according to Rogowski & Yake (2005). Since the data pool in this regard was small (n=9), parametric tests could not be preformed. The Spearman rank order correlation was executed to determine whether a correlation between these factors existed. This test showed no statistically meaningful correlation between the variables with a $p > 0.05$. Marked correlations in this test is only significant at $p<0.05$.

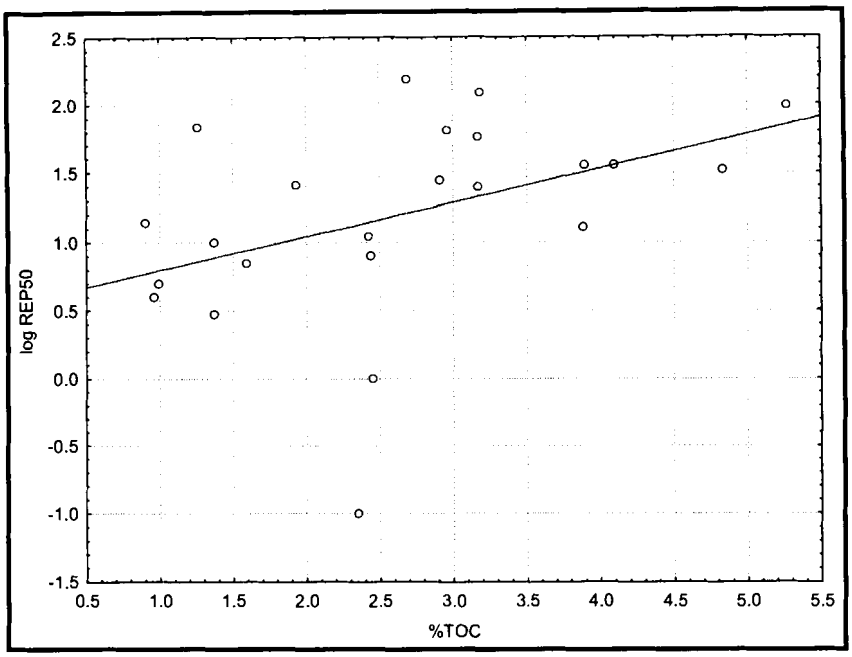

Figure 3.5: The regression analysis of the log REP50 value of the samples surrounding the active incinerator at the ARC against the %TOC found at the corresponding sampling sites. The 0 values were excluded from the data set (R^2 = 0.173; p = 0.048).

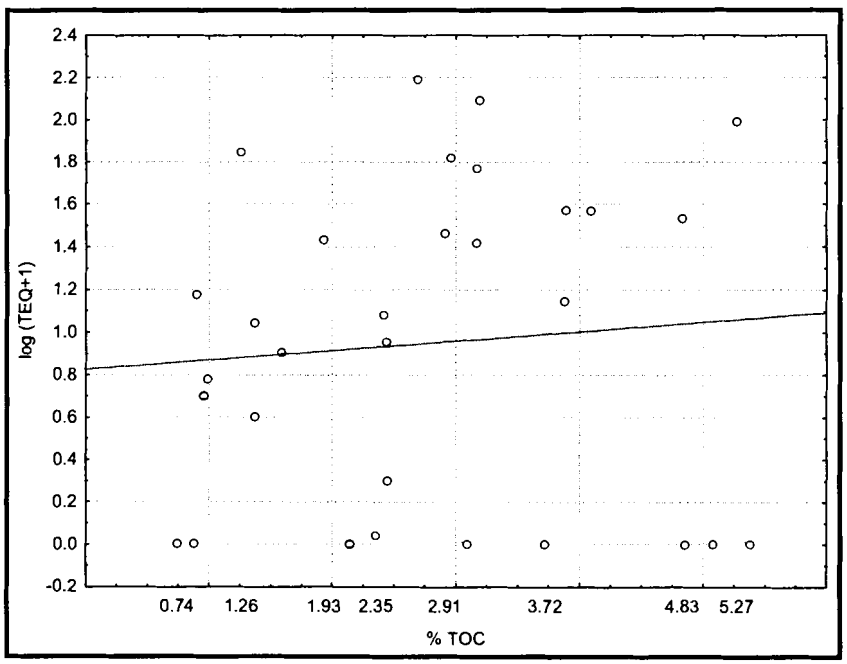

Figure 3.6: The regression analysis of the log (TEQ + 1) value of the samples surrounding the active incinerator at the ARC against the %TOC found at the corresponding sampling sites. The 0 values included into the data set (R^2 = 0.0073; p = 0.65).

To determine whether the TOC and TEQ had a combined effect on the TEQ, a multiple regression analysis was done. No meaningful relationship was found ($p > 0.05$).

3.5. Meteorological data.

Monthly average climate data was obtained from the South African Weather Service. This data includes the average precipitation (Table 3.4 and Table 3.5), the average minimum (min) and maximum (max) temperatures and an analysis of the wind direction prevalence.

Table 3.4 indicates that the highest precipitation in Potchefstroom occurred between November and March with the lowest rainfall during the winter months in June and July. Table 3.5 shows the minimum and maximum temperatures for 2004, the year in which sampling occurred.

Table 3.4: The average monthly rainfall (mm) for the Potchefstroom area from 1994-2004 (South African Weather Service, 2005).

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1994	119	195.5	49.5	25	0.8	0	1	0	0	23	63.5	87
1995	68	5	59	36	16	0	0	3	39	75.7	84.9	165.9
1996	133.5	188.5	46.5	164	40	0	8.5	20	5.7	73.3	80.1	180.3
1997	48	39	125.5	80.5	107.8	0	1.7	10.6	31.5	22.7	104.5	47.7
1998	80.1	61.3	50.1	21	0	0	0	0	25.5	56.9	93	152.4
1999	17.1	~	6	13.2	26.2	0	0	0	0	1.5	12.5	82.2
2000	54.9	~	257.7	13	0	0	0	0	56	83.6	45.3	~
2001	25.5	80	24.7	73.4	26	20.7	0	17.5	8	61.6	167	142.1
2002	94.9	84.5	36.6	28	19	0	0	39.5	0	32.5	~	167.8
2003	76	59.7	67.4	0	0	0	0	19	0	41	94.1	23.3
2004	81	179.5	100	61	0	20	4.4	15	0	44.8	38.4	~
Average	72.55	99.22	74.82	46.83	21.36	3.7	1.42	11.33	15.06	46.96	78.3	116.52

~ Missing data values

Table 3.5: The average monthly max and min temperatures for Potchefstroom for 2004 (South African Weather Service, 2005).

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
max 2004	30	28.3	25.9	24.9	23.8	19.7	18.4	24.1	25.4	28.8	32.1	30.1
min 2004	17	16.2	14.3	9.6	4.2	0.8	-0.8	5.7	7	12.1	15.5	16.5

The preceding tables are summarised in Figure 3.7. This figure shows that the highest temperatures also coincide with the highest precipitation and that the lowest temperatures were in June and July, the months with the lowest precipitation.

Furthermore, the fluctuation between the maximum temperatures was relatively low with the greatest variation between November and July. These two months' maximum temperatures differed by 13.7 °C. According to Table 3.6 the main direction of wind in the Potchefstroom area was from the North followed by North-North-East. This is shown graphically in Figure 3.8.

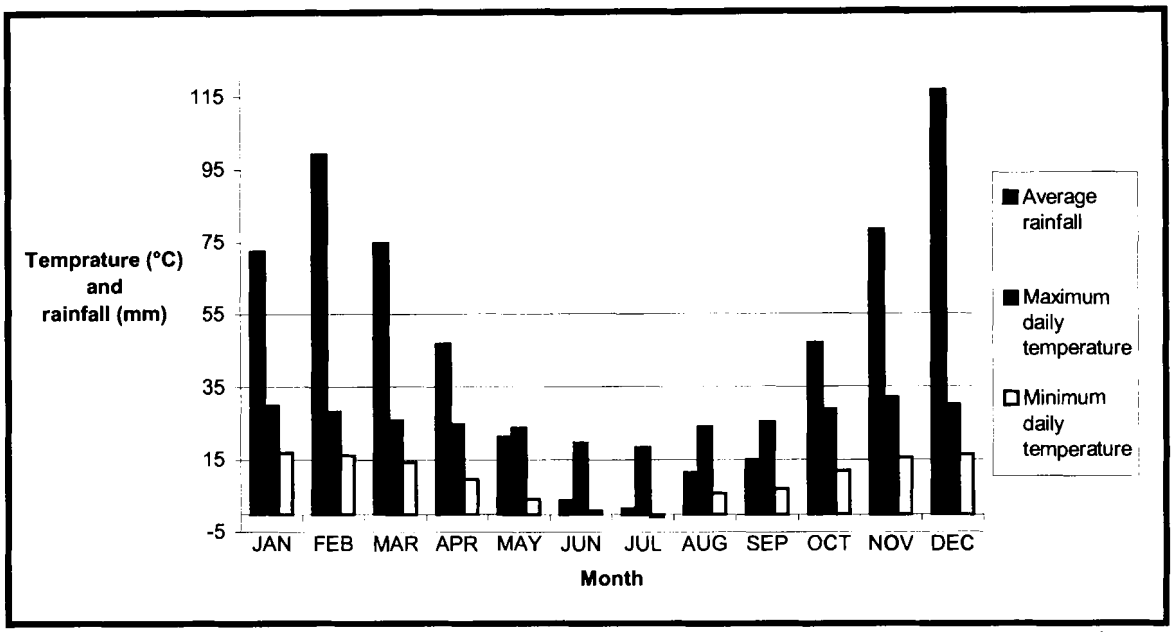

Figure 3.7: Average monthly rainfall (1994-2004) as well as the maximum and minimum temperatures (2004) for the Potchefstroom area.

Table 3.6. The wind's directional prevalence (%) in Potchefstroom calculated from 1990-2004 (South African Weather Service, 2005).

MONTH	Calm	N	NNE	NE	ENE	E	ESE	SE	SSE	S	SSW	SW	WSW	W	WNW	NW	NWW
JAN	6	21	13	8	9	5	1	1	1	3	4	5	4	5	3	4	7
FEB	8	18	13	12	11	8	2	1	1	3	3	4	3	4	2	3	4
MARCH	11	21	15	10	8	6	2	1	1	3	3	4	3	4	2	3	4
APRIL	10	27	17	7	3	2	1	1	1	3	3	4	5	5	3	3	5
MAY	16	23	15	5	3	2	1	1	1	3	3	6	6	6	2	3	4
JUN	13	25	18	6	3	2	1	1	1	2	4	5	6	4	2	3	4
JUL	9	27	18	6	4	3	1	1	1	2	4	5	5	4	2	3	4
AUG	8	31	14	5	2	2	1	1	1	3	5	6	6	4	3	4	5
SEP	5	29	11	6	5	4	1	1	1	2	4	6	5	6	4	5	6
OCT	4	27	14	8	8	5	2	1	1	3	3	3	4	5	3	5	7
NOV	4	26	12	8	7	4	1	1	1	3	3	4	4	5	4	5	7
DEC	4	28	12	8	6	4	1	1	1	3	3	3	3	5	4	6	9
Total	98	303	172	89	69	47	15	12	12	33	42	55	54	57	34	47	66
% ff	8.13	25.15	14.27	7.39	5.73	3.9	1.24	0.996	0.996	2.7	3.49	4.56	4.48	4.73	2.82	3.9	5.48

N	North	ESE	East-South-East	SW	South-West
NNE	North-North-East	SE	South-East	WSW	West-South-West
NE	North-East	SSE	South-South-East	W	West
ENE	East-North-East	S	South	WNW	West-North-West
E	East	SSW	South-South-West	NW	North-West
NWW	North-West-West				

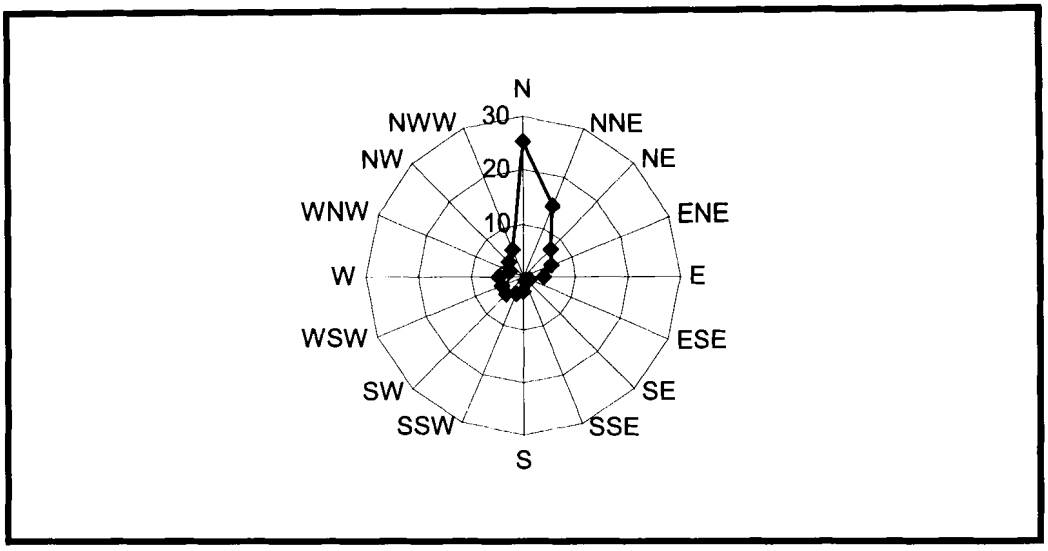

Figure 3.8: The percentage frequency (%) for each wind direction in Potchefstroom from data collected between 1990 and 2004 by the South African Weather Service. Calm conditions prevailed 8.13% of the time.

Chapter 4: Discussion

4.1. The distribution of dioxin-like chemicals in soil surrounding an active incinerator.

The levels of dioxin-like chemicals in environmental samples are highly variable and depend on a number of factors (Meneses, *et al.*, 2004). Mainly the concentration and the accumulation of dioxin-like chemicals in soil are depended upon the deposition of the chemicals as well as the uptake by plants, background concentrations, and the loss of pollutants through processes such as volatilisation and leaching (Meneses, *et al.*, 2004). The amount of total organic carbon and the characteristics of the soil have also been known to affect the soil's capacity to absorb pollutants (Rogowski & Yake, 2005). During the current investigation, soil characteristics other than the TOC were not taken into account. Neither was past land-use or historical levels accounted for.

The normalised TEQ compensated for the differences in carbon content that occurred at the different sampling sites. This facilitated the compilation of a distribution map that accounted for the TOC variable and illustrated what concentration of dioxin-like chemicals would likely be in an area, if all the sites had 100% TOC value. Both Figures 3.2 and 3.3 showed the same tendency in TEQ distribution. However, the normalised TEQ does not represent the risk the environment or humans are exposed to in these areas, since it is not the actual concentration of the dioxin-like chemicals at a site. To estimate the physical risk and exposure, the measured TEQ values should be used.

4.1.1. The effect of tillage and TOC content on the distribution of dioxin-like chemicals.

As can been seen in the distribution map (Figure 3.2) the levels of dioxin-like chemicals were quite variable, localised, and with no clear distribution pattern. What became apparent was that the levels of dioxin-like chemicals were generally very low in agricultural regions. Samples 28 and 29 were exceptions (Table 3.1). Sample 28 had the highest measured TEQ in the entire grid. There can be a number of reasons for this observation. The field was being used for grazing at the time of sampling, thus does not exclude possible pesticide applications in the past that could still be affecting the data. Another possibility is that the field itself could have been burned or that ash could have been applied as a fertiliser, although the TOC was not markedly

higher than the surrounding area. The TEQs were very low or fell under the detection limit of the bio-assay for the rest of the samples taken in agricultural ecosystems. Lower levels of dioxin-like chemicals in agricultural ecosystems could be attributed to dioxin-like chemicals being diluted during tilling and subsequent erosion of soil surface layers (Rogowski & Yake, 2005). Sample 16 that was below the detection limit of this method was taken from an ant mound since no other soil could be sampled in the region. This sample did not represent the topsoil concentration like the other samples collected, since the earth was brought from deeper regions by the activity of the ants. This value could represent an under estimation of the true dioxin concentration of the area.

Figure 3.4 shows that the amount of tillage had a statistically significant influence on the TEQ calculated in soil. As the likelihood of tillage increased, the concentration of dioxin-like chemicals in the area decreased. The standard deviation indicated in Figure 3.4 is very large. One of the possible reasons for this observation is the variation in the individual TEQs measured at each site. This intrinsic variation in the TEQ caused a large standard deviation to occur when determining the average for the three sites.

When visually comparing the distribution maps of the TOC (Figure 3.1), and the TEQ from the REP50s (Figure 3.2), there did not seem to be a clear relationship between the TOC and the amount of dioxin-like chemicals present in the soil. Regression analysis was done to assess the relationship between the %TOC in the soil and the concentration of dioxin-like chemicals present (REP50 ngTEQ/g) (Hassanin *et al.*, 2005). The influence the TOC had on the REP50 values for the 23 sites evaluated were low since the R^2 value was only 0.173 (Figure 3.5). There is a slope present in Figure 3.5 since the p-value is less than 0.05. This showed that a statistically significant correlation did exist between the TOC and REP 50 values however the correlation was not very strong and factors other than the %TOC, such as soil tillage, could have affected the concentration of dioxin-like chemicals in the soil (Hassanin *et al.*, 2005). The combined effect of tillage and TOC on the TEQ was analysed through the use of a multiple regression analyses. This regression showed that these two factors had no meaningful combined influence on the TOC.

When 0 values were included in the analysis (Figure 3.6) the influence of the TOC on the TEQ became negligible, with a slope equivalent to 0 ($p > 0.05$). The 0 values are not necessarily 0. The possibility exists that dioxin-like chemicals were present in the

area at concentrations too low to be detected with this method. The large effect that the 0 values had on the statistical analysis of the data has to be investigated further. The use of half the detection limit could be a better option.

The weak correlation between the TOC and TEQ levels is unusual since organic-rich terrestrial surfaces have a higher capacity for the sorption of pollutants from the atmosphere (Prevedouros *et al.*, 2004). However, Rogowski & Yake (2005) found that there was not a significant correlation between %TOC and TEQs from open areas. The sampling sites in this study area were relatively open. The residential areas, however, could not be described as open since there was a concentration of buildings. For this reason the relationship between %TOC and REP 50 values were separately calculated for the residential areas. The results of the analysis showed that there was no correlation between the %TOC and the TEQ in the residential sites. It is possible that a higher correlation would have been found between the %TOC and the TEQ if all the sampling sites were exposed to the same amount of dioxin-like chemicals. This was not the case due to the presence of many different diffuse sources in the Potchefstroom area. Each sampling site was exposed to a different potential amount of dioxin deposition from industrial boilers in the industrial area, the burning of waste and the effect of backyard burning (Wevers, De Frè, Desmedt, 2004) in the urban areas. These factors made statistical analyses very difficult given that all these factors could not be converted into quantitative data.

4.1.2. Transport and deposition of dioxin-like chemicals from the waste incinerator stack.

The highest TEQ levels for dioxin-like chemicals occurred on the outskirts of the area (Figure 3.2) and seemed to indicate that the transport of dioxin-like chemicals might have been further than initially expected, or that localised sources were involved. Domingo *et al.* (2001) had a similar study design with the furthest point 1500 m from a municipal waste incinerator in Spain (the basis of the design of this study). However, Lohman & Seigneur (2001) stated that these chemicals tend to be transported further than 100 km from the source, with the exception of medical waste incinerators. The majority of dioxin-like chemicals released from medical waste incinerators are deposited locally (Lohman & Seigneur, 2001). This indicates that the grid design might have been clustered too closely to the point of origin to be an estimate of the effect of the incinerator. Sites further from the incinerator should have been included in this study. It is as yet unclear at what radius the greatest deposition

will occur. A better experimental design therefore could be sampling a grid with a greater scale (at least 1 to 2 km between sampling sites) with reference points close to the incinerator. Plume temperature models could help to predict the area most likely affected by deposition. In South Africa it has been shown that dioxin-like chemicals are formed as they leave the stack tip (Brent & Rogers, 1990). This could influence the distance of deposition from the source.

According to Figure 3.2 there are higher concentrations of TEQs in the residential areas of Potchefstroom. These areas could also have various other sources of dioxin-like chemicals including: backyard burning, industrial activities, exhaust fumes and the incinerator itself. In many cases, these areas also have a larger historical load of dioxin-like chemicals. Numerous other studies have found elevated levels of dioxin-like chemicals in industrial and urban areas (New Zealand Ministry for the Environment, 2002; Rogowski & Yake, 2005) compared to other land uses. A possible explanation for this observation could be the differences in soil composition and TOC caused by different land uses.

The levels of dioxin-like chemicals varied between non-detectable and 154 ngTEQ/kg. These values are lower than can be expected from an area associated with a mixed medical and carcass incinerator. The estimated emission release factor was determined using the *UNEP Standardized Toolkit for identification and quantification of dioxin and furan release* (UNEP, 2003). The toolkit expresses the expected dioxin emission from a point source and only indicates the range of possible emissions. These values were calculated through UNEP-recommended procedures as listed in the Toolkit; a value of 83750000 ngTEQ/annum released to air was calculated for the incinerator. This value is extraordinarily high since the furnace had no APCS and functioned in a batch-type operation. These conditions often lead to poor combustion conditions and increased dioxin formation (McKay, 2002; UNEP, 2003). Although this value gave valuable information on the magnitude of release to the environment, it had the limitation of not being able to access the fate of the dioxin-like chemicals after release (UNEP, 2003). The calculated value was also just an estimate and could vary greatly from the actual release levels. The only way to determine the physical dioxin release was through direct measurement of gas and ash emission. In this type of incineration, a one-time measurement will also not give a complete picture of dioxin release since each incineration batch differed in composition and subsequent emission. The low measurable values in the

environment can be caused by a number of different factors influencing the dioxin-like chemicals concentrations in soil.

4.1.2.1 Meteorological conditions and the deposition of dioxin-like chemicals

Firstly, the concentration of dioxin-like chemicals in environmental compartments has been linked to seasonal and meteorological conditions (Moon *et al.*, 2005). It has been observed that there are higher dioxin concentrations in the winter months. Although there is a greater amount of household combustion processes in winter, the main emission sources (incinerators and industrial processes) stay constant. The effect of ambient temperature thus seems to play an important role in the amount of dioxin-like chemicals that are deposited onto soil. It has been found that with higher temperatures, less dioxin-like-chemicals are deposited (Moon *et al.*, 2005). The maximum daily temperature for Potchefstroom during 2004 varied between 32.1 and 18.4 °C (Table 3.4). Clearly the average ambient temperature in Potchefstroom is relatively high. One of the reasons that less dioxin-like chemicals are deposited in areas with high temperatures is because of the removal of dioxin-like chemicals during their movement in the atmosphere through chemical degradation processes such as OH^- radical reactions (Moon *et al.*, 2005). High temperatures could also cause the volatilisation from soil of these chemicals leading to long-range transport of the contaminants (Gouin, Mackay, Jones, Harner & Meijer, 2004).

Deposition processes are also greatly influenced by precipitation. There are two main deposition processes that affect dioxin-like chemicals: dry and wet deposition. The process of dry deposition is a result of gravitational forces as well as turbulent and molecular diffusion (Meneses, *et al.*, 2004). Lower chlorinated dioxin congeners are associated with coarse particles and predominantly deposited through dry deposition processes and gaseous deposition (Welsch-Pausch & McLachlan, 1998; Moon *et al.*, 2005). The higher chlorinated congeners are associated with finer particles and are mainly deposited through wet deposition (Moon *et al.*, 2005). Since the toxicity of dioxin-like-chemicals is restricted to the congeners that are chlorinated in the 2,3,7,8 positions of the molecule (Stanmore, 2004) and higher chlorinated congeners, wet deposition should lead to the deposition of a higher margin of toxic congeners. These highly-chlorinated congeners are also the dioxin-like chemicals that are most stable in the environment. The highest rainfall in Potchefstroom occurs during the summer months, in particular during December with an average rainfall of 118 mm (Figure 3.7). The least rainfall occurs during the colder winter months (Figure 3.7). Thus, in

the winter months, when the greatest amount of deposition can occur, since there is less temperature-dependant degradation of dioxin-like chemicals, there is little or no rainfall to facilitate wet deposition. When the ambient temperature is highest and the greatest amount of degradation occurs, wet deposition can occur. The meteorological condition in the central regions of South Africa that is dry with high ambient temperatures will not favour a great amount of dioxin deposition. Another factor influencing the wet deposition is the location of the incinerator. Since the incinerator is positioned outdoors, burning activity will not occur during periods of rain, further decreasing the chance that wet deposition will occur.

The wind direction did not seem to influence the distribution of dioxin-like chemicals in the grid that was sampled. There is no visible association between the main wind direction, North (Figure 3.8), and the highest concentration of dioxin-like chemicals (Figure 3.2).

4.1.3. Bio-availability

Another factor that could have influenced dioxin concentrations was the bio-availability of the toxicants in the samples. The bio-availability can be influenced by a number of factors such as ageing of the chemicals in soil. Ageing processes move the chemicals into compartments that are inaccessible and there is a reduction in extractability (Reid *et al.*, 2000). This could also play a role in the measurable concentration of dioxin-like chemicals in environmental soil samples.

4.1.4. The effect of cytotoxicity and additional sources of dioxin-like chemicals.

In this data set sample 10 showed cytotoxicity at all concentrations. When the sample was diluted, there was too little compounds binding to the AhR receptor and only a weak response was noted. The sample then fell below the detection limit of the bio-assay. The reaction of the cells caused by the dioxin-like chemicals was masked by a substance in the extract that caused the cells to die. Cytotoxic responses will be discussed further in section 4.3. Since this sample was given a default value of zero plus it could be an underestimation of the true dioxin content of soil in the area.

It is hard to determine the effect of one point source in a city like Potchefstroom. Some of the sampling locations are exposed to multiple possible dioxin sources as

discussed earlier. Potchefstroom has industrial sources such as power generating boilers, brick production plants, vehicle exhaust gases, backyard burning, veld fires and other uncontrolled combustion processes. These sources also have the potential to release dioxin-like chemicals into the atmosphere and can contribute to the current dioxin load in soil surrounding the Potchefstroom area.

4.2. Soil concentration compared to international findings.

In a study of dioxin concentrations over a large area in Washington State, USA, including urban, industrial, agricultural and forestry land-uses, normalised results were reported (Rogowoski & Yake, 2005). Their study found different TEQs for different land uses. The highest concentrations were found in urban areas and the lowest concentrations in agricultural settings. The concentrations reported were similar to those found in this study although the distribution of the TEQs over different land-uses did not overlap. The highest concentration found in this study occurred in agricultural land and not an urbanised area. The total dioxin concentrations reported by Rogowoski & Yake (2005) were between 42 and 610 ngTEQ/kg (depending on the land-use), falling in a similar range to this study's values, between non-detectable and 154 ngTEQ/kg. Both these values fell below the USA's action level of 1000 ngTEQ/kg but within the range for evaluation between 50-1000 ng TEQ/kg (according to New Zealand Ministry for the Environment, 2002). The range of evaluation indicates that an area needs further study and risk assessment due to a relatively-high dioxin soil content. Canada on the other hand has more stringent standards as presented in Table 1.1. According to Canadian soil guidelines the residential soil criterion is 4 ngTEQ/kg (New Zealand Ministry for the Environment, 2002). The levels found in the present study were higher than the Canadian guideline and fell within the USA's range for evaluation. Especially sample 5 (97 ngTEQ/kg), sample 1 (122 ngTEQ/kg), and sample 28 (154 ngTEQ/kg) need further investigation into the high dioxin levels. These international guidelines indicate that further research concerning dioxin-like chemicals in South Africa is crucial to determine the risk that the population is being exposed to. Even though Potchefstroom is well developed, there are fewer industries and possible sources of dioxin-like chemicals in the area when compared to South Africa's industrial centres.

4.3. Incinerator ash results

Very little could be deduced from the data of the analyses of the incinerator ash and soil within a close proximity to the incinerators themselves. The main reason for this was the cytotoxicity within the samples as illustrated by the dose response curve in Figure 4.2. In this dose response curve, the two highest concentrations caused the H4IIE cells to die and this led to low RLU readings. The maximum response only occurred at lower concentrations. This made accurate data analysis impossible. There are often compounds present in environmental samples that can cause cytotoxicity. Environmental samples normally consist of complex mixtures that contain elements such as sulphur, which is a highly cytotoxic constituent (Giesy *et al.*, 2002). To prevent cytotoxic responses these elements have to be removed before the bio-assay is performed.

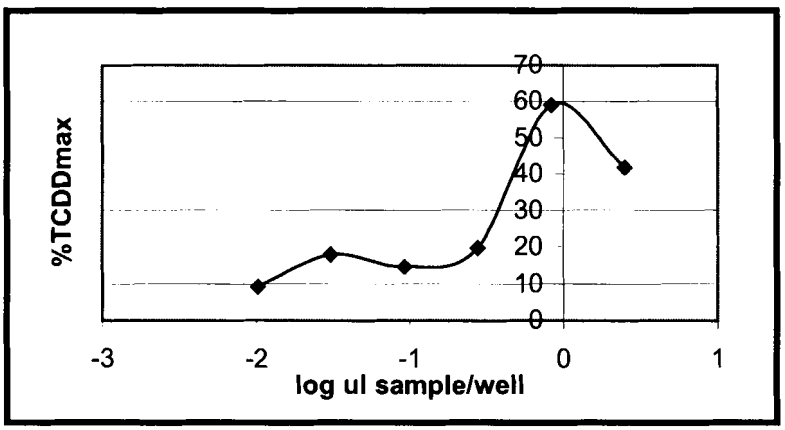

Figure 4.1: The dose response curve for the ash collected at site C2GA showing clear signs of cytotoxicity.

One method that can be used is the removal of sulphur utilising activated copper (U.S. EPA, 1996b). These samples could also undergo fractionation with magnesium silicate (U.S. EPA, 1996c; Koh *et al.*, 2005). This method is commonly used in the fractionation of organochlorine pesticides and polynuclear hydrocarbons (U.S. EPA, 1996c). The main purpose of sample cleanup procedures is to separate complex mixtures of complex analysts form interferences that cause the following phenomena in results (U.S. EPA, 1996c):

- errors in quantification, due to masked reactivity;
- false positive; and
- false negative readings.

These techniques will also help to achieve better R^2 values for samples 6 and 22 by removing any interfering compounds.

To eliminate the possible effect of cytotoxicity that cannot be visually detected it is essential to measure cell viability in all assays dealing with complex environmental mixtures (Giesy *et al.*, 2002). One of the methods that can be employed is the 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test. During this assay the surviving cell numbers are indirectly determined through the reduction of tetrazolium dye (MTT). This yellow chemical dye is reduced by living cells to a purple fromazan product. The amount of purple formazan produced can be determined spectrophotometrically once dissolved in dimethyl sulfoxide. The ratio of surviving cells can then be determined through comparing the response of untreated control cells to the cells exposed to the sample extracts (Langdon, 2004).

The only incinerator sample that delivered a usable dose response curve was an incinerator that had been out of use for an extended period. The substances that caused the cytotoxic response could have been degraded or diluted with time or the incinerator could have been run with a high efficiency.

4.3.1. The treatment of data below the detection limit of the method.

Non-detectable levels of pollutants are often found in more than 40% of samples (Baccarelli *et al.*, 2005). In the distribution data 37.84% of the data consisted of data that fell below the detection limit of the H4IIE bio-assay. These non-detectable values were substituted with zero values. Although substitution is an easy method to use in the handling of non-detectable values, it is normally an underestimation of the true pollutant concentration. An undetectable value does not mean that there are no dioxin-like chemicals present; it only indicates that the concentration is between 0 and the detection limit of the H4IIE bio-assay. The U.S. EPA does not recommend this method if more than 15% of data falls under the detection limit (Baccarelli *et al.*, 2005). Other statistical methods such as distribution models become a more accurate statistical solution (Baccarelli *et al.*, 2005). For the purposes of this study the substitution was adequate since an estimation of the dioxin distribution was the main aim and not the physical quantification of all soil samples. In future, however, these new statistical methods could only improve on the current experimental design, since 0 values make meaningful statistical analyses difficult.

4.4. Potential risk associated with food intake.

The TEQ values in the Ikageng residential area were relatively high. The REP50 values for the two sites sampled in the region were 33 and 69 ngTEQ/kg respectively. In this residential area there was a percentage of inhabitants (the exact amount is unknown at this time) that had vegetable gardens, poultry and other animals on their properties. In one section of Ikageng, where 30 houses were assessed, 7 houses with chicken coops were found. Of the 7 coops only 2 currently have chickens. One family owns 18 chickens and do consume both eggs and the chickens themselves. The other family owns 8 chickens that are still immature and it is not known how the poultry will be utilised (Phanzi, personal communication). As discussed in Section 1.8, dioxin-like chemicals present in soil can enter the food chain by being absorbed directly through dust deposition on vegetation or indirectly from animals feeding on vegetation (Anon, 2005d). Vegetation, however, can also be exposed to dioxin-like chemicals through direct deposition onto the plants (wet deposition, dry gaseous deposition and particles deposition) as well as adsorption through the root systems and foliar uptake (Smith & Jones, 2000).

In a USA study it was found that people eating residentially raised meat and eggs had between a 2- and 6-fold increased dioxin levels in serum when compared to a group consuming commercially produced food (Goldman, Harnly, Flattery, Patterson & Needham, 2000). Residentially reared animals tend to have a higher dioxin content due to their diets and increased exposure to soil, especially in the case of poultry (Goldman *et al.*, 2002). When chickens are raised commercially, the animals do not come into contact with much soil and are fed on commercial feed. In contrast to this, poultry reared on small scale at the home are likely to come into contact with soil and insects that are in the area, increasing dioxin exposure (Goldman *et al.*, 2000; Kijlstra, 2004). These observations indicate that contaminated food contribute to the body burden of dioxin-like chemicals in humans.

If it were assumed that chickens consume between 2 and 10 g of soil a day and that 25% of the dioxin-like chemicals present in the soil were transferred to the chicken (Kijlstra, 2004), the egg dioxin levels of these two sites (33 and 69 ngTEQ/kg) would be between 2.75 - 13.75 and 5.75 - 28.75 pgTEQ/g egg fat respectively. This is of serious concern since the E.U. has set the maximum limits for dioxin-like chemicals in hen eggs and egg products at 3 pgTEQ/g fat (E.U. Press release, 2002).

Table 4.1. The concentration of dioxin-like chemicals in free-range eggs collected at sites located between 0.2 and 4.5 km from a possible source of dioxin-like chemicals.

Country	Total WHO-TEQ (pg/g fat)	References
Belarus	13.46-13.74	DiGangi & Petrlík, 2005
Bulgaria	69.57	"
Czech Republic°	3.21/ 3.70 / 3.35-4.12	"
Egypt	137.52	"
India°	15.08/29.20	"
Kenya	31.02	"
Mexico	26.32	"
Mozambique	9.45	"
Pakistan	3.65-3.71	"
Philippines	12.98	"
Russia°	21.76/63.06	"
Senegal	38.56	"
Slovakia	16.12	"
Tanzania	3.63-3.73	"
Turkey	4.30	"
Uruguay	5.93	"
USA	7.41-7.71	"
Italy*	0.045	Taioli, Marabelli, Scortichini, Migliorati, Pedotti, Cigliano, Caporale, 2005
Spain#	0.5	Bocio & Domingo, 2005

* Eggs and mayonnaise not specifically free range eggs and not sampled close to possible source.
\# Samples not specifically free range eggs and not collected near a possible source.
° Values from different areas in the country, each areas TEQ separated by a forward slash.

The WHO standards state that a TDI for dioxins and furans is between 1-4 pg TEQ/kg body weight (Pereira, 2004). For a human weighing 60 kg the TDI would be between 60-240 pg TEQ. The E.U. commission's weekly tolerable intake for dioxin-like chemicals including the 12 dioxin-like PCBs is 14 pg TEQ/kg body weight (Pereira, 2004) that is 840 pgTEQ for a person weighing 60 kg. If we assume that a standard egg contains 6 g of fat (Kijlstra, 2004), an egg in the two Ikageng sample areas, from a chicken consuming 10g of soil, would contribute 82.5 pgTEQ and 172.5 pgTEQ, respectively, to the daily intake. Thus one of these eggs would equal the WHO TDI for dioxins and furans, and the consumption of between five and ten eggs

would equal the E.U. commission's weekly tolerable intake. The estimated daily intake of the general population in Tarragona Spain living near a hazardous waste incinerator from egg consumption was 1.3 pg TEQ/day (Bocio & Dominigo, 2005). This value is much lower than the estimated value for the sites measured in Ikageng. However, the concentration of dioxin-like chemicals in eggs as summarised in Table 4.1 for other areas close to a possible point source, were higher than the E.U maximum limit. The concentration of dioxin-like chemicals in free-range eggs is therefore a major concern to the amount of dioxin-like chemicals ingested daily by a population living in close proximity to a point source of dioxin-like chemicals.

Eggs are not the only source of dioxin-like chemicals in food products. As previously mentioned the uptake of dioxin-like chemicals through vegetation is the main route through which dioxin-like chemicals enter the food chain. Beef and milk fats, however, remain the major contributors of dioxin-like chemicals in the human diet where this has been established. The EU has set the dioxin limit on ruminant meat and milk (including butter) at 3 pgTEQ/g fat (E.U. Press release, 2002). Since relatively-high soil concentrations were found in the area examined, further studies will be crucial to determine the amount of dioxin-like chemicals entering the population through their diet.

Chapter 5: Conclusion and recommendations

The results of the ash and ground samples taken in direct proximity to the incinerator were unsatisfactory due to cytotoxicity in the bio-assay. It is crucial to exclude this phenomenon from the results through cleanup and fractionation processes of extracts during the extraction phase. The cytotoxic response should also be determined for every complex environmental sample through the use of viability test such as the MTT-test. Even with the appearance of the above-mentioned phenomena, the H4IIE bio-assay was a viable method to determine the distribution of dioxin-like chemicals surrounding an active incinerator.

Another improvement on the current experimental design would be the statistically sound handling of non-detectable data points. The simple substitution of non-detects with 0 can lead to underestimations of the true dioxin level in environmental samples. For this reason the distribution model alternative should be investigated further.

From the maps in Chapter 3 (Figure 3.1 and Figure 3.2) it is clear that there was no definite pattern to the distribution of dioxin-like chemicals in the Potchefstroom area. There is, however, an indication that the higher levels was on the outer margins of the sampling grid and not centered on the incinerator. This could indicate that the pollutants were deposited further from the point source than initially expected. The study design of similar projects in the future would be improved by increasing the distance between sampling points to at least between 1 and 2 km, simultaneously retaining reference points within these distances to look at the effect of local deposition. The %TOC had a small to negligible effect on the concentration of dioxin-like chemicals in this study. However, the TEQ increased with a corresponding decrease in tillage. Further research to determine the correlation between the %TOC, tillage and the TEQ can elucidate the importance of these two factors in the retention of dioxin-like chemicals in soil of Southern Africa.

Even though the U.S. regards medical waste and carcass incineration as two of the major sources linked to dioxin release (Van Overmeire *et al.*, 2001), the concentration in the surrounding soil was not dramatically high. The levels were lower than expected from estimated release values and literature. The most likely cause for this finding would be the climatic characteristics of the area. Since Potchefstroom has a relatively high ambient temperature, volatilisation and chemical

degradation processes could be favoured. In the winter months when higher deposition is expected (due to less degradation and volatilisation as well as an increase in household combustion processes), there is almost no precipitation in the area. Precipitation is an important factor in the deposition of higher chlorinated dioxin congeners. The lack of precipitation would lead to a lower margin of deposition, especially to those congeners that would lead to a higher TEQ due to their increased chlorination and stability in the environment. Coupled with the likelihood that no incineration will be initiated when it does start to rain (due to the open nature of the facility), precipitation is unlikely to be a major factor in wet deposition.

To truly assess the effect of climate and temperature variation, a continuous study should be undertaken to measure the amount of dioxin-like chemicals in flue gases and soil at different times during the year. This study would take into account the effect of seasonal variation as well as the effect of precipitation on the TEQ of soil samples.

Dioxin-like chemicals are present in the soils of Potchefstroom and these levels measured are within and exceeding international levels of concern. Especially levels in residential areas, where food is produced at homes, are of serious consequence. The consumption of free-range eggs in this area could contribute between 13.75 and 28.75 pgTEQ/g egg fat. This is elevated when compared to the EU limit for dioxin-like chemicals in hen eggs of 3 pgTEQ/g egg fat. Since the local population is being exposed to these persistent organic pollutants, further investigation is crucial not only in the Potchefstroom area, but in similar and possibly more polluted parts of South Africa. The measurement and assessment of dioxin-like-chemicals and other POPs is important for South Africa, not only to determine the effect of these chemicals on humans and the environment, but also to fulfil this country's international obligations. This study should be seen as contributing in this regard.

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