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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).

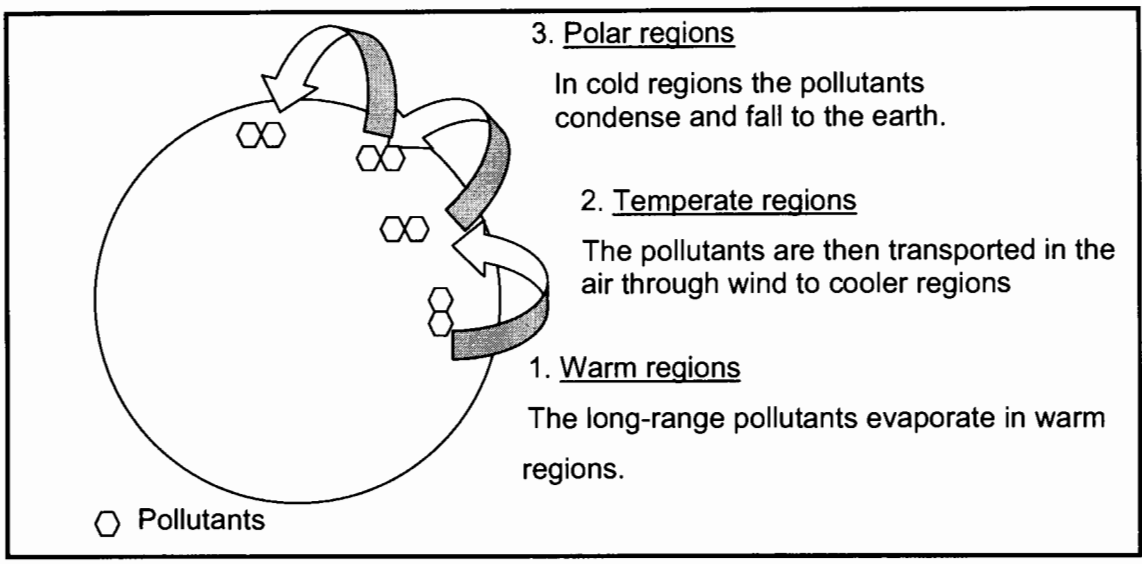

Figure 1.1: The multi-hop (grasshopper) movement of organic chemicals from a warm region to a polar region (adapted from Anon, 2005a).

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.

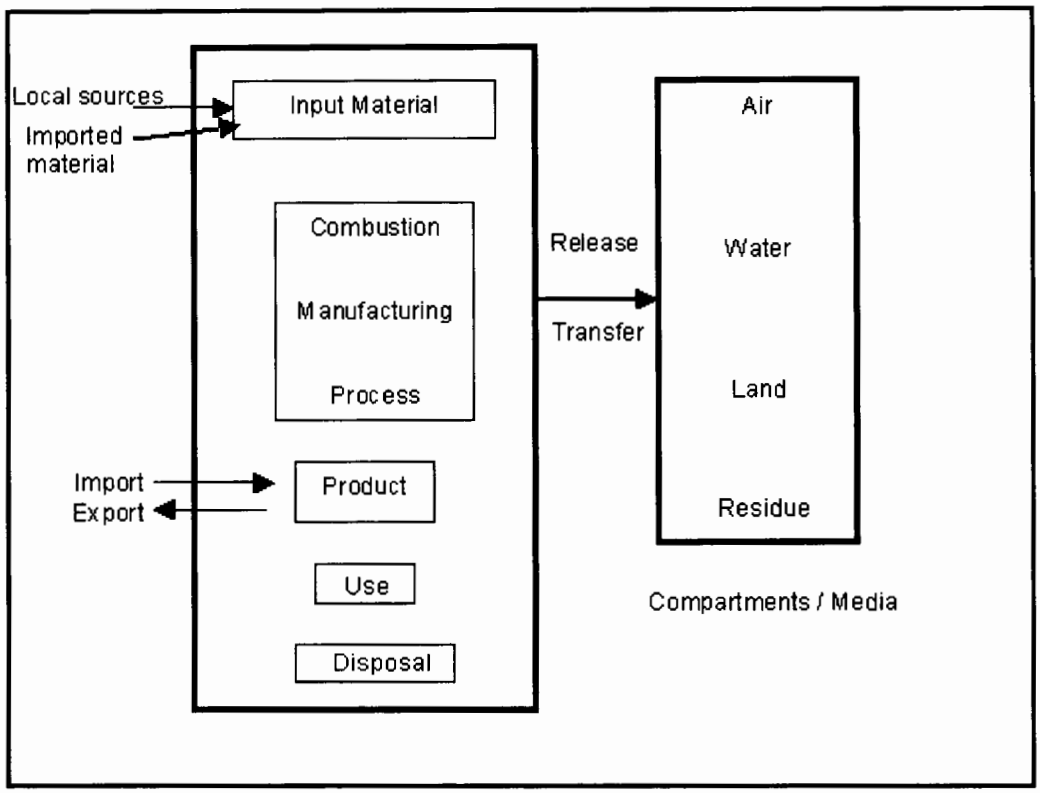

Figure 1.2: The life cycle and distribution of the dioxin group of persistent organic pollutants (UNEP, 2003).

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).

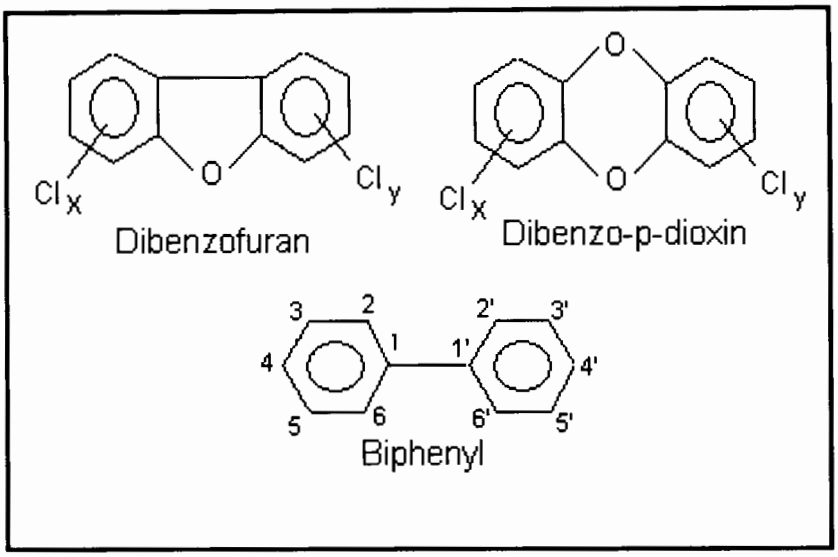

Figure 1.3: The chemical structure of dibenzo-*p*-dioxins, dibenzofurans and dioxin like-PCBs (adapted from Anon, 2004b).

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).

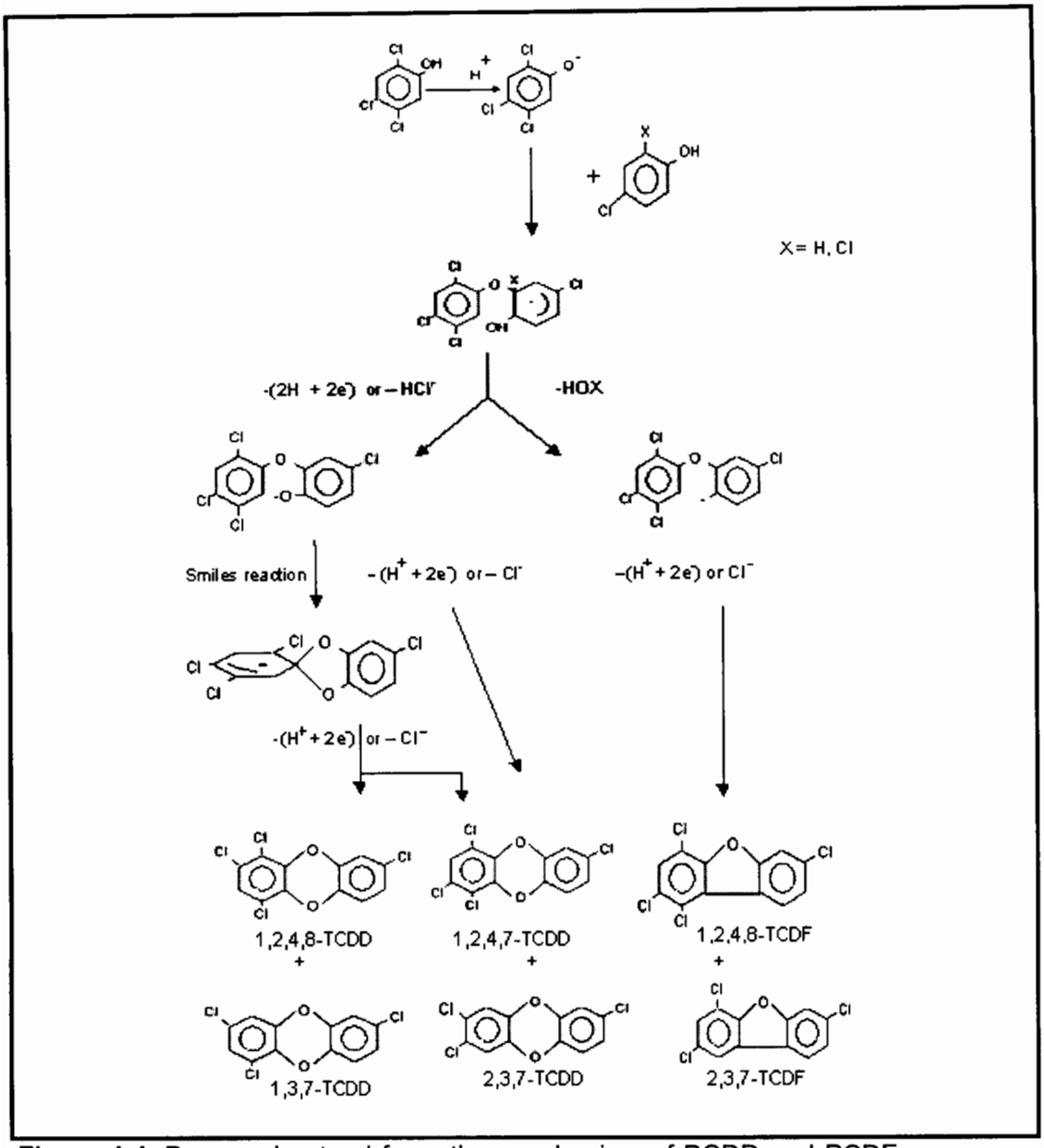

Figure 1.4: Proposed natural formation mechanism of PCDD and PCDF congeners mediated by peroxidase (Hoekstra *et al.*, 1999).

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).

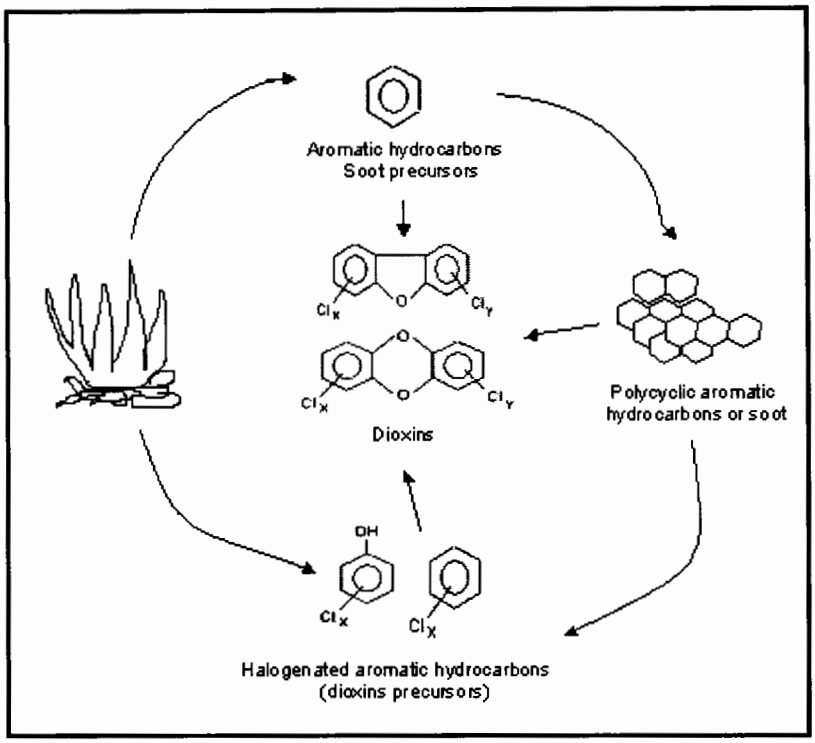

Figure 1.5: The formation pathway of dioxins during combustion processes (adapted from Anon, 2004c).

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).

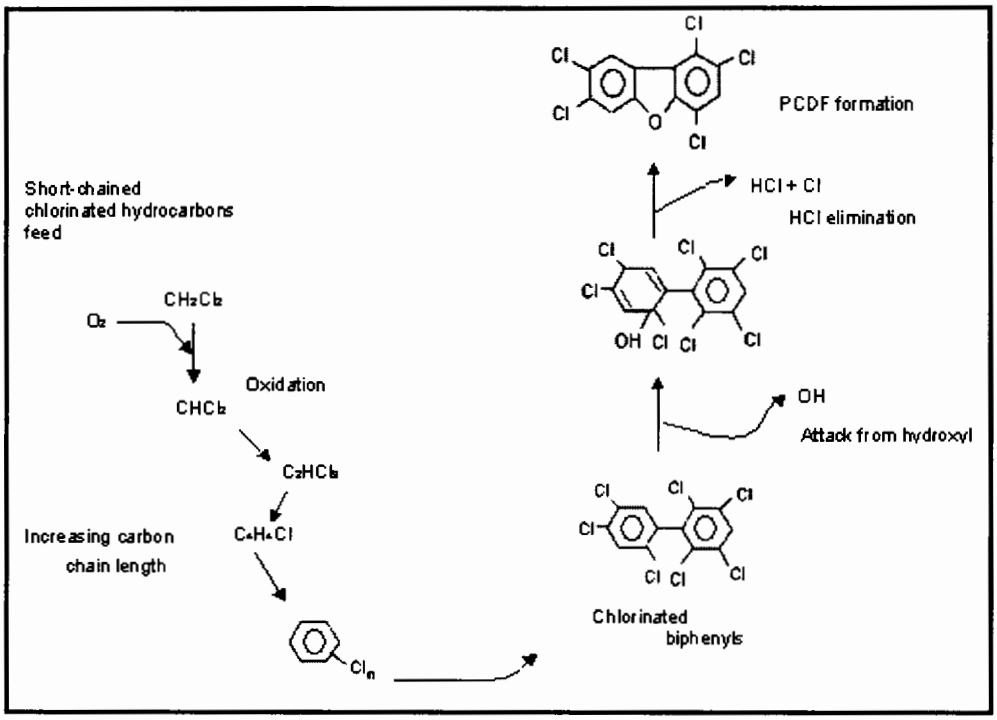

Figure 1.6: Mechanism of the homogenous pathways for PCDD/F formation (Environment Australia, 1999).

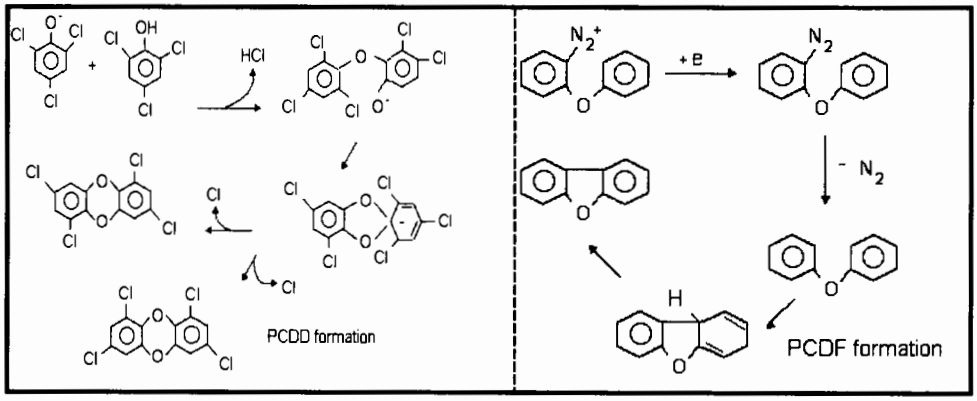

Figure 1.7: Mechanistic view of the formation of PCDD and PCDF in the fly-ash catalysed precursor pathway (Environment Australia, 1999).

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-HxCDF	2,3,3',4,4',5-HxCB
1,2,3,4,7,8-HxCDD	1,2,3,7,8,9-HxCDF	2,3,3',4,4',5'-HxCB
1,2,3,6,7,8-HxCDD	1,2,3,6,7,8-HxCDF	2,3',4,4',5,5'-HxCB
1,2,3,7,8,9-HxCDD	2,3,4,6,7,8-HxCDF	3,3',4,4',5,5'-HxCB
	1,2,3,4,6,7,8-HpCDF	2,3,3',4,4',5,5'-HpCB
1,2,3,4,6,7,8-HpCDD	1,2,3,4,7,8,9-HpCDF	
OCDD	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.

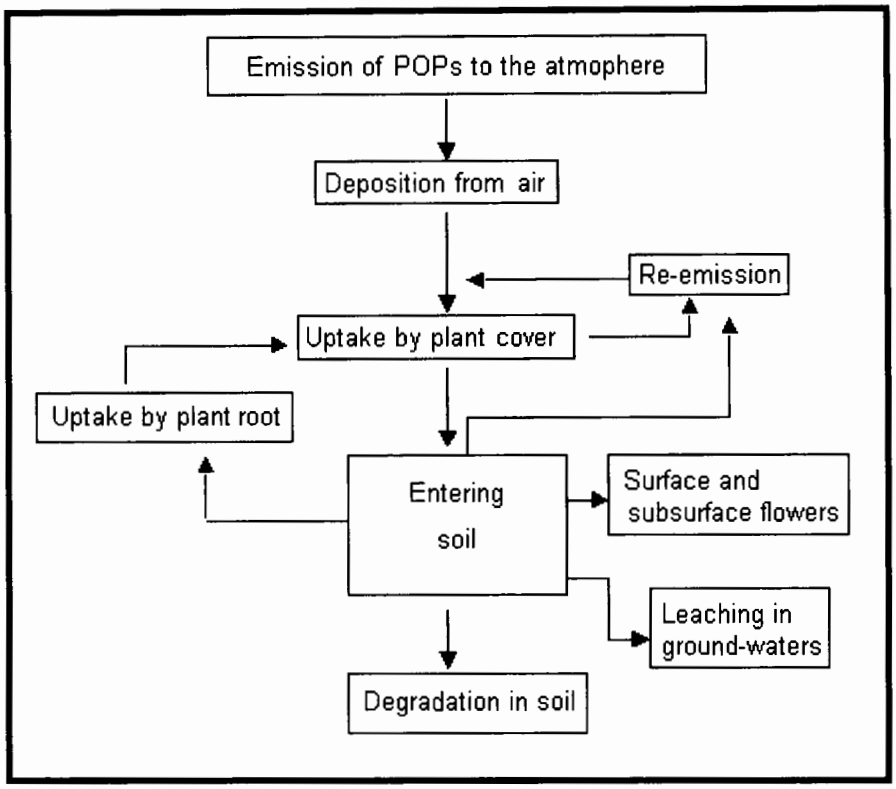

Figure 1.8: Conceptual model of the behaviour of POPs in the air-plant-soil system (Galiulin, Bashkin & Galiulina, 2002).

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).

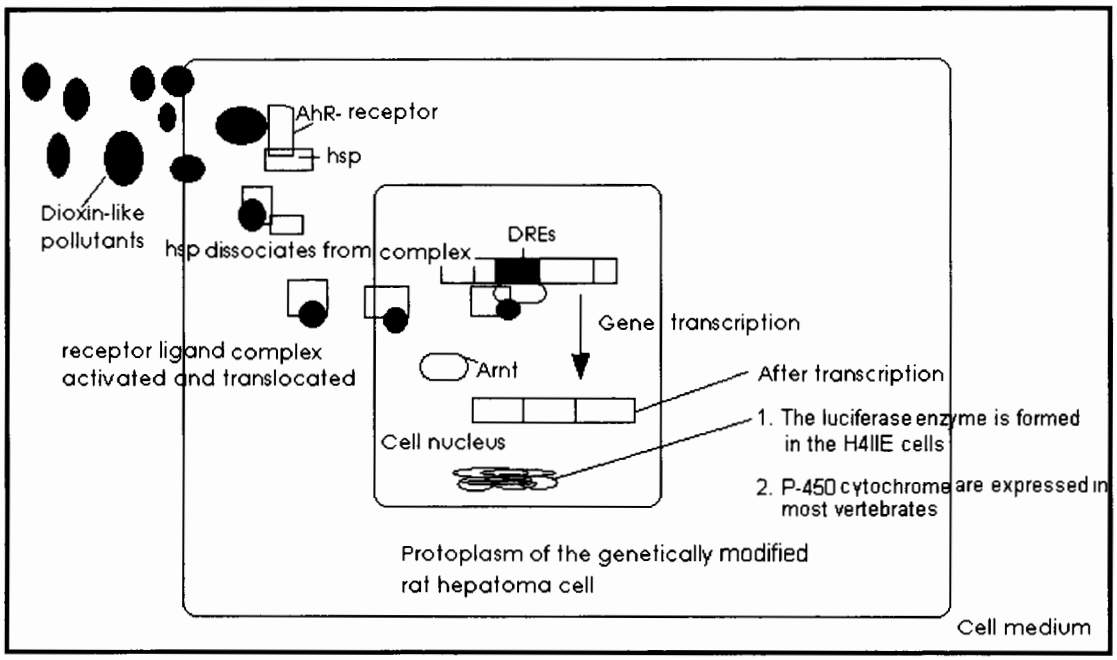

Figure 1.9: The AhR-mechanism of dioxin-like chemical activity.

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

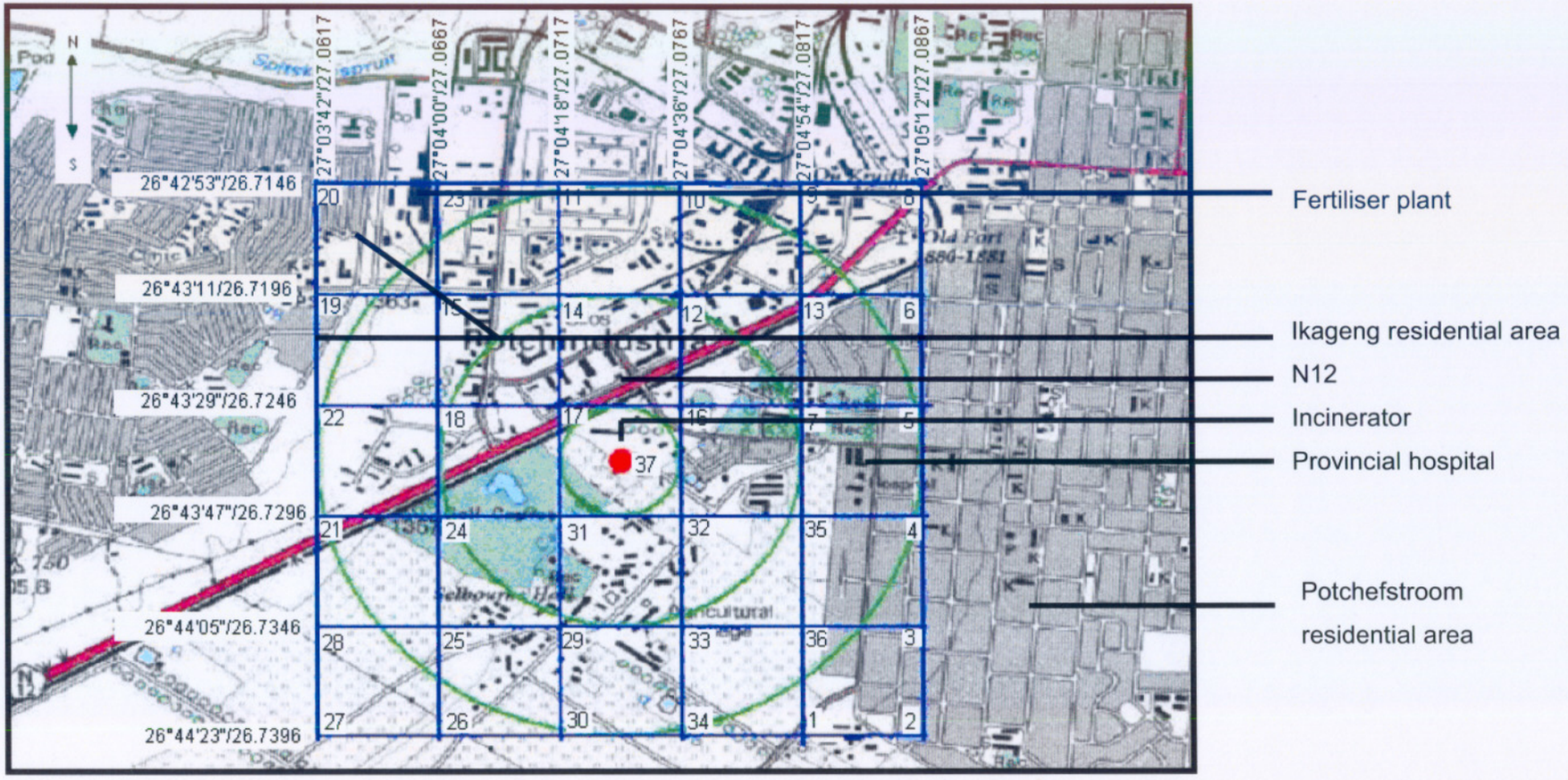

Figure 2.2: A map (scale 1cm: 263.15 m) showing the sampling grid, and co-ordinates in the area surrounding the incinerator of the Veterinary Diagnostic Services. The sampling points were located close to the intersections of the grid, and the edges of the grid are 2.5 km in length.