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Persistent Organic Pollutants (POPs) Associated With A Platinum Mine In The Limpopo Province, South Africa

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Table of Contents

	pp
Abstract	5-7
Opsomming	8-10
Acknowledgments	11
Abbreviations and Acronyms	12-13
Chapter 1	
Introduction and literature review	
1.1 Introduction	
Persistent organic pollutants	14-16
1.1.1 The Stockholm Convention (SC)	16-18
1.1.2 Current legislation of South Africa concerning dioxins, furans and PCBs	18-19
1.1.3 The aims and objectives of the project	19-20
1.2 Literature review	
Dioxin-like chemicals (dioxins, furans and PCBs)	20-21
1.2.1 Dioxins and furans	21-22
1.2.2 PCBs	22-23
1.2.3 Chemical and physical characteristics of PCDD/Fs and PCBs	23-25
1.2.4 Sources and formation PCDDs/Fs	25-26
1.2.4.1 Thermal processes	26-27
1.2.4.2 Wet chemical manufacturing processes	27
1.2.5 Environmental fate of PCDD/Fs and PCBs	27-29
1.2.6 Contamination of food sources by PCDD/Fs and PCBs	29-30
1.2.7 Potential risks and health effects of PCDD/Fs and PCBs	30-31
1.2.8 Toxic equivalency factor (TEF) and the toxic equivalency quotient (TEQ)	31-33
1.3 Detection of PCBs, dioxins and furans	34
1.3.1 Analysis techniques	34
1.3.1.1 Chemo-analysis	34
1.3.1.2 Bio-analytical methods	34-35
1.3.2 The biochemical background of the H4IIE bio-assay	35
1.3.2.1 Aryl hydrocarbon receptor (AhR) and the mode of action	35-37
1.3.2.2 H4IIE *luc* bio-assay	37-39
Chapter 2	
Platinum mining in South Africa	

2.1 History of the Bushveld Complex and platinum 40-42
2.2 Platinum mining and POPs 42
2.2.1 Platinum mining activities associated with Union Section 42-43
2.2.1.1 Ore extraction 43-46
2.2.1.2 Ore concentration 46-49
2.2.1.3 Smelting 49-52
2.2.1.4 Refining 52

Chapter 3

Methodology

3.1 Study design 53
3.2 Site sampling and methods 54-59
3.3 Treatment of samples 59
3.3.1 Extraction and clean-up of samples 59-60
3.3.2 Storage of samples 61
3.3.3 Determining the total organic carbon (TOC) 61-62
3.4 H411E reporter gene bio-assay 62
3.4.1 Cell culture 62
3.4.2 Bio-assay 62-63
3.4.2.1 An outline and example of how data was analysed 63-66

Chapter 4

Results and discussion

4.1 Results 67-70
4.2 Discussion 70-73
4.2.1 Slag 73-76
4.2.1.1 Possible TEQ formation during processing 76-77
4.2.1.2 Proposed source reduction measures 77-78
4.2.1.3 Emissions and releases of dioxin-like chemicals 78-79
4.2.1.4 Possible health and environmental risks 79
4.2.2 Dumpsite 79-80
4.2.2.1 Possible formation of dioxin-like chemicals 80-81
4.2.2.2 Soil as sink and secondary source 81-82
4.2.3 Tailings dams 82
4.2.3.1 Potential contributing factors to the presence and/or absence of dioxin- like chemicals 82-83

4.2.4 Woodchips 83-84
4.2.5 Proposed treatment of cytotoxic samples 84-85
4.2.6 Normalised data 85
4.2.7 Summary 85-86

Chapter 5

Conclusion and recommendations

5.1 Conclusion 87-88
5.2 Recommendations 88-90

Chapter 6

References 91-105

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Abstract

South Africa ratified the Stockholm Convention (SC), which became legally binding on 17 May 2004. This Convention targets 12 particularly toxic persistent organic pollutants (POPs) for virtual elimination. The Convention also requires parties to reduce the release of organochlorine pesticides and the intentionally- and unintentionally-produced POPs such as dioxins, furans and polychlorinated biphenyls (PCBs) (referred to as dioxin-like chemicals).

Dioxins are a heterogeneous mixture of chlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) congeners. These substances were never intentionally produced but are produced as by-products of industrial processes (such as metallurgical processes and bleaching of paper pulp). They can also be formed during natural processes such as volcanic eruptions and forest fires. The largest contributor to releases of PCDD/Fs in the environment is incomplete combustion from waste incinerators leading to the unintentional production of these compounds. Polychlorinated biphenyls (PCBs) are used in transformers and capacitors, but can also be formed unintentionally during industrial and thermal processes. Dioxin-like chemicals (PCDD/Fs and/or PCBs) are classified as persistent because of the following characteristics: lipophilicity and hydrophobicity; resistance to photolytic, chemical and biological degradation and they are able to travel long distances. As South Africa is a semi-arid region, POPs will be less prone to travel here because these substances favour colder regions with high soil organic matter.

Fish, predatory birds, mammals (including humans) absorb high concentrations of POPs through the process of bio-concentration, leading to bio-accumulation of these substances in the fatty tissue. PCDD/Fs occur as unwanted trace contaminants in air, water, land, in residues and products (such as consumer goods e.g. paper and textiles). The distribution of these chemicals into various matrices is problematic since they cause damage to the environment and human health. These chemicals pose a threat to human health when found in high concentrations that may lead to acute hepatoxicity and dermal toxicity (chloracne). Long–term exposure to low concentrations of these substances might lead to chronic effects such as reproductive problems and carcinogenicity.

Since ferrous and non-ferrous metal production is a source of dioxin-like chemicals, a platinum mine in the Limpopo Province, South Africa, was selected for this investigation. The aim of the study was to determine if there are dioxin-like chemicals associated with platinum mining and processing, and if the H4IIE reporter gene bio-assay could be used to semi-quantify and assess the potencies of the complex environmental and process samples by determining their Toxic Equivalency Quotients (TEQ). The implications of the sources to the formation of dioxin-like chemicals regarding the SC were investigated and recommendations were made to improve this study.

Samples were collected from tailings dams, woodchips, a dumpsite and slag from the smelter at Union Section. Samples were extracted with the Soxhlet apparatus using hexane as solvent. The percentage total organic carbon (%TOC) was determined for each sample to normalise the data. The method used was the Walkley-Black method.

In determining the TEQ of each sample, the H4IIE *luc* cell line was used. The cells of the H4IIE *luc* line are genetically modified rat hepatoma cells stably transfected with a luciferase firefly gene. The luciferase gene is activated by the presence of dioxin-like compounds and the concentration of the enzyme is measured as relative light units (RLUs). The amount of RLUs is directly proportional to the dioxin load in the extract. This method is rapid, cost and time-effective in determining the TEQ when compared to chemical analysis.

The TEQ_{20}-values in the various samples, as determined with the H4IIE *luc* cell line, ranged from 0.007 ngTEQ/kg to 54.06 ngTEQ/kg. Thermal processes at the smelter, sorption of hydrophobic organic compounds (HOCs) to soil and tailings, and external sources such as anthropogenic activities contributed to high TEQ_{20}-values. Climatic conditions, wind, precipitation, and solubility of HOCs into surfactants lead to low TEQ_{20}. The smelter at Union Section had a very high TEQ_{20} of 44.62 ngTEQ/kg compared to Impala Platinum mine (5.15 ngTEQ/kg). This implies that workers at Union Section are possibly exposed to low and high concentrations of dioxin-like chemicals. Long-term exposure to these compounds could lead to bio-accumulation in the fatty tissue of the mine workers, leading to chronic effects such as reproductive problems and cancer. The air emission of the furnace at the smelter was 0.03 gTEQ/annum and the release of the PCDD/Fs into the slag was 0.60 gTEQ/annum. By effectively managing the smelter it is possible to reduce the TEQ.

The TEQ of each sample increased due to normalising the data. The normalised TEQ_{20} ranged from 0.94 ng TEQ/kg to 42497.48 ngTEQ/kg.

Dioxin-like chemicals are present on a platinum mine, but at varying quantities and the effects of these compounds might be detrimental to the environment and the workers at the platinum mine. Further analyses of the health impacts associated with the platinum mine are needed. The H4IIE reporter gene bio-assay could be used to effectively determine the TEQ of each sample. Although this investigation has identified the formation and presence of dioxin-like chemicals at certain stages of mining and processing, not all of the processes were investigated. Some of these processes have the potential to add, and even destroy, these chemicals, affecting potential human exposure and amounts released to the environment. This, however, requires further investigation.

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Keywords: Dioxin-like chemicals; H4IIE reporter gene bio-assay; hydrophobic organic compounds (HOCs); normalised TEQ; PCBs; PCDD/Fs; platinum mine; South Africa; Soxhlet extraction; Stockholm Convention; TEQ; Walkley-Black.

Persisterende Organiese Besoedelstowwe (POB's) wat met 'n Platinummyn in die Limpopo-Provinsie, Suid-Afrika geassosieer word

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Opsomming

Suid-Afrika het die Stockholmkonvensie wat op 17 Mei 2004 bindend geword het, bekragtig. Hierdie konvensie teiken 12 besondere toksiese persisterende organiese besoedelstowwe (POB's). Die konvensie verwag van ondertekenaars om die vrystelling van organochloorpestisiede en die opsetlike en toevallige geproduseerde POB's, soos dioksiene, furane en poligechloreerde bifeniele (PCB's) (verwys na dioksien-agtige stowwe) te verminder.

Dioksiene is 'n heterogene mengsel van gechloreerde dibenso-*para*-dioksiene en dibensofurane (PCDD/F's). Hierdie stowwe is nooit doelmatig geproduseer nie, maar is die newe-produkte van industriële prosesse (soos metallurgiese prosesse en bleik van papier en pulp). Hulle kan ook deur natuurlike prosesse soos vulkaniese uitbarstings en bosbrande gevorm word. Die grootse bydrae tot PCDD/F's-vrystelling in die omgewing is die onvolledige verbranding van afval in verbrandingsoonde wat lei tot die newe-produksie van die verbindings. PCBs word in transformators en kapasitore gebruik, maar word ook as newe-produkte in industriële en termiese prosesse gevorm. Dioksien-agtige stowwe (PCDD/F's en/of PCB's) word as persisterend geklassifiseer omdat dit oor die volgende eienskappe beskik: lipofilies en hidrofobies; bied weerstand teen fotolitiese, chemiese en biologiese afbraak; en is in staat om oor lang afstande vervoer te word. Omdat Suid-Afrika 'n half-woestyngebied is, sal POB's nie maklik hierheen versprei word nie omdat hierdie stowwe 'n voorkeur het vir koue streke met hoë organiese inhoud in grond.

Visse, predatoriese voëls en soogdiere (insluitend die mens) absorbeer hoë konsentrasies van POB's deur die proses van bio-konsentrasie wat tot bio-akkumulasie van die stowwe in die vetweefsel lei. PCDD/F's kom voor as spoor-kontaminante in die lug, water, op land, as residue en produkte (verbruikersgoedere soos papier en tekstielware). Die verspreiding van hierdie stowwe in verskeie omgewingsmatrikse kan menige ernstige gevolge vir die mens en omgewing inhou. Blootstelling aan hoë konsentrasies van dioksien-agtige stowwe kan tot akute hepatoksisiteit en velprobleme (chlooraknee) lei. Langtermynblootstelling aan lae

konsentrasies van hierdie stowwe kan tot kroniese effekte soos voortplantingsprobleme en vatbaarheid vir kanker lei.

Omdat metaal- en nie-metaalproduksie 'n bron van dioksien-agtige stowwe is, is 'n platinummyn in Limpopo-Provinsie, Suid-Afrika, vir hierdie ondersoek gekies. Die doel van die studie was om te bepaal of dioksien-agtige stowwe met die ontginning en prosessering van platinum geassosieer word en of die H4IIE-*luc*-weefselkultuursellyn gebruik kan word om die omgewingsmonsters te semi-kwantifiseer en om die potensie van die monsters te analiseer deur die Toksiese Ekwivalensie Kwosiënt (TEK) vir elke monster te bereken. Die implikasies van die bronne van dioksien-agtige stowwe ten opsigte van die Stockholmkonvensie is ondersoek en aanbevelings is gemaak om die studie te verbeter.

Slikdammonsters, houtsplintermonsters, 'n asgatmonster en metaalskuimmonsters (slak) van die smelter by Union-afdeling is versamel. Die monsters is in 'n Soxhlet-apparaat met heksaan as oplosmiddel geëkstraheer. Die persentasie van totale organiese koolstof (%TOK) is vir elke monster bepaal om die data te normaliseer. Die metode wat gebruik is, was die Walkley-Black metode.

Om die TEK van elke monster te bepaal is daar van die H4IIE-*luc*-weefselkultuursellyn gebruik gemaak. Die selle van die H4IIE-*luc*-weefselkultuursellyn is genetiesgemanipuleerde rot-hepatoomselle wat stabiel met die vuurvlieglusiferasegeen getransfekteer is. In die teenwoordigheid van dioksiene word die produksie van lusiferase geaktiveer en word die konsentrasie van die ensiem as relatiewe-lig-eenhede (RLE) gemeet. Die RLE's is direk eweredig aan die teenwoordigheid van die dioksiene in die ekstrak. In vergelyking met chemiese analisetegnieke, is hierdie metode vinnig, koste- en tyd-effektief in die bepaling van TEK-waardes.

Die TEK_{20}-waardes bepaal deur die weefselkultuursellyn het gewissel vanaf 0.007 ngTEK/kg tot 54.06 ngTEK/kg. Termiese prosesse van die smelter, sorpsie van hidrofobiese organiese stowwe (HOS) aan grond en sediment, sowel as eksterne bronne soos antropogeniese aktiwiteite, dra tot die hoë TEK_{20}-waardes by. Klimaattoestande, wind, presipitasie, erosie en oplosbaarheid van HOS in surfaktante, lei tot lae TEK_{20}-waardes. Die smelter van Unionafdeling het 'n baie hoë TEK_{20}-waarde (44.62 ngTEK/kg) in vergelyking met die Impala Platinummyn (5.15 ngTEK/kg) getoon. Dit impliseer dat werkers by Union-afdeling moontlik aan lae en hoë konsentrasies van dioksien-agtige stowwe blootgestel word. Langtermynblootstelling aan die besoedelstowwe mag tot die bio-akkumulasie van die stowwe in die vetweefsel van die mynwerkers lei wat tot kroniese effekte soos

voortplantingsprobleme en kanker kan lei. Die lugvrystelling van die smelter by Union-afdeling is 0.3 gTEK/jaar terwyl die vrystelling van dioksien-agtige stowwe in die slak 0.60 gTEK/jaar is. Die waardes kan egter verminder word as effektiewe beheer en kontrole op die smelter toegepas word.

Die TEK-waarde het verhoog toe die data genormaliseer was. Die genormaliseerde TEK_{20} het gewissel vanaf 0.94 ngTEK/kg tot 42497.48 ngTEK/kg.

Dioksien-agtige stowwe is teenwoordig op die platinummyn, maar is teenwoordig in wisselende hoeveelhede en die effekte van hierdie stowwe kan nadelig vir die omgewing en die werkers op die platinummyn wees. Verdere analise vir die gesondheidsimpak met die platinummyn geassosieer, word benodig. Die H4IIE-*luc*-weefselkultuursellyn kan gebruik word om die omgewingsmonsters se TEK-waardes te bepaal. Alhoewel hierdie ondersoek die teenwoordigheid en vorming van dioksien-agtige stowwe geïdentifiseer het in verskeie stadiums van die ontginning en prosessering van platinum is nie al die prosesse ondersoek nie. Sommige van die prosesse het die potensiaal om dioksien-agtige stowwe te vorm en / of te vernietig, wat potensiële blootstelling aan die mens en die vrystelling na die omgewing beïnvloed. Dit benodig egter verdere ondersoek.

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Sleutelwoorde: Dioksien-agtige stowwe; H4IIE-*luc*-weefselkultuursellyn; Hidrofobiese organiese besoedelstowwe; Genormaliseerde TEK; PCBs; PCDDs; PCDF; Platinummyn; Soxhlet-apparaat; Suid-Afrika; Stockholmkonvensie; TEK; Walkley-Black.

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Acronyms and Abbreviations

AEL	African explosives limited
AhR	Aryl hydrocarbon receptor
ANFEX®	Ammonium nitrate fuel oil products
Arnt	Aryl hydrocarbon nuclear translocator
AR	Androgen receptor
BDH	Barium diphenylamine sulphonate
BDL	Below detection limit
bHLH-PAS	Basic-helix-loop-helix, Per-Arnt-Sim
BL	Blank control
CALUX®	Chemical-activated luciferase expression
C	Carbon
Ca	Calcium
Cd	Cadmium
CV	Coefficient of variance
CO_2	Carbon dioxide
$CuSO_4$	Copper sulphate
CYP	Cytochrome P450 enzyme system
CYP1A1	Cytochrome P450 1A
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DEA&T	Department of Environmental Affairs and Tourism
DMEM	Dulbecco's Modified Eagle's Medium
DRE	Dioxin responsive element
DWAF	Department of Water Affairs and Forestry
ECEH-WHO	European Centre of Environmental Health of the World Health Organisation
EC	Effective concentration
EDTA	Ethylene-diamine-tetraaceticacid
ER	Estrogen receptor
EU	European Union
EROD	Ethoxyresorufin-O-deethylase
$Fe(NH_4)_2(SO_4)_2$	Ferrous (II) ammonium sulphate
Fe^{3+}	Ferric iron
FBS	Fetal bovine serum
FT	Tank cells
GC	Gas chromatography
GPS	Global positioning system
H_2SO_4	Sulphuric acid
HAHs	Halogenated aromatic hydrocarbons
HCB	Hexachlorobenzene
HGG	Hot Gas Generator
HOCs	Hydrophobic organic compounds
HRGC-HRMS	High –resolution gas chromatography-high-resolution mass spectrometry
HSP90	Heat shock proteins
HeptaCDD	Heptachlorodibenzo-*p*-dioxin
HeptaCDF	Heptachlorodibenzofuran
HPLC	High performance liquid chromatography
HexaCDD	Hexachlorodibenzo-*p*-dioxin
HexaCDF	Hexachlorodibenzofuran
HexaCB	Hexachlorobiphenyl
ICMESA	Industrie Chimiche Meda Societa Azionaria
ILP	Immunophilin-like proteins
INC	Intergovernmental negotiating committee
IPCS	International Program on Chemical Safety
ISO 14001	International Standard Organisation
I-TEF	International toxicity equivalency factors
I-TEQ	International toxicity equivalent
L	Ligand
LRAT	Long-range atmospheric transport
K_{oc}	Soil distribution coefficient
K_{ow}	Octanol-water partitioning coefficient
$K_2Cr_2O_7$	Potassium dichromate
Mg	Magnesium

MVA	Mega Volt Amps
MTT cytotoxic assay	3-[4,5-dimethylthiazol-2yl]-2,5-dipheyl tetrazolium bromide
NC	Not calculated
NIP	National implementation plan
NP	Nonylphenol
NRF	National Research Foundation
OctaCDD	Octachlorodibenzo-*p*-dioxin
OctaCDF	Octachlorodibenzofuran
OCs	Organochlorines
OM	Organic matter
o/s	Oversized particles
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
PBDEs	Polybrominated diphenyl ethers
PBS	Phosphate free buffered saline
PCBs	Polychlorinated biphenyl
PCDD/Fs	Polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran
PCDDs	Polychlorinated dibenzo-*p*-dioxin
PCDFs	Polychlorinated dibenzofuran
PentaCDD	Pentachlorodibenzo-*p*-dioxin
PentaDDF	Pentachlorodibenzofuran
PentaCB	Pentachlorobiphenyl
PGMs	Platinum group metals
PHAH	Polyhalogenated aromatic hydrocarbons
PHHs	Polycyclic halogenated hydrocarbons
PLC	Programmable logic controller
POPs	Persistent organic pollutants
ppt	Parts per trillion
PVC	Polyvinyl chloride
RLU	Relative light units
REP	Relative potencies
R^2	Correlation coefficient
SA	South Africa
S	Solvent control
SC	Stockholm Convention
SIBX	Sodium isobutyl xanthate
SOM	Soil organic matter
SD	Standard deviation
SU	Sump
SVOCs	Semi-volatile organic compounds
2,3,7,8-TCDD	2,3,7,8–Tetrachlorodibenzo-*p*-dioxin
TetraCDF	Tetrachlorodibenzofuran
TetraCB	Tetrachlorobiphenyl
TEF	Toxic equivalency factor
TEQ	Toxic equivalency quotient
%TOC	Percentage total organic carbon
TOC	Total organic carbon
UCZ	Upper critical zone
UG2	Upper group 2
UK	United Kingdom
UNEP	United Nations Environmental Program
US	United States
US.EPA	United States Environmental Protection Agency
u/s	Undersized particles
UV	Ultra violet
WHO	World Health Organisation
WHO-TEF	World Health Organisation toxicity equivalency factors
WRC	Water Research Commission
2,4,5-T	2,4,5-trichloracetic acid

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Persistent Organic Pollutants

Persistent Organic Pollutants (POPs) are organic compounds of natural or anthropogenic origin (Godduhn & Duffy, 2003) that possess a wide variety of characteristics. These characteristics include: toxicity; lipophilicity and hydrophobicity; resistance to photolytic, chemical and biological degradation; persistence within the environment for extended periods (Kitamura, Takazawa, Hashimoto, Choi, Ito & Morita, 2004) and these pollutants are able to travel long distances (Safe, 1995; Bouwman, 2003; Caza & Bailey, 2005). POPs bio-accumulate up in the food chain. This process implies that the pollutants are readily absorbed by fatty tissue of fish, predatory birds, mammals and humans (Persistent Organic Pollutants, 2001). These substances bio-concentrate in living organisms by absorbing high concentrations of these substances from water that can be greatly magnified, up to 70 000 times the background levels (United Nations Environmental Programme (UNEP), 2004).

POPs, as defined in the Stockholm Convention (SC), can be divided into two main categories: pollutants´ produced intentionally as products or intermediates (such as polychlorinated biphenyls (PCBs)), and those pollutants produced as incidental by-products of anthropogenic activities such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Breivik, Alcock, Li, Bailey, Fiedler & Pacyna, 2004).

Indisputable evidence has shown that persistent semi-volatile organic compounds (SVOCs) including organochlorines (OCs), PCBs, polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and dioxins and furans can circulate globally by means of long-range atmospheric transport (LRAT) and regional/global scale re-distribution after their release into the environment (Welch, 1995; Gouin, Mackay, Jones, Harner & Meijer, 2004; Hassanin, Lee, Steinnes & Jones, 2005). These compounds exist as vapours or are associated with particles/aerosols, depending on physical-chemical properties and temperature (Breivik & Heimstad, 2005). These pollutants become geographically widely distributed through processes known as the 'grasshopper effect' and the 'one hop effect' (UNEP, 2004: Gouin *et al.,* 2004). The 'grasshopper effect' and the 'one hop effect' are graphically illustrated in Figure 1.1, as it would apply to POPs. The 'grasshopper effect' implies that pollutants are released in one part of the world (warmer parts) and can, through multiple cycles of evaporation and condensation, be transported through the atmosphere to regions (colder parts) far away from the original sources due to multiple hops (Hanberg, 1996). The 'one hop effect' is partially the same as the grasshopper effect, but does not undergo multiple hops,

only one hop. A 'one hop effect' can occur for most radio-nuclides, heavy metals (e.g. Lead (Pb) and Cadmium (Cd)) and to a lesser extent some heavier and relatively in-volatile organic chemicals (Breivik & Heimstad, 2005).

During the 'one hop effect', pollutants are transported to a region and become trapped for extended periods in the specific ecosystem by not having the capability to re-enter the atmosphere to the same extent as the 'grasshopper effect' pollutants. Relatively high concentrations of these contaminants can be found in areas with little human activity (Corsolini, Kannan, Imagawa, Focardi & Giesy, 2002; Börga, Wolkers, Skaare, Hop, Muir & Gabrielsen, 2005) such as the Arctic and Antarctic polar regions.

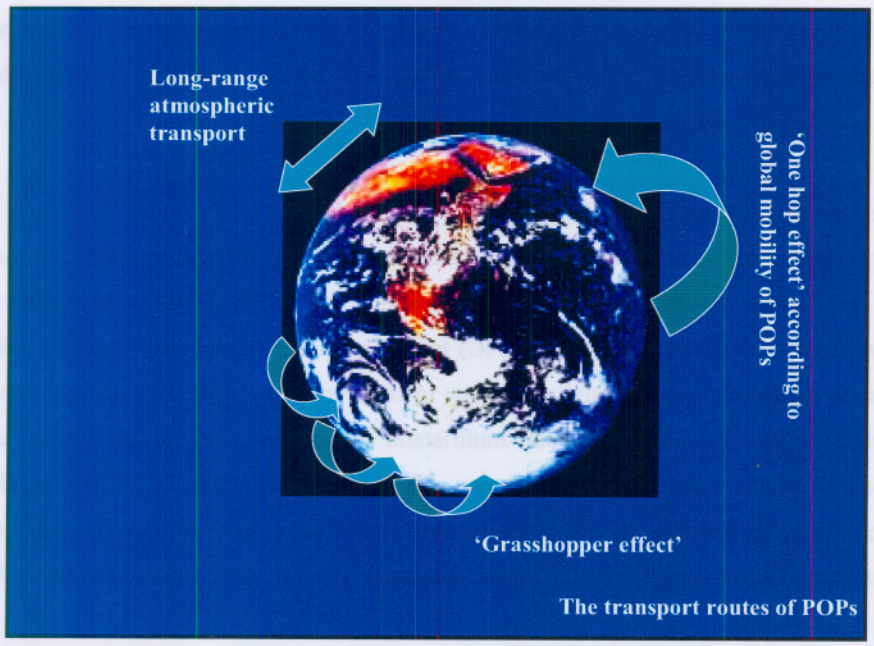


Figure 1.1: The transport routes of POPs during long-range transport. The figure represents the 'grasshopper effect' and the 'one hop effect'. The 'one hop effect' illustrates the movement of POPs according to global mobility (low to high) and the 'grasshopper effect' indicates the movement of POPs from hotter, equatorial regions to colder regions.

Global circulation patterns, low temperatures, winter darkness, slow degradation rates and low evaporation rates contribute to the high concentrations of these substances found in colder climates (Caza & Bailey, 2005). The ocean currents can also transport these pollutants, as well as migratory animals (such as the polar skua, other seabirds, and whales) (Corsolini *et al.*, 2002), where they enter the trophic webs, bio-accumulate and bio-magnify into the fatty tissue of living organisms when eaten, or through decay after death.

Since South Africa is semi-arid and subtropical, persistent organic pollutants will be less prone to travel here because these substances prefer colder regions with high soil organic matter (Hanberg, 1996; Gouin *et al.,* 2004). POPs have a high affinity for soil organic matter (SOM) and will be less readily emitted from soil of high organic matter (OM) content and low temperature regions, than from low OM soil and hot regions (Dalla Valle, Jurado, Dachs, Sweetman & Jones, 2005).

Our climate and rainfall will play an important role on the soil's holding-capacity for these pollutants. The fate and behaviour of these hydrophobic organic pollutants in soil are governed by several factors such as soil characteristics, compound properties, and environmental factors such as temperature and precipitation (Reid, Jones & Semple, 2000; Dalla Valle *et al.,* 2005). These compounds tend to absorb onto particulates, and are removed from the atmosphere by photo-degradation and dry and wet deposition (Kaupp & McLachlan, 1999).

1.1.1 The Stockholm Convention (SC)

The ability of these pollutants to be transported long distances globally by air and water, pose a threat to human health and the environment. In 1997, UNEP mandated an intergovernmental negotiating committee (INC) to prepare an internationally legally binding instrument to implement international action due to risks posed by these pollutants (Caza & Bailey, 2005). The aim of this global, multilateral agreement is to protect human and environmental health from POPs (Bouwman, 2004). On 22 May 2001, the world's governments met in Sweden to adopt an international treaty, the SC, that curtails bio-accumulation and bio-magnification of these pollutants (UNEP, 2005; Godduhn & Duffy, 2003).

In 2002, South Africa (SA) ratified the SC (Anon, 2005a) during the World Summit on Sustainable Development in Johannesburg, which became legally binding on 17 May 2004. This Convention immediately targeted 12 particularly toxic POPs for virtual elimination (Godduhn & Duffy, 2003) and required parties to reduce the total release of the intentional and unintentional produced POPs such as dioxins, furans and PCBs (Anon, 2005b) and organochlorine pesticides. The 12 toxic POPs known as the 'Dirty Dozen' (Bouwman, 2004; Anon, 2005a; Anon, 2005b; Caza & Bailey, 2005) are listed in Table 1.1.

The SC can be summarised as 5 aims (UNEP, 2005).

Aim no 1: Eliminate dangerous POPs, starting with the 12 compounds as listed in Table 1.1. This is done to protect human and environmental health from the harmful effects of these

pollutants. Many of these substances are already banned in countries that ratified the SC. PCBs containing equipment must be totally phased out by 2025 and recovered PCBs must be treated and eliminated by 2028. The use of DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] must be limited, seeking alternatives to control disease-carrying vectors. Steps must be taken to reduce the release of dioxins, furans, PCBs and hexachlorobenzene as by-products of combustion or industrial production. This Convention also requires parties to develop national implementation plans within two years to exchange information on POPs and their alternatives.

Table 1.1: The 12 chemicals listed by the SC, as well as their application/formation in various agricultural and industrial sectors (Bouwman, 2004; UNEP, 2005).

Chemicals	Application/Formation
Aldrin	Applied to soil to kill insect pests.
Chlordane	Broad-spectrum insecticide on agricultural crops.
DDT	Used to control malaria in developing countries transmitted by disease vectors.
Dieldrin	Control of termites and textile pests as well as control of insect-borne diseases in agricultural soils.
Dioxins	Unintentionally produced during incomplete combustion processes, incineration and chemical-industrial processes. These compounds are found in automobile exhaust, tobacco smoke, wood and coal smoke. Not used commercially.
Endrin	Control of mice, voles and other rodents.
Furans	Unintentionally produced from the same processes that release dioxins, and are found in commercial mixtures of PCBs.
Heptachlor	Control of crop pests and disease vectors.
Hexachlorobenzene (HCB)	Applied to kill fungi that affect food crops. This substance is released as by-product during chemical-manufacturing processes.
Mirex	Applied as an insecticide to reduce fire ants and other types of ants and termites. Mirex has been used as a fire retardant in plastics, rubber and electrical goods.
PCBs	Used in industries for several applications such as heat exchange fluids and lubricants.
Toxaphene (Camphechlor)	Applied to cotton, cereal grains, fruits, nuts and vegetables. Used to control ticks and mites in livestock.

Aim no 2: Support the transition to safer alternatives. Many of these POPs targeted in the SC are already virtually obsolete and replacement chemicals and techniques are in place. The

challenge, however, is to find the leftover stocks and prevent them from being used and to find alternatives whose benefits outweigh their risks.

Aim no 3: Target additional POPs for action. This can only be done by taking precautionary action to curtail exposures to harmful chemicals that are to some degree persistent, bio-accumulating, toxic and mobile. However, governments can identify a potential candidate pollutant to add to the listing of POPs by stating the reason for its concern. The Review Committee of the SC will evaluate the pollutant by using scientific data to determine if the pollutant's chemical properties warrant its inclusion in the treaty. Recommendations will be made to the parties of the SC, who will decide as a group, how to list this pollutant. This will take on the form of an amendment, and each of the parties needs to ratify it.

Aim no 4: Clean-up old stockpiles and equipment containing POPs. Strategies must be developed to identify stockpiles and waste sites. These sites must be managed in a safe, responsible and environmentally-sound manner so that leakage of pollutants into the environment will be reduced and ultimately cleaned up.

Aim no 5: Work together as a nation to reduce POPs. This would include: National action plans, information exchange between national focal points, launching of educational programmes and specialist training. Further research of these pollutants should be encouraged and established, to increase public awareness of human and environmental effects of POPs.

1.1.2 Current legislation of South Africa concerning dioxins, furans and PCBs

The current SA regulation on POPs is developing gradually, since SA ratified the SC. However, SA does not have stern mechanisms in the form of dedicated legislation (Anon, 2005c; Anon, 2005d) or self-regulation in place to monitor POPs. Currently there are control measures to regulate dioxins and furans from the stack emissions of two classes of incinerators, which should not exceed 80 ng/m^3, measured over 6 to 16 hours (0.2 ng/m^3 International Toxic Equivalent (I-TEQ)). According to this legislation, there are two classes of incinerators. The first includes incinerators in which the waste serves as the fuel or supplementary fuel in an industrial process e.g. the use of cement kilns or any other industrial boilers or furnaces for the disposal of noxious or hazardous materials. The second class includes incinerators for the disposal of waste that contains hazardous or potentially hazardous waste (Department of Water Affairs and Forestry (DWAF), 2005). For other possible sources of dioxins and furans there are currently no emission laws in SA. Due to SA

ratifying the SC, the removal of PCBs and PCB-containing equipment need to be phased out by 2025, as it is a restricted chemical listed in Annex III of the Rotterdam Convention (Rotterdam Convention, 2005). Amendment of section 44 of the National Environmental Management Act 107 of 1998 prohibits, restricts, and controls activities, which are likely to have detrimental effects on the environment, which includes restricted chemicals such as PCBs (Government Gazette, 2005a; Government Gazette 2005b).

Research on POPs in SA is currently done by tertiary institutions such as the North-West University (Potchefstroom Campus) and the University of Pretoria, supported by funding from the National Research Foundation (NRF) and Water Research Commission (WRC), that will contribute significantly towards the ability of SA to meet the requirements of the Stockholm Convention.

The Department of Environmental Affairs and Tourism (DEA&T) held a workshop on 27 January 2003 to launch the National Implementation Plan (NIP) of the Stockholm Convention (Anon, 2005c). The NIP will assess sources of POPs in the country, the impacts of these pollutants, and the infrastructure and additional capacity available to manage this issue. Currently, no inventories have been developed for emission factors of these substances, although SA is responsible to develop and implement an action plan according to article 5 (a) of the SC (UNEP, 2003). Subparagraph (i) of Annex A specifies that an action plan shall include source inventories and release estimates. These source inventories that should be established (Eduljee & Dyke, 1996; Dyke, Foan, Wenborn & Coleman, 1997) have several functions:

- The systematic screening of industrial and non-industrial processes that identifies possible sources of POPs;
- To estimate annual POPs emissions from identifiable source processes;
- To identify future trends of releases and inter-matrix effects; and
- To make recommendations on establishing inventories.

1.1.3 The aims and objectives of the project

The aim of this project was to determine if a platinum mine and its associated activities will result in the production of dioxin-like chemicals, using a reporter gene bio-assay. This will be useful information for future use, which will help in decision-making on policies concerning these substances and thereby contributing towards inventories as management tools. The objectives of this study were:

- To determine whether there are POPs, especially dioxin-like chemicals such as polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans (PCDD/Fs), and polychlorinated biphenyls (PCBs) present on a platinum mine in the Limpopo Province, SA;
- To determine whether the H4IIE *luc* reporter gene bio-assay can be used to investigate the possible formation and subsequent releases of dioxin-like chemicals;
- To determine the TEQ of each sample of selected environmental matrices on the platinum mine using the H4IIE reporter gene bio-assay;
- To investigate the implications of this source regarding the Stockholm Convention; and
- To develop recommendations regarding further research on this potential source.

The hypothesis was that dioxin-like compounds are present on a platinum mine due to thermal processes associated with platinum mining, and that the H4IIE reporter gene bio-assay can be used to detect these substances in different matrices associated with the operation of a platinum mine.

By establishing the hypothesis, aims and objective of the project different attributes concerning POPs especially dioxin-like chemicals need to be considered. The traits of dioxin-like compounds include the physical-chemicals characteristics as well as the sources from which they are formed. However, the formation, environmental fate and behaviour, and the possible health effects these compounds might cause should also be thoroughly studied. The next section will address the different traits of dioxin-like compounds.

1.2 Literature review

Dioxin-like chemicals (dioxins, furans and PCBs)

Many persistent organic pollutants pose a serious health hazard to both the environment and humans. An increase of a series of ecological and environmental contamination episodes and identification of dioxins in industrial waste (Davy, 2004) led to the American and European public's steadily-growing concern of POPs, especially dioxins. Two good examples are the weed killer and defoliant, Agent Orange used by the United States (U.S.) military during the Vietnam War that led to dioxin poisoning of humans (U.S.-Vietnam Cooperative Research, 2005), and the explosion at the Industrie Chimiche Meda Societa Azionaria (ICMESA) plant in Seveso, Italy releasing approximately 30 kg of these chemicals, affecting the health of communities in the area (Davy, 2004; Corliss, 2005).

The emphasis for this project was placed on identifying possible sources of POPs, especially dioxin-like chemicals, due to these environmental accidents. Very little is known on the concentrations, routes of exposure and the health effects that dioxin-like chemicals might cause in SA and it is necessary to attain the research skills to detect the presence of these compounds and their behaviour in the South African environment.

1.2.1 Dioxins and furans

Dioxins and furans are some of the most toxic chemicals known to man and are present in the environment as unwanted trace contaminants in the air we breath, water (fresh, ocean, estuarine that are deposited in the sediments), on land, in residues (liquid wastes, sludge and solid residues) and consumer products (paper and textiles) (Corsolini, Ademollo, Romeo, Greco & Focardi, 2005). These substances are also found in many industrial and thermal processes (Fiedler, 1996). Dioxins and furans are a heterogeneous mixture of chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans (PCDD/Fs) (Parzefall, 2002). PCDD/Fs are two classes of "quasi-planar" tricycles aromatic ethers with 210 different compounds (congeners) in total (De Souza Pereira, 2004). Of these, 17 congeners, including the most toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), pose a major health risk to the environment and man (Table 1.2).

Table 1.2: The 17 toxic congeners of PCDD/Fs.

PCDDs Congener:	**PCDFs Congener:**
2,3,7,8-TetraCDD	2,3,7,8-TetraCDF
1,2,3,7,8-PentaCDD	1,2,3,7,8-PentaCDF
1,2,3,4,7,8-HexaCDD	2,3,4,7,8-PentaCDF
1,2,3,6,7,8-HexaCDD	1,2,3,4,7,8-HexaCDF
1,2,3,7,8,9-HexaCDD	1,2,3,6,7,8-HexaCDF
1,2,3,4,6,7,8-HeptaCDD	1,2,3,7,8,9-HexaCDF
OctaCDD	2,3,4,6,7,8-HexaCDF
	1,2,3,4,6,7,8-HeptaCDF
	1,2,3,4,7,8,9-HeptaCDF
	OctaCDF

(Adapted from: Baars, Bakker, Baumann, Boon, Freijer, Hoogenboom, Hoogerbrugge, van Klaveren, Liem, Traag & de Vries, 2004; Van den Berg, Birnbaum, Bosveld, Brunström, Cook, Feeley, Giesy, Hanberg, Hasegawa, Kennedy, Kubiak, Larsen, van Leeuwen, Liem, Nolt, Peterson, Poellinger, Safe, Schrenk, Tillit, Tyskind, Younes, Waern, Zacharewski, 1998).

Abbreviations: CDD, chlorinated dibenzodioxins; CDF, chlorinated dibenzofurans.

2,3,7,8-TCDD is an environmental pollutant that is the most potent halogenated aromatic hydrocarbon (HAHs) originating as a by-product from the chemical industry or waste incineration, which causes low-level accumulation in the general population via the food

chain (Denison & Heath-Pagliuso, 1998; Geusau, Abraham, Geissler, Sator, Stingl & Tschachler, 2001; De Souza Pereira, 2004). This compound became known to the general public as being the main contaminant found in 2,4,5-trichloracetic acid (2,4,5-T), the so-called "Agent Orange" in the Vietnam War and was also the main contaminant in the "Seveso Episode" (Matsumura, 1983; De Souza Pereira, 2004). Besides being formed as unintentional by-products of manufacturing or disposal processes, PCDD/Fs may also be introduced into processes as contaminants in raw materials (UNEP, 2003).

1.2.2 PCBs

PCBs are the most widespread global pollutant that contaminates the atmosphere up to altitudes of about 6000 meters (Langer, 1998). The 12 toxic congeners of PCBs are listed in Table 1.3 that contributes to the pollution of the global atmosphere. Large amounts of PCBs have been found in all world oceans, in the Arctic and Antarctic and in the middle of all deserts. Several areas, which are heavily polluted, are now called PCB reservoirs, among them the Baltic See, Hudson Bay and Great Lakes in North America (Langer, 1998). PCBs have been produced commercially for some five decades, starting from 1920, by direct chlorination of biphenyl (Baars *et al.,* 2004). Commercial PCBs and environmental PCB residues contain complex mixtures of congeners and elicit a broad spectrum of biological responses (Langer, 1998). The various (commercial) technical PCB-mixtures are characterised by their chlorine content and include brand names such as 'Aroclor' (produced in Unites States of America), 'Chlophen' (produced in Germany), 'Phenoclor' (produced in France), 'Fenclor' (produced in Italy) and 'Kanechlor' (produced in Japan) (Baars *et al.,* 2004).

Table 1.3: The 12 toxic congeners of non-ortho and mono-ortho PCBs (Van den Berg *et al.,* 1998; Fiedler, 2003; Baars *et al.,* 2004).

Non-ortho PCBs congeners	Mono-ortho PCBs congeners
3,3',4,4'-TetraCB (77)	2,3,3',4,4'-PentaCB (105)
3,4,4',5-TetraCB (81)	2,3,4,4',5-PentaCB (114)
3,3',4,4'5-PentaCB (126)	2,3',4,4',5-PentaCB (118)
3,3',4,4'5,5'-HexaCB (169)	2',3,4,4,5'-PentaCB (123)
	2,3,3',4,4',5-HexaCB (156)
	2,3,3',4,4',5'HexaCB (157)
	2,3',4,4',5,5'-HexaCB (167)
	2,3,3',4,4',5,5'-HexaCB (189)

Abbreviations: CB, chlorinated biphenyl.

These mixtures were used in a wide scale of open applications (UNEP, 1999), such as coatings, ink solvents in carbonless copy paper, flame retardants, as additives in paints, in sealants, and in plastics because of their favourable characteristics such as:

- high chemical stability;
- low flammability;
- good heat conduction; and
- low electrical conductivity.

Due to their persistent nature, and their toxicity in the environment, many countries decided to ban the use of PCBs in open applications (Baars *et al.,* 2004), but PCBs are still used in electronic appliances, heat transfer systems, hydraulic fluids and dielectric fluid in capacitors and transformers, although this use will decrease over time (Jiang, Li, Chen & Jin, 1997; UNEP, 1999; Baars *et al.,* 2004). These compounds were used in great quantities because of their favourable characteristics and are found in the environment due to the intentional production and chemical accumulation in biological matrices (UNEP, 1999). Because these substances could be produced by South Africa as by-products during industrial processes, a need therefore arises to investigate these compounds' different characteristics.

1.2.3 Chemical and physical characteristics of PCDD/Fs and PCB

PCDD/Fs are a group of chlorinated tricyclic aromatic compounds with related physical and chemical properties, but with different biological potencies, and are found as complex mixtures in the environment (Huang & Buekens, 1996; De Souza Pereira, 2004). PCDDs consist of two benzene rings inter-connected by two oxygen atoms, but in the case of PCDFs the benzene rings are inter-connected by a carbon bond and an oxygen bridge (Figure 1.2) (McKay, 2002).

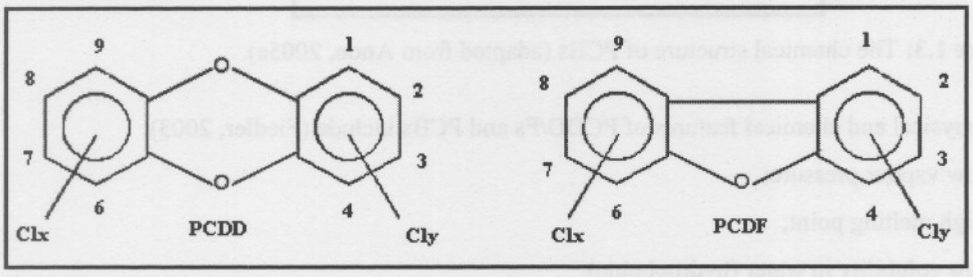


Figure 1.2: The general structure of PCDD/Fs indicating the 2 benzene rings connected with oxygen or carbon atoms as described above (adapted from Anon, 2005e).

PCDD/Fs can be chlorinated in the 2-, 3-, 7-and 8 position of the molecule (Hanberg, 1996; Fiedler, 2003). Different chlorination on the aromatic ring's structure can occur, leading to

75 PCDD and 135 PCDF isomers in total (Huang & Buekens, 1996; Rappe, 1996) as indicated in Table 1.4. There are more PCDFs than PCDDs, because the single oxygen atom makes the furan molecule less symmetrical than the corresponding dioxin (Rappe, 1996).

Table 1.4: Number of possible PCDD/F isomers (adapted from Rappe, 1996).

Number of chlorine atoms	Number of PCDD isomers	Number of PCDF isomers
1	2	4
2	10	16
3	14	28
4	22	38
5	14	28
6	10	16
7	2	4
8	1	1
Total	**75**	**135**

PCBs are aromatic, synthetic compounds, which do not occur in the natural environment. They consist of a biphenyl structure with two linked benzene rings (Figure 1.3) in which some or all of the hydrogen atoms have been substituted by chlorine atoms (De Souza Pereira, 2004; Ross, 2004). The chemical formula of PCBs is $C_{12}H_{10-n}Cl_n$ where n ranges from one to ten and a molecular weight between 189 and 499 $g.mol^{-1}$ (De Souza Pereira, 2004).

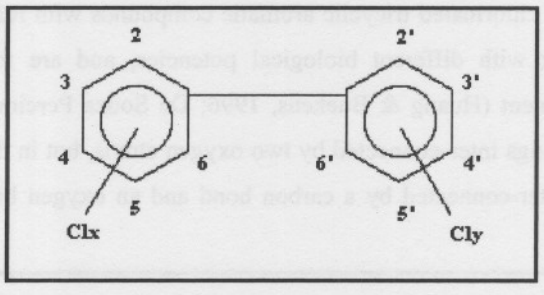


Figure 1.3: The chemical structure of PCBs (adapted from Anon, 2005e).

The physical and chemical features of PCDD/Fs and PCBs include (Fiedler, 2003):

- low vapour pressure;
- high melting point;
- low solubility in water (hydrophobic);
- high solubility in organic/fatty matrices (lipophilic);
- good stability and affinity for non-polar conditions;
- accumulation and bio-magnification in the food chain;
- preference to bind to organic matter in soil and sediments; and
- resistance to photolytic, chemical, biological and metabolic degradation.

Due to their hydrophobic nature, PCDD/Fs and PCBs tend to adhere to sediments, soils or biological tissues from aquatic living organisms (De Lima Ribeiro & Ferreira, 2003). Clay minerals are the most important inorganic component in soils for sorption of contaminants, due to a large exchange capacity, and appreciable internal and external surfaces accessible to organic and inorganic molecules (Lee, Kim, Chung & Jeong, 2004). Molecules that are not so hydrophobic have the tendency to solubilise in an aqueous phase (De Lima Ribeiro & Ferreira, 2003). The mobility of PCDD/Fs and PCBs in soils is controlled by the equilibrium sorption/desorption process among several compartments like air, water, mineral and organic matter (Wu, Schramm, Xu & Kettrup, 2002). For these non-polar compounds the equilibrium favours sorption to organic carbon in soil (Brzuzy & Hites, 1995). Therefore, the soil distribution coefficient (K_{oc}) for PCDD/Fs can be predicted from their high octanol-water partition coefficient (K_{ow}) values that range between 10^6 and 10^8 (Wu *et al.,* 2002; De Lima Ribeiro & Ferreira, 2003). The hydrophobicity of these chemicals is expressed by the K_{ow}, which estimates the solubility of these substances in both aqueous and organic phase (De Lima Ribeiro & Ferreira, 2003), and does not readily undergo metabolic transformation (De Kock & Lord, 1989). Since PCDD/Fs are non-polar and non-ionic, the soil pH does not affect the sorption process (Wu *et al.,* 2002). It should be realised that certain sources contribute to formation of POPs and will be discussed in the next section.

1.2.4 Sources and formation of PCDD/Fs

Environmental contamination of PCDD/Fs can be attributed to a series of primary sources. These primary sources of PCDD/Fs can be divided into several categories including (Eduljee & Dyke, 1996; Fiedler, 1996; Rappe, 1996; Fiedler, 2003; Hays & Aylward, 2003):

- chemical-industrial reactions such as bleaching of paper and pulp with chlorine gas and dry cleaning distillation residues;
- thermal or combustion processes, which involve burning of chlorinated organic or inorganic compounds, incineration of municipal solid waste, medical waste incineration, hazardous waste incineration, sewage sludge incineration and sintering plants. Since platinum mining involves thermal processes during the formation of precious metals it might be a possible source of PCDD/Fs and PCBs (see Chapter 2 for platinum mining);
- photochemical reactions under atmospheric conditions or aerial transport can result in the formation of PCDD/Fs;
- non-biological and biological processes can result in the formation of PCDD/Fs; examples include the mixing of chlorophenols, hydrogen peroxide and peroxidase at room temperature. These reactions can occur under *in vivo* or experimental conditions such as in sewage sludge and compost; and

- reservoirs including sewage sludge, compost, and contaminated soils.

PCDDs/Fs are formed unintentionally as by-products of many industrial processes such as: waste incineration; chemical and pesticide manufacturing; and pulp and paper bleaching, but have never been intentionally produced for human usage (Tuppurainen, Halonen, Ruokojärvi, Tarhanen & Ruuskanen, 1997; Stanmore, 2004). These pollutants are formed during the combustion of organic matter in the presence of chlorine and metals and in the presence of hydrogen chloride (Tuppurainen *et al.,* 1997). PCDD/Fs are also formed during volcanic eruptions and forest fires (Fiedler, 1996; Baars *et al.,* 2004).

PCDD/Fs formed at high temperatures (760-800°C) are more stable than those being formed at 350-380°C (Stanmore, 2004). The formation routes of both PCDD/Fs can be divided into 2 groups (UNEP, 2003):

- thermal processes (Section 1.2.4.1); and
- formation in wet chemical processes (Section 1.2.4.2).

1.2.4.1 Thermal processes.

These substances are formed in trace quantities during homogeneous (gas-phase) reactions and heterogeneous (solid-phase) reactions. The homogeneous reactions occur at temperatures between 500-800°C, during the pyrolytic rearrangement of chlorinated precursors, which include four principal pathways (Stanmore, 2004):

- the cyclisation of polychlorobiphenyls;
- the cyclisation of polychlorodiphenyl ethers;
- the chlorination of dibenzofurans; and
- the dechlorination of OctaCDF.

The heterogeneous reaction is a catalytic reaction that occurs between 200-400°C that includes two primary routes that play an important role in the formation of PCDD/Fs (Altwicker, 1996; UNEP, 2003; Stanmore, 2004):

- *De novo* synthesis where PCDDs/PCDFs are formed from elemental carbon;
- Precursor formation/reaction via aryl structures derived from incomplete aromatic oxidation or cyclisation of hydrocarbon fragments.

There are in general four conditions, which favour the generation of PCDD/Fs in thermal processes (UNEP, 2003):

- high temperature processes;

- incomplete combustion;
- organic carbon; and
- chlorine.

1.2.4.2 Wet chemical manufacturing processes.

There are many chemical processes that favour the generation of PCDD/F formation such as the manufacturing of pulp and paper when bleaching is carried out with chlorine and the chemical industry that manufacture chlorinated phenols and their derivates, chlorinated aromatics, chlorinated aliphatic chemicals and chlorinated catalyst and inorganic chemicals (UNEP, 2003; Stanmore, 2004). There are several conditions that favour the generation of PCDD/Fs (UNEP, 2003):

- high temperatures (> 150°C);
- alkaline conditions; and
- ultraviolet radiation or other radical starters.

By determining how PCDD/Fs and PCBs are formed, future pollution by these substances will be prevented. It is however important to be acquainted with these substances environmental fate and behaviour which will be discussed in the next section.

1.2.5 Environmental fate and behaviour of PCDD/Fs and PCBs

As a result of their chemical nature (addressed in 1.2.3), PCDD/Fs, primarily bind to particulate and organic matter in soils and sediments as well as the fatty tissue of biota (Geyer, Schramm, Feicht, Behecti, Steinberg, Brüggemann, Poiger, Henkelmann & Kettrup, 2002; Fiedler, 2003). There are two primary pathways for dioxins when entering the food chain. These pathways include (Van Overmeire, Clark, Brown, Chu, Cooke, Denison, Baeyens, Srebrnik, & Goeyens, 2001; Galiulin, Bashkin & Galiulina, 2002):

- Air-to-plant-to-soil–to-animal/human (Figure 1.4);
- Water/sediment-to-fish.

Another possible pathway of dioxin and dioxin-like chemicals to enter the food web is through the accidental contamination incidents due to inappropriate handling and processing of feed and food substances (Van Overmeire *et al.,* 2001).

In the air, these semi-volatile compounds can exist in both gaseous phase and bound to particles (Fiedler, 2003). Many factors influence and control the ambient air concentrations

of PCDD/Fs (Figure 1.5) such as inputs (primary and secondary), losses (deposition, reactions), and mixing/dilution processes (Alcock, Sweetman & Jones, 2001).

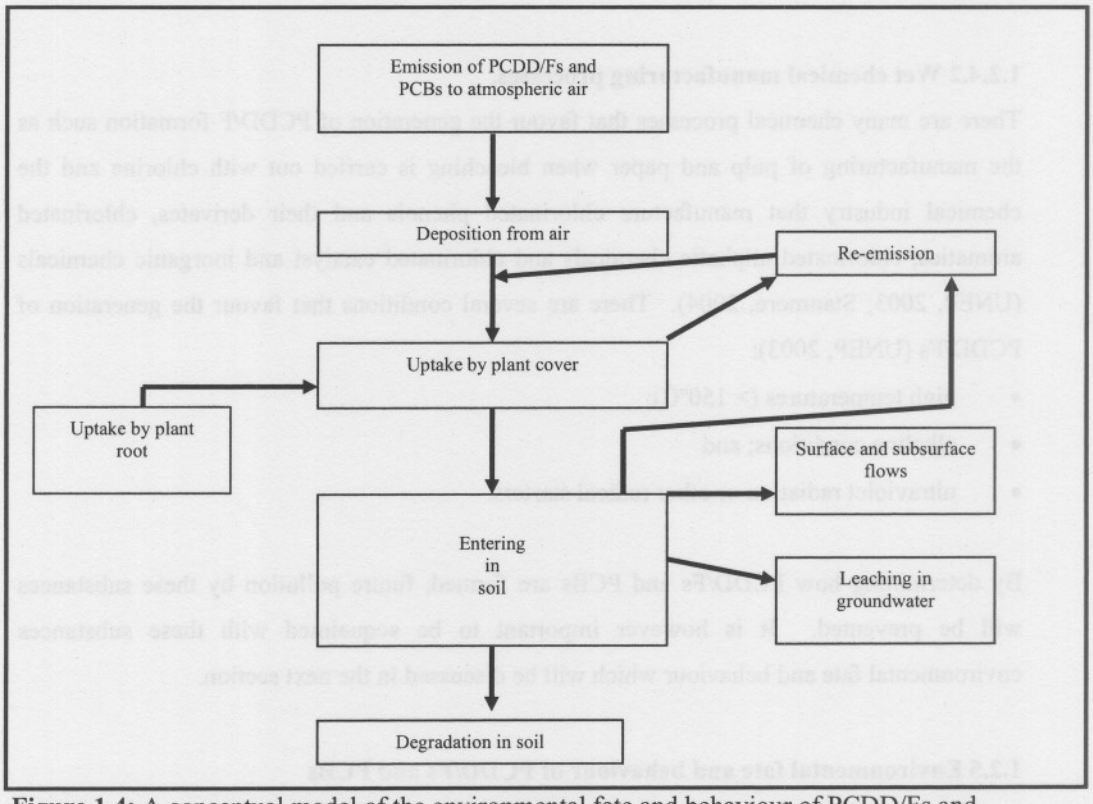


Figure 1.4: A conceptual model of the environmental fate and behaviour of PCDD/Fs and PCBs in the air-plant-soil system (adapted from Galiulin *et al.,* 2002).

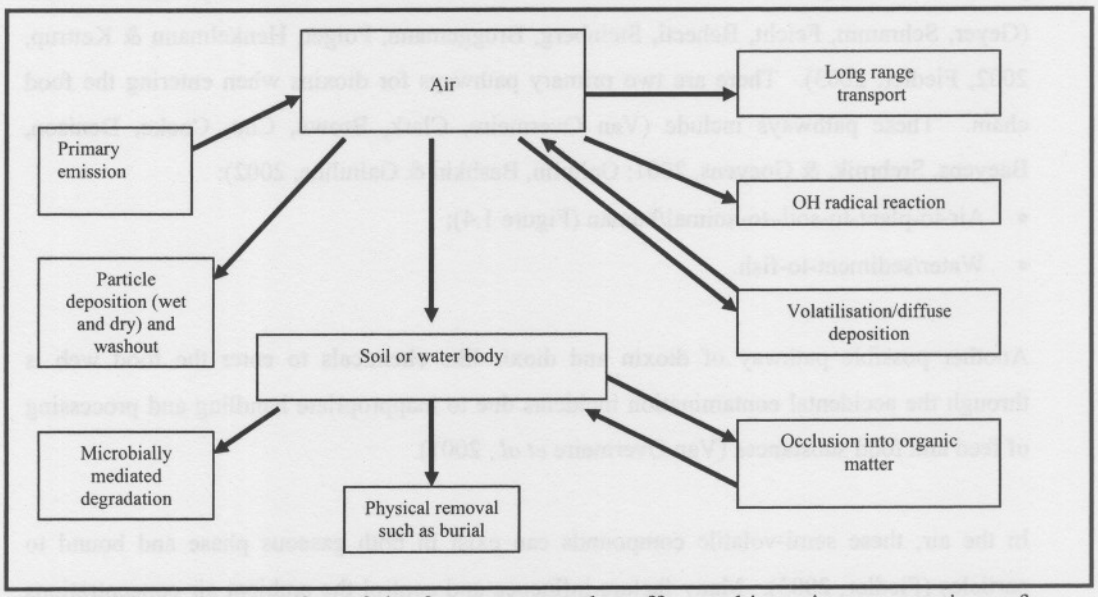


Figure 1.5: A representation of the fate processes that affect ambient air concentrations of PCDD/Fs in the environment (adapted from Alcock *et al.,* 2001).

PCDD/Fs in the vapour phase can undergo photochemical transformation with de-chlorination processes that will lead to more toxic congeners (Galiulin *et al.,* 2002). These pollutants are emitted to air by various routes such as evaporation from plants and soils and by wind erosion of soils (Galiulin *et al.,* 2002). PCDD/Fs can be deposited on plant surfaces via wet deposition, via dry deposition of chemicals bound to atmospheric particles or via diffuse transport of gaseous chemicals in the air to the plant surface (Fiedler, 2003).

The behaviour of these pollutants in the plant-soil subsystem involves a spectrum of processes (Galiulin *et al.,* 2002):

- Absorption by plant roots;
- Re-emission;
- Migration (leaching); and
- Degradation in soil and water.

Because these chemicals are found in various environmental compartments, these chemicals pose a threat to human health and the environment. It is therefore very important to determine the potential risks and health effects these pollutants might cause to the environment and humans.

1.2.6 Contamination of food by PCDD/Fs and PCBs

PCDD/Fs are environmental contaminants which are found worldwide in a variety of samples including air particulate matter, soil, clay, sediments, sludge, mussels, fish, seals, whales, dolphins, pigs, cattle, cows-milk, chickens, eggs, human adipose tissue and breast milk (Hayward, Nortrup, Gardner & Marion Clower, 1999; Geyer *et al.,* 2002;). As a result of the human activity inputs, the PCDD/F concentration could increase in soil (Anon, 2005f). Since the half-life of PCDD/Fs in natural sediments is greater than 100 years, the environmental fate and behaviour of these chemicals must be known (Stanmore, 2004).

The transfer of PCDD/Fs into human food occurs by particle-bound distribution on grass and other fodder plants and into the aquatic food chains (Parzefall, 2002). Humans are exposed to PCDD/Fs and PCBs through contaminated food and water intake, inhalation and skin contact (Langer, 1998; Parzefall, 2002). Humans are exposed to dioxins mainly (>95%) through contamination of food (Parzefall, 2002). The highest exposure of humans to PCDD/Fs occurs in infants via breast-feeding. The major food sources are fish and seafood, meat and meat products and milk and diary products, each group accounting for a third of total human intake

of PCDD/Fs and PCBs under industrialised conditions in developed countries (Parzefall, 2002; Guruge, Seike, Yamanaka & Miyazaki, 2005; Pirard & De Pauw, 2005).

1.2.7 Potential risks and health effects of PCDD/Fs and PCBs

The average half-life of 2,3,7,8-TCDD in rats is 19 days, but in humans it is about 150 times that of rats. The half-life of 2,3,7,8-TCDD in humans range from about 5-11 years, with the best estimated average of seven to eight years (Hays & Aylward, 2003). The half-life of 2,3,7,8-TCDD is dependent on the total body fat content of the organism and the amount of non-absorbable lipid compounds in the diet of the organism (Geyer *et al.*, 2002). The PCDD/Fs and dioxin-like PCBs elicit a broad spectrum of responses that are specific for the age, sex, strain and species of animal studied. The effects these chemicals have on the environment and humans include (Poland & Knutson, 1982; Matsumura, 1983; Hanberg, 1996): hepatoxicity, decrease in food consumption leading to weight loss, atrophy of the thymus gland, developmental and reproductive toxicity, immunotoxicity, embryo-toxicity, carcinogenic, tumour promotion, and dermal toxicity such as chloracne (Figure 1.6).



Figure 1.6: Possible dioxin poisoning of the Ukrainian presidential candidate Viktor Yushchenko that led to acute chloracne (Anon, 2005g).

Other effects that can occur is wasting syndrome, diverse effects on hormones and growth factors, induction of phase I and phase II drug-metabolising enzyme activities, teratogenicity, and alterations in neural development (Loonen, Van de Guchte, Parsons, De Voogt & Govers, 1996; Hilscherova, Machala, Kannan, Blankenship & Giesy, 2000; Geusau *et al.*, 2001; Fiedler, 2003). The toxico-kinetic behaviour of PCDD/Fs in humans (and other primates) depends on three major properties (De Souza Pereira, 2004):

1. lipophilicity;

2. metabolism; and
3. binding to responsive genes in the liver.

The different characteristics of PCDD/Fs and the effects they cause in organisms and in the environment have lead to the development of the Toxic Equivalency Factors (TEF) for these substances, which will be explained in the next section.

1.2.8 Toxic equivalency factor (TEF) and the toxic equivalency quotient (TEQ)

The complex nature of PCDD/F and PCB mixtures complicates risk evaluation for humans, fish and wildlife. For this purpose the concept of TEF was developed (Van den Berg *et al.,* 1998). The introduction of this concept was to facilitate risk assessment to animals and humans and regulatory control of exposure to these mixtures (WHO, 1998; Toyoshiba, Walker, Bailer & Portier, 2004).

The European Centre of Environmental Health of the World Health Organisation (ECEH-WHO) and the International Program on Chemical Safety (IPCS) first introduced the TEF approach in Stockholm on 15-18 June 1997 (Van Overmeire *et al.,* 2001). This approach is the most common method based on the toxicity characterisation of dioxin-like compounds (Van den Berg *et al.,* 1998; Toyoshiba *et al.,* 2004). ECEH-WHO/IPCS developed TEF values (WHO-TEF) for PCDD/Fs and dioxin-like compounds such as PCBs (Eljarrat, Caixach & Rivera, 2003). At the same time the United Nations Environmental Program (UNEP) also developed an International TEF scheme (I-TEF) for PCDD/Fs, but not for PCBs (Dyke & Stratford, 2002). These values do not differ significantly from the WHO-TEF values and is termed the I-TEF values.

The TEF values have been used in two different ways (Van den Berg *et al.,* 1998): 1) as a relative potency value that is based on the results of several *in vivo* and *in vitro* studies; or 2) as a relative potency of a compound to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) to cause a particular toxic or biological effect. TEFs can be defined as the ratio of the toxicity of a reference compound (2,3,7,8-TCDD) to the toxicity of an index congener (dioxin congeners) as stated in Dixon, Clemons & Bols (1997) and Toyoshiba *et al.* (2004).

TEF values have been developed for invertebrates, mammals, fish, and birds (Van den Berg *et al.,* 1998). The values derived for mammals were considered to be applicable to human risk assessment. Table 1.5 and 1.6 list the WHO-TEFs for human risk assessment and mammals for PCDD/Fs, PCBs and congeners.

Table 1.5: WHO-TEFs for PCDD/Fs for human risk assessment and mammals based on the WHO meeting in Stockholm, Sweden, 15-18 June 1997 (Van den Berg *et al.,* 1998; Stanmore, 2004).

Congener	TEF value
Dibenzo-*p*-dioxins	
2,3,7,8-TetraCDD	1
1,2,3,7,8-PentaCDD	1
1,2,3,4,7,8-HexaCDD	0.1
1,2,3,6,7,8-HexaCDD	0.1
1,2,3,7,8,9-HexaCDD	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01
OCDD	0.0001
Dibenzofurans	
2,3,7,8-TetraCDF	0.1
1,2,3,7,8-PentaCDF	0.05
2,3,4,7,8-PentaCDF	0.5
1,2,3,4,7,8-HexaCDF	0.1
1,2,3,6,7,8-HexaCDF	0.1
1,2,3,7,8,9-HexaCDF	0.1
2,3,4,6,7,8-HexaCDF	0.1
1,2,3,4,6,7,8-HeptaCDF	0.01
1,2,3,4,7,8,9-HeptaCDF	0.01
OctaCDF	0.0001

Abbreviations: CDD, chlorinated dibenzodioxins; CDF, chlorinated dibenzofurans.

TEFs for dioxin-like compounds apply only to aryl hydrocarbon receptor (AhR) mediated responses and they do not consider modulating effects of compounds that are not Ah receptor ligands (Van den Berg *et al.,* 1998; WHO, 1998). The criteria for including a compound in the TEF scheme for dioxin-like compounds (Van den Berg *et al.,* 1998; Behnisch, Hosoe, Sakai, 2001) are that the compound must:

- show structural relationship to the PCDD/Fs;
- bind to the AhR;
- elicit Ah receptor-mediated biochemical and toxic responses; and
- be persistent and accumulate in the food chain.

TEFs and relative potency (REP) have been used synonymously. The REP is defined as species-, endpoint-, and assay-specific determinations of potency expressed relative to some standard such as 2,3,7,8-TCDD (Van den Berg *et al.,* 1998; Villeneuve, Richter, Blankenship & Giesy, 1999). The REP of samples are usually calculated as the amount of standard (reference toxicant) giving the same response as the sample, commonly based on the amount

of sample needed to produce 50% of the maximal standard response (Giesy, Hilscherova, Jones, Kannan & Machala, 2002). TEFs are defined as consensus values based on REP determination across multiple species and/or endpoints. The TEFs have been used during risk assessment and are generally order of magnitude estimates in comparison with REP, which are more precisely defined and are needed for bio-assay-directed –mass-balance analysis of complex mixtures (Villeneuve *et al.,* 1999). According to Giesy *et al.* (2002), the REP of samples are calculated as the amount of the standard (2,3,7,8-TCDD), which gives the same response as samples based on the amount needed to produce 50% of the maximal response which equals the TEQ of the sample.

Table 1.6: WHO-TEFs for PCBs for human risk assessment and mammals (Van den Berg *et al,* 1998).

Congener	TEF value
***Non-ortho PCBs**	
PCB77	0.0001
PCB 81	0.0001
PCB126	0.1
PCB169	0.01
***Mono-ortho PCBs**	
PCB105	0.0001
PCB114	0.0005
PCB118	0.0001
PCB123	0.0001
PCB156	0.0005
PCB157	0.0005
PCB167	0.00001
PCB189	0.001

*: Refer to Table 1.3 for explanation of the congeners

The TEQ of a sample is calculated by multiplying TEF values by the concentration of a specific congener. The total sum equals the TEQ per mass (WHO, 1998; Hilscherova *et al.,* 2000). TEQ concentrations in samples are calculated by using the following equation (Van den Berg *et al.,* 1998; WHO, 1998):

$$TEQ = \Sigma (PCDD_i \times TEF_i) + \Sigma (PCDF_i \times TEF_i) + \Sigma (PCB_i \times TEF_i)$$

This in turn makes the TEQ approach very essential in assessing exposure levels of dioxin-like compounds to humans and the environment (Maruyama, Yoshida, Tanaka & Nakanishi, 2003).

1.3. Detection of dioxin-like chemicals

1.3.1 Analysis techniques

The detection and quantification of dioxin levels in food, feed and in environmental samples require two analysis techniques. To determine the TEQ of food, feed and environmental samples two analysis approaches could be used (Van Overmeire *et al.*, 2001):

- congener specific analyses (chemical-analysis) by using TEFs to address and facilitate risk assessment (Section 1.3.1.1); and
- bio-analytical methods, which provide an overall TEQ value (Section 1.3.1.2).

1.3.1.1 Chemical-analysis

The only accepted technique at this moment, as indicated by Van Overmeire *et al.* (2001) that allows for a determination of congener specific concentration at low levels (part per trillion (ppt)), is high-resolution gas chromatography combined with high-resolution mass spectrometry (HRGC-HRMS). This is a standard method for determining TEQs and has several advantages (Van Overmeire *et al.*, 2001):

- Provides information on dioxin congener distribution and level;
- Identifying sources of contaminants responsible for increased TEQ levels;
- It is a very useful risk management tool.

Not only do chemical-analyses have advantages, but limitations as well. These limitations include (Schwirzer, Hofmaier, Kettrup, Nerdinger, Schramm, Thoma, Wegenke & Wiebel, 1998; Van Overmeire *et al.*, 2001; Giesy *et al.*, 2002):

- Very expensive and time consuming;
- Requires sophisticated instrumentation;
- Considerable effort in the preparation of samples. These samples may contain compounds undetected by chemo-analysis and the toxicity of the samples are overlooked;
- TEFs are available for only a limited number of congeners;
- Analysis time is not negligible;
- Chemical analysis does not take into account chemical interactions such as synergism and antagonism.

1.3.1.2 Bio-analytical methods

Biological assay of xenobiotics may be divided into two major groups: those utilising living material (micro-organism, insects, animals, plants), and those exploiting bio-molecular approaches such as immuno-assays and tissue culture assays (Roy, Mysior & Brzezinski, 2002). These assays are based on very specific and selective bio-chemical interactions such

as ligand-receptor or ligand-antibody recognition (Roy *et al.,* 2002). Bio-analytical methods yield overall TEQ values without providing information on the levels of individual dioxin congeners (Van Overmeire *et al.,* 2001). There are a number of bio-assays that can be used to determine dioxins and dioxin-like compound potencies, including the H4IIE reporter gene bio-assay or, as it is also known, chemical-activated luciferase expression (CALUX®), ethoxyresorufin-O-deethylase (EROD) and immuno-assay (Behnisch, Hosoe, & Sakai, 2002). *In vitro* luciferase/reporter gene bio-assays such as the H4IIE are advantageous for several reasons (Schwirzer *et al.,* 1998; Van Overmeire *et al.,* 2001; Giesy *et al.,* 2002):

- These techniques measure the biological responses such as enzyme activity, transcription and expression of reporter genes, ligand-receptor binding or antigen-antibody reactions;
- Bio-analysis allows for a higher sample throughput;
- Cost effective;
- Time effective when screening complex mixtures;
- Simplistic;
- Can distinguish between agonists and antagonists;
- Distinguishes between stable and unstable AhR agonists;
- Is tissue- and species-specific; and
- Valuable screening method.

Bio-assay not only has advantages but there are disadvantages that are summarised as follows (Behnisch *et al.,* 2001):

- A luminometer and stable transfected cells are needed;
- Possible tissue factors could be missing due to transformation into recombinant cells;
- Leaves out outer signal pathways;
- Detects only AhR ligands with no information on biological activity; and
- High TEQ/false-positive results could be reported because of induction of any compound capable of binding to the AhR without clean-up.

As a result of the above-mentioned advantages of *in vitro* luciferase/reporter gene bio-assay, the biochemical background of the H4IIE bio-assay will be discussed in the next section.

1.3.2 The biochemical background of the H4IIE bio-assay

1.3.2.1 Aryl hydrocarbon receptor (AhR) and the mode of action

The AhR is a member of the bHLH-PAS (basic-helix-loop-helix, Per-Arnt-Sim) protein family and is a ligand-dependent transcription factor located in the cytosol of the cell (Figure 1.7). It complexes with heat shock proteins (Hsp90) of a molecular weight of 90 kDa and

immunophilin-like proteins (ILP) (Shimba, Todoroki, Aoyagi, & Tezuka, 1998; Giesy *et al.,* 2002; Janošek, Hilscerová, Bláha & Holoubek, 2005; Ramadoss & Perdew, 2005).

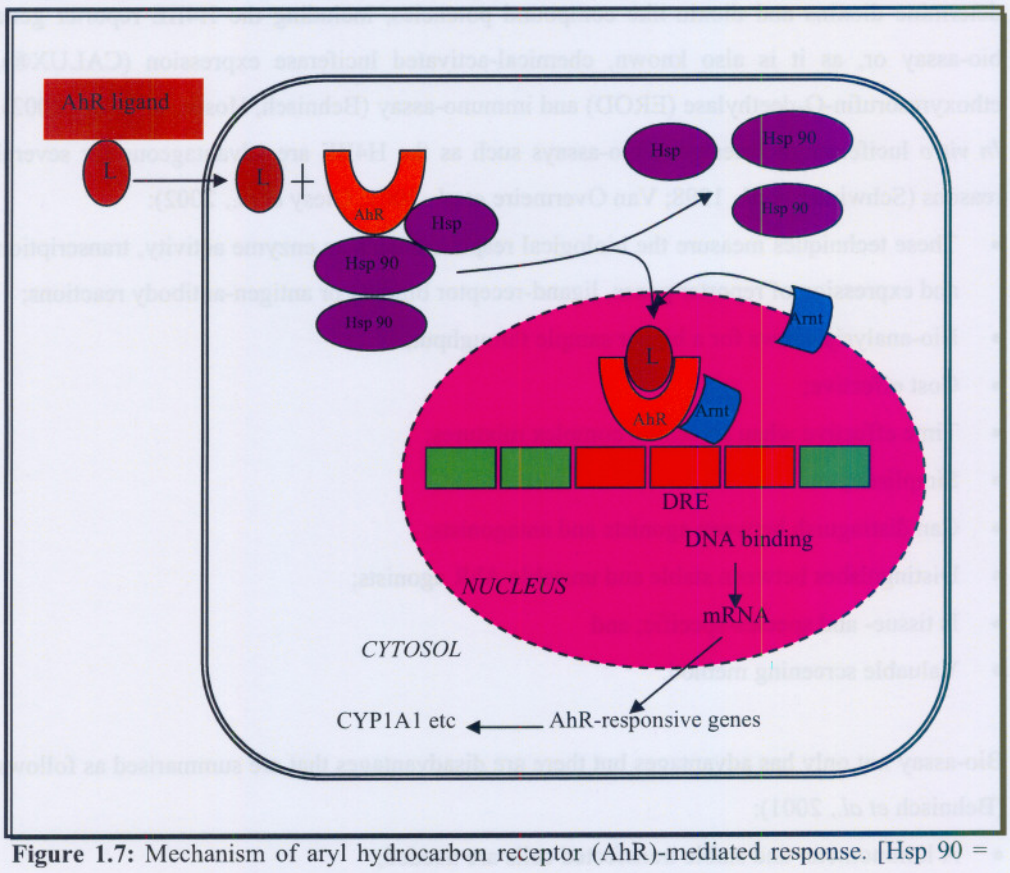


Figure 1.7: Mechanism of aryl hydrocarbon receptor (AhR)-mediated response. [Hsp 90 = heat shock proteins; L = ligand; DRE = dioxin response element; Arnt = [Ah- receptor nuclear translocator] (adapted from Hilscherova *et al.,* 2000).

The AhR is the receptor for 2,3,7,8-TCDD and related compounds, but under normal physiological conditions, the AhR is involved in the regulation of xenobiotic metabolism and the maintenance of homeostatic function such as the development of the liver, heart and spleen (Shimba *et al.,* 1998; Wang, Wang, Wen, Buhler & Hu, 2004).

The strength to which congeners may bind to the AhR is directly proportional to the toxicity, enhanced gene transcription and enzyme activities mediated by the AhR mechanism (Safe, 1995; Giesy *et al.,* 2002). Most of the toxic effects of dioxin-like compounds are caused through the AhR mechanism (Safe, 1995; Hilscherova *et al.,* 2000).

The mechanism of the AhR-mediated response is illustrated in Figure 1.7. After the dioxins and dioxin-like chemicals diffuse into the cell it binds with high affinity to the cytosolic AhR (Safe, 1995; Shimba *et al.,* 1998). The receptor ligand complex is activated and translocated to the nucleus where Hsp90 dissociate from the complex to form a heterodimer with the Ah receptor nuclear translocator (Arnt) (Shimba *et al.,* 1998; Hilscherova *et al.,* 2000; Nie, Blankenship, & Giesy, 2001; Behnisch *et al.,* 2002).

The heteromeric ligand AhR:Arnt complex binds with high affinity to specific DNA sequences including: 5'-T-GCGTG-3' / 3'-A-CGCA-5'. These DNA sequences are termed the dioxin responsive element (DRE) (Hankinson, 1995; Sanderson, Aarts, Brouwer, Froese, Denison & Giesy, 1996). Binding to the DRE results in DNA bending, disruption of chromatin and nucleosome, and thus leading to increased promoter accessibility, and transcriptional activation of adjacent responsive genes such as the cytochrome P451A1 (Hankinson, 1995; Safe, 1995; Hilscherova *et al.,* 2000; Giesy *et al.,* 2002). The cytochrome P450 enzyme system (CYP) plays and important role in regulating a variety of endogenous substances such as hormones (Börga *et al.,* 2005).

Diverse synthetic and natural-occurring chemicals such as HAHs and non-halogenated PAHs can lead to activation of the AhR (Lewis, Ioannides. & Parke, 1986; Wang *et al.,* 2004). Other different compounds that could bind to the AhR are (Hilscherova *et al.,* 2000):

- Indoles;
- Tryptophan-derived compounds;
- Oxidised carotenoids;
- Heterocyclic amines; and
- Some pesticides of drugs with various structures such as imidazols and pyridines.

1.3.2.2 H4IIE *luc* bio-assay

The H4IIE *luc* bio-assay is an *in vitro* test and is used to detect and semi-quantify specific contaminants, and measure the catalytic activity of cytochrome P450 1A (CYP1A) (Whyte & Tillitt, 2004). This bio-assay consists of rat hepatoma cells that contain a stably transfected vector (the pGudLuc1.1 vector) of the firefly luciferase gene under the transcriptional control of DRE (Nie *et al.,* 2001; Van Overmeire *et al.,* 2001). Stable transfection of genes incorporates genes of interest, such as the luciferase firefly gene, which becomes a permanent part of the genome of the hepatoma cell. These cells are in turn suitable for long-term experiments and the results are more reproducible (Hilscherova *et al.,* 2000). By exposing these recombinant cell lines to dioxins or dioxin-like compounds, a luciferase gene will be

transcribed, which, upon addition of a substrate, luciferin, catalyses a light-producing reaction (Figure 1.8). The amount of photons released during this process is referred to as relative light units (RLU) and are proportional to the concentration of the pollutant mixture extracted from the environmental matrix. The luminescent endpoint can be measured with a luminometer to provide a sensitive measure of AhR-mediated gene expression potency (Villeneuve *et al.,* 1999). Some chemicals such as polyhalogenated aromatic hydrocarbons (PHAHs) including 2,3,7,8-TCDD may induce many different proteins such as CYP1A1 activity (Otsuka, 2002). These chemicals cause competitive inhibition and reduce activity at greater concentrations (Dixon *et al.,* 1997).

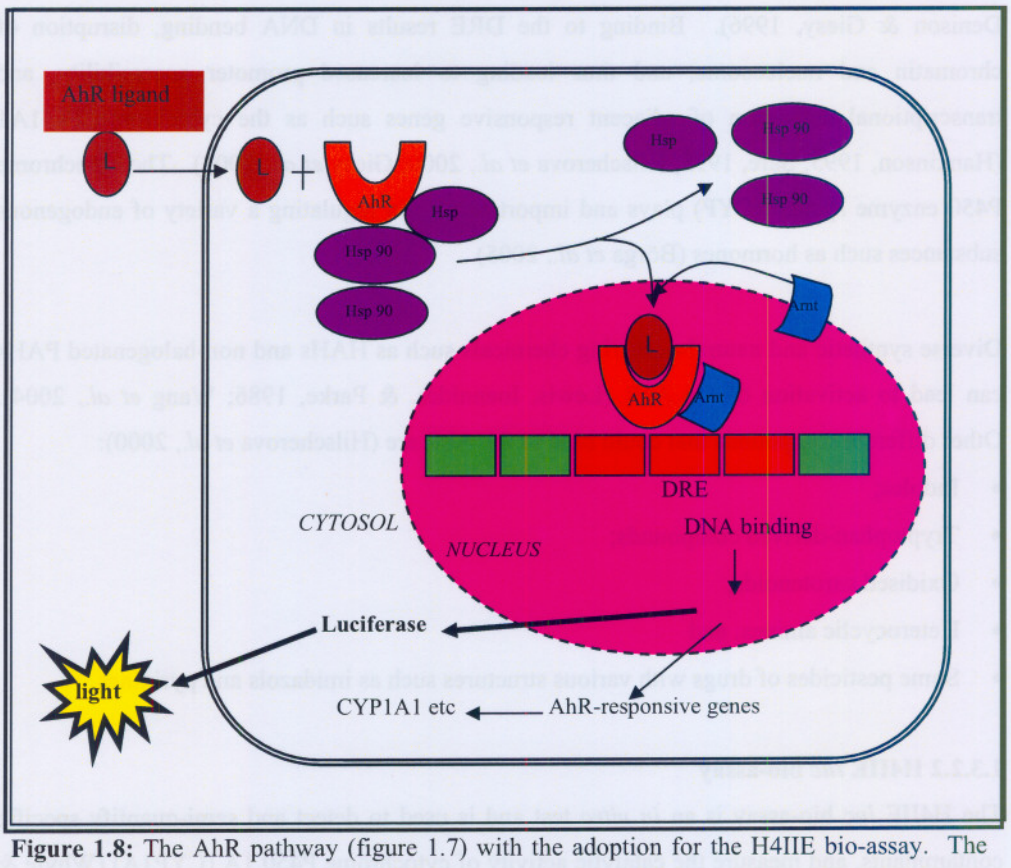


Figure 1.8: The AhR pathway (figure 1.7) with the adoption for the H4IIE bio-assay. The pathway is indicated as the parts in bold (adapted from Hilscherova *et al.,* 2000).

The value and utility of the H4IIE *luc* bio-assay can be summarised as follows (Whyte & Tillitt, 2004):

- The H4IIE bio-assay is rapid and a highly-sensitive test that can indicate the presence of polycyclic halogenated hydrocarbon (PHHs) and related compounds in environmental samples;

- It can determine the potential cumulative toxicity of PCDD/Fs and PCBs to organisms;
- It can provide information on biological interactions of PHHs when used in concert with analytical chemistry methods;
- It can be used in the assessment of toxic impacts on wildlife;
- The bio-assay is among the Tier 1 or "workhorse methods" recommended for screening environmental samples for dioxin-like activity in virtually all habitats; and
- It can detect very low concentrations of contaminants that are of high priority.

These characteristics of the H4IIE *luc* bio-assay makes it an ideal tool for evaluating the toxic potential of environmental and process samples (Whyte & Tillitt, 2004) on a platinum mine and determining if there is a possible threat that these compounds pose to the environment and the workers at the platinum mine.

Chapter 2

PLATINUM MINING IN SOUTH AFRICA

2.1 History of the Bushveld Complex and platinum

The Bushveld Complex (Figure 2.1) is an approximately 2 000 million-year-old layered igneous intrusion formed by the repeated injection of lava (or magma) into a sub-volcanic, shallow-level chamber (Cawthorn, 1999; Matthey, 2005). This intrusion has a large surface area with outcrop extremities of approximately 450 km east-west and 300 km north-south. (Implats, 2005). It is quadrilobate in shape with lobes in the form of inverted cones (not illustrated). The Complex comprises an array of diverse igneous rocks that consists of the Swartklip subfacies and the Rustenburg subfacies as seen in Figure 2.1. The Rustenburg Layered Suite (Figure 2.2), consist of both the Merensky Reef (named after geologist Dr. Hans Merensky) and the Upper Group 2 (UG2) chromitite layer (Cawthorn, 1999). These two horizons are exploited for their platinum group metals (PGMs) and associated metals content.

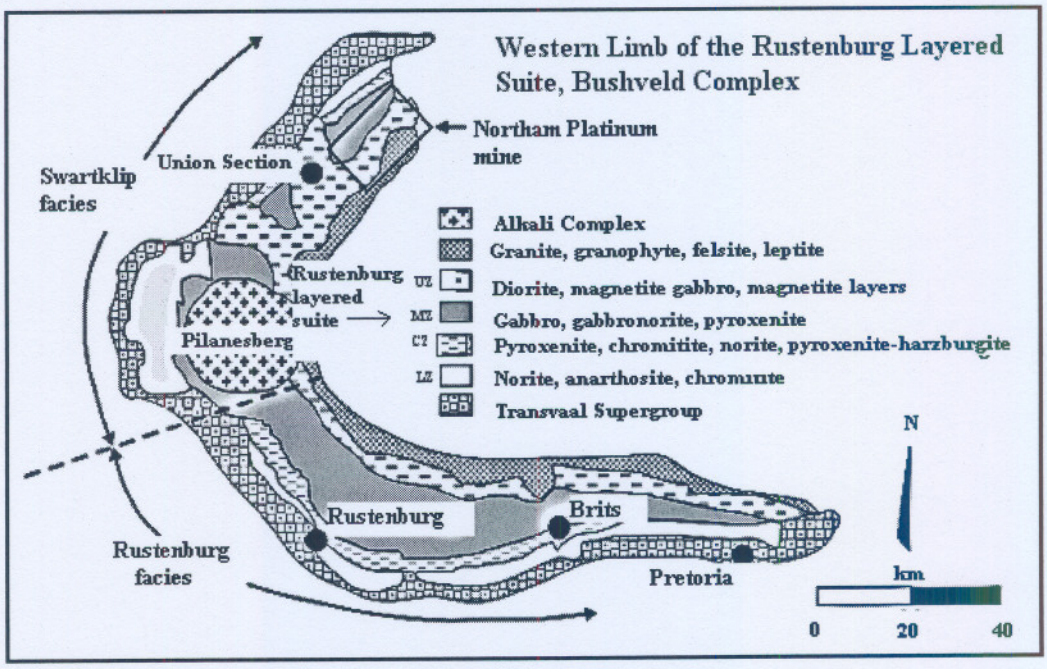


Figure 2.1: The western limb of the Bushveld Complex showing two main facies of the Rustenburg Layered Suite, the Swartklip facies and the Rustenburg facies (adapted from Smith, Basson & Reid, 2003). The study area, Union Section occurs within the Swartklip facies.

The platinum mine called Union Section at Swartklip forms part of Rustenburg Platinum Mines Limited, contributing about 13.3% of platinum ounces produced in South Africa. Rustenburg Platinum Mines Limited is a subsidiary to Anglo American Platinum Corporation

Limited (Amplats) together with Potgietersrus Platinums Limited, Lebowa Platinum Mines Limited, Rustenburg Base Metals Refiners Limited, and Precious Metals Refiners (Cawthorn, 1999; Anglo Platinum, 2004). This Group is the world's largest primary producer of platinum and accounts for about 38% of the world's production (Anglo Platinum, 2004).

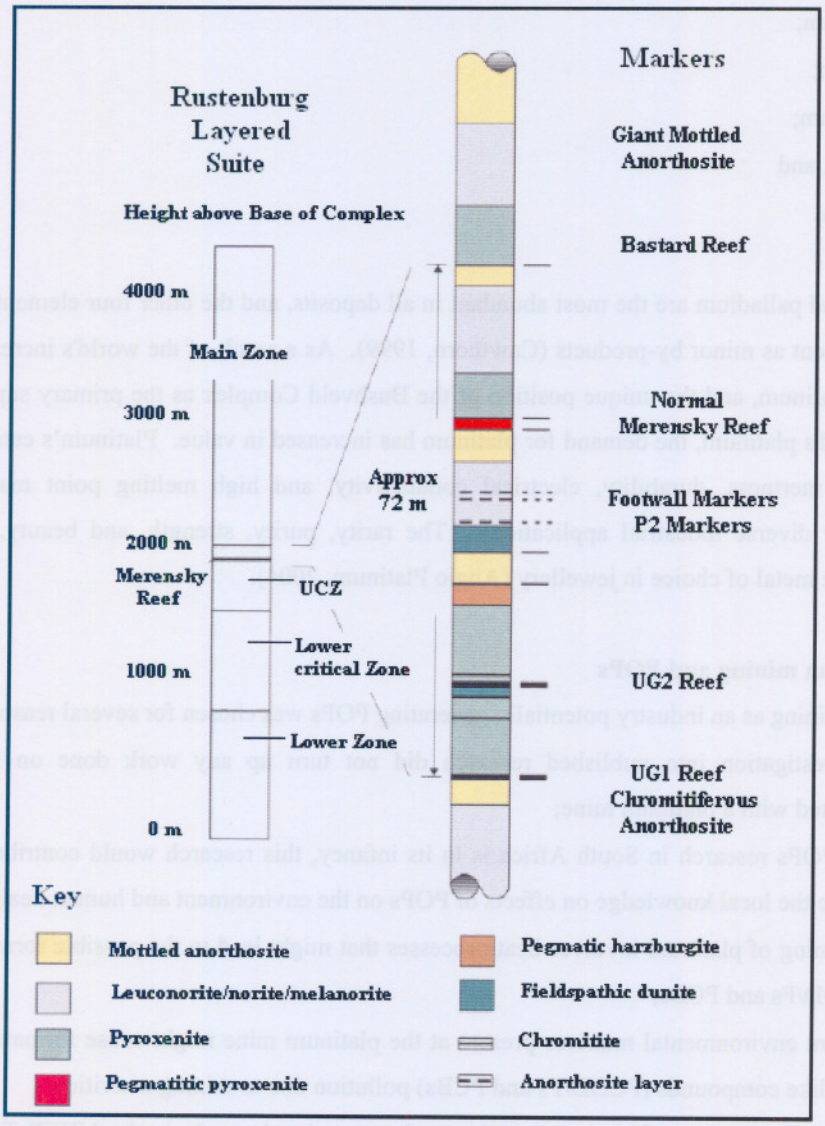


Figure 2.2: Simplified stratigraphy of the Rustenburg Layered Suite. The upper portion of the Upper critical zone (UCZ) contains several chromitite-rich layers including the Merensky and UG2 Reef (Smith *et al.*, 2003).

The name platinum originated from the Spanish word "platina" meaning silver and was first discovered by Antonio de Ulloa in 1735 in South America. Dr. Hans Merensky discovered

the largest deposits of platinum ever found in South Africa in 1924 and this led to the birth of the Merensky Reef (Platinum, 2005). Amplats mines, processes, refines and markets platinum group metals (PGMs) and produces gold, nickel, copper and cobalt (Anglo Platinum, 2004). The PGMs include six base metals (Cawthorn, 1999):

- platinum;
- palladium;
- rhodium;
- ruthenium;
- iridium; and
- osmium.

Platinum and palladium are the most abundant in all deposits, and the other four elements are always present as minor by-products (Cawthorn, 1999). As a result of the world's increasing need for platinum, and the unique position of the Bushveld Complex as the primary supplier of the world's platinum, the demand for platinum has increased in value. Platinum's catalytic properties, inertness, durability, electrical conductivity, and high melting point make it suitable for diverse industrial applications. The rarity, purity, strength, and beauty, also makes it the metal of choice in jewellery (Anglo Platinum, 2004).

2.2 Platinum mining and POPs

Platinum mining as an industry potentially generating POPs was chosen for several reasons:

- An investigation into published research did not turn up any work done on POPs associated with a platinum mine;
- Since POPs research in South Africa is in its infancy, this research would contribute to increase the local knowledge on effects of POPs on the environment and human health;
- The mining of platinum involves heat processes that might lead to the possible formation of PCDD/Fs and PCBs;
- Different environmental matrices present at the platinum mine might cause formation of dioxin-like compounds (PCDD/Fs and PCBs) pollution due to mining activities;
- No specific mention of platinum mining and processing is made in the UNEP Toolkit (UNEP, 2003). Since SA is the major global supplier, this information will assist SA in its inventory and NIP, as well as contribute towards the SC.

2.2.1 Platinum mining activities associated with Union Section

Union Section is situated 20 km due north of the Pilanesberg Alkaline Complex, and 18 km west of the town of Northam in the north-western sector of the Rustenburg Layered Suite,

Bushveld Complex (Smith *et al.,* 2003). Union Section's lease area covers about 5 708 hectares. The strike length exposed is 9.5 km and the average dip of the ore is 18° is towards the southeast (Du Plessis, 2005 pers. comm.). Mining operations commenced here in the early 1940s on the Merensky Reef, on the farm Swartklip and Turfbult (Northam, 2004).

To understand the potential formation and fate of POPs during platinum mining and processing, a good understanding of the whole cycle is required. This will aid in identifying the potential processes that could lead to POP formation, as well as sections during the process where they might accumulate, be released, or cause potential human exposure.

2.2.1.1 Ore extraction

The Merensky and UG2 reefs at the Swartklip Platinum mine (Union Section) are narrow, less than a meter thick. The working areas such as in the stopes are too cramped to permit the use of mechanised equipment. The miners use hand-held pneumatic drills to bore holes, which are then filled with explosives. The explosives used in the stopes are supplied by African Explosives Limited (AEL) and are ammonium nitrate fuel oil products packaged in plastic (composition unknown, possibly Polyvinyl chloride (PCV)) that are referred to as ANFEX® (AEL, 2005). To prevent the collapse of the mine roof, support is given by wooden beams (Jordaan, 2005 pers. comm.). After blasting, the ore is removed from the stope using scrapers attached to winches. The ore is then transported through a series of ore passes to the bottom of the shaft, and hauled to the surface.

Woodchips from the wooden beams are produced as a by-product during the ore extraction process due to the blasting activities. The result is that the ore and the woodchips are processed together during the extraction phase (Figure 2.3. The woodchips are removed from the ore by screening (sieving), prior to platinum extraction, but the small particles of wood not removed are milled together with the ore before initial concentration. Woodchip samples were collected from the UG2 and Merensky process at the Mortimer Plant of Union Section for this project.

Green arrows in Figure 2.3 indicate the processing stages where samples were collected from the UG2 process. At the Merensky process (Figure 2.4) woodchip samples were collected and green arrows indicate the point of collection in the process. As a result of the removal of woodchips from the ore, a dumpsite near Mortimer Plant was investigated. Two soil samples were collected because the small particles of woodchips are disposed of here (Figure 2.5).

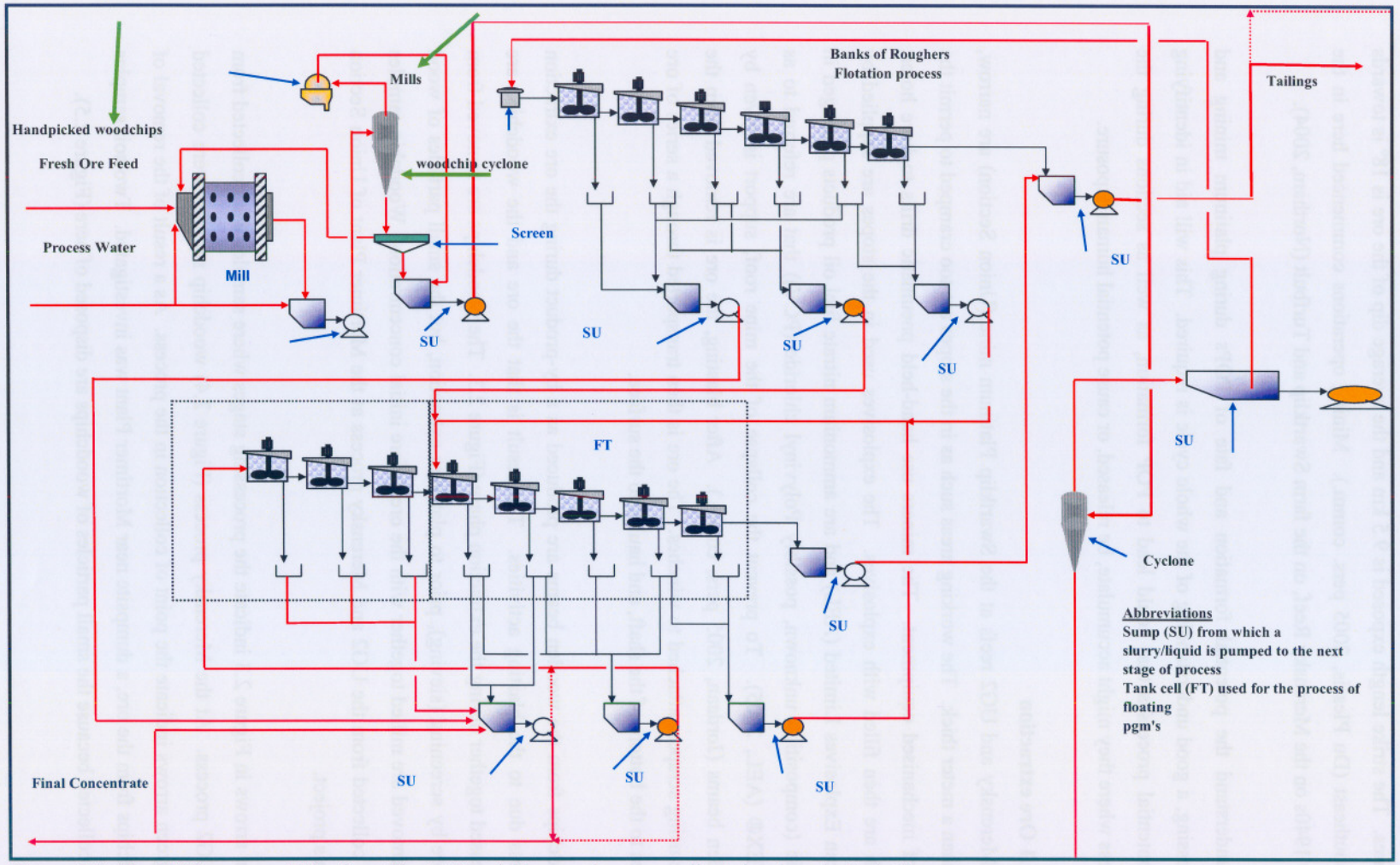


Figure 2.3: The UG2 process at Mortimer Plant. Woodchip samples were collected and are indicated by green arrows.

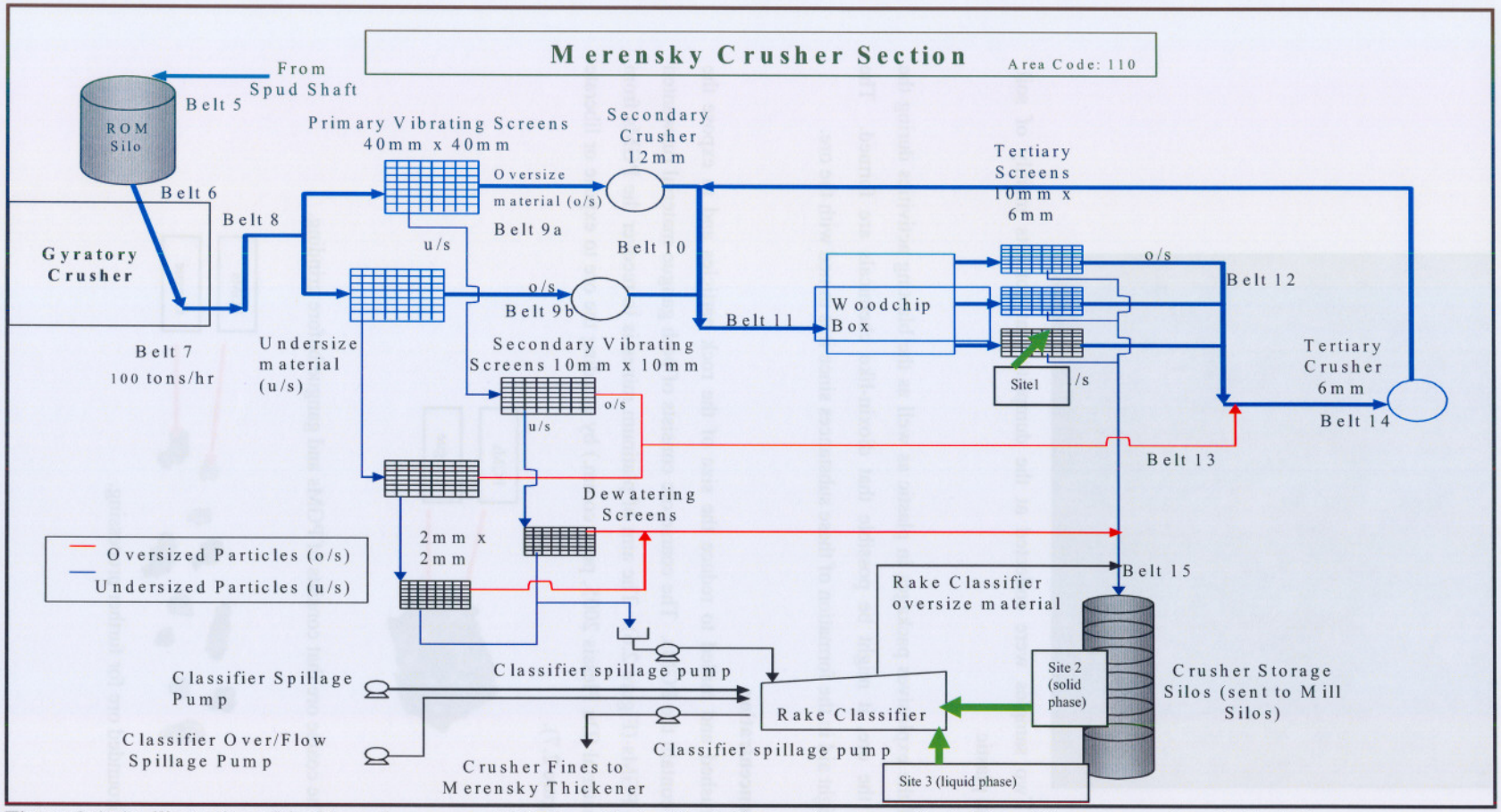


Figure 2.4: An illustration of the Merensky process at Mortimer Plant. Woodchip samples were collected from this process and are indicated by green arrows.



Figure 2.5: Two samples were collected at the dumpsite that consists mainly of soil, woodchips and plastic.

As a result of the explosives packages in plastic as well as the blasting activities during the extraction of the ore, it might be possible that dioxin-like chemicals are formed. The woodchips might aid in the formation of these substances since it is mined with the ore.

2.2.1.2 Ore concentration

The ore is crushed and milled to reduce the size of the rock particles and to expose the minerals that contain the PGMs. The coarse ore consists of both gangue material (unwanted material) and PGMs (Figure 2.6). The aim of platinum mines is to recover the PGMs from the gangue material (Du Plessis 2005, pers. comm.) by grinding the ore to expose or liberate the PGMs (Figure 2.7).

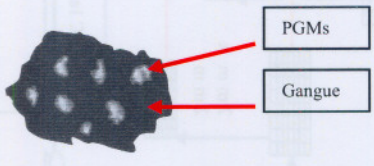


Figure 2.6: The coarse ore that consists of PGMs and gangue before grinding.

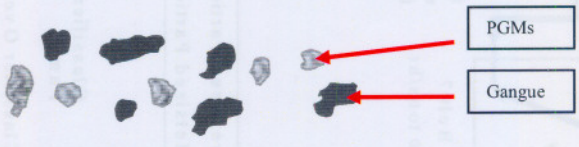


Figure 2.7: Grounded ore for further processing.

The ore is crushed and milled to assist the flotation process to separate and liberate the valuables. The pulp from these mills is pumped to the flotation cells. Surfactants are added to produce a froth containing the valuable minerals in the rougher flotation cells. The surfactants used by Mortimer Plant during the flotation process are summarised below in the Table 2.1.

Table 2.1: Surfactants used during the flotation process at Mortimer Plant (Du Plessis, 2005 pers. comm.).

Surfactants Product Name	Unit of delivery	Application at Mortimer Plant	Hazard category (*See key below)	Name of supplier
Sodium Isobutyl Xanthate (SIBX)	Bulk liquid	Collector	C/F/E/T	Senmin
Sascol 95	Bulk liquid	Promoter	C/F/E/T	Sasol-Minchem
Sascol 65	Bulk liquid	Promoter	C/E/T	Senmin
Gempolym M47	50 kg bag/ powder	Depressant	E/T	Gempolym
Gempolym KU9	50 kg bag/ powder	Depressant	E/T	Gempolym
Copper sulphate ($CuSO_4$)	25 kg bag/ powder	Activator	F/E/T	Kimleigh Chemicals
Magna floc 156	25 kg bag/ powder	Thickening		*
Bulab 5031 coagulant	240 kg drum liquid	Thickening		*
Aero +3302 cyanamid	200 kg drum liquid	Promoter	F/E/T	CYTEC
XP 200	200 kg drum liquid	Frother	T = mild irritant	Pelichem

Key to hazard classification:
C = Corrosive
E = Environmental pollution
F = Flammable and/or Explosive
T = Toxic to humans
*: Unknown

The surfactants used during the flotation process can increase the solubility of hydrophobic organic compounds (HOCs) by partitioning it into the hydrophobic cores of the micelles (Cho, Choi, Goltz & Park, 2002). A micelle is an aggregate of amphiphilic molecules (often fatty acids) whose polar, hydrophilic ends are in contact with water that forms the outer surface of a sphere, and the inside of the sphere that consists of non-polar, hydrophobic molecular ends (Anon, 2005h). During the processes of flotation, the air is blown into the pulp through the agitator in the floatation cells (Figure 2.8). This aids to disperse the air throughout the pulp. As the air bubbles rise to the surface, they collect the PGMs and overflow into the concentrate launder.

Surfactants are very important in the flotation process, as they cause the valuable PGMs to become hydrophobic, to attach themselves to an air bubble and to float. The surfactants added during the flotation process aids to make the gangue become hydrophilic which reports to the underflow/tails (Du Plessis, 2005 pers. comm.). By not adding surfactants, separation of PGMs from gangue will not take place, no matter how much air is pumped into the pulp.

Different types of surfactants with a variety of functions are added during the flotation process that has various functions (Du Plessis, 2005 pers. comm.):

1. A collector such as SIBX binds itself onto the PGMs and then to an air bubble as it rises through the pulp. The reaction of the collector used at Union Section is illustrated in Figure 2.9.

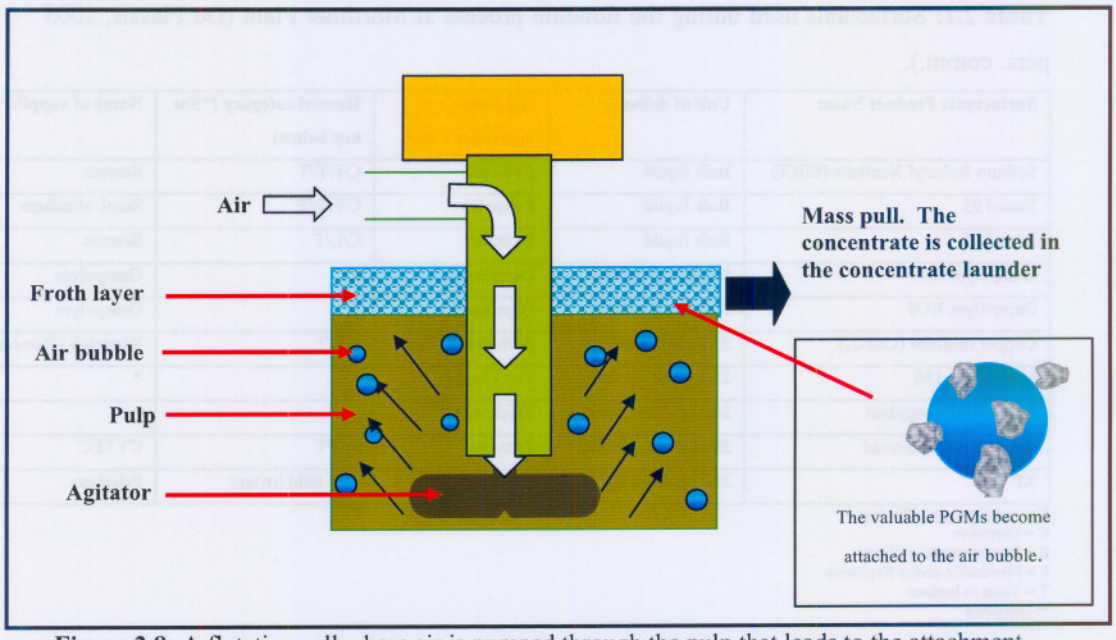


Figure 2.8: A flotation cell where air is pumped through the pulp that leads to the attachment of the PGMs to the air bubble.

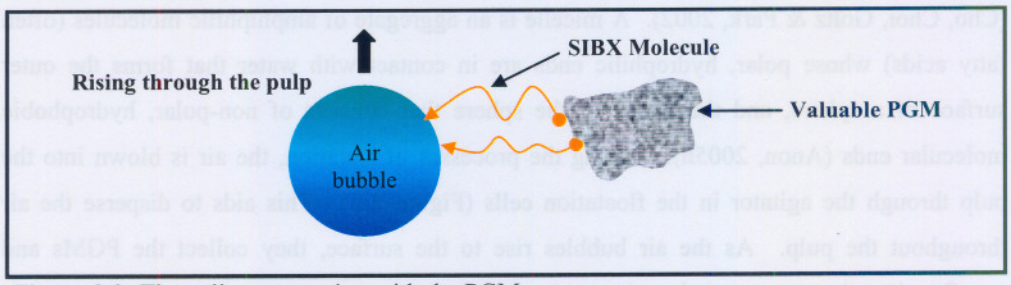


Figure 2.9: The collector reaction with the PGMs.

2. The activator used during the flotation process at Union Section is $CuSO_4$. The $CuSO_4$ reacts with the PGM surface to form an insoluble compound that makes the molecule more hydrophobic. Its function is to create a more stable surface onto which the collector can bind. Another function of the activator is to create a strong bond between the collector and PGM surface to withstand the drag forces as the bubble rises to the surface.

3. The promoter (Sascol 95 and 65) creates a surface area on the PGM molecule that is more attractive to collector molecules that enhance the packing density.
4. A frother adsorbs onto the bubble surface, decreasing the surface tension and stabilising the bubbles. This signifies that the bubbles are strong enough to withstand drag forces and do not burst. The frother used during the flotation process is XP200.
5. The depressants used by Union Section are M47 and KU9. The function of the depressant is to prevent the gangue from floating. It is added to depress the unwanted particles by making them hydrophilic and not attractive to collector molecules.
6. Flocculants (Magna floc 156) are designed to bring together solids in order to produce a bundle of solids with a high density that will settle faster than individual solid particles.
7. pH modifiers are used to regulate the pH of the pulp so that the optimum conditions for collection, activation or depression are achieved.

The metal content is increased in the froth produced during the flotation process. The froth from the cells is then pumped to the thickeners for setting of solids. The gangue (waste materials) formed during the flotation process are transported to the tailings dams. The tailings are formed as a waste stream during the platinum mineral processing that is a biologically-sterile medium with limited water holding capacity and high base saturation percentage (Du Plessis, 2005 pers. comm.). The tailings consist mainly of sand (75%) and silt (20%). The remaining 5% of the tailings are represented as clay fraction and organic fraction. Tailings dams pose significant environmental dangers that include air, dust and groundwater pollution (Maboeta & Van Rensburg, 2003) due to containing high concentrations of manganese, iron and sulphur (Du Plessis, 2005 pers. comm.). Woodchips are a major source of organic carbon and nitrogen that would be ideal forms of essential nutrients and organic matter to be used as a revegetating medium of tailings (Maboeta & Van Rensburg, 2003).

Since woodchips is a major source of organic carbon, the woodchips could contribute to the formation of dioxin-like chemicals. The different surfactants used during the flotation process could also aid in the formation of dioxin-like chemicals, because some of these surfactants contain chlorine (not applicable to Anglo Platinum, Union Section).

2.2.1.3 Smelting

The wet flotation concentrates are bought by the Smelter at Union Section (Figure 2.10) for further processing. During the smelting process, the flotation concentrates are treated. The moisture content of the concentrate received ranges between 18 and 25% (Du Plessis, 2005 pers. comm.). An overhead crane and grab are used to load the wet concentrate into the

double screw feed bin. The double screw discharges the concentrate onto the Flash Drier feed belt, which is in turn discharged into the paddle mixer/back mixer.

The concentrate discharges through a steeply inclined 'drop box' system into an accelerator/disintegrator, which in turn distributes the product evenly. The disintegrator further breaks the concentrate up, feeding it into the hot air-stream from the Hot Gas Generator (HGG). The HGG supplies the Flash Drier with hot gas at a temperature of ±500°C for drying of the concentrate. Ducting conveys the material into a dust-air separation system where the final drying takes place. The separation system consists of twin primary cyclones in parallel from where the hot gas and remaining entrained dust is ducted to a multi-clone. The dried concentrate is transported to the bag-house. The exhaust air from the bag-house is at a temperature of 100°C and is discharged through the Flash Drier stack. The dried concentrate from the separation system flows from the bag-house to the product bin. This 'bone-dry' concentrate is batched together with lime (5% by mass) in the feedstock hoppers. The material is dropped into the pneumatic transfer vessels and conveyed to the furnace feed bins. The feed is delivered via the side of the furnace and the quantity is controlled using a programmable logic controller (PLC) in the furnace control room.

The concentrate is melted by an electric arc furnace (ELKEM 19 500 Mega Volt Amps (MVA)) electric arc furnace (Du Plessis, 2005 pers. comm.). This furnace is rectangular in shape and has six in-line Söderberg electrodes positioned in two distinct rows that generate electrical currents to generate the required temperatures. The function of the Soderberg electrode is to pass an electric current into molten bath via the electrodes (Anon, 2005i). The furnace operates at different temperatures depending on the sequence in the smelting process and is operated 24 hr/day for 365 days/year (Du Plessis, 2005 pers. comm.). The 'bone-dry' concentrate mixed with the limestone is melted, and forms two distinct layers within the furnace. The bottom layer is composed of the heavier *matte,* mainly copper, nickel and iron sulphides. The *matte* contains more than 99% of the valuable metals. The *matte* is tapped at 1200-1600°C and the slag around 1400°C (Du Plessis, 2005 pers. comm.). The upper layer comprises the slag (by-product formed during smelting), which consists of oxides of silica, magnesia, iron and other impurities. The slag assists in the smelting temperature control and acts as waste removal mechanism during metal smelting (Du Plessis, 2005 pers. comm.). The Smelter treats approximately 500 metric tons of material per day. Of this, roughly 20% is *matte* and the remaining 80% is slag. The annual average concentrated *matte* produced is 1500 metric ton, and 13500 metric ton slag. A slag sample was collected from the Smelter, indicated by a green arrow in the schematic representation of Figure 2.10.

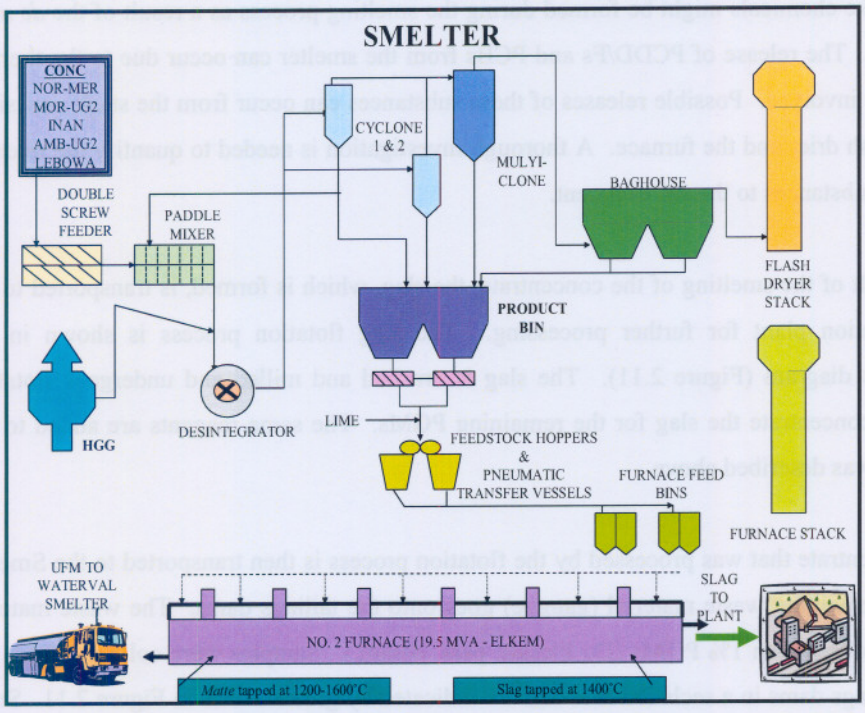


Figure 2.10: A schematic diagram of the Smelter process. A slag sample was collected at the point where the green arrow is pointing.

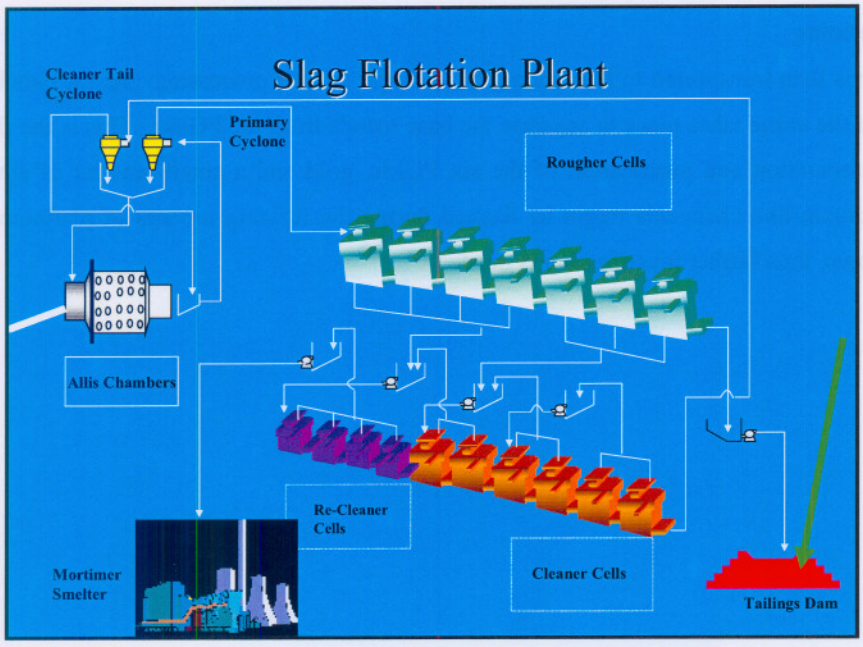


Figure 2.11: Graphic representation of the slag flotation process. The froth produced during this process is transported to the smelter for further processing. Samples were collected from the tailings dams and are indicated by a green arrow.

Dioxin-like chemicals might be formed during the smelting process as a result of the *de novo* synthesis. The release of PCDD/Fs and PCBs from the smelter can occur due to the thermal processes involved. Possible releases of these substances can occur from the stack emissions of the flash drier and the furnace. A thorough investigation is needed to quantify the releases of these substances to the environment.

As a result of the smelting of the concentrate, the slag, which is formed, is transported to the slag flotation plant for further processing. The slag flotation process is shown in the schematic diagram (Figure 2.11). The slag is crushed and milled and undergoes flotation again to concentrate the slag for the remaining PGMs. The same reagents are added to the slag that was described above.

The concentrate that was processed by the flotation process is then transported to the Smelter and the remaining waste material (gangue) goes onto the tailings dams. The waste material consists of less than 1% PGMs (Du Plessis, pers. comm.). Samples were collected from the three tailings dams in a secluded area and are indicated by green arrows in Figure 2.11. Strict regulations were followed in terms of not disturbing the natural habitats and the animals in this area.

2.2.1.4 Refining

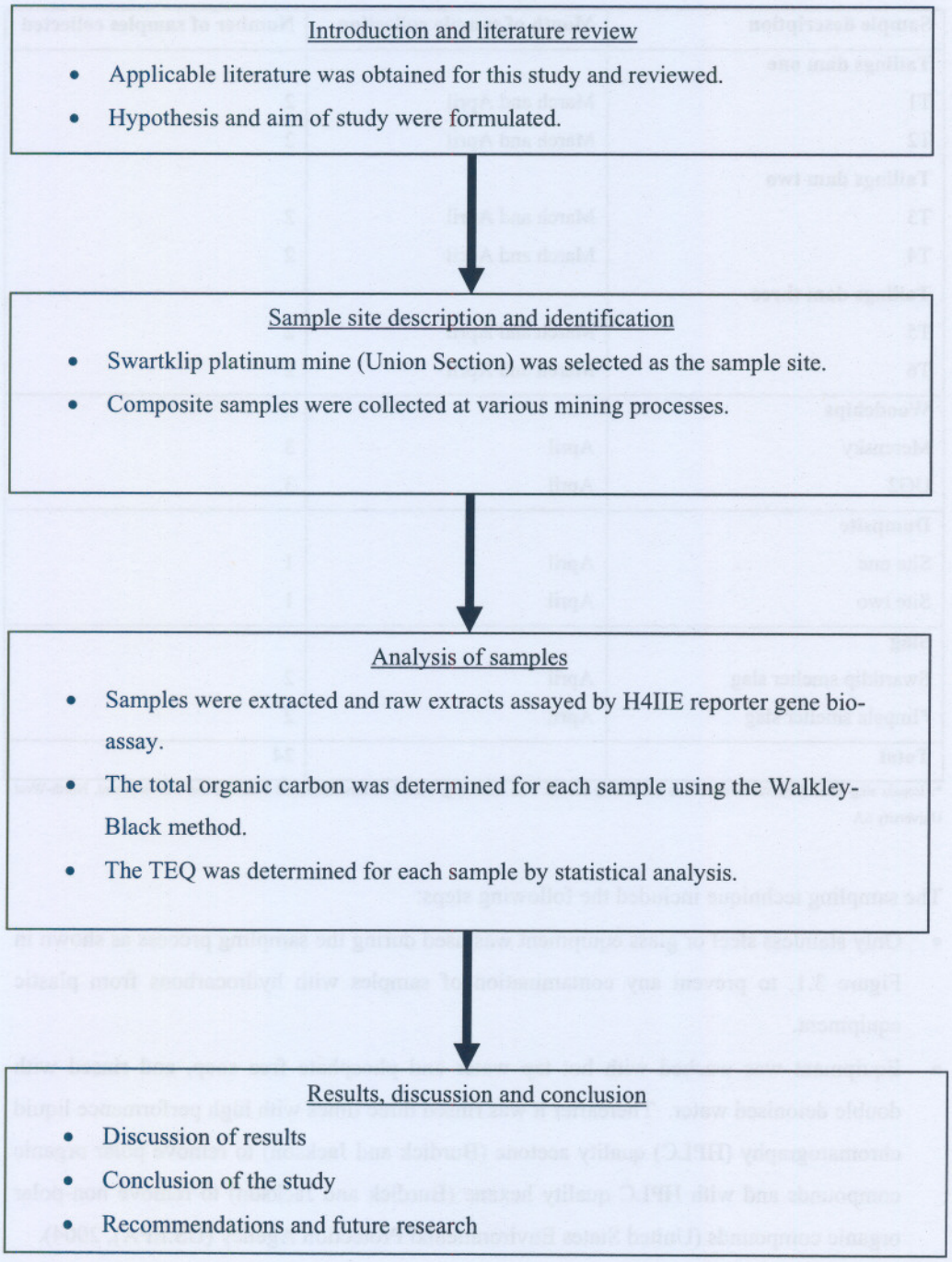
The *matte* is then transported to Rustenburg Smelter for further processing. At the Smelter, refining of the *matte* takes place to separate the base metals from the PGMs. This is the final stage for separation and purification of the six PGMs, gold and a small amount of silver. Whether dioxin-like chemicals might be formed during the refining process of platinum, is still unknown, thus further investigation is needed.

Chapter 3

METHODOLOGY

3.1 Study design

Based on the information and objectives of the study presented in Chapters 1 and 2, the study was designed as follows:



3.2 Site sampling and methods

Environmental and process samples (Table 3.1) were collected from Swartklip Platinum mine (Union Section) (see Chapter 2) by obtaining the necessary permission to enter the mining area and to take photographs of the sites.

Table 3.1: Samples collected from the platinum mine during March and April (2004).

Sample description	Month of sample collection	Number of samples collected
Tailings dam one		
T1	March and April	2
T2	March and April	2
Tailings dam two		
T3	March and April	2
T4	March and April	2
Tailings dam three		
T5	March and April	2
T6	March and April	2
Woodchips		
Merensky	April	3
UG2	April	3
Dumpsite		
Site one	April	1
Site two	April	1
Slag		
Swartklip smelter slag	April	2
*Impala smelter slag	April	2
Total		**24**

*: Impala slag from Waterval Smelter was donated by Prof L. Van Rensburg, School of Environmental Sciences and Development, North-West University SA

The sampling technique included the following steps:

- Only stainless steel or glass equipment was used during the sampling process as shown in Figure 3.1, to prevent any contamination of samples with hydrocarbons from plastic equipment.
- Equipment was washed with hot tap water and phosphate free soap, and rinsed with double deionised water. Thereafter it was rinsed three times with high performance liquid chromatography (HPLC) quality acetone (Burdick and Jackson) to remove polar organic compounds and with HPLC quality hexane (Burdick and Jackson) to remove non-polar organic compounds (United States Environmental Protection Agency (US.EPA), 2004).

- At a specific site, environmental and process samples were collected from several separate locations and a composite sample was made by thoroughly mixing together equal quantities from the different locations. Foreign objects such as sticks, leaves and rocks were removed (US. EPA, 1996). Composite samples were placed in the glass bottles and covered with brown paper bags to prevent microbial degradation, volatilisation and to prevent degradation of sample by means of heat (Schumacher, 2002). The same cleaning protocol as for the equipment was followed for the glass bottles. Glassware can contribute to contamination of the sample if not properly cleaned and the removal of analytes by adsorption on the glass surface could take place (US.EPA, 1994). The samples were transported at 4°C (US. EPA, 1994).

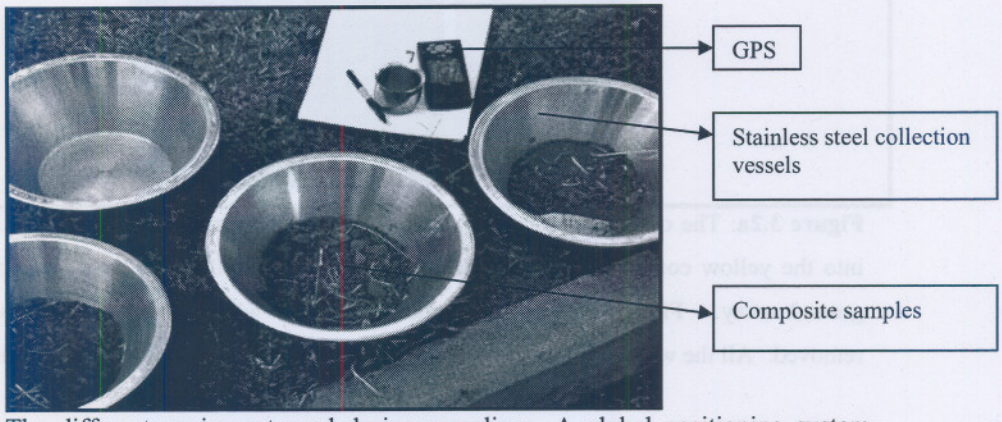


Figure 3.1: The different equipment used during sampling. A global positioning system (GPS) was used to determine the sample site co-ordinates. Composite samples were made in stainless steel collection vessels.

At various localities on the Mortimer Plant, samples in the form of woodchips were collected. Mortimer Plant consists of the UG2 and Merensky process as well as the slag flotation process. At the UG2 process, the first woodchip sample (Figure 3.2a) was collected from the conveyer belts before the coarse ore passes through the crushers and screens to produce finer ore. The second woodchip sample was collected from the mill's discharge (Figure 3.2b) and the third sample was collected at the woodchip screen (cyclone) as shown in Figure 3.2c. See Chapter 2 for Mortimer UG2 process and sample sites indicated by the green arrows.

At the Merensky process, samples were collected from the woodchip box, which consists of tertiary vibrating screens as shown in Figure 3.3. Two woodchip samples were also collected from the rake classifier (Figure 3.4a and b). See Chapter 2 for the Merensky process and sample sites indicated by the green arrows.



Figure 3.2a: The oversized woodchips that are removed from the conveyer belts and placed into the yellow containers. **Figure 3.2b**: The discharge mill where smaller woodchips are ground away. **Figure 3.2c**: The woodchip cyclone where small sized woodchips are removed. All the woodchip samples were collected from the UG2 process at Mortimer Plant.

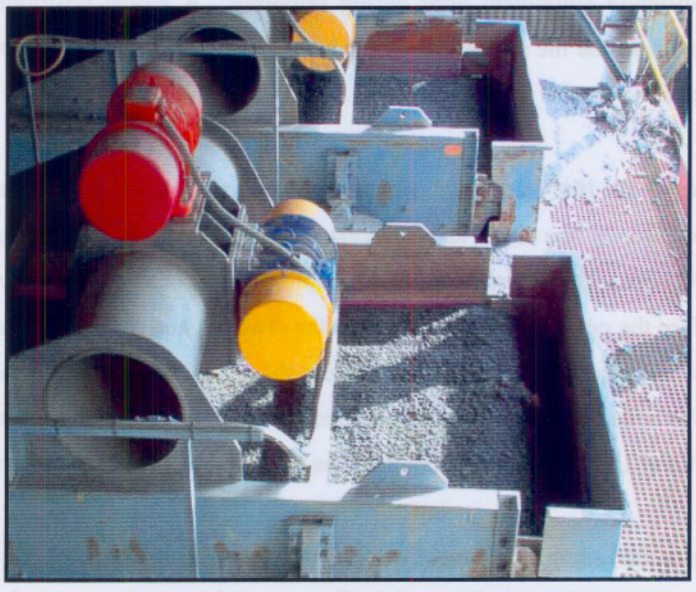


Figure 3.3: Woodchip box with tertiary vibrating screens during the Merensky process at Mortimer Plant. Visible particles of wood were collected from the screens.



Figure 3.4a: Samples were collected from the discharge end (solid phase) during the Merensky process. **Figure 3.4b**: The liquid phase of the rake classifier where samples were collected (Merensky process).



Figure 3.5: A slag sample was collected from the Smelter. The slag is fed to the slag plant mill for further processing. The photo illustrates a slag spillage at the Smelter.

During the mining process, the visible, smaller-sized woodchips are taken from the cyclone and vibrating screens, and transported to the waste dumps where they are set on fire or buried. The larger portions of the woodchips from the conveyer belts are collected by an outside contracting company and disposed of. Two composite samples were collected at the dumpsite. After the concentrate from the Mortimer Plant is transported to the Smelter, it is melted to form the *matte* and slag. A slag sample (Figure 3.5) was collected from the Smelter plant's electrical arc furnace before the slag was transported to the slag flotation plant for further processing. See Chapter 2 for the smelter plant process and sample sites indicated by green arrows.

Samples were collected from three secluded tailings dams at Union Section for analysis. Tailings dam one was the latest tailings area. Deposits on the tailings dam started in February

2002. When samples were collected in 2004 (site T1 and T2), the tailings dam stood about 6 meters high as indicated in Figure 3.6.



Figure 3.6: The latest tailings dam at Union Section that was established in 2002.



Figure 3.7: The older tailings dam that was established in 1973 is currently being rehabilitated.



Figure 3.8: The oldest tailings dam currently being re-mined. This tailings dam was commissioned in 1945. Extensive rehabilitation can be seen on the oldest tailings dam.

Tailings dam two (Figure 3.7) was established after Mortimer Plant was commissioned in 1973 (Du Plessis, 2005 pers. comm.). Sites T3 and T4 were on tailings dam two that was older than tailings dam one. This tailings dam is currently used for cementation for mining activities. By using the cement, underground tunnels are made safer by preventing the collapse of roofs (Du Plessis, 2005 pers. comm.). Tailings dam three was the oldest tailings dam (Figure 3.8) and was commissioned in 1945 after the Ivan Concentrator was established. Ivan Concentrator forms part of the different plants associated with Union section. Sites T5 and T6 were on the third tailings dam. Re-mining of this tailings dam started in 2002/3 and was still underway in 2004. Union section is ISO (International Standard Organisation) 14001 certified (Du Plessis, 2005 pers. comm.).

3.3. Treatment of samples

3.3.1 Extraction and clean-up of samples

The samples collected from the platinum mine during March and April 2004 were extracted prior to the H4IIE reporter gene bio-assay (Table 3.1). The extraction process consisted of several phases:

- Freeze-drying. Prior to extraction, each sample was freeze dried (Schwirzer *et al.,* 1998; Besselink, Schipper, Klamer, Leonards, Verhaar, Felzel, Murk, Thain, Hosoe, Schoeters, Legler & Brouwer, 2004) for approximately 48 hours. Conventional drying by oven heating could not be done because dioxins and furans in the sample may degrade. Freeze drying is also necessary to be able to accurately determine the weight of the samples before extraction.
- Soxhlet extraction. After freeze drying, the samples were sieved using a 0.5 mm sieve. Twenty gram of sample was mixed with equal quantities of anhydrous sodium sulphate (Merck; UnivAR®) to remove all traces of water. The equipment was cleaned as described in paragraph 3.2 with HPLC quality chemicals (US.EPA, 2004). The entire mixture was placed inside a Soxhlet extractor on top of cleaned glass wool (extracted with dichloromethane:hexane 3/1) to prevent the samples from siphoning into the hexane (Figure 3.9). Hexane was used as extraction solvent. Samples were extracted for 16 hours (Besselink *et al.,* 2004) with hexane that circulated at a rate of three percolations/hour.
- Concentration step 1: The sample was concentrated to the barest minimum volume using a rotary evaporator. It was transferred into clean test tubes and the volume of the extract adjusted to 10 ml with hexane. If any particles were visible in the extract, it was funnelled through anhydrous sodium sulphate and flushed three times with hexane (Besselink *et al.,* 2004). The eluted extract would then be re-concentrated as before.

Caution was taken not to accidentally evaporate the entire sample in which case the extraction procedure with new sample was repeated.

- Clean-up of the samples included the removal of all polar or non-polar interferences, such as PAHs, by using acid as the clean-up reagent (US. EPA, 2004). Ten ml of hexane-extract was carefully mixed with 10 ml concentrated sulphuric acid (Merck; 95-99%) and left for a minimum of one hour for the phases to separate properly. The acid phase was discarded. This step was repeated until the acid phase was clear. To remove any acid traces, the sample was washed with water only once. The water phase was discarded after one hour. Water traces from the sample were removed by funnelling the hexane through glass wool topped with a small amount of anhydrous sodium sulphate.
- Concentration step 2: The concentrated cleaned sample extract was further concentrated to near dryness (0.5 ml) under a gentle stream of nitrogen. The needles through which the gas was blown over the surface of the hexane were rinsed three times with acetone and hexane before being lowered into the test tubes. The test tubes were kept in a water bath at room temperature to prevent a drop in solvent temperature because of the evaporation process. This also prevents the condensation of H_20 into the extract (US. EPA, 1996).

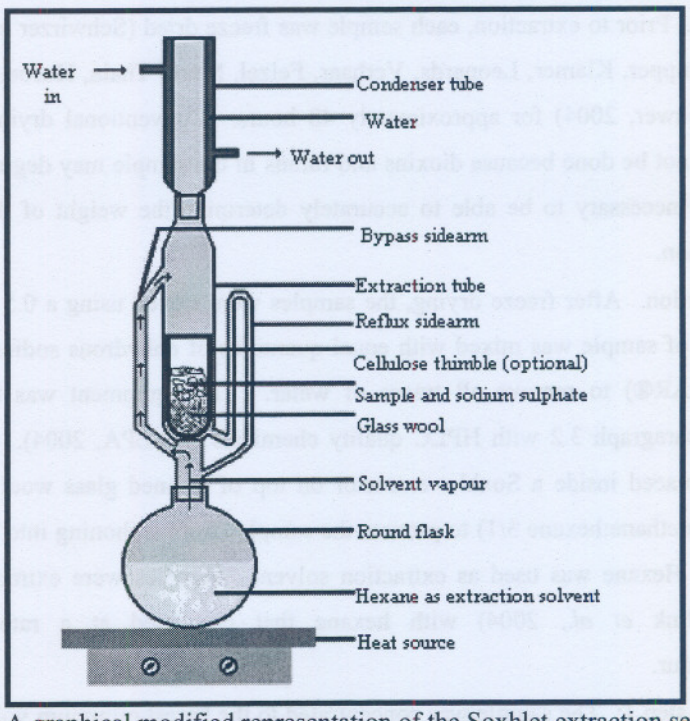


Figure 3.9: A graphical modified representation of the Soxhlet extraction setup (adapted from Anon, 2005j). Hexane was used as solvent during extraction. The sample was placed on top of glass wool only, and not inside thimbles as is indicated in the figure. Thimbles could be used for very finely ground samples to prevent the sample from dropping into the round bottom flask.

3.3.2 Storage of samples

The 0.5 ml concentrated sample's volume was adjusted to 1 ml with the addition of hexane and transferred to a 2 ml amber gas chromatograph (GC) vial. The GC vial caps were covered with Para-film® to prevent undue evaporation of solvent and were stored at 4°C (US.EPA, 1994).

3.3.3 Determining the total organic carbon (TOC)

Three basic forms of carbon (C) are present in soil and sediment: elemental C, inorganic C and organic C. Determining the TOC forms an essential part of site characterisation (Schumacher, 2002). The percentage TOC (%TOC) present in the samples was determined using the Walkley-Black method (Walkley & Black, 1934). The samples were oxidised with potassium dichromate ($K_2Cr_2O_7$) and sulphuric acid (H_2S0_4) as given in equation 1 and the unreacted Cr^{2+} was titrated against ferrous (II) ammonium sulphate [$Fe(NH_4)_2(SO_4)_2$] (Azad, Ishikawa, Chowdhury, Ansari & Faruk-E-Azam, 2003) shown in equation 2.

Equation (1) $2Cr_2O_7^{2-} + 3C + 16H^+ \leftrightarrow 4Cr^{3+} + 3CO_2 + 8H_2O$

Equation (2) $6Fe^{2+} + Cr^{6+}{}_2O_7 + 14H^+ \leftrightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$

The following assumptions are made when using this method (Schumacher, 2002; Organic carbon by wet oxidation, 2005):

- The reduction of dichromate is equivalent to the organic carbon present in the samples;
- The recovery of organic carbon range from 60-80%, with a mean recovery being 76%, that makes the results approximate but adequate for the purpose of this investigation.

Finely ground samples of 0.5-1 g (10 g of sample may be used depending on organic carbon content) were transferred into 500 ml Erlenmeyer flasks. 20 ml 0.167 mol/dm^3 $K_2Cr_2O_7$ supplied by Merck was added. 20 ml concentrated H_2S0_4 (Merck; 95-99%) was added to the solution under a ventilation box and swirled thoroughly for one minute. An exothermic reaction occurred in the Erlenmeyer flask and was left to cool for half and hour (Schumacher, 2002). After the solution was cooled, 150 ml distilled water was added to the solutions to halt the reaction. 10 ml chemically pure orthophosphoric acid (Merck; UniLAB®) was added to help eliminate interferences from the ferric (Fe^{3+}) iron that might be present in the sample. 1 ml barium diphenylaminesulphonate indicator (BDH) was added to the solution prior to the titration. This indicator was treated with care because it is carcinogenic (Schumacher, 2002). The solution was titrated with 0.5 mol/dm^3 $Fe(NH_4)_2(SO_4)_2$ supplied by Merck, until the solution turned purple/blue to green. A reagent blank was also made to standardise the Fe^{2+} solution.

The %TOC was determined and calculated using the following equation:

$$\%TOC = \frac{(B-S) \times M \times 0.3 \times F}{\text{Mass of sample}}$$

B = volume of Fe^{2+} solution used to titrate blank

S = volume of Fe^{2+} solution used to titrate sample

M = Concentration $Fe(NH_4)_2(SO_4)_2$ in mol/ℓ

F = Empirical correction factor of 1.4 was used to compensate for amount of organic carbon that is recovered, see second bullet on previous page (Schumacher 2002).

3.4 H4IIE reporter gene bioassay

3.4.1 Cell culture

The H4IIE *luc* cells were received as a gift from J. Giesy (Michigan State University 2003) and were used to determine the TEQ of each sample. These are rat hepatoma cells, stably transfected with a luciferase reporter gene under control of DRE (Nie *et al.,* 2001). The H4IIE *luc* cells were grown in sterile conditions (pH 7.4) in a humidified carbon dioxide (CO_2) incubator at 37°C, 5/95%CO_2/air (Sanderson *et al.,* 1996; Nie *et al.,* 2001; Masunaga, Morinaka, & Nakanishi, 2002). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma Aldrich) with L-glutamate (1000 mg/ℓ glucose) without phenol red and sodium bicarbonate. The sodium bicarbonate (Sigma Aldrich) was added when the media was prepared from powder form. DMEM without phenol red was used because phenol red might act as a ligand to AhR thus creating a false response that absorbs some of the light emitted during the luciferase assay.

The DMEM was supplemented with 10% heat inactivated foetal bovine serum (FBS) (Gibco). The cells were cultured in tissue culture dishes (Greiner Bio-One, 100, 0/20 mm, vented, sterile) with a working volume of 12 ml. Trypsin (0.25%Trypsin/0.1%Versene, calcium (Ca^{2+}) and magnesium (Mg^{2+}) in free phosphate buffered saline (PBS), EDTA (Ethylene-diamine-tetra acetic acid)) was used during cell passage.

3.4.2 Bio-assay

The H4IIE *luc* cell suspension was made up in DMEM supplemented with FBS stripped from its hormones. The cells for the bio-assay were plated into the 60 interior wells of 96-well culture plates (250µl per well) at a density of approximately 50 000 cells per well (adapted from Koh, Khim, Villeneuve, Kannan, Johnson, & Giesy, 2005). The outer wells were seeded with PBS (sans Ca^{2+} and Mg^{2+}) to create an even humidity around the wells. A typical plate layout is represented in Figure 3.10. The cells were incubated overnight prior to dosing. Test and control wells were dosed with 2.5 µl of the appropriate extract or solvent (hexane)

and the blank wells received no dose (Koh *et al.,* 2005). Three replicate wells were analysed for each sample dilution.

Abbreviations: BL = Blank; S = Solvent Control; DS = Dilution series of samples; PBS, T = 2,3,7,8-TCDD dilution series, Re = Raw extract

Plate: A

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	DS1:4				DS1:3			DS1:2			
B		T1:0.048 µl	T1	T1	BL	Re 1:2.5 µl	Re 1	Re 1	Re 2:2.5 µl	Re 2	Re 2	
C		T2:0.012 µl	T2	T2	BL	DS1 :0.833 µl	DS1	DS1	DS1 :1.25 µl	DS1	DS1	
D		T3:0.003 µl	T3	T3	BL	DS2:0.2778 µl	DS2	DS2	DS2:0.625 µl	DS2	DS2	
E		T4:0.0075 µl	T4	T4	S	DS3:0.0925 µl	DS3	DS3	DS3:0.3125 µl	DS3	DS3	
F		T5:0.0001875 µl	T5	T5	S	DS4:0.0308 µl	DS4	DS4	DS4:0.156 µl	DS4	DS4	
G		T6:0.0000468 µl	T6	T6	S	DS5:0.0102 µl	DS5	DS5	DS5:0.078 µl	DS5	DS5	
H												

Figure 3.10: A typical plate layout.

For dose-response characterisation, a dilution series consisting of six concentrations of samples were prepared by three-fold or two-fold serial dilutions that were tested (three replicate wells per concentration) on the cells. The luciferase assay was conducted after 72 h of exposure to extracts (Koh *et al.,* 2005). Cells were inspected visually for viability and differences in confluency, and cytotoxicity were noted. The plates were washed three times with PBS (with Ca^{2+} and Mg^{2+}) using an eight-channel vacuum manifold. 100 µl of substrate for the luciferase enzyme (Luclite® supplied by Perkin-Elmer) and PBS (with Ca^{2+} and Mg^{2+}) were added to the wells and incubated for 10 minutes. The RLU were registered with a luminometer (Biotek Flx800 Microplate fluorescence reader). After the bio-assay was completed, sample responses were converted to relative light units expressed as a percentage of the maximum response observed for 2,3,7,8-TCDD (Ultra Scientific) (Koh *et al.,* 2005). This was done to normalise responses for day-to-day variation in response magnitude, and enabling the comparability of the results obtained to other *in vitro* bio-assay results reported in the literature (Khim, Villeneuve, Kannan, Lee, Snyder, Koh, & Giesy, 1999; Koh *et al.,* 2005). The method used in determining the TEQ of each sample will be discussed in the next section.

3.4.2.1 An outline and example of how data was analysed

The following is an example how the data sets were analysed to determine TEQs for each sample collected at the Swartklip Platinum mine (Union Section). Plate A (Figure 3.11) is a raw data set that includes RLUs for TCDD, and two samples (T2 and T3) as well as solvent

control (S), blank control (BL), and PBS. Complete dose-response curves were obtained for the standard and the samples. The standard that was used was TCDD. The pgTCDD/well ranged from 120-0.12. The dilution series of the TCDD was 1:4 (Figure 3.10). Samples were diluted either 1:2 or 1:3 depending on initial results (Figure 3.10). The samples were analysed at sensitivities that ranged from 160-200. The sensitivity is a setting of the plate reader.

Plate A

PBS X	TCDD	TCDD	TCDD		T2	T2	T2	T3	T3	T3	PBS
X	X	X	X	X	X	X	X	X	X	X	X
X	345.79	385.38	328.7	S	401.2	228.9	178.3	81.5	56.29	56.08	X
X	276.45	274.38	304.8	S	141.3	125.6	81.79	154.8	52.82	58.86	X
X	230.31	233.6	281.5	S	88.59	67.23	70.57	45.34	47.14	60.27	X
X	247.91	161.93	169.3	BL	37.01	57.9	54.3	44.53	43.14	64.46	X
X	81.041	129.09	97.36	BL	39.8	36.78	98.75	35.16	66.03	75.65	X
X	31.636	94.994	33.13	BL	47.68	37.13	32.7	63.13	42.52	49.65	X
X	X	X	X	X	X	X	X	X	X	X	X

Figure 3.11: A raw data set that includes TCDD, PBS, S, BL and two samples.

The 1:2 dilution series ranged from 2.5- 0.078125 µℓ sample/well, and the ranges for the 1:3 dilution series were from 2.5-0.01 µℓ sample/well. If the solvent control were to be subtracted from the RLU of the samples, the response would have become negative and of no use. For this reason the solvent control was not subtracted from the sample and the standard. The standard deviation (SD) and the coefficient of variance (CV) were also calculated for the samples and TCDD. If the CV was greater than 20%, one of the RLUs was left out of the calculations in order to bring the CV lower than 20% (Besselink *et al.,* 2004). This makes the CV an indicator of effective data generation. The percentage %TCDD max was calculated to express relative potency for both standard and samples. The %TCDD max values were used in the dose-response curves of the samples and the standard (illustrated in Figure 3.12 and 3.13).

The effective concentrations (EC) of the TCDD and sample eliciting a 20%, 50% and 80% response from the cells, were calculated on the basis of the amount of sample needed to produce a response equivalent to 2,3,7,8-TCDD (Hilscherova, Kannan, Nakata, Hanari, Yamashita, Bradley, Mccabe, Taylor & Giesy, 2003). The EC was determined from the straight line section of the dose-response curve by calculating the slope and the intercept of the straight line using suitable functions of the Excel computer program. Because the TEQ values are ratios between the EC of the TCDD and the EC of the sample, and the fact that the slopes of the straight lines differ, different values are calculated for the $TEQ_{20\text{-}80}$. This explains why in some instances there is an increase in the $TEQ_{20\text{-}80}$ and in others a decrease.

If the slope of the sample straight line and the slope of the standard were to be the same, all three TEQ_{20-80} values would have been identical.

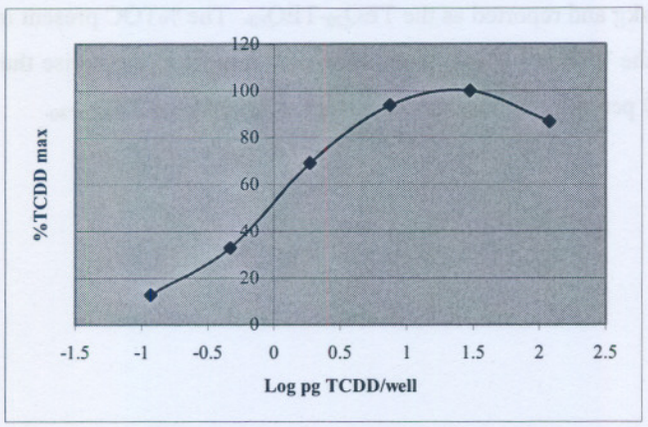


Figure 3.12: A standard TCDD dose-response curve with %TCDD max plotted against the log TCDD (pg/well).

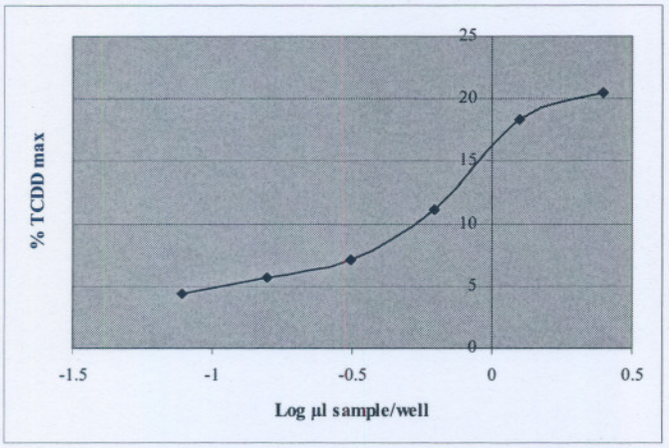


Figure 3.13: A dose-response curve of a sample with the %TCDD max plotted against log sample (µℓ sample/well).

Range estimates are more appropriate than single point estimates because of non-parallelism of the dose-response curves (the lower the range, the more parallel the curves). Extrapolations of EC_{20} should be made for samples not reaching 20% efficacy (Hilscherova *et al.,* 2003), signifying that samples are below the detection limit of the method (Whyte, Schmitt & Tillitt, 2004). This indicates that these samples cannot be reported as an accurate number (Baccarelli, Pfeiffer, Consonni, Pesatori, Bonzini, Patterson, Bertazzi & Landi, 2005). As reported in Sanderson *et al.* (1996), the minimum detection limit of the luciferase assay is 0.0064 pg TCDD/well and a maximum detection limit of 0.448 pg TCDD/well. The measured minimum detection limit of the H4IIE bio-assay performed during this study fell between the reported ranges (0.117 pg TCDD/well). EC_{20}-EC_{80} values were used for the

samples that reached 20% of usefulness. TEQs were calculated using H4IIE cell line specific REPs (c.f. Section 1.2.8) that ranged from REP_{20}-REP_{80}. The REP_{20}-REP_{80} units were converted to ngTEQ/kg and reported as the TEQ_{20}-TEQ_{80}. The %TOC present in the samples was determined by the Walkley-Black method for each sample to normalise the REP values, thus expressing TEQ per unit organic carbon present as normalised $TEQ_{20\text{-}80}$.

Chapter 4

RESULTS AND DISCUSSION

4.1 Results

Both raw extracts after Soxhlet extraction and acid-treated extracts of several mine samples were examined for their ability to elicit AhR-mediated activity *in vitro* using the H4IIE reporter gene bio-assay. These samples are listed in Table 4.1. As indicated in Table 4.1 different numbers of bio-assay runs were completed for each sample in an attempt to determine the best curves so that accurate TEQs could be calculated.

Table 4.1: Different platinum mine samples, the treatment of the samples and the number of bio-assays completed.

Sample No	Treatment of samples	Number of bio-assay runs
Tailings dams		
Tailings dam one		
T1 March	^	1
T1 April	^	2
T2 March	^	2
T2 April	^	1
Tailings dam two		
T3 March	^	2
T3 April	^	3
T4 March	^	2
T4 April	^	3
Tailings dam three	^	
T5 March	^	3
T5 April	^	5
T6 March	^	3
T6 April	^	1
Woodchips		
Merensky		
M1	^	2
M2	^	2
M3	^	3
UG2		
UG2.1	^	3
UG2.2	^	2
UG2.3	^	3
Dumpsite		
Site one (DS1)	^	2
Site two (DS2)	^	2
Slag		
Swartklip slag (SW1)	^	2
Swartklip slag (SW2)	∞	4
Impala slag (IS1)	^	2
Impala slag (IS2)	∞	2

^: Samples extracted and cleaned-up prior to bio-assay; ∞: Samples extracted but no clean-up prior to bio-assay

Of al the samples exposed to the bio-assay, the raw extracts as well as the acid treated extract of the slag samples were bio-assayed. Of the other samples only the acid treated extract was used in the bio-assay to determine if the target compounds are present. Prof L. van Rensburg (School of Environmental Sciences and Development, North-West University, SA) provided a slag sample from another mine as a gift and the slag samples will be discussed further in Section 4.2.1. Dose-response curves were obtained for the standard and the samples. The dose-response curve for each sample was calculated as discussed in Chapter 3. The TEQ of each sample was calculated (listed in Table 4.2).

Table 4.2: The TEQs obtained for environmental samples from the platinum mine.

			ngTEQ/kg		
Sample No.	**R^2**	**%TCDD max**	**TEQ_{20}**	**TEQ_{50}**	**TEQ_{80}**
Tailings					
T1 (M)	0.99	13.27	*0.31	NC	NC
T2 (M)	0.99	20.86	1.76	*0.23	NC
T3 (M)	0.97	9.20	*0.01	NC	NC
T4 (M)	0.98	9.50	*0.07	NC	NC
T5 (M)	0.97	20.46	1.55	*0.17	NC
T6 (M)	0.91	44.02	2.41	5.58	*12.89
T1 (A)	0.99	19.32	1.62	*0.44	NC
T2 (A)	0.99	59.93	54.06	74.26	*102.01
T3 (A)	0.94	9.08	*0.007	NC	NC
T4 (A)	0.93	11.05	*0.11	NC	NC
T5 (A)	0.99	23.08	1.87	*0.07	NC
T6 (A)	0.94	25.06	10.62	*1.03	NC
Woodchips					
M1	0.97	14.14	0.81	*0.00	NC
M2	0.96	9.93	*0.01	NC	NC
M3	0.87	41.10	3.26	*4.20	NC
UG 2.1	0.97	21.66	0.80	*0.19	NC
UG 2.2	0.98	43.41	11.42	*0.59	NC
UG 2.3	0.99	14.92	*0.13	NC	NC
Slag					
SW (1)	0.96	97.28	44.62	106.72	255.25
SW (2)	0.95	39.40	1.76	*0.40	NC
Dumpsite					
DS1	0.96	70.66	30.51	54.16	*96.13
DS2	0.99	27.90	5.31	*0.23	NC

Refer to Table 3.1 for explanation of samples. Slag sample from the Smelter: The (1) indicates a sample washed with sulphuric acid and (2) indicates a sample not washed with acid. TEQ: Relative potency of sample in terms of TCDD (ngTEQ/kg); R^2: Correlation coefficient obtained from the straight line of the dose-response curve; *: Extrapolated values; %TCDD max: Express relative potency for both standard and samples in terms of the highest response elicited by the standard; NC: Non-calculable.

The CV values for the samples and the standard were between 0.1 and 25%. The %TCDD max for the samples ranged from 9% to 97.28%. Concentrations of TEQ_{20} ranged from 0.007

ngTEQ/kg to 54.06 ngTEQ/kg that was calculated for each of the 24 samples listed in Table 4.1 using REP values. Table 4.2 lists the TEQ_{20-80} of each sample, the correlation coefficient (R^2), %TCDD max for each sample. Impala slag was not included in Table 4.2 and Table 4.3, because it does not form part of the process at Union Section.

Table 4.3: REP values normalised by %TOC and reported as normalised TEQ_{20-80}.

		ngTEQ/kg				ng/kg		
Sample	**Response (%)**	**TEQ_{20}**	**TEQ_{50}**	**TEQ_{80}**	**%TOC**	**Normalised TEQ_{20}**	**Normalised TEQ_{50}**	**Normalised TEQ_{80}**
Tailings								
T1 (M)	BDL	*0.31	NC	NC	0.08	*378.50	NC	NC
T2 (M)	+	1.76	*0.23	NC	0.16	1050.10	*137.31	NC
T3 (M)	BDL	*0.01	NC	NC	0.18	*6.46	NC	NC
T4 (M)	BDL	*0.07	NC	NC	0.21	*37.43	NC	NC
T5 (M)	+	1.55	*0.17	NC	0.29	528.40	*13.00	NC
T6 (M)	+	2.41	5.58	*12.88	0.23	1046.90	2416.9	*5579.5
T1 (A)	+	1.62	*0.0	NC	0.33	482.70	*0.09	NC
T2 (A)	59.93	54.06	74.26	*102.01	0.31	17163.00	23576.00	*32385
T3 (A)	BDL	*0.007	NC	NC	0.33	*2.09	NC	NC
T4 (A)	BDL	*0.11	NC	NC	0.16	*66.46	NC	NC
T5 (A)	+	1.87	*0.07	NC	0.23	808.30	*30.30	NC
T6 (A)	+	10.62	*1.03	NC	0.35	2976.00	*290.76	NC
Woodchips								
M1	+	0.81	*0.00	NC	N/A	N/A	NC	NC
M2	BDL	*0.01	NC	NC	0.25	*3.07	NC	NC
M3	+	3.26	*4.20	NC	N/A	N/A	NC	NC
UG 2.1	+	0.80	*0.19	NC	N/A	N/A	NC	NC
UG 2.2	43	11.42	*0.59	NC	5.50	207.65	*10.79	NC
UG 2.3	BDL	*0.13	NC	NC	14.07	*0.94	NC	NC
Slag								
SW (1)	97.28	44.62	106.72	255.25	0.10	42497.48	101641.69	243097.50
SW(2)	+	1.76	*4.54	NC	0.10	1681.33	*4330.19	NC
Dump site								
DS1	70	30.51	54.16	*96.13	0.73	4151.63	7369.10	13080.10
DS2	+	5.31	*0.23	NC	1.74	304.80	*13.3	NC

Refer to Table 3.1 and Table 4.1 for explanation of samples. +: Response from the cells that were above 20%, BDL: Below detection limit of the method (Baccarelli *et al.*, 2002; Whyte *et al.*, 2004) (c.f. Section 4.2.1), TEQ: Relative potency of sample in terms of TCDD (ngTEQ/kg); N/A: Not applicable since no sample was available to determine %TOC; Normalised TEQ_{20-80}: The TEQ value divided by the %TOC; *: Extrapolated values; NC: Non-calculable.

It was a once-off gift, to compare for interest sake the slags of the two mines and will be discussed further in Section 4.2.1. Non-calculable as presented in Table 4.3 only indicates that no values were determined for the samples since the standard range of response was TEQ_{20}. Many of these samples were below the detection limit of the method (BDL) and

TEQ_{20} was extrapolated. In addition to TEQ_{20} extrapolations, dose-response curves could however not be determined for these samples.

As presented in Table 4.3, not all of the samples reached 20% efficacy and therefore EC_{20} extrapolation were made for these samples. This implies that the sample TEQ levels were below the detection limit of the method (Whyte *et al.,* 2004). Samples that reached 20% efficacy indicated the definite presence of mixtures of dioxin-like chemicals in the environmental and process samples.

It is important to determine the total organic carbon present in soils and sediments because it forms an essential part of any site characterisation. Sorption and desorption of both natural-occurring and anthropogenically-introduced organic compounds can be determined by the presence or absence of organic carbon that markedly influence how dioxin-like chemicals, such as dioxins and furans, react with soil and sediments (Schumacher, 2002). The TEQ values were normalised by %TOC present in the samples (Table 4.3). The normalised TEQ_{20} ranged from 0.94 ngTEQ/kg to 42497.48 ngTEQ/kg. These values were determined by dividing the TEQ values with the %TOC. The normalised TEQ is higher than the TEQ because it represents a TEQ at a particular site as if the sample consisted of organic carbon only. By normalising the data, the difference between the TEQ values and the normalised $TEQ_{20\text{-}80}$ can be compared.

4.2. Discussion

Investigation of the literature led to the conclusion that non-ferrous metal production was a source of dioxin-like chemicals (UNEP, 2003). However, based on this study's results, Union Section releases only a small amount of dioxin-like chemicals to air, water and land. Table 4.2 and Table 4.3 showed that dioxin-like chemicals were detected in the platinum mine samples by using the H4IIE reporter gene-bioassay as bio-analytical screening method. The TEQ_{20} of these substances present in the environmental samples ranged from 0.007 ngTEQ/kg to 54.06 ngTEQ/kg, as listed in Table 4.2. Since the H4IIE reporter gene bio-assay was used to determine the presence of dioxin-like chemicals, TEQ values for each sample could be determined (Table 4.2).

Currently, South Africa does not have any quality guideline levels for dioxins, but does have control measures to regulate dioxins and furans from stack emissions of incinerators as discussed in Chapter 1. Because South Africa ratified the SC, it is of great need to develop these guidelines and to implement it in our environmental law. Other countries that have developed quality guidelines for PCDD/Fs are indicated in Table 4.4. These guidelines were

established to protect human health and the environment (Anon 2005k). As presented in Table 4.4, the guidelines for different countries vary greatly.

Table 4.4: Quality guideline levels for dioxins in several countries (Adapted from Anon, 2005k: units reported as in the original publication, and are dry weight).

AIR*	**Levels of TEQs^**
Canada	0.1 ng/m^3
United Kingdom (U.K.)	1.0 ng/m^3
Ireland	0.1 ng/m^3
US	0.2 ng/m^3
Japan	0.1-5 ng/m^3
SOILS	
Canada	4 ng/kg
USA	1000 ng/kg
Japan	1 ng/kg
Finland	2 ng/kg
Germany	1000 ng/kg
Italy	5000 ng/kg
The Netherlands	1000 ng/kg
Sweden	250 ng/kg
WATER	
UK	0.01 ng/L
US	0.3 ng/L
Japan	0.001 ng/L
SEDIMENTS	
UK	55 ng/kg for both saltwater and freshwater sediments
Japan	150 ng/kg
The Netherlands	100 ng/kg
HUMAN INTAKE	
Canada	10 pgTEQ/kg bodyweight (bw)/day
UK	10 pgTEQ/kg bw/day
USA	0.006 pgTEQ/kg bw/day
Japan	4 pgTEQ/kg bw/day
Austria	10 pgTEQ/kg bw/day
Denmark	5 pgTEQ/kg bw/day
Finland	5 pgTEQ/kg bw/day
France	1 pgTEQ/kg bw/day
Germany	1 pgTEQ/kg bw/day
Italy	10 pgTEQ/kg bw/day
The Netherlands	1 pgTEQ/kg bw/day
Sweden	5 pgTEQ/kg bw/day

* Air emission limits for sources

^ The expression of TEQs of dioxins in different units by countries. The levels for environmental media are given in ng/m^3 or ng/kg and in pg/kg for human intake

The soil and sediment guidelines in Table 4.4 were used to compare to soil and sediment samples from the platinum mine such as the tailings dams and the dumpsite if it were to be assumed that the values were TEQ_{20} (Table 4.4). The tailings dams and the dumpsite were also compared to the other stages of the mining processes (Figure 4.1). The other stages include woodchip extraction from the Merensky and UG2 plant and the formation of slag in the smelter.

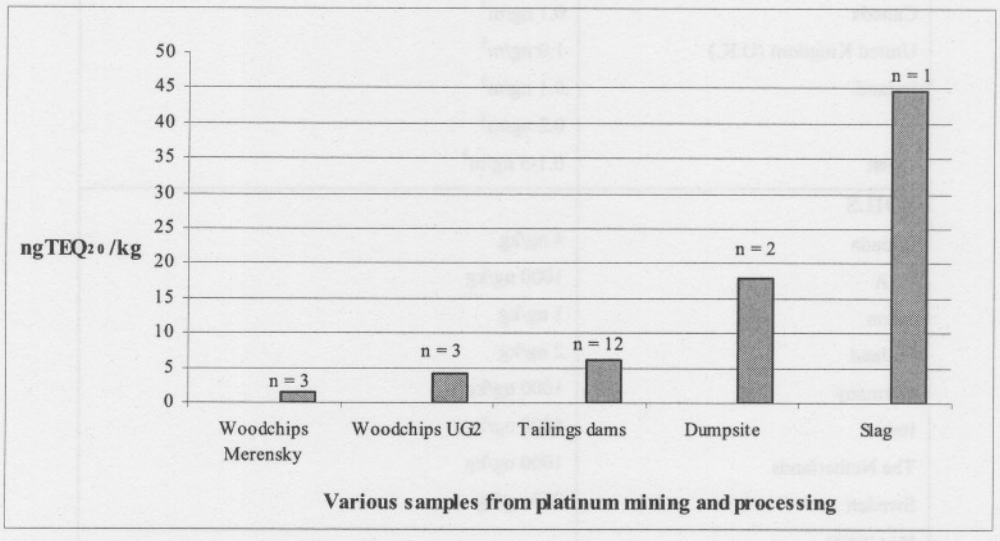


Figure 4.1: The mean TEQ_{20} of the samples collected from different environmental matrices at Mortimer plant. The number of samples used to determine the TEQ is also indicated. (SD: Merensky - 1.69; UG2 - 6.33; Tailings dams - 15.33; Dumpsite - 17.78).

Table 4.5: The mean TEQ_{20} associated with each environmental matrix and process from the platinum mine.

Samples	Mean TEQ_{20} (ngTEQ/kg)
Tailings dams	
Tailings dam 1	14.44
Tailings dam 2	0.052
Tailings dam 3	4.112
Mean of the 3 tailings dams	**6.20**
Woodchips	
Merensky	**1.36**
UG2	**4.12**
Dumpsite	
DS1	30.5
DS2	5.31
Mean of Dumpsite	**17.91**
Swartklip slag (1)	**44.62**

The soil quality guidelines ranged from 1 ng/kg to 5000 ng/kg as presented in Table 4.4, which indicates that the TEQ values at the dumpsite of Swartklip Platinum mine (Union

Section) exceeded the guidelines of some countries. The sediment guidelines ranged from 55 ng/kg to 150 ng/kg, indicating that the tailings fell between the ranges, although tailings are not strictly sediments.

Figure 4.1 represents the different matrices from which samples were collected. Each of these matrices were compared to the soil quality guidelines as listed in Table 4.4, since guidelines for slag and woodchips do not exist in any published material. The two soil quality guidelines that were used were 1ng/kg and 250 ng/kg. This implies that the samples collected from Union Section were between 1 ng/kg for Japan and the 250 ng/kg regarded by Sweden as safe (Table 4.4). USA, Germany, Italy and the Netherlands have much higher guideline levels, which were not considered for the study. The samples of the various mining processes and their TEQ_{20} will now be considered in the following sections.

4.2.1. Slag

Slag is formed during the combustion process (temperature of 1400°C) of the dried concentrate to produce valuable PGMs, which are transported to the refineries at Rustenburg for further processing (as addressed in Chapter 2). Slag from the Impala Platinum mine (Rustenburg, North-West Province) was provided as a gift, and was compared to the Swartklip slag (Union Section). Because the Impala slag was not originally part of the investigation (i.e. a completely different mine and therefore can not be compared to the TEQs of the other processes already discussed), raw extracts as well as acid treated extracts were bio-assayed. Four samples in stead of two were bio-assayed for more accurate results. Although the clean-up process removes all other PAHs, we were interested to see if there were chemicals present in the raw extract that would elicit an AhR-mediated response. Table 4.6 gives the TEQ_{20-80} of each of the slag samples.

Table 4.6: TEQ_{20-80} of the slag samples from Swartklip platinum mine (Union Section) and Impala platinum mine.

Sample	Response	TEQ_{20} (ng/kg)	TEQ_{50} (ng/kg)	TEQ_{80} (ng/kg)
Swartklip slag acid washed	97.28%	44.62	106.72	255.25
Swartklip slag not acid washed	+	1.76	*0.40	NC
Impala slag acid washed	+	5.15	*0.98	NC
Impala slag not acid washed	BDL	*0.00	NC	NC

+: Positive response from the cells that were above 20%.; BDL: Below detection limit of method (Baccarelli *et al.*, 2002; Whyte *et al.*, 2004);
*:Extrapolated values; NC: Not calculated.

The samples were extracted as described in Chapter 3, but one half of each extract was also treated with sulphuric acid to keep the target compounds. By not treating the other half of the extract with sulphuric acid, PAHs were retained. Of all the slag samples, only the slag from

Swartklip (acid washed) gave a high enough response (97.28% of the standard) to enable a TEQ_{20-80} to be calculated from the dose-response curve. For Swartklip slag (not acid washed) and the Impala slag (acid washed), the response was so low, that only a TEQ_{20} could be calculated. The other TEQ values in the table (marked with *) are extrapolated values and were thus not directly measured by the method. Impala slag (not acid washed) was below the detection limit (Baccarelli *et al.*, 2002; Whyte *et al.*, 2004). Those samples indicated by + showed a response higher than 20% at some of their concentrations, but proper dose-response curves could not be created because of cytotoxicity of high concentration samples (Figure 4.2).

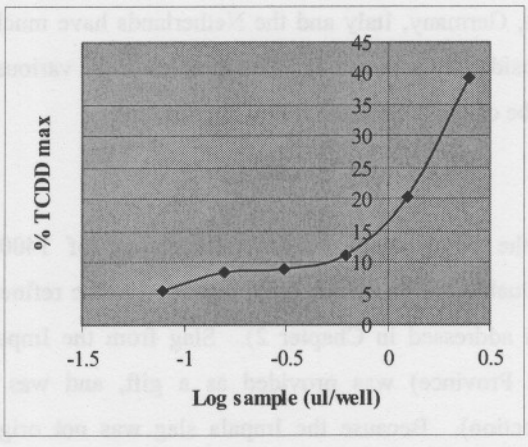


Figure 4.2: A dose-response curve to indicate that less than 3 points greater than 20% detection limit of the method fell on the straight section of the graph and no straight-line equation was possible.

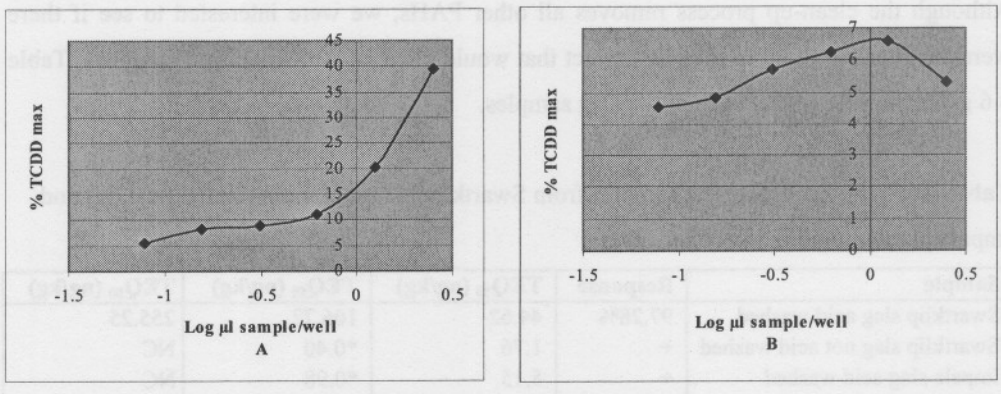


Figure 4.3: Dose-response curves of Swartklip slag, not acid washed (A) and Impala slag, not acid washed (B).

If one was to assume additive effect (synergism), a higher TEQ was to be expected for samples not treated with sulphuric acid, thus still containing all PAHs. It is possible, however, that these samples (Figure 4.3) had very complex mixtures of PAHs that were

responsible for an inhibition effect on the cells, or these PAHs might have been more bio-available, out-competing the less abundant dioxins to the Ah-receptor, but eliciting a weaker response from the cells. The inhibition effect indicates that the PAHs present were persistent to a lesser extent, and possibly also weak agonists (Safe, 1995; Till, Riebniger, Schmitz & Schrenk, 1999; Hamers, van Schaardenburg, Felzel, Murk & Koeman, 2000). These PAHs might have been bio-transformed to non-active metabolites during extraction that could have caused a low response in the cells due to competitive substrate inhibition (Hilscherova *et al.,* 2000). Those extracts, from which all PAHs, except the target compounds had been removed, showed a higher TEQ (Figure 4.4), probably because the compounds responsible for inhibition were removed, and those present in the mixture had an additive effect on the cells (Hamers *et al.,* 2000; Masunaga *et al.,* 2002).

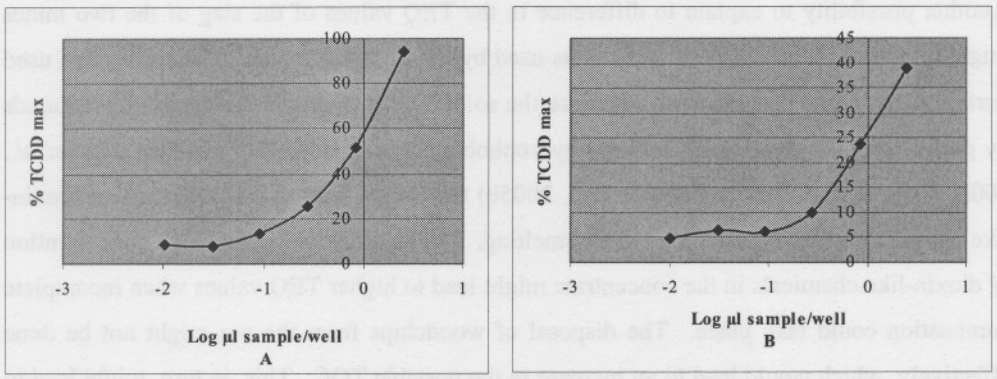


Figure 4.4: Dose-response curves of Swartklip slag, acid washed (A) and Impala slag, acid washed (B).

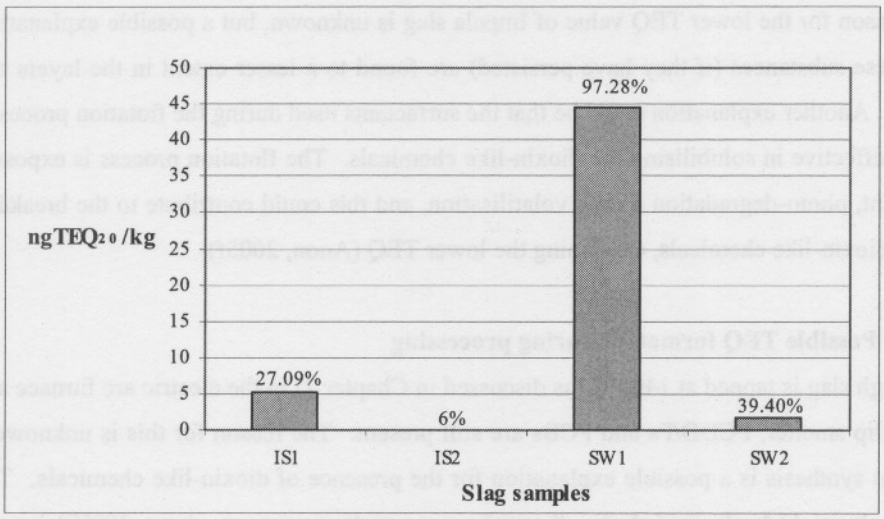


Figure 4.5: The TEQ_{20} of each type of slag. The maximum % response elicited from the bio-assay is also indicated on each bar. (SW = Swartklip slag; IS = Impala slag).

From Figure 4.5 it is clear that the slag from the Swartklip Mine contributed more to the possible dioxin-like chemical load than slag from Impala Mine. This was a once-off finding and before any definite conclusions can be made, a thorough investigation needs to be undertaken. It is, however, possible that the furnace at the Swartklip Mine (Union Section) is not as effective in eliminating dioxin-like chemicals as the furnace at the Impala Mine. Due to the Merensky and UG2 reef being formed by repeated injection of lava (or magma), the layers being mined at Swartklip might contain higher concentrations of dioxin-like chemicals, since volcanic activities lead to the natural formation of these compounds (Fiedler, 1996; Baars *et al.,* 2004), although it is not known if these will remain in the rock, and for how long they will persist. It does, however, indicate the possible presence of chlorine.

Another possibility to explain to difference in the TEQ values of the slag of the two mines might be found in the different surfactants used by the different mines. The surfactants used during the flotation process might increase the solubility of hydrophobic organic compounds by partitioning the compounds into the hydrophobic cores of surfactant micelles (Cho *et al.,* 2002; Zhou & Zhu, 2005a; Zhou & Zhu, 2005b) that might lead to concentration of dioxin-like chemicals in the concentrate before smelting. The implications of the high concentration of dioxin-like chemicals in the concentrate might lead to higher TEQ values when incomplete combustion could take place. The disposal of woodchips from the ore might not be done effectively, which would lead to an increase in the possible TOC. This, in turn, might lead to an increase in the capacity of the slag to sorb dioxins, leading to higher TEQ values.

The reason for the lower TEQ value of Impala slag is unknown, but a possible explanation is that these substances (if they have persisted) are found to a lesser extent in the layers being mined. Another explanation could be that the surfactants used during the flotation process are not as effective in solubilising the dioxin-like chemicals. The flotation process is exposed to UV light, photo-degradation and/or volatilisation, and this could contribute to the breakdown of the dioxin-like chemicals, explaining the lower TEQ (Anon, 2005f).

4.2.1.1 Possible TEQ formation during processing

Although slag is tapped at 1400°C (as discussed in Chapter 2) in the electric arc furnace at the Swartklip smelter, PCDD/Fs and PCBs are still present. The reason for this is unknown. A *de novo* synthesis is a possible explanation for the presence of dioxin-like chemicals. These chemicals might be formed during thermal processes (temperatures above 300°C) in which chlorine-containing substances are burned together with carbon and a suitable catalyst (possibly copper and even other metals) in the presence of O_2 as the gases are cooled (Fiedler,

2003; Anon, 2005k; Huang & Buekens, 1996; Stanmore, 2004). The source of chlorine is unknown.

Other mechanisms that may contribute to the formation of dioxin-like chemicals are incomplete destruction of dioxin-like chemicals present in feedstock material (such as slag sent for reprocessing) and 'in-furnace' formation via condensation of chemically related precursor compounds (Anon, 2005m).

Incomplete combustion implies that if combustion is inefficient, dioxin-like chemicals present in the materials being combusted can be emitted. Good combustion conditions rely on sufficient temperature, time and turbulence (oxygen) to ensure that any of the compounds present are completely destroyed (Anon, 2005m). 'In-furnace' formation indicates that during combustion, intermediate combustion products such as ring-structured hydrocarbon species (precursors) are formed. If chlorine is present, it can react to form PCDD/Fs (Anon, 2005m). To reduce the amount of the dioxin-like chemicals present at the platinum mine, certain reduction measures could be implemented.

4.2.1.2 Proposed source reduction measures

Although no documentation regarding the current operational effectiveness (temperature, residence times, etc.) of the Swartklip smelter could be released, preliminary recommendations of possible primary and secondary measures are presented below to reduce the formation of dioxin-like chemicals at the smelting facility (Anon, 2005l):

- The use of hydrometallurgical processes. This implies that the desired metals are separated from the unwanted material in aqueous acid solutions.
- Quality control of feed material. The presence of oils, plastics and chlorine compounds in the feed should be avoided to reduce the generation of PCDD/Fs. By effective milling and grinding techniques, with good dust extraction and abatement, the formation of dioxin-like chemicals would decrease.
- Effective process control of the furnace should take place by monitoring temperature, residence time, gas components and fume collection damper controls. In maintaining these variables such as residence time etc, optimum-operating conditions would take place for the reduction of dioxin-like chemicals.
- Effective pollution control should take place by choosing a process that entails lower energy usage and lower emissions from the furnace stack.
- Air emission should be controlled at all stages of the process, from handling, smelting and material transfer points, to emission control of dioxin-like chemicals.

- High volumes of particulate matter with large surface areas are generated during the smelting process. The particulate matter that are generated are surfaces on which the dioxin-like chemicals adsorb. By removing the dust by high-efficiency dust control, the formation of dioxin-like chemicals could be reduced.

4.2.1.3 Emissions and releases of dioxin-like chemicals

Non-ferrous metal production could lead to possible releases of dioxin-like chemicals to air, water and in residues (UNEP, 2003). The possible calculated release of dioxin-like chemicals in the slag (residue) from the electric arc furnace is 0.6023 gTEQ/annum (listed in Table 4.7). Emissions from the Smelter to air, releases to slag (residue) and to *matte* (product) were calculated by using the UNEP Toolkit (2003), H4IIE bio-assay and the emission factor calculated from the release of the slag. The estimated emission factor of the product (*matte*) is 0.0004 gTEQ/ton *matte* produced per year. The release for the slag was calculated by multiplying the H4IIE bio-assay determined TEQ (44 ngTEQ/kg) with the tonnage of slag produced per year (13 500 metric ton). The emission factor for the *matte* was calculated by dividing the emission of slag (0.6023 gTEQ/annum) with annual tonnage *matte* (1500 metric ton) produced. An assumption is made that there are little or no dioxin-like chemicals present in the *matte*. The air emission from the electric arc furnace is 0.03 gTEQ/annum. This value was calculated from the default emission factor of 2 µgTEQ/t (other non-ferrous metal production category) given in the *Standardized Toolkit for the Identification and Quantification of dioxin and furan release* (UNEP, 2003) and the tonnage of material produced at the smelter for one year (15000 metric ton).

Table 4.7: Possible releases of dioxin-like chemicals in various environmental matrices from the smelter at Union Section.

Union Section Smelter	Emission to air (gTEQ/annum)	Release to water (gTEQ/annum)	Emission factor of product (gTEQ/t)	Release of slag (gTEQ/annum)
Flash drier	*	*	*	*
Electric arc furnace	0.03[a]	^	0.0004[c]	0.6023[b]

*: The emission of the flash drier to air, and releases to product, residue and water is not known and this is a field for further investigation

^: The emission of the furnace to water is not yet known

[a]: Determined by using the *Standardized Toolkit for the Identification and Quantification of dioxin and furan release* (UNEP, 2003)

[b]: Determined from the H4IIE bio-assay

[c]: Determined from the emission of the slag

The release to water could not be estimated or calculated, but some releases are likely. Other possible emission from the smelter that could be determined is the emissions from the flash

drier to air (indicated with * in Table 4.7), since the temperature of the flash drier is favourable (± 500°C) for the formation of dioxin-like chemicals (as addressed in Chapter 2).

Eduljee & Dyke (1996) reported values of dioxin-like chemicals, especially PCDD/F emission for a non-ferrous metal plant, to range between 5 and 35 gTEQ/annum in the U.K. Emission from non-ferrous metals in New-Zealand reported emission values that ranged between 0.000012 gTEQ/annum to 0.394gTEQ/annum (Anon, 2005l). This indicates that the emissions from the smelter are between the values determined in U.K and New-Zealand. Many sources contribute to the world's releases of dioxin-like chemicals to the environment. However, non-ferrous metals contribute only 8% of these compounds released to the environment (Roots, Henkelmann & Schramm, 2004). This implies that the furnace at the smelter only contributes to a small portion of the global formation of these chemicals, but it could be a significant local source.

4.2.1.4 Possible health and environmental risks

Workers at the Swartklip smelter are potentially at risk when exposed to these compounds, especially when taking the normalised $TEQ_{20\text{-}80}$ values into consideration (Table 4.3). The impact of the high normalised $TEQ_{20\text{-}80}$ could have detrimental health effects for the workers working at and nearby the smelter. Such effects might include reproductive problems and cancer. The environment surrounding the smelter is also possibly at risk by being exposed to the relatively high concentration of dioxin-like chemicals (based on TOC normalised values) that takes years to break down (Kitamura *et al.,* 2004).

Mine workers, in addition to dioxin-like chemical exposure (that are endocrine disruptive), may also be exposed to other endocrine-disrupting chemicals. Reagents, such as polyglycol-ethers (alkylphenol poly-ethoxylates, APn-Eos) are used as depressants during the flotation process at many platinum mines, and these compounds may cause endocrine disruptive activities. Nonylphenol (NP) has been widely utilised over the past few decades as a non-ionic surfactant in both industrial and domestic applications (Oh, Park & Chung, 2005). Nonylphenol is an endocrine-disrupting compound, which interferes with the proper functioning of the endocrine system that causes changes in sex ratio, embryonic damage and reduced fecundity (Oh *et al.,* 2005). Platinum mine workers might be exposed to this chemical that could pose potential harm to workers.

4.2.2 Dumpsite

Two samples were collected from one dumpsite as described in Chapter 3. The two samples (DS1 & DS2) that were collected consisted of soil, woodchips and plastics. The reason two

samples were collected at the dumpsite was because woodchips together with waste were disposed of here by the mine, and to investigate the impact of anthropogenic activities at the dumpsite. Of the two samples at the dumpsite, only DS1 gave a response (70.66% of the standard) high enough so that TEQ_{20-50} could be calculated from the dose-response curve (Table 4.3). The TEQ_{80} was extrapolated from the dose-response curve. The TEQ_{20} was 30.5 ngTEQ/kg and the TEQ_{50} was 54.16 ngTEQ/kg for dumpsite DS1 (Table 4.3). For DS2, the response was so low that only a TEQ_{20} (5.31 ngTEQ/kg) could be calculated. The average TEQ_{20} for the dumpsite was 17.91 ngTEQ/kg (Table 4.5).

4.2.2.1 Possible formation of dioxin-like chemicals

As mentioned by the *Standardized Toolkit of Identification and Quantification of dioxin and furan release* (UNEP, 2003), waste not handled and disposed of effectively, can lead to the formation and release of dioxin-like chemicals into the environment. Degradation of organic material takes place on landfills and at dumpsites that result in the formation of gases (with methane as a major constituent). If water passes through the waste, it results in a leachate. If no proper collection system is installed at the landfills or dumpsite, landfill gases and leachates tend to escape in an uncontrolled manner, leading to the formation of dioxin-like chemicals in the surroundings. Domestic burning to produce heat from wood, bio-mass and old tyres collected from the dumpsite could also contribute to the formation of these substances and may lead to elevated TEQ values at dumpsites, such as at the dumpsite nearby Mortimer Concentrator.

The difference in TEQ_{20} values between the two sites (DS1 and DS2) might be explained by differences in woodchip heaps that differ in age, the extent of bio-degradation that have taken place at that specific point on the site, anthropogenic activities such as burning of bio-mass and wood, and differences in processes and conditions relating to the production of these substances.

Since fires at the landfill or dumpsite may serve as a source of dioxin-like chemicals (Roots *et al.,* 2004), this site needs to be effectively managed. All of these activities contribute to the possible formation of dioxin-like chemicals that are emitted to the air, and released to land and water, if not effectively controlled. This could be the main reason for the high TEQ present at the dumpsite. Sorption of hydrophobic organic compounds could contribute to the high dioxin-like chemical load at dumpsite DS1 due to presence and distribution of organic carbon during production of heat at the dumpsite (Brzuzy & Hites, 1995; Wu *et al.,* 2002).

An additional explanation for the high TEQ_{20} value (30.5 ngTEQ/kg), is that soil is a reservoir with a large capacity for dioxin-like chemicals since these compounds have long half-lives in soil, and tend to sorb strongly to soils with high organic carbon content (Brzuzy & Hites, 1995; Wu *et al.,* 2002; Roots *et al.,* 2004; Dalla Valle *et al.,* 2005). Other contributing factors to the high dioxin-like chemical load at the dumpsite could be migratory birds and wind which transport these compounds from other sources to the dumpsite, but this is highly unlikely, as it means very high levels elsewhere. The response of dumpsite DS2 is very low (27.90% as indicated in Table 4.3)), but dioxin-like chemicals are present.

4.2.2.2. Soil as sink and secondary source

The low TEQ of DS2 (5.31 ngTEQ/kg) could be that these compounds had moved from accessible soil compartments into less or inaccessible compartments (Reid *et al.,* 2000), making it difficult to determine the true TEQ of this site. It is possible that dioxin-like chemicals at DS2 were not at the surface layers from where samples were collected, but deeper in the soil layers due to leaching (Wu *et al.,* 2002), and implies a need for further investigation. Another possible reason for the low value is the potential loss of dioxin-like chemicals by photo-degradation and/or volatilisation that depends on several factors including exposure to sunlight and climatic conditions (Anon, 2005f).

Table 4.8: Dioxin-like chemical concentration of the dumpsite for this study and concentrations of dioxin-like chemicals in soil presented as ngTEQ/kg from EU Member States (adapted from Roots *et al.,* 2004).

Country	Any type (ngTEQ/kg)	Forest (ngTEQ/kg)	Pasture (ngTEQ/kg)	Arable (ngTEQ/kg)	Rural (ngTEQ/kg)	Contaminated (ngTEQ/kg)
This study	-	-	-	-	-	**5.96-30.5**
Austria	-	0.01-64	1.6-14	-	-	332
Belgium	2.7-8.9	-	-	-	2.1-2.7	-
Finland	-	-	0.004-30	-	-	85 000
Germany	0.1-42	10-30	-	0.03-25	1	30 000
Greece	2-45	-	0.8-13	-	-	1144
Ireland	0.15-8.6	4.8	0.1-43	-	-	-
Italy	0.057-0.12	-	-	1.9-3.1	-	-
Luxembourg	1.8-20	6.0	-	-	1.4	-
The Netherlands	2-55	-	-	-	2.2-17	98 000
Spain	0.63-8.4	-	-	-	0.1-8.4	-
Sweden	-	-	-	-	0.11	11.446
United Kingdom	0.87-87	-	-	-	0.78-20	1585

-: No values

The dioxin-like chemical concentration in the soil of the dumpsite (5.31-30.5 $ngTEQ_{20}/kg$) was within the TEQ ranges found for contaminated soils from several European Union (EU)

members as indicated by Roots *et al.* (2004) (Table 4.8). By being within the range of the landfill concentrations found by Roots *et al.* (2004), it however does not imply that the dumpsite should be neglected. Restriction of many of the human activities on the dumpsite (such as the burning of wood, plastics and tyres) should take place, since it would contribute to the reduction in the formation of dioxin-like chemicals.

4.2.3 Tailings dams

Twelve samples were collected from three tailings dams. TEQ for each sample was determined as presented in Table 4.2 and Table 4.3). Little of the substances under investigation were present on the tailings dams. Of all the tailings dam samples, only tailings dam one (T2 collected during April) gave a response high enough (59.93% of the standard) so that TEQ_{20} and TEQ_{50} could be calculated from the dose-response curve. The TEQ_{20} and TEQ_{50} ranged from 10.25 ngTEQ/kg to 54.06 ngTEQ/kg. TEQ_{80} of T2 was extrapolated. Dioxin-like chemicals were expected to be at low concentrations at the tailings dams, since it does not form part of the possible sources of these compounds (see Chapter 1). Some of the samples were below the detection limit of the method, and are therefore indicative of low levels of these substances TEQ in the tailings dams. Many of the samples gave a + response, but the extracts need to be treated with activated copper and fractionated because cytoxicity was noticed (c.f. Section 4.2.5), before further determinations can be made.

4.2.3.1 Potential contributing factors to the presence and/or absence of dioxin-like compounds on tailings dams

The highest TEQ value for the tailings dams was T2 with a value of 54.06 ng/kg. This sample was collected during April 2004, but a sample collected at the same spot during March 2004 only gave a TEQ value of 1.76 ngTEQ/kg. Many factors could contribute to the dioxin-like chemical load of the tailings dams. Such factors include climate, precipitation, migratory birds, wind (deposition), burning of bio-mass and anthropogenic activities (Breivik *et al.,* 2004; Reid *et al.,* 2000). The three tailings dams was compared to each other by calculating the mean of each tailings dam, to determine which tailings dam had the highest TEQ_{20} (Figure 4.6). The mean TEQ_{20} for tailings dam one is 14.44 ngTEQ/kg, which is the highest of the three tailings dams due to the high TEQ value of T2 obtained during April 2004. The reason for this is not clear. It is known that clay minerals in soil play an important role in the sorption of contaminants, since these compounds are non-polar (Wu *et al.,* 2002; Lee *et al.,* 2004; Zhou & Zhu, 2005b). However, the clay fraction and composition was not determined in this case. The composition of the tailings is sand, silt, a clay fraction and an organic fraction (discussed in Chapter 2). Clay minerals are found on the tailings that have large exchange capacities, and appreciable internal and external surfaces accessible to organic

molecules (dioxin-like chemicals) or inorganic molecules (Lee *et al.,* 2004). The difference in the clay content of the tailings (not measured) could help explain the differences in TEQ. This tailings dam could act as a reservoir of dioxin-like chemicals to the environment.

At tailings dams one and three, the presence of dioxin-like chemicals is low (Table 4.3). The reason for this is the possible loss of dioxin-like chemicals by photo-degradation and/or volatilisation that are dependent on exposure to sunlight and precipitation and winds or subsequent erosion of the topsoil (Anon, 2005h; Rogowski & Yake, 2005). In addition, the tailings dams were not expected to have very high levels since they are not associated with a potential source process. When comparing the TEQ values determined for the tailings dams (Figure 4.6) to the guidelines for soils and sediments of other countries, all of these dams would have been regarded as polluted at some stage by more than one of the countries listed in Table 4.4 and Table 4.8.

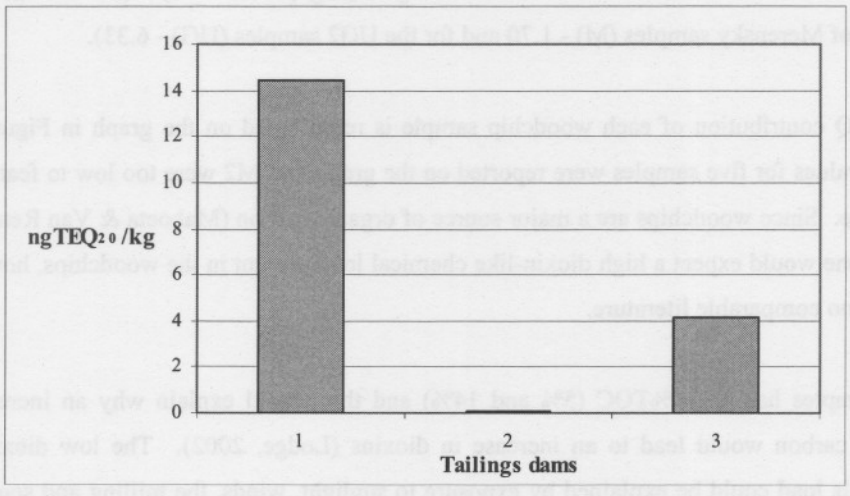


Figure 4.6: The mean TEQ_{20} of the three tailings dams. (SD: Tailings dams one - 26.42; Tailings dam two - 0.051; Tailings dam three - 4.35).

4.2.4 Woodchips

Six woodchip samples were collected from the UG2 and Merensky process at Mortimer Plant (Union Section). Table 4.3 represents the calculated TEQs for the samples. The dioxin-like chemical load of the woodchips was low. The UG2.2 sample, however, had a higher response – 43% of the maximum response. The TEQ_{20} values ranged from 0.01 ng/kg to 11.42 ng/kg. The woodchip sample that gave the highest TEQ (11.42ngTEQ/kg) was UG2.2. TEQ_{20} could be calculated from the dose-response curve for UG2.2.

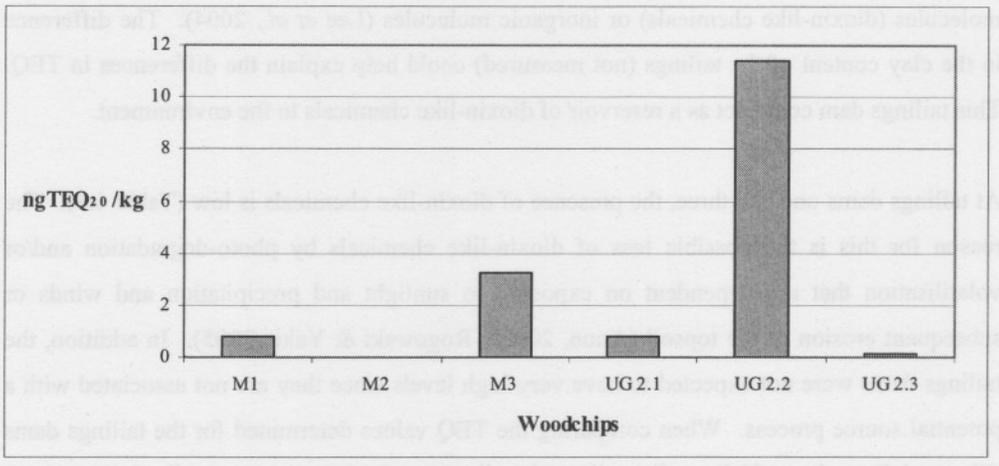


Figure 4.7: The TEQ_{20} of each woodchip sample. All the woodchip samples are included in the figure. The TEQ_{20} for Merensky ranged from 0.007ng TEQ/kg to 3.27 ngTEQ/kg and the TEQ_{20} for UG2 were between 0.80 to 11.42 ngTEQ/kg (Table 4.11). (SD for the mean TEQ20 of Merensky samples (M) - 1.70 and for the UG2 samples (UG) - 6.33).

The TEQ contribution of each woodchip sample is represented on the graph in Figure 4.7. TEQ_{20} values for five samples were reported on the graph, but M2 were too low to feature on this scale. Since woodchips are a major source of organic carbon (Maboeta & Van Rensburg, 2003), one would expect a high dioxin-like chemical load present in the woodchips, however, there is no comparable literature.

Two samples had high %TOC (5% and 14%) and this would explain why an increase in organic carbon would lead to an increase in dioxins (Lodge, 2002). The low dioxin-like chemicals load could be explained by exposure to sunlight, winds, the milling and screening of the woodchips during extraction of platinum (as discussed in Chapter 2). By milling and screening the woodchips, the sorbtion capacity of the woodchips for dioxin-like chemicals could become altered.

4.2.5 Proposed treatment of cytotoxic samples

The best treatment for samples that show cytotoxic response is to first remove any compounds present in the complex mixtures that could be cytotoxic to the cells. For example, sulphur is a major cytotoxic constituent in sediment and soils that should be eliminated from the extracts prior to performing tests for dioxin-like activities (Giesy *et al.,* 2002). To eliminate sulphur from the extracts, the sample extracts need to be treated with activated copper. In an attempt to remove remaining cytotoxic compounds, the extract of the sample should be fractionated (on the grounds of polarity, for instance) and each fraction should be

exposed to the cells (Behnisch *et al.,* 2001). Fractionation is done to limit cytotoxic effects from the sample matrix and could be used to discriminate between specific classes of compounds into separate fractions or to destroy non-stable classes of toxicants (Behnisch *et al.,* 2001). The viability of the cells in the bio-assay was not determined other than through microscopic investigation. The method for determining the viability of the cells is the MTT cytotoxic assay (3-[4,5-dimethylthiazol-2yl]-2,5-dipehnyl tetrazolium bromide) which is being implemented in the tissue culture laboratory (Bláha, Hecker, Murphy, Jones, Newsted & Giesy, 2004). Refining the methods during this study would contribute significantly towards the thorough assessment of dioxin-like compounds.

4.2.6 Normalised data

As listed in Table 4.3, the normalised TEQ_{20} ranged from 0.94 ngTEQ/kg to 42497.48 ngTEQ/kg. The values of the normalised TEQ_{20} were higher than the measured TEQ_{20} (0.007 ngTEQ/kg to 54.06 ngTEQ/kg) (listed in Table 4.3). In managing the mining processes more effectively regarding dioxin formation, the risks posed to the environment and human health would consequently be minimised (c.f. Section 4.2.1.2). The normalised values could, in future, be used to assess the effectiveness of any mitigation measures implemented to reduce the formation of dioxin-like chemicals of the activities associated with platinum mining and processing.

4.2.7 Summary

The hypothesis of this study was that dioxin-like chemicals are present on a platinum mine due to thermal processes associated with platinum mining and processing, and that the H4IIE reporter gene bio-assay can be used to detect these chemicals in different matrices associated with the operation, of a platinum mine. The results obtained during this study indicated that dioxin-like chemicals are present on the platinum mine especially at the Smelter. Certain factors might influence the formation, transport, fate and behaviour of the substances.

To investigate the total organic carbon of each of the environmental matrices, explanations for the dioxin-like chemicals' formation would be more accurately assessed. As mentioned in the SC, parties are required to virtually eliminate dioxin-like chemical formation, such as PCDD/Fs and/or PCBs, by reducing the release of these substances to the environment. The H4IIE reporter gene bio-assay was also successfully used to detect the presence of these substances in the environmental samples of the platinum mine.

I have assessed certain aspects of platinum mining and processing. Although this investigation has identified the formation and presence of dioxin-like chemicals at certain

stages of mining and processing, not all of the processes were investigated. Some of these processes have the potential to add, and even destroy, these chemicals, affecting potential human exposure and amounts released to the environment. There is therefore a need to investigate the mining processes thoroughly to identify and quantify the possible source processes of dioxin-like chemicals.

Chapter 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study aimed to:

- Determine if POPs, especially the dioxin-like chemicals (PCDD/Fs and/or PCB), are present at a platinum mine;
- Determine if the H4IIE reporter gene bio-assay can be used to assess the environmental and process samples associated with the platinum mine;
- Determine TEQs for each sample using the H4IIE bio-assay;
- Investigate the implications of this source regarding the SC;
- Develop recommendations regarding further research on this potential source.

In summary, it can be concluded that dioxin-like chemical formation processes are associated with platinum mining and processing, but, as far as was investigated, at relatively-low overall concentrations. The reporter gene bio-assay can therefore be used to assess and semi-quantify the environmental samples of the platinum mine by determining the TEQ of most samples. Dioxin-like chemicals were present on platinum mines at various quantities due to many factors influencing the deposition, transport and fate of these chemicals. Temperature processes probably played a major role in the varying TEQ values.

The TEQ_{20} values varied from 0.007 ngTEQ/kg to 54.06 ngTEQ/kg. Dioxin-like chemicals are evident at the thermal process on the mine such as at the smelter (44.62 ngTEQ/kg). This could be explained by *de novo* synthesis in the presence of chlorine together with carbon and a suitable catalyst (possibly copper or even other metals) at temperatures above 300°C in the presence of excess air or oxygen.

The lowest TEQ_{20} value was found in the woodchips (0.007 ngTEQ/kg) that could explain that low concentrations of these substances were already present and that photo-degradation and/or volatilisation, sunlight, wind and solubility of the hydrophobic organic compounds contributed to a much lower concentrations of dioxin-like chemicals at the site.

The project has shown that the H4IIE reporter gene bio-assay can be used for the detection of dioxin-like chemicals of samples related to platinum mining and processing. The method is effective, rapid and highly sensitive and detects low concentrations of contaminants that are of high priority in environmental and process samples. This assay can also be used to assess

toxic impacts on wildlife by providing an indication of potential health risk associated with organisms being exposed to contaminated matrices.

Workers exposed daily to dioxin-like chemicals from the smelter may accumulate these substances in their fatty tissue. This could lead to chronic and acute effects such as chloracne, cancer, weight loss and reproductive problems. By effectively monitoring these substances in various environmental matrices on the platinum mine, and by identifying the formation processes and sites, the formation of these chemicals could be reduced with appropriate mitigation measures. It appears that the risk is minimal if compared to the Seveso incident. However, biological monitoring will be required to substantiate this inference.

5.2 Recommendations

A thorough knowledge of the mining processes is essential for a better understanding of possible contributions to the formation of dioxin-like chemicals. The platinum mining processes should be assessed, since these processes might be possible sources of dioxin-like chemicals. It should also be thoroughly analysed by collecting samples from process materials, air, sediment, soil, vegetation and water. This will aid in determining the TEQs of dioxin-like chemicals at various stages of the mining processes. In-turn, this will enable the assessment of the risks these chemicals might pose to the environment and human health.

Chemical analysis of selected samples should also be considered to determine congener specific concentrations. The method to be used during chemical analysis is HRGC-HRMS. This method is very expensive, time consuming and requires sophisticated instrumentation, but it provides information on dioxin congener distribution and identifies possible sources of contaminants that are responsible for increased TEQ levels. HRGC-HRMS is a very useful risk management tool. By using this technique and biological methods such as the H4IIE reporter gene bio-assay, environmental matrices could be thoroughly assessed. However, the H4IIE bio-assay is a very useful first tier assay that identifies areas that pose a potential dioxin risk to human health and the environment.

During this study, cytotoxicity were observed from some of the samples, but these samples were not fractionated or treated with activated copper, since protocols are currently being investigated. To analyse these environmental samples accurately and effectively, they need to be fractionated to determine which fraction of the sample might contain the dioxin-like chemicals, and treated with activated copper to eliminate any traces of sulphur that might be present in the samples. Sulphur containing samples are cytotoxic to the cells.

The Walkley-Black method was successful in determining the %TOC of each sample. This method is rapid, simple and has minimal equipment needs, but this procedure has been shown to lead to incomplete oxidation of organic carbon and it is particularly poor for digesting elemental carbon forms. To compensate for this, the method of loss-on-ignition could be used.

The H4IIE reporter gene bio-assay was useful in determining if dioxin-like chemicals were present in the environmental and process samples, but the use of several other bio-assays will enable the assessment, detection and semi-quantification of endocrine activity. Many POPs cause endocrine activities and might be found in various abiotic matrices. Further investigation of endocrine activity should take place on the mine. This will aid in the better understanding of the fate, transport, behaviour and effects of these compounds on the platinum mines. The assays capable of detecting compounds that act through, estrogen receptor (ER)-mediated mechanism and androgen receptor (AR)-mediated mechanism. This will help in the further characterisation and evaluation of the toxic potential of the various environmental matrices.

The viability of the H4IIE cells was not tested, but the MTT cytotoxic assay is being implemented. This assay determines the viability of the cells measuring the activity of mitochondrial dehydrogenase.

Environmental quality guidelines for dioxin-like chemicals in different environmental matrices should be developed, since South Africa does not have these guidelines. These guidelines should be developed to protect human health and the environment and be implemented in South African legislation. These guidelines would aid in assessing and determining if a possible source of pollution is of major concern. South Africa should include in its legislation emission limits and tolerable daily intake of these substances.

Awareness programmes on POPs and their health effects are some of the measures that need to be promoted to reduce the release of POPs such as dioxins, which should be aimed at the public, state and industries. The necessary infrastructure should be developed to improve research on POPs, especially dioxin-like chemicals such as PCDD/Fs and PCBs that could be promoted at various institutes in this country. The knowledge gained by researching the behaviour, fate and transport of these chemicals in a Southern African environment will contribute significantly to international science.

The recommendations could be summarised as follows:

- A thorough knowledge of the mining process to identify possible sources of dioxin-like chemicals;
- Chemical analysis of samples;
- Fractionation of the samples to determine which fraction contains dioxin-like chemicals;
- Elimination of sulphur with activated copper to reduce cytoxicity;
- Walkley-Black method used together with the loss-on-ignition method;
- To determine if products and by-products have an influence on endocrine activities associated with platinum mining and processing, using assays capable of detecting compounds that act through, estrogen receptor (ER)-mediated mechanism and androgen receptor (AR)-mediated mechanism;
- Test the viability of the cells;
- Development of environmental quality guidelines to protect human health and the environment. The guidelines need to be implemented in South African legislation;
- To reduce the release of dioxin-like chemicals by educating the public, state and industries; and
- The development of infrastructure to promote future research that would significantly contribute to the international science community.

This research should be considered as contributing towards the aims of the SC.

Chapter 6

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