PERSISTENT ORGANIC POLLUTANTS (POPS) IN THE WATER ENVIRONMENT

Report to the

WATER RESEARCH COMMISSION

by

Claudine Roos[1], Rialet Pieters[1], Bettina Genthe[2], Henk Bouwman[1]

[1]School of Environmental Sciences and Development, North-West University, Potchefstroom, South Africa

[2]CSIR, Stellenbosch, South Africa

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EXECUTIVE SUMMARY

BACKGROUND

According to the National State of the Environment Report (DEAT, 1999), South Africa is a water-poor country with only 8.6% of its rainfall being available as surface water. Groundwater resources are also relatively limited compared to that of other countries. Currently, South Africa's available freshwater resources are nearly fully-utilised and under severe stress. The quantity and quality of available water will increasingly become a limiting resource, and supply will become a major factor restricting the country's future socio-economic development. Many of our freshwater environments (rivers, dams and wetlands) are polluted by industrial, domestic and commercial effluents, sewage, acid mine drainage, agricultural run-off and litter, and many of South Africa's rivers have eutrophication problems. Poor water quality does not only affect associated sediments and aquatic life, but has an effect on terrestrial ecosystems and the economy as well. In 2009, South Africa's agricultural sector nearly suffered a severe knock when possible restrictions were announced on exports of fruit and vegetables to key markets, due to the concerns about produce being irrigated with contaminated water. Polluted water may also pose health threats to recreational and domestic water users.

RATIONALE

The Stockholm Convention on Persistent Organic Pollutants (SC POPs), of which South Africa is a party, carries a number of obligations and expectations. Based on the obligation to develop a National Implementation Plan (NIP), the State is obliged to reduce or terminate all sources of POPs within the SC provisions. This therefore implies that a state should know the environmental levels of these POPs whereby priority sources and hotspots can be targeted for interventions. Since much of this information is either old or completely lacking, research needs to be undertaken.

This study, therefore, focussed on a group of highly persistent, toxic pollutants which is ubiquitous in terrestrial and aquatic environments all over the world. Here, we characterised the scale and significance of certain organic pollutants (OPs), especially persistent organic pollutants (POPs) in selected water bodies of South Africa, specifically targeting sediments as matrix, which are the main reservoirs of these pollutants in aquatic environments. POPs are highly stable, toxic, hydrophobic and lipophilic compounds, with the ability to accumulate in biological tissues.

Previous Water Research Commission (WRC) funded research showed that dioxin-like POPs are present in the aquatic environments of South Africa, with the highest concentrations of these substances measured in industrialised areas. This study was designed to get an overall picture of presence and distribution of the original 12 POPs of the Stockholm Convention. This study also concluded that of all media that can be sampled (water, sediment, fish, and other biota), sediment was the best, as not all the others were always present, ideal, or recoverable. The current study concept and design was based on the completed WRC project mentioned above, thereby using the capacity, infrastructure, and experience gained.

AIMS AND OBJECTIVES

The main aim of this study was to assess the scale and significance of certain OP and POPs pollution in South African waters.

Objective 1

To assess with higher confidence the scale and significance of the occurrence of POPs in the water environment in South Africa, the potential short-term and long-term impacts on water resources and water-linked ecosystems and the associated threats to sustainability of water resources and water use.

Objective 2

To better identify and quantify the fate and effect of selected POPs in the water environment.

Objective 3

To guide and inform the development of appropriate policy and regulatory measures that will support implementation of the requirements of the Stockholm Convention, and substantially contribute to the protection of water resources and water-linked ecosystems with regard to POPs.

METHODOLOGY

Phase I of this study aimed at investigating the extent of polychlorinated dibenzo-*para*-dioxin (PCDD), polychlorinated dibenzo-furan (PCDF) and polychlorinated biphenyl (PCB) pollution in the Vaal Triangle, by targeting aquatic sediments and biota. Sediment and fish samples were collected from selected rivers in the Vaal Triangle area and screened for the presence of dioxin-like compounds (DLCs) by use of the H4IIE-*luc* reporter gene bio-assay. This bio-assay is a rapid, sensitive and relatively cost-effective method, which measures the effects of dioxin-like compounds on rat hepatoma cells, transfected with the firefly luciferase gene.

Selected samples were analysed with gas chromatography/mass spectrometry (GC/MS) to confirm results.

The levels of DLCs at the majority of the sites were below the detection limit of the assay. It was not so for sites 4, 9, and 21. The levels of DLCs at sites 4 and 9 exceeded many of the European and USA quality guidelines proposed for sediments. No DLCs were found in fish tissues. The absence of PCDD/Fs and PCBs in aquatic sediments and fish tissues from the Vaal Triangle area might be due to the climatic conditions of the area, dilution effects in streams, and degradation of these compounds by UV-radiation and microbial organisms.

Phase II of the project focussed on a broader spectrum of compounds, which included various organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), non-dioxin-like PCBs and polybrominated diphenyl ether, in addition to DLCs. Sampling regions included the mainly industrial cities – Cape Town, Richards Bay, Durban and Bloemfontein. Low-income high-density residential areas surrounding a wetland in Soweto/Lenasia and Botshabelo were also included. Rivers flowing into neighbouring countries, rivers in the vicinity of paper and pulp producers and high altitude rivers were also included.

Sediment sampled from these sites was firstly screened for the presence of DLCs using the H4IIE-*luc* bio-assay. Sites eliciting quantifiable responses were selected for further chemical analysis. Of the 96 sites, only 23 had quantifiable levels of DLCs. These sites were mainly of industrial, semi-industrial or low-income residential origin. PAHs were the predominant compound at most of the sites, while OCPs and PCBs were present at intermediate concentrations and PBDEs at minor concentrations. The concentrations of compounds measured at South African sites were generally intermediate when compared to concentrations measured elsewhere in the world, but the normalised concentrations of certain compounds at some of the sites exceeded the Canadian sediment quality guidelines.

CONCLUSIONS

All of the compounds tested for were found in South African sediments; however not all of them were detected at all sites. The levels of DLCs in soils and sediments were generally low, with only 23 of the 96 sites eliciting quantifiable responses when screened with the H4IIE-*luc* bio-assay. Of the 23 sites, 77% was of industrial or semi-industrial origin, 15% was industrial-residential combinations, 6% was high-density low-income residential areas and 2% was residential-agricultural combinations. TOC content, seasonal and meteorological conditions (high temperatures, low precipitation and long summers), photodegradation, sedimentation shifts, effects of dilution, and degradation by micro-organisms were identified

as the possible causes for the low levels of DLCs in the South African environment. A loss in cell viability caused by the cytotoxicity of some of the samples could also have contributed to reduced relative light/luminescence units (RLUs).

Chemical analyses results indicated that PAHs were the most abundant of all the groups of compounds investigated, and were present at the highest levels of all the compounds analysed. OCPs and PCBs were present in intermediate concentrations, while PBDEs were the least abundant and present in the lowest concentrations. Aldrin and chlordene were not detected at any of the sites, whereas nonachlor, chlordane and oxychlordane were present at only a few of the sites in minor concentrations. HCB, HCH and DDT were the predominant OCPs, while heptachlor and mirex were present in lower concentrations. This might be ascribed to the fact that HCB is still produced for industrial applications, and HCH and DDT are presently applied as pesticides in some parts of the country, while the use of heptachlor and mirex has been banned.

In general, 4-ringed PAHs were the most abundant, followed by 5-ringed congeners and finally either by 3- or 6-ringed congeners. Two-ringed PAHs were the least abundant. HMM-PAHs are less susceptible to biodegradation and loss from soil or sediment, which may explain the high prevalence of these congeners. CB-153, -138, -118 and -101 were the major PCB congeners in South African sediments. The lighter PCBs were less abundant, because they are rapidly biodegraded in the environment. PBDEs were generally present at low concentrations, with BDE-153 being the predominant PBDE.

Principal component analysis (PCA) distinguished between two main groups of chemicals – OCPs and industrially associated compounds. Separate PCAs for each group of compounds illustrated strong associations between:

- Lim 4 and *o,p'*-DDT indicating current use of the pesticide,
- D14 and the metabolites, DDE and DDD, indicating historic use of DDT,
- Some S/L sites and heptachlor,
- RB1, 3 and 4 and HCH and HCB,
- BO4, Lim4 and PBDEs,
- RB 1, 3 and 4, BF7, D14 and the lighter PCBs,
- CT8, S/L8 and the heavier PCBs,
- S/L 1, 4 and 8 and the lighter PAHs and
- Croc1b, RB2, Lim4 and the heavier PAHs.

Additionally, An(An+Ph) and Fl(Fl+Py) ratios indicated that the PAHs at the majority of the sites were of pyrogenic origin, or neither solely pyrogenic or petrogenic ("mixed origin").

The normalised concentrations (1% TOC) of the compounds of interest at a few of the sites exceeded the Canadian sediment quality guidelines. The concentrations of OCPs and PCBs were generally below the proposed sediment quality guidelines, while the concentrations of PAHs and DLCs at many of the sites exceeded the guidelines.

The concentration of pollutants measured in South African soils and sediments were intermediate when compared to the levels measured in some European, Asian and Scandinavian countries, with the exception of a few sites where exceptionally high levels of certain compounds were measured.

A screening risk assessment determined whether possible human health effects might be anticipated based on chemical contaminants detected in sediments. In order to determine whether this is possible, a human health risk assessment was conducted modelling the chemical contaminant concentrations expected in fish based on levels detected in sediments. Trans-media calculations (sediment to fish) were conducted based on individual chemical parameters as described in the particular sections.

The screening risk assessment identified the chemicals that could be responsible for adverse health effects if fish were to be eaten over a 30 year period. Although not present at the highest concentrations, the chemicals that were of principal concern were identified as benzo(a)pyrene, total PCBs, and heptachlor. The type of adverse effect that might result was also identified as predominantly carcinogenic, with no toxic effects being anticipated, as the predicted doses were well below those considered safe by the WHO and US EPA. Therefore, this screening risk assessment highlighted that possible health risks can be anticipated resulting from ingestion of fish on a regular basis. However, these risk estimates were based on maximum concentrations detected for each chemical to represent reasonable maximum exposure and identify areas of concern. This has highlighted the need for more detailed analyses.

There are many uncertainties in any health risk assessment, and this study presents a screening or rapid human health risk assessment. Seasonal and spatial variations are not considered as the various sampling points were tested on only one occasion. This was used to provide an indication of potential health risks and should be investigated in more detail.

In addition to sample variation, dose calculations also represent uncertainty, based on the assumption of the number of times a year that people eat fish and the amount of fish eaten. This again illustrates that a more detailed study is needed to improve on the certainty of the result of the risk assessment, especially for the identified sites.

The aims and objectives of Phase II were:

- To assess the scale and significance of the occurrence of POPs and other OPs in the water environment in South Africa,
- To better identify and quantify the fate and effect of selected POPs and other OPs in the water environment, and
- To guide and inform the development of appropriate policy and regulatory measures that will support implementation of the requirements of the SCPOPs, and substantially contribute to the protection of water resources and water-linked ecosystem with regard to POPs, by:
    - Identifying and quantifying selected POPs and other OPs in the water environment,
    - Assessing the levels and distribution of these compounds,
    - Determining the possible sources and releases to the environment, and
    - Assessing the effects on human health to identify communities possibly at risk.

The project has achieved all aims and objectives. In addition, it has also contributed towards the establishment of analytical capacity for these compounds in South Africa by NMISA, an outcome not originally anticipated.

RECOMMENDATIONS

Specific research recommendations

Further investigation is recommended into the sources and levels of certain POPs and organic pollutants at the following sites:

- D14 (Umgeni mouth – possibly receiving effluent from various industries up-stream), where the levels of heptachlor, DDE, DDT and DLCs were all above the ISQGs;
- RB1 and RB2 (Richards Bay industrial sites): At RB1 the ISQGs of several PAH-congeners and γ-HCH were exceeded, while the ISQG and PEL of DLCs were exceeded. At RB2, PAHs and PCBs were present at such concentrations that the ISQGs of these compounds were exceeded, and for γ-HCH and DLCs the PELs were exceeded as well;

- BF6, BF7 and BF8 (Bloemfontein industrial and low-income residential sites): The concentrations of some of the PAH-congeners at BF6 and BF7 where higher than the proposed ISQG, and the DLC concentrations at all three of the sites exceeded the ISQGs and the PELs;
- Lim4 (Low-income residential site in the Limpopo Province – a malaria-endemic area): The ISQGs of DDE, DDT and DLCs were exceeded at Lim4. Lim4 was identified as the only site where South Africa's contribution of POPs into a neighbouring country was substantial. This site should therefore be treated as a high priority.
- Croc1b (near the premises of a paper mill in Mpumalanga): This site had the largest amount of PAH-congeners exceeding the ISQGs, PELs and LELs. The concentration of DLCs at this site was above the ISQG. The sites situated down-stream of Croc1b and closer to the borders of neighbouring countries had less significant concentrations of organic pollutants.

Further investigations are also recommended for the following sites where the highest potential health risks were calculated:

- Soweto/Lenasia wetland sites, S/L1, 6 to 9, and 12;
- Cape Town industrial sites, CT2 and 7;
- Umgeni River mouth in Durban, D14;
- Richards Bay industrial sites, RB1, 2 and 3;
- Bloemfontein sites, BF6, 7 and 8,
- Botshabelo, BO4;
- The international rivers, Olif4 and Croc1b.

General recommendations

- It is recommended that other cell-based bio-assays are performed to determine if sediments are capable of eliciting estrogenic and androgenic responses. MVLN- and MDA bio-assays, where stably transfected human breast cancer cell lines are employed, are suggested.

- It is suggested that sediment samples should in the future be analysed by HRGC/HRMS for the presence of DLCs as well, to compare biologically and chemically derived results.

- The sites identified should be subjected to a much closer scrutiny as to actual POPs sources and distribution. This will provide indications as to how management interventions may abate pollution. This study was only a snapshot of pollutant concentrations as sampled at the time of sediment sampling. Spatial and temporal changes are very likely and nothing is known about this. Time-based sampling of compounds of interest, as identified here, should be conducted in future studies.

- The workshop held on 26 January 2011 with stakeholders on the findings of this project suggested that there should be regular monitoring of problem sites as well as continued and extensive surveys to detect other relevant areas in need of closer monitoring. This monitoring scheme results should feed into the National Toxicity Monitoring Programme (NTMP) of the Department of Water Affairs (DWA). A fairly extensive list of the nature of the matrices to be monitored and/or surveyed was suggested by the workshop delegates. These matrices were selected on the grounds of their usefulness to identify point source and non-point source pollution as well as to determine the levels of these compounds in wildlife and humans and to determine bio-accumulation.

- Attention should also be given to heavy metal levels at these sites as these may pose as significant co-stressors.

- The sites identified as being of concern should also be investigated as to actual levels of pollutants in aquatic organisms such as fish and birds, as well as to actual human fish consumption patterns, and thereby determine human and environmental health risk.

- Stress and effects due to POPs in fish and other aquatic biota at the identified sites should also be investigated.

- The results of this study should be published and, where possible, presented at appropriate fora. Note should also be taken of the appropriate comments on communication in Appendix 2.

- It is recommended that the results from this study be used to guide and inform the development of appropriate policy, development, scientific, and regulatory measures that will support the implementation of the requirements of the SCPOPs and thereby

contribute to the protection of water resources and water-linked ecosystems with regard to POPs and similar organic chemicals. In this regard:

- This report should be considered by the process that is completing the NIP for South Africa.
- This report should also be considered by Provincial and National authorities as well as all other stakeholders.
- Various other stakeholders will also find value from this report as well as the NIP.
- As and when candidate POPs are considered by the SCPOPs, samples from all sites should be analysed so as to inform the SA position during negotiations prior to decisions. The present set of sites seems adequate (although more could be added) for this purpose. Hexabromocyclododecane (HBCD), endosulfan and short-chained chlorinated paraffins are currently on the agenda (in 2011). For some compounds, these are the only data available in Africa.
- Some compounds have almost no representation in data. For instance, PFOA (perfluoro-octanoic acid), also a 'new' POP, has almost no data for the whole of Africa. South Africa can play a major role as scientific leader for the continent in this regard.
- The presence of these compounds, albeit at relatively low general levels, shows that these compounds are present in the environment. However, almost no data, except for some organochlorine pesticides, are available about its levels in humans. Breast milk would make an ideal first matrix to assess the presence, levels, and possible health risks these compounds may pose. Again, South Africa could show scientific leadership.
- The analytical capacity in South Africa and Africa for these compounds is low. Except for the NMISA that developed the techniques to generate the data, few other laboratories seem to have the capacity, willingness, and scope to implement the high-level analytical procedures required. Although there are laboratories that can be capacitated to do so (trained personnel and equipment seems to be available), the financial incentives are not in place for them to add these compounds to their accredited repertoires. The lack of a centralised, well equipped, well funded, and well managed national laboratory for POPs analysis in SA was discussed at length at the workshop (Appendix 2). Special mention was made of the need for well trained staff, and retaining of that staff in the laboratory and in the country (Appendix 2).

- The bio-analytical technique employed in this study was successful in determining those samples that should go for dedicated chemical analysis. The costs would otherwise be prohibitive. The capacity and trained personnel developed via this study should be nurtured and encouraged to fulfil a similar role in future projects. There is also good scope to add additional bio-analytical techniques. The workshop participants also suggested inclusion of testing representatives of various trophic levels (Appendix 2). It is also conceivable that the bio-analytical technique can also be considered as a technique within a regulatory context.
- As there is no centralised entity in South Africa dedicated towards following scientific and international developments regarding chemical pollution issues, the establishment of such a function should be considered. Such a function could *inter alia* pro-actively inform policy, determine knowledge gaps and, identify scientific needs.
- South Africa has no sediment quality guidelines. Sediment quality guidelines of the compounds considered here as well as others such as heavy metals may be developed based on our data as well as others that are or may become available. However, the international SQG that we have used here may be used in the interim for comparative purposes.
- Although source reduction would be of prime importance in mitigation, there may be sites that would require remediation. However, this would entail an in-depth assessment of each site, as well as following international guidelines on remediation. These guidelines are currently in development by the Stockholm Convention and associated agencies.

Additional workshop recommendations

The recommendations below were suggested by the workshop:

- One of the needs identified is for a national scientific society/forum on POPs, that might be affiliated with existing associations/societies. Another suggestion was the creation of a national committee consisting of representatives from DWA, WRC, DEA, DoH, DoA, RQS, Defence Force, and scientists that could inform Parliament on POPs, recent international research findings, and new developments regarding POPs in SA (Appendix 2).

Another big gap that needs to be filled by such a committee would be to address communication of POPs-related issues – from informing communities at risk of POPs exposure, to educating through various printed and electronic media.

Delegates from the workshop were unanimous that the effectiveness of such a national committee would very much depend on a champion that could drive such an initiative.

- There is a need for a national database on all POPs related research in SA. The location of such a database needs to be determined.

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Mr HM du Plessis	Water Research Commission (Past Chairman)
Mr C Moseki	Water Research Commission (Chairman)
Dr S Jooste	Department of Water Affairs
Mr P Viljoen	Department of Water Affairs
Mr B Madikizela	Department of Water Affairs
Ms S Bollmohr	Department of Water Affairs
Dr P Kempster	Department of Water Affairs
Dr D Odusanya	Department of Water Affairs
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Parts of this work have already been published:

Nieuwoudt, C., Quinn, L.P., Pieters, R., Jordaan, I., Visser, M., Kylin, H., Borgen, A.P., Giesy, J.P., Bouwman, H. 2009. Dioxin-like chemicals in soil and sediment from residential and industrial areas in central South Africa. Chemosphere. 76:774-783.

Quinn, L., Pieters, R., Nieuwhoudt, C., Borgen, A.R., Kylin, H., Bouwman, H. 2009. Distribution profiles of selected organic pollutants in soils and sediments of industrial, residential and agricultural areas of South Africa. Journal of environmental monitoring. 11:1647-1657.

Nieuwoudt, C., Pieters, R., Quinn, L,P., Kylin, H., Borgen A,R., Bouwman H. 2011. Polycyclic aromatic hydrocarbons (PAHs) in soil and sediment from industrial, residential, and agricultural areas in central South Africa: An initial assessment. Soil and sediment contamination. 20:188-204.

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ABBREVIATIONS AND ACRONYMS

A

ACGIH	American Conference of Governmental Industrial Hygienists
ADD	Average Daily Dose
AhR	Aryl hydrocarbon receptor
Arnt	Aryl hydrocarbon nuclear translocator
ASE	Accelerated Solvent Extractor
ATSDR	Agency for Toxic Substances and Disease Registry
AWER	Aquatic, Watershed and Earth Resources

B

BF	Bloemfontein (sampling sites)
BFR	Brominated flame retardants
BO	Botshabelo (sampling sites)

C

CBD	Central business district
CCME	Canadian Council of Ministers of the Environment
CCMS	Committee on Challenges of Modern Society
CF	Condition factor
CLRTAP	Convention on Long-range Transboundary Air Pollution
Croc	Crocodile River (sampling sites)
CSIR	Council for Scientific and Industrial Research
CT	Cape Town (sampling sites)
CV	Coefficient of variation

D

D	Durban (sampling sites)
DCM	Dichloromethane
DDA	Bis(dichlorodiphenyl) acetic acid
o,p'-DDD	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
p,p'-DDD	1,1,-Dichloro-2,2-bis(*p*-chlorophenyl)ethane
o,p'-DDE	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene
p,p'-DDE	1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene
o,p'-DDT	1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane

p,p'-DDT	1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane
DEAT	Department of Environmental Affairs and Tourism
DLCs	Dioxin-like compounds
dm	dry mass
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulphoxide
DRE	Dioxin response element
Drknberg	Drakensberg rivers (sampling sites)
DWAF	Department of Water Affairs and Forestry
DWEA	Department of Water and Environmental Affairs

E

EC_{20-80}	Effective concentrations eliciting 20%, 50% and 80% response in cells
ECHA	European Chemical Agency
EDC	Endocrine disrupting chemicals
EU	European Union

F

FBS	Foetal bovine serum
FHAI	Fish Health Assessment Index

G

GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GC-TOF-MS	Gas chromatography-time of flight-mass spectrometer
GDP	Gross domestic product
GHS	Globally Harmonized System
GPC	Gel permeation chromatography

H

HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HEPA	High efficiency particulate air
HEPES	[4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer
HMW-PAH	High molecular weight polycyclic aromatic hydrocarbons
HpCCD/F	Heptachlorodibenzo-*para*-dioxins/furans

HxCB/DD/DF	Hexachlorobiphenyls/dibenzo-*para*-dioxins/dibenzofurans
HPLC	High pressure liquid chromatography
HRGC-HRMS	High resolution gas chromatography high resolution mass spectrometry
HSP	Heat shock proteins

I

IARC	International Agency for Research on Cancer
INCHEM	International Programme on Chemical Safety
ISQG	Interim sediment quality guidelines
I-TEFs	International toxic equivalency factors
IUPAC	International Union of Pure and Applied Chemistry

K

Komati	Komati River (sampling sites)
KZN Riv	KwaZulu-Natal rivers (sampling sites)

L

LADD	Lifetime Average Daily Dose
LC	Lipid content
LEL	Lowest effect level
Lim	Limpopo River (sampling sites)
LMW-PAHs	Low molecular weight polycyclic aromatic hydrocarbons
LOD	Limit of detection
Log K_{ow}	Log of the octanol and water partition coefficient

M

MS	Mass spectrometry
MTT	3-[4,5-dimethyltiazol-2yl]-2,5-diphenyl tetrazolium bromide

N

NADH	Nicotinamide-adenine dinucleotide
NADPH	Nicotinamide-adenine dinucleotide phosphate
NATO	North Atlantic Treaty Organization
ND	Not detected
NILU	Norwegian Institute for Air Research
NIP	National Implementation Plan (of the SC)

NMISA	National Metrology Institute of South Africa
NTMP	National Toxicity Monitoring Programme (South Africa)
NTP	US National Toxicology Program

O

OCDD/F	Octachlorodibenzo-*para*-dioxins/furans
OCPs	Organochlorine pesticides
Olif	Olifants River (sampling sites)
OPs	Organic pollutants
OXC	Oxidisable organic carbon

P

PAH	Polycyclic aromatic hydrocarbons
PBDE	Polybrominated diphenylether
PBS	Phosphate buffered saline
PCA	Principal component analysis
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-*p*-dioxins
PCDF	Polychlorinated dibenzofurans
PEL	Probable effect level
Pongola	Pongola River (sampling sites)
POPs	Persistent Organic Pollutants

R

RB	Richards Bay (sampling sites)
REP	Relative potency
RLU	Relative light/luminescence units

S

SA	South Africa
SCCP	Short-chained chlorinated paraffins
SCPOPs	Stockholm Convention on Persistent Organic Pollutants
SEL	Severe effect level
S/L	Soweto/Lenasia (sampling sites)
SvC	Solvent control

T

TCDD	2,3,7,8-tetrachloro dibenzo-*para*-dioxin
TCDD-EQ	TCDD-equivalent
TEF	Toxic equivalency factors
TEQ	Toxic equivalent quotient
TLTC	Too low to calculate
TOC	Total organic carbon

U

UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
UV	Ultra violet

W

WHO	World Health Organization
WHO-TEFs	World Health Organization toxic equivalency factors
WRC	Water Research Commission
WWTW	Waste water treatment works

1 INTRODUCTION AND OBJECTIVES

1.1 South Africa's water crisis

South Africa is an arid to semi-arid region, receiving a mean annual rainfall of less than 500 mm, compared to a world mean of approximately 860 mm (South African Weather Service, 2009). The Department of Water Affairs and Forestry (DWAF) [now the Department of Water Affairs (DWA)] predicted that the water demand of the country will exceed the water supply by 2040 (DWAF, 1986), but some believe that it might happen by as early as 2025 (Institute for Futures Research, 2009), or even by 2013 (Ogutu, 2007) if the current situation persists. It is not only the quantity of water that is important; the quality of water is of equal significance. Anthropological practices generating a myriad of pollutants could have an immense impact on water quality, and it is therefore vital that this scarce resource is protected, to prevent a severe water crisis from occurring in the country.

In South Africa, many water quality surveys have been conducted, mainly focussing on heavy metals such as copper, arsenic, mercury and lead (Jackson *et al*., 2001; Van Aardt & Erdmann, 2004; Pretorius *et al*., 2001), and other industrial and agricultural pollutants (Gravelet-Blodin *et al*., 1997; Du Preez *et al.,* 2005), but not much is known about organic pollutants (OPs), especially persistent organic pollutants (POPs), in the country.

1.2 Persistent organic pollutants (POPs)

POPs are highly stable, toxic compounds, which persist in the environment by resisting photolytic, biological and chemical degradation. Many POPs can be lethal in high concentrations but their greatest detrimental effects lie in their chronic toxicity, leading to dermal effects, liver and kidney disease, defects of the immune-, reproductive-, nervous-, and endocrine systems and even cancer (Schecter *et al*., 2006). As a result of their lipophilic nature, these pollutants tend to accumulate in matrices rich in organic matter, such as soil, sediment and biota, and can bio-accumulate in food chains (Schecter *et al*., 2006). Their physical and chemical properties enable the compounds to undergo long-range transport, allowing the pollutants to become widely distributed geographically, even to regions where they have never been used or produced (Ritter *et al.,* 2005).

POPs have been a global focus of social and scientific concern, and to take action against these pollutants, the United Nations Environment Programme (UNEP) initiated the Stockholm Convention on Persistent Organic Pollutants (SCPOPs) in May 1995 (UNEP, 2001).

1.3 The Stockholm Convention on POPs (SCPOPs)

The SCPOPs is an international, legally-binding treaty initially focussing on the reduction and elimination of the twelve most harmful POPs, also known as the “dirty dozen” (UNEP, 2001). These POPs include certain chlorinated pesticides [aldrin, chlordane, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), dieldrin, endrin, heptachlor, mirex and toxaphene], two groups of industrial chemicals – hexachlorobenzene (HCB) and polychlorinated biphenyls (PCB), and unintentional combustion by-products known as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (UNEP, 2005a). Recently, the SCPOPs have added more chemicals to their list: α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH (lindane), polybrominated diphenyl ethers (PBDEs), pentachlorobenzene, perfluorooctane sulfonate, chlorodecone, hexabromobiphenyl and octabromodiphenyl ether. A formal body of the Convention, the POPs Review Committee, has several other compounds on their agenda considered for inclusion in the SCPOPs. These include short-chained chlorinated paraffins (SCCPs), and endosulfan, (SCPOPs, 2009). Almost all of these pollutants have been banned in most countries of the world, although the use of DDT still occurs in developing countries, among them South Africa, for the control of pests. There are, however, many sites, which are already contaminated with these chemicals that continue to release POPs into the environment (UNEP, 2005a).

The SCPOPs is best understood as having five essential **objectives** or **aims**:

- The first aim of the Convention is to **terminate** the **release and use** of the POPs included in the SCPOPs. The Convention **bans and limits** the **production and use** of the **intentionally produced POPs** and it aims at **reducing releases** of the **unintentionally produced POPs,** which are formed as by-products of combustion and industrial processes (UNEP, 2005a).

- Secondly, the Convention **supports** the **replacement** of **harmful POPs** with **safer**, **cost-effective alternatives**. This process may pose a challenge to developing countries, as they may lack the financial and technological resources to use and manufacture less threatening chemicals and develop new techniques. The Convention calls on developed nations to share their knowledge and lend financial support to developing countries in aiding their transition to more suitable alternatives (UNEP, 2001).

- In addition to the POPs listed in Section 2.3, there might be other POP-like chemicals that could harm human health and the environment. The third aim of the SCPOPs is to **identify** these **additional POPs** and to aim at the reduction and elimination of these substances (UNEP, 2005a).

- Fourthly, the Convention aims at **cleaning up stockpiles** and **equipment containing POPs**. Stockpiles and waste sites should be identified and managed in an environmentally safe manner (UNEP, 2005a).

- The fifth aim of the Convention is to **increase public awareness** and **provide information regarding these pollutants** through educational programmes and other national action plans. The Convention calls on industries, public interest groups, politicians and scientists to work together to establish a global partnership as a component of the SCPOPs (UNEP, 2005a).

South Africa played a major role in the negotiations and implementation of the SCPOPs. The final text of the Convention (SCPOPs, 2009) was successfully negotiated in Johannesburg in December 2000, and on 22 to 23 May 2001 the world's governments held a conference in Stockholm, Sweden and adopted the SCPOPs. South Africa signed and ratified the treaty on 4 September 2002, and the Convention entered into force on 17 May 2004, ninety days after the deposit of the fiftieth instrument of ratification, acceptance, approval or accession by a country to the Convention (Bouwman, 2004). As a party to the Convention, South Africa is legally obligated to abide by the objectives of the treaty, and is encouraged to support research on POPs.

Despite the fact that South Africa has the largest economy and industrial base in Africa (World Bank, 2009), the levels of OPs are not well known. Some research has been done on the pesticide POPs, such as DDT (Bouwman *et al.,* 2006), and the intentionally produced PCBs (Greichus *et al*., 1977; Grobler *et al.,* 1996), but there is still very much to learn about POPs in the country. This study will therefore contribute towards a better understanding of POPs and other OPs in major South African waters.

1.4 Scope and aims of the study

The main aim of this study was to assess the scale and significance of certain OP and POPs pollution in South African waters.

The motivation for the initiation and the aims of the two phases of the WRC POPs II project were as follows:

1.4.1 Phase I

A previous WRC-funded study, "Survey of certain persistent organic pollutants in major South African waters" (WRC Report no 1213/1/05; Vosloo & Bouwman, 2005), focussing on the levels of dioxin-like compounds (DLCs) in South African sediments, indicated that PCDD/Fs and PCBs were present in selected aquatic environments throughout the country (Vosloo & Bouwman, 2005). Of the 22 aquatic sites included in the study, the highest levels of DLCs were measured in the Vaal Triangle region, Gauteng. Therefore, this project, WRC POPs II, Phase I focussed on determining the levels of DLCs in selected waterbodies of this area.

It is important to determine the extent of dioxin-like pollution in the Vaal Triangle area, since the rivers of this region drain into the Vaal Dam (27°00' S, 28°19' E), which provides potable water for the region. This means that a large number of people may be exposed to dioxin-contaminated water, and some even to contaminated fish.

The aim of the present study was to do a more comprehensive investigation of dioxin-like persistent organic pollution in the Vaal Triangle area.

The objectives of Phase I were:

- To gain a better understanding of dioxin-like pollution in the aquatic environment of the Vaal Triangle region by determining the presence of these pollutants in sediment and fish tissue.
- To quantify the amount of PCDD/Fs and PCBs in sediment and fish tissue by calculating TCDD-equivalents using the H4IIE-*luc* reporter gene bio-assay.
- To determine bio-accumulation of PCDD/Fs and PCBs in biota by comparing the quantities of DLCs in sediment and fish tissue to one another.
- To compare TCDD-equivalent values, obtained with the H4IIE-*luc* bio-assay, with results obtained from chemical analysis, as an additional measure to confirm the levels of DLCs measured with bio-analysis.

1.4.2 Phase II

The second phase of this project focussed on determining the levels of various OCPs in sediments of industrial and high-density low-income residential areas in South Africa. These areas were identified as priority areas for Phase II of this project, since the results of previous studies and Phase I have indicated the presence of DLCs and certain other POPs and OPs in South African soils and sediments, with the highest levels of pollutants mainly associated with industrial and high-density residential areas (Vosloo & Bouwman, 2005; Nieuwoudt, 2006; Nieuwoudt *et al*. 2009; Quinn *et al*., 2009).

The analytes included in the study were the organochlorine pesticides (OCPs) – hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), aldrin, heptachlor, chlordane and its metabolites oxychlordane and chlordene, nonachlor, mirex, DDT and its metabolites DDD and DDE; and the industrially associated pollutants – PBDEs, PCBs, DLCs (PCDDs, PCDFs and dioxin-like PCBs) and PAHs. For some of the compounds that were measured, such as the brominated flame retardant, PBDE, they were the first such analyses done for sediment in South Africa. The majority of the pollutants analysed (except for PAHs) are classified as POPs or emerging POPs by the SCPOPs. Although PAHs are not listed in the SCPOPs, they are included in the Convention on Long-range Transboundary Air Pollution (CLRTAP) and the sixteen most frequently-occurring and/or dangerous PAHs are classified as priority pollutants by the United States Environmental Protection Agency (US EPA) (Zhang & Tao, 2009). Even though South Africa did not ratify the CLRTAP, we should aim to reduce our emissions of these troublesome pollutants.

The aims and objectives of Phase II were:

- To assess the scale and significance of the occurrence of POPs and other OPs in the water environment in South Africa,
- To better identify and quantify the fate and effect of selected POPs and other OPs in the water environment, and
- To guide and inform the development of appropriate policy and regulatory measures that will support implementation of the requirements of the SCPOPs, and substantially contribute to the protection of water resources and water-linked ecosystems with regard to POPs, by:
    - Identifying and quantifying selected POPs and other OPs in the water environment,
    - Assessing the levels and distribution of these compounds,

- Determining the possible sources and releases to the environment, and
- Assessing the effects on human health to identify communities possibly at risk.

2 LITERATURE REVIEW

2.1 Organochlorine pesticides (OCPs)

Of the OCPs included in Phase II of the study, only HCB, HCH, heptachlor, mirex and DDT were present at measurable levels at the majority of the sites. Aldrin and chlordene were not detected at any of the sites, while nonachlor, chlordane and oxychlordane were present at only a few of the thirty sites, at insignificant concentrations.

In the following sections the physical and chemical properties, sources, environmental fate, and toxicity of HCB, HCH, heptachlor, mirex and DDT will be discussed briefly. Although the majority of this study's sampling sites were situated in areas where these OCPs have not been applied for many years, residues may still remain due to the highly persistent nature and long-range transport of these chemicals.

2.1.1 Hexachlorobenzene (HCB)

Until 1965 HCB (C_6Cl_6) was widely used as a fungicide on the seeds of onions, sorghum, wheat and other grains. It was also used in the production of fireworks, ammunition and synthetic rubber (Sala *et al*., 2001). Currently, its production is banned in most countries and it is included in the SCPOPs (UNEP, 2005a).

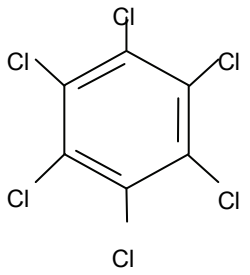


Figure 2.1. The chemical structure of HCB.

2.1.1.1. Physical and chemical properties

HCB is an organochlorine compound (chlorinated hydrocarbon) (Fig. 2.1) that is widespread in the environment, highly lipophilic and bio-accumulative. Technical agricultural grade HCB contains 98% HCB, 1.8% pentachlorobenzene and 0.2% tetrachlorobenzene (ATSDR, 2002a). It has a molecular mass of 284.76 and is practically insoluble in water (0.005 mg/l). HCB has a vapour pressure of 2.3 x 10^{-3} Pa at 25°C and a log K_{ow} of 3.93 to 6.42 (ATSDR, 2002a).

2.1.1.2. Sources

Although its use as a fungicide was banned in the 1960's, HCB is still used as an industrial chemical and it is an unintended by-product of several processes, such as the production of chlorinated solvents (Bailey, 2001). Presently, the major sources of HCB are emissions from metal industries, combustion processes and chemical processes such as

perchloroethylene-, chlorobenzene- and chlorinated organics production (Euro Chlor, 2002). It is also a trace contaminant in certain pesticides or may remain in the environment due to historic use as a fungicide (ATSDR, 2002a).

2.1.1.3. Environmental fate

Since HCB is lipophilic and highly persistent, the compound is relatively stable in soil with half-lives ranging from 2.7 to 7.5 years (Augustijn-Beckers *et al*., 1994). The compound may be degraded aerobically and anaerobically, but its low water solubility causes HCB to have a low mobility in the soil environment. Once in the aquatic environment, HCB is broken down rapidly. Experimental results on hydro-soil have shown almost complete degradation of HCB to pentachlorophenol and related compounds in less than 5 days (Augustijn-Beckers *et al*., 1994). Breakdown in vegetation also seems to be rapid (only 1% of initial amount remaining after 15 days) (Beall, 1976).

2.1.1.4. Toxicity

The most prominent health effects caused by HCB are reproductive toxicity. Cam (1963) and Jarrel and Gocmen (2000) have reported on the effects of HCB on a Turkish population accidentally exposed to high levels of HCB-treated seeds. Children that were breastfed by mothers exposed to HCB developed a lethal disease, “Pembe yara”, or porphyria, which included blistering or scarring of the skin, light sensitivity, skin infection and osteoporosis (Edwards *et al*., 1991). It was also reported that there were some villages without children below the age of 5 years, which would qualify HCB as one of the most potent reproductive toxicants. While some human reproductive health studies have shown a positive correlation between HCB exposure and spontaneous abortion, decreased birth mass, decreased crown-rump length and reduced gestational length (Jarrel *et al.*, 1998, Schade & Heinzow, 1998; Fenster *et al.*, 2006), others have reported no or non-linear associations (Gladen *et al*., 2003; Sagiv *et al*., 2007; Khanjani & Sim, 2006). Although no neurological symptoms have been reported for humans, rodents exhibit symptoms such as tremors, paralysis, muscle incoordination, weakness and convulsions at high doses of HCB exposure (Edwards *et al*., 1991).

Increased lung-, thyroid-, liver- and spleen tumours were noted in animals chronically exposed to HCB, but the potential for the chemical to cause carcinogenic effects in humans is not known (Edwards *et al*., 1991; ATSDR 2002a).

2.1.2. Hexachlorocyclohexane (HCH)

HCH ($C_6H_6Cl_6$) was previously used as an insecticide to control cotton insects, leaf hoppers, stem borers and wireworms on cotton, cereals, sugar beets, oilseed and hardwood logs (INCHEM, 2001). It is also used to treat head and body lice and scabies. The technical product consist of a mixture of isomers (α-, β-, γ-, δ- and ε-HCH) (Fig. 2.2) of which γ-HCH (also known as lindane) is the major component and the only isomer that possesses insecticidal activity (Willet *et al*., 1998). Although not initially included in the SCPOPs, the α-, β- and γ-isomers are now considered as "emerging POPs" (UNEP, 2005a) and should therefore eventually be banned.

2.1.2.1. Physical and chemical properties

HCH is a cyclohexane substituted by six chlorine atoms. The compound is volatile, hydrophobic and bio-accumulative (ATSDR, 2007a). Technical HCH is a white or yellowish powder or solid flakes with a persistent musty odour (INCHEM, 2001). It consists of approximately 40-45% of the γ-isomer, and 20-22%, 18-22%, 4% and 1% of the δ-, α-, β- and ε-isomers, respectively (INCHEM, 2001). The isomers are differentiated by variations in the axial-equatorial positions of chlorine around a ring of 6 carbons (Willet *et al*., 1998) (Fig. 2.2). Compared to other OCPs, HCH is more water soluble and more volatile (Table 2.1).

Table 2.1. The physical and chemical properties of α-, β- and γ-HCH (adapted from Willet *et al*., 1998).

HCH-isomer	Molecular mass	Water solubility (mg/l)	Vapour pressure (mmHg)	Log K_{OW}
α -HCH	290.8	10	0.02	3.80
β -HCH		5	0.005	3.78
γ-HCH (lindane)		7.3	9.4×10^{-6}	3.61-3.72

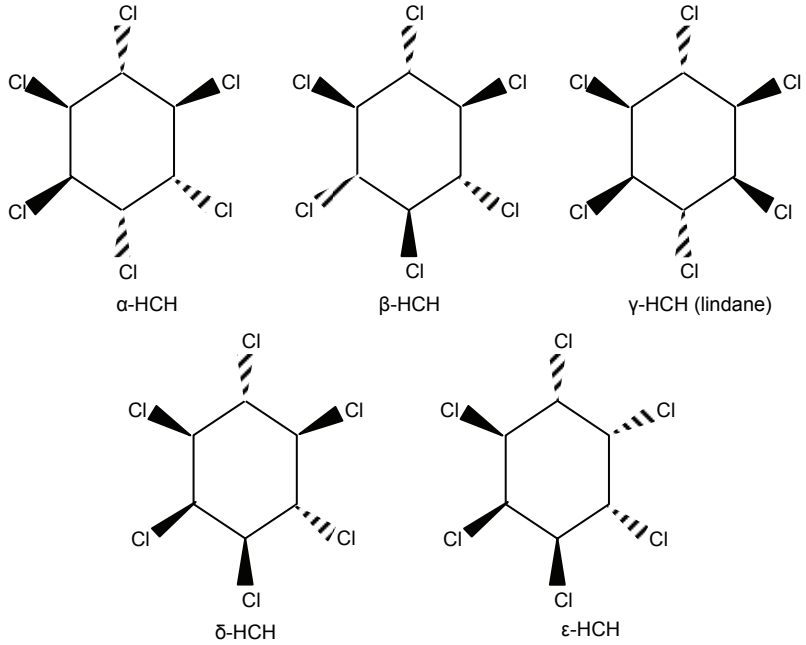


Figure 2.2. The chemical structures of the HCH-isomers.

2.1.2.2. Sources

Despite the ban on technical grade HCH and restricted use of lindane, its residues are still entering the environment. HCH still remains in the environment due to its extensive historical use, present use of lindane (for the control of lice and mites) in several countries, unused stockpiles from earlier manufacturing, as well as leachates from waste disposal sites (Bhatt *et al*., 2009; ATSDR, 2007a).

2.1.2.3. Environmental fate

HCH is highly persistent in the environment. In air, HCH can exist in the vapour phase or may be bound to particulate matter, such as soil and dust. It may undergo long-range transport, but the vapour phase is degraded more rapidly than the particulate phase (ATSDR, 2007a). Because of its low polarity, HCH tends to associate with soil and

sediment (Andreu & Picó, 2004) where it is highly persistent (half-life of approximately 15 months), but eventually broken down to less toxic substances by algae, fungi and bacteria. In the aquatic environment, HCH is highly stable and resistant to photodegradation (ATSDR, 2007a; Wauchope *et al*., 1992).

2.1.2.4. Toxicity

γ-HCH is considered to be the most acutely toxic of the HCH-isomers (Smith, 1991). The main target organ of HCH is the central nervous system (CNS). While α- and γ-isomers are CNS stimulants, the β- and δ-isomers are CNS depressants (Gosselin *et al.*, 1984). Acute health effects associated with high level HCH exposure include impairment of the CNS, excitation, clonic and tonic convulsions, increased respiratory rate and hyper-irritability (Smith, 1991). Other health effects associated with HCH exposure include blood disorders, dizziness, headaches, seizures and changes in the levels of sex hormones (ATSDR, 2007a). Although experimental results are contradictory, some tests on animals suggest that lindane and other HCH isomers are "reasonably anticipated to be human carcinogens" (Smith, 1991; ATSDR, 2007a).

2.1.3 Heptachlor

Heptachlor ($C_{10}H_5Cl_7$) was extensively used in the 1960s and 1970s to control termites, ants and soil insects on seed grains and crops. It was also used by exterminators and home owners to kill termites. In South Africa, its registration was withdrawn in 1976 (South African Department of Agriculture, 2008) and its use is currently banned in most countries. Heptachlor is also included in the SCPOPs, but commercial use is still permitted for the control of fire ants in underground power transformers (ATSDR, 2007b).

2.1.3.1. Physical and chemical properties

Heptachlor is a chlorinated dicyclopentadiene (Fig. 2.3). Technical heptachlor consists of approximately 72% heptachlor and about 28% related compounds, such as *trans*-chlordane and *trans*-nonachlor. Its available formulations included dusts, wettable powders, emulsifiable concentrates and oil solutions and it was sold under the trade names Biarbinex, Drinox, Fennotox, Heptox, Termide and Velsicol 104, amongst others (ATSDR, 2007b).

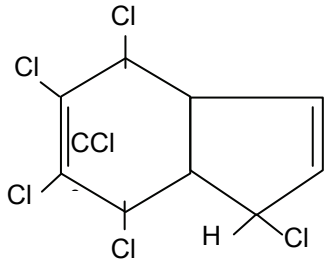


Figure 2.3. The chemical structure of heptachlor.

Heptachlor (molecular mass = 373.32) has a water solubility of only 0.056 mg/l, and it is soluble in acetone, alcohol, benzene, cyclohexanone, kerosene and xylene (Kidd & James, 1991). It has a vapour pressure of 3.99 x 10^{-2} Pa at 25°C, and a log K_{ow} of between 6.1 and 6.13 (Simpson *et al*., 1995).

2.1.3.2. Sources

There are no natural sources of heptachlor, but heptachlor epoxide is formed by abiotic or biotic transformation of heptachlor in the environment (WHO, 2006). As with the other banned OCPs, heptachlor still exists in the environment due to historical use, unused stockpiles and leachates from disposal sites (ATSDR, 2007b). Additionally, heptachlor is also a component in plywood glues and the pesticide, chlordane (which is currently still used for the control of termites) (WHO, 2006).

2.1.3.3. Environmental fate

Heptachlor is broken down to heptachlor epoxide in the environment. The metabolite is more likely to be found in the environment than heptachlor (ATSDR, 2007b) and is resistant to biodegradation, photolysis, oxidation and hydrolysis (Smith, 1991).

Heptachlor and heptachlor epoxide are subjected to long-range transport, and are removed from the atmosphere by wet and dry deposition (WHO, 2006). Soil and sediment are the predominant environmental compartments for heptachlor. Both the mother compound and the metabolite are moderately bound to, and highly persistent in soils and sediments (Augustijn-Beckers *et al*., 1994). Depending on the soil type, half-lives for heptachlor may range between 150 and 290 days (Augustijn-Beckers *et al*., 1994). The major route of loss of heptachlor from soil surfaces is volatilisation. Because heptachlor is almost insoluble in water, it may enter surface waters mainly through surface run-off. In the aquatic environment, heptachlor is rapidly degraded to heptachlor epoxide by hydrolysis and degradation by microorganisms (Augustijn-Beckers *et al.,* 1994). Volatilisation, adsorption to sediments and photodegradation may also contribute towards the loss of heptachlor and heptachlor epoxide from the water environment (Smith, 1991; Matsumura, 1985).

2.1.3.4. Toxicity

Like most OCPs, heptachlor may interfere with nerve transmission (Ecobichon, 1991). The health effects associated with heptachlor epoxide (the main and most persistent metabolite of heptachlor) may be greater than the effects associated with heptachlor. Health effects due to exposure to heptachlor or its metabolites may include hyperexcitation of the CNS,

liver damage, lethargy, incoordination, tremors, convulsions, stomach cramps and coma (Smith, 1991; ATSDR, 2007b). Exposure to heptachlor or heptachlor epoxide may also cause reproductive effects. Animal studies have shown infertility and improper development of offspring in mice and rats (Smith, 1991). Some experiments suggest that heptachlor may promote the development of tumours in rats (Smith, 1991), but evidence is insufficient to assess the potential of heptachlor to cause cancer in humans.

2.1.4 Mirex

Mirex ($C_{10}Cl_{12}$) is a chlorinated hydrocarbon which was used as an insecticide to control fire ants and leaf cutter ants (mostly in South America), mealybugs (Hawaii) and harvester termites (South Africa) (ATSDR, 1995) . Its use was prohibited in 1976 by the US EPA (UNEP, 2005a).

2.1.4.1. Physical and chemical properties

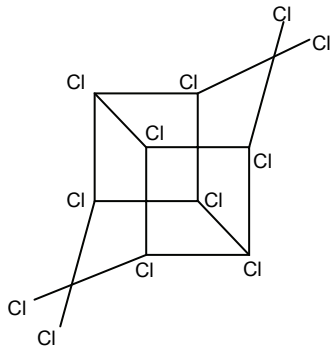


Figure 2.4. The chemical structure of mirex.

Similar to the other OCPs, mirex is highly persistent, toxic and resistant to degradation (ATSDR, 1995). The compound is a white crystalline solid, which is a derivative of cyclopentadiene (C_5H_6). It is odourless, inflammable and practically insoluble in water (0.6 mg/l at 25°C) (Kenaga, 1980). It is, however, soluble in dioxane, xylene, benzene and methyl ethyl ketone (ATSDR, 1995). Mirex has a vapour pressure of 3 x 10^{-7} mmHg at 25°C and a log K*ow* of 5.28 (Verschueren, 1983). The structural formula is given in Fig. 2.4.

2.1.4.2. Sources

Mirex does not occur naturally in the environment, but is produced as a result of the dimerisation of hexachlorocyclopentadiene in the presence of an aluminium chloride catalyst (Sittig, 1980). Although mirex is mostly known for its insecticidal properties, it was also extensively used as a flame retardant in plastics, rubber, paint, paper and electrical equipment (ATSDR, 1995). Its use as an insecticide and fire retardant was banned in the 1970's, but residues of this compound may still remain in the environment due historical use and disposal via accidental spillages, fires and volatilisation from old stockpiles.

2.1.4.3. Environmental fate

Mirex binds strongly with organic matter in soil, sediment and water. When bound to particulate matter, it can be transported far before partitioning. Adsorption and volatilisation are the most important environmental fate processes for mirex, while atmospheric transport may also play a role (ATSDR, 1995). Due to its lipophilic nature (high log K_{OW}) and persistence, mirex is bio-accumulated and bio-magnified in food chains.

The compound is highly resistant to chemical and biological degradation in soil and sediment (half life of >10 years). The primary process responsible for the degradation of mirex (to photomirex) is photolysis (Carlson *et al*., 1976). During anaerobic degradation mirex in soil and sediment is dechlorinated, while aerobic biodegradation plays a minor role (Carlson *et al.*, 1976).

2.1.4.4. Toxicity

Data on human health effects are insufficient. Animal studies have linked mirex exposure to harmful effects on the stomach, intestines, liver, kidneys, eyes, thyroid, nervous system and reproductive system (ATSDR, 1995). In rats, mirex exhibits toxic effects on foetuses, including cataract formation and liver hypertrophy (UNEP, 2002). It is classified as a Group 2B possible human carcinogen by the US EPA, but experimental results are inconclusive.

2.1.5 DDT

Although its use was banned in 1983 (South African Department of Agriculture, 2008), the application of DDT is still permitted in certain parts of South Africa to control the *Anopheles sp* vector of the malaria parasite (Bouwman, 2004).

2.1.5.1. Physical and chemical properties

DDT ($C_{14}H_9Cl_5$), DDE ($C_{14}H_8Cl_4$) and DDD ($C_{14}H_{10}Cl_4$) are organochlorine substances consisting of two attached aromatic phenyl rings with chlorine atoms covalently bonded in the *ortho*- or *para* positions (Fig. 2.5). Commercial DDT is a mixture of these closely related compounds, with *p,p'*-DDT being the principal component (65 to 80%), and *o,p'*-DDT and *p,p'*-DDD being present in smaller amounts (15 to 21%, and about 4%, respectively) (Beard, 2006). In its pure form DDT is a colourless crystalline solid with a weak, chemical odour, and it has been marketed under the trade names Anofex, Cesarex, Chlorphenothane, Didimac, Digmar, Dicophane, ENT 1506, Genitox, Gexarex, Gyron, Ixodex, Micro DDT 75, Neocidol, Pentachlorin, Rukseam, R50 and Zerdane (ATSDR,

2002b). The pesticide is available in several different forms including aerosols, dustable powders, emulsifiable concentrates, granules and wettable powders (ATSDR, 2002b).

DDT has an extremely low volatility, and as reflected by their log K_{OW}, DDT and its metabolites are insoluble in water, making these chemicals persistent in soils and aquatic sediments (Table 2.2) (ATSDR, 2002b). It is highly lipophilic and soluble in most organic solvents, fats and oils, and therefore has the potential to bio-concentrate and bio-accumulate in the environment (Beard, 2006; Zhu *et al.,* 2005).

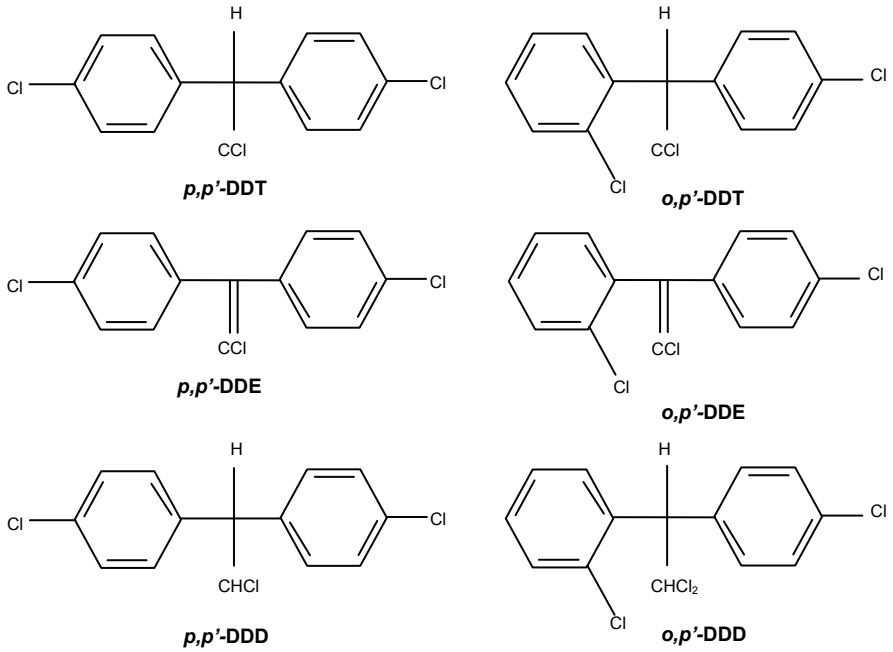


Figure 2.5. The chemical structures of *o,p'*- and *p,p'*-DDT, -DDE and -DDD.

Table 2.2. The IUPAC names and physical and chemical properties of DDT and its metabolites (adapted from ATSDR, 2002b).

Compound	IUPAC name	Molecular mass	Water solubility (mg/l)	Vapour pressure (Pa)	Log K_{OW}
p,p'-DDT	1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)-ethane	354.49	0.025	1.6×10^{-7}	6.91
o,p'-DDT	1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-ethane	354.49	0.085	1.1×10^{-7}	6.79
p,p'-DDE	1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene	318.03	0.12	6.0×10^{-6}	6.51
o,p'-DDE	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene	318.03	0.14	6.2×10^{-6}	6.00
p,p'-DDD	1,1,-Dichloro-2,2-bis(*p*-chlorophenyl)-ethane	320.05	0.09	1.35×10^{-6}	6.02
o,p'-DDD	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane	320.05	0.1	1.94×10^{-6}	5.87

DDT is stable under most environmental conditions and relatively resistant to degradation. Its less toxic metabolite, DDE, has a stability equal to, or greater than, the parent compound. Half lives reported for DDT range between 2 and 15 years for soil and as much as 150 years in the aquatic environment (ATSDR, 2002b; Hooper *et al*., 1997).

2.1.5.2. Sources

DDT is produced by the reaction of chloral (CCl_3CHO) and chlorobenzene (C_6H_5Cl) in the presence of sulphuric acid as catalyst (ATSDR, 2002b). It was first synthesised in 1874 by a chemist named Zeidler, but its insecticidal properties were only discovered in 1939 by P.H. Mueller (WHO, 1979; US EPA 1975). DDT was initially used by the military during the second World War for public health purposes to control malaria, typhus, body lice and bubonic plague (WHO, 1979). In addition to its public health uses, DDT was also applied to a variety of food crops, including beans, cotton, soybeans, sweet potatoes, peanuts, cabbage, tomatoes, cauliflower, corn and other crops (Casida & Quistad, 1998).

Due to the concern over carcinogenicity, bio-accumulation and health effects on wildlife (Kumar *et al*., 2008; Lee *et al*., 2001), the use of DDT is prohibited in most countries, but is still legally manufactured for the use in malaria-endemic areas. In South Africa, the widespread use of DDT was banned in 1983 (DEAT, 2005), but it is currently applied for vector control in confined areas in the northern and eastern parts of Limpopo, the north-eastern parts of Mpumalanga and northern KwaZulu-Natal (Bouwman *et al*., 1992; Sharp & Le Sueur, 1996; Coetzee & Hunt, 1998). Most of the DDT still found in the environment

in areas where use had been banned, are due to the highly persistent nature of the chemical, and potential for long-range transport (Gong *et al*., 2007; Hung *et al*., 2007).

Because the processes used to synthesise DDT and dicofol are similar, dicofol is often contaminated with DDT. Dicofol, a non-systemic acaricide used for the control of mites on crops and orchards, is still registered for use in South Africa, and could therefore be an additional source of DDT contamination to the environment (Clark *et al*., 1990; Qiu *et al.,* 2005).

2.1.5.3. Environmental fate

DDT is highly persistent in the environment, and because it tends to associate with organic matter, DDT is relatively immobile in soils. Routes of loss and degradation in the terrestrial environment include runoff, volatilisation, photolysis and biodegradation (Beard *et al.*, 2000). However, this will only happen over long periods of time (ATSDR, 2002b). DDE and DDD are major metabolites and breakdown products of DDT in the environment. The metabolites are also persistent and their physical and chemical properties are similar to that of DDT (ATSDR 2002b, Table 2.2). DDT released into water adsorbs to particulate matter and sediment is the main sink for DDT in the aquatic environment (Zeng *et al.,* 1999). Its lipophilic property, combined with an extremely long half-life, is responsible for its high potential for bio-accumulation in aquatic organisms, and DDT progressively bio-magnifies in food chains (Ford & Hill, 1991). DDT, DDE and DDD present in water may be transformed by hydrolysis, photodegradation and biodegradation (Coulson, 1985).

DDT gets into the atmosphere as a result of emissions or volatilisation. Volatilisation of DDT, DDE and DDD is known to account for considerable losses of these compounds from soil surfaces and water (Wania & MacKay, 1993). Volatilisation loss will depend on the amount of DDT applied, proportion of soil organic matter, proximity to soil-air interface (depth) and the amount of sunlight (Zhu *et al.,* 2005). In the atmosphere, approximately 50% of DDT is adsorbed to particulate matter and 50% exists in the vapour phase (Bidleman, 1988). Long-range transport of DDT in the atmosphere is dependent on airflow patterns. DDT is removed from the atmosphere by precipitation, wet and dry deposition and diffusion into water bodies, or degraded by photochemically by hydroxyl radical reactions (Woodwell *et al*., 1971).

2.1.5.4. Toxicity

DDT is very slowly transformed to DDE and DDD in the human body. Although DDD is excreted rapidly, DDE and DDT are stored in the fatty tissue, excreted very slowly and

may bring about health effects. DDT and its metabolites are ultimately transformed into bis(dichlorodiphenyl) acetic acid (DDA) and excreted via the urine (ATSDR, 2002b).

Acute effects due to low to moderate exposure to DDT may include nausea, diarrhoea, increased liver enzyme activity, irritation, depression and excitability. Higher doses may lead to tremors and convulsions (Van Ert & Sullivan, 1992). Studies on experimental animals have shown that DDT may cause chronic effects on the nervous system, liver, kidneys and immune system (ATSDR, 2002b). There is also evidence that DDT may cause reproductive effects, due to endocrine disruption (Zeng *et al*., 1999). According to Bornman and co-workers (2009) a study conducted during 2004 to 2006 in the Limpopo Province in South Africa revealed that women who lived in villages sprayed with DDT gave birth to 33% more boys with urogenital birth defects than women in unsprayed villages. Studies on rats and mice have shown decreased embryo implantation, miscarriage and decreased foetal mass as a result of DDT exposure (Chowdhury *et al*., 1990). It appears that DDT may have the potential to cause genotoxic effects in humans. Blood cell cultures of men occupationally exposed to DDT showed an increase in chromosomal damage (ATSDR, 2002b). The evidence regarding the carcinogenicity of DDT is unclear. It has been shown to cause increased production of tumours of mainly the liver and lung in test animals. Significant association between DDT exposure and pancreatic cancers in chemical workers has also been found (ATSDR, 2002b).

DDT has also been shown to have negative impacts on animals, especially birds, where it has directly been linked to eggshell thinning, and it is highly toxic to many aquatic invertebrate species (Beard, 2006).

2.2 Industrially associated organic pollutants

2.2.1. Unintentionally produced organic pollutants

PCDDs ($C_{12}H_{8-x}Cl_xO_2$), PCDFs ($C_{12}H_{8-x}Cl_xO$) and co-planar PCBs ($C_{12}H_{10-x}Cl_x$), collectively known as DLCs, as well as PAHs, are not currently produced deliberately for any purpose except scientific research. They are formed as by-products of incomplete combustion during industrial and thermal processes (Schecter *et al*., 2006). PCBs had been manufactured for industrial purposes since the early 1930s, but their production and use were banned in the 1980s. The deliberately produced non-dioxin-like PCBs (discussed in section 2.2.2) are still released into the environment due to historical use and volatilisation from stockpiles, while dioxin-like PCBs are formed unintentionally similarly to PCDD/Fs (Koppe & Keys, 2001). The physical and chemical properties, sources, environmental fate,

and toxicity of DLCs (PCDD/Fs and PCBs) and PAHs will be discussed in sections 2.2.1.1 and 2.2.1.2.

2.2.1.1. Dioxin-like compounds – Polychlorinated dibenzo-*p*-dioxins (PCDDs), -dibenzo-furans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs)

2.2.1.1.1. Physical and chemical properties

PCDDs and PCDFs are two groups of planar tricyclic compounds with similar chemical structures (Fig. 2.6) and properties. They consist of twelve carbon atoms forming two aromatic phenyl rings, attached to one another by two oxygen bonds in dioxins and by one oxygen bond and one carbon-carbon bond in furans. Both PCDDs and PCDFs may contain between one and eight chlorine atoms in the hydrogen atom position (Fig. 2.6) (Schecter *et al*., 2006). PCBs are aromatic compounds formed by two benzene rings bonded by a single carbon-carbon bond. The hydrogen atoms on the biphenyl molecule may be replaced by one to up to ten chlorine atoms. The two benzene rings can rotate along the carbon-carbon bridge axis, enabling PCBs to assume a propeller-like conformation or a co-planar conformation similar to PCDDs (Fig. 2.6) (Schecter *et al*., 2006). Co-planar PCBs will also be referred to as “dioxin-like PCBs” from here on. Theoretically, there are 75, 135 and 209 possible congeners for PCDDs, PCDFs and PCBs, respectively, but only the seven PCDD, ten PCDF and twelve PCB congeners listed in Table 2.4 (in Section 2.2.1.1.4) are of toxicological interest (WHO, 1997).

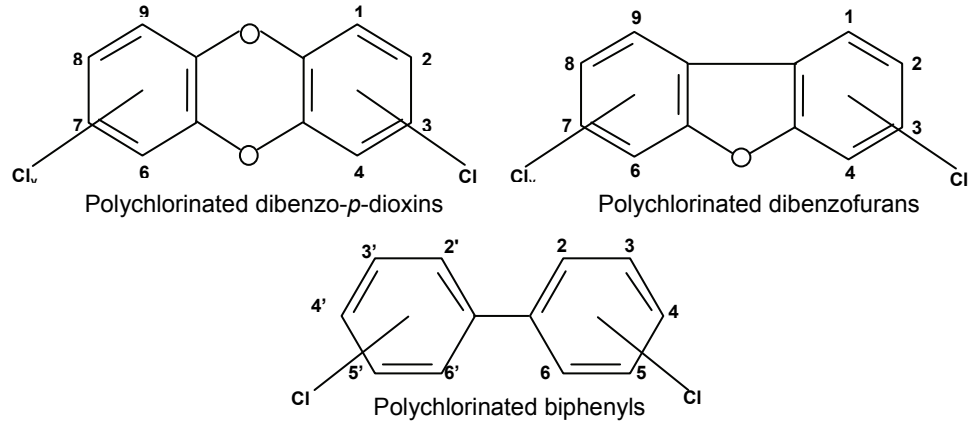


Figure 2.6. The chemical structures of PCDDs, PCDFs and PCBs.

Table 2.3. The physical and chemical properties of PCDD/Fs and PCBs (adapted from Whylie *et al.*, 2003; Henry & De Vito, 2003; Sinkkonen & Paasivirta, 2000; Syracuse, 2007).

PCDD/F and PCB congeners	Molecular mass		Water solubility (mg/l)	Vapour pressure (Pa)	Log K_{OW}
	PCDD	PCDF			
Monochlorodibenzo-*p*-dioxins/ dibenzofurans	218.6	212.6			
Dichlorodibenzo-*p*-dioxins/ dibenzofurans	253.1	237.1			
Trichlorodibenzo-*p*-dioxins/ dibenzofurans	287.5	271.5			
Tetrachlorodibenzo-*p*-dioxins/ dibenzofurans	322	306	2×10^{-10} - 2.78×10^{-1}	1.09×10^{-10} - 5.3×10^{-1}	4.52 - 13.37
Pentachlorodibenzo-*p*-dioxins/ dibenzofurans	356.4	340.4			
Hexachlorodibenzo-*p*-dioxins/ dibenzofurans	390.9	374.9			
Heptachlorodibenzo-*p*-dioxins/ dibenzofurans	425.3	409.3			
Octachlorodibenzo-*p*-dioxins/ dibenzofurans	459.8	443.8			
	PCBs				
PCB 81 - 3,4,4',5-TeCB	292				6.3
PCB 77 - 3,3',4,4'-TeCB	292		1.8×10^{-1}	4.4×10^{-7}	6.0 - 6.6
PCB 126 - 3,3',4,4',5-PeCB	326.43				7.0
PCB 169 - 3,3',4,4',5'-PeCB	326.43		3.6×10^{-5} - 1.2×10^{-2}	4.0×10^{-7}	7.4
PCB 105 - 2,3,3',4,4'-PeCB	326.43		3.4×10^{-3}	6.5×10^{-6}	7.0
PCB 114 - 2,3,4,4',5-PeCB	326.43		1.6×10^{-2}	5.5×10^{-6}	7.0
PCB 118 - 2,3',4,4',5-PeCB	326.43		1.3×10^{-2}	9.0×10^{-6}	7.1
PCB 123 - 2',3,4,4',5-PeCB	326.43				7.0
PCB 156 - 2,3,3'4,4',5-HxCB	360.88		5.3×10^{-3}	1.6×10^{-6}	7.6
PCB 157 - 2,3,3',4,4',5'-HxCB	360.88				7.6
PCB 167 - 2,3',4,4',5,5'-HxCB	360.88		2.2×10^{-3}	5.8×10^{-7}	7.5
PCB 189 - 2,3,3',4,4',5,5'-HpCB	395.32		7.5×10^{-4}	1.3×10^{-7}	8.3

The physical and chemical properties (Table 2.3), and therefore also the fate and toxicity of these compounds, are determined by the number and structural position of chlorine atoms on the molecules. DLCs are generally relatively insoluble in water, sorb strongly to soil and organic matter and have a high potential for bio-accumulation. As the degree of chlorination increases, DLCs become less soluble and volatile, but more lipophilic and strongly associated with organic matter, making the higher chlorinated compounds more stable and persistent in the environment (Mackay *et al*., 1992).

2.2.1.1.2. Sources

DLCs are formed as unintentional by-products of industrial and thermal processes, under the optimal conditions of carbon, oxygen and chlorine availability in the presence of metal catalysts at temperatures ranging from 400 to 700°C. These optimal conditions commonly occur during incomplete combustion processes (Fiedler *et al*., 1996). In developing countries where regulations have not yet been established against all of the potential sources of PCDD/Fs and co-planar PCBs, mixed waste incineration is identified as the largest contributor towards DLC pollution, with the most significant sources being the combustion of municipal, hazardous and medical waste (Ritter *et al*., 2005). In developed countries where regulations to minimise incineration processes and other possible sources of POPs have been established and enforced, uncontrolled combustion processes seem to be the major culprit. A survey conducted by the US EPA during 2002 to 2004 revealed that backyard trash burning was the largest source of DLCs, contributing towards an estimated 56% of the total DLC pollution in the US. The other 44% comprised of incineration processes, paper and pulp industry, residential and industrial wood burning and vehicle exhaust emissions. According to the New York State Department of Environmental Conservation (2004) the lack of control regulations on backyard trash burning attributed towards the problem, emphasising the importance of establishing and enforcing regulations on POPs emissions.

In summary, the most common sources of DLCs include waste incineration, ferrous and non-ferrous metal production, cement production, power generation and heating, mining and the production of mineral products, vehicle exhaust emissions, uncontrolled combustion, chemical- and petrochemical industry and paper and pulp manufacturing. Smaller non-point sources include burning wood in stoves and fireplaces, landfill fires, open burning on the ground, and natural processes such as forest fires and volcanoes (Ritter *et al*., 2005; UNEP, 2005b).

2.2.1.1.3. Environmental fate

PCDD/Fs and dioxin-like PCBs are distributed ubiquitously in the environment (Carey *et al.*, 1998). These compounds are semi-volatile and can occur in both the gaseous and particulate phase in the atmosphere. DLCs are not atmospherically persistent in the vapour phase, but they are persistent when associated with particulate matter, and can be transported over long distances before being removed from the atmosphere by wet and dry deposition (Mackay *et al*., 1992).

In terrestrial and aquatic compartments, DLCs are also associated with particulate matter. In soils, they are bound tightly to the solid phase and are therefore relatively immobile, resistant to degradation and persistent. The more volatile, less chlorinated PCDD/Fs and dioxin-like PCBs are lost from soils to the atmosphere through volatilisation (Gao *et al*., 2005) or may be broken down by photodegradation (Isosaari, 2004). Due to their high soil-water partition coefficients and low water solubilities (Table 2.3), PCDD/Fs and PCBs in aquatic environments tend to associate with organic matter in sediments. Photolysis and biological degradation are the main transformation processes affecting the persistence of DLCs in water, but once associated with sediments, they are more resistant to degradation and persistent (Henry & De Vito, 2003). Aquatic sediments may therefore be an important environmental sink for PCDD/Fs and PCBs. (The degradation of DLCs will be discussed more comprehensively in Results and Discussion).

PCDD/Fs and PCBs can be redistributed from abiotic compartments to bio-accumulate in lipid-rich tissues of biota (Carey *et al*., 1998; Fiedler, 2003). The more chlorinated congeners (tetra- and above) generally have higher bio-concentration and bio-accumulation factors, and the presence of DLCs in human breast milk (Fuerst *et al.,* 1987; Kayama *et al*., 2003; Schecter *et al*., 2002) and in animals at the top of the food chain (Storelli & Marcotrigiano, 2003; Scott *et al*., 2009; Malisch & Baum, 2007) demonstrate that these substances can bio-magnify (Ritter *et al*., 2005). Humans are mainly exposed to PCDD/Fs and PCBs through their diet. Once food is digested, these substances bind to lipoproteins in blood and are transported to different parts of the body. DLCs have a tendency to bio-accumulate in fatty tissues as well as the liver, bone marrow and cerebral tissue of mammals and may cause significant health effects (Carey *et al*., 1998).

2.2.1.1.4. Toxicity

Lateral chlorines (in the 2, 3, 7 and 8 positions) are essential for toxicity (Carey *et al.,* 1998), and the PCDD/Fs and PCBs listed in Table 2.4 is seen as toxicologically important (WHO, 1997). These congeners have assigned toxic equivalency factors (TEFs) to indicate their toxic potencies relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), the most toxic dioxin. TEFs are used to calculate the toxic potential of these DLCs on birds, mammals and fish.

The toxic responses of DLCs are through the aryl hydrocarbon receptor (AhR) inside the cytoplasm of animals (Carey *et al*., 1998). DLCs are lethal in high concentrations but their greatest detrimental effects lie in their chronic toxicity, leading to dermal effects, defects of

the immune-, reproductive-, nervous-, and endocrine systems and even cancer (Schecter *et al*., 2006).

Table 2.4. The IUPAC names of the most toxic PCDD/F and PCB congeners, according to the WHO (1997).

Specific isomers	Compound name	Specific isomers	Compound name
PCDDs		**PCBs**	
2,3,7,8 –TCDD	Tetrachlorodibenzo-*p*-dioxin		
1,2,3,7,8-PeCDD	Pentachlorodibenzo-*p*-dioxin	**Non-*ortho* substituted PCBs**	
1,2,3,4,7,8-HxCDD	Hexachlorodibenzo-*p*-dioxin	3,3',4,4'-TeCB	Tetrachlorobiphenyl (PCB 77)
1,2,3,4,6,7,8-HxCDD	Hexachlorodibenzo-*p*-dioxin	3,4,4',5-TeCB	Tetrachlorobiphenyl (PCB 81)
1,2,3,7,8,9-HxCDD	Hexachlorodibenzo-*p*-dioxin	3,3'4,4',5-PeCB	Pentachlorobiphenyl (PCB 126)
1,2,3,4,6,7,8-HpCDD	Heptachlorodibenzo-*p*-dioxin	3,3',4,4',5,5'-HxCB	Hexachlorobiphenyl (PCB 169)
1,2,3,4,5,6,7,8-OCDD	Octachlorodibenzo-*p*-dioxin		
PCDFs			
2,3,7,8-TeCDF	Tetrachlorodibenzofuran	**Mono-*ortho* substituted PCBs**	
1,2,3,7,8-PeCDF	Pentachlorodibenzofuran	2,3,3',4,4'-PeCB	Pentachlorobiphenyl (PCB 105)
2,3,4,7,8-PeCDF	Pentachlorodibenzofuran	2,3,4,4',5-PeCB	Pentachlorobiphenyl (PCB 114)
1,2,3,4,7,8-HxCDF	Hexachlorodibenzofuran	2,3',4,4',5-PeCB	Pentachlorobiphenyl (PCB 118)
1,2,3,6,7,8-HxCDF	Hexachlorodibenzofuran	2',3,4,4',5-PeCB	Pentachlorobiphenyl (PCB 123)
1,2,3,7,8,9-HxCDF	Hexachlorodibenzofuran	2,3,3',4,4',5-HxCB	Hexachlorobiphenyl (PCB 156)
2,3,4,6,7,8-HxCDF	Hexachlorodibenzofuran	2,3,3',4,4',5'-HxCB	Hexachlorobiphenyl (PCB 157)
1,2,3,4,6,7,8-HpCDF	Heptachlorodibenzofuran	2,3',4,4',5,5'-HxCB	Hexachlorobiphenyl (PCB 167)
1,2,3,4,7,8,9-HpCDF	Heptachlorodibenzofuran	2,3,3',4,4',5,5'-HpCB	Heptachlorobiphenyl (PCB 189)
1,2,3,4,5,6,7,8-OCDF	Octachlorodibenzofuran		

A condition commonly seen in industrial workers chronically exposed to DLCs is chloracne. This condition is perceived as the hallmark of dioxin exposure and can be described as the visible acne-like eruption of blackheads, cysts and pustules occurring on the cheeks, behind the ears, in the armpits and in the groin region (Schecter *et al*., 2006). Other dermal effects, such as hyperpigmentation and hypertrichosis (excessive hair growth) can also be associated with exposure to DLCs.

PCDD/Fs and dioxin-like PCBs are also classified as endocrine disrupting chemical (EDCs). Because they are structurally similar to natural hormones, EDCs can interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones, disrupting a number of natural reactions in various systems (Kavlock *et al*., 1996). By exerting their effects on the endocrine system, DLCs largely affect the reproduction and growth of animals and humans (Birnbaum, 1995). A study on rats has shown that relatively high doses of TCDD and related chemicals can cause testicular and ovarian degeneration. PCDD/F and PCB exposure have also been linked to other reproductive effects such as the inability to maintain pregnancy, decreased fertility,

reduced sperm counts, increased endometriosis, and lowered testosterone levels (Moore *et al*., 1985; Birnbaum, 1995).

Thyroid hormones, which are critical for normal growth and differentiation of cells, also appear to respond to dioxin exposure. DLCs interfere with normal growth regulation by decreasing thyroid hormone levels, causing several developmental deficits in animals and humans (Koppe *et al*., 1991). The developmental effects of PCDD/Fs and PCBs are especially evident on the unborn embryo or foetus (Koppe *et al*., 1991). Studies done on infants from Japan and Taiwan whose mothers consumed DLC contaminated rice oil while pregnant showed premature births, decreases in birth mass, discolouration of the skin and nails, and abnormal teeth and gums. Along with these obvious physical differences, many of the children showed neurological and behavioural changes, such as unresponsiveness and lowered short-term memory, when compared to normal, unaffected children (Koppe *et al*., 1991; Tanabe, 1988).

Other effects of PCDD/Fs and PCBs on the nervous system may include decreases in visual recognition, learning deficits and changes in brain activity, which may lead to depression and personality changes (Corsolini *et al*., 2002). In addition to the neurological effects of PCDD/Fs and PCBs, these substances can have serious effects on the immune system. Studies on Rhesus monkeys, which have immune systems similar to humans and other animals, have revealed that DLC exposure can cause a decrease in the thymus gland, reducing the ability to produce cell-killing T-lymphocytes, which is important for immunity (Brouwer *et al*., 1999).

Finally, some DLCs might have the ability to cause cancer. In 1985, the US EPA characterised mixtures of dioxin-like substances as “probable human carcinogens”, but their reassessment in 2003 concluded that dioxins are better characterised as “likely human carcinogens”. The most toxic and best studied member of the dioxin family, 2,3,7,8-TCDD, is classified as a “known human carcinogen” by the International Agency for Research on Cancer (US EPA, 2008). Although a number of cancerous effects, such as liver, lung and bladder tumours have been reported in animals exposed to DLCs, it is more difficult to prove that these substances cause cancer in humans (Birnbaum, 1995).

2.2.1.2. The polycyclic aromatic hydrocarbons (PAHs)

2.2.1.2.1. Physical and chemical properties

PAHs are a large group of semi-volatile organic compounds consisting of fused aromatic rings in linear, angular or clustered arrangements (Nadal *et al*., 2004) (Fig. 2.7). They have a lipophilic nature and have high affinity for organic matter (Morillo *et al*., 2007). As shown in Table 2.5, individual PAHs differ significantly in their physical and chemical properties with properties such as water solubility and vapour pressure ranging in five and twelve orders of magnitude, respectively (Lundstedt, 2003). Low molecular weight PAHs (LMW-PAHs) are generally more volatile and more water soluble than the high molecular weight PAHs (HMW-PAHs) (Table 2.4). HMW-PAHs, on the other hand, have higher hydrophobicity and lipophilicity, making them less susceptible to degradation and more persistent in the environment (as shown by their Log K_{OW}; Table 2.5).

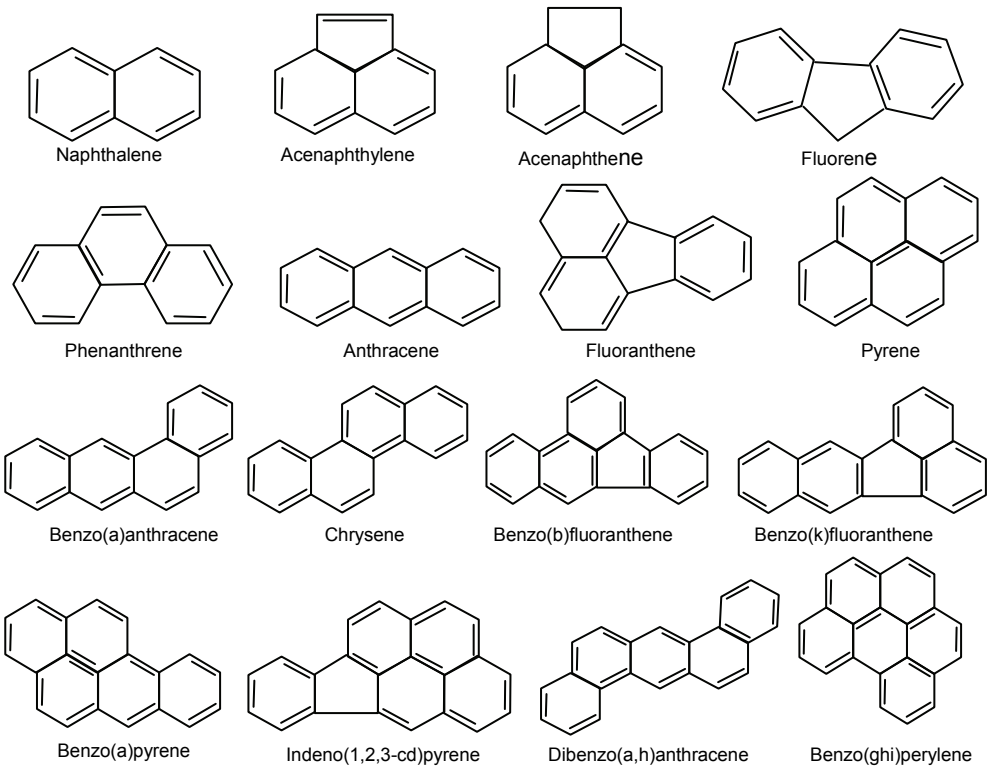


Figure 2.7. The chemical structures of the 16 US EPA PAHs.

There are more than a hundred different PAHs, but for the purpose of this study, we only focussed on the 16 PAHs identified by the US EPA as requiring priority monitoring action within the framework of environmental quality control (Zhang & Tao, 2009). The structural

representations and physical and chemical properties of these US EPA PAHs are shown in Fig. 2.7 and Table 2.5.

2.2.1.2.2. Sources

Anthropogenic activities are generally recognised as the most important source of PAH release to the environment (Nadal *et al*., 2004). The PAHs measured during this study are mainly of *petrogenic* or *pyrogenic* origin. This means that they are generally formed as by-products during processes such as petroleum refining, burning of fossil fuels (mainly crude oil) and other petrochemical processes, or as a result of inefficient combustion of organic materials such as wood, coal and oil. Domestic heating, power generation, incineration or vehicle exhaust emissions are also sources of PAHs. Some of the lighter PAHs such as acenapthene, fluorene and anthracene are also produced from wood treatment using creosote (Masih & Taneja, 2006; Culotta *et al.,* 2006).

Perylene, retene and phenanthrene homologues are generally formed by natural processes such as fires and volcanic eruptions. Some homologues can also be derived from biogenic precursors such as pigments and steroids under anaerobic conditions (Culotta *et al.*, 2006).

Table 2.5. The molecular mass and some physical and chemical properties of the 16 US EPA PAHs (adapted from Lundstedt, 2003, originally from Mackay *et al.,* 1992).

		Molecular mass	**Water solubility (mg/l)**	**Vapour pressure (Pa)**	**Log K_{ow}**
2-ringed PAHs	Naphthalene	128	31	1.0×10^{2}	3.37
3-ringed PAHs	Acenaphthylene	152	16	9.0×10^{-1}	4.00
	Acenaphthene	154	3.8	3.0×10^{-1}	3.92
	Fluorene	166	1.9	9.0×10^{-2}	4.18
	Phenanthrene	178	1.1	2.0×10^{-2}	4.57
	Anthracene	178	0.045	1.0×10^{-3}	4.54
4-ringed PAHs	Fluoranthene	202	0.26	1.2×10^{-3}	5.22
	Pyrene	202	0.13	6.0×10^{-4}	5.18
	Benzo(a)anthracene	228	0.011	2.8×10^{-5}	5.91
	Chrysene	228	0.006	5.7×10^{-7}	5.91
5-ringed PAHs	Benzo(b)fluoranthene	252	0.0015	_	5.80
	Benzo(k)fluoranthene	252	0.0008	5.2×10^{-8}	6.00
	Benzo(a)pyrene	252	0.0038	7.0×10^{-7}	5.91
6-ringed PAHs	Indeno(1,2,3-cd)pyrene	276	0.00019	_	6.50
	Dibenzo(a,h)anthracene	278	0.0006	3.7×10^{-10}	6.75
	Benzo(ghi)perylene	276	0.00026	1.4×10^{-8}	6.50

2.2.1.2.3. Environmental fate

The semi-volatility of PAHs makes them highly mobile through the environment by deposition and re-volatilisation between air, soil, water and sediments (Nadal *et al*., 2004). The LMW two- and three-ringed PAHs are mainly present in gaseous form, while the heavier four- to six-ringed PAHs are largely associated with particulate matter. Both gaseous and particulate forms are transported in the atmosphere over short and long distances (Chen *et al*., 2004). Their semi-volatile nature and aqueous solubility make LMW-PAHs highly available for various degradation processes, volatilisation and leaching. In contrast, HMW-PAHs are less available for degradation because they are primarily associated with particles in the atmosphere and water (Wild & Jones, 1995). In aquatic environments, PAHs rapidly tend to become associated with organic matter in sediments. The degree of sorption generally increases as the number of benzene rings in the PAH molecule increases, since this implies higher lipophilicity (Weissenfels *et al*., 1992). Due to their hydrophobicity and persistence, PAHs may be retained in sediments for many years. Therefore, sediment represents the most important reservoir of PAHs in aquatic environments (Culotta *et al.,* 2006).

Degradation of PAHs in the environment occurs through biological, chemical and photochemical processes. Biological degradation, where microorganisms such as bacteria and fungi transform PAHs to inorganic end products, appears to be the main process responsible for the breakdown of PAHs in soils and sediments (Wilson & Jones, 1993), however, oxidation and photochemical reactions may be equally important degradation pathways (Kochany & Maguire, 1994).

2.2.1.2.4. Toxicity

One of the greatest concerns regarding PAH exposure is the fact that some of these compounds are mutagenic and carcinogenic. Exposure of humans to PAHs may lead to elevated levels of DNA mutation, reproductive defects and an increased risk of cancer and other adverse health effects (US EPA, 2002; Zhang & Tao, 2009). Epidemiological studies have shown that exposure to some PAHs may cause increased incidences of leukemia, bone-, brain-, bladder- and scrotal cancers and adverse pregnancy outcomes (Nadal *et al*., 2007; Mastrangelo *et al*., 1996). Studies done on exposed workers of aluminium smelters and coke ovens have shown increased incidences of lung and bladder cancer (Boffetta *et al*., 1997; Mastrangelo *et al*., 1996; Negri & Vecchia, 2001).

The carcinogenic effects of PAHs depend on the specific structure and physical and chemical properties of the PAH, and HMW-PAHs such as benzo(a)pyrene,

benzo(a)anthracene, dibenzo(a,h)anthracene are more potent carcinogens (Eickhoff *et al*., 2003). The US EPA classified benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene and indeno(1,2,3-c,d)pyrene as Group B2 probable human carcinogens (US EPA, 2002).

2.2.2. Intentionally produced industrial chemicals

PBDEs and PCBs are intentionally produced industrial chemicals. PBDEs are brominated flame retardants (BFR) which are applied to various combustible materials such as plastics, wood, paper and textiles to increase the product's fire resistance (De Wit, 2002). They are additive flame retardants, which are simply blended with the polymers and therefore likely to enter the environment by leaching out of the products (Alaee *et al*., 2003).

PCBs were produced for industrial purposes as insulating materials in electrical equipment, plasticisers (softening materials) in plastic products, hydraulic fluids, adhesives, lubricants, fire retardants and dielectrics in transformers (Koppe & Keys, 2001). Due to their toxicity, the production and use of PCBs were banned in the 1980s, but they are still released into the environment via accidental spillages, fires and volatilisation from old stockpiles (Koppe & Keys, 2001). Co-planar or dioxin-like PCBs are still unintentionally formed in the same way as PCDD/Fs, as described in section 2.2.1, but this section will focus only on the non-dioxin-like PCBs.

2.2.2.1. Polybrominated biphenyl ethers (PBDEs)

2.2.2.1.1. Physical and chemical properties

PBDE ($C_{12}H_{10-x}Br_xO$) consists of two benzene rings linked with an oxygen atom, with one to ten bromine atoms attached to the benzene rings (Hellström, 2000) (Fig. 2.8). Theoretically, there are 209 possible PBDE congeners, divided into ten congener groups (monoBDE to decaBDE). The majority of BFRs are higher brominated (penta to deca) compounds, and PBDEs with less than four bromine atoms are generally not found in commercial PBDE products (Darnerud *et al*., 2001). Technical mixtures of PBDEs include mainly pentaBDE, octaBDE and decaBDE, consisting of different mixtures of tetra- to decaBDE (De Boer *et al*., 2003). Consequently, almost no data are available on mono, di-, tri-, hexa- and nona-BDEs.

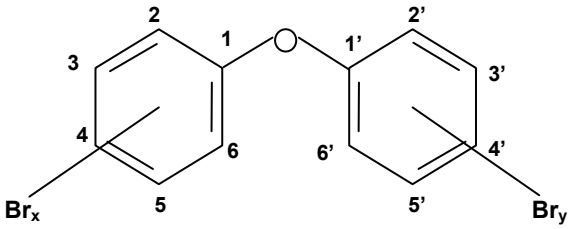


Figure 2.8. The chemical structure of PBDEs.

Table 2.6. The physical and chemical properties of PBDE (De Wit, 2002; Darnerud *et al.,* 2002; Hellström, 2000; Hunziker *et al*., 2004, ECHA, 2008;

Compound	Molecular mass	Water solubility (µg/l)	Vapour pressure (Pa)	Log K_{OW}
PBDEs				
Tetra-BDE	485.8	10.9	2.7 - 3.3 x 10^{-4}	5.9 - 6.2
Penta-BDE	564.8	2.4	2.9 - 7.3 x 10^{-5}	6.5 - 7.0
Octa-BDE	801.5	0.5	1.2 - 2.7 x 10^{-7}	8.4 - 8.9
Deca-BDE	959.2	<0.1	4.6 x 10^{-6} - 5.8 x 10^{-11}	10

PBDEs are extremely hydrophobic and resistant to environmental degradation processes. Because of their low volatility, low water solubility (Table 2.6) and strong adsorption to sediments, the higher brominated compounds are less mobile in the environment (Watanabe & Tatsukawa, 1990). With an increasing degree of bromination, the aqueous solubility and vapour pressure of BDE congeners decrease, while the hydrophobicity increases (Zegers *et al*., 2003) (Table 2.6).

2.2.2.1.2. Sources

PBDE products are produced by brominating diphenyl ether in the presence of a catalyst (De Wit, 2002). The various PBDE technical products are applied to different materials as flame retardants. PentaBDE are mainly used in epoxy resins, phenol resins, polyesters, polyurethane foam and textile. OctaBDE are generally applied to acrylonitrile butadiene styrene, polycarbonate and thermosets; whereas DecaBDE products are used in most types of synthetic materials including textiles and polyester used in circuit boards (De Wit, 2002).

The most evident sources of BFRs into the environment are effluents from plants producing BFRs, flame-retarded polymers and other plastic products (Sellström & Jansson, 1995). Because they are “additive flame retardants”, which are mixed into the polymers and not chemically bound to the plastics or textiles, PBDEs may separate or

leach from the surface of their product applications into the environment. Polyurethane foam and electronic equipment, such as television sets and computers may be significant sources of BFRs (Watanabe & Sakai, 2003). Additionally, the sources responsible for the formation of polybrominated and mixed brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans could also be responsible for the formation of BFRs. Possible emission sources include municipal, hospital and hazardous waste incineration, facilities recycling plastics and metals from electronic equipment, accidental fires and disposal sites (WHO, 1998).

2.2.2.1.3. Environmental fate

Commercial PBDEs are relatively resistant to physical, chemical and biological degradation (Darnerud *et al*., 2001). PBDEs are only slightly volatile (Table 2.6), and have a low potential to evaporate from aqueous surfaces. They have a high adsorption potential to suspended matter, and especially the higher brominated BFRs tend to accumulate in sediments or particulate matter near their sources of emission (ECHA, 2008). The lower brominated compounds, especially the break-down products of PBDE, such as polybrominated and mixed brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans, may on the other hand be transported further from their sources of emission (Watanabe & Sakai, 2003).

While the higher brominated BFRs are persistent in the environment, the lower brominated PBDEs are more susceptible to degradation. Photodegradation and microbiologic degradation are the main means of PBDE break-down, but incineration may also play a significant role (Darnerud *et al*., 2001). Due to their very low water solubilities, hydrolysis is not likely to be a significant route of environmental degradation (ECHA, 2008).

2.2.2.1.4. Toxicity

Although their greater molecular size may inhibit movement across biological membranes, the similarity in molecular structure of PBDEs with that of PCBs and PCDD/Fs, and their resistance to degradation, give rise to concern that they may lead to similar environmental problems and health effects (Allchin *et al*., 1999). Toxicological data have demonstrated that some BFRs may lead to serious health effects, such as thyroidogenic, estrogenic and dioxin-like activities (Bergman & Ulrika, 2001). Studies on mammals have also shown effects on the liver and reduced embryonic development (Hellström, 2000).

Documented effects of PBDE on human health are deficient. PBDEs may cause chloracne and effects on the liver. Deca-BDE has been classified as “possibly carcinogenic to

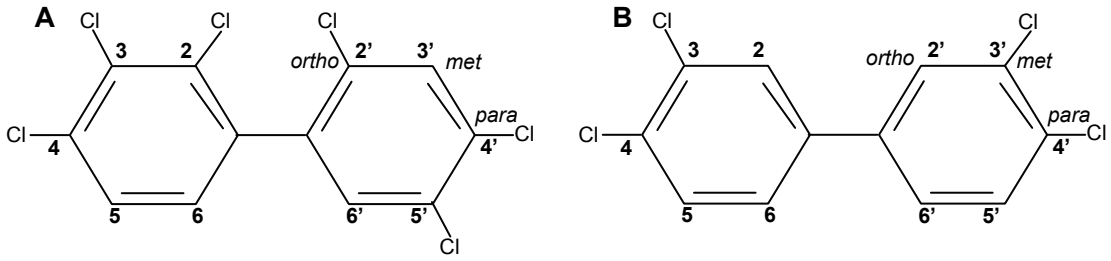
humans" by the US EPA, while tetra-, penta- and octa-BDE has been classified as not carcinogenic (Hellström, 2000). There is evidence linking PBDE exposure to the induction of cancer by a non-mutagenic mechanism (Helleday *et al.*, 1999). PBDE may also have negative effects on the thyroid hormone system and may cause developmental neurotoxic effects such as aberrations in spontaneous behaviour, learning, memory and function (Eriksson *et al.,* 2002).

2.2.2.2. Polychlorinated biphenyls (PCBs)

The 197 PCB congeners which were not listed in Table 2.4 are collectively referred to as "non-dioxin-like", "non-coplanar" or "*ortho*-substituted" congeners, and are discussed a separately from the dioxin-like PCBs because of their deliberate production.

2.2.2.2.1. Physical and chemical properties

Although dioxin-like and non-dioxin-like PCBs have similar physical and chemical properties (referred to in section 2.2.1.1 and summarised in Table 2.3), they can be distinguished in terms of their sources, structure and mechanism of action (Fischer *et al*., 1998). In short, dioxin-like PCBs are unintentionally produced as by-products of industrial processes, while the non-dioxin-like PCBs were purposefully manufactured in the past for industrial purposes (Ritter *et al*., 2005). Regarding their structures, coplanar or dioxin-like PCBs lack *ortho* substitution, whereas non-coplanar PCBs contain chlorine atoms in one or more of the four *ortho* positions (closest to the biphenyl bond) (Fig. 2.9) (Fischer *et al.* 1998). Where coplanar PCBs binds with affinity to the AhR, non-coplanar PCBs are poor ligands for the AhR and the mechanism of their biological effects are in many cases unknown (Rogan & Gladen, 1992).



Non-coplanar/*ortho*-substituted/non-dioxin-like PCBs.

Coplanar/non-*ortho*-substituted/dioxin-like PCBs.

Figure 2.9. The chemical structures of non-dioxin-like and dioxin-like PCBs, with 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138) being representative of non-dioxin-like PCBs (A), and 3,3',4,4'-tetrachlorobiphenyl (PCB 77) being representative of dioxin-like PCBs (B).

Table 2.7. The physical and chemical properties of PCBs (adapted from Whylie *et al.*, 2003; Henry & De Vito, 2003; Sinkkonen & Paasivirta, 2000; Syracuse, 2007).

PCB congeners	Molecular mass	Water solubility (mg/l)	Vapour pressure (Pa)	Log K_{ow}
Monochlorobiphenyl	188.65	1×10^{-5} - 1×10^{-7}	2.1×10^{-4} - 4×10^{-3}	4.30 - 8.26
Dichlorobiphenyl	233.1			
Trichlorobiphenyl	257.54			
Tetrachlorobiphenyl	292			
Pentachlorobiphenyl	326.43			
Hexachlorobiphenyl	360.88			
Heptachlorobiphenyl	395.32			
Octachlorobiphenyl	429.77			
Nonachlorobiphenyl	464.21			
Decachlorobiphenyl	498.66			

In summary, PCB congeners that contain more chlorine atoms and have fewer *ortho* substitutions are less volatile, less soluble in water, have a higher potential to bind to organic matter, and are more susceptible to anaerobic dechlorination processes (Carey *et al*., 1998). These congeners typically have a higher potential for bio-accumulation, and are more abundant in soils and sediments and less abundant in water and the atmosphere (Henry & De Vito, 2003). On the other hand, the congeners that contain less chlorine atoms and have more *ortho* substitution are more volatile, more water soluble and are more readily metabolised by animals. These congeners are therefore more prominent in the atmosphere and surface waters (Henry & De Vito, 2003).

2.2.2.2.2. Sources

PCBs had been manufactured from the early 1930s for industrial purposes. These compounds are chemically stable, resistant to heat, non-flammable and have low vapour pressures and high dielectric constants (Ritter *et al.*, 2005), which made them excellent as insulating materials in electrical equipment, plasticisers (softening materials) in plastic products, hydraulic fluids, adhesives, lubricants, fire retardants and dielectrics in transformers (Koppe & Keys, 2001). Although their production and use were banned, they are still released into the environment as a consequence of historical use and disposal via accidental spillages, fires and volatilisation from old stockpiles (Henry & De Vito, 2003).

2.2.2.2.3. Environmental fate

In the environment, PCBs are always found as mixtures of both dioxin-like and non-dioxin-like PCBs. The environmental fate of PCBs is dependent on their physical and chemical properties (Ritter *et al*., 2005), and are discussed in section 2.3.

2.2.2.2.4. Toxicity

Although the mechanism of toxicity and the toxicological effects of dioxin-like PCBs are well known, it was originally thought that *ortho*-substituted, non-dioxin-like PCBs are biologically inactive, but is now known that they can exhibit toxic effects (Fischer *et al*., 1998). Their exact mechanism of action is uncertain, but it is known that toxic effects of non-dioxin-like PCBs occur via multiple toxicity pathways not involving the AhR (Henry & De Vito, 2003). Studies have shown that certain *ortho*-substituted PCB congeners are partially responsible for neurotoxic effects, which include decreased catecholamine levels in certain regions of the brain and may cause behavioural changes (Shain *et al.,* 1991; Schantz *et al*., 1995) and learning deficits (Chen & Hsu, 1994). Other effects of PCBs include the stimulation of insulin increase (Rogan & Gladen, 1994) and neuroendocrine, endocrine, immunological and carcinogenic effects. Endocrine disruptive effects of non-dioxin-like PCBs include abnormal thyroid gland development, a decrease in serum thyroxine concentrations and various effects on the reproductive system (Brouwer *et al*., 1998).

2.3 Environmental transport of OPs and POPs

Once POPs or OPs (discussed in sections 2.1 to 2.2) are formed, they may be released into atmospheric, aquatic or terrestrial compartments, depending on their emission sources. When present in one of these compartments they can cycle between the others, because their physical and chemical properties allow them to partition between gaseous, liquid and particulate phases (Carey *et al*., 1998). POPs and other OPs may be transported through various routes including ocean currents, rivers, strong winds and migratory animals such as birds, whales, dolphins and salmon, as well as anthropogenic practices such as trade (Krümmel *et al*., 2003). Due to the great volumes involved and their massive movements, oceanic- and riverine transport may contribute significantly in transferring pollutants all over the world. Although the majority of the OPs included in the study are insoluble in water, these substances adsorb onto sediment particles or are taken up by biota, which carry them over long distances (Whylie *et al.,* 2003). Since the majority of POPs are semi-volatile they are capable of long-range transport, leading to their

occurrence in places where they have never been produced (Henry & De Vito, 2003), therefore making POPs a global issue.

In Polar regions where the production and emission sources of POPs are limited, significant levels of these substances are found in breast milk of women and in fatty tissues of dolphins and whales. This phenomenon can be explained by the "cold condensation" or "grasshopper" effects (Corsolini *et al.*, 2002) – as explained by Figure 2.10.

At regions with higher temperatures, POPs evaporate into the atmosphere with ease because of their volatility. Once in the atmosphere, these substances are carried by wind and air currents. When there are alterations in atmospheric conditions; POPs descend to the earth in precipitation. In this process POPs are transferred further north (Fig. 2.10) (Corsolini *et al.*, 2002).The cycle repeats itself and in colder Polar regions POPs descend to the earth as rain or snow. The low temperatures at these locations prevent evaporation, trapping POPs at these regions.

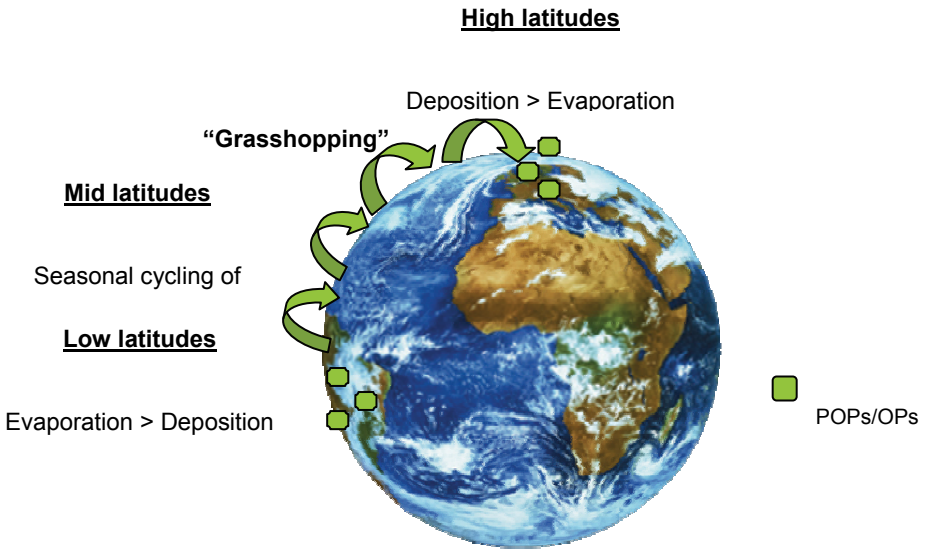


Figure 2.10. A diagrammatic representation illustrating long-range transport of OPs or POPs known as the "grasshopper-effect".

This sequence of events may be repeated several times (Corsolini *et al.*, 2002). During the "grasshopper-effect" a part of the pollutant load may be lost through degradation, deposition in sediment or soil, absorption into vegetation, or algae taking up pollutants (Tysklind *et al.,* 1993).

2.4 Methods used to measure OPs in this study

Presently, gas chromatography mass spectrometry (GC/MS) or high-resolution GC high-resolution MS (HRGC/HRMS) are the most generally applied chemical methods used for the detection and quantification of POPs. The major advantage of GC/MS- and HRGC/HRMS analyses are the techniques' abilities to determine the identity and concentrations of numerous individual chemicals and congeners with precision (Safe, 1995). However, the technique is laborious and extremely expensive for a developing country such as South Africa. Also, chemical analyses merely determine the concentrations of congeners and do not account for interactions among chemicals, which can alter the toxic potential of complex mixtures (Hilscherova *et al*., 2000).

Due to the shortcomings in standard chemical methods, biological methods such as bio-markers, cell- or organ-based bio-assays and protein binding assays have been introduced during the last decade (Behnisch *et al*., 2001). For the purpose of this study, a cell-based bio-assay, the H4IIE-*luc* bio-assay was used. This bio-assay is rapid, sensitive, and relatively cost-effective in comparison to chemical analysis. A disadvantage of the assay is its inability to identify individual compounds with precision; however, they measure the toxic effects of specific mixtures on organism level accurately (Chutter, 1998). The assay also integrates possible interactions among chemicals, detecting the disturbances on organisms and the environment that might have been missed by performing chemical analysis alone (Hilscherova *et al*., 2000).

When bio-assays are used to measure the amount of DLCs, the toxicity is reported as TCDD-equivalents (TCDD-EQs). TCDD-EQs are the equivalent to toxic equivalency quotients (TEQs) calculated from chemical analysis results (Schecter *et al*., 2006). To calculate the TEQ of a compound using chemical analysis, the concentration of each isomer is multiplied with its TEF value, which is ultimately added to give the total TEQ for the sample (Safe, 1995).

Since both chemical and biological methods have limitations and advantages it is best to use these methods in combination with one another (Kannan *et al*., 2001). For Phase I of this study, samples were firstly screened for the presence of DLCs, whereafter selected samples were chemically analysed to confirm the bio-assay results.

For Phase II, all of the samples were first screened with the H4IIE-*luc* bio-assay to determine the amount of DLCs present in each sample, since bio-analysis is a cost-

effective means to screen a large number of samples. Thereafter only the samples eliciting quantifiable responses with the assay were further analysed chemically for other OPs (pesticides, PCBs, PBDE and PAHs). Although the H4IIE-*luc* bio-assay only detects DLCs (PCDD/Fs and dioxin-like PCBs), it is thought to be an effective method to eliminate the sites with insignificant amounts of industrial pollutants, since PAHs, BFRs and PCBs have similar sources (industrially associated) than PCDD/Fs. It is realised and it should be noted that some sites with insignificant amounts of PCDD/Fs, but containing significant amounts of PAHs, BFRs and PCBs may be eliminated for further analysis. Nonetheless, considering the financial implications of chemically analysing a large amount of samples, this was perceived as the best possible means of eliminating samples for further analysis.

2.5 Mechanism of the H4IIE-*luc* tissue culture bio-assay

The H4IIE-*luc* bio-assay is a recently developed process measuring the effects of DLCs on rat hepatoma cells, stably transfected with a luciferase reporter gene under control of dioxin-response elements (DREs) (Hilscherova *et al*., 2000). This bio-assay measures cytochrome P450 induction, which is an endpoint in an AhR-mediated response and an indicator of toxic exposure (Stegeman, 1992). The AhR, also known as the dioxin receptor, is a transcription factor, which is a member of the basic helix-loop-helix family of transcription regulators. This receptor is complexed with heat shock proteins (HSP) and is located in the cytosol of cells (Carey *et al*., 1998). Upon binding of the ligand (in this case DLCs) to the AhR, conformational changes occur resulting in the translocation of the AhR-ligand complex to the nucleus and dissociation of HSP from the receptor (Elferink, 2003) (Fig. 2.11). In the nucleus, the AhR-ligand complex heterodimerises with the AhR nuclear translocator (Arnt) protein (Pocar *et al*., 2005). The binding of the ligand-AhR-Arnt transcriptionally active complex to the DRE, results in increased transcription of cytochrome P450 (Fiedler, 2003; Eisen *et al*., 1983) and in the case of the H4IIE-*luc* cell line, it results in an up-regulation of luciferase transcription (Fig. 2.11). Once luciferin is added to the cells, a light-producing reaction is catalysed, which is equivalent to their toxicant exposure and can be measured with a luminometer (Denison *et al*., 1996; Hilscherova *et al*., 2000).

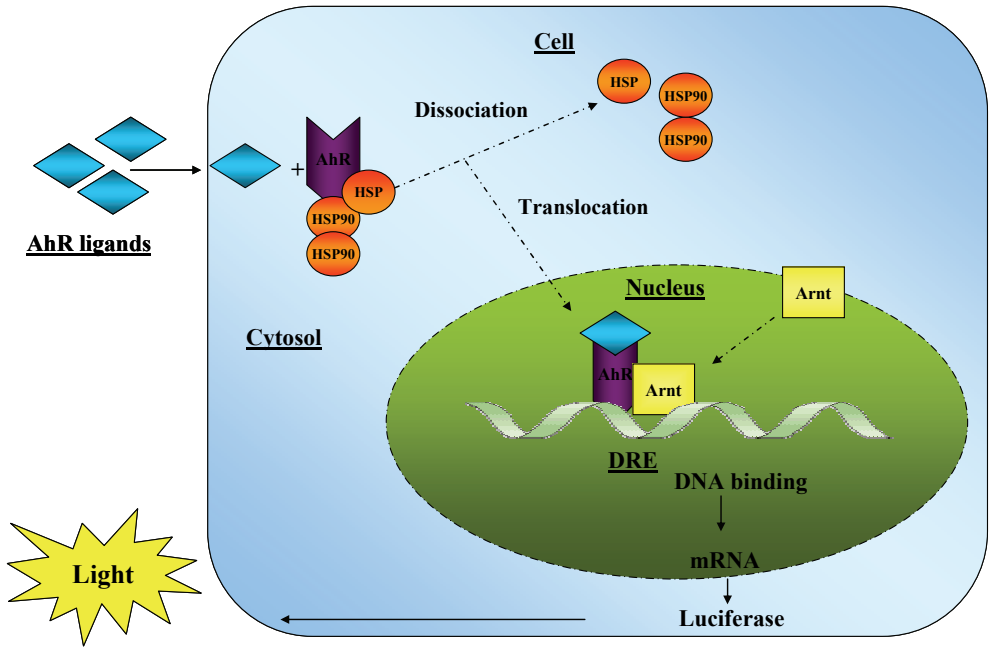


Figure 2.11. Schematic explanation of the mechanism of the H4IIE-*luc* bio-assay.

The protocol of the H4IIE-*luc* bio-assay is described in Section 3.1.4.

3 SAMPLING SITES, MATERIALS, AND METHODS

3.1 Phase I

3.1.1 Site selection

The motivation for choosing the Vaal Triangle as a sampling area for DLC pollution was based on previous work done by Vosloo & Bouwman (2005). During their study, 22 aquatic sites were selected throughout South Africa to establish the presence and levels of PCDD/F and PCB. Their sites were chosen down-stream from possible PCDD/F and PCB sources. Results indicated that DLCs were present in all 22 of the sites, with the highest TCDD equivalent values calculated for the Vaal Triangle site. This project aimed to investigate and evaluate dioxin-like pollution in the Vaal Triangle more extensively by targeting water bodies in this area for analysis.

3.1.1.1. Sediment sampling sites

To cover a large part of the Vaal Triangle river system, several rivers in this region were selected as sediment sampling sites. These rivers included the Klip River, Natal Spruit, Riet Spruit (draining into the Vaal River at the Barrage), Blesbok Spruit, Taaibos Spruit, Leeu Spruit and Suikerbosrand River. The accessibility of the rivers played an important role in site selection, and sites were chosen that were easily accessible. The sites were selected near potential emission sources of PCBs and PCDD/F. Suikerbosrand River was chosen as a reference river, because of its expected low potential for POPs pollution due to its location.

Blesbok Spruit was represented by Sites 1, 8, 9, 11, 12, 13 and 15 (Fig. 3.1). Whereas the smaller water bodies, Taaibos Spruit and Leeu Spruit, were represented by only one site each, Site 20 and Site 22 (Fig. 3.2), respectively. Sites 16, 17 and 18 were the Suikerbosrand reference sites (Fig. 3.3. and Table 3.1). Natal Spruit was represented by Sites 2 and 3, the Klip River by Sites 4 to 7, and 14, and Riet Spruit by Site 10, 19 (Riet Spruit North) and 21 (Riet Spruit South). A second batch of sediment samples were collected from Sites 14, 15, 16, 17, 19 and 20, during June 2006 and shipped away for GC/MS analysis.

3.1.1.2. Fish sampling sites

Of the main study, only three rivers were selected for collecting fish: the Blesbok Spruit, Klip River and Suikerbosrand River. This included one major river for each of the sub-studies and Suikerbosrand River as the reference river (Table 3.1). In addition to fish samples, composite sediment samples were collected at these sites. The samples would

be used to compare the amount of POPs in the sediment to those in the fish to determine bio-accumulation. The sites were chosen where the conditions for fishing were favourable.

The rivers had to be accessible for fishing by means of gill nets, line fishing and/or electro-fishing. This implied that the rivers had to be deep enough and sustain sufficient water flow. Site selection was assisted by previous work done by Rand Water, where the same sites were used to sample fish (Du Preez, 2005). Suikerbosrand River and Blesbok Spruit were represented by one site each, Site 1F and Site 2F (Fig. 3.1), respectively.

Site 1F (Suikerbosrand River reference site) was selected in an area where POPs pollution was expected to be minimal. The site was situated 13 km South of Heidelberg on a cattle farm. No industries or factories were seen in this area. The Blesbok Spruit fish-sampling site (Site 2F) was situated in a recreational park in Heidelberg. This sampling site was located down-stream of the industrial areas of Heidelberg, Brakpan, Springs and Nigel, which were expected to produce industrial waste that might contain POPs (Fig. 3.3).

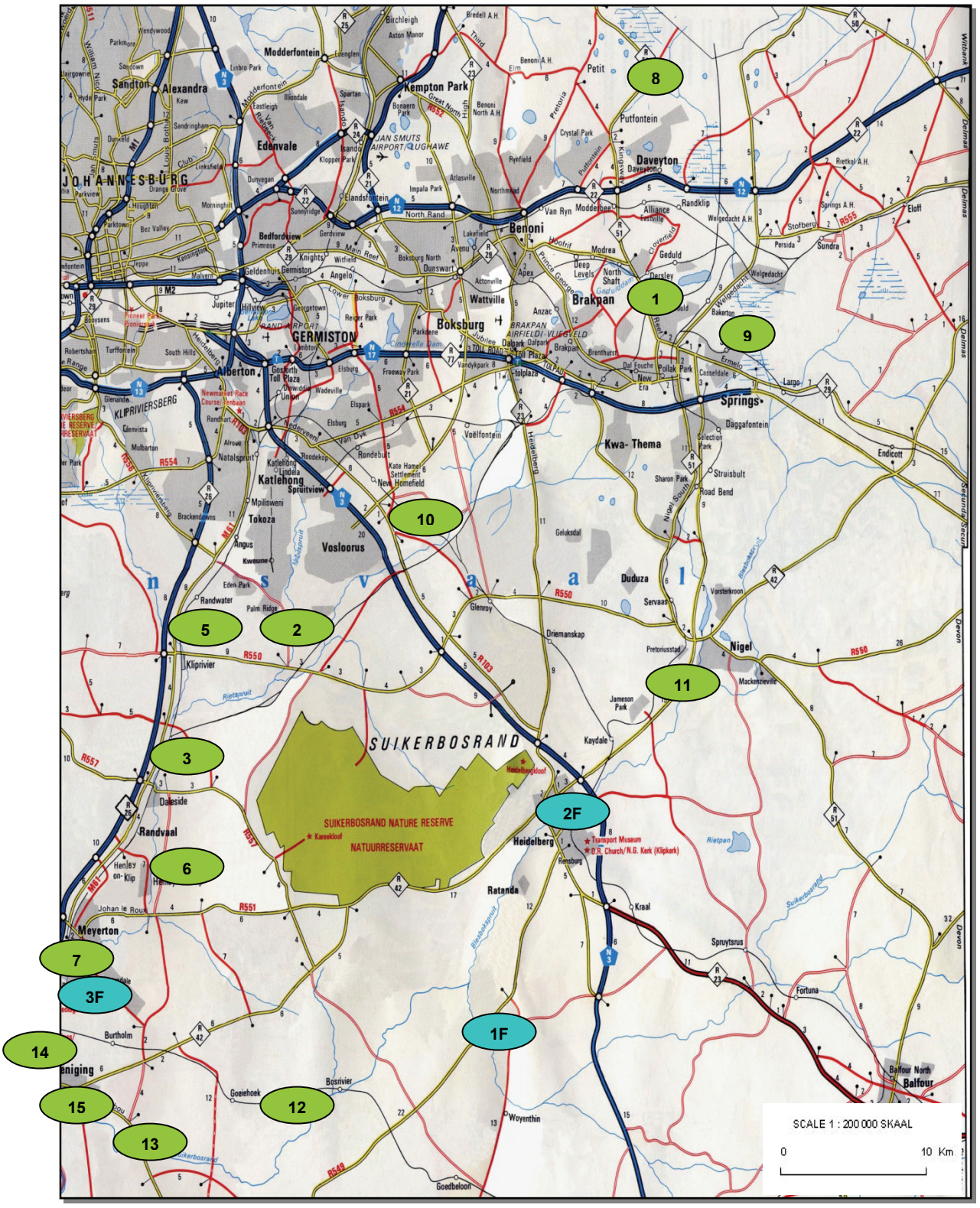


Figure 3.1. A map of the Vaal Triangle indicating Blesbok Spruit, Natal Spruit, Klip River and Riet Spruit sediment sampling sites (Green), as well as the Blesbok Spruit (1F), Suikerbosrand River (2F) and Klip River (3F) fishing sites (Blue and F) (Map Studio, 1990).

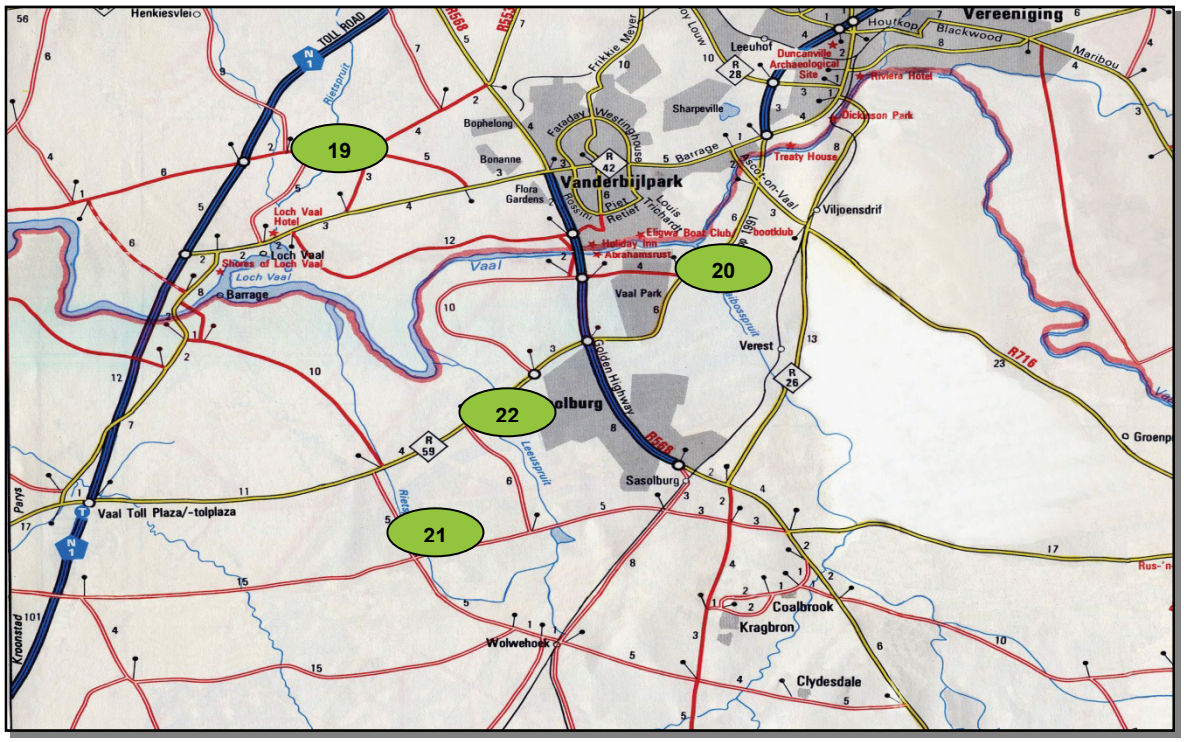


Figure 3.2. Map of the Vaal Triangle indicating Site 19 (Riet Spruit North), 20 (Taaibos Spruit), 21 (Riet Spruit South) and Site 22 (Leeu Spruit) (Map Studio, 1990).

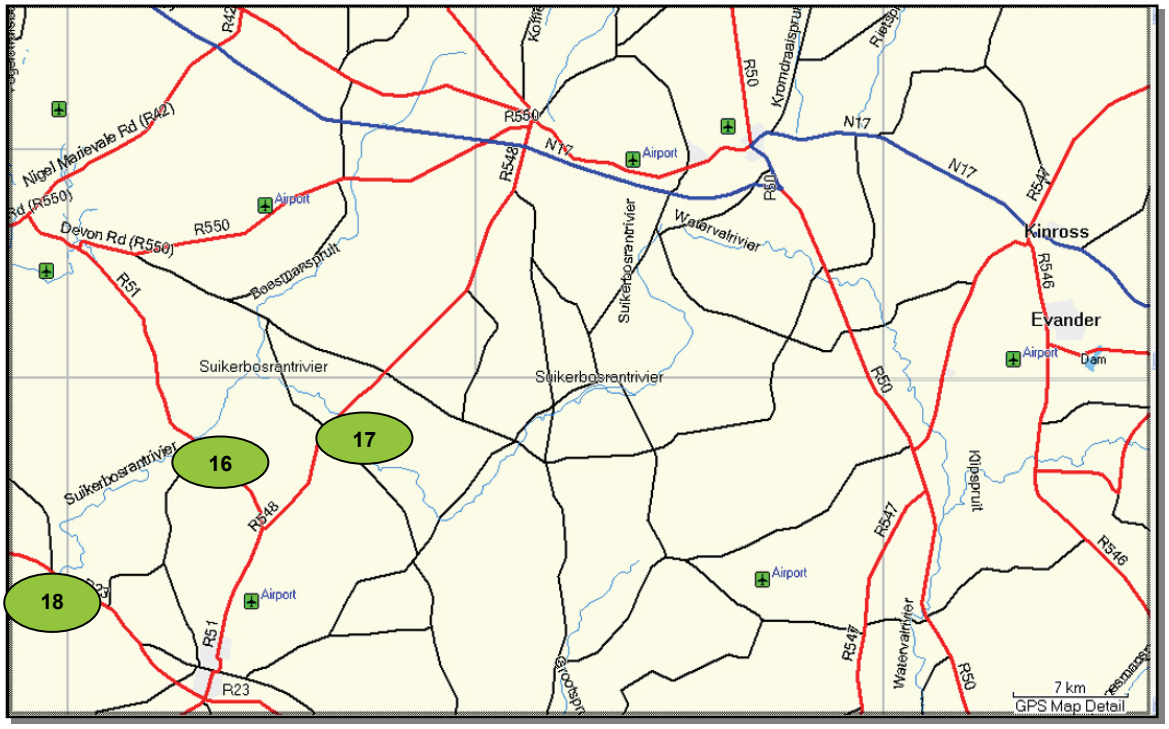


Figure 3.3. Map of the Suikerbosrand River reference area (Sites 16-18) (Garmap, 2002).

Table 3.1. Site description of the sediment samples collected in the Vaal Triangle.

Site	Coordinates	Location	Date	River	Nearby industries
1	S 26°12.992' E 28°26.501'	Geduld (Brakpan) ± 200 m West of the R51-M2 intersection	21/04/2005	Blesbok Spruit	Mines and tailings dams Pulp and paper producer Non-ferrous metal producer Paper treatment plant
2	S 26°25.756' E 28°10.902'	Palm Ridge. North of the Suikerbosrand Nature Reserve	19/04/2005	Natal Spruit	None
3	S 26°26.588' E 28°07.607'	Daleside	19/04/2005	Natal Spruit	Paper milling activities
4	S 26°20.176' E 27°54.161'	Near Lenasia residential area	19/04/2005	Klip River	Mainly residential. Downstream of various industries.
5	S 26°25.051' E 28°05.609'	Klip River. South of Rand Water on the R 550	19/04/2005	Klip River	Brick works
6	S 26°25.051' E 28°05.609'	Henley on Klip	19/04/2005	Klip River	None
7	S 26°36.548' E 27°59.856'	South of Meyerton off the R26	20/04/2005	Klip River	Generation of electricity
8	S 26°05.224' E 28°25.772'	Putfontein	21/04/2005	Blesbok Spruit	Residential area
9	S 26°13.167' E 28°28.981'	Grootvaly Blesbok Spruit Wetland Nature Reserve	19/04/2005	Blesbok Spruit	Tailings dams Sewage works Residential area
10	S 26°22.339' E 28°14.686'	South of Vosloorus	21/04/2005	Riet Spruit	Mainly residential

Site	Coordinates	Location	Date	River	Nearby industries
11	S 26°26.505’ E 28°28.903’	Nigel	21/04/2005	Blesbok Spruit	Residential area Waste dumps
12	S 26°41.648’ E 28°06.433’	Goeiehoek	20/04/2005	Suikerbosrand River after confluence with Blesbok Spruit	Power station
13	S 26°38.422’ E 28°13.818’	Platkoppies smallholdings	20/04/2005	Suikerbosrand River after confluence with Blesbok Spruit	None in close proximity
14°	S 26°39.807’ E 27°57.206’	South-West of Vereeniging	20/04/2005	Klip River	Residential area.
15°	S 26°40.259’ E 28°00.998’	Vereeniging – Drie Riviere residential area: Fish eagle drive	20/04/2005	Suikerbosrand River after confluence with Blesbok Spruit	Residential area: being developed at the time. Near a ferrous metal producer.
16°	S 26°32.042’ E 28°34.626’	On the R51, 8 km North West of the R51-R548 intersection	04/05/2005	Suikerbosrand River reference site	In the vicinity of farms
17°	S 26°31.302’ E 28°39.960’	On the R548, 9 km North East of the R51-R548 intersection	04/05/2005	Suikerbosrand River reference site	In the vicinity of farms
18	S 26°36.063’ E 28°29.586’	On the R23, 100 m West of the Poortjie intersection	04/05/2005	Suikerbosrand River reference site	In the vicinity of farms
19°	S 26°41.925’ E 28°29.586’	North of the Barrage – Loch Vaal	29/06/2005	Riet Spruit North	Steel refinery
20°	S 26°45.190’ E 27°52.495’	On the R59, North East of Sasolburg	29/06/2005	Taaibos Spruit	Oil and gas refinery

Site	Coordinates	Location	Date	River	Nearby industries
21	S 26°49.694’ E 27°45.221’	South West of Sasolburg	29/06/2005	Riet Spruit South	None
22	S 26°48.128’ E 27°47.907’	On the R59, North East of Sasolburg	29/06/2005	Leeu Spruit	Oil and gas refinery Power plant Ferrous metal producer

° Sediment samples were collected again, during June 2006, at Sites 14, 15, 16, 17, 19 and 20 to be analysed with GC/MS.

Table 3.2. Site description of fish samples collected in the Vaal Triangle.

Site	Coordinates	Location	Date	River
1F	S 26°37’47.8” E 28°17’48.2”	± 13 km South of Heidelberg on the R549 on a cattle farm.	05/12/2005	Suikerbosrand River
2F	S 26°30’16.1” E 28°21’44.4”	In Heidelberg residential area. In a park across from a fuelling station (Total).	07/12/2005	Blesbok Spruit
3F	S 26°36’26.8” E 28°00’10.6”	Rothdene residential area (caravan park)	09/12/2005	Klip River

3.1.2 Sediment and fish sampling

Sediment was collected from the surface layers (top 1 to 10 cm) by means of a brass grab sampler or a metal spade and deposited into stainless steel containers. Composite samples were assembled by combining equal volumes of sediment from five random points within each of the aquatic sites. The sub-samples were mixed together briskly for approximately one minute to obtain a homogenous sample and transferred to labelled glass containers. The lids of the containers were lined with aluminium foil to prevent possible sample contamination due to contact with the plastic lining of the lid. To prevent ultra violet (UV) break-down and bio-degradation of the compounds of interest, the containers were covered in brown paper bags and stored at -4°C until air-dried prior to extraction (Hilscherova *et al.,* 2003).

Sampling was done with pre-cleaned steel or glass equipment, according to US EPA method 1613 (US EPA, 1994). This entails that each of the containers and utensils, which came into contact with the sample was washed with phosphate-free soap (Liquinox, Merck) and rinsed with tap- and ultra pure water (18 MΩ) prior to sampling. The containers and utensils were also rinsed with high pressure liquid chromatography-grade (HPLC-grade) acetone and hexane (both from Burdick & Jackson™) to remove polar and non-polar particles, respectively. Glass or stainless steel equipment was used at all times, avoiding plastic to prevent sample contamination (Koh *et al*., 2005).

To determine if POPs were present, and if bio-accumulation and bio-magnification occurred in fish tissue, two fish species were selected for sampling namely *Labeo umbratus* and *Labeo capensis.* These fish species belong to the family Cyprinidae, which includes minnows and carps (Skelton, 2001). *L. umbratus* and *L. capensis* were chosen because they are bottom feeders and it is known that POPs associate with the organic carbon particles of sediment (Schumacher, 2002). Bottom-feeding species are in direct physical contact with sediment and they can bio-accumulate high concentrations of chemical contaminants (Heath *et al*., 2004).

L. umbratus and *L. capensis* are abundant in the Vaal Triangle area and relatively easy to capture (South African Institute for Aquatic Biodiversity, 2005). According to Heath *et al.* (2004) these two fish species are among the test species recommended for freshwater contaminant investigations in South Africa. These species are caught

by recreational and subsistence fisherman and would give an indication of human exposure to PCDD/Fs and PCBs through fish consumption.

L. capensis (Fig. 3.4), better known as the Orange River mud fish, is dispersed throughout the Orange-Vaal system. They can be identified by a depressed head and a mouth with papillate outer lips and two pairs of thin barbells. Adults are a dark greyish colour and they prefer to graze on surfaces of rocks and plants (Skelton, 2001).

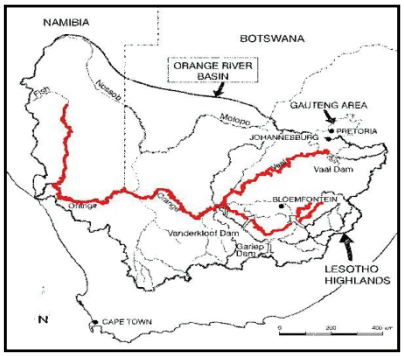


Figure 3.4. Map of southern Africa showing the distribution of *L. capensis* (adapted from Skelton, 2001) (Fish image: FOW, 2006; Map: adapted from DWAF, 2005).

Moggels (*L. umbratus*) (Fig. 3.5) are often mistaken for *L. capensis*, because of their similar appearances. They are both greyish in colour and are similar in shape and size. Moggels can be distinguished from the Orange River mud fish by their rounded heads, mouths with sub-terminal lips and their two small barbells (South African Institute for Aquatic Biodiversity, 2005). *L. umbratus* can be found in the Orange-Vaal system, South- and South-east Cape coastal regions as well as some parts of the Eastern Cape and Mpumalanga (Nussey *et al*., 2000).

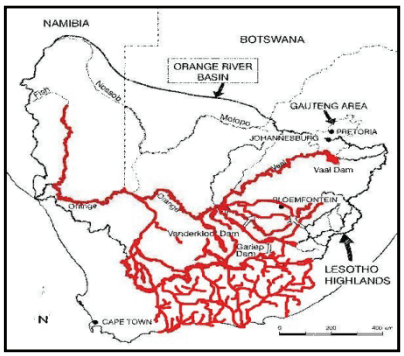


Figure 3.5. A map of southern Africa showing the distribution of *L. umbratus* (adapted from Skelton, 2001) (Fish image: FOW, 2006; Map: adapted from DWAF, 2005).

Hook and line, electro fishing, and gill nets were attempted as sampling methods, with gill nets being the most effective. A single fish was caught by hook and line and only young fish were captured by electro fishing. Gill nets with mesh sizes of 75, 90 and 110 mm were used for sampling, concentrating on larger/older fish. Both *L. umbratus* and *L. capensis* were caught at Blesbok Spruit (Site 2F) and Suikerbosrand River (Site 1F). Fish of both sexes were sampled. After the fish were removed from the nets, they were identified, measured (total length), weighed and a rapid fish health assessment was done (Adams *et al*., 1993).

The fish health assessment index (FHAI), created by Goede & Barton (1990) and refined and adapted for South African conditions by Avenant-Oldewage (2001), was used during the study. This is a rapid, cost effective method used to evaluate the condition of water bodies according to the health of fish. The FHAI includes a detailed assessment of the external and internal features as well as blood analysis of fish. The examination of external features includes the skin, fins, eyes, opercula, gills and ectoparasites. The thymus, mesenteric fat, liver, spleen, hindgut, kidneys and bile should be scored as part of the internal assessment. An in-depth FHAI requires blood analysis for haematocrit, blood plasma protein, leukocrit and white blood cell count.

During the study, only selected variables were measured to determine the general health of fish. A numerical value was assigned to the condition of the eyes, skin, fins, opercula and gills, according to the criteria in Table 3.3. The colour and appearance of the liver, bile and spleen were examined and scored too. Along with the FHAI-values, the condition factors (CF) of fish were calculated with the following formula:

$$CF = \frac{\text{Mass (g)} \times 10^5}{[\text{Length (mm)}]^3}$$

The CF is a mass-to-length ratio value indicating the condition of individual fish. Higher CF values indicate a higher body mass to length ratio, being a sign of good fish condition. This method provided a quantitative basis in order to compare the overall health and condition of fish to one another (Heath *et al.*, 2004). It was not one of the aims of this study to evaluate the water bodies, but by scoring the morphology of fish structures and organs, a quantitative impression of the fish's health can be determined. This would be valuable in linking possible POPs exposure to the general health of fish.

Fish were stunned by a forceful blow to the head, before severing the spinal cord, and dissected on pre-cleaned stainless steel trays. Each of the utensils that came into contact with the fish tissue was washed with distilled water and rinsed with acetone and hexane (Horst *et al.,* 2002).

Table 3.3. Score values assigned to variables according to the Fish Health Assessment Index (FHAI) (adapted from Heath *et al*., 2004).

Variable	Field code	FHAI value
Eyes:		
Normal – No aberrations	N	0
Exophthalmia - Protrusion of one (E1) or both (E2) eyes	E1/E2	30
Haemorrhagic – Blood in eye	H1/H2	30
Blind – Eyes are dull, opaque coloured	B1/B2	30
Missing – Eye is missing	M1/M2	30
Other – Any other eye conditions	OT	30
Skin:		
Normal – No aberrations	0	0
Mild – Mild skin aberrations	1	10
Moderate – Moderate skin aberrations	2	20
Severe – Severe skin aberrations	3	30
Fins:		
No active erosion – Normal or healed lesions	0	0
Mild active erosion – Active erosion, no haemorrhage or infection	1	10
Severe active erosion – Active erosion, haemorrhage and/or secondary infection	2	20
Opercula:		
Normal – Gills are covered, no shortening	0	0
Mild shortening – Small portion of the gill is uncovered	1	10
Severe shortening – Large portion of the gill is uncovered	2	20
Gills:		
Normal – No apparent aberration	N	0
Frayed – Tips of gill lamellae are eroded	F	30
Clubbed – Tips of lamellae are swollen	C	30
Marginate – Gill has a discoloured margin	M	30
Pale – Gills are light in colour	P	30
Other – Other observations	OT	30
Liver:		
Normal – Good, solid red colour	A	0
Light red	B	0
Fatty liver – light tan colour (“coffee with cream”)	C	30
Nodular – White mycobacterial cysts and incipient nodules	D	30
Focal discolouration	E	30
Colour change in whole liver	F	30
Other – Aberrations not mentioned above	OT	30
Bile:		
Yellow/Straw colour – Bladder empty/partially full	0	-
Yellow/Straw colour – Bladder full, distended	1	-
Light green/Grass green	2	-
Dark green/Dark blue green	3	-

Variable	Field code	FHAI value
Spleen:		
Black – Very dark red colour	B	0
Red – Red colouration	R	0
Granular – Rough appearance	G	0
Nodular – Contains nodules and cysts of varying sizes	NO	30
Enlarged – Significantly larger than normal	E	30

The liver, gonads and fillets of the fish were removed on site by dissection. The fish tissue was weighed, wrapped in pre-cleaned aluminium foil, placed in waterproof plastic bags and labelled (Heath *et al.*, 2004). The samples were cooled immediately and transported and stored at -4°C (Hilscherova *et al.*, 2003). Livers and gonads, which are lipid-rich tissues, were selected for analysis, because it is known that PCDD/Fs and PCBs are lipophilic (Ritter *et al*., 2005). The section of fish most commonly consumed by humans is fillet tissue. Fillets were extracted and analysed to establish the potential effects of contaminated fish consumption on humans (Heath *et al*., 2004).

3.1.3 Sample extraction and clean up

Both Soxhlet and Accelerated Solvent Extraction (ASE) methods are accepted by the US EPA for the extraction of PCDD/Fs and dioxin-like PCBs (Grochowalski & Maślanka, 2003), and both apparatus have shown to be comparable in their extraction efficiency (Dionex, 2002). Sediment samples (excluding the fishing sites' sediment samples) were extracted by ASE, since this apparatus is more time- and cost-effective. The Soxhlet apparatus was used to extract fish tissue, because the volumes of fish tissue, especially fillet tissues, were too large to be extracted with the ASE. To determine bio-accumulation, sediment- and fish tissue samples, collected at the same site, had to be compared to one another. Therefore, sediment samples collected at fish sampling sites were also extracted with the Soxhlet apparatus, to eliminate any possible variables in extraction method.

To remove water from the sediment samples, they were freeze-dried for three days. Small stones, leaves and twigs were removed from the dried sediment sample by hand. The samples were then ground and sieved (0.5 mm mesh size) to obtain a homogenous sample. Forty grams (40 g) of sediment was mixed with an equal volume of anhydrous sodium sulphate (Merck, univAR) to remove any possible moisture still present in the sample. The sediment was placed on top of pre-cleaned

glass wool and extracted with a 3:1 mixture of HPLC-grade DCM and hexane mixture for 16 to 24 hours (Hilscherova *et al.,* 2001).

The ASE (Dionex, ASE 100) was used for sediment extraction. Sediments were extracted with a 3:1 mixture of DCM and hexane under the following parameters: 1500 psi, 100°C, five minute static and heat time, a flush volume of 60%, 100 seconds nitrogen purge time and 2 cycles (McCant *et al.*, 1999). The extract was collected in a pre-cleaned collection bottle and allowed to cool. After extraction, the extract was rotary evaporated to almost dryness. The remaining extract was transferred to a pre-cleaned test tube and adjusted to 10 ml with hexane (Hilscherova *et al.*, 2001).

Some sediment extracts may contain high levels of elemental sulphur, which might be cytotoxic to the rat hepatoma cells used in the bio-assay. To remove sulphur, all of the extracts were treated with freshly activated copper shavings (US EPA, 1986). Copper was added to the extracts until it did not turn black any longer. The sample was quantitatively transferred to separation funnels to perform an acid treatment (US EPA, 2000).

During the acid clean-up, the extract was treated with concentrated sulphuric acid (98%, Merck) to remove all traces of polycyclic aromatic hydrocarbons which are not dioxins, furans or PCBs (Vondráček *et al.*, 2001). Fifteen millilitres (15 ml) of sulphuric acid was added to the extract, carefully mixed, ventilated often, and left to stand for at least an hour for the two phases to separate. The acid was then tapped off and fresh sulphuric acid was added. This step was repeated until the acid phase was clear (3 to 5 times). To remove all traces of acid from the extract, the sample was washed with a 5% sodium chloride (NaCl) solution and left for an hour for the phases to separate. This step was followed by a 20% potassium hydroxide (KOH) solution treatment. KOH is a strong base with the ability to break down the compounds of interest. Therefore, the KOH-solution was tapped off at the instant the two phases separated. This step was followed by a second 5% NaCl-solution treatment. The remaining extract was filtered through glass wool covered with anhydrous sodium sulphate to remove all traces of water (US EPA, 1994b). The crude extract was further concentrated to approximately 0.5 ml by a gentle stream of nitrogen gas and made up to 1 ml with hexane. The extract was stored in amber coloured gas chromatography (g.c.) vials at –4°C until used in the bio-assay.

Each of the utensils and apparatus used during sample extraction was washed beforehand with phosphate-free soap, rinsed with ultra-pure water (double deionised; 18MΩ) and rinsed with acetone and hexane (Vondráček *et al.*, 2001).

The air-dried sediments, collected during June 2006 for the purpose of comparing biological and chemical methods, were shipped to the Norwegian Institute for Air Research. The Institute was responsible for the extraction and analysis of samples (Method ISO/IEC-17025). High-resolution gas chromatography/mass spectroscopy (GC/MS) was used to measure the concentrations of DLCs in the sediment samples.

On the day after sample collection, fish tissues were divided into composite groups, of two or three individuals per group. Individuals in a composite group had to belong to the same species, gender and age/size class (Heath *et al.*, 2004, US EPA, 2000). Composite samples were prepared from gonads, liver and fillet tissue, respectively.

The tissue samples were freeze-dried for three days and the dry mass of samples were determined afterwards (Adams *et al.*, 1993). Because of the nature of the tissues, fillets and ovaries were homogenised with a blender, and a mortar and pestle were used for livers and male gonads. All of the livers and gonads, but only 10 g of fillet tissue, were extracted. The tissues were mixed with anhydrous sodium sulphate to remove any moisture remnants (Corsolini *et al.*, 2002).

The Soxhlet extraction method was used to extract fish tissue, and followed by an acid wash (as described earlier). The sample was transferred to a test tube and concentrated to 0.5 ml by a nitrogen gas purge. The concentrated extract was made up with hexane to a volume of 1 ml, and stored in g.c. vials at -4°C until analysis using the H4IIE-*luc* bio-assay (Koh *et al.*, 2005). The lipid content was also determined to express the results per lipid mass.

3.1.4 The H4IIE-*luc* bio-assay

Sterile techniques were employed during maintenance, culturing and passaging of the cell line, working in laminar-flow bio-safety hoods and incubators with high efficiency particulate air (HEPA)-filters. All of the equipment used was autoclaved and cleaned with 70% ethanol, and all cell media were filtered through Vacucap® filter units (0.8/0.2 µm, LifeSciences) prior to use.

The H4IIE-*luc* bio-assay uses a rat hepatoma cell line stably transfected with a firefly luciferase reporter gene. The cells were a gift from Prof. John Giesy, then from the Michigan State University in the USA. Cells were maintained in a water-jacketed incubator (ThermoForma series II, Labotec) at a temperature of 37°C in a humidified air:carbon dioxide mixture (95% air/5% CO_2). They were grown in tissue culture dishes (100/20 mm, Greiner Bio-one) using Dulbecco's Modified Eagle's Medium (DMEM) without phenol red (Sigma cat no. D2906), supplemented with 10% foetal bovine serum (FBS; Hyclone, Thermo Scientific). The dishes were inspected regularly for cell density, microbial activity and other signs of contamination, such as turbidity of the media, the presence of floating objects in media, and the occurrence of microscopic objects with different morphology than the cells (McFarland *et al*., 1998). Cells were passaged as soon as the tissue culture dish was confluent (Villeneuve *et al*., 1999; McFarland *et al*., 1998).

The bio-assay was performed over a period of five days, during which the cells were grown in DMEM, supplemented with 10% hormone-free foetal bovine serum. On the first day, 96-micro well plates (400 µl, Nunc) were seeded with a 0.25 ml cell suspension (50 000 cells/ml). The outer wells of each plate were not seeded, but contained 0.25 ml PBS (without Ca^{2+} and Mg^{2+}), acting as a buffer area to prevent a hydrostatic pressure from having an effect on cells. The plates were incubated for 24 hours at 37°C and an air/CO_2 concentration of 95%/5% (Hilscherova *et al*., 2003).

On day two, the cells were dosed in triplicate with 2.5 µl of the reference compound (2,3,7,8-TCDD) or sample extract at six different concentrations. The 2,3,7,8-TCDD were dosed at a four-times dilution series (120.0, 30.0, 7.50, 1.88, 0.47 and 0.12 pg TCDD/well) and the extract were dosed at a two-times dilution series (1:1, 1:2, 1:4, 1:8, 1:16 and 1:32). A solvent control (SvC) row (containing hexane) and blank row (containing cells only) separated the wells dosed with 2,3,7,8-TCDD from the wells dosed with extracts, to prevent cross-communication of cells (Giesy *et al*., 1997; Hilscherova *et al.,* 2001). The plates were incubated for 72 hours.

On the fifth and final day of the assay, the plates were inspected microscopically for confluency and viability. Additionally, a viability test (described in Section 3.1.5) was performed. The culture medium was removed and the cells were washed with PBS with Ca^{2+} and Mg^{2+}. After adding 100 µl of Luclite™ reagent (Perkin Elmer) to each well, the plates were incubated for 10 minutes and placed in a luminometer

(Microplate fluorescence reader FLX 800, Bio-Tek Instruments Inc.) to measure the amount of light emitted by the cells (expressed as relative light units or RLU's). Generally, the sensitivity of the instrument was set at 180, but it was adjusted when necessary to create optimum deviation from the background (Hilscherova *et al*., 2001).

Results obtained from the assay were processed with Microsoft Excel XP, calculating the mean RLU's, standard deviation, coefficient of variation (CV) and the percentage maximal induction relative to 2,3,7,8-TCDD (% TCDD max) for each reference compound- and sample series. Since solvents may contribute to cell response, the mean RLU's of the SvC wells should be subtracted from the RLU's of the samples and TCDD (Besselink *et al.,* 2004). However, when this was done the RLU's produced by samples had negative values and were inadequate; therefore, the effect of the solvent was not taken into consideration. Furthermore, the CV had to be less than, or as close as possible to 20 to have acceptable variation. When this value was greater than 20, one of the RLU's was dropped from the calculations (Whyte *et al*., 2006).

Dose-response curves were plotted for the TCDD-reference compound series with the amount of TCDD (log pg TCDD/well) plotted on the x-axis and % TCDD max on the y-axis. Three data points lying on the linear part of the sigmoid curve (beyond 20% TCDD max, but below the point where the curve flattened) were used to calculate the slope, y-intercept and correlation coefficient (R^2), using the equation y = mx + c (Schramm *et al*., 2001). The corresponding x-intercepts for amount of TCDD responsible for 20%, 50% and 80% response in cells were consequently calculated, yielding the effective concentrations or $EC_{20,}$ $EC_{50\ and}$ EC_{80}. The data obtained from the sediment or soil samples were processed in the same way as the reference compound, compiling dose-response curves for each sample, with the amount of sample (log µl sample/well) on the x-axis and the % TCDD max on the y-axis. $EC_{20\text{-}80}$ values were calculated in the same way as the TCDD $EC_{20\text{-}80}$ values. This indirect assay assumes that doses of TCDD and sample that elicit the same magnitude of response, can be defined as equally effective doses (Finey, 1971), or relative effect potencies (REP). REPs were calculated from the reference compound and sample's $EC_{20\text{-}80}$ values (using $REP_{20\text{-}80} = EC_{20\text{-}80\ TCDD}/EC_{20\text{-}80\ sample}$) to address the phenomenon of non-parallelism between the reference dose-response curve and the sample dose-response curve. It cannot be assumed that the complete mixtures from the

environment will exhibit equal efficacy to TCDD; therefore, the % TCDD max of the samples had to be greater than 20 (Villeneuve *et al*., 2000). Since the levels of DLCs in the South African sediment and soil samples generally did not elicit responses of 50% or greater, only REP_{20} were converted into TCDD-EQ_{20} values by back-calculation, based on the volume of the extract assayed, and the degree of concentration during the extraction procedure (Koh *et al.,* 2006).

The limit of detection (LOD) for the bio-assay was calculated by determining the mean EC_0 (TCDD concentration at which no response was elicited from cells) for the entire study's TCDD dose-response curves. The 95% confidence interval was subsequently determined, added to the mean (Thomsen *et al*., 2003), and converted to ng TCDD-EQ/kg dw.

3.1.5 The MTT viability bio-assay

Cell viability and proliferation greatly affects the reliability of results produced by *in vitro* assays. The MTT bio-assay is a reliable approach to examine cell proliferation, determining if cell responses were possibly affected by the sample extract. The bio-assay functions on the principle of tetrazolium salt reduction (Oh *et al*., 2004; Vistica *et al*., 1991). Yellow tetrazolium salt (3-[4,5-dimethyltiazol-2yl]-2,5-diphenyl tetrazolium bromide) or MTT in short, is metabolically reduced by active cells [through nicotinamide-adenine dinucleotide phosphate (NADPH) and nicotinamide-adenine dinucleotide (NADH)] resulting in the formation of intracellular purple formazan, which can be dissolved by a tissue culture medium and quantified spectrophotometrically (Oh *et al.,* 2004).

A duplicate set of plates were prepared alongside the luminescence assay, dosed exactly the same. On the fifth and final day, plates were washed with PBS with Ca^{2+} and Mg^{2+}, and 100 µl freshly prepared MTT solution was added to each cell containing well and to three control wells without cells (Blaha *et al*., 2004). The plates were incubated for 30 minutes at 37°C and a 5%/95% CO_2/air concentration. The MTT solution was replaced with 200 µl DMSO (Saarchem, uniLAB) per well and left for 30 minutes to allow the crystals to dissolve. The absorbencies were measured at 492 nm and 645 nm (background) with a spectrophotometer (PowerWave X, Bio-Tek Instruments Inc.) (Blaha *et al*., 2004). Each plate was read thrice at both absorbencies and the mean of the three readings was used to calculate cell viability.

The true absorbance for each well was determined by subtracting the reference absorbance from the absorbencies at 492 nm as well as the absorbency of MTT (wells with no cells, but MTT only). Viability was expressed as a percentage of the absorbance of the control cells (Vistica *et al.,* 1991). These calculations were performed in Microsoft Excel XP. If the cells were less than 80% viable, it was assumed that cell viability might have affected the credibility of the luminescence assay's results.

3.1.6 Determination of oxidisable and total organic carbon

Because persistent organic pollutants and toxicants preferentially associate with organic carbon particles, the total organic carbon (TOC) content of samples had to be determined (Schumacher, 2002). The oxidisable organic carbon (OXC) content of samples was determined with the Walkley-Black method and converted to TOC using the method described below.

The Walkley-Black method is a quantitative, destructive technique based on the principle of wet oxidation followed by ferrous ammonium sulphate titration. The method is fairly simple to use and time- and cost-effective (Chan *et al*., 2001; Schumacher, 2002). During this process carbon is oxidised by the dichromate ion ($K_2Cr_2O_7 + 3C + 16H^+ \rightarrow 4\ Cr^{3+} + 3CO_2 + 2H_2O$), and excess dichromate ions are back titrated with ferrous ions ($6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$). To calculate the OXC content, Schumacher (2002) proposed the following equation:

$$\text{OXC (\%)} = \frac{[\text{Fe(NH}_4)_2(\text{SO}_4)_2 \text{ used for blank (ml)} - \text{Fe(NH}_4)_2(\text{SO}_4)_2 \text{ used for sample (ml)}] \times M \times 0.3 \times f}{\text{Mass of sediment sample (g)}}$$

Where: M was the concentration of $Fe(NH_4)_2(SO_4)_2$ of 0.5 mol.dm^{-3}, and
f was the correction factor of 1.4.

The TOC content was ultimately calculated from the percentage OXC, using the equation recommended by Sánchez-Monedero and co-workers (1996): TOC (%) = 1.23(OXC) + 0.35. The results from biological and chemical analysis were normalised to 1% TOC to allow for comparison with environmental quality guidelines proposed by other countries.

3.2 Phase II

3.2.1 Site selection

Based on the results of previous studies (Vosloo & Bouwman, 2005; Nieuwoudt, 2006; Nieuwoudt *et al.* 2009; Quinn *et al.,* 2009), it is apparent that POPs and other industrial pollutants are present in South African soils and sediments. Although some of these pollutants, (especially PCDD/Fs, PCBs and some OCPs) may generally be present at very low concentrations, hot-spots remain, mostly associated with industrial hubs and high-density residential areas, which was the main focus of this study. Also included in the study were water bodies located near paper mills, rivers flowing into neighbouring countries, and high-altitude rivers. The sampling regions or locations included in each of these categories are listed in Table 3.4.

Table 3.4. The site source categories and the locations, regions, or rivers included in each category.

Sampling site source categories	Locations/regions/rivers
Industrial areas	Soweto and Lenasia, Cape Town, Richards Bay, Bloemfontein and its associated high-density residential area, Botshabelo.
Low-income high-density areas	Soweto and Lenasia, Cape Town, Richards Bay, Bloemfontein and its associated high-density residential area, Botshabelo.
Rivers flowing to neighbouring countries	Limpopo-, Komati-, Pongola-, Olifants- (Mpumalanga) and Crocodile (east) Rivers
High-altitude rivers	Drakensberg area: Mzimkhulu- and Mkomazi Rivers, and one stream of which the name is uncertain.
Paper mills	KwaZulu-Natal: Mhlathuze-, Tugela- and Mvoti Rivers

3.2.2 Soweto and Lenasia

With a population of nearly one million, Soweto is the most densely populated black urban residential area in South Africa (Statistics South Africa, 2008). The area is mainly residential, with only a few small industries. Lenasia, on the other hand, is more industrial with the main industries specialising in the manufacturing of plastic and resin, furniture and steel products.

The Klip River wetland system, possibly one of the most economically important wetlands in Africa, meanders through the western parts of Soweto, and the northern

and eastern parts of Lenasia (McCarthy *et al*., 2007). Preliminary results of Phase I, showed the highest levels of DLCs thus far recorded for South African sediments (380 ng TCDD-EQ/kg, dm) in sediment collected from this wetland, in an area situated south of Soweto and south-east of Lenasia (Fig. 3.6, S/L 9 and 12). It was therefore decided that a section of the wetland (further referred to as the "Soweto & Lenasia wetlands") should be included in the current study to determine the scale and significance of selected organic pollutants in this wetland more extensively.

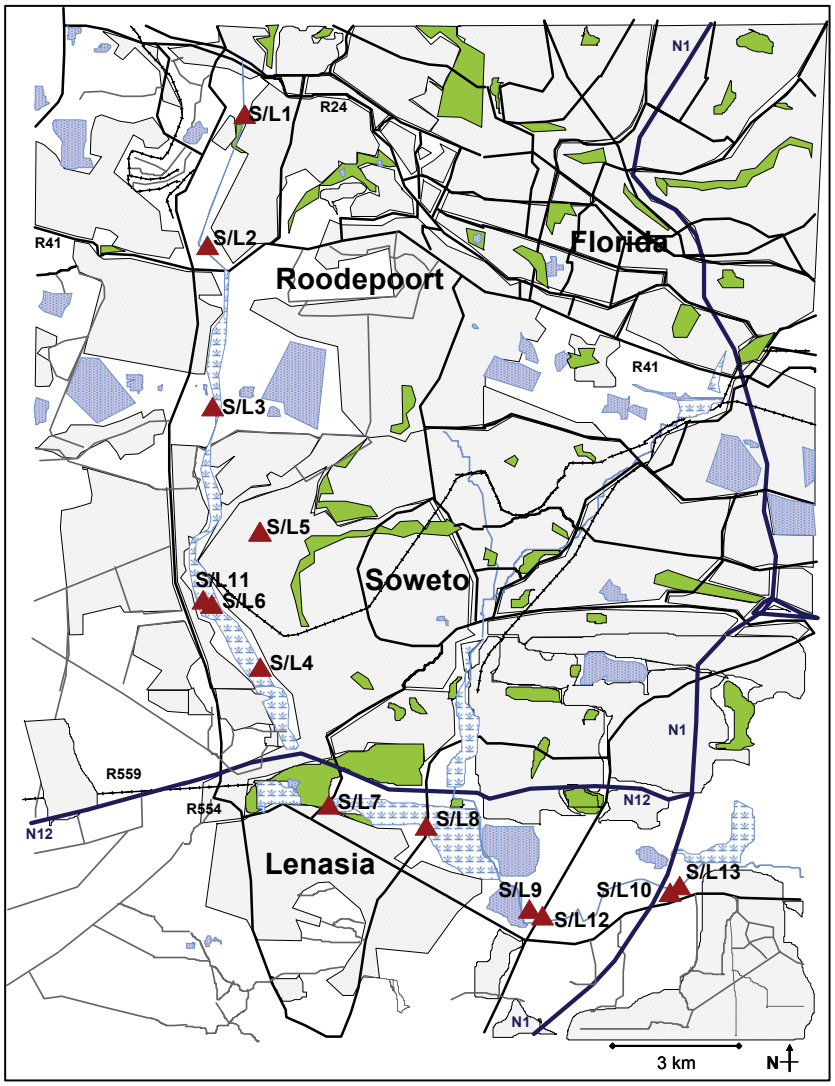


Figure 3.6. Sediment and soil sampling sites in the Soweto/Lenasia wetland.

The first samples were collected north and up-stream of Soweto (S/L1 to 3). Samples were also collected in the residential and industrial areas of Soweto and Lenasia (S/L5 to 8, and 11), and finally at a location down-stream of both areas (S/L9, 10, 12 and 13) (Fig. 3.6 and Table 3.5). Although Soweto and Lenasia are not extremely industrialised, the wetland receives polluted effluent from the western section of the

Witwatersrand urban-industrial-mining complex (McCarthy *et al*., 2007). Domestic activities such as open burning for cooking or heating may also contribute to organic pollutants in the wetland.

Table 3.5. Description of sediment and soil sampling sites from Soweto and Lenasia (S/L) wetlands.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
S/L1	17/01/07	S26°08'10.6" E27°49'24.5"	Roodepoort: Mindalore, east of Chamdor Road (R558)	Residential area, near old gold mine tailings dams.	Wetland reeds
S/L2	17/01/07	S26°10'09.0" E27°49'03.1"	Witpoortjie. North-east of R558-R41 intersection	Residential and several industries	Wetland reeds
S/L3	17/01/07	S26°12'35.1" E27°48'50.9"	Parallel with Adcock Street (R558) near Jameson Raid monument. North-west of Thulani.	Approximately 300-400 m away from residential area, near a gold mine tailings dam	Wetland reeds
S/L4	17/01/07	S26°16'26.4" E27°49'39.2"	Protea Glen: Protea Boulevard, east of R558-Protea Blvd intersection.	Residential area. No industries.	Wetland reeds
S/L5	17/01/07	S26°14'25.3" E27°49'37.1"	Naledi: Milkplum Street, next to Adcock Street (R558)	Residential area. No industries.	Wetland reeds
S/L6	17/01/07	S26°15'30.5" E27°48'51.5"	Naledi: Pitse Street, next to Adcock Street (R558)	Residential area. No industries.	Wetland reeds
S/L7	17/01/07	S26°18'26.3" E27°50'43.7"	Nirvana Drive bridge. R554 crossing.	Residential area. Coal burning	Wetland reeds
S/L8	17/01/07	S26°18'48.2" E27°52'22.0"	M10: Klip Spruit Valley bridge	Down-stream of residential area. Small industries.	Wetland reeds, next to river
S/L9	17/01/07	S26°20'08.3" E27°54'09.5"	On the R553 near bridge: Approximately 200 m north of the R554-R553 intersection.	Downstream of sewage works.	Wetland reeds

Table 3.5. *Continued*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
S/L10	17/01/07	S26°19'44.2" E27°56'34.1"	On the R554: East of the R554-N1 intersection.	Near an old bridge, in the vicinity of farm/small-holdings	Wetland reeds, next to river
S/L11*	17/01/07	S26°15'29.3" E27°48'46.8"	Naledi: Pitse Street, next to Adcock Street (R558)	Residential area	Grass/rocky area next to S/L 6 wetland
S/L12*	17/01/07	S26°20'08.3" E27°54'09.5"	On the R553 near bridge: Approximately 200 m north of the R554-R553 intersection.	Downstream of sewage works.	Soil
S/L13*	17/01/07	S26°19'44.2" E27°56'34.1"	On the R554: East of the R554-N1 intersection.	Near an old bridge, in the vicinity of farm/small-holdings	Soil

*Associated soil samples

3.2.3 Cape Town

Cape Town is the second most populous city in South Africa (Statistics South Africa, 2008) and has a large industrial complex consisting of oil refining, diamond cutting, shipbuilding and -repair, food processing, printing, and the production of paper, chemicals, fertilisers, cement, clothing, plastics, and leather goods. The city also has a large national railway, an international airport, and a harbour complex. Unfortunately, the steep incline and depth of the harbour made it inaccessible for sampling. The nuclear power station, Koeberg, provides electricity for the majority of Cape Town's needs. The Western Cape is well-known as an agricultural region, generating 25% of the total gross income of the country's agricultural sector (South African Info, 2009). Sediments were sampled from industrially-, residentially- and agriculturally impacted areas to determine the scale and significance of POPs pollution associated with these areas. The sites CT2, 4 to 8, 10, 14, 19 and 20 were selected because of their close proximity to industry, mainly focusing on a petrochemical plant, a paper and pulp treatment plant, an oil- and gas refinery, manufacturers of chemicals and fertilisers, and other smaller industries (Figs. 3.7-3.9, Table 3.6).

CT1, 9 and 17 were situated in medium- to high-income-, and high-density, low-income residential areas, whereas sites 11, 12, 15, 16 and 18 were selected in areas that would give an indication of the impacts associated with industrial-residential combinations. Finally, CT3 and 13 were located in the vicinity of agriculturally impacted areas, mostly vineyards (Figs. 3.7-3.9, Table 3.6). Information regarding the sampling sites is listed in Table 3.6, and site locations are indicated in Figures 3.7 to 3.9.

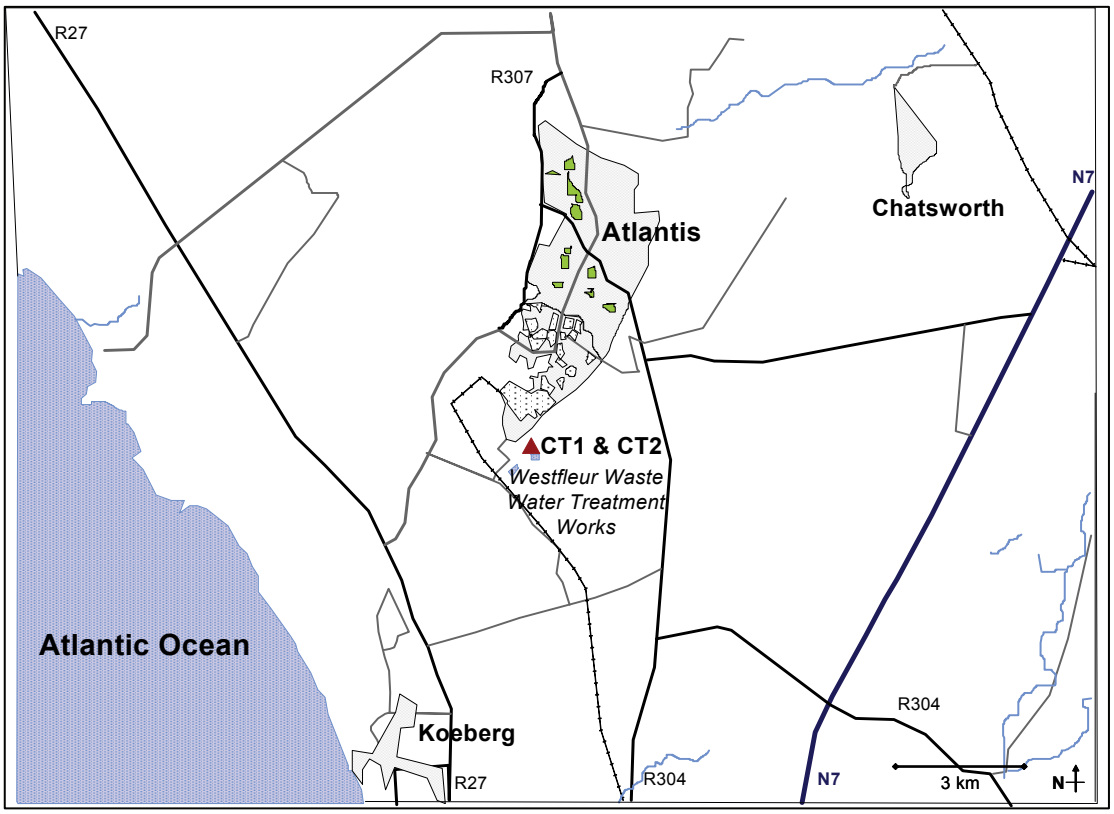


Figure 3.7. Sampling locations of CT1 and CT2.

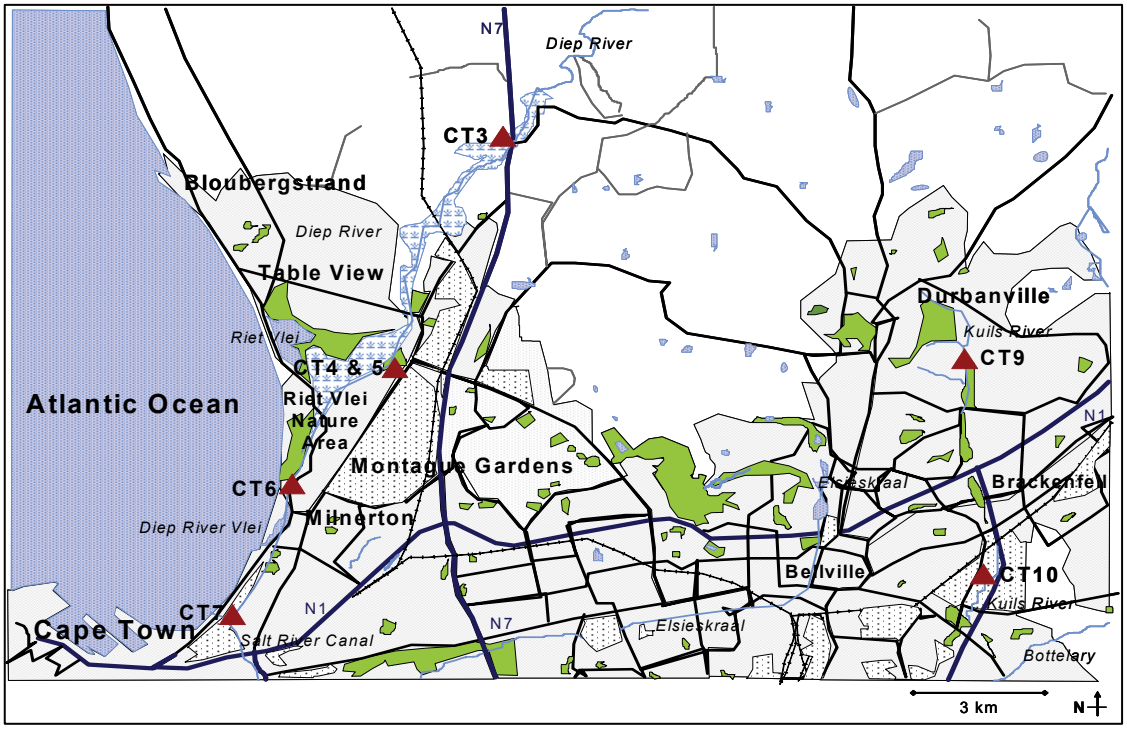


Figure 3.8. The northern parts of Cape Town and its suburbs indicating the sampling locations of CT3 to 7 and CT9 and 10.

Figure 3.9. The southern parts of Cape Town and its suburbs indicating the sampling locations of CT8 and CT11 to 20.

Table 3.6. Description of sediment sampling sites from Cape Town (CT) and its suburbs.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
CT1	16/04/07	S33°36'34.8" E18°28'41.2"	Atlantis: Westfleur waste water treatment works (WWTW).	Final sewage effluent containing **domestic waste**.	Sediment settled in cement canal leading to waste water pond – drains to aquifer which supplies drinking water.
CT2	16/04/07	S33°36'34.8" E18°28'41.2"	Atlantis: Westfleur WWTW	Same system as mentioned above at CT1, but contains **industrial waste**.	Same as CT1
CT3	16/04/07	S33°47'25.6" E18°32'52.8"	Vissershok: Vissershok Road off the N7.	Upper part of Diep River. Agriculturally impacted (river flows through vineyards).	River was fairly dry (seasonal flow) and wetland-like with many reeds.
CT4	16/04/07	S33°50'53.4" E18°31'10.8"	Theo Marais Park/Sports fields. Off Koeberg Road (M5).	Highly industrialised area collecting waste water from many industries, including a paper and pulp treatment plant.	Unlined canal collecting industrial waste water and storm water drainage.
CT5	16/04/07	S33°50'53.4" E18°31'10.8"	Theo Marais Park/Sports fields. Off Koeberg Road (M5).	Located in the same area as, and only a few metres away from CT4. Also collects industrial waste water, but from other industries including a fertiliser manufacturer and an oil-and gas refinery.	Unlined canal collecting industrial waste water and storm water. The two canals (CT4 & CT5) join at a point where it flows into the Diep River.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
CT6	16/04/07	S33°52'38.0" E18°29'28.1"	Milnerton: Next to the R27, adjacent to Milnerton Golf club and the Canoe club.	Located down-stream of the Riet Vlei Nature area, a few kilometres down-stream of CT4 and CT5.	Lower part of Diep River (wide and deep), just up-stream of the estuary.
CT7	16/04/07	S33°54'29.8" E18°28'36.5"	Paarden Eiland industrial. Vrystaat Road.	Highly industrialised area located near a petrochemical plant.	Part of the Diep River Vlei. Wetland-like area with many hyacinths.
CT8	16/04/07	S33°57'08.6" E18°29'42.7"	Mowbray: Rondebosch Golf course.	The sample was taken on the Golf course from the Black River, down-stream of Athlone WWTW and other industries.	Black River (slow flow).
CT9	17/04/07	S33°50'46.4" E18°40'06.9"	Dubanville: On Fairview Road off Fairtrees Road.	Open field near residential area. Only residential areas and vineyards are located up-stream of this site.	Kuils River (slow flow). Contains a lot of reeds and water plants.
CT10	17/04/07	S33°53'58.6" E18°40'21.0"	Kaymor Industria: Cilmor Street off the M31.	Industrial area. A new storm water canal was being built at the time with a steep downward slope leading to river. May receive industrial storm water.	Kuils River. Sample was collected between reeds in the shallow part of the river – near the riverbed.
CT11	17/04/07	S33°57'03.4" E18°40'10.2"	Kalkfontein/Gersham: Residential area north-west of Nooiensfontein & Stellenbosch Road.	Residential area. Down-stream of Bellville's WWTW, industries and a residential area. Down-stream of the Bottelary- and Kuils River confluence.	Kuils River (wide). Cattle feeding and drinking at site.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
CT12	17/04/07	S34°04'10.5" E18°45'52.3"	Macassar Beach: Off Macassar Road – at pump station.	Industrial area, down-stream of industries and residential areas and Macassar WWTW. Beyond the Kuils- and Eerste River confluence.	Eerste River (medium flow) after Kuils River confluence.
CT13	17/04/07	S34°03'54.4" E18°46'19.1"	Faure area: R102	Mainly vineyards up-stream.	Eerste River (slow flow) before confluence with Kuils River.
CT14	17/04/07	S33°58'16.3" E18°34'42.7"	Airport Industria: Manhattan Street. Open lot surrounded by industries.	Highly industrialised area which may receive a lot of storm water due to downwards slope leading to the pond.	Relatively stationary artificial pond. Wetland-like and enclosed by reeds.
CT15	17/04/07	S34°00'04.9" E18°33'05.6"	Sand Industria: Near M7 off ramp to the M9. Edith Stephens Wetland Park.	Down-stream of residential and industrial areas. The wetland is used to control storm drainage and to prevent flooding.	Wetland. Relatively stationary with many hyacinths.
CT16	17/04/07	S34°00'37.7" E18°31'36.5"	Hanover Park: Springfield Street.	The river flows through many industries and low-income, high-density residential areas.	Ottery River. Flows through a canal that is lined with bricks. Water from this river is used for the irrigation of vegetables.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
CT17	17/04/07	S34°04'22.0" E18°31'06.9"	Zeekoevlei: Zeekoevlei Rd.	Surrounded by low-income residential areas. Sediment was collected near the Cape Flats WWTW. Receives flow from the Bottelary River and receives discharge from the Rondevlei.	Zeekoevlei: Large vlei with slow water flow. Frequent blue-green algal blooms.
CT18	17/04/07	S34°04'34.9" E18°31'06.5"	Muizenberg: Uxbridge Road. Zandvlei yacht club.	Residential area. Vlei receives flow from rivers draining industrial areas.	Zandvlei: Tidal flow - estuary area that forms a vlei.
CT19	17/04/07	S34°04'34.9" E18°27'41.8"	Frogmore Estate: Aberfeldy Road.	Residential area situated directly down-stream of Retreat Industrial Area. The river flows directly through this industrial area.	River with medium flow. Beyond Spaanschemat- and Keysers River confluence.
CT20	17/04/07	S34°02'50.9" E18°28'26.2"	Heathfield: Willowmere Road.	Residential area surrounded by industries in. Receives flow from the Diep River.	Little Princess vlei: Vlei with many reeds and water plants.

3.2.4 Durban

Durban has a large and diverse economy consisting of industrial, tourism and transportation sectors, and one of South Africa's foremost harbours. The harbour is a major importer of raw materials and an exporter of many products such as manganese, chrome ore, coal, sugar and grain. Significant industries in Durban include textiles, railroad repair, machine works, oil refineries and the manufacturing of soap, paint, dye, and fertiliser. Many high-density, low-income residential areas are located in the vicinity of the city. Umlazi is one of the biggest of these types of settlements, accommodating more than 300 000 inhabitants (Statistics South Africa, 2008).

In Durban, several river systems were targeted to represent highly polluted, less polluted and theoretically "pristine" areas. Sediment was sampled from several industrial areas (D1 to 5, 8 to 11 and 14) and close to the harbour at the yacht club (D6), as well as near the dry docks (D7), where old paint is stripped from ships. Reference samples were also collected from rivers flowing through residential areas (D12 and 13), expected to receive minimal industrial effluent, due to the lack of industries up-stream of these areas (Fig. 3.10). Site descriptions follow in Table 3.7.

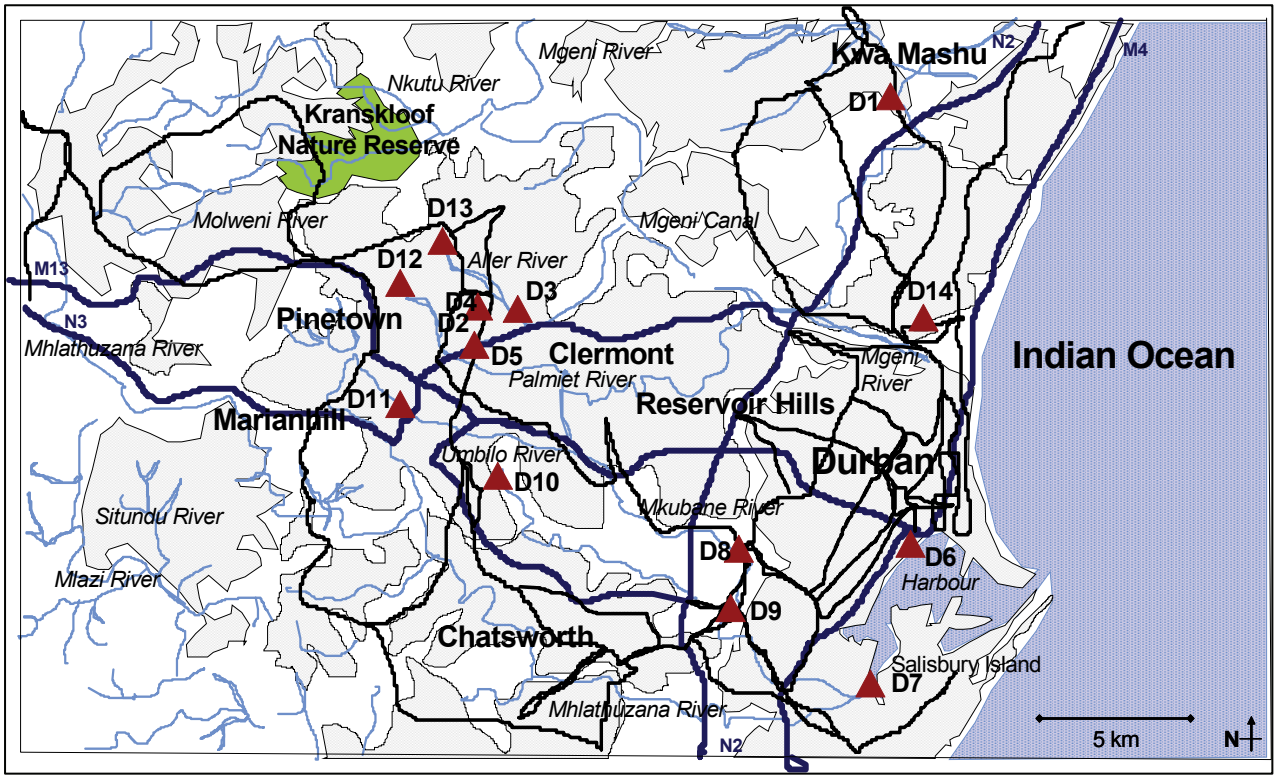


Figure 3.10. Map of Durban and its suburbs indicating the sampling sites of this area (D1 – 14).

Table 3.7. Description of sediment sampling sites from Durban (D) and its suburbs.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
D1	20/02/07	S29°45'03.8'' E31°00'36.7"	Inanda/Phoenix: On R102. Stone age cement construction site	Draining various industries, mainly textile and building materials manufacturers.	Umhlagane River. Medium/slow flowing stream.
D2	20/02/07	S29°48'21.9'' E30°53'49.3"	New Germany WWTW (downstream of effluent)	Low-income residential area, receiving effluent from various industries including steelworks and textile industry.	Aller River. Medium/slow flowing stream receiving treated sewage effluent.
D3	20/02/07	S29°48'21.9'' E30°53'49.3"	New Germany WWTW (upstream of effluent)	(See D2)	Aller River. Medium/slow flowing stream sampled up-stream of effluent pipes.
D4	20/02/07	S29°48'17.2'' E30°53'04.5"	Falcon industrial park, off Eskom Road at Valleyview RD.	Mainly textile industry. Up-stream of New Germany WWTW.	Aller River. Slow, narrow stream.
D5	20/02/07	S29°48'53.2'' E30°52'55.6"	Down-stream of Pinetown and New Germany industria. Genie Sand distributors.	Various industries.	Palmiet River. Medium/rapid flowing stream.
D6	21/02/07	S29°51'56.3'' E31°01'03.4"	Durban Harbour: At yacht club across from Wilson wharf.	Down-stream of industrial areas.	Sea – harbour.
D7	21/02/07	S29°53'58.5'' E31°00'17.2"	Durban Harbour: Bayhead Park. Bayhead Road 550. Trawlers wharf.	Down-stream of dry docks and industrial areas.	Harbour.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
D8	21/02/07	S29°52'04.2'' E30°57'53.0"	Umkumbaan: Next Cato Manor Sports Field. Wentlock Rd.	Down-stream of various industries. Historically crematoria site.	Mkubane River tributary. Slow flowing, narrow river stream.
D9	21/02/07	S29°52'55.9'' E30°57'46.5"	Carrington Heights: Wright Place. Under bridge off Francois Road- Grosvenor Road intersection.	Industrial area.	Mkubane and Umbilo River confluence. Slow flowing, narrow stream.
D10	21/02/07	S29°50'51.4'' E30°53'22.0"	Pinetown: Umbilo WWTW.	Down-stream of WWTW, receiving effluent from textile and dye industries.	Umbilo River. Slow flowing river stream.
D11	21/02/07	S29°49'44.5'' E30°51'30.8"	Pinelands: Trim Park (recreational park). Winston Churchill Drive – Merrifield Road crossing.	Directly down-stream of various industries.	Umbilo River. Shallow river stream.
D12	21/02/07	S29°47'56.8'' E30°51'36.9"	Pinetown: Padfield residential area. Padfield Road.	Residential area. Palmiet River "reference site". Site is up-stream of industries and expected to be "pristine".	Palmiet River. Slow flowing, narrow river stream
D13	21/02/07	S29°47'17.5'' E30°52'22.2"	Reservoir Hills residential area.	Residential area. Aller River "reference site". Site is up-stream of industries and expected to be "pristine".	Aller River. Shallow river stream.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
D14	21/02/07	S29°48'28.2'' E31°01'19.4"	Umgeni Mouth: Umgeni Park (Riverside). Riverside Road, between Butter and Brown's Drive turn-offs.	Umgeni River mouth: After confluence of Palmiet-, Aller- and Mbongokazi Rivers with Umgeni. May contain various industrial effluents.	Umgeni River mouth. Deep, wide river mouth.

3.2.5 Richards Bay

Richards Bay is home to the country's largest harbour, which was converted into a deep water harbour in 1976. The city has an extensive industrial complex composed of aluminium smelters, a fertiliser plant, paper and pulp manufacturers and woodchip producers. Iron ore, titanium oxide and zircon are mined from sand dunes located close to the estuary. The city's exports include coal, aluminium, titanium, granite, ferrochrome, paper, pulp, woodchips and phosphoric acid (Statistics South Africa, 2008).

Only nine samples were collected from Richards Bay, since the area is very concentrated with water bodies situated close to one another. Sediment was sampled from the harbour and its surrounding areas, including the small craft harbour (RB5), Naval Island recreational area (RB6) and water canals under harbour authority (RB2 and 3; Fig. 3.11). Where accessible, samples were also collected from other industrially impacted areas, specifically targeting the two aluminium smelters, a fertiliser plant, a woodchip manufacturer and other less significant industries (RB1 and 4; Fig. 3.11). Sediment from RB1 was very black and had a distinct noxious odour. The site was located in the direct vicinity of a large industrial complex and seemed to be heavily polluted with industrial effluent. Sites RB7 to 9 were chosen in residential areas (Fig. 3.11). Table 3.8 contains site descriptions and other information on the sampling areas.

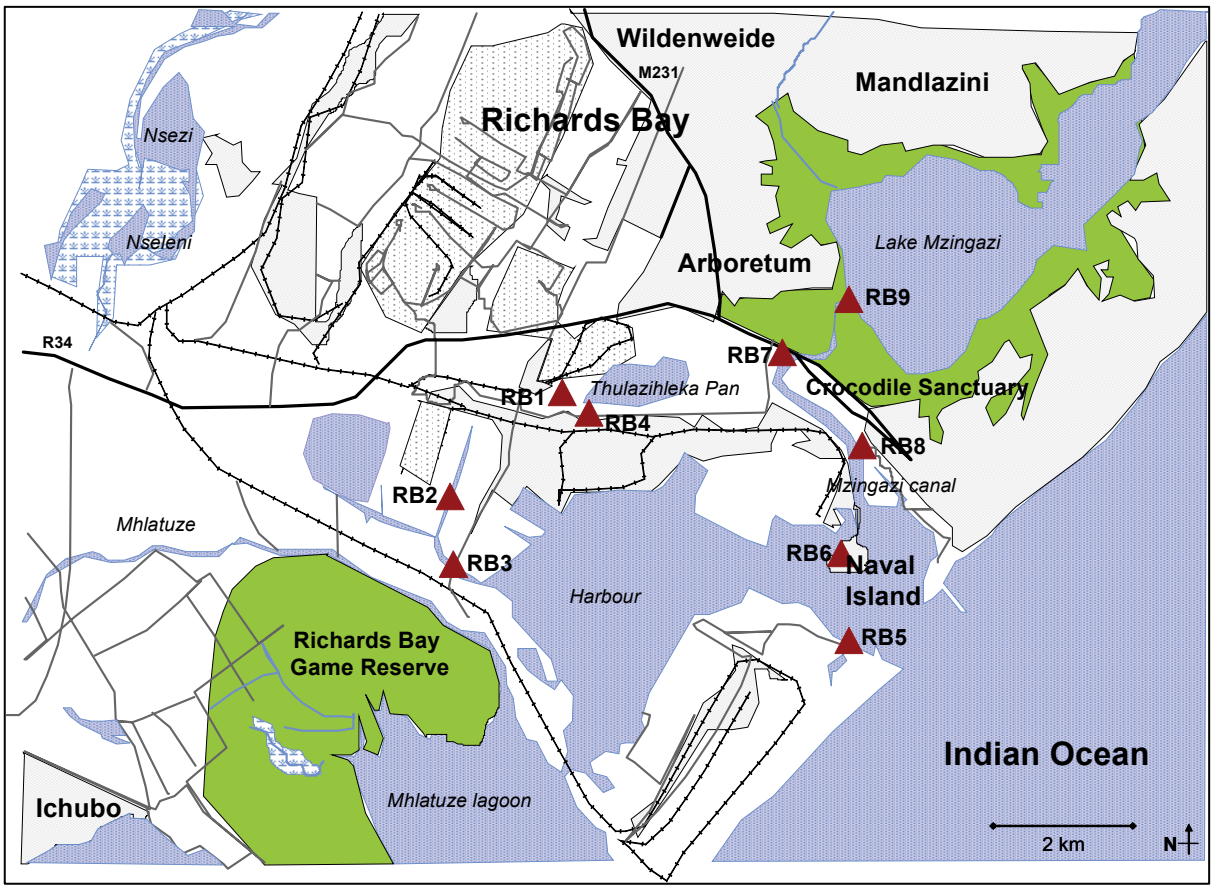


Figure 3.11. Map of Richards Bay indicating sediment sampling sites.

Table 3.8. Description of sediment sampling sites from Richards Bay (RB).

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
RB1	19/02/07	S28°46'44.2'' E32°02'07.9"	Richards Bay industrial: Gravel road off Harbour Arterial.	Industrial area. Includes woodchip production, aluminium smelters, fertiliser plant etc.	Wetland area with many reeds.
RB2	19/02/07	*S28°47'33.40'' E32°01'03.32"	Manzamyana Canal off Urania Road, close to railway.	Various industries. In close proximity of aluminium smelters.	# Manzamyana Canal.
RB3	19/02/07	*S28°48'03.48'' E32°01'05.21"	Urania Road. After confluence of Manzamyana- and Bhizolo Canals.	Down-stream of various industries. Near aluminium smelters.	# Manzamyana- and Bhizolo Canal confluence.
RB4	19/02/07	S28°46'54.0'' E32°02'22.5"	Thulazihleka Pan: Bird Sanctuary.	Down-stream of various industries. Near woodchip producers, aluminium smelters etc.	Pan. Sample was taken near reeds.
RB5	19/02/07	*S28°48'37.70'' E32°04'51.15"	Richards Bay Harbour. Sample taken at location between small craft harbour and combi terminal.	Near industrial area. Sample taken inside harbour.	Harbour (Sea).
RB6	18/02/07	S28°47'58.5'' E32°04'45.4"	Naval Island. Recreational area.	Near harbour.	Lagoon-like, tidal area.
RB7	18/02/07	S28°46'26.6'' E32°04'09.4"	Ngodweni Canal. After Medway Road, before joining with Mzingazi Canal.	Residential development.	# Ngodweni Canal.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
RB8	18/02/07	S28°47'09.4'' E32°04'55.9"	Mzingazi canal. Meerensee boatclub.	Residential area.	# Canal.
RB9	18/02/07	S28°46'02.2'' E32°04'46.9"	Lake Mzingazi: Private Road.	Upstream from future residential area under construction.	Lake.

Unlined canals, which look like natural streams.

* GPS coordinates uncertain due to poor satellite reception.

3.2.6 Bloemfontein and Botshabelo

As with the other cities included in the study, Bloemfontein has a large industrial centre containing railroad workshops, metal works, food-processing plants, and manufacturers of clothing, furniture, plastics and glassware. The city is home to a power generation plant and has many transportation services, including a railway and an airport. Mining, livestock farming (cattle and sheep) and dry-land maize and wheat cultivation are the main occupations in the area surrounding the city.

In total, ten sediment samples were collected from Bloem Spruit and Modder River, as well as from other dams and ponds in the area. The sampling locations once again included industrially- (BF4 to 6 and 8), residentially/recreationally- (BF3, 7, 9 and 10) and agriculturally (BF1 and 2) impacted areas (Table 3.9; Figs. 3.12 & 3.13).

Botshabelo, the largest township in the Free State Province, is located about 50 km east of Bloemfontein along the N8 national road (Fig. 3.12). Botshabelo is mainly residential, but it has an industrial complex consisting of more than 150 factories specialising in the manufacturing of plastics and textile, amongst others. Samples were taken from a wetland system meandering through the township, passing through industrial- and residential areas (BO1 to 4; Fig. 3.12, Table 3.10). During the sampling period, it was evident that many parts of the township had poor municipal services with inadequate water supply and sanitation.

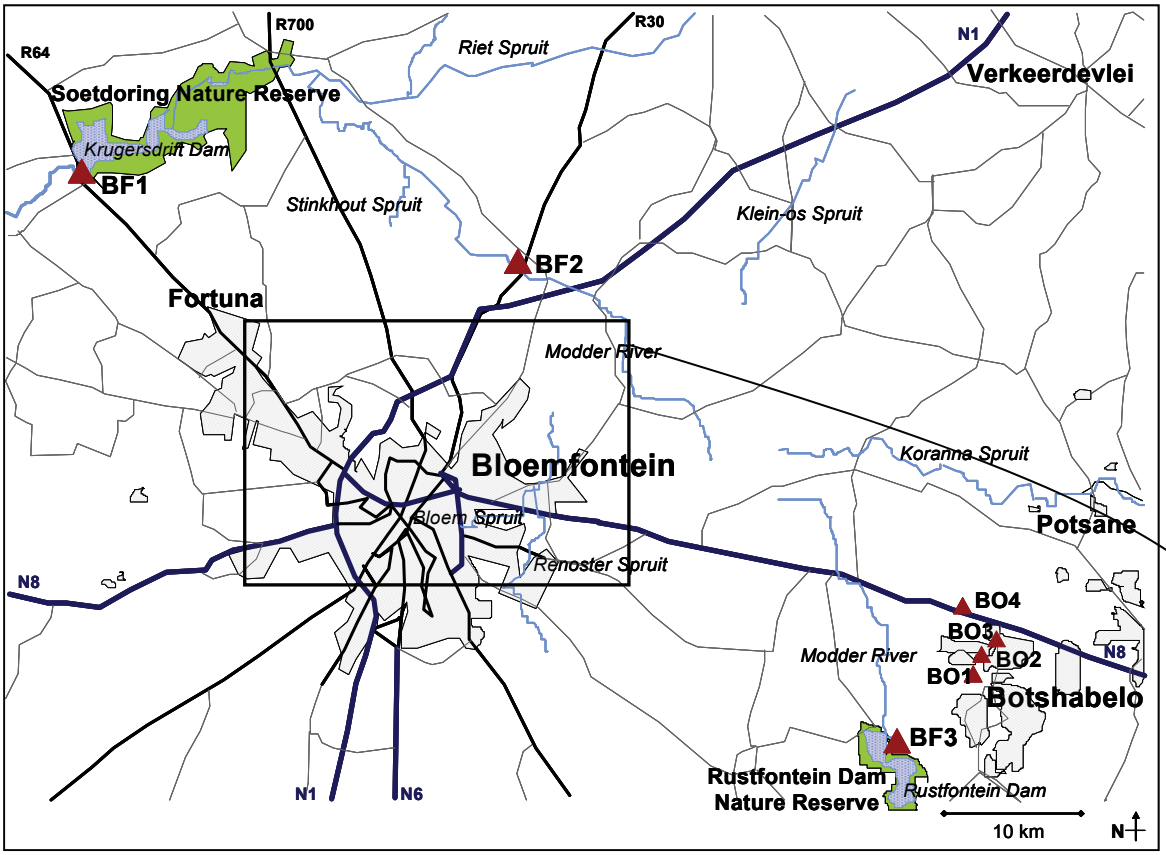


Figure 3.12. Map of Bloemfontein and its surrounding areas indicating the sites sampled from Botshabelo and the outskirts of Bloemfontein.

Figure 3.13. Map of Bloemfontein indicating sediment sampling sites.

Table 3.9. Description of sediment sampling sites from Bloemfontein (BF).

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
BF1	19/04/07	S28°53'06.5" E25°57'18.3"	On the R64 near the wall of the Modder Dam/Krugersdrift Dam.	Agricultural area.	Modder River tributary that flows from the Modder Dam. Rocky riverbed with many reeds and little sediment.
BF2	19/04/07	S28°56'44.8" E26°19'01.5"	On the R30 off the N1.	Undeveloped/agricultural area.	Modder River tributary down-stream of Krugersdrift Dam. Slow flowing tributary with clayish sediment.
BF3	19/04/07	S29°16'42.9" E26°37'27.5"	Off the N8 Rustfontein turn off.	Rustfontein Dam recreational area. No industries in close proximity. May be impacted on by Botshabelo.	Large dam. Used for recreational purposes, including fishing.
BF4	19/04/07	S29°07'20.1" E26°16'05.0"	Of the M10 (Rudolf Greyling) south of the intersection with the N8 in Bloemfontein.	Across from Bloemfontein Golf Club, near Ooseinde industrial area.	Bloem Spruit: River tributary flowing through deep gully.
BF5	19/04/07	S29°07'12.2" E26°14'15.6"	Buitesig: McGreggor Street.	River flows through the CBD, many industries and a low-income residential area.	Bloem Spruit: Shallow, narrow, rapid flowing river with many rocks..
BF6	19/04/07	S29°07'53.5" E26°14'15.2"	Off McGreggor Street, turn into Dr. Belcher Street.	The stream flows through an industrial- and a low-income residential area.	Unknown river stream than joins with Bloem Spruit at a down-stream locality. Shallow river medium/slow water flow.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
BF7	19/04/07	S29°07'44.1" E26°14'48.9"	Ooseinde: McKenzie Street.	Industrial area with an informal settlement located on the river bank.	Part of the same system as BF6. The stream is narrow, shallow and rocky.
BF8	19/04/07	S29°06'47.5" E26°12'26.5"	Loch Logan: Across from the waterfront on municipal grounds.	Situated within Bloemfontein's CBD. Near a shopping mall. Mostly businesses located near the area.	Loch Logan: Pond. Receiving water from inflow pipes.
BF9	19/04/07	S29°09'14.2" E26°13'18.2"	Hamilton: Mill Street	Low-income, high-density residential area (Bochabella*) and a large industrial complex (Hamilton).	Blou Dam: relatively large dam with many reeds and abundant bird life. The area was flooded during the time of sampling.
BF10	19/04/07	S29°11'09.5" E26°10'53.1"	Lourierpark: Geelhout Street	Residential/Recreational area.	Rooi Dam: Large Dam with abundant bird life and many plants.

* Not to be confused with Botshabelo, the large township situated 50 km east of Bloemfontein.

Table 3.10. Description of sediment sampling sites from the township of Botshabelo (BO).

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
BO1	19/04/07	S29°13'47.1" E26°41'31.8"	Off of main road, open field.	Residential/industrial area.	Wetland with a narrow stream. Raw sewage was present in water.
BO2	19/04/07	S29°13'02.1" E26°42'01.2"	Off of main road on right hand side.	Residential area.	Wetland. Sampled from a small pond-like area.
BO3	19/04/07	S29°12'18.8" E26°42'31.7"	Main Road close to the Shell garage.	Industrial area.	Wetland area – a narrow stream with many reeds.
BO4	19/04/07	S29°11'28.9" E26°41'35.6"	On the N8 – near a small bridge.	Near national road (N8) surrounded by grasslands.	Wetland area with narrow stream..

3.2.7 International and other rivers

One of the project's objectives was to estimate the scale of POPs transport to neighbouring countries. For this purpose, several international rivers were sampled, including the Olifants- (Mpumalanga), Crocodile- (Mpumalanga), Komati-, Pongolo-, and Limpopo Rivers. The Olifants-, Crocodile- (Fig. 3.14), and Pongola (Fig. 3.15) Rivers flow from South Africa into Mozambique, and the Komati River (Fig. 3.15) runs into Swaziland. The Limpopo River (Fig. 3.16) is a large river system running through South Africa and Mozambique, forming a border between South Africa and Zimbabwe, and South Africa and Botswana. Samples were collected from three to six locations distributed within each river system, and at least one sediment sample was taken as close as possible to the borders of neighbouring countries. The majority of sites were located down-stream of, or in close proximity to industries such as paper and pulp manufacturers, petrochemical plants and brick works. Many sites were situated in the vicinity of agricultural lands, primarily sugar cane plantations.

To determine if selected POPs are transported via long-range transport and deposited in the colder climates of high altitude areas, sediment was sampled from the Mzimkhulu- and Mkomzi Rivers and one unknown stream located in the Drakensberg, a high altitude mountain in South Africa (Fig. 3.17). No major industries are located in the area, and the main agricultural practice is farming with cattle and crops, therefore no industrial POPs are expected to be found in these sediments. If these pollutants are present in sediments collected from these localities, it could possibly be a consequence of long-range transport. However, chlorinated pesticides, which may be applied to crops, were expected in these sediments.

En route from Richards Bay to Durban opportunistic samples were taken from three water bodies: the Mhlathuze-, Tugela- and Umvoti Rivers. These samples were collected down-stream of, or in close proximity to paper and pulp manufacturers to assess the potential contribution of these industries to the presence of organic pollutants in sediments. The samples were taken in Felixton, Mandini and Aldinville (Fig. 3.13).

Site descriptions are listed in Table 3.11 and sites maps are given in Figures 3.14 to 3.17.

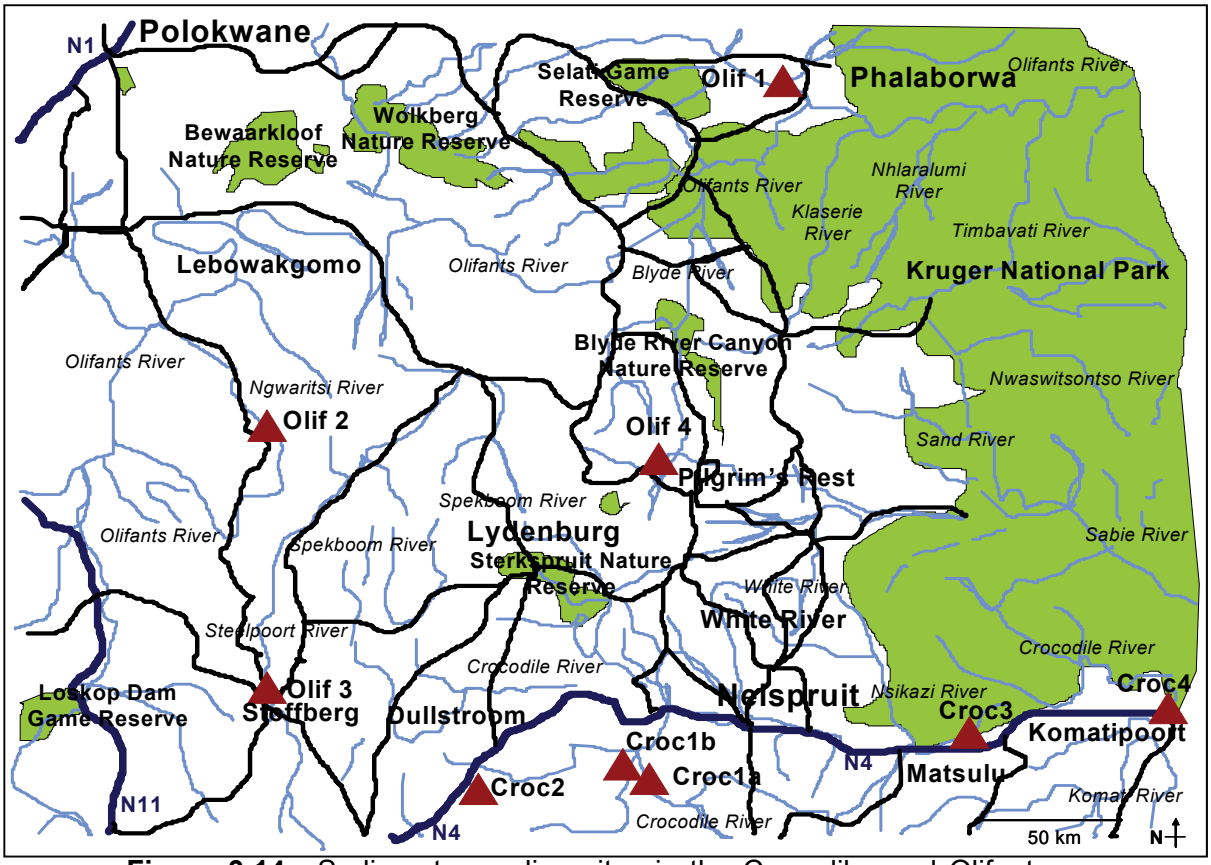


Figure 3.14. Sediment sampling sites in the Crocodile- and Olifants

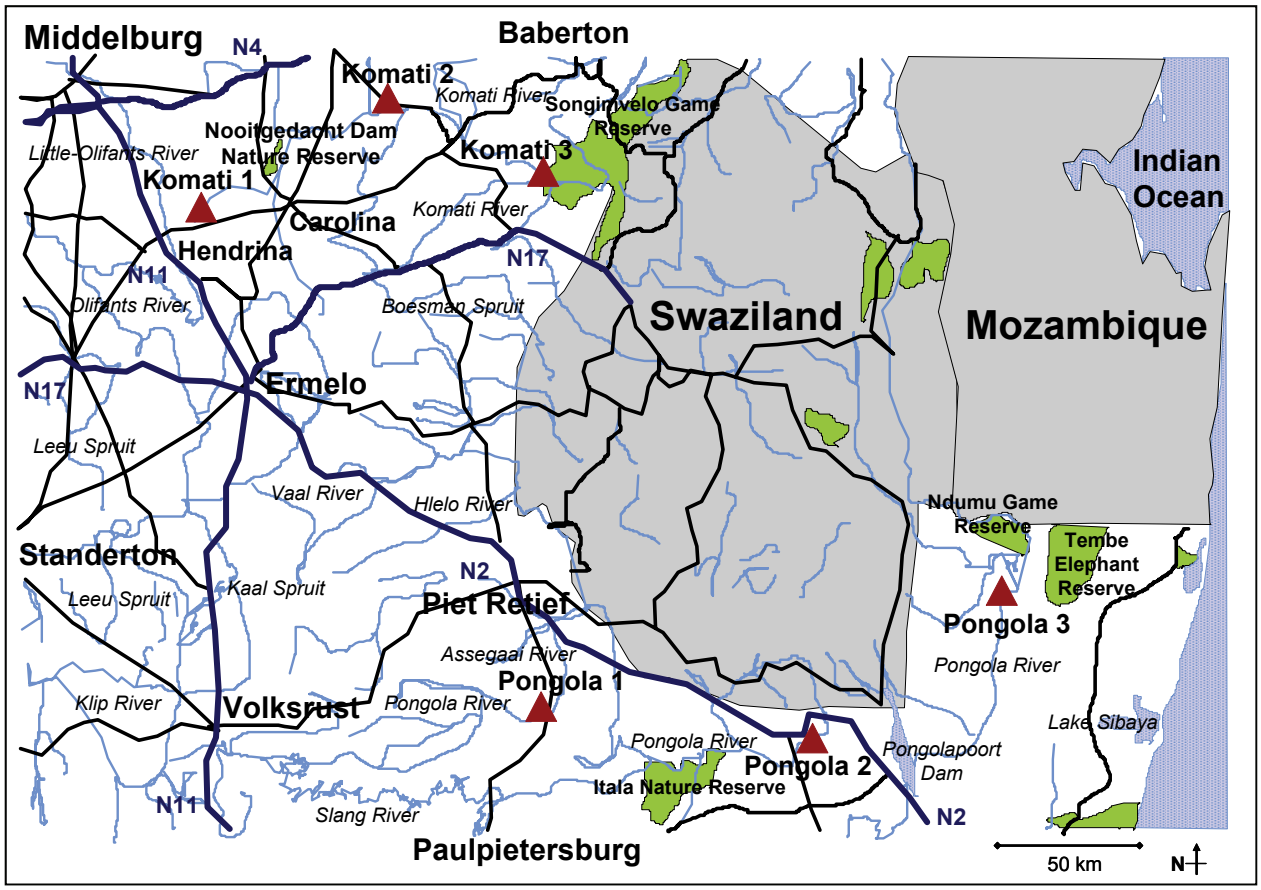


Figure 3.15. Sediment sampling sites in the Komati- and Pongola

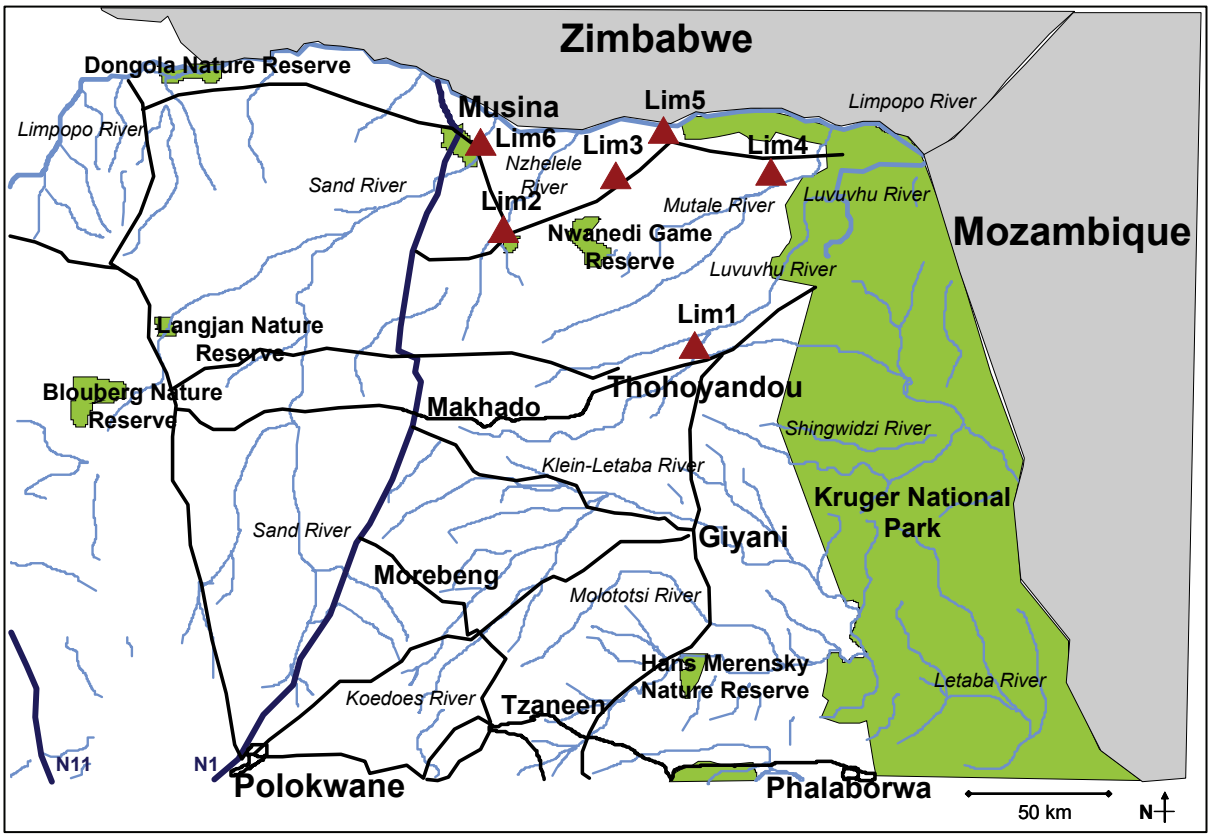


Figure 3.16. Sediment sampling sites in the Limpopo River.

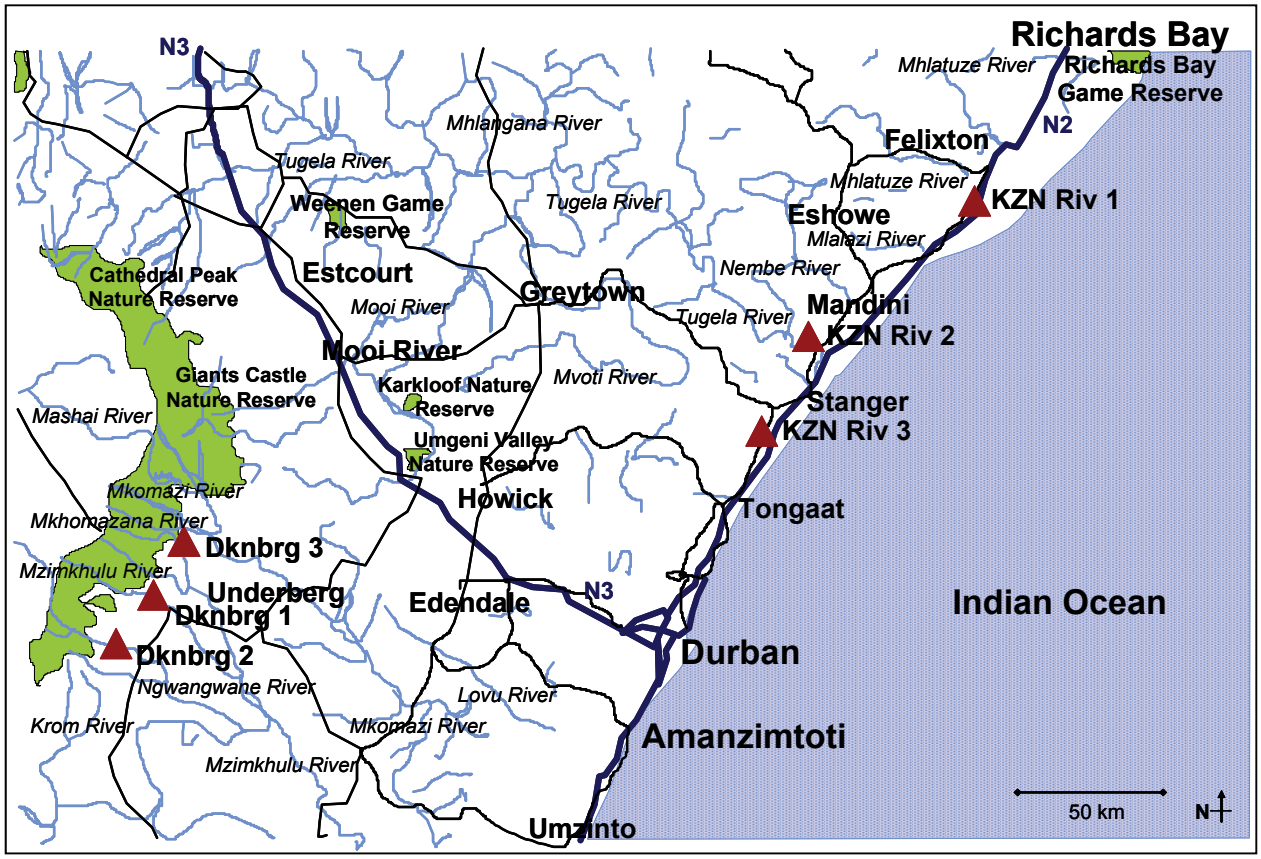


Figure 3.17. High-altitude rivers and rivers sampled near KZN paper mills.

Table 3.11. Description of sediment sampling sites from international- [Limpopo- (Lim), Olifants- (Olif), Crocodile- (Croc), Komati- and Pongola Rivers] and high-altitude rivers (Drknberg), and KwaZulu-Natal (KZN) rivers associated with paper mills.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
Lim1	07/03/07	S22°54'071" E30°41'729"	North east of Thohoyandou on R524.	Agricultural (cattle drinking place). Local housing 50 km away.	Luvuvhu River.
Lim2	08/03/07	S22°36'524" E30°09'942"	Near Tshipise on R508, north of R525-R508 intersection.	Agricultural (citrus farms).	Nzhele River.
Lim3	08/03/07	S22°28'260" E30°27'796"	On R525 – North of Nwanedi Game Reserve.	Bridge and traffic crossing.	Nwanedi River.
Lim4	07/03/07	S22°28'407" E30°52'823"	Gravel road south of Masisi.	Local housing in the vicinity agricultural activities. Laundry and bathing in river.	Mutale River.
Lim5	08/03/07	S22°21'063" E30°35'489"	Gravel road north of R525.	Gholf course, lucern and palm tree plantation. Fishing spot / game farms.	Nwanedi River.
Lim6	08/03/07	S22°23'931" E30°05'950"	On R508: South-east of Messina.	Adjacent to Musina Nature Reserve.	Sand River.
Olif1	15/02/07	S23°58'35.5'' E31°04'26.5"	On R530 near Phalaborwa, 46 km from Mica.	Industrial area. Close proximity of oil and gas refinery, powerplant, mines.	Olifants River: Slow/stationary river tributary.
Olif2	15/02/07	S24°47'00.0'' E29°50'05.5"	On R579 near Jane Furse. Bridge over road.	Agricultural land and close to many informal settlements.	Olifants River: Rapid flowing, rocky river tributary.
Olif3	15/02/07	S25°23'55.0'' E29°49'31.9"	2 km north of Stoffberg. Under bridge 2871, Blinkwater.	Agricultural area. Steel and coal works.	Olifants River: Relatively rapid flowing water river tributary.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
Olif4	16/02/07	S24°52'38.4'' E30°45'36.6"	Pilgrim's Rest: Secondary Road. (Blyde River)	Paper and pulp plantations and mining industries.	Olifants River tributary: Very rocky river with rapid flow.
Croc1a	17/02/07	S25°35'57.0'' E30°45'30.5"	Secondary road north-west of Kaapsehoop. Water under road.	Paper mill – downstream of Ngodwana paper and pulp plant. Many plantations in area.	Crocodile River: Wetland-like tributary. Slow flowing with a lot of plant material.
Croc1b	17/02/07	S25°34'04.8'' E30°39'43.9"	Ngodwana paper mills. Tar road off of bridge.	Ngodwana paper mills.	Crocodile River: Fast flowing tributary. Soil and sediment composite.
Croc2	17/02/07	S25°38'22.0'' E30°19'37.6"	Waterval Boven: Bridge located in Abattoir Road.	Town, agricultural lands and plantations.	Crocodile River: Rocky, fast flowing river.
Croc3	16/02/07	S25°29'17.7'' E31°30'11.9"	Malelane: Monte Vista Estate	Sugar cane and plantations.	Crocodile River: Relatively slow flowing river stream.
Croc4	16/02/07	S25°26'31.8'' E31°57'50.6"	Komatipoort: Bridge over river. Right turn-off of Rissik Street. .	Sugar cane and plantations.	Crocodile River: Slow, wide river with many reeds.
Komati1	17/02/07	S26°07'58.8'' E29°54'09.8"	On the R38 between Carolina and Hendrina. (Vaalbank)	Agricultural area with coal mines.	Komati River tributary. Almost dry, very slow flowing river.
Komati2	17/02/07	S25°49'35.1'' E30°25'23.9"	On R541 approximately 15 km north of Badplaas.	Agricultural.	Komati River tributary: Rocky, rapid flowing tributary.
Komati3	16/02/07	S26°00'32.4'' E30°52'09.7"	Josefdal. Off R40 from Baberton. Mlomati Sappi plantations.	Sugar cane and paper plantations.	Komati River tributary: Slow flowing tributary with many reeds.

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
Pongola1	17/02/07	S27°18'33.8'' E30°53'53.1"	On R33 near Commondale.	Sappi plantations.	Komati River: Wide, deep river with sandy sediment.
Pongola2	18/02/07	S27°25'01.5'' E31°42'50.7"	Illovo farms near Pongola.	Sugar refinery. Agricultural, mainly sugar cane plantations.	Komati River: Wide river with heavy flow and sandy soil.
Pongola3 ***Sample lost***	18/02/07	S27°02'12.7'' E32°15'56.4"	50 km north of Jozini on Khosi-bay road bridge.	Agricultural. Many informal settlements and brick works near site.	Komati River: Wide river with medium flow. (Swimming and bathing)
Drknberg1	22/02/07	S29°46'27.9'' E29°28'29.2"	Drakensberg: On R617. At farm 4 km South of Underberg.	Farming/Plantations.	Upper part of Mzimkhulu River.
Drknberg2	22/02/07	S29°54'20.3'' E29°20'11.6"	Drakensberg: Right turn-off off R617. Approximately 20 km from Bushmen's neck.	Farming.	Mountain stream.
Drknberg3	22/02/07	S29°39'10.7'' E29°32'45.8"	Secondary gravel road. 20 km north of Himeville.	Farming.	Upper part of Mkomazi River.
KZN Riv1	20/02/07	S28°50'19.3'' E31°54'02.7"	Felixton. Paper mills.	Down-stream of paper and pulp plant. Many agricultural lands (mainly sugar cane) in the area.	Mhlathuze River. Wide river, with medium to rapid flow.
KZN Riv2	20/02/07	S29°09'05.9'' E31°24'35.1"	Mandini: Off R102, road into town by bridge.	In close proximity of paper mills and sewage works.	Tugela River. Narrow part of river, with slow to medium flow.
KZN Riv3	20/02/07	S29°22'59.1'' E31°15'05.9"	Aldinville: South of Stanger. Gravel road, under bridge.	In close to paper mills, plantations, brickworks and sand mining.	Umvoti River. Wide river, with medium flow.

3.2.8 Sediment sampling

Soil was collected on only a few instances (less than five samples in total), and the sampling method for soil was similar to the method used for sediment sampling. The method described in 3.1.2 was used.

3.2.9 Sample extraction and clean-up

All the samples were first screened for the presence of DLCs with the H4IIE-*luc* reporter gene bio-assay at the North-West University in Potchefstroom. Only those samples eliciting significant responses in the assay were selected for chemical analysis (assuming that the compounds included in the study have similar sources to DLCs) at the National Metrology Institute of South Africa (NMISA) based on the grounds of the Council for Scientific and Industrial Research (CSIR), Pretoria or Norwegian Institute for Air Research (NILU) in Kjeller, Norway.

3.2.9.1. Sample extraction and clean-up for analysis with H4IIE-luc bio-assay

Prior to extraction, the samples were air-dried for a week in an isolated, darkened room that was free from any other activity (Mai *et al.,* 2007). Small stones, leaves and twigs were carefully removed from the dried sediment or soil. The samples were ground with pre-cleaned mortars and pestles to increase the reactivity surfaces, and homogenised with a copper sieve (0.5 mm mesh size). An Accelerated Solvent Extractor (ASE; Dionex, ASE 100) was used to extract DLCs from the samples. The ASE operates on the principle of pressurised fluid extraction by using elevated temperatures and pressure to achieve rapid, effective extraction (Grochowalski *et al*., 2004).

The method of extraction can be summarised as follows: A 40 g sub-sample was mixed with an equal volume of anhydrous sodium sulphate (Na_2SO_4) (Merck, UniVAR) to remove residual moisture (Hilscherová *et al*., 2003). Depending on the volume of the sample, it was packed in a 66 ml or 100 ml stainless steel ASE extraction cell, sandwiched between two cellulose filters (30 mm, Dionex). A 3:1 (v/v) mixture of dichloromethane (DCM) and hexane (HPLC grade; Burdick and Jackson) was used to extract the samples using two cycles with the following parameters: 10 342 kPa, 100°C, 5 minute static and heat time, 60% flush volume and 100 seconds nitrogen purge (McCant *et al*., 1999; Hölscher *et al*., 2004). The extracts were collected in pre-cleaned collection bottles and allowed to cool, where after they were

evaporated to 1 ml under a gentle stream of nitrogen gas (ultra pure grade, Afrox) at 40˚C inside a TurboVap II (Caliper).

The volumes were adjusted to 10 ml with hexane and the extracts were subjected to an acid clean-up to prevent non-target compounds, such as PAHs from contributing to the response elicited from the H4IIE-*luc* cells (US EPA, 1999; Vondráček *et al*., 2001).

Elemental sulphur was removed from the extracts by gel permeation chromatography (GPC) or by treatment with activated copper. Exclusion of sulphur by GPC was the preferred method, but when the instrument was temporarily out of order, activated copper was used. To exclude sulphur from the samples, the extracts were evaporated to almost dryness and reconstituted to 2 ml with DCM, subsequent to the acid clean-up. After being pre-filtered through a 1 µm glass fibre filter (Ascrodisc, LifeSciences), the extract was injected into the GPC using the same parameters as for the reference mixture. The extract was collected in TurboVap II flasks from 0 to 22 minutes after injection to eliminate sulphur. Alternatively, a copper treatment (US EPA, 1986) was done. The extracts were finally evaporated to 1 ml and stored in amber coloured gas chromatography (GC) vials at -4°C until used in the bio-assay. On the day of the bio-assay, a two-time dilution series were prepared from the extracts using hexane (Khim *et al*., 1999).

All utensils and apparatus used during sample extraction and clean-up were washed beforehand with phosphate-free soap, rinsed with ultra-pure water (18MΩ) and rinsed with HPLC-grade acetone and hexane (Vondráček *et al.*, 2001).

3.2.9.2 Sample extraction and clean-up for chemical analysis

Extraction and clean-up method for OCPs, PBDEs and PCBs

Sample extraction and analysis for OCPs, PBDEs and intentionally produced PCBs were done under Norwegian accreditation by the Norwegian Institute for Air Research (NILU) in Kjeller, Norway. Analytical details are given by Knutzen *et al*. (2003) and Bengtson Nash *et al.* (2008). In short, dried sediment samples were spiked with ^{13}C-labelled analogs of the analytes and extracted with cyclohexane. The majority of the sample matrix was removed with size exclusion chromatography followed by cleanup on silica and alox columns. Sulphuric acid was also used for

cleanup prior to analysis. Suitable recovery control standards were added to each sample before quantification.

Extraction and clean-up method for PAHs

The extraction and clean-up for PAHs were done at the North-West University, Potchefstroom, whereafter the extracts were sent to the National Metrology Institute of South Africa (NMISA), Pretoria, for analysis. Extraction and clean-up were done using a similar method as depicted in Section 4.4.1. In summary, 40 g of dried sample were mixed with an equal volume of Na_2SO_4, and spiked with perdeuterated surrogate standards prior to extraction. The samples were extracted with a 3:1 mixture of DCM and hexane with the ASE^{TM} (Dionex), using the same parameters as listed in Section 4.1.1. Elemental sulphur was removed from the extracts with the GPC (Waters), after which the extracts were cleaned on activated Florisil® PR (60-100 mesh, Fluka) columns (US EPA, 1996b). Suitable recovery control standards were added to each sample before quantification.

3.2.10 Sample analysis

3.2.10.1 H4IIE-luc bio-assay

Refer to Section 3.1.4.

3.2.10.2 The MTT viability bio-assay

Refer to Section 3.1.5.

3.2.10.3 Chemical analysis of samples

Chemical analysis of samples for OCPs, PBDEs and PCBs

Chemical analyses of samples for OCPs, PBDEs and PCBs were done by NILU in Kjeller, Norway. Analysis were done with HRGC/HRMS using an Agilent 6890 N gas chromatograph coupled to an Autospec (Micromass Waters, Manchester UK) mass spectrometer. The mass spectrometer was operated at a resolution of >10 000 using electron ionisation. Procedural blanks were run throughout using the criteria that blank levels of target analytes should be below the detection limit (signal-to-noise ratio 3:1) or less than 10% of the lowest expected sample concentration.

This method is accredited after ISO/IEC 17025 – “General requirements for the competence of testing and calibration methods”. The following conditions had to be

met for quality assurance for an unequivocal identification and quantification of analytes:
(1) the retention time had to be correct;
(2) the isotope ratio had to be correct;
(3) the signal-to-noise ratio had to be more than 3:1 for identification;
(4) the recovery of internal standard had to be within 40 to 120%; and
(5) blank values had to be determined for the complete clean-up and quantification procedures.

Chemical analysis of samples for PAHs

Chemical analysis for PAHs was done by the NMISA based in Pretoria, South Africa. The Pegasus III Gas Chromatography-Time of Flight-Mass Spectrometer (GC-TOF-MS) (Leco) consisting of an Agilent 7683B series injector, Agilent Technologies 6890N Network GC system, and a Pegasus III time of flight mass spectrometer run on ChromoTof version 3.32 software, were used. The column configuration consisted of a 2 m long column with a 0.53 mm ID deactivated guard column, coupled to a 12 m, 0.25 mm RESTEK column with a film thickness of 0.15 µm, fitted with a press-tight fitting. Helium was used as the carrier gas.

For quality control purposes, procedural blanks were run throughout and the acquisition system was adjusted with instrument optimisation and automatic leak check.

3.2.11 Determination of oxidisable and total organic carbon

Refer to Section 2.1.7.

3.2.12 Statistical analysis

Basic statistics

To do the fundamental calculations and to draw bar and line graphs and pie charts Microsoft Excel was used. STATISTICA version 8 was used for basic comparisons between two or more sampling sites, sampling regions and land-use types. Here, the Post-hoc Tukey one-way ANOVA, Kruskal-Wallis ANOVA or the non-parametric Mann-Whitney U test were applied, depending on the amount of data points available for comparison. For these tests a *p*-value of less than 0.05 ($p < 0.05$) was considered as statistically significant.

For correlations, Pearson's correlation coefficient (R^2) was used to determine effect size. The correlation was not significant if $R^2 \leq 0.13$. If R^2 was greater than 0.13, but smaller or equal to 0.3, the correlation was significant; and if R^2 was greater than 0.3, the correlation was practically important or statistically practically significant (Ellis & Steyn, 2003).

Multivariate data analysis

Principle component analysis (PCA) was performed to determine the distribution patterns of pollutants at the various sites. The software package CANOCO 4.5 was used, taking only the sites where chemical analyses results were available into account.

Individual concentrations were expressed as a portion of the sum of all other pollutant concentrations at each sampling site (Alcock *et al*, 2002; Masunaga *et al*., 2003). In other words, compositional data were considered. The compositional data were log-transformed according to the method proposed by Howel (2007), prior to applying the PCA. The motivation for the method is as follows: The linear dependence of proportions in compositional data leads to an absence of an interpretable covariance structure. This causes the apparent strength and direction of the association between two proportions to appear to depend on the number of elements in the total composition and to differ for different subsets of elements. The ratio of proportions should, however, remain the same and not be influenced by how many pollutants there are, since compositional data attempt to describe the relative magnitudes of pollutants. To address the absence of this property, the centred log-ratio of each proportion (p) was determined by dividing each proportion by the geometric mean across the sample: $\log(p_{ij}/g(p_j))$ where $g(p_j) = (p_{1j}/p_{2j}\ldots\ldots,p_{dj})^{1/d}$, where d pollutants are measured in n samples so that p_{ij} is the proportion of pollutant i in sample j (Howel, 2007). The PCA was performed without any further transformations of the data. Where the levels of compounds were below the detection limit, the half-LODs were used.

The principal components loading plot partially explains the importance of each congener in determining the positions of samples along the principal component axis. The compounds with the highest contributions towards the differences observed between samples are located furthest from the origin of the axes. The greater the distance from the origin of the axes, the more the compound contributes to the variation accounted for along that axis. A negative or positive position along the axis

indicates whether the compound is negatively or positively correlated with the principal component (Wenning *et al*., 1992).

4 RESULTS AND DISCUSSION

4.1 PHASE I

4.1.1. Bioanalytical results

The H4IIE-*luc* bio-assay was used to quantify the amount of DLCs in sediment and fish tissue samples, by measuring the amount of light emitted by cells. The relative light units (RLUs) were converted to TCDD equivalent values.

4.1.1.1. Sediment samples

The sediment sample extracts produced the following results when analysed with the H4IIE bio-assay (Table 4.1). The detection limit for sediment extracts was 1-2 ng TEQ/kg.

Although some of the samples produced one data point beyond 20% TCDD maximum this was not sufficient for calculating TCDD-equivalents. This is what is referred to as “too low to calculate” (TLTC) in Table 4.1. Since the same amount of sediment (40 g) was extracted from each site, the % TCDD maximum values produced by each site’s sediment extract are comparable. The only samples that triggered responses in the cells, which were high enough to quantify, were sites 4 (Klip River), 9 (Blesbok Spruit) and 21 (Riet Spruit South). The other sediment samples produced insignificant cell responses, with the samples’ highest TCDD maximum values ranging from 35% to undetectable (Table 4.1).

Since PCDD/Fs and PCBs bind to the organic components of sediments, the organic carbon content of each site’s sediment had to be taken into consideration (Schumacher, 2002). A high TOC suggests that sediments would have the potential to hold higher concentrations of DLCs than sediments containing less organic carbon. The Walkley-Black method was used to calculate the percentage TOC of sediments (Table 4.1). Site 9 had the highest TOC and also produced a quantifiable amount of response from the cells. However, since the other sites produced little response, it was impossible to determine the correlation of the carbon content of a sample to its TCDD equivalent value. The other two sites which produced quantifiable responses had TOC contents of below 3%.

Table 4.1. Information regarding the TOC, cell viabilities, correlation coefficient and amount of DLCs in sediment sample extracts.

Site nr.	Total organic carbon content (%)	Cell viability	TCDD-equivalents (ng TEQ/kg sample)
1	5.06	CT (43%)	TLTC+
2	4.17	N	TLTC+++
3	1.58	CT (41%)	TLTC+
4	2.12	CT (40%)	TCDDeq20 = 161.24
			TCDDeq50 = 249.63
			TCDDeq80 = 386.49*
5	3.92	N	TLTC+++
6	2.82	CT (44%)	TLTC+
7	1.88	N	TLTC+
8	4.90	N	TLTC+
9	14.58	N	TCDDeq20 = 25.81
			TCDDeq50 = 52.35
			TCDDeq80 = 106.19*
10	5.05	CT (58%)	TLTC+++
11	4.92	CT (46%)	TLTC+
12	3.22	N	TLTC++
13	1.54	N	TLTC+
14	5.58	CT (26%)	TLTC+
15	2.71	N	TLTC+
16	2.92	CT (72%)	TLTC+
17	4.06	N	TLTC++
18	1.77	N	TLTC+++
19	2.16	CT (51%)	TLTC+++
20	0.75	N	TLTC+++
21	2.95	N	TCDDeq20 = 4.31
			TCDDeq50 = 5.35
			TCDDeq80 = 66.35*
22	1.02	N	TLTC+

TLTC: Too low to calculate: The amount of DLCs in the samples was too low to quantify.

The sample elicited a maximum cell response of: $^{+}$ below 20%, $^{++}$ 20% ≥ 25% or $^{+++}$ 25% ≥ 35% (Table 4.2).

Cell viability: N = normal (above 80%), CT = cytotoxic - % viability indicated in brackets.

- No values calculated. * Extrapolated values.

Another factor which was taken into account during the analysis of samples was the viability of cells, which was determined via microscopic inspection and an MTT-viability bio-assay. The amount of light emitted by cells with viabilities less than 80% could not be considered to produce meaningful RLU values, since these RLUs are not accurate indications of the amount of DLCs in the sample. Fewer living cells would produce lower emissions of light compared to a 100% viable well. In this instance the low response would not be due to low DLC concentrations, but rather

fewer cells. The viability of the majority of the cells were not affected, but samples 1, 3, 4, 6, 10, 11, 14, 16 and 19 appeared to be toxic to cells, since cells subjected to the highest dosing concentrations (2.5 µL sample/well) of these samples had viabilities below 80% (Table 4.1).

Because the EC80's of sites 4, 9 and 21 were extrapolated, the EC50 was reported. These values were exceptionally high when compared to the other eleven samples of which the amount of DLCs was undetectable. The Agency for Toxic Substances and Disease Register of the USA advises further investigation into a site if the amount of dioxins in soils or sediments exceeds 50 ng/kg (Fiedler, 2003). Other proposed environmental quality guidelines for aquatic sediments are listed in Table 4.2. The TCDD equivalent value of samples 4 and 9 exceeded the limits of six out of the eight countries' advised quality guidelines. The levels measured at Site 4 were the highest yet for DLCs measured in South Africa.

Table 4.2. Proposed environmental quality guidelines for aquatic sediments (adapted from Ianuzzi, Bonnevie & Wenning, 1995).

Source	TEQ guideline values (ng/kg)
United States Army Corps of Engineers	1000
United States Environment Protection Agency (US EPA)	60-100
New York State Department of Environmental Conservation	10-100
Wisconsin Department of Natural Resources	1
International Joint Commission, Great Lakes Science Advisory Board	10
Environment Canada/Pacific Yukon Region	10
Hamburg Department of Environment, Germany	5-10
Proposed Dutch guidelines	15-100

Taking the extrapolated TCDD equivalent values of 386 ng/kg and 106 ng/kg (EC80) into consideration, the large amount of DLCs in sediments from sites 4 and 9 is reason for concern.

Site 4 was located in the Klip River Wetland located south of the low-income, high-density residential area, Soweto. This site might receive PCDD/Fs formed as by-products of backyard burning, since inhabitants of this area rely on open fires for cooking and heating. The site might also receive effluent from various industries that might have contributed to the high dioxin-like load. Site 9 was located approximately

5 km North-East of Springs in the Grootvaly Blesbok Spruit Wetland Nature Reserve. Although not as many major dioxin-producing industries were observed in the direct vicinity of this sampling site as in the more industrialised areas of Vereeniging, Vanderbijlpark and Sasolburg, there were several mines and tailings dams, as well as a sewage works and informal residential settlements located within a 10 km radius from Site 9. Mining activities may lead to the production of dioxins, furans and PCBs (US EPA, 2002b). Numerous gold and coal mines, and tailings dams from these mines, are located in Welgedacht, Geduld, Brakpan, Springs, and Daveyton, surrounding this nature reserve. In fact, this sampling site was approximately 500 m removed from an old tailings dam, probably of a gold mine. The tailings dam situated near the site was not the sole source of possible dioxin pollution; many informal residential settlements were located close to the sampling area. Since many of these informal types of settlements are not supplied with electricity and municipal services, residents are dependent on open burning for preparing food, supplying warmth and incinerating waste. The burning of wood and incineration of waste containing various substances, such as plastics, rubber and other compounds, may produce large concentrations of DLCs (UNEP, 2003).

PCDD/Fs and PCBs produced by these emission sources are generally deposited locally (Lohman & Signeur, 2001), and it is possible that the sources from the informal settlement contributed to the high dioxin load of Site 9. Since it is unknown if the sewage works emitted its effluent up-stream from the site, it was not certain if this possible source contributed to dioxin-like pollution at this site.

The low levels of DLCs measured in sediments were not anticipated, since all of the sites, except for the Suikerbosrand River reference sites (Site 16, 17 and 18), were located in close proximity to many potential sources of DLCs. In fact, many of the sites were located even closer to possible dioxin-producers. Why would sites 4 and 9 have such high levels of DLCs, while the amounts of PCDD/Fs and PCBs at the other sites were undetectable, if all the sites were located in close proximity to highly industrialised dioxin-producing areas? The answer most probably lies in the nature of the two sites. Sites 4 and 9 were the only sites situated in wetlands, while the locations of the other sites were in streams.

The water in wetlands is more stationary when compared to faster flowing river streams, which has a huge impact on the movement of sediments (Davies & Day, 1998). It implies that sediments from sites 4 and 9 are not subjected to constant river

flow, which could wash away sediments with PCDD/Fs and PCBs bound to it, as in the other eleven sites. The hydrology of wetlands allows these water bodies to function as water purification systems, by trapping and filtering pollutants through sediments (Davies & Day, 1998, Maltby, 1991). This means that the water flowing from wetlands would be “purified”, whereas the sediments of the wetland would have higher loads of DLCs. Wetlands are generally very rich in flora. The abundance of plants in wetlands helps to stabilise sediments, increasing sediment capture rates (Davies & Day, 1998). Plants may also have the additional function of protecting sediments from UV-radiation, which may degrade DLCs present in sediment. These properties of Site 9 might have been responsible for the higher levels of PCDD/Fs and PCBs present at this site.

A high dioxin-load was expected for the other Vaal Triangle sites, since they were situated near industries such as ferrous and non-ferrous metal producers, power generation plants, paper and pulp- producers and treatment plants, and an oil and gas refinery. All of the above-mentioned industries are classified as possible dioxin- and furan producers by UNEP (UNEP, 2003). Of the major industries located in the Vaal Triangle, ferrous and non-ferrous metal producers are responsible for the largest contribution of PCDD/F and PCB emissions (about 32%). Oil and gas refineries and power generation plants also play a significant role, contributing 12% and 6% of dioxin-like emissions, respectively. These sources discharge their emissions through high smokestacks. According to Lohman & Signeur (2001), only about 10% of PCDD/F and PCB emitted by these types of sources are deposited locally, the other 90% of emissions are transported and deposited beyond 100 kilometres from the source. This might explain why very low concentrations of DLCs were present in sediments at sites located near these highly industrialised areas. Other possible motivations for the low dioxin-levels measured at these sites, will be discussed at the end of this section.

The low % TCDD maximum responses elicited by Sites 1, 3, 4, 6, 10, 11, 14, 16 and 19 (Table 4.1) were due to cell deaths. The highest concentrations of these extracts were cytotoxic and only 26-51% of cells were viable. Cell populations in wells with viabilities lower than 80%, could not respond accurately to PCDD/Fs and PCBs, as explained earlier. The highest % TCDD maximum responses will therefore be lower than would be expected for wells with 100% viable cell populations, since cytoxicity masks possible dioxin-like presence in extracts. Generally, elemental sulphur present in sediments may be toxic to cells (US EPA, 1986), but since a copper treatment was

performed to remove the sulphur from the extracts, it is likely that other compounds were responsible for cell necrosis. The samples were extracted only once, but the bio-assay was performed twice or thrice to confirm the consistency of results. These samples produced the same cytotoxic results in each of the bio-assay repetitions. However, the compound/s in the sediment extracts that caused the cell deaths is/are unknown. It is known that DCM, one of the solvents used in the extraction, may be toxic to cells and could have been considered as a possible reason for cell necrosis (Arietta *et al*., 2003). It was for this reason that the extracts were evaporated to almost dryness, after which they were reconstituted with hexane. At the low volumes of hexane used in the wells, it had no toxic effects on cells, as was proven by the solvent control wells. If DCM was the cause of toxicity, it would have caused cell necrosis in all of the assays, which it did not (Table 4.1).

The Suikerbosrand River sites (Sites 16, 17 and 18) were selected on the grounds of its geographical location, outside of the Vaal Triangle area. These sites were selected to be the reference sites, since minimal DLCs were expected to be found at these sites. The results corresponded to what we expected, since the concentrations of PCDD/Fs and PCBs present at these sites were too low to quantify. Samples 17 and 18 elicited responses in cells of 23% and 30% TCDD maximum, respectively. When the % TCDD maximum values of these two reference sites were compared to all of the other Vaal Triangle sites, Sites 18 and 17 had the second and fourth largest dioxin loads, respectively. The highest TCDD maximum value produced by Site 16's sediment was 11.82%. This value was relatively low when compared to the other two sites in the same river, but since only 72% of the cells were viable, this low grade cytotoxicity could have masked PCDD/Fs and PCBs present in the sediment extract.

As the samples were collected in the vicinity of smallholdings and farms, and no dioxin-producing industries were located in close proximity to the sampling sites, it is expected that natural sources, such as veld fires, might have been responsible for the dioxin-like contamination of these sediments. It is also a possibility that PCDD/Fs and PCBs were transported to these sites by winds, as discussed earlier. Although the Suikerbosrand River was initially considered as an ideal reference site, it seemed that this was not the case, since the levels of DLCs measured at Site 17 and 18 were generally higher than at the other sites. Since no quantifiable dioxin-like responses were detected in eleven of the sites, and the responses from the reference sites were lower than that of Site 9, the Suikerbosrand River sites could be considered as valid for this study.

4.1.1.2.. Determination of bio-accumulation

One of the aims of Phase I was to investigate the bio-accumulation of PCDD/Fs and PCBs, reported for by these chemicals in literature. This was done by comparing the quantities of DLCs in sediment and fish tissue to one another. For this purpose, sediment and fish samples were collected, on the same day, from the Suikerbosrand River (1F), Blesbok Spruit (2F) and Klip River (3F). The samples were analysed with the H4IIE bio-assay.

Sediment samples

Again, the responses from raw extracts were so low that no TCDD-equivalents could be calculated (Table 4.3). The response of the three sites can be compared by referring to the TCDD maximum values of each site. This is allowable because the same mass (40 g) of sediment was extracted for both sites. The TOC values ranged between 2.69 and 8.01%. Results from the MTT-viability assay indicated that the samples did not inhibit cell viability (Table 4.3).

Table 4.3. Information on sediment samples collected from Site 1F and Site 2F.

Site nr.	% TOC	Cell viability	Response elicited by highest dosing concentration of the sample (% TCDD maximum)
1F Suikerbosrand River	5.11	N	11.84
2F Blesbok Spruit	8.01	N	14.52
3F Klip River	2.69	N	26.40

Cell viability: N = normal (above 80%).

Site 2F's sediment sample elicited a response in cells equal to 14.52% TCDD maximum. This value was not much higher than the 11.84% TCDD maximum value produced by the reference site (Site 1F). Site 3F had the highest concentration of PCDD/Fs elicting a response equal to 26.40 %TCDD maximum. Considering the location of the Suikerbosrand River reference site, minimal DLCs were expected to be found at this site. The sampling site was located on a cattle farm, far away from potential dioxin-producing industries. Thus, the low levels of DLCs measured at this site, corresponded well to what we anticipated. The total organic carbon content of only 5.11% indicated that these sediments had a relatively low dioxin-binding capability (Table 4.3).

The Blesbok Spruit sampling site (2F) was situated down-stream of potential dioxin-producing industries, such as paper mills, ferrous metal producers and power

stations. There were especially many gold and coal mines located within a 20 km radius from the site. The sediment was collected from a recreational park in the residential area of Heidelberg. The road close to the site carried heavy traffic, and exhaust emissions might have been an additional source of DLCs. However, the bio-assay results indicated that the highest dosing concentration of this site's sediment extract, elicited a low response of only 14.52% TCDD maximum (Table 4.3). The low carbon content of sediment (8.01%) could have contributed towards the minute quantities of PCDD/Fs and PCBs present at Site 2F.

The Klip River sampling site (3F) was located in a caravan park in Rothdene. The town of Meyerton is located only a few kilometres North of the site. It was expected that the sediment at the site would contain some traces of DLCs, since the river meanders through Meyerton, which has several industries which have the potential to produce DLCs. It was also expected that exhaust fumes could contribute to DLCs. Yet, the levels of DLCs at the site were unquantifiable, with the highest response in cells being 26.4% TCDD maximum (Table 4.3). As with the other two sites, the low carbon content of sediment (2.69%) could have contributed to the low levels of DLCs found at the site.

Fish tissue samples

A total of 18 fish were caught at Site 1F (Suikerbosrand River), of which nine were *L. umbratus* (6 females, 3 males) and nine *L. capensis* (nine males). From the Blesbok Spruit sampling site (2F), only ten fish were sampled, of which eight were *L. umbratus* (6 females, 2 males) and two were *L. capensis* (females). Fifteen fish were caught from the Klip River (3F). Eleven were *L. umbratus* (6 females, 5 males) and 4 were *L. capensis* (1 female, 3 males). The fish were caught during spawning season and most of the fish were sexually mature.

To minimise intra-species variation, it would have been ideal to have ten individuals per composite group, with three replicates of each group (Heath *et al*., 2004). However, since the amount of fish caught were a limiting factor, fish were divided into composite groups consisting of two to three individuals each. Some composite groups were alike (Group 1 and 2 and Group 6 and 7) containing individuals belonging to the same species, gender and size class (Table 4.4).

a. Fish health assessment

During the study, a limited Fish Health Assessment was performed in order to link possible POPs exposure to the general health of fish. According to the health assessment performed during the study, the lowest possible FHAI-value a fish could obtain was 0 and the highest possible score was 190. Compared to lower FHAI scores, higher scores indicate that fish had possibly been subjected to water of poorer quality (Table 4.4).

Table 4.4. Fish health assessment index and other information regarding fish sampled from the Blesbok Spruit and Suikerbosrand River.

Blesbok Spruit										
Composite group	**Fish ID**	**Eyes**	**Skin**	**Fins**	**Opercula**	**Gills**	**Liver**	**Bile**	**Spleen**	**FHAI score**
Group 1	1	0	10	20	0	0	0	0	0	30
L. umbratus	2	0	10	20	0	0	0	0	0	30
Females	3	0	10	10	0	0	0	0	0	20
Group 2	4	0	10	10	0	0	0	0	0	20
L. umbratus	5	0	10	10	0	0	0	0	0	20
Females	6	0	10	20	0	0	0	0	0	30
Group 3	7	0	0	10	0	0	0	0	0	10
L. umbratus	8	0	10	10	0	0	0	0	0	20
Males										
Group 4	9	0	10	10	0	0	0	0	0	20
L. capensis	10	0	0	10	0	0	0	0	0	10
Females										
Mean										**21.00**
Standard deviation										**7.38**
Suikerbosrand River										
Group 5	11	0	10	0	0	0	30	0	0	40
L. umbratus	12	0	0	0	0	0	0	0	0	0
Males	13	0	30	20	0	0	0	0	0	50
Group 6	14	0	10	10	0	0	0	0	0	20
L. umbratus	15	0	10	20	0	0	0	0	0	30
Females	16	0	10	10	0	0	0	0	0	20
Group 7	17	0	10	20	0	0	0	0	0	30
L. umbratus	18	0	0	20	0	0	0	0	0	20
Females	19	0	10	20	0	0	0	0	0	30

Group 8	20	0	0	10	0	0	0	0	0	10
L. capensis	21	0	10	0	0	0	30	0	0	40
Males (small)	22	0	0	0	0	0	30	0	0	30
Group 9	23	0	0	10	0	0	30	0	0	40
L. capensis	24	0	10	10	0	0	0	0	0	20
Males (medium)	25	0	10	10	0	0	0	0	0	20
Group 10	26	0	10	20	0	0	0	0	0	30
L. capensis	27	0	10	10	0	0	0	0	0	20
Males (large)	28	0	10	20	0	0	0	0	0	30
Mean										**26.67**
Standard deviation										**11.89**
Klip River										
Group 11	29	0	10	0	0	0	0	0	0	10
L. capensis	30	0	10	10	0	0	0	0	10	30
Males	31	0	20	10	0	0	0	0	10	40
Group 12	32	0	10	10	0	0	0	10	0	30
L. umbratus	33	0	0	10	0	0	0	0	0	10
Females	34	0	20	30	0	0	0	0	0	50
Group 13	35	0	10	10	0	0	0	10	0	30
L. umbratus	36	0	10	10	0	0	0	0	0	20
Females	37	0	10	10	0	0	0	10	0	30
Group 14	38	0	10	10	0	0	0	0	0	20
L. umbratus	39	0	10	10	0	0	0	10	0	30
Males										
Group 15	40	0	10	10	0	0	0	0	0	20
L. umbratus	41	0	10	20	0	0	0	0	0	30
Males	42	0	10	10	0	0	0	10	0	30
Mean										**27.14**
Standard deviation										**10.69**

FHAI-values = Fish Health Assessment Index values

The eyes of all fish were normal and only mild skin aberrations were observed in most of the fish, except for fish 13, which suffered severe skin aberrations. Twenty five of the fish had badly damaged fins with active erosion, haemorrhage and/or secondary infection occurring in eight of these cases. Both the opercula and gills were in good condition. The bile and spleen of fish were normal, but four of the fish had fatty livers (Table 4.4).

A mean FHAI score of 21.00 was calculated for Blesbok Spruit (2F), 26.67 for Suikerbosrand River (1F) and 27.14 for Klip River (3F). The FHAI-value of individuals varied between 10 and 30 for Site 2F, and between 0 and 50 for Site 1F and 3F (Table 4.4).

b. CFs of fish

The total length and mass of fish ranged from 350-510 mm and 350-1400 g at Site 2F, 170-510 mm and 54.3 g-1500 g at Site 1F, and from 385-590 mm and 600-2000 g at Site 3F, indicating greater variance in fish collected from Site 1F (Table 4.5). The CF values of fish sampled from Site 2F varied from 0.816-1.635, with a mean CF of 1.059, and Site 3F from 0.88-1.349, with a mean of 1.062. These values were similar to the CF values calculated for the reference site. Fish collected from the Site 1F had CF values distributed between 0.843-3.52, with a mean value of 1.222. The standard deviation for Site 1F was 0.574, Site 2F was 0.220 and Site 3F was 0.149. Although the values calculated for the majority of fish collected from the Suikerbosrand River were generally similar, one individual (fish nr. 24) had a high CF, which affected the mean value of Site 1F (Table 4.5).

Table 4.5 The CFs calculated from the length and mass of fish sampled from Blesbok Spruit and Suikerbosrand River.

Blesbok Spruit				
Composite group	**Fish ID number**	**Total length (mm)**	**Mass (g)**	**CF**
Group 1	1	510	1400	1.055
	2	460	1000	1.027
	3	510	1200	0.905
Group 2	4	430	1300	1.635
	5	480	1000	0.904
	6	495	1400	1.154
Group 3	7	390	600	1.011
	8	510	1200	0.905
Group 4	9	440	1000	1.174
	10	350	350	0.816
Mean				**1.059**
Standard deviation				**0.221**

Suikerbosrand River				
Group 5	11 12 13	460 490 500	1000 1300 1200	1.027 1.105 0.960
Group 6	14 15 16	480 510 510	1200 1500 1500	1.085 1.131 1.131
Group 7	17 18 19	520 460 510	1500 1000 1300	1.067 1.027 0.980
Group 8	20 21 22	180 180 170	76.8 64.3 54.3	1.317 1.103 1.105
Group 9	23 24 25	230 250 390	113 550 500	0.929 3.52 0.843
Group 10	26 27 28	490 410 460	1200 800 1450	1.019 1.161 1.489
Mean				**1.222±0.574**

Klip River				
Composite group	**Fish ID number**	**Total length (mm)**	**Mass (g)**	**CF**
Group 11	29 30 31	550 530 500	1600 1500 1100	0.962 1.007 0.88
Group 12	32 33 34	590 445 520	2000 950 1450	0.974 1.078 1.031
Group 13	35 36 37	510 510 440	1300 1250 1100	0.98 0.942 1.291
Group 14	38 39	495 500	1100 1400	0.907 1.12

Group 15	40 41 42	385 440 390	600 1100 800	1.051 1.291 1.349
Mean				**1.062±0.149**

Because the general health and physical condition of fish is an indication of stress, including POPs exposure, a limited Fish Health Assessment was done and the CFs of fish were calculated.

The majority of fish from Sites 1F, 2F and 3F were in good condition, indicating that neither of the water bodies was extensively polluted, however the reference site appeared to be in a poorer state than the industrialised site. Since the amount of DLCs present at both of the sites were barely detectable, the high FHAI-values may probably be attributed to other than dioxin-related factors. Numerous factors may lead to injury or bad health of fish: availability of food, presence of predators, or variables in the external environment (e.g. temperature, pH, etc).

In comparison to other variables, the fins and skin of fish were the most severely injured. It has to be taken into account that fish were caught by means of gill nets, which might have caused extensive damage to the skin. When trapped in gill nets, fish are immobile and vulnerable to attacks from predators, such as larger fish or crabs, which may also lead to injury. Thus, taking the method of sampling and the environmental conditions into account, the damage to fish was within the usual variance expected for wild populations (Table 4.5).

CFs of Site 1F, 2F and 3F indicated that fish were generally in good condition: The majority of fish had high body masses relative to their lengths (Table 4.5). The CF values might not be accurate reflections of the fish's condition, as the fish were collected during spawning season, and the extensively developed gonads would have contributed considerably to the mass of each fish. One would gain a more accurate reflection of the fish's condition if fish were caught before or after spawning season, eliminating any contributions to the actual mass of each individual. Both the FHAI- and CF values showed similar tendencies, with little difference in the general health of fish sampled from the industrialised site and the reference site.

c. Bio-analytical results

Since the sediment samples had shown no detectable TCDD equivalent values (Table 4.1), it was not surprising to have found no detectable TCDD-equivalents in any of the fish tissue samples either. In order to compare TCDD maximum values, the same mass of tissue had to be extracted. This was not the case for gonadal and hepatic tissue. Equal masses of fillet tissue (10 g) were extracted for both of the sites.

In order to facilitate the comparison of fish tissues to one another, and fish tissues to sediment extracts, extrapolation of % TCDD maximum values potentially produced by 40 grams of each tissue was attempted. Since none of the assayed liver and gonad samples produced responses in cells beyond 20% TCDD maximum, these values could not be used as reliable indications of PCDD/F or PCB presence in extracts, and must be treated with caution. Even cells dosed with solvent only (solvent control wells) may have produced % TCDD maximums such as these. The % TCDD maximum values of fish tissue could not be compared to that of the sediment, because of the difference in mass of extracted matrices.

The detection limit for fish samples ranged from 3-7 ng TEQ/kg for fillet tissues, 3-165 ng TEQ/kg for livers, and 2-23 ng TEQ/kg and 3-60 ng TEQ/kg, for female and male gonads, respectively. The detection limit is dependent of the extracted mass, and very small masses of liver tissue were extracted (Table 4.6 and 4.7). The smaller the mass of the tissue extracted, the bigger the limit of detection.

The levels of DLCs in fish tissue were low, eliciting cell responses ranging from 4.82-14.47% TCDD maximum (Table 4.6 and 4.7). The lipid content of tissue samples ranged from 5.72-13.03% for fillet tissues, 6.05-27.29% for livers, and 3.46-89.29% for gonads (Table 4.6).

Table 4.6. A summary of the lipid content determination, MTT-cell viability assay and the H4IIE bio-assay results for fish collected from Blesbok Spruit (2F) and Klip River (3F).

Composite group nr.	Lipid content (%)	Cell viability	Highest response elicited (%TCDD max)
1. *L. umbratus* females			
Fillet tissue	5.89	N	8.16
Liver	7.61	N	7.15
Gonads	3.46	N	5.28
2. *L. umbratus* females			
Fillet tissue	6.51	N	6.63
Liver	6.05	N	5.13
Gonads	3.56	N	4.82
3. *L. umbratus* males			
Fillet tissue	5.82	N	9.11
Liver	17.45	N	5.77
Gonads	5.73	N	6.60
4. *L. capensis* females			
Fillet tissue	5.95	N	7.30
Liver	23.74	N	7.86
Gonads	45.88	N	6.60
11. *L. capensis* males			
Fillet tissue	5.41	N	9.6
Liver	14.07	N	6.8
Gonads	16.82	N	9.1
12. *L. umbratus* females			
Fillet tissue	6.56	N	13.7
Liver	7.45	N	7.4
Gonads	3.07	N	7.2
13. *L. umbratus* females			
Fillet tissue	6.00	N	15.9
Liver	3.53	N	6.8
Gonads	5.59	N	5.3
14. *L. umbratus* males			
Fillet tissue	2.51	N	9.6
Liver	12.27	N	7.1
Gonads	11.83	N	7..6
15. *L. umbratus* males			
Fillet tissue	6.29	N	7.7
Liver	4.58	N	11.1
Gonads	10.86	N	8.2

Cell viability: N = Normal; ¤ = A part of the sample was lost because of accidental spillage during extraction.

Table 4.7. A summary of the lipid content determination, MTT-cell viability assay and the H4IIE bio-assay results for fish collected from Suikerbosrand River (1F).

Composite group nr.	Lipid content (%)	Cell viability	Highest response elicited (%TCDD max)
5. *L. umbratus* males			
Fillet tissue	6.52	N	5.34
Liver	14.95	N	6.86
Gonads	5.88	N	8.03
6. *L. umbratus* females			
Fillet tissue	6.16	CT (72%)	14.47
Liver	11.29	N	7.82
Gonads	5.70	N	12.17
7. *L. umbratus* females			
Fillet tissue	5.85	N	10.05
Liver	10.39	N	8.45
Gonads	4.59	N	12.71
8. *L. capensis* males (small)			
Fillet tissue	13.03	N	8.62
Liver	27.29	N	11.06
Gonads	89.29	N	8.79
9. *L. capensis* males (medium)			
Fillet tissue	5.72	N	6.13
Liver	10.23	N	7.48
Gonads	24.59	N	8.76
10. *L. capensis* males (large)			
Fillet tissue	5.96	N	10.21
Liver	10.82	N	9.89
Gonads	9.70	N	13.34

Cell viability: N = Normal, CT = cytotoxic - % viability indicated in brackets.

Most of the sample extracts had no effect on cell viability, but the extract from composite group 6's fillet tissue sample was cytotoxic to cells, resulting in a 72% viability of cells.

In general, the fish were in a good condition and it appeared as if the fish were not significantly exposed to any form of chemical pollution. This was confirmed with the bio-analysis which had shown that very low quantities of DLCs were present in composite fillet-, liver- and gonad samples.

To facilitate the comparison of DLCs in samples, the probability of extrapolating % TCDD maximum values, which would have been produced by 40 grams of each tissue, were investigated. But because only small sample masses were extracted, samples contained such low concentrations of DLCs that the cell responses produced by sample extracts were barely distinguishable from cell responses produced by pure solvent. Cell responses produced by livers and gonads were extremely low and irregular and the extrapolated values would have been invalid reflections of dioxin-like activity in these samples.

The lipid content of fish-tissues ranged from 3.46 to 89.29%. Liver- and gonadal tissues had the highest lipid contents, which suggested that these tissues had high capabilities of binding to PCDD/Fs and PCBs, if these substances were present in aquatic environments (Tables 4.6 and 4.7). Low levels of dioxins were expected to be found in sediments and fish tissues collected from the Suikerbosrand River (1F), since this site was used as a reference site, and it was expected that higher levels of DLCs would be present in sediments and fish tissue of the Blesbok Spruit (2F) and Klip River (3F) sites, as these sites were located near to, and down-stream of, many potential sources of dioxins.

With the very low levels of PCDD/Fs and PCBs in the sediment, one would not expect to find very high levels of these substances in bottom-feeding fish. The two fish species collected, *L. umbratus* and *L. capensis*, are both bottom-feeding species and therefore they may ingest dioxin-contaminated sediment particles. They are also in direct physical contact with potentially contaminated sediments (Carey *et al*., 1998). Therefore, the expectation was to find levels slightly higher than that of the sediment. Although higher levels of PCDD/Fs and PCBs were anticipated in sediments as well as fish tissues collected from the Blesbok Spruit (2F) and Klip River (3F), the amounts of these substances were very low at all of the sites. Because the levels of PCDD/Fs and PCBs in sediments, as well as in fish tissues, were too low to quantify, sediment and biota samples could not be compared to each other, and bio-accumulation could not be determined.

4.1.2. Comparison of chemical analysis and bio-analysis results

Some sediment samples were chemically analysed to confirm the results of the H4IIE bio-assay. Sediment was collected from Site 14, 15, 16, 17, 19 and 20 during June 2006. The motivation for choosing these six sites for GC/MS analysis was the

following: Site 16 and 17 were selected as the reference sites for minimal dioxin-like pollution, and Site 14, 15, 19 and 20 were chosen due to their locality in close proximity of potential dioxin-producers. Selection of samples to be sent away for chemical analysis was done prior to H4IIE-analysis of the initial sediment samples. For this reason, Sites 4, 9 and 21 the only sites with quantifiable amounts of DLCs, were not targeted for chemical analysis. The samples were shipped to the Norwegian Institute for Air Research (NILU) to be analysed with GC/MS. Only a few samples were analysed by GC/MS, because of high costs. The methods for analysis employed by NILU were accredited according to ISO/IEC – 17025.

The same samples were also extracted and analysed with the H4IIE-*luc* bio-assay, allowing for comparison of results produced by these methods.

Bio-analytical results

Bio-analysis of the six samples produced the following results:

Table 4.8. The amount of DLCs in samples analysed with the H4IIE bio-assay.

Site nr.	Cell viability	TCDD-equivalents (ng TEQ/kg sample)	Highest response elicited by cells (% TCDD max)
14	Normal	Too low to calculate	37.9
15	Normal	Too low to calculate	14.52
16	Normal	Too low to calculate	14.90
17	Normal	Too low to calculate	14.20
19	Normal	Too low to calculate	21.9
20	Normal	Too low to calculate	15.96

The amount of DLCs in sediment samples was too low to calculate. The highest % TCDD maximum values produced by these samples ranged from 14.20 to 15.96 (Table 4.8). The limit of detection of the assay for these sediments was 1 ng TEQ/kg.

Chemical analysis results

Note: Additional analytical results are presented in Appendix 1.

GC/MS analysis is a sensitive method which can accurately measure the concentrations of each individual congener. The TEQ values of each of the dioxin-like congeners were calculated for mammals, fish and birds, by multiplying the concentration of each congener by its WHO TEF value (Tables 4.9-4.10). To

determine the total TEQ of each site's DLCs, the TEQ values of the congeners were totalled, assuming an additive effect.

OCDD and OCDF were in many instances responsible for the greatest contribution to PCDD/Fs, but because of their low WHO-TEF values, they contributed very little to the TEQ. On the other hand, TCDD and PeCDD generally had low concentrations, but due to their high TEF-values (of 1) these compounds had high contribution to the TEQ.

Site 14 and Site 17, had the highest and lowest total TEQ values, respectively. The total TEQs of the six sites ranged from 0.09-1.33 ng/kg for mammals (Table 4.10). When comparing the sites, it was apparent that the two reference sites had much lower concentrations of DLCs than the other sites.

The total TEQs of each congener group, for mammals of the six sites were compared with one another. Except for Site 15, where the TEQ of PCDF was slightly higher, PCDD was responsible for the highest TEQ through-out the other sites' TEQ values. PCDFs were the most toxic congener group to mammals, yielding a higher TEQ relative to its concentration, when compared to PCDDs and PCBs.

Table 4.9 The concentration of PCDD/F and PCB congeners in samples analysed by GC/MS analysis, measured in ng/kg.

Congener	Site 14 (ng/kg)	Site 15 (ng/kg)	Site 16 (ng/kg)	Site 17 (ng/kg)	Site 19 (ng/kg)	Site 20 (ng/kg)
Dioxins						
2,3,7,8-TCDD	0.03	0.02	0.03	0.03	0.02	0.01
1,2,3,7,8-PeCDD	0.14	0.04	0.03	0.03	0.08	0.04
1,2,3,4,7,8-HxCDD	0.16	0.04	0.03	0.02	0.12	0.04
1,2,3,6,7,8-HxCDD	0.58	0.09	0.04	0.03	0.37	0.05
1,2,3,7,8,9-HxCDD	0.33	0.11	0.03	0.03	0.22	0.05
1,2,3,4,6,7,8-HpCDD	14.70	0.96	0.25	0.13	9.08	0.45
OCDD	146.00	7.34	2.64	0.72	75.10	2.15
Total	**161.94**	**8.60**	**3.05**	**0.99**	**84.99**	**2.79**
Furans						
2,3,7,8-TCDF	0.43	0.07	0.03	0.03	0.27	0.07
1,2,3,7,8/1,2,3,4,8PeCDF	0.36	0.14	0.06	0.04	0.18	0.09
2,3,4,7,8-PeCDF	0.29	0.06	0.03	0.02	0.12	0.06
1,2,3,4,7,8/1,2,3,4,7,9-HxCDF	0.43	0.12	0.03	0.02	0.19	0.09
1,2,3,6,7,8-HxCDF	0.29	0.07	0.02	0.02	0.13	0.05
1,2,3,7,8,9-HxCDF	0.09	0.22	0.09	0.05	0.08	0.03
2,3,4,6,7,8-HxCDF	0.34	0.08	0.03	0.02	0.16	0.04
1,2,3,4,6,7,8-HpCDF	3.39	0.71	0.12	0.08	1.37	0.29
1,2,3,4,7,8,9-HpCDF	0.26	0.07	0.05	0.02	0.08	0.03
OCDF	7.54	0.76	0.24	0.11	1.29	0.41
Total	**13.42**	**2.30**	**0.7**	**0.14**	**3.87**	**1.16**
PCBs						
3,3',4,4'-TeCB (PCB-77)	76.10	2.22	0.77	0.77	49.7	5.12
3,4,4',5-TeCB (PCB-81)	3.02	0.11	0.06	0.04	1.54	0.21
3,3',4,4',5-PeCB (PCB-126)	3.12	0.44	0.05	0.04	1.29	0.39
3,3',4,4',5,5'-HxCB (PCB-169)	0.43	0.10	0.03	0.02	0.18	0.03
Total	**82.67**	**2.87**	**0.91**	**0.87**	**52.71**	**5.75**
Σ (PCDD, PCDF, PCB)	**258.03**	**13.77**	**4.66**	**2.27**	**141.57**	**9.70**

Table 4.10 The TEQs of PCDD/F and PCB congeners in samples analysed by GC/MS analysis. TEQs were calculated according to WHO TEF-values for humans (WHO, 1997).

Congener	Site 14 (ng/kg)	Site 15 (ng/kg)	Site 16 (ng/kg)	Site 17 (ng/kg)	Site 19 (ng/kg)	Site 20 (ng/kg)
Dioxins						
2,3,7,8-TCDD	0.03	0.02	0.03	0.03	0.02	0.01
1,2,3,7,8-PeCDD	0.14	0.04	0.03	0.03	0.08	0.04
1,2,3,4,7,8-HxCDD	0.02	0.00	0.00	0.00	0.01	0.00
1,2,3,6,7,8-HxCDD	0.06	0.01	0.00	0.00	0.04	0.01
1,2,3,7,8,9-HxCDD	0.03	0.01	0.00	0.00	0.02	0.00
1,2,3,4,6,7,8-HpCDD	0.15	0.01	0.00	0.00	0.09	0.00
OCDD	0.15	0.00	0.00	0.00	0.08	0.00
Total	**0.57**	**0.09**	**0.08**	**0.06**	**0.34**	**0.07**
Furans						
2,3,7,8-TCDF	0.04	0.01	0.00	0.00	0.03	0.01
1,2,3,7,8/1,2,3,4,8PeCDF	0.02	0.01	0.00	0.00	0.01	0.00
2,3,4,7,8-PeCDF	0.15	0.03	0.02	0.01	0.06	0.03
1,2,3,4,7,8/1,2,3,4,7,9-HxCDF	0.04	0.01	0.00	0.00	0.02	0.01
1,2,3,6,7,8-HxCDF	0.03	0.01	0.00	0.00	0.01	0.01
1,2,3,7,8,9-HxCDF	0.01	0.02	0.01	0.01	0.01	0.00
2,3,4,6,7,8-HxCDF	0.03	0.01	0.00	0.00	0.02	0.00
1,2,3,4,6,7,8-HpCDF	0.03	0.01	0.00	0.00	0.01	0.00
1,2,3,4,7,8,9-HpCDF	0.00	0.00	0.00	0.00	0.00	0.00
OCDF	0.01	0.00	0.00	0.00	0.00	0.00
Total	**0.37**	**0.10**	**0.04**	**0.03**	**0.17**	**0.07**
PCBs						
3,3',4,4'-TeCB (PCB-77)	0.08	0.00	0.00	0.00	0.05	0.00
3,4,4',5-TeCB (PCB-81)	0.00	0.00	0.0	0.00	0.00	0.00
3,3',4,4',5-PeCB (PCB-126)	0.31	0.04	0.01	0.00	0.13	0.04
3,3',4,4',5,5'-HxCB (PCB-169)	0.00	0.00	0.00	0.00	0.00	0.00
Total	**0.40**	**0.04**	**0.01**	**0.00**	**0.18**	**0.04**
Σ (PCDD, PCDF, PCB)	**1.33**	**0.23**	**0.13**	**0.09**	**0.69**	**0.18**

Both of the analytical methods measured low quantities of DLCs in sediments of the six sites. When results were compared to one another (Table 4.11), chemical analysis confirmed the very low concentrations of PCDD/Fs and PCBs. Although GC/MS results were generally an order of magnitude lower, there was a good correlation between biological and chemical analysis results ($R^2 = 0.96$).

Table 4.11 Comparison of bio-analytical and chemical analysis results

Site	TCDD-EQ (ng/kg)	%TCDD-max	WHO-TEQ (ng/kg)
Site 14	Could not be calculated	37.9	1.33
Site 15	Could not be calculated	14.52	0.23
Site 16	Could not be calculated	14.90	0.13
Site 17	Could not be calculated	14.20	0.09
Site 19	Could not be calculated	21.9	0.69
Site 20	Could not be calculated	15.96	0.18

4.1.3. Possible reasons for low levels of DLCs

Although all of the potential sources for PCDD/Fs and PCBs do occur in South Africa, either concentrated in industrial parks or distributed in residential and rural landscapes, the levels of these compounds were generally low in South African sediments. Even though reduced concentrations were anticipated for the agriculturally and residentially impacted sites, measurable concentrations were expected for the industrial sites. The factors possibly contributing towards the low levels of DLCs measured in the majority of the South African sediments will be debated in this section.

Seasonal and meteorological conditions

South Africa's climate is characterised by high temperatures, little precipitation and long summers, different from the more moderate conditions in Europe, North America and elsewhere where most of the POPs research has been conducted. These conditions could influence the distribution and degradation of organic pollutants, especially in abiotic matrices, such as soil and sediment (Quinn *et al*., 2009).

Variations in seasonal and meteorological conditions, such as ambient temperature, rainfall, wind speed and direction, solar intensity and humidity have been linked to the occurrence of DLCs in environmental compartments (Lohmann *et al.,* 1999). Moon and co-workers (2005) established that DLC concentrations in Korean soils and sediments were higher in the winter months than during the summer. Their study was performed in Daeyeon-dong and Gijang-gun in Busan, Korea. Basan is a summer rainfall region with mean ambient temperatures ranging between 4.1 and 14.5˚C during the winter, and between 16.2 and 25.3˚C during the summer. The typical weather conditions of the area are similar to the climates of the majority of the sampling regions included in our study.

Moon and co-workers (2005) concluded that ambient temperature and the amount of precipitation were the most probable factors affecting the seasonal variation in PCDD/F and PCB deposition. High ambient temperatures may facilitate the volatilisation of DLCs from surfaces and may lower the amount of DLCs available for deposition through chemical degradation processes such as OH^- radical reactions, leading to lowered PCDD/F and PCB concentrations in the environment (Meneses *et al.,* 2003). Therefore, lower ambient temperatures occurring during the winter might be responsible for higher dioxin-loads in sediments and soils. Since DLCs, especially the more chlorinated compounds, are dependent on wet deposition, the amount of precipitation may also affect the amount of PCDD/Fs and PCBs deposited in the environment.

South Africa generally has warm summers (December to February) with mean maximum temperatures of the sampling regions reaching 22.1 to 33.9˚C. The minimum temperatures occurring during the summer months were also relatively high, ranging from 9.5 to 22.1˚C. Temperatures were mild during spring and autumn, but winter months (June-August) were generally cooler with maximum temperatures varying between 17.2 and 31.1°C, and minimum temperatures of as low as -2.6 to 17˚C often prevailing (South African Weather Service, 2009).

When considering the combinations of meteorological conditions, generally, the high temperatures prevailing during the summer months were probably responsible for the volatilisation and degradation of DLCs, resulting in low concentrations of these compounds being available for deposition when sufficient precipitation occurred. Conversely, during the colder winter months when the conditions were favourable for the deposition of DLCs, there was very little or no precipitation to facilitate deposition.

Photodegradation of DLCs

As mentioned in the previous section, South Africa has hot summers with long sunny days, receiving approximately 12 to 13 hours of sunlight on mean (South African Weather Service, 2009). This implies that the environment is exposed to solar rays for extended periods of time, potentially leading to the degradation of DLCs through UV radiation.

DLCs have optimal UV absorption wavelengths ranging from < 270 nm to 290 nm, and sunlight can therefore act as an important radiation source to degrade these compounds through photolysis by cleavage of the carbon-chlorine- or carbon-oxygen

bonds (Isosaari, 2004). It has been established that photolysis is one of the few environmentally significant degradation mechanisms for DLCs in water, air, soils and sediments (Isosaari, 2004; Kim & O'Keefe, 2000). UV-light irradiation leads to the preferential loss of chlorines from the peri positions (positions 1, 4, 6 and 9), rather than from the more stable lateral positions (positions 2, 3, 7 and 8) through carbon-chlorine cleavage. Carbon-oxygen cleavage, on the other hand, is an important degradation pathway for DLCs containing four or less chlorines, producing dihydroxybiphenyls or hydroxydiphenylethers and lower chlorinated PCDD/Fs as end-products, with hydrobenzoic acid being the ultimate aromatic photodegradation product of PCDD/Fs (Kim & O'Keefe, 2000).

Several experiments have been conducted on the photodegradation of DLCs in pure- and natural waters, such as rivers and ponds. It has been found that the degradation half-lives of PCDD/Fs and PCBS in natural waters (4 to 6 hours) are much shorter than in pure water (1.2 to 6.5 days). This can be attributed to the presence of natural organics in natural waters acting as sensitisers, facilitating degradation processes (Isosaari, 2004). Although sunlight easily penetrates water, it only penetrates the top few millimetres of sediments and only the compounds in this layer can be degraded through photolysis. Although the degradation of DLCs in sediments occurs at a slower rate (approximately 5 to 8 days), photodechlorination processes in sediments and water are similar with peri-substituted chlorines being cleft preferentially and lateral chlorines being less reactive (Isosaari, 2004).

Since the sediments in the study areas were subjected to a high degree of UV-radiation, it is possible that DLCs present in the sediments were degraded by photodechlorination or cleavage of the carbon-oxygen bond. For this study, sediment samples were collected from the upper sediment layer (top 1 to 10 cm) at locations which were easily accessible for collection by hand. This implied that samples were generally collected at locations where the water level was about ankle- to knee-deep. At this depth, UV-rays can penetrate the water to irradiate the top sediment layer, potentially leading to the degradation of PCDD/Fs.

Sedimentation shifts and effect of dilution

Although precipitation promotes the deposition of DLCs, large amounts of precipitation may lead to lowered levels of DLCs in water and sediments. During periods of high rain- or snowfall large quantities of water may enter a water body/system, diluting the concentrations of DLCs and shifting sediments to down-

stream localities (Davies & Day, 1998). This implies that the DLCs which are bound to sediments are also carried down-stream, decreasing the concentrations of PCDD/Fs and PCBs in the upstream parts of rivers.

Degradation by microorganisms

Microorganisms may also be responsible for the degradation of DLCs. Bio-degradation and bio-transformation of PCDD/Fs by microorganisms has even been considered as bio-remediation options for polluted environments.

Certain aerobic bacteria containing aromatic hydrocarbon dioxygenases have broad substrate specificity and have the ability to degrade the ring structures of PCDDs and related compounds. In addition to this type of bio-degradation, reductive dechlorination by anaerobic microorganisms and fungal degradation has also been recognised as modes of PCDD bio-transformation (Halden *et al*., 1999). Microbial reductive dechlorination of PCDD/Fs has been established in sediments and soils. This dechlorination process may degrade highly chlorinated congeners, which are generally barely attacked by aromatic hydrocarbon dioxygenases (Wittich, 2004). *Dehalococcoides* and *Dehalococcoides ethenogenes* are two strictly anaerobic strains of bacteria which have been shown to be able to dechlorinate selected PCDD/F congeners. It is also presumed that members of the *Dehalococcoides* group are capable of dechlorinating a tetra-chlorinated PCB congener, chlorobenzene and trichlorodibenzo-p-dioxin. Members of the *Dehalococcoides* group are widely dispersed in nature and play a major role in the transformation of chlorinated substances (Wittich, 2004). *Sphingomonas wittichii* is another bacterial strain with the unique ability to mineralise PCDD/Fs by the key enzyme, dioxin dioxygenase (Halden *et al*., 1999). Many other microorganisms and fungi may be responsible for the degradation of DLCs. Although water and sediments were not analysed for the presence of microorganisms, during the study, it has to be taken into account that DLCs may possibly be degraded by this pathway.

Any one of the factors discussed in this section, or combinations thereof might have been responsible for the unexpectedly low levels of DLCs measured in sediments associated with highly industrialised areas, containing many potential sources of PCDD/Fs and PCBs.

4.2 Phase II

4.2.1 Biological analysis results

H4IIE-luc- and MTT bio-assay results and TOC content: A summary

In this section the results of the H4IIE-*luc*- and MTT bio-assays will be reported. Since the amount of organic carbon present in sediment or soil may affect its potential to retain DLCs (Schumacher, 2002), the OXC- and TOC contents of samples will be reported as well. The correlation between the percentage TOC and amount of DLCs in the samples will be established to verify if a statistically significant relationship exists for the samples investigated during this study. Finally, the levels of DLCs measured at the South African sites will be discussed and compared to one another.

The levels of PCDD/Fs and dioxin-like PCBs measured with the H4IIE-*luc* bio-assay ranged from < LOD to 103 ng TCDD-EQ/kg, dm. The LOD of the bio-assay was 1.93 ng TCDD-EQ/kg, dm, and where TCDD-EQs could not be quantified the half-LOD (of 0.97 ng TCDD-EQ/kg, dm) was reported (Table 4.12). Only 23 of the 96 samples elicited responses beyond 20% TCDD max and could be quantified. Sixteen of these samples were from industrially impacted areas, three were from residentially impacted areas (mostly associated with low-income, high-density residential settlements), and the remaining four samples were associated with residential-agricultural or industrial-residential combinations. The majority of the samples were of such a nature that the viability of the cells was not affected (80-100% viable, in 47 of the 96 cases) or only slightly affected (60-79% viable, in 26 of the 96 cases) (Table 4.12). However, in 23 of the 96 cases the viability of cells were moderately (40-59% viable), highly (20-39% viable) or severely (0-19% viable) affected (Table 4.12).

Decreased cell viability or cytotoxicity could result in false negative results for dioxin-like TCDD-EQ, since wells with fewer living cells would emit less light than 100% viable wells. In such instances, the reduced response in cells (decrease in % TCDD max) would not necessarily be due to little DLCs, but rather to fewer cells. The causes for the loss in cell viability are uncertain. Since only some of the wells, mostly at the highest sample concentrations in a dilution series, were affected, it was likely that a compound or compounds present in the extract was the main culprit.

Table 4.12 The % OXC and TOC, and results from the H4IIE-*luc* and MTT bio-assays.

Site abbreviation	Type of land-use	Carbon content		H4IIE-*luc* bio-assay results		MTT assay results
		OXC (%)	[a]TOC (%)	TCDD-EQ_{20} values (ng TCDD-EQ/kg, dm)	[b]Normalised TCDD-EQ_{20}'s (ng TCDD-EQ/kg, dm)	[c]Cell viability
S/L1	Residential	5.10	6.63	**7.56**	**1.14**	Moderately affected
S/L2	Residential	3.75	4.96	*0.97*	*0.20*	Moderately affected
S/L3	Residential	4.25	5.57	*0.97*	*0.17*	Moderately affected
S/L4	Residential	5.91	7.62	*0.97*	*0.13*	Not affected
S/L5	Residential	2.27	3.14	*0.97*	*0.31*	Not affected
S/L6	Residential	4.84	6.30	*0.97*	*0.15*	Not affected
S/L7	Residential	6.03	7.76	**6.27**	**0.81**	Highly affected
S/L8	Semi-industrial	5.43	7.03	**4.33**	**0.62**	Slightly affected
S/L9	Semi-industrial	10.81	13.64	**86.63**	**6.35**	Slightly affected
S/L10	Residential/Agricultural	4.97	6.47	**15.02**	**2.32**	Moderately affected
S/L11	Residential	1.88	2.66	*0.97*	*0.36*	Slightly affected
S/L12	Semi-industrial	4.52	5.91	**13.86**	**2.34**	Slightly affected
S/L13	Residential/Agricultural	7.46	9.52	*0.97*	*0.10*	Not affected
CT1	Residential	1.12	1.73	*0.97*	*0.56*	Slightly affected
CT2	Industrial	4.30	5.64	**16.15**	**2.86**	Slightly affected
CT3	Agricultural	0.36	0.79	*0.97*	*1.23*	Moderately affected
CT4	Industrial	0.10	0.47	*0.97*	*2.08*	Slightly affected
CT5	Industrial	0.49	0.95	*0.97*	*1.02*	Not affected
CT6	Industrial	0.79	1.32	**12.34**	**9.33**	Not affected
CT7	Industrial	3.58	4.75	**11.93**	**2.51**	Slightly affected
CT8	Industrial	0.79	1.32	**11.49**	**8.67**	Moderately affected
CT9	Residential	0.26	0.66	*0.97*	*1.46*	Moderately affected
CT10	Industrial	1.32	1.98	*0.97*	*0.49*	Slightly affected
CT11	Industrial/Residential	1.01	1.59	*0.97*	*0.61*	Highly affected
CT12	Industrial/Residential	1.41	2.08	*0.97*	*0.47*	Highly affected
CT13	Agricultural	0.92	1.48	*0.97*	*0.66*	Severely affected
CT14	Industrial	1.91	2.70	*0.97*	*0.36*	Severely affected
CT15	Industrial/Residential	0.39	0.84	*0.97*	*1.16*	Slightly affected
CT16	Industrial/Residential	1.09	1.68	*0.97*	*0.58*	Moderately affected
CT17	Residential	0.37	0.81	*0.97*	*1.20*	Highly affected
CT18	Industrial/Residential	0.69	1.19	*0.97*	*0.81*	Slightly affected
CT19	Industrial	1.12	1.72	*0.97*	*0.56*	Slightly affected
CT20	Industrial	0.62	1.11	*0.97*	*0.87*	Severely affected
D1	Industrial	0.24	0.65	*0.97*	*1.49*	Not affected
D2	Industrial	0.70	1.22	*0.97*	*0.80*	Not affected
D3	Industrial	0.32	0.74	*0.97*	*1.31*	Not affected
D4	Industrial	0.58	1.07	*0.97*	*0.91*	Not affected
D5	Industrial	0.24	0.64	*0.97*	*1.51*	Not affected
D6	Harbour – industrial	0.13	0.51	*0.97*	*1.89*	Moderately affected
D7	Harbour – industrial	0.26	0.67	*0.97*	*1.46*	Not affected
D8	Industrial	0.32	0.74	*0.97*	*1.30*	Slightly affected
D9	Industrial	0.82	1.36	*0.97*	*0.71*	Not affected
D10	Industrial	0.37	0.81	*0.97*	*1.20*	Moderately affected

D11	Industrial	0.69	1.19	*0.97*	*0.81*	Moderately affected
D12	Residential	0.79	1.32	*0.97*	*0.73*	Slightly affected
D13	Residential	0.71	1.23	*0.97*	*0.79*	Moderately affected
D14	Industrial	2.61	3.56	**28.89**	**8.12**	Not affected
RB1	Industrial	1.25	1.88	**103**	**54.72**	Moderately affected
RB2	Harbour – industrial	0.29	0.71	**28.01**	**39.49**	Not affected
RB3	Harbour – industrial	0.89	1.44	**18.42**	**12.75**	Not affected
RB4	Industrial	4.53	5.92	**7.48**	**1.26**	Not affected
RB5	Harbour – industrial	0.78	1.31	*0.97*	*0.74*	Not affected
RB6	Harbour - recreational	0.23	0.64	*0.97*	*1.52*	Not affected
RB7	Residential	0.32	0.74	*0.97*	*1.30*	Not affected
RB8	Residential	0.49	0.95	*0.97*	*1.02*	Not affected
RB9	Residential	0.38	0.82	*0.97*	*1.19*	Not affected
BF1	Agricultural	1.92	2.71	*0.97*	*0.36*	Not affected
BF2	Agricultural	0.81	1.34	*0.97*	*0.72*	Not affected
BF3	Recreational	1.13	1.75	*0.97*	*0.56*	Slightly affected
BF4	Industrial	0.72	1.23	*0.97*	*0.79*	Slightly affected
BF5	Industrial	0.41	0.85	*0.97*	*1.14*	Slightly affected
BF6	Industrial	0.73	1.25	**44.32**	**35.46**	Not affected
BF7	Industrial/Residential	1.38	2.05	**45.17**	**22.01**	Not affected
BF8	Industrial	1.20	1.82	**47.45**	**26.03**	Slightly affected
BF9	Industrial/Residential	5.17	6.71	**10.34**	**1.54**	Not affected
BF10	Residential/Recreational	2.61	3.56	*0.97*	*0.27*	Not affected
BO1	Industrial/Residential	1.77	2.53	*0.97*	*0.38*	Slightly affected
BO2	Residential	2.82	3.82	*0.97*	*0.25*	Not affected
BO3	Industrial	3.29	4.40	*0.97*	*0.22*	Not affected
BO4	Industrial/Residential	4.31	5.65	**36.05**	**6.38**	Not affected
Lim1	Agricultural	0.40	0.84	*0.97*	*1.15*	Not affected
Lim2	Agricultural	0.37	0.80	*0.97*	*1.21*	Not affected
Lim3	Residential	0.57	1.05	*0.97*	*0.93*	Slightly affected
Lim4	Residential	2.32	3.21	**22.95**	**7.16**	Not affected
Lim5	Recreational	0.51	0.97	*0.97*	*1.00*	Not affected
Lim6	Recreational	0.33	0.75	*0.97*	*1.29*	Not affected
Olif1	Industrial	1.12	1.73	*0.97*	*0.56*	Not affected
Olif2	Residential/Agricultural	1.03	1.61	*0.97*	*0.60*	Not affected
Olif3	Industrial/Agricultural	0.24	0.65	*0.97*	*1.49*	Not affected
Olif4	Industrial	1.56	2.27	**33.31**	**14.70**	Not affected
Croc1a	Industrial	1.26	1.90	*0.97*	*0.51*	Slightly affected
Croc 1b	Industrial	0.93	1.49	**8.02**	**5.39**	Not affected
Croc2	Agricultural	2.73	3.70	*0.97*	*0.26*	Moderately affected
Croc3	Agricultural	1.68	2.42	*0.97*	*0.40*	Slightly affected
Croc4	Agricultural	1.07	1.67	*0.97*	*0.58*	Highly affected
Komati1	Agricultural	0.21	0.61	*0.97*	*1.58*	Highly affected
Komati2	Agricultural	3.87	5.11	*0.97*	*0.19*	Slightly affected
Komati3	Agricultural	0.51	0.97	*0.97*	*1.00*	Not affected
Pongola1	Agricultural	0.41	0.85	*0.97*	*1.14*	Slightly affected
Pongola3	Residential/Agricultural	0.89	1.45	*0.97*	*0.67*	Not affected
Drknberg1	Agricultural	0.86	1.41	*0.97*	*0.69*	Slightly affected
Drknberg2	Agricultural	0.60	1.08	*0.97*	*0.90*	Not affected
Drknberg3	Agricultural	0.97	1.55	*0.97*	*0.63*	Not affected

KZNRiv 1	Industrial	0.56	1.04	*0.97*	*0.94*	Not affected
KZNRiv 2	Industrial	0.82	1.35	*0.97*	*0.72*	Not affected
KZNRiv 3	Industrial	0.49	0.96	*0.97*	*1.01*	Slightly affected

[a]The TOC was converted from the OXC as described in section 4.6. [b]The TCDD-EQ20s were normalized to 1% of the TOC.

Bold: Measured TCDD-EQ20's. *Italics: The half-LOD was reported for responses below 20% TCDD-max.*

[c] *not affected: 80-100% viable; only slightly affected: 60-79% viable and severely affected: 0-19% viable*

It is known that elemental sulphur and other mixtures of compounds present in sediments may be cytotoxic to H4IIE-*luc* cells (Hilscherova *et al*., 2000; Yoo *et al*., 2006), but since sulphur was eliminated from the extracts it is uncertain which compound(s) was/were responsible for cytotoxicity.

In addition to the percentage viability of cells, the TOC content of the samples had to be taken into consideration before comparing sites to one another. To establish if there was a statistically significant relationship between the percentage TOC and TCDD-EQs of samples, the OXC determined with the Walkley-Black wet oxidation method was converted to TOC (Table 4.12) and the correlation between the amount of organic carbon and levels of DLCs was determined. In literature, both strong (Shimizu *et al.,* 2003; Wisconsin Department of Natural Resources, 2003; Wevers *et al*., 2004.) and weak (Hilscherova *et al*., 2003; Nieuwoudt *et al.,* 2009; Suarez *et al*., 2006) correlations have been found. For the samples included in the current study, the percentage TOC generally correlated weakly with the TCDD-EQ. When all 96 of the samples were included in the calculation (using half-LODs for sites at which the level of DLCs was below the assay's LOD), the R^2 was equal to 0.0997, which means that there was only a 9.97% correlation between the amount of organic carbon and the amount of DLCs in the samples. When only the 23 samples eliciting quantifiable responses in cells were considered, the correlation was even weaker (R^2 = 0.002), but when the outlier, RB 1, was removed from the equation the correlation improved slightly to R^2 = 0.0435, which was still not statistically significant ($p > 0.05$).

For this reason, normalised TCDD-EQ_{20}s will not be used to compare sites to one another; however, the normalised data (Table 4.12) is useful for comparison of our results to environmental sediment quality guidelines (Wisconsin Department of Natural Resources, 2003).

4.2.2 Comparison of DLC levels in South African sediments

Bar graphs were plotted with the sites arranged in decreasing magnitude of TCDD-EQ_{20} (Fig. 4.1, Table 4.12). The same was done for the data normalised to 1% TOC (Fig. 4.2, Table 4.12). Although the patterns were not identical, some similarities were noted. Although the non-normalised TCDD-EQ_{20}s were generally higher, RB1 had the highest, and S/L7 and 8 had the lowest TCDD-EQ_{20}s for both normalised (Fig. 4.2) and non-normalised (Fig. 4.1) data. Furthermore, the industrial and less industrial sites were randomly distributed in the continuum of greater to lesser concentrations of DLC pollution, i.e. not all industrial sites had concentrations greater than the residential-, recreational-, industrial/residential- or residential/agricultural sites (Figs. 4.1 & 4.2). This may indicate to different sources of DLCs throughout the study area, such as domestic fires and alternative fuel usage for cooking and heating in the low-income residential areas.

When considering all 96 sites, and weighing the mean concentration of TCDD-EQ_{20}s in sediments from the various sampling regions against each other, Richards Bay (18.51 ng TCDD-EQ/kg, dw; n = 9) followed by Bloemfontein (15.89 ng TCDD-EQ/kg, dw; n = 10) and the Soweto/Lenasia wetland (11.32 ng TCDD-EQ/kg, dw; n = 13) had the greatest concentrations of DLCs, with the TCDD-EQ_{20}s from the other areas being less substantial, but the differences were not statistically significant ($p = 0.54318$, Post-hoc Tukey test, one-way ANOVA). Since the majority of the towns or cities (except for the reference sites) were selected on the grounds that they are potential hot-spots for DLC pollution, significant differences in the concentration of TCDD-EQ_{20} were not expected.

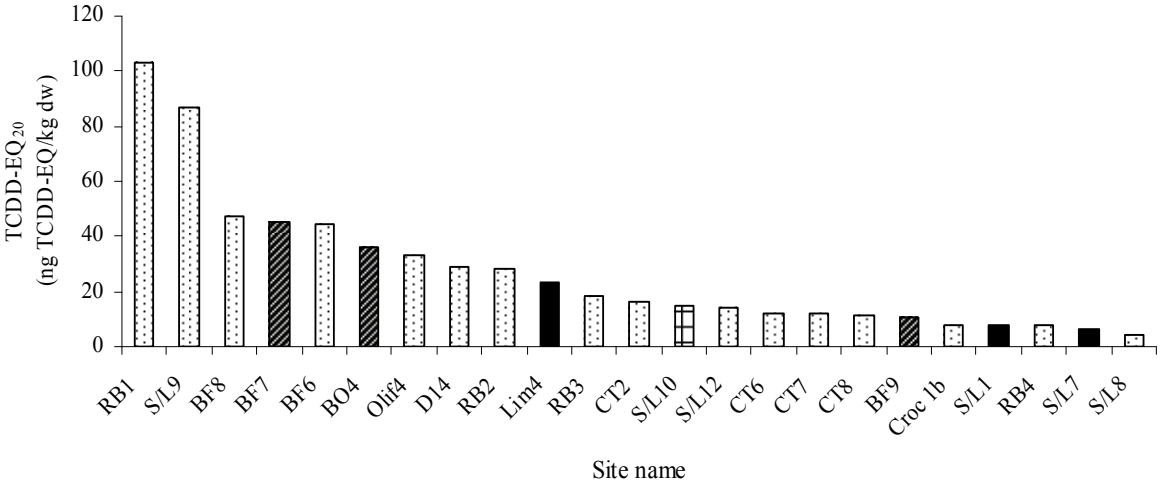


Figure 4.1. The 23 South African samples eliciting quantifiable responses, arranged from greatest to least TCDD-EQ_{20}. ⬚ = Industrially or semi-industrially-, ▨ = industrial/residentially-, ■ = residentially-, and ⊞ = residential/agriculturally impacted

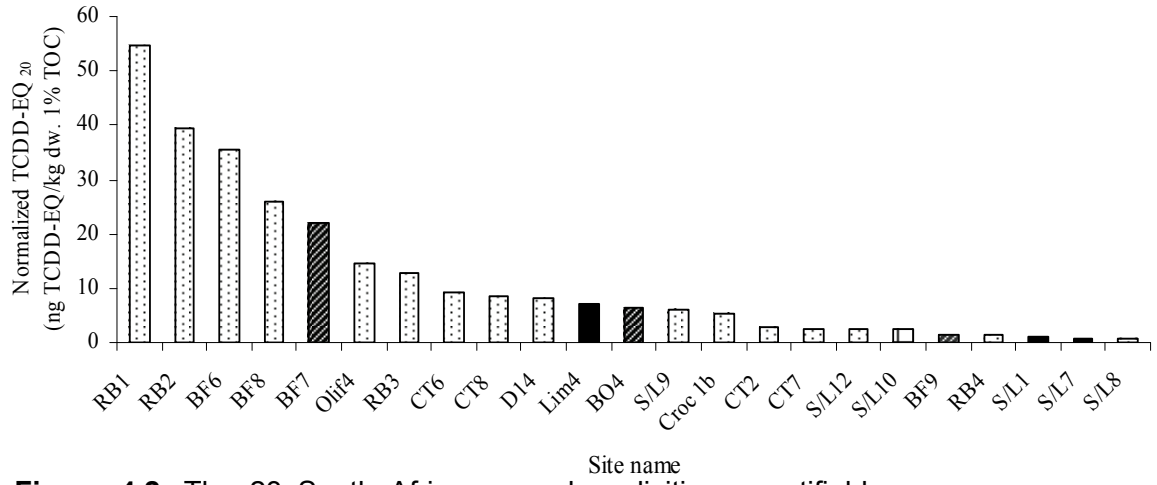


Figure 4.2. The 23 South African samples eliciting quantifiable responses, arranged from greatest to least normalized TCDD-EQ_{20}. ⬚ = Industrially or semi-industrially-, ▨ = industrial/residentially-, ■ = residentially-, and ⊞ = residential/agriculturally impacted

It was, however, anticipated that there would be a significant difference in the concentrations of TCDD-EQ_{20} associated with different land-uses. Industrial areas, followed by low-income, high-density residential areas are generally expected to be greater potential sources of PCDD/Fs than agricultural and recreational areas. Incomplete combustion occurring during industrial and thermal processes (which includes smaller non-point sources such as domestic burning of wood, landfill fires and open burning frequently occurring in low-income residential settlements) may continuously produce and emit DLCs, leading to greater concentrations of TCDD-EQ_{20} in the associated areas. As anticipated, the industrial areas had slightly greater concentrations of DLCs than the residential, agricultural and recreational areas (see land-use classification in Table 4.1; 12.1 versus 3.5, 1.9 and 1.9 ng TCDD-EQ/kg, dm, respectively), but no statistically significant difference was observed ($p = 0.08081$, Post-hoc Tukey test, one-way ANOVA). It should be noted, though, that all 96 of the sites, those with unquantifiable concentrations of TCDD-EQ_{20} as well (where the half-LOD was reported), were included in the ANOVA, decreasing the mean TCDD-EQ values of the sampling area or land-use type as a whole.

When only the 23 samples eliciting responses beyond 20% TCDD max were considered (Table 4.13), the industrial and semi-industrial areas were responsible for a statistically significantly higher ($p < 0.05$, Kruskal-Wallis ANOVA) contribution towards the total concentration of DLCs measured at the South African sites. Industrially impacted areas were responsible for 77% of the contribution towards the total TCDD-EQ concentration measured at the 23 sites, while residential, residential/agricultural- and industrial/residential combinations contributed towards 6%, 2%, and 15%, respectively (Fig 4.3).

Although the industrially impacted sites dominated in their contribution with respect to the total TCDD-EQ, it was expected that a greater fraction of the 96 sites selected in industrial areas would have measurable TCDD-EQ_{20}s.

For example, only one of the 12 industrial sites sampled in Durban elicited a cell response beyond 20% TCDD max (Table 4.12). In the next sub-sections each of the sampling regions will be considered separately, and the scale and significance of the occurrence of DLCs will be reflected on.

Table 4.13 A summary of the bio-assay data (TCDD-EQ_{20} values), land-use categories and percentage contribution to the total TCDD-EQ of the 23 sites eliciting quantifiable responses.

Land-use category and sites	TCDD-EQ_{20} values (ng TCDD-EQ/kg, dm)	Land-use category and sites	TCDD-EQ_{20} values (ng TCDD-EQ/kg, dm)
Industrial and semi-industrial		**Industrial/residential**	
S/L8	4.33	BF7	45.17
S/L9	86.63	BF9	10.34
S/L12	13.86	BO4	36.05
CT2	16.15	**Sum TCDD-EQ_{20}**	**91.56**
CT6	12.34	**Contribution to total TCDD-EQ_{20}**	**15%**
CT7	11.93		
CT8	11.49	**Residential**	
D14	28.89	S/L1	7.56
RB1	103	S/L7	6.27
RB2	28.01	Lim4	22.95
RB3	18.42	**Sum TCDD-EQ_{20}**	**36.78**
RB4	7.48	**Contribution to total TCDD-EQ_{20}**	**6%**
BF6	44.32		
BF8	47.45		
Olif4	33.31	**Residential/agricultural**	
Croc1b	8.02	S/L10	15.02
Sum TCDD-EQ_{20}	**475.63**	**Sum TCDD-EQ_{20}**	**15.02**
Contribution to total TCDD-EQ_{20}	**77%**	**Contribution to total TCDD-EQ_{20}**	**2%**
Sum TCDD-EQ_{20} of all categories			**618.99**

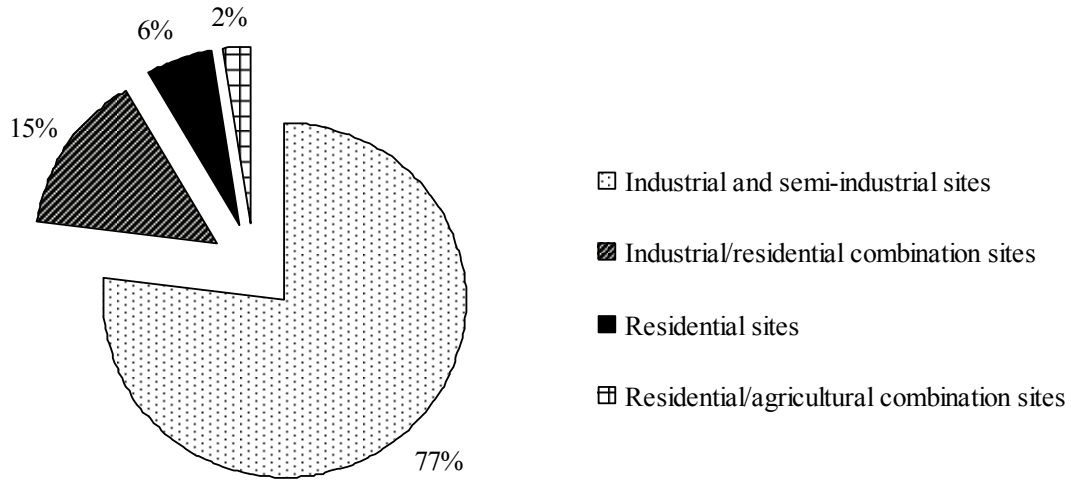


Figure 4.3. The percentage contribution of the various land-use types towards the total concentration of TCDD-EQ20 measured at the 23 quantifiable South African sediment sites.

4.2.3 Soweto and Lenasia sites

As stated earlier, Soweto and Lenasia are mainly residential areas, with only a few industries located in their direct vicinity. Of the 13 samples (ten sediments and three associated soils) collected in the area, six brought about significant cell responses. The greatest concentrations of TCDD-EQ_{20} were measured at the semi-industrial site, S/L9, (86.63 ng TCDD-EQ/kg, dm) and its associated soil site, S/L12 (13.86 ng TCDD-EQ/kg, dm). A sediment sample collected at the same location during 2005 contained levels of DLCs reaching 380 ng TCDD-EQ/kg, dm, which was the greatest level measured yet for South African sediments. S/L9 and 12 were collected beyond the point where the Klip River, the wetland draining the areas north of Soweto, and the wetland draining the east of Soweto merges, and although the immediate surroundings of S/L9 and 12 are not highly industrialised, the location may potentially receive polluted effluents from some of these areas. The wetlands drain the south of Johannesburg and meander through the west of the outskirts of Roodepoort, both highly industrialised areas with many industries, such as chemical- and petrochemical plants, ferrous- and non-ferrous metal smelting operations, paper and pulp industries, cement production and fuel combustion, having the potential to produce DLCs. The wetland also receives run-off from urban areas, discharge from sewage treatment plants, and polluted water from the Witwatersrand's mining complexes, mostly contaminated by acid mine drainage (McCarthy *et al*., 2007). S/L8, also collected in a semi-industrial area situated south of Soweto produced a TCDD-EQ20 of 4.33 ng TCDD-EQ/kg, dm.

Of the residentially associated samples, S/L1 and S/L7 (collected up-stream of S/L9) yielded concentrations of 7.56 and 6.27 ng TCDD-EQ/kg, dm, respectively (Table 4.12). A TCDD-EQ_{20} of 15.02 ng TCDD-EQ/kg, dm was measured at S/L10, a residential/agriculturally impacted site. Most of the residences in the area are well developed and supplied with potable water and electricity, but many of the low-income, high-density residential settlements are not. Residents are dependent on domestic fires for cooking and heating, which are potential sources for PCDD/F and PCB releases (UNEP, 2005b), possibly explaining the relatively high levels of DLCs. Sites S/L7 and S/L10 are located in close proximity to two national roads (the N1 and N12) carrying heavy traffic, with vehicle exhaust emissions also probably contributing to DLC pollution (UNEP, 2005b).

Finally, wetlands are also known for their filtering and purifying properties (Davies & Day, 1998; Maltby, 1991), trapping pollutants in their sediments. They are generally very rich in flora and the abundance of plants help to stabilise sediments, increasing sediment capture rates (Davies & Day, 1998). Plants may also have the additional function of protecting sediments from UV-radiation which may degrade DLCs present in sediment. These properties of the Soweto and Lenasia wetland might explain the relatively great levels of PCDD/Fs and PCBs at these sites.

4.2.4 Cape Town sites

The levels of DLCs measured at the Cape Town sites ranged between 11.49 and 16.15 ng TCDD-EQ/kg, dm, (Table 4.12) with only four of the 20 samples eliciting quantifiable responses. Agriculturally and residentially impacted samples' TCDD-EQs were generally below the assay's LOD. The samples with measurable TCDD-EQ_{20}s were collected in industrial areas and it was therefore expected that the sediments would be polluted with DLCs to some extent. It was, however, anticipated that more of the industrially-associated sites would produce quantifiable responses in the H4IIE-*luc* cells. Both the samples CT4 and 5 were collected from canals containing industrial effluents from a paper and pulp manufacturer, a fertiliser company, chemical industry and an oil and gas refinery. Also CT10, 12, 14, 16 and 20 were sampled in highly industrialised areas or close to low-income, high-density residential areas. The reason for the low concentrations of DLCs at these sites is uncertain. Only a few of the samples (CT12, 14, 16 and 20) were of such a nature that the assay's results could have been affected by a loss in cell viability (Table 4.12). Other possible causes for the relatively low concentrations of DLCs may include seasonal

and meteorological conditions, photodegradation and degradation by microorganisms, which were explained earlier in this Chapter under Section 4.1.3. Although dilution may be a possible contributor towards low DLC levels, it was not considered to have a significant effect on Cape Town's sediments, since the CT samples were collected during the dry season (April 2007).

4.2.5 Durban sites

Only D14, the sample collected from the Umgeni River mouth, had a quantifiable TCDD-EQ_{20} of 28.89 ng TCDD-EQ/kg, dm. This sample was collected beyond the confluence of the Palmiet-, Aller- and Mbongokazi Rivers with the Umgeni River. This site potentially receives various industrial effluents from textile industry, oil refineries and soap-, paint-, dye- and fertiliser manufacturers situated up-stream. It is uncertain why so few of the industrially impacted samples had quantifiable levels of DLCs. According to the MTT bio-assay's results (Table 4.12) cell viability generally had little or no effect on the H4IIE-*luc* bio-assay's outcome. Although the samples were preferably collected from rivers or streams with slow to medium flow, sedimentation shifts could have had an effect on DLC levels in sediments. The samples were collected in February 2007 during Durban's rainy season, implying that DLCs which are bound to sediments may be diluted or "washed-away" to down-stream localities, as large quantities of water enter the system. Other factors, discussed in Section 4.1.3 could also be potential causes of low DLC levels at the majority of the Durban sites.

4.2.6 Richards Bay sites

Richards Bay has a highly concentrated industrial complex with ample industrial activity. Four of the nine samples had measurable levels of TCDD-EQ_{20} ranging from 7.48 to 103 ng TCDD-EQ/kg, dm. The area had the greatest mean concentration of DLCs of all the sites included in the study, and the second highest TCDD-EQ_{20} measured thus far for South African sediments was measured at RB1 (103 ng TCDD-EQ/kg, dm) (Table 4.12). RB1 was collected from a wetland-like area in the heart of Richards Bay industria, possibly receiving effluent of deposition from aluminium smelters, a woodchip producer and a fertiliser plant, all potential sources of DLCs (UNEP, 2005b). The sample had a deep black colour and distinct noxious odour, and the site seemed to be free from any bird life. RB2 and 3 were collected near the harbour from the Manzamyana Canal, and beyond the Manzamyana Canal's confluence with the Bhizolo Canal.

Figure 4.4. A photograph of the site RB 1 (A) and its immediate surroundings

These are man-made canals, which are not lined with cement but packed with loose rocks and stones covered with sediment, appearing similar to natural river tributaries. The canals pass through the industrial area and are closely associated with the aluminium smelter. TCDD-EQ_{20}s measured for RB2 was 28.01 ng TCDD-EQ/kg, dm, and RB3 was 18.42 ng TCDD-EQ/kg, dm. The site RB4 was situated in the Thulazihleka Pan Bird Sanctuary, also located near the industrial area, in particular the woodchip manufacturer. A TCDD-EQ_{20} of 7.48 ng TCDD-EQ/kg, dm was measured for sediments from this pan. The other industrially impacted site, RB5, and the recreationally impacted site, RB6, were sampled in lagoon-like tidal areas, not directly associated with the industrial area. The levels of DLCs at RB5 and 6, and the residential sites RB7, 8 and 9 were below the LOD of the bio-assay.

4.2.6 Bloemfontein and Botshabelo sites

The agricultural (BF1 and 2) and recreational (BF3 and 10) areas sampled in Bloemfontein generally had unquantifiable levels of DLCs. However, the levels of DLCs at two of the industrial- (BF6 and 8) and two of the industrial/residential combination sites (BF7 and 9) were quantifiable and varied between 10.34 and 47.45 ng TCDD-EQ/kg, dm. The impacted residential sites were mostly of a low-income, high-density nature and emissions from domestic fires and fuels used for cooking and heating probably contributed to DLC pollution. At the industrial sites the power generation- and food-processing plants, metal works, and manufacturers of clothing, furniture, plastics and glassware might have been responsible for DLC emissions.

Although the Botshabelo sites seemed highly polluted (distinct smell and polluted appearance of water and sediment), only one of the four sites was polluted with

DLCs. A TCDD-EQ_{20} of 36.05 ng TCDD-EQ/kg, dm was measured at BO4. The site is situated next to a national road (the N8) and vehicle exhaust emissions, along with domestic processes, and to a smaller extent industrial activities are suspected to be the main culprits of DLC pollution. It should also be noted that BO4 is the most down-stream of the four Botshabelo sites and that DLCs might have concentrated through the wetland system before being deposited, leading to elevated levels at this point.

4.2.7 International and other rivers

Of the international rivers it was only one site in the Limpopo River (Lim4), one site in the Olifants River (Olif4) and one site in the Crocodile River (Croc1b) which elicited quantifiable TCDD-EQs of 22.95, 33.31 and 8.02 ng TCDD-EQ/kg, dm, respectively. The six samples collected from the Limpopo River were from agricultural (Lim1 and 2), residential (Lim3 and 4) and recreational (Lim5 and 6) origin. Little or no DLC pollution was expected for these sites, with the exception of Lim4, which was located near a larger low-income, high-density residential area. Greater concentrations of TCDD-EQ were anticipated for the Olifants River sites, since even the agriculturally associated sites were located in the vicinity of coal and steel works (Olif3) and low-income, high-density residential settlements (Olif2). Olif1 and 4 were sampled in close proximity to various industries, but only Olif4, situated close to plantations and paper and pulp manufacturers, and mining industries elicited a measurable response in the H4IIE-*luc* cells. The samples from the Crocodile River were mostly collected near to agricultural lands and sugarcane plantations (Croc2 to 4). Two samples were also collected close to paper mills near Nelspruit (Croc1a and 1b). Croc1b, a sediment and soil composite sample, collected on the premises of the paper mills contained quantifiable amounts of DLCs, while Croc 1b, situated a few kilometres from the plant did not. The Komati- and Pongola Rivers were mostly sampled in agricultural areas, where there might be quantifiable levels of OCPs, but the levels of DLCs were below the assay's detection limit.

To assess the possible role of long-range transport from distant sources, sediments were collected from high-altitude rivers in the Drakensberg near the Lesotho border. The three sites (Drknberg1 to 3) were located in agricultural areas, with no industrial activities in the vicinity. The absence of PCDD/Fs and PCBs (Table 4.12) indicated that long-range transport played a negligible role in carrying these compounds from their sources to the high-altitude sites.

The levels of DLCs in sediments sampled from the sites associated with paper and pulp manufacturers (KZN Riv1 to 3), namely the Mhlathuze-, Tugela- and Umvoti Rivers, were also below the LOD of the bio-assay (Table 4.12). This was unexpected, since some level of DLC pollution was expected at sites which are so closely connected to significant potential sources of PCDD/Fs and PCBs (UNEP, 2005b). The low concentrations of TCDD-EQ_{20} might be ascribed to seasonal and meteorological conditions, photodegradation, sedimentation shifts, dilution or degradation by microorganisms, as explained in Section 4.1.3.

4.2.8 Chemical analysis results

To establish the levels of OCPs, PAHs, PBDEs, and PCBs present in the samples, 30 of the original 96 samples were selected for chemical analysis. This included the 23 samples eliciting quantifiable responses with the H4IIE-*luc* bio-assay (in bold in Table 4.12), the two high-altitude reference samples (Drknberg 1 and 3) for minimal industrial pollution, and all of the Soweto and Lenasia (S/L) wetland sediment samples (Table 4.12).

Although only six of the 13 S/L samples produced measurable TCDD-EQ_{20}'s, a full assessment (biological and chemical analysis) was performed on the area's sediments, since it is the area where the highest concentration of DLCs yet recorded for South African sediments was measured, possibly having the potential to pose threats to human and environmental health. Furthermore, it was the sampling location where the closest interaction existed between humans and the possibly contaminated sediments, with many residents (especially in the poorer low-income residential settlements) playing, swimming, fishing and washing their clothes in parts of the wetland and river system.

Chemical analysis results: A summary

The chemical analysis results of the 30 sites are summarised in Tables 4.15 to 4.17. Where the levels of compounds were below the detection limit, the half-LOD was reported. Due to too little sample being available for extraction, S/L 13 was not analysed for OCPs, PCBs and PBDE, and the high-altitude reference sites Drknberg1 and 3 was not analysed for PAHs.

Of the various groups of compounds analysed, only the PAHs were present in all of the samples, and the levels of PAHs were statistically significantly higher ($p < 0.05$,

Mann-Whitney U test) than the levels of the other pollutants when considering all 30 sites. OCPs (Table 4.15) and PCBs (Table 4.17) had intermediate concentrations, and PBDE were the least abundant, with low concentrations of the compound at only a few of the sites (Table 4.17). Table 4.14 contains a summary of abbreviations of chemical compound names that will be used in tables and figures from this point forward.

Table 4.14. Abbreviations of chemical compound names.

OCPs		**PAHs**	
Compound name	**Abbreviation**	**Compound name**	**Abbreviation**
HCB	HCB	Naphthalene	Naph
α-HCH	α-HCH	Acenaphthylene	Anaphthy
β-HCH	β-HCH	Acenaphthene	Anaphthe
γ-HCH	γ-HCH	Fluorene	Fluor
Heptachlor	Hpchlor	Phenanthrene	Phen
Mirex	Mirex	Anthracene	Anthr
o,p′-DDE	*o,p′*-DDE	Fluoranthene	Fluoran
p,p′-DDE	*p,p′*-DDE	Pyrene	Pyr
o,p′-DDD	*o,p′*-DDD	Benzo(a)anthracene	B(a)A
p,p′-DDD	*p,p′*-DDD	Chrysene	Chrys
o,p′-DDT	*o,p′*-DDT	Benzo(b)fluoranthene	B(b)Fl
p,p′-DDT	*p,p′*-DDT	Benzo(k)fluoranthene	B(k)Fl
		Benzo(a)pyrene	B(a)Pyr
		Indeno(1,2,3-cd)pyrene	In(1,2,3-cd)Pyr
		Dibenz(a,h)anthracene	Dib(a,h)A
		Benzo(ghi)perylene	B(ghi)Per

OCPs: The detection limit for OCPs was 10 ng/kg and the mean recovery percentages were between 46% and 92%. Aldrin and chlordene were not detected at any of the sites, whereas low levels of nonachlor, chlordane (20 to 120 ng/kg, dm) and oxychlordane (5 to 130 ng/kg) were found at only five of the 30 sites. HCB, HCH and DDT were the predominant OCPs, with concentrations ranging between 50 and 6 800 ng/kg, dw; < LOD to 2 300 ng/kg, dw; and 5 and 11 000 ng/kg, dw; respectively. Heptachlor and mirex were present at lower concentrations (Table 4.15). This can be prescribed to the fact that HCB is still currently produced for

industrial applications, and HCH (specifically γ-HCH) and DDT are presently used as pesticides in some parts of the country, while the use of heptachlor and mirex are largely prohibited in South Africa (Bouwman, 2004; Nel *et al*., 2002).

Of the HCH-isomers, γ-HCH (lindane) was the most prevalent, contributing towards 55 to 99% of the Σ-HCH (Table 4.15). Although lindane was added to the SC POPs in 2009, it is currently registered and used in agricultural and domestic gardens (Nel *et al*., 2002). Lindane consists of a mixture of all HCH isomers with approximately 90% γ-HCH (Gong *et al*., 2007). The large contribution of γ-HCH at the sites, therefore points to recent applications of lindane, rather than technical HCH (consisting of 55 to 80% α-HCH) or historic inputs, since γ-HCH degrades more rapidly than α-HCH, under aerobic and anaerobic conditions (Wu *et al*, 1997).

DDT is degraded to DDE and DDD in the environment. To assess whether the DDT measured in sediments was due to recent or historic use, the ratio of DDT to (DDE + DDD) was calculated. DDT:(DDE + DDD) ratios greater than one would indicate recent application, while ratios of less than one would indicate historic use (Gong *et al.*, 2007). The ratios were less than one at the majority of the sites, indicating historic use of DDT. At S/L8 and Lim4, the ratio exceeded one and suggested recent application. Lim 4 was sampled in a malaria endemic area where DDT is sprayed for vector control. The suggestion of recent DDT application at the semi-industrial site, S/L8, was, however, unexpected. It should be noted, though, that the levels of *o,p'*- and *p,p'*-DDT were only 840 and 470 ng/kg, dm at this site.

The sites with the highest pesticide loads were D14, the Umgeni River mouth (ΣOCPs = 23 300 ng/kg, dm), followed by a residential site in the Limpopo Province, Lim4 (ΣOCPs = 22 400 ng/kg, dm) and the Soweto wetland site, S/L9 (ΣOCPs = 12 900 ng/kg, dm) (Table 4.15, Fig. 4.5). At Lim4 and D14, ΣDDT were mainly responsible for the high OCP-loads, while HCB, ΣDDT, and HCH had about equal contributions towards the ΣOCPs at S/L9 (Fig 4.5).

PAHs: The concentration of the 16 US EPA priority PAHs were above the detection limit at all of the sites, except for the two sites, RB1 and RB4, where acenaphthylene could not be quantified due to interferences on the chromatograms (Table 4.16). The distribution profiles of PAHs were similar at the majority of the sites (Fig 4.6).

In general, 4-ringed PAHs [fluoranthene, pyrene, benzo(a)anthracene and chrysene] were the most abundant, followed by 5-ringed congeners [benzo(b)fluoranthene, benzo(k)fluoranthene and chrysene] in most instances, and finally, either by the 3-[acenaphtylene, acenaphtene, fluorene, phenanthrene and anthracene] or 6-ringed congeners [indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and benzo(ghi)perylene]. The 2-ringed PAHs were generally the least abundant (except at the sites S/L4, S/L8, S/L13 and Olif4) (Fig. 4.12). Due to their physical and chemical properties, HMW-PAHs are less susceptible to biodegradation or loss from soil through processes such as evaporation, leaching and dissolution (Manoli & Samara, 1999), which might explain the higher prevalence of the HMW-PAH species at the majority of the sites.

The concentration ranges were as follows: 2-ringed PAHs: 11 000 to 1 100 000 ng/kg, dw; 3-ringed PAHs: 270 to 700 000 ng/kg, dw; 4-ringed PAHs: 2 600 to 920 000 ng/kg, dw; 5-ringed PAHs: 5 100 to 800 000 ng/kg, dw; and 6-ringed PAHs: 1 100 to 1 600 000 ng/kg, dm.

The mean recovery for PAHs was between 10% and 60%. The highest concentrations of ΣPAHs were measured at Croc1b, situated on the premises of a papermill (ΣPAHs = 8 992 000 ng/kg, dm), S/L8, situated within a residential area (ΣPAHs = 5 528 000 ng/kg, dm, and CT7, a highly industrialised area in the vicinity of a petrochemical plant (ΣPAHs = 3 040 000 ng/kg, dm) (Fig. 4.6). Source characterisation will be dealt with later in this chapter.

PCBs: CB-153, -138, -118 and -101 were the predominant PCB congeners, while the others were present at lower concentrations (Fig. 4.7). The concentrations of the seven PCB congeners analysed for varied between < LOD and 3 100 ng/kg, dm for CB-28, < LOD and 1 900 ng/kg, dm for CB-52, < LOD to 9 400 ng/kg, dm for CB-100, 160 and 14 000 ng/kg, dm for CB-118, 120 and 18 000 ng/kg, dm for CB-138 and CB-153, and < LOD and 4 700 ng/kg, dm for CB-180 (Table 4.17). The detection limit for PCBs was 10 ng/kg and recoveries for these congeners ranged from 58 to 92%. The highly industrialised site, RB1, had the highest concentration of ΣCBs at 57 300 ng/kg, dm, followed by S/L9 (ΣCBs = 48 580 ng/kg, dm), D14 (ΣCBs = 46 180 ng/kg, dm) and RB2 (ΣCBs = 39 790 ng/kg, dm), also associated with industrial activities (Fig. 4.7).

PBDE: The detection limit for PBDE was 1 ng/kg, dm and the half-LOD was reported where concentrations of PBDE were below the detection limit (Table 4.17).

Recoveries for PBDE were between 66 and 72%. BDE-28, -47 and -209 were not detected at any of the sites. The concentrations of BDE-99, -153 and -183 ranged between < LOD to 60, 110 and 20 ng/kg, dm, respectively, with BDE-153 being the predominant PBDE (Table 4.17, Fig. 4.8). The compound were generally present at low levels with the highest concentration of ΣBDE measured at D14 being 179 ng/kg, dm. Other sites with significant concentrations of BDE included CT7 (ΣBDE = 87 ng/kg, dm) and S/L9 (ΣBDE = 60 ng/kg, dw; Fig. 4.8).

Table 4.15. The concentrations (ng/kg, dm) of certain OCPs at the sites selected for further chemical analysis.

	HCB	α-HCH	β-HCH	γ-HCH	*ΣHCH*	Hpchlor	Mirex	*o,p'*- DDE	*p,p'*-DDE	*o,p'*- DDD	*p,p'*-DDD	*o,p'*- DDT	*p,p'*-DDT	*ΣDDT*
S/L 1	760	5*	5*	780	*790*	70	40	50	340	5*	80	70	290	*835*
S/L 2	2100	5*	5*	1400	*1410*	20	30	80	650	30	350	30	340	*1480*
S/L 3	540	5*	5*	360	*370*	40	60	30	540	5*	160	40	340	*1115*
S/L 4	140	250	20	330	*600*	50	40	50	590	10	240	50	380	*1320*
S/L 5	100	5*	5*	640	*650*	20	20	70	480	5*	550	30	440	*1575*
S/L 6	160	5*	5*	210	*220*	5*	70	20	410	20	130	70	190	*840*
S/L 7	50	5*	5*	900	*910*	90	50	10	340	20	140	90	190	*790*
S/L 8	230	5*	5*	1100	*1110*	50	10	5*	440	60	110	840	470	*1925*
S/L 9	4100	430	30	2100	*2560*	360	230	120	2600	150	750	380	1300	*5300*
S/L 10	80	5*	5*	1300	*1310*	120	50	70	1100	90	90	140	30	*1520*
S/L 12	3500	5*	5*	1400	*1410*	430	150	110	2900	90	290	420	5*	*3815*
CT2	5700	5*	5*	320	*330*	140	20	90	780	5*	210	190	390	*1665*
CT6	3200	5*	5*	5*	*15*	40	40	40	810	5*	170	200	330	*1555*
CT7	6800	5*	5*	80	*90*	90	10	20	430	5*	130	180	440	*1205*
CT8	560	5*	5*	350	*360*	30	5*	30	660	5*	160	90	330	*1275*
D14	2600	5*	5*	560	*570*	2800	330	190	8600	130	890	1300	4100	*15210*
RB1	5600	800	5*	2100	*2905*	40	20	5*	290	5*	20	20	90	*430*
RB2	1800	5*	5*	1300	*1310*	80	20	5*	190	5*	40	30	140	*410*
RB3	2600	5*	5*	1300	*1310*	30	30	5*	300	5*	5*	5*	50	*370*
RB4	3300	5*	5*	1400	*1410*	80	20	5*	220	5*	5*	5*	100	*340*
BF6	4800	5*	5*	670	*680*	10	20	10	980	20	230	360	750	*2350*
BF7	5300	5*	5*	890	*900*	60	40	30	1500	40	170	140	410	*2290*
BF8	1300	5*	5*	90	*100*	150	80	20	660	40	200	40	490	*1450*

	HCB	α-HCH	β-HCH	γ-HCH	*ΣHCH*	Hpchlor	Mirex	*o,p'*- DDE	*p,p'*-DDE	*o,p'*- DDD	*p,p'*-DDD	*o,p'*- DDT	*p,p'*-DDT	*ΣDDT*
BF9	3600	5*	5*	340	*350*	20	30	50	1200	10	200	30	450	*1940*
BO4	1400	5*	5*	5*	*15*	5*	5*	5*	790	5*	70	140	290	*1300*
Lim 4	60	5*	5*	30	*40*	170	5*	220	7800	180	340	2500	11000	*22040*
Olif 4	960	5*	5*	5*	*15*	5*	30	5*	190	5*	5*	5*	90	*300*
Croc 1b	2100	5*	5*	5*	*15*	5*	60	5*	280	5*	5*	5*	90	*390*
Drknberg 1	50	5*	5*	2300	*2310*	70	5*	40	1200	5*	170	130	570	*2115*
Drknberg 3	90	5*	5*	920	*930*	210	40	40	990	5*	190	130	460	*1815*

* Where concentrations were below the detection limit, the half-LOD was reported.

Table 4.16. The concentrations (ng/kg, dm) of the 16 US EPA PAHs at the sites selected for further chemical analysis.

	Naph	Anaphthy	Anaphthe	Fluor	Phen	Anthr	Fluoran	Pyr	B(a)A	Chrys	B(b)Fl	B(k)Fl	B(a)Pyr	In(1,2,3-cd)Pyr	Dib(a,h)A	B(ghi)Per
S/L1	141 000	10 000	4 600	93 000	210 000	26 000	220 000	170 000	270 000	110 000	120 000	62 000	30 000	8 700	1 100	14 000
S/L2	63 000	2 600	2 500	7 100	60 000	6 200	100 000	89 000	67 000	77 000	90 000	38 000	69 000	85 000	11 000	90 000
S/L3	26 000	1 100	1 200	3 900	30 000	2 300	33 000	25 000	12 000	14 000	16 000	7 000	11 000	15 000	2 200	15 000
S/L4	180 000	10 000	2 900	13 000	93 000	6 600	53 000	36 000	12 000	18 000	18 000	7 300	10 000	23 000	2 600	26 000
S/L5	70 000	3 200	2 800	5 000	63 000	5 500	91 000	70 000	39 000	56 000	50 000	23 000	41 000	38 000	7 800	37 000
S/L6	180 000	6 500	1 600	15 800	200 000	17 000	250 000	170 000	120 000	170 000	180 000	70 000	78 000	120 000	20 000	120 000
S/L7	140 000	7 300	13 000	23 000	180 000	25 000	240 000	200 000	150 000	140 000	160 000	75 000	140 000	202 000	29 000	190 000
S/L8	1 100 000	37 000	40 000	93 000	700 000	90 000	700 000	500 000	300 000	400 000	320 000	140 000	390 000	250 000	78 000	270 000
S/L9	66 000	2 900	6 000	14 000	120 000	11 000	180 000	150 000	90 000	100 000	120 000	55 000	93 000	120 000	18 000	120 000
S/L10	61 000	2 700	1 400	4 800	46 000	3 200	43 000	31 000	17 000	20 000	30 000	13 000	18 000	34 000	3 800	36 000
S/L12	120 000	4 000	5 300	7 100	150 000	7 600	260 000	230 000	127 000	150 000	130 000	82 000	140 000	170 000	23 000	140 000
S/L13	120 000	3 500	2 200	5 300	56 000	4 300	55 000	40 000	20 000	34 000	40 000	16 000	24 000	36 000	5 600	42 000
CT2	96 000	1 000	25 000	44 000	230 000	29 000	280 000	260 000	180 000	160 000	150 000	77 000	23 000	150 000	24 000	120 000
CT6	13 000	290	900	3 000	14 000	2 500	15 000	20 000	7 200	8 300	10 000	5 100	7 700	10 000	1 100	14 000
CT7	80 000	10 000	21 000	67 000	320 000	50 000	220 000	240 000	280 000	340 000	330 000	130 000	200 000	310 000	42 000	310 000
CT8	22 000	500	1 400	4 200	23 000	4 300	34 000	36 000	18 000	28 000	21 000	9 000	20 000	19 000	3 000	2 200
D14	46 000	2 600	4 600	12 000	51 000	10 000	61 000	84 000	34 000	58 000	60 000	84 000	47 000	59 000	9 700	74 000
RB1	28 000	§	4 900	12 000	110 000	22 000	180 000	160 000	100 000	160 000	140 000	65 000	97 000	120 000	20 000	120 000
RB2	11 000	270	4 600	4 300	38 000	3 200	92 000	80 000	52 000	65 000	87 000	36 000	63 000	68 000	12 000	70 000
RB3	180 000	4 900	14 000	26 000	180 000	19 000	190 000	160 000	97 000	180 000	220 000	83 000	110 000	110 000	21 000	120 000
RB4	66 000	§	3 300	11 000	38 000	6 200	46 000	34 000	22 000	29 000	40 000	15 000	49 000	28 000	4 800	30 000
BF6	44 000	1 800	3 300	8 700	56 000	6 500	91 000	79 000	51 000	63 000	62 000	27 000	51 000	59 000	8 900	57 000
BF7	110 000	5 000	7 100	13 000	130 000	13 000	140 000	130 000	100 000	110 000	130 000	51 000	90 000	120 000	22 000	120 000
BF8	15 000	610	660	2 700	26 000	3 000	58 000	52 000	27 000	44 000	37 000	16 000	31 000	37 000	5 500	39 000
BF9	69 000	1 600	3 000	9 400	52 000	5 200	80 000	71 000	36 000	53 000	73 000	32 000	40 000	73 000	9 200	73 000

	Naph	Anaphthy	Anaphthe	Fluor	Phen	Anthr	Fluoran	Pyr	B(a)A	Chrys	B(b)Fl	B(k)Fl	B(a)Pyr	In(1,2,3-cd)Pyr	Dib(a,h)A	B(ghi)Per
BO4	59 000	17 000	5 100	43 000	140 000	13 000	240 000	180 000	100 000	150 000	180 000	81 000	120 000	140 000	28 000	180 000
Lim4	11 000	480	590	2 900	23 000	2 400	48 000	43 000	20 000	23 000	37 000	18 000	37 000	52 000	6 000	49 000
Olif4	93 000	2 500	1 400	6 200	31 000	6 500	33 000	31 000	23 000	20 000	29 000	11 000	20 000	21 000	4 200	29 000
Croc1b	69 000	15 000	17 000	16 000	390 000	44 000	650 000	700 000	730 000	920 000	920 000	520 000	800 000	1 600 000	11 000	1 300 000

§ No peak: The sites D14 and RB4 had no peak for acenaphthylene (Anaphthy) on the chromatogram and it could not be quantified.

Table 4.17. The concentrations (ng/kg, dm) of certain non-dioxin-like PCBs and PBDE at the sites selected for further chemical analysis

	PCBs							PBDE		
	CB-28	**CB-52**	**CB-101**	**CB-118**	**CB-138**	**CB-153**	**CB-180**	**BDE-99**	**BDE-153**	**BDE-183**
S/L1	120	90	1200	890	4100	2200	340	5	2	2
S/L2	80	230	790	1300	5600	4200	120	0.5*	0.5*	1
S/L3	20	110	580	930	4400	4500	410	0.5*	1	2
S/L4	5*	90	990	1500	2200	2100	90	0.5*	0.5*	0.5*
S/L5	5*	40	660	1100	3000	1200	370	0.5*	0.5*	0.5*
S/L6	5*	30	560	1100	1400	3900	210	0.5*	0.5*	0.5*
S/L7	5*	10	680	2200	1400	1900	550	0.5*	0.5*	0.5*
S/L8	5*	10	1300	3500	4200	3700	1300	0.5*	0.5*	0.5*
S/L9	190	590	2900	14000	8200	18000	4700	10	30	20
S/L10	5*	140	560	2800	1200	1200	130	0.5*	0.5*	0.5*
S/L12	160	810	640	2100	5900	3700	1100	0.5*	6	1
CT2	130	320	4700	4300	5800	3400	430	8	0.5*	0.5*
CT6	340	200	4600	7200	8100	2900	1300	10	0.5*	0.5*
CT7	90	450	5800	5800	9200	4900	980	30	50	7
CT8	5*	5*	3900	6100	2200	4900	170	0.5*	0.5*	0.5*
D14	1300	980	8800	7400	6600	17000	4100	60	110	9
RB1	3100	1900	9400	6700	18000	14000	4200	40	60	9
RB2	120	370	8200	7800	9200	12000	2100	10	3	3
RB3	230	690	4400	5100	3900	5500	260	0.5*	0.5*	0.5*
RB4	210	230	6100	6200	5700	3600	1100	0.5*	0.5*	0.5*
BF6	120	230	3200	1700	4200	2900	250	3	5	0.5*
BF7	300	530	2900	1700	3400	3800	30	0.5*	3	0.5*
BF8	260	5*	890	3600	2100	4700	5*	0.5*	2	0.5*
BF9	140	220	530	4900	5100	1300	410	0.5*	2	0.5*

	PCBs							PBDE		
	CB-28	**CB-52**	**CB-101**	**CB-118**	**CB-138**	**CB-153**	**CB-180**	**BDE-99**	**BDE-153**	**BDE-183**
BO4	5*	5*	5*	160	330	410	230	0.5*	0.5*	0.5*
Lim4	5*	5*	5*	240	120	120	90	0.5*	0.5*	0.5*
Olif4	5*	5*	330	980	3000	1100	130	0.5*	6	0.5*
Croc1b	5*	5*	530	690	1400	2600	150	0.5*	3	0.5*
Drknberg1	5*	5*	5*	1800	1300	1900	30	0.5*	0.5*	0.5*
Drknberg3	5*	5*	5*	960	1100	970	70	0.5*	0.5*	0.5*

* Where concentrations were below the detection limit, the half-LOD was reported.

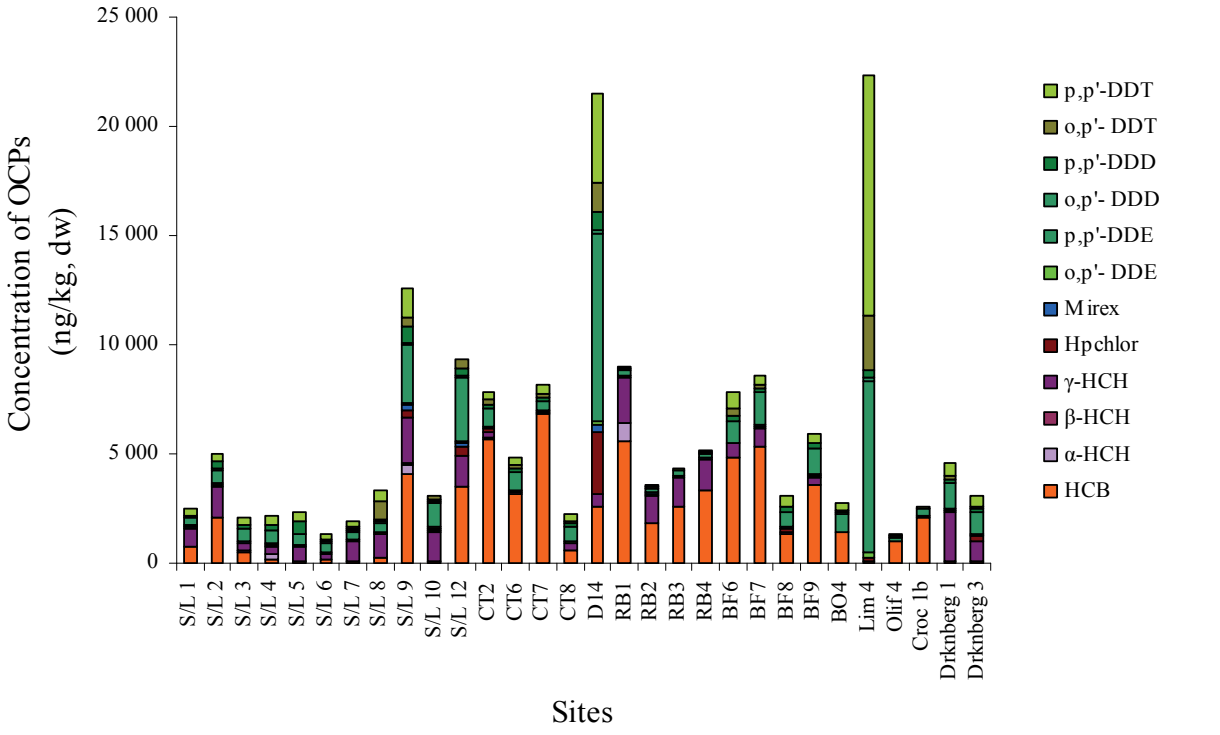


Figure 4.5 The contribution of HCB, HCH, heptachlor, mirex, DDE, DDD and DDT to the ΣOCPs measured at each site.

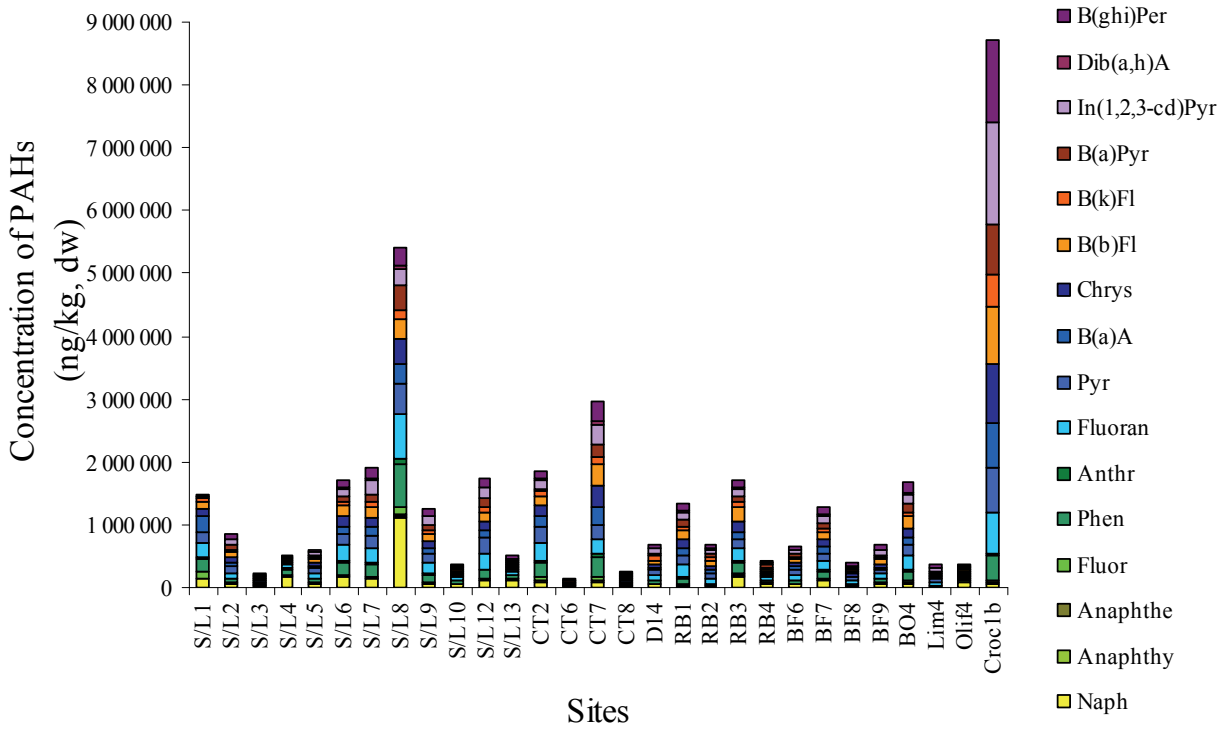


Figure 4.6 The contribution of the 16 US EPA priority PAHs towards the ΣPAHs measured at each site. (Note that acenaphthylene was not included at RB1 and RB4).

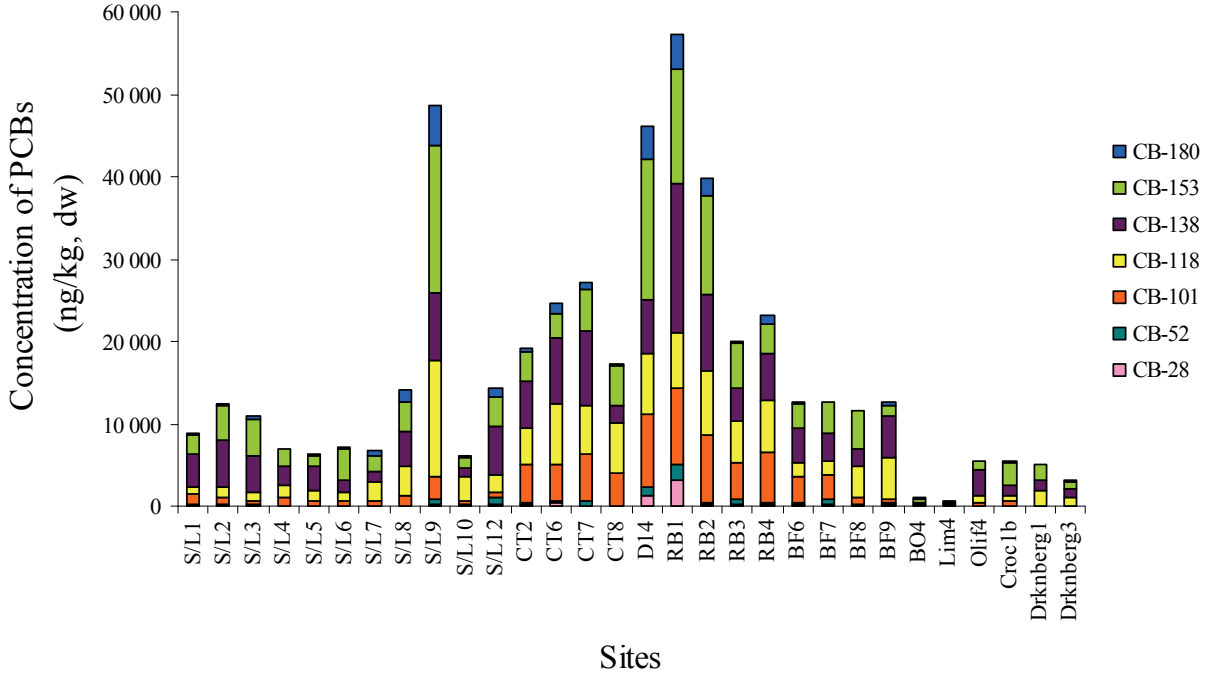


Figure 4.7 The contribution of the seven PCB congeners to the ΣPCBs measured at each site.

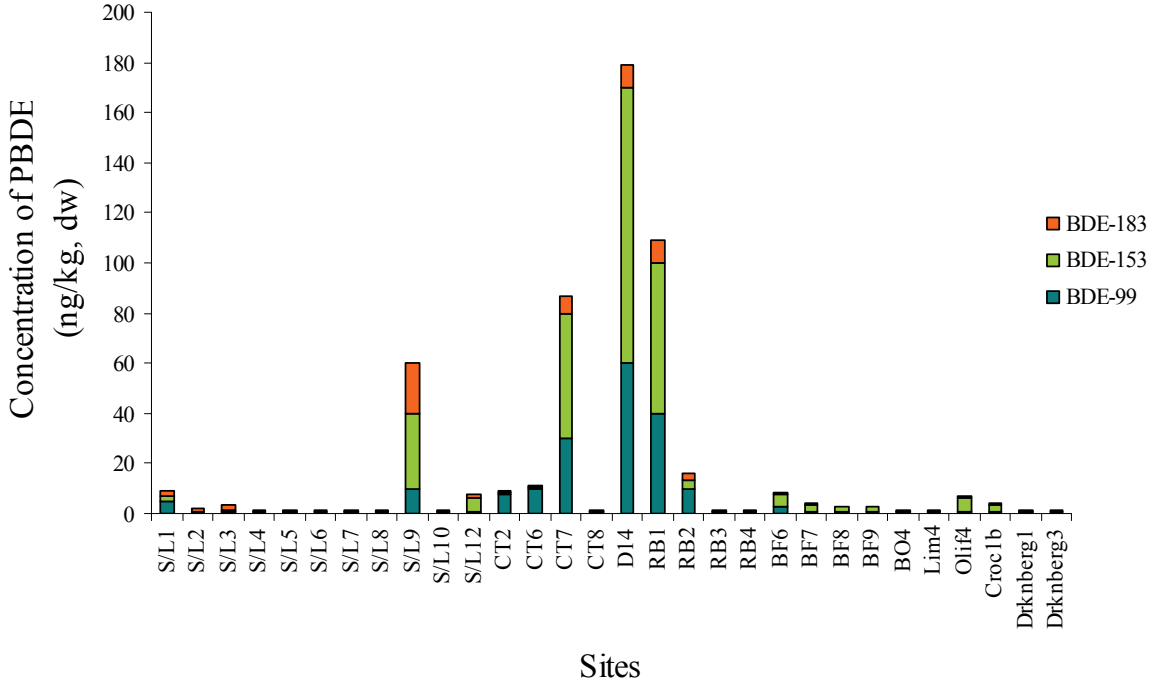


Figure 4.8 The contribution of BDE-183, -153 and -99 towards the Σ-PBDE measured at each site.

4.2.9 Chemical analysis results: Principal component analysis

PCA was used to investigate similarities and differences in the pollution profiles of the sites, which included industrial, semi-industrial, residential and agricultural areas (or combinations thereof) from different sampling regions. In the industrial areas, industrially-associated compounds such as PAHs (unintentionally produced) and PCBs and PBDE (intentionally produced) were anticipated, whereas the agricultural areas were expected to be characterised by the predominance of OCPs. At the low-income residential areas (such as the S/L and BO sites) an additional source of organic compounds (mostly DLCs and PAHs) were expected, due to domestic and backyard (open) burning used for heating and cooking.

Only chemical analysis data were included in the PCAs. The three sites, S/L 13 and Drknberg 1 and 3 did not participate in the PCAs, because the chemical analysis data for these sites were incomplete. Also, data for the PAH, acenaphthylene, was excluded from the PCAs, since the compound could not be quantified at two of the sites (no 0-values are allowed during PCA).

Separate PCA's were run for the following compounds or groups of compounds:

- **All compounds:** This included the OCPs (HCB, ΣHCH, mirex, heptachlor and ΣDDT), ΣPAHs, ΣPCBs and ΣPBDE. The purpose of this PCA was to determine the general distribution of the sites with all of the compounds included, to test the hypothesis that industrial sites will associate more with the industrial pollutants (PAHs, PCBs and PBDE), while the agricultural sites will be characterised by a predominance of OCPs.
- **OCPs only:** Here, the concentrations of HCB and mirex, and the congener or isomer-specific data for HCH and DDT were used in the PCA. The intention was to consider the distribution of the OCPs in more detail.
- **PAHs only:** While the LMW-PAHs are mainly of petrogenic origin, exist in gaseous form, and would deposit further from their sources of emission, the HMW-PAHs are mainly of pyrogenic origin, associate with particulate matter, and would deposit closer to their sources of emission. Due to their differences in properties, it is expected that the various PAH congeners will have distinct distribution profiles. The purpose of this PCA was to establish these differences and to determine the sources of PAHs.
- **PCBs and PBDEs only:** As for the PAHs, differences in the distribution of the heavier, more chlorinated, and lighter, less chlorinated PCBs were expected.

Since both PCBs and PBDE are intentionally produced industrial compounds, they participated in the same PCA.

PCA including all compounds

Factor 1 explained 34% and factor 24% of the variance in the data. Factor 1 was mainly a contrast between heptachlor (Hpchlor), ΣDDT and ΣHCH with positive loadings, and HCB, ΣPBDE and ΣPCBs with negative loadings. Factor 2 also distinguished between ΣDDT and heptachlor on the positive axis, and ΣHCH and ΣPCBs on the negative axis (Fig. 4.9).

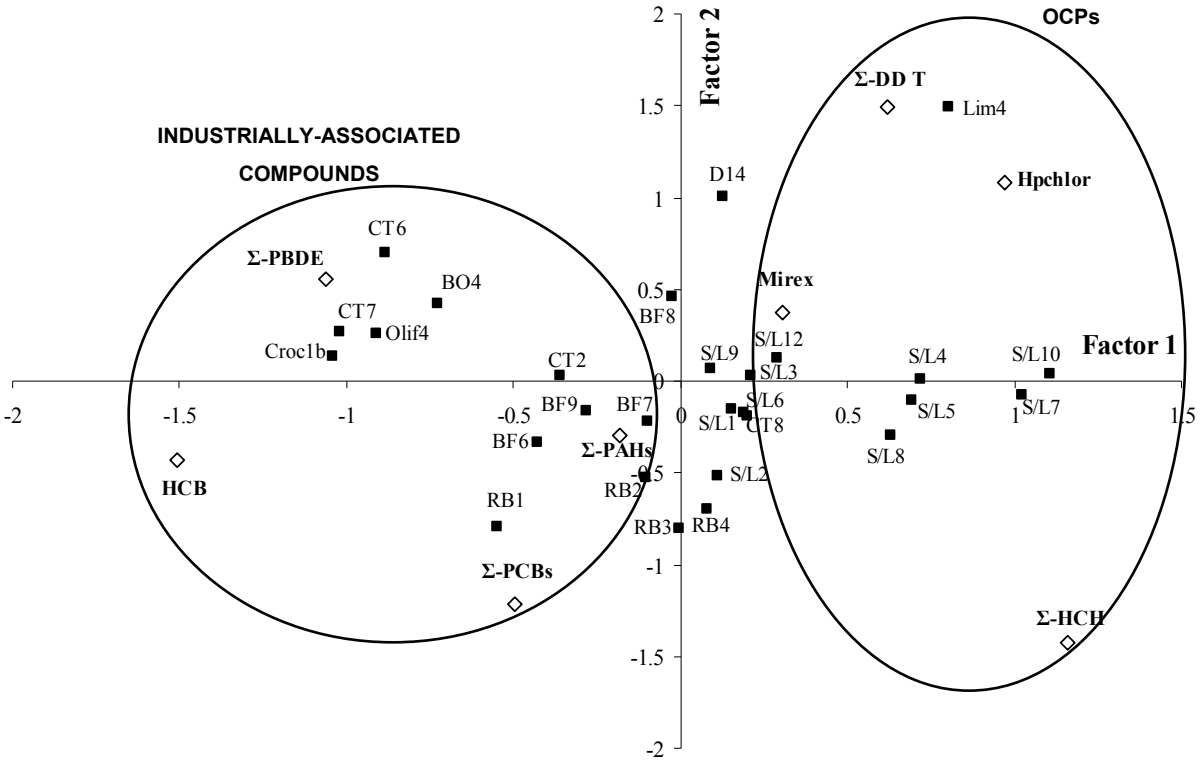


Figure 4.9 A PCA biplot of factor 1 and factor 2, showing the entire set of compounds.

The OCPs (except for HCB, which is presently used for industrial applications) had a tendency to group in the right quadrants, while the industrially-associated compounds (PCBs, PAHs, PBDE and HCB) tended to group in the left quadrants (Fig. 4.9). Sites originating from the same sampling regions clustered together, possibly indicating common sources of pollution (see RB 1 to 4, BF 6 to 9, CT 6 and 7, and the S/L sites, Fig. 4.9).

Lim4 and D14 (the sites with the highest ΣOCP-loads, Fig. 4.5) showed strong association with ΣDDT and heptachlor. Lim4 is situated in the Limpopo Province, in an area impacted by farming activities, where DDT is still commonly applied for the control of malaria. The strong association with the compound was, therefore, anticipated. The high levels of OCPs at D14 were, however, not foreseen, but it could be explained by the fact that the site might receive pesticides associated with up-stream farming activities (mainly sugar cane). The Palmiet-, Aller- and Mbongokazi Rivers, which may be polluted by pesticides from farming activities, converge with the Umgeni River and might explain the high concentrations of pesticides at D14. There existed no pertinent relationships between ΣHCH and HCB and any of the sites (Fig. 4.9). The largely industrial RB sites grouped around ΣPCBs and ΣPAHs, while there was also a close association between ΣPAHs and the industrial sites BF6, BF7, BF9 and CT2, and the low-income residential site, S/L1. PAHs could be produced as by-products of a myriad of industrial processes, or by open burning, thus, the occurrence of PAHs at these sites were anticipated. Some of the S/L sites grouped around the insecticide, mirex, while CT6, CT7, Croc1b, Olif4 and BO4, associated with the flame-retardant ΣPBDE (Fig. 4.9), commonly used in many industrial processes and applied to most synthetic materials.

PCA with OCPs only

The biplot mainly distinguished between DDE, DDD and DDT in the upper and lower right quadrants, and the HCH congeners in the upper and lower left quadrants (Fig. 4.10). Factor 1 explained 32% of the variance in data with *o,p'*-DDT, *p,p'*-DDD and *pp'*-DDE being the main contributors on the positive end of the axis, and α- and γ-HCH and HCB on the negative end. Factor 2 explained 22% of the variance and was dominated by HCB, *p,p'*-DDT and β-HCH with positive loadings, and γ-HCH, heptachlor and *o,p'*-DDD with negative loadings (Fig. 4.10). Clustering of sites originating from the same sampling regions was once again evident (see RB, S/L and BF sites, Fig 4.10).

The strong association of Lim4 and D14 with the DDT-congeners (shown in Fig 4.9) was confirmed by the second biplot with OCPs only. Lim 4 was largely characterised by the presence of *o,p'*-DDT, whereas D14 was characterised by the predominance of the metabolites, *o,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD. The majority of the S/L sites (especially S/L5, 7, 8, 10 and 12) were grouped around *o,p'*-DDD and heptachlor (Fig. 4.10), while RB1, RB3 and RB4 were clustered around mirex.

There were no further significant positive correlations between any of the other OCPs (HCB, α-, β-, and γ-HCH, p,p'-DDT and p,p'-DDE) and any of the sites (Fig. 4.10).

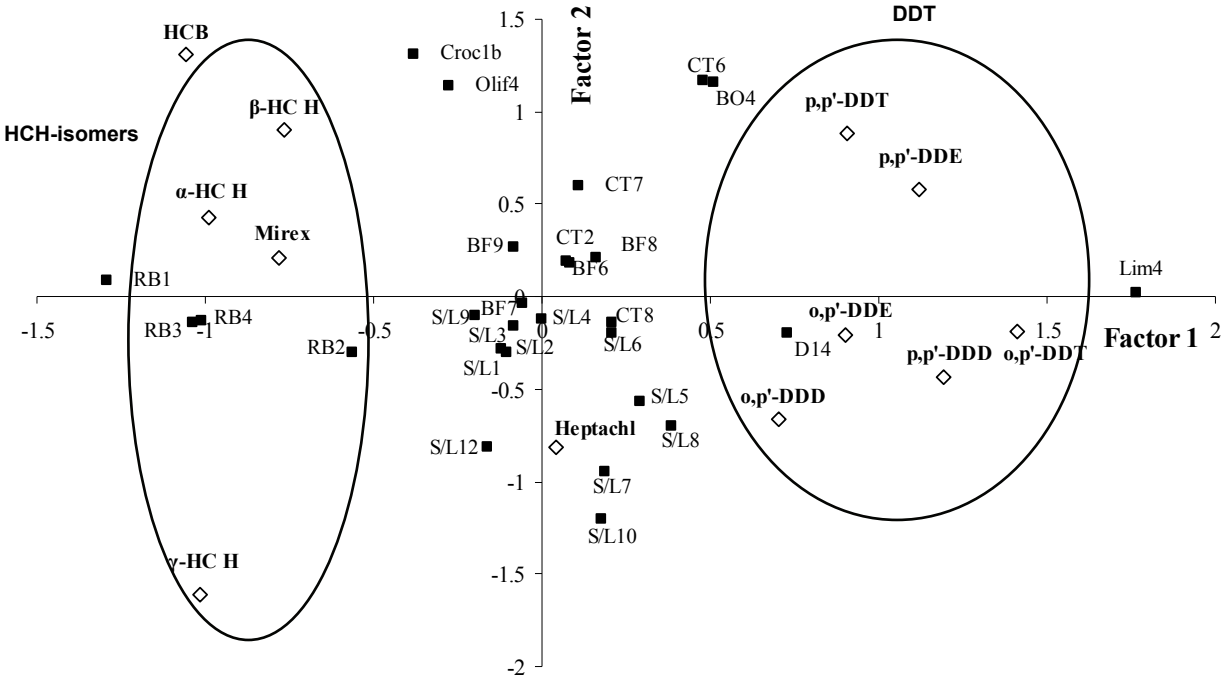


Figure 4.10. A PCA biplot of factor 1 and factor 2, with only the OCPs included.

The presence of DDD and DDE in samples is generally representative of historical application of DDT, while DDT itself indicates possible recent use. The results of the PCA corresponded with the current situation in South Africa, where DDT application was banned in most parts of the country, except for malaria endemic areas, which include the Limpopo Province (Lim4). The presence of DDT at sites where the substance is not actively applied could be indicative of long-range transport, old stockpiles, or the use of the pesticide, dicofol (contaminated by DDT). Mirex and heptachlor were mostly used for the control of termites in South Africa, but their use is currently banned. It is suspected, but cannot be confirmed, that mirex was probably used to combat termites at the facility producing wood chips (RB1) and could have spread to the surrounding sites (RB2 to 4). Since both mirex and heptachlor have long half-lives in sediment, residues of these compound may still remain in the environment from historical use and disposal via accidental spillages, fires and volatilisation from old stockpiles.

PCA with PAHs only

For the PAH biplot, 47% of the variance in the data was explained by factor 1, and 19% by factor 2. The 3-ringed PAHs (anthracene, fluorene and phenanthrene) with positive loadings, and the 6-ringed PAH [indeno(1,2,3-cd)pyrene and benzo(ghi)perylene] with negative loadings, were the main contributors towards factor 1 (Fig. 4.11). The 4-ringed congeners [benzo(a)anthracene and chrysene] with positive loadings, and the 6-ringed congener [benzo(a,h)anthracene] with a negative loading, were the main contributors to factor 2 (Fig. 4.11). The lighter 2- and 3-ringed PAHs grouped in the right half of the biplot, while the heavier 4-, 5- and 6-ringed congeners grouped on the left, with the various groups forming distinct clusters (Fig. 4.11).

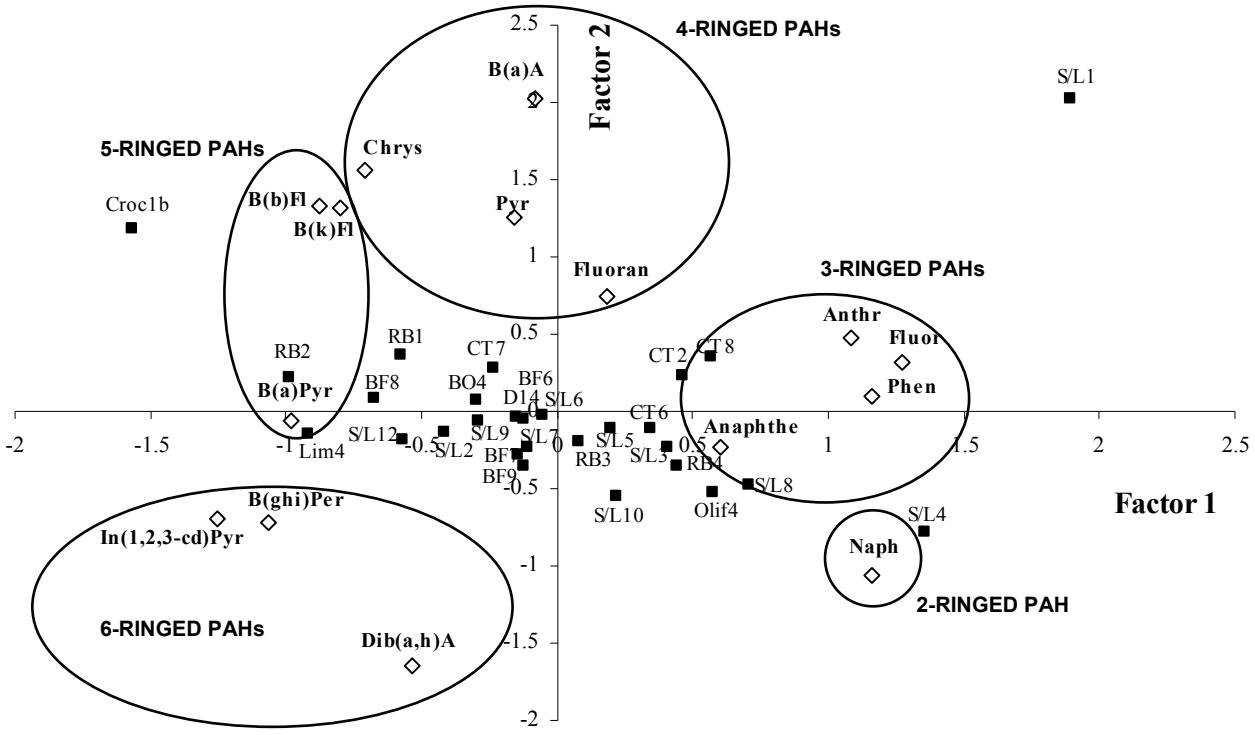


Figure 4.11 A PCA biplot of factor 1 and factor 2, with only the PAH-congeners included.

The majority of the sites grouped around the origin of the axes, without any highly significant associations with any of the congeners. This was probably due to the fact that the composition of the PAH-congeners was almost evenly distributed at the majority of the sites (Fig. 4.6). The few strong associations between PAH-congeners and sites included: benzo(a)pyrene with RB2, Lim4 and BF8; naphthalene with S/L4; and acenaphthene with RB4, S/L8, Olif4 and S/L3 (Fig. 4.11).

Since no sound conclusions on the possible sources and the distribution of PAHs could be made based on the results of the PCA, it was decided to compare concentration ratios of certain PAH-congeners, to identify their origins (Pies *et al*., 2008).

Because of the many possible sources and processes that PAHs may undergo prior to their deposition in soil or sediment, it is difficult to accurately identify their origins (Wilcke, 2007). However, source identification is made possible by comparing the concentration ratios of certain PAHs (Pies *et al*., 2008). Anthracene/phenanthrene [An/(An + Ph)] and fluoranthene/pyrene [Fl/(Fl + Py)] ratios can be used to determine whether the sources of PAHs are of petrogenic or pyrogenic origin (Gschwend & Hites, 1981; Budzinski *et al*., 1997; Pies *et al*., 2008).

Petrogenic processes promote the formation of phenanthrene, which is thermodynamically more stable than anthracene, leading to lowered An/(An + Ph) ratios (<0.1), whereas the high temperatures during pyrogenic processes facilitate the formation of anthracene, increasing the An/(An + Ph) ratio (>0.1) (Pies *et al*., 2008). Also, a Fl/(Fl + Py) ratio of less than 0.5 usually indicates petroleum sources, while a ratio of greater than 0.5 indicates combustion (Yunker *et al*., 2002). However, the petroleum boundary seems to be closer to 0.4 than 0.5, thus ratios between 0.4 and 0.5 are more characteristic of liquid fossil fuels and ratios greater than 0.5 are characteristic of grass, wood or coal combustion (Yunker *et al*., 2002).

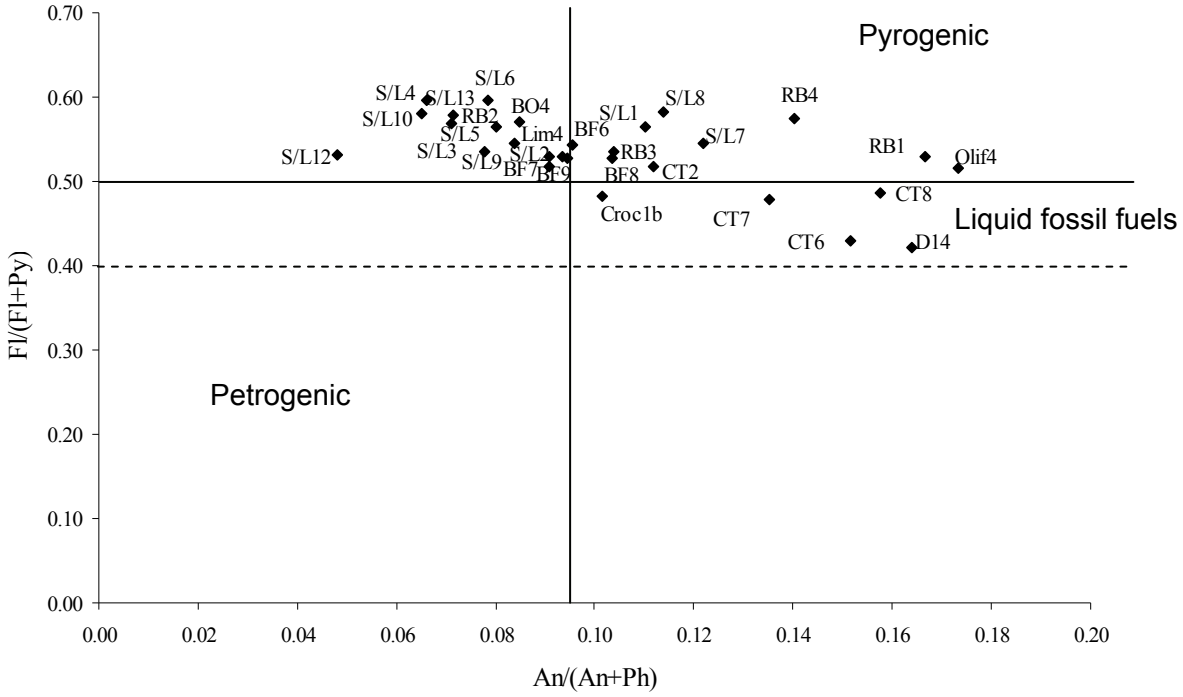


Figure 4.12. A cross-plot with An/(An+Ph) on the x-axis, and Fl/(Fl+Py) on the y-axis, where An/(An+Ph) < 0.1 and Fl/(Fl+Py) < 0.4 indicates petrogenic sources, 0.4 < Fl/(Fl+Py) < 0.5 indicates the use of liquid fossil fuels, and An/(An+Ph) > 0.1 and Fl/(Fl+Py) > 0.5 indicates pyrogenic sources.

None of the sites were solely indicative of petrogenic sources [no sites had both An/(An+Ph) ratios of less than 0.1 and Fl/(Fl+Py) ratios of less than 0.4] (Fig. 4.12).

At five of the sites, the use of liquid fossil fuels was suggested as the main source of PAHs. These sites included Croc1b, located on the premises of a paper mill, CT7, 8 and 9, situated in mixed industrial areas (paper mill, petrochemical plant and other industries), and D14, the Umgeni River mouth, situated down-stream of numerous industries (textile industry, metal producers, building material manufacturers). In addition to liquid fossil fuels used in the above-mentioned industrial processes, vehicle exhaust emissions may also have contributed to the “fossil fuel” origin of PAHs at these sites, since most of the sites are situated in high-traffic areas.

The An/(An+Ph) and Fl/(Fl+Py) ratios of RB1, 3 and 4, Olif4, BF6 and 8, SL, 1, 7 and 8, and CT2 ranged between 0.10 and 0.17, and 0.52 and 0.58, respectively, indicating pyrogenic processes as the major source of PAHs at these sites (Fig. 4.12). This means that the inefficient incineration of organic materials such as wood,

coal and oil were primarily responsible for the PAHs at these sites (Masih & Taneja, 2006; Culotta *et al.,* 2006). Industrial combustion processes during aluminium smelting, wood chip production (RB1, 3 and 4), ferrous and non-ferrous metal production (CT2), paper and pulp manufacturing (Olif4) and coal-based power generation (BF8) were considered as the major contributor towards pyrogenic sources at these sites. On the other hand, domestic combustion (backyard burning or open fires used for cooking and heating) were the most likely sources of petrogenic PAHs at the low-income residential sites S/L1 and 7 and BF6. In fact, the burning of coal was noted at S/L7 during sampling.

The PAH origin at the remainder of the sites (S/L2 to 6, 9, 10, 12 and 13, RB2, BO4, BF7 and 9, and Lim4) were neither classified as entirely pyrogenic nor petrogenic. The An/(An+Ph) ratios were less than 0.1, indicating petrogenic origins, whereas the Fl/(Fl+Py) ratios were larger than 0.5, pointing towards pyrogenic sources (Fig. 4.12). All of these sites, except for RB2 (which was located in an industrial area), were situated in or affected by low-income residential areas or settlements. It is speculated that the "mixed" origin of the PAHs at these sites are primarily due to open or backyard burning, used for cooking and heating, where various substances may be used to ignite (oil, petrol, diesel, paraffin) and maintain (wood, plastic, charcoal, paper) fires.

PCA with PCBs and PBDE only

Factor 1 explained 32% and factor 2 explained 26% in the variance of data. Factor 1 was largely characterised by the PBDE-congeners (BDE-99, -153 and -183) with positive loadings and the lighter molecular mass, lower chlorinated PCB congeners (CB-28, -52 and -101) with negative loadings. Factor 2, on the other hand, distinguished between the heavier molecular mass, higher chlorinated PCBs (CB-118, -138 and -153) with positive loadings, and CB-52, BDE-153 and BDE-183 with negative loadings. There was a distinct separation between the PBDEs (lower right quadrant), lighter PCBs (lower left quadrant) and heavier PCBs (upper left quadrant) (Fig. 4.13).

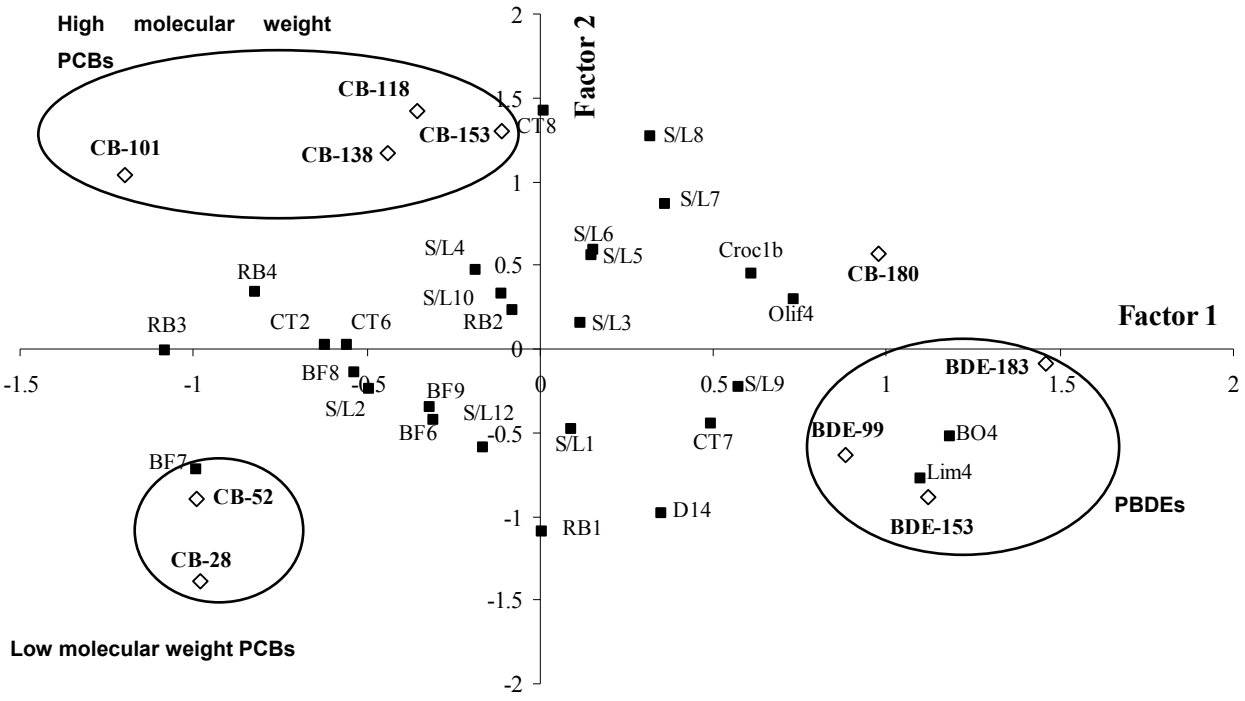


Figure 4.13. A PCA biplot of factor 1 and factor 2, with only the PCB- and PBDE-congeners included.

There were strong associations between BO4, Lim4 and the PBDEs, Croc1b, Olif4 and CB-180, CT8 and the heavier PCBs, and BF7 and the light PCBs. The other sites were scattered around the origin of the axes without any significant correlations between the sites and any PBDE- or PCB-congeners (Fig. 4.13).

BO4 and Lim4 are low-income residential sites, and their positive correlation with the PBDE congeners was at first unexpected. PBDEs are mainly industrially associated, and the most evident sources of BFRs into the environment are effluents from plants producing BFRs, flame-retarded polymers and other plastic products (Sellström & Jansson, 1995). However, the sources responsible for the formation of polybrominated and mixed brominated/ chlorinated dibenzo-*p*-dioxins and dibenzofurans could also be responsible for the formation of PBDE (WHO, 1998). Backyard or open burning of municipal waste, plastics and products containing flame retardants, might have been responsible for the formation of PBDEs at these sites.

PCBs were produced for industrial purposes as insulating materials in electrical equipment, plasticisers (softening materials) in plastic products, hydraulic fluids, adhesives, lubricants, fire retardants and dielectrics in transformers, but their production and use were banned in the 1980s. Currently, they are primarily released

into the environment due to historical use, accidental spillages, fires and volatilisation from old stockpiles (Koppe & Keys, 2001). The heavier PCB congeners are more abundant in soils and sediments and less abundant in water and the atmosphere, while the lighter congeners are less prominent is soils and sediments, and more abundant in the atmosphere and surface waters (Carey *et al*., 1998; Henry & De Vito, 2003). This was the case for the South African sites, with CB-28 and -52 being present at much lower levels than the heavier PCBs (Fig. 4.13). The industrial sites, BF7 and CT8, were associated with the low molecular mass and the high molecular mass PCBs, respectively. The concentrations of the light PCBs at BF7 were between 300 and 530 ng/kg, dm, and the concentrations of the heavy PCBs at CT8 were between 170 and 6100 ng/kg, dm. Both sites were located down-stream of mixed industrial areas, which might have contributed to the PCB loads at these sites.

4.2.10 South Africa's position in the global POPs issue

A comparison with sediment quality guidelines

Compared to the northern hemisphere, knowledge of the concentrations of OPs, especially POPs, in South Africa are very limited. There are currently no guidelines or regulations in place to govern the levels of POPs in the South African environment. Canadian soil and sediment quality guidelines (considered as highly protective) were therefore used to assess the levels of POPs for the study area. The results of this study were normalised to 1% TOC to allow comparison with the quality guidelines (Tables 4.19 a & b), proposed by the Canadian Council of Ministers the Environment (CCME, 2002) and the Ontario Ministry of Environment and Energy (1998). None of the soil sites (S/L11, 12 and 13) exceeded any soil quality guidelines. Therefore, only the sediment quality guidelines (Table 4.18) are reported from this point onwards. There are no Canadian sediment quality guidelines available for PBDE and the compound was not included in this assessment.

Table 4.18. Sediment quality guidelines (reported in ng/kg, dm) proposed by the CCME (2002) [§] and Ontario Ministry of Environment and Energy (1998) [§§].

Compound group	Compound name	Canadian Sediment Quality Guidelines[§]		Ontario Sediment Quality Guidelines[§§]	
		Interim freshwater sediment quality guidelines (ISQG)	Probable Effect Level (PEL)	Lowest Effect Level (LEL)	Severe Effect Level (SEL)
OCPs	HCB			20 000	24 000 000
	γ-HCH (Lindane)	940	1 380	3 000	12 000 000
	Heptachlor epoxide (metabolite of Heptachlor)	600	2 740	5 000	5 000 000
	Mirex			7 000	130 000 000
	DDD (*o,p'*- and *p,p'*-)	3 540	8 510	8 000	6 000 000
	DDE (*o,p'*- and *p,p'*-)	1 420	6 750	5 000	19 000 000
	DDT (*o,p'*- and *p,p'*-)	1 190	4 770	7 000	12 000 000
DLCs	PCDD/Fs and dioxin-like PCBs	0.85 ng TEQ/kg	21.5 ng TEQ/kg		
PAHs	Naphthalene	34 600	391 000		
	Acenaphthylene	5 870	128 000		
	Acenaphthene	6 710	88 900		
	Fluorene	21 200	144 000	190 000	160 000 000
	Phenanthrene	41 900	515 000	560 000	950 000 000
	Anthracene	46 900	245 000	220 000	370 000 000
	Fluoranthene	111 000	2 355 000	750 000	1 020 000 000
	Pyrene	53 000	875 000	490 000	850 000 000

	Compound name	Canadian Sediment Quality Guidelines[§]		Ontario Sediment Quality Guidelines[§§]	
		Interim freshwater sediment quality guidelines (ISQG)	Probable Effect Level (PEL)	Lowest Effect Level (LEL)	Severe Effect Level (SEL)
	Benzo(a)anthracene	31 700	385 000	320 000	1 480 000 000
	Chrysene	57 100	862 000	340 000	460 000 000
	Benzo(b)fluoranthene	No guidelines available		No guidelines available	
	Benzo(k)fluoranthene			240 000	1 340 000 000
	Benzo(a)pyrene	31 900	782 000	370 000	1 440 000 000
	Indeno(1,2,3-cd)pyrene			200 000	320 000 000
	Dibenz(a,h)anthracene	622 000	135 000	60 000	130 000 000
	Benzo(ghi)perylene			170 000	320 000 000
PCBs	Total PCBs	34 100	277 000	70 000	530 000 000
PBDE	Total PBDE	No guidelines available		No guidelines available	

Table 4.19a. The percentage TOC, and the levels of PAHs measured at each site normalised to 1% TOC and reported in ng/kg, dm to allow comparison to sediment quality guidelines (Table 4.18).

Sites	% TOC	PAHs														
		Naph	Anaphthy	Anaphthe	Fluor	Phen	Anthr	Fluoran	Pyr	B(a)A	Chrys	B(b)Fl	B(k)Fl	B(a)Pyr	In(1,2,3-cd)Pyr	Dib(a,h)A	B(ghi)Per
S/L1	6.63	21 272	1 509	694	14 030	31 681	3 922	33 190	25 647	**40 733***	16 595	18 104	9 354	4 526	1 313	166	2 112
S/L2	4.96	12 693	524	504	1 430	12 089	1 249	20 148	17 932	13 499	15 514	18 133	7 656	13 902	17 126	2 216	18 133
S/L3	5.57	4 665	197	215	700	5 383	413	5 921	4 486	2 153	2 512	2 871	1 256	1 974	2 691	395	2 691
S/L4	7.62	23 620	1 312	381	1 706	12 204	866	6 955	4 724	1 575	2 362	2 362	958	1 312	3 018	341	3 412
S/L5	3.14	22 267	1 018	891	1 591	20 040	1 750	28 947	22 267	12 406	17 814	15 905	7 316	13 042	12 088	2 481	11 770
S/L6	6.30	28 571	1 032	254	2 508	31 745	2 698	39 681	26 983	19 047	26 983	28 571	11 111	12 381	19 047	3 175	19 047
S/L7	7.76	18 032	940	1 674	2 962	23 184	3 220	30 912	25 760	19 320	18 032	20 608	9 660	18 032	26 018	3 735	24 472
S/L8	7.03	**156 482***	5 263	5 690	13 230	**99 579***	12 803	99 579	**71 128***	**42 677***	56 902	45 522	19 916	**55 480***	35 564	11 096	38 409
S/L9	13.64	4 838	213	440	1 026	8 797	806	13 195	10 996	6 598	7 331	8 797	4 032	6 818	8 797	1 320	8 797
S/L10	6.47	9 432	418	216	742	7 113	495	6 649	4 794	2 629	3 093	4 639	2 010	2 783	5 257	588	5 567
S/L12	5.91	20 293	676	896	1 201	25 367	1 285	43 969	38 895	21 477	25 367	21 984	13 867	23 675	28 749	3 890	23 675
S/L13	9.52	12 600	368	231	557	5 880	452	5 775	4 200	2 100	3 570	4 200	1 680	2 520	3 780	588	4 410
CT2	5.64	17 017	177	4 431	7 799	40 769	5 140	49 631	46 086	**31 906***	28 361	26 588	13 649	4 077	26 588	4 254	21 271
CT6	1.32	9 829	219	680	2 268	10 585	1 890	11 341	15 121	5 444	6 275	7 561	3 856	5 822	7 561	832	10 585
CT7	4.75	16 844	2 105	4 421	14 107	**67 374***	10 527	46 320	50 531	**58 953***	**71 585***	69 480	27 371	**42 109***	65 269	8 843	65 269
CT8	1.32	16 606	377	1 057	3 170	17 361	3 246	25 664	27 174	13 587	21 135	15 851	6 793	15 096	14 342	2 264	1 661
D14	3.56	12 928	731	1 293	3 372	14 333	2 810	17 143	23 607	9 555	16 300	16 862	23 607	13 209	16 581	2 726	20 797
RB1	1.88	14 876		2 603	6 376	**58 442***	11 688	95 633	**85 007***	**53 129***	**85 007***	74 381	34 534	**51 535***	63 755	10 626	63 755
RB2	0.71	15 506	381	6 485	6 062	**53 568***	4 511	**129 690***	112 774	**73 303***	**91 629***	122 642	50 748	**88 809***	95 858	16 916	98 677
RB3	1.44	**124 594***	3 392	**9 691***	17 997	**124 594***	13 152	**131 516***	110 750	**67 142***	**124 594***	152 281	57 452	**76 141***	76 141	14 536	83 063
RB4	5.92	11 140		557	1 857	6 414	1 046	7 764	5 739	3 713	4 895	6 751	2 532	8 270	4 726	810	5 064
BF6	1.25	**35 199***	1 440	2 640	6 960	**44 800***	5 200	72 799	**63 200***	**40 800***	50 400	49 600	21 600	**40 800***	47 200	7 120	45 600

Sites	% TOC	PAHs															
		Naph	Anaphthy	Anaphthe	Fluor	Phen	Anthr	Fluoran	Pyr	B(a)A	Chrys	B(b)Fl	B(k)Fl	B(a)Pyr	In(1,2,3-cd)Pyr	Dib(a,h)A	B(ghi)Per
BF7	2.05	**53 596***	2 436	3 459	6 334	**63 341***	6 334	68 213	**63 341***	**48 724***	53 596	63 341	24 849	**43 851***	58 468	10 719	58 468
BF8	1.82	8 228	335	362	1 481	14 261	1 646	31 813	28 522	14 810	24 134	20 295	8 776	17 004	20 295	3 017	21 392
BF9	6.71	10 290	239	447	1 402	7 754	775	11 930	10 588	5 368	7 904	10 886	4 772	5 965	10 886	1 372	10 886
BO4	5.65	10 442	3 009	903	7 610	24 778	2 301	42 476	31 857	17 698	26 547	31 857	14 336	21 238	24 778	4 956	31 857
Lim4	3.21	3 431	150	184	905	7 174	749	14 973	13 413	6 239	7 174	11 541	5 615	11 541	16 220	1 872	15 285
Olif4	2.27	**41 031***	1 103	618	2 735	13 677	2 868	14 559	13 677	10 147	8 824	12 795	4 853	8 824	9 265	1 853	12 795
Croc1b	1.49	**46 348***	**10 076***	**11 419***	10 747	**261 968***	29 555	**436 614***	**470 200***	**490 351*~^**	**617 976*^**	617 977	**349 291^**	**53 7371*^**	**1 074 742^**	7 389	**873 227^**

* Levels exceeding ISQG ~Levels exceeding the PEL ^Levels exceeding the LELNone of the sites exceeded the SEL.

Table 4.19b. The percentage TOC and the levels of OCPs, DLCs and PCBs measured at each site normalised to 1% TOC and reported in ng/kg, dm to allow comparison to sediment quality guidelines (Table 4.18).

Sites	%TOC	OCPs							DLCs	PCBs
		HCB	γ-HCH (Lindane)	Hpchlor	Mirex	ΣDDD	ΣDDE	ΣDDT	Total DLCs (bio-assay)	Total PCBs
S/L1	6.63	115	118	11	6	13	59	54	**1.14***	1 349
S/L2	4.96	423	282	4	6	77	147	75	0.20	2 482
S/L3	5.57	97	65	7	11	30	102	68	0.17	1 965
S/L4	7.62	18	43	7	5	33	84	56	0.13	915
S/L5	3.14	32	204	6	6	177	175	150	0.31	2 028
S/L6	6.30	25	33	1	11	24	68	41	0.15	1 144
S/L7	7.76	6	116	12	6	21	45	36	0.81	869
S/L8	7.03	33	156	7	1	24	63	186	0.62	1 994
S/L9	13.64	301	154	26	17	66	199	123	**6.35***	3 561
S/L10	6.47	12	201	19	8	28	181	26	**2.32***	933
S/L12	5.91	592	237	73	25	64	509	72	**2.34***	2 437
CT2	5.64	1 010	57	25	4	38	154	103	**2.86***	3 382
CT6	1.32	2 419	4	30	30	132	643	401	**9.33***	18 629
CT7	4.75	1 432	17	19	2	28	95	131	**2.51***	5 731
CT8	1.32	423	264	23	4	125	521	317	**8.67***	13 043
D14	3.56	731	157	**787***	93	287	**2 470***	**1 518***	**8.12***	12 978
RB1	1.88	2 975	**1 116***	21	11	13	157	58	**54.72*~**	30 443
RB2	0.71	2 537	**1 833*~**	113	28	63	275	240	**39.49*~**	**56 091***
RB3	1.44	1 800	900	21	21	7	211	38	**12.75***	13 899
RB4	5.92	557	236	14	3	2	38	18	**1.26***	3 906
BF6	1.25	3 840	536	8	16	200	792	888	**35.46*~**	10 080
BF7	2.05	2 582	434	29	19	102	745	268	**22.01*~**	6 168
BF8	1.82	713	49	82	44	132	373	291	**26.03*~**	6 341
BF9	6.71	537	51	3	4	31	186	72	**1.54***	1 879
BO4	5.65	248	1	1	1	13	141	76	**6.38***	203
Lim4	3.21	19	9	53	2	162	**2 502***	**4 211***	**7.16***	182
Olif4	2.27	424	2	2	13	4	86	42	**14.70***	2 449
Croc 1b	1.49	1 411	3	3	40	7	191	64	**5.39***	3 614
Drknberg1	1.41	36	**1 634*~**	50	4	124	881	497	0.69	3 584
Drknberg3	1.55	58	595	136	26	126	667	382	0.63	2 016

*Levels exceeding ISQG ~Levels exceeding the PEL

^Levels exceeding the LEL None of the sites exceeded the SEL

Sites exceeding the sediment quality guidelines are printed in bold in Tables 3.19a and b. None of the compounds were present at such concentration that the severe effect level (SEL) was exceeded.

The levels of HCB, mirex and DDD measured at the South African sites did not exceed any of the sediment quality guidelines (Table 4.19b). The interim sediment quality guidelines (ISQG) for γ-HCH were above the proposed levels at RB1, RB2

and Drknberg1. At RB2 and Drknberg1, the probable effect level (PEL), which is the level above which adverse effects are expected to occur on a frequent basis, were exceeded as well (Table 4.19b). Since there are no Canadian sediment quality guidelines available for heptachlor, the ISQG of the metabolite, heptachlor epoxide, was used (Table 4.18). D14 was the only site with a heptachlor level above the recommended ISQG of 600 ng/kg, dm. The concentrations of ΣDDE and ΣDDT at D14 and Lim4 exceeded the ISQG, but were below the PEL (Table 4.19b).

Of the industrially-associated compounds, the ISQG for DLCs were exceeded at 21 of the 30 sites. Of this 21 sites, five (RB1, RB2, BF6, BF7 and BF8) had DLC concentrations of above the PEL as well (Table 4.19b). It should be taken into account that the biological data of DLCs was used for this assessment. Although a positive correlation between biological and chemical results generally exists, biological results may be an order of magnitude higher (Nieuwoudt *et al*., 2009; Carbonnelle *et al*., 2004; Vanderperren *et al*., 2004; Van Wouwe *et al*., 2004) and might therefore be an over estimation of DLC levels. The concentrations of the PAH congeners were above the sediment quality guidelines at eleven of the sites. The ISQGs of some PAH congeners were exceeded at S/L1, S/L8, CT2, CT7, RB1, RB2, RB3, BF6, BF7 and Olif4, while some of the congeners' ISQGs, PELs and LELs were exceeded at Croc1b.

The following sites were identified as "priority" areas for this study, due to the high concentrations of certain compounds or the amount of compounds exceeding the Canadian environmental quality guidelines which are recognised as highly protective:

- **D14 (Umgeni mouth – possibly receiving effluent from various industries up-stream)**, where the levels of heptachlor, DDE, DDT and DLCs were all above the ISQGs;
- **RB1 and RB2 (Richards Bay industrial sites):** At RB1 the ISQGs of several PAH-congeners and γ-HCH were exceeded, while the ISQG and PEL of DLCs were exceeded. At RB2, PAHs and PCBs were present at such concentrations that the ISQGs of these compounds were exceeded, and for γ-HCH and DLCs the PELs were exceeded as well;
- **BF6, BF7 and BF8 (Bloemfontein industrial and low-income residential sites):** The concentrations of some of the PAH-congeners at BF6 and BF7 where higher than the proposed ISQG, and the DLC concentrations at all three of the sites exceeded the ISQGs and the PELs;

- **Lim4 (Low-income residential site in the Limpopo Province – a malaria-endemic area):** The ISQGs of DDE, DDT and DLCs were exceeded at Lim4;
- **Croc1b (On the premises of a paper mill in Mpumalanga):** This site had the largest amount of PAH-congeners exceeding the ISQGs, PELs and LELs. The concentration of DLCs at this site was above the ISQG.

A comparison with the levels found in other countries

Table 4.20 compares the minimum and maximum concentrations of pollutants measured during the current study to previous South African studies and studies done elsewhere in the world. When considering the levels of OPs and POPs measured at the 30 South African sites, the levels were generally lower than, or within the same range as that measured in other countries. The maximum concentrations of certain pollutants measured at some sites were, however, higher than that measured in some of the other countries (Table 4.20). This study focussed mainly on sediment sites and only one soil sample was chemically analysed for OCPs, PCBs and PBDE and included in the comparison.

The maximum level of HCB measured during the current study was higher than that measured during the previous South African study, but lower than the maximum levels measured in China, Germany and Australia (Table 4.20). The maximum level of ΣHCH, measured at RB1, was also higher than that measured during the previous study. It exceeded the levels measured near an old pesticide factory, residue store, residential and reference areas in Spain, but it was one to two orders of magnitude lower than samples collected in the vicinity of a chemical plant at Ya-Er Lake and the Haihe and Dagu Draingage Rivers in China (Yang *et al*., 2005; Wu *et al.*, 1997).

This was the first analysis for heptachlor and mirex in South African soils and sediments done by our research group. In both matrices, the maximum concentrations of heptachlor were much lower than the levels measured in soil and sediments of other countries such as Mexico, Australia and the USA (Table 4.20). On the other hand, the concentrations of mirex were relatively high, with the maximum concentration in sediment reaching 330 ng/kg, dm and soil reaching 150 ng/kg, dm. This was less than the concentrations measured in Brazil, but higher than the concentrations measured in Korea, India and the Czech Republic (Table 4.20).

Compared to the levels of ΣDDT found in other countries, the maximum concentrations of ΣDDT in South African sediment were also relatively high, with

concentrations exceeding the levels measured in China, the USA and Russia. The maximum concentration measured during the current study was also an order of magnitude higher than that measured in the previous South African study (Table 4.20). This is mainly due to the site located in the Limpopo Province (Lim4) where DDT is still actively sprayed for the control of malaria. Levels of ΣDDT measured in Canadian sediments in a study area which included urban, suburban and rural sites were an order of magnitude higher than the maximum level measured during this study (Wong *et al*., 2009; Table 4.20). The very high ΣDDT = 472 000 ng/kg (Table 4.20) was measured at an urban site and was attributed to "organochlorine and sources" in the sewershed since the waterbody also received storm water inputs (Wong *et al*., 2009).

Weighing the concentrations of DLCs measured with the bio-assay against the levels of DLCs measured elsewhere in the world (either by chemical or bio-analysis), the concentrations found in South African soils and sediments were relatively low. The concentrations of DLCs measured in the USA, Spain, Norway, Sweden and the Netherlands were one to three orders of magnitude higher than the maximum concentration measured during the current study (Table 4.20). This was the same case for ΣPBDEs, with the levels of ΣPBDEs measured in Italy, the Netherlands, Portugal, Sweden, Norway and the United Kingdom, being two to three orders of magnitude higher than the concentrations measured in South African soils and sediments (Table 4.20).

At 8 992 000 ng/kg, dm, the maximum level of ΣPAHs measured during this study was high when compared to the levels measured in Germany, Canada and the previous South African study, but it was lower than the levels measured in an industrial area, impacted on by coal mining in Germany. The maximum concentration of ΣPCBs exceeded the concentrations measured in Yugoslavia and China, but was less than the concentrations measured in Romania (Table 4.20).

Considering the levels of the above-mentioned pollutants, the concentrations measured in South African soil and sediment were generally intermediate when compared to other countries, with the exception of a few sites (listed in this Section) exceeding the pollutant levels measured elsewhere in the world. Combined with probably much lower overall emissions from a smaller industrial base in South Africa when compared with China and other industrialised countries, another probable major reason for the lower levels of these pollutants found in South African matrices

is the country's climate, characterised by high temperatures, low precipitation, and long summers, different from the more moderate conditions in Europe, North-America and elsewhere where most of the POPs research has been conducted.

Table 4.20 The levels of certain OPs and POPs measured in soil and sediment from the current and previous South African studies and other countries.

	Site description	Sediments (ng/kg, dm)		Soil (ng/kg, dm)		Reference
		Min	Max	Min	Max	
HCB						
Current study (South Africa)		**<50**	**6 800**	**3 500***		
Previous study (South Africa)	Central South Africa. Sampling areas included industrial, agricultural and residential sites.	30	320	530	15 000	Quinn *et al.*, 2009
China	Ya-Er Lake area in the vicinity of a chemical plant.	31 500 000	57 100 000	35 400 000	37 700 000	Wu *et al.*, 1997
Germany	Upper Rhine River	ND	60 000			Breitung *et al.*, 2008
Australia	Northern Australia – sites along the east coast of Queensland	<500	28 000			Müller *et al.*, 1999
ΣHCH[a]						
Current study		**15**	**2 900**	**1 410***		
Previous study (South Africa)	Central South Africa.	200	1 700	320	1 860	Quinn *et al.*, 2009
China	Ya-Er Lake area in the vicinity of a chemical plant.	1 460	18 260	980	4 660	Wu *et al.*, 1997
Spain	Sampling areas included area near an old pesticide factory, residue store, residential and reference areas.	ND	ND	4.31	80 693	Concha-Graña *et al.*, 2006
China	Haihe and Dagu Drainage River (industrial and urban areas)	1880	141 030			Yang *et al.*, 2005

	Site description	Sediments (ng/kg, dm)		Soil (ng/kg, dm)		Reference
		Min	Max	Min	Max	
Heptachlor						
Current study		**ND**	**2 800**		**430***	
Mexico	North-western Mexico: coastal lagoons and agricultural drains	1 160	49 000			González-Farias *et al.*, 2002
Australia	Port Jackson, Sydney	ND	24 400			Birch & Taylor, 2000
USA	Corn belt agricultural area			ND	56 000	Aigner *et al.*, 1998
Mexico	Natural soil form south-eastern region of Argentina			740	4 420	Miglioranza *et al.*, 2003
Mirex						
Current study		**ND**	**330**		**150***	
India	West Bengal - Hugli estuary	ND	<100			Guzzella *et al.*, 2005
Korea	Han River		ND			Kim *et al.*, 2009
Czech Republic	Along the Czech border with Poland, Germany and Slovakia			ND	<48	Shegunova *et al.*, 2007
Brazil	Northeastern part of São Paulo State			ND	2 780	Rissato *et al.*, 2006
ΣDDT[b]						
Current study		**300**	**22 000**		**3820***	
Previous study (South Africa)	Central South Africa.	270	4 620	480	6 700	Quinn *et al.*, 2009
Canada	Sampling areas included urban, suburban and rural sites.	200	472 000	1 000	18 000	Wong *et al.*, 2009
China	Mainly industrial, but sites include agricultural and suburban areas.			56 000	1 335 000	Gong *et al.*, 2004
China	Yangtze Estuary and Hangzhou Bay	<60	6 040			Yang *et al.*, 2005
USA	San Francisco Bay	<100	9 000			Pereira *et al.*, 1994
Russia	Lake Baikal	14	2 700			Iwata *et al.*, 1995

	Site description	Sediments (ng/kg, dm)		Soil (ng/kg, dm)		Reference
		Min	Max	Min	Max	
ΣPCDD/Fs and dioxin-like PCBs						
Current study	**(Bio-assay results - ng TCDD-EQ/kg)**	**ND**	**103**	**ND**	**14**	
Previous study (South Africa)	Central South Africa.	0.12	32	0.34	20	Nieuwoudt *et al*., 2009
USA	South Mississippi: rural area.	12.7	615	0.08	22.6	Rappe *et al*., 1997
Spain	Montcada, Barcelona: near a municipal solid waste incinerator.			0.15	29.27	Domingo *et al*., 1999
Norway	Grenlandsfjords: industrial area.	25 000	730 000			Ishaq *et al*., 2009
Sweden	Background and industrial sites.	<1 - 200	1 700	<1	11 000	Fiedler *et al*., 1999; Yoon *et al*., 2004
The Netherlands	Background and industrial sites.	1 - 10	4 000	2.2 - 16	98 000	Fiedler *et al*., 1999; Yoon *et al*., 2004
ΣPAHs[c]						
Current study (South Africa)		**134 690**	**8 992 000**	**517 900**	**1 877 000**	
Previous study (South Africa)	Central South Africa.	44 000	2 799 000	201 000	38 846 000	Quinn *et al*., 2009
Canada	Sampling areas included urban, suburban and rural sites.	42 000	3 300 000	58 000	3 200 000	Wong *et al*., 2009
Italy	Marsala, Stagnone coastal lagoon: industrial area.	72 000	18 381 000			Culotta *et al*., 2006
Germany	Mossel and Saar Rivers: Impacted by industries and coal mining.	12 200	31 200			Pies *et al*., 2008
Slovakia	Industrial area in the vicinity of an aluminium plant.			40 000	200 000	Wilcke *et al*., 1996

	Site description	Sediments (ng/kg, dm)		Soil (ng/kg, dm)		Reference
		Min	Max	Min	Max	
ΣPBDEs						
Current study[g]		**1.5**	**179**	**7.5***		
Italy[d]	Northern Italy: Lake Maggiore	60	27 000			Mariani *et al.*, 2008
Netherlands[e]	Background and industrial sites.	4 600	527 600			De Boer *et al.*, 2003
Portugal[e]	Major river basins.	500	21 000			Lacorte *et al.*, 2003
Sweden	Reference soils from agricultural research stations.			29	95	Sellström *et al.*, 1998
United Kingdom and Norway[f]	Latitudinal transect which includes both countries: rural woodlands.			65	12 000	Hassanin *et al.*, 2004
United Kingdom	Rural area.			1 000	12 000	
ΣPCBs						
Current study		**585**	**57 300**	**14 410***		
Previous study (South Africa)	Central South Africa. Sampling areas included industrial, agricultural and residential sites.	460	8 550	1 480	38 320	Quinn *et al.*, 2009
Yugoslavia	Residential, recreational and industrial areas.	ND	320	ND	410	Skrbic *et al.*, 2007
Romania	Sampling areas included forested zones, waste-disposal sites and sediments from the Bahlui River.	24 000	158 000	8 000	1 132 000	Dragan *et al.*, 2006
China	Urban lakes in Wuhan, Central China.	900	46 140			Yang *et al.*, 2009

[a] Sum of alpha-, beta- and gamma-HCH.

[b] Sum of *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT and *p,p'*-DDT.

[c] The 16 US EPA PAHs.

[d] Sum of BDE-28, -47, -100, -99, -154, -153, -183 and -209.

[e] Sum of BDE-47, -99, -153 and -209.

[f] Sum of BDE-47, -99, -100, -153 and -154.

[g] Sum of BDE-99, -153, and -183. BDE-28, -47 and -209 were present at levels below the LOD.

ND = Not detected (below the detection limit).

*Only one **soil** sample was chemically analysed for OCPs, PCBs and PBDE. No min or max values.

4.3 Human health risk assessment

A human health risk assessment was conducted to provide an indication of whether or not the organic chemicals detected in the sediment samples may cause adverse health effects to humans if fish from these areas are consumed. Therefore, the sediment is considered as proxy for human exposure by assuming that the pollutants, by nature of their physical and chemical properties, will accumulate in fish that will be consumed by humans. The methodology used to asses this potential human health risk was that described by the US-EPA (1988, 1996) and the WHO (2002). The health risk assessment consists of a 4-step process including hazard identification, dose-response assessment, exposure assessment, and lastly risk characterisation.

Hazard Quotient

For agents that cause non-cancer toxic effects, a Hazard Quotient (H.Q.) was calculated, comparing the expected exposure to the agent to an exposure that is assumed not to be associated with toxic effects.

For oral or dermal exposures, the Average Daily Dose (ADD) was compared to a Reference Dose (RfD):

$$\text{H.Q.} = \text{Average Daily Dose} / \text{Reference Dose} \qquad \text{Equation 1}$$

Any Hazard Quotient less than 1 is considered to be safe for a lifetime exposure.

Cancer risks

For chemicals that may cause cancer if ingested, risk is calculated as a function of the Oral Slope Factor and Dose and can be calculated as follows:

$$\text{Risk} = 1 - e^{-(\text{Oral Slope Factor} * \text{Lifetime Average Daily Dose})} \qquad \text{Equation 2}$$

4.3.1 Approach

The maximum concentration detected in all sample sites was used as a worst case scenario to determine what risks (if any) were involved as a screening risk assessment. If a chemical was found to be responsible for risks considered by the US-EPA and WHO to be unacceptably high, a more detailed assessment for that chemical was investigated, making use of the spread of the data, averages, and identifying which sampling site was responsible

for the highest concentrations detected. Figs. 4.14 and 4.15 illustrate the maximum concentration of the chemicals detected at 31 sampling sites used in the primary screening in the human health risk assessment.

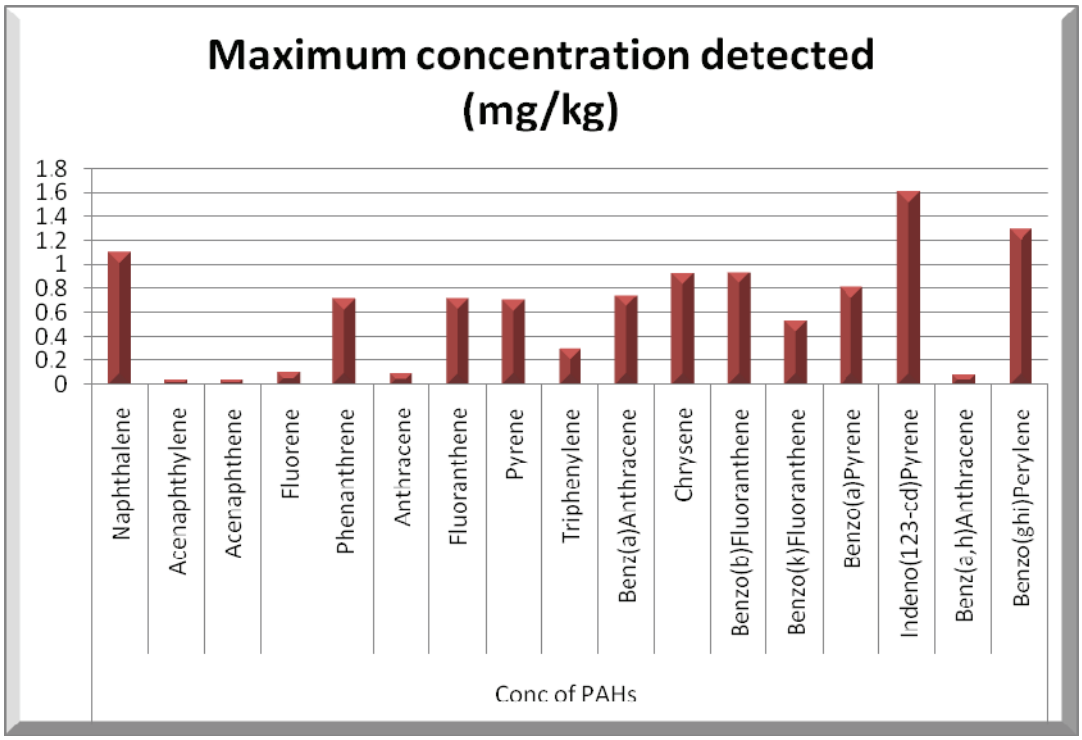


Figure 4.14 Maximum PAH concentrations (mg/kg) detected at the sites tested

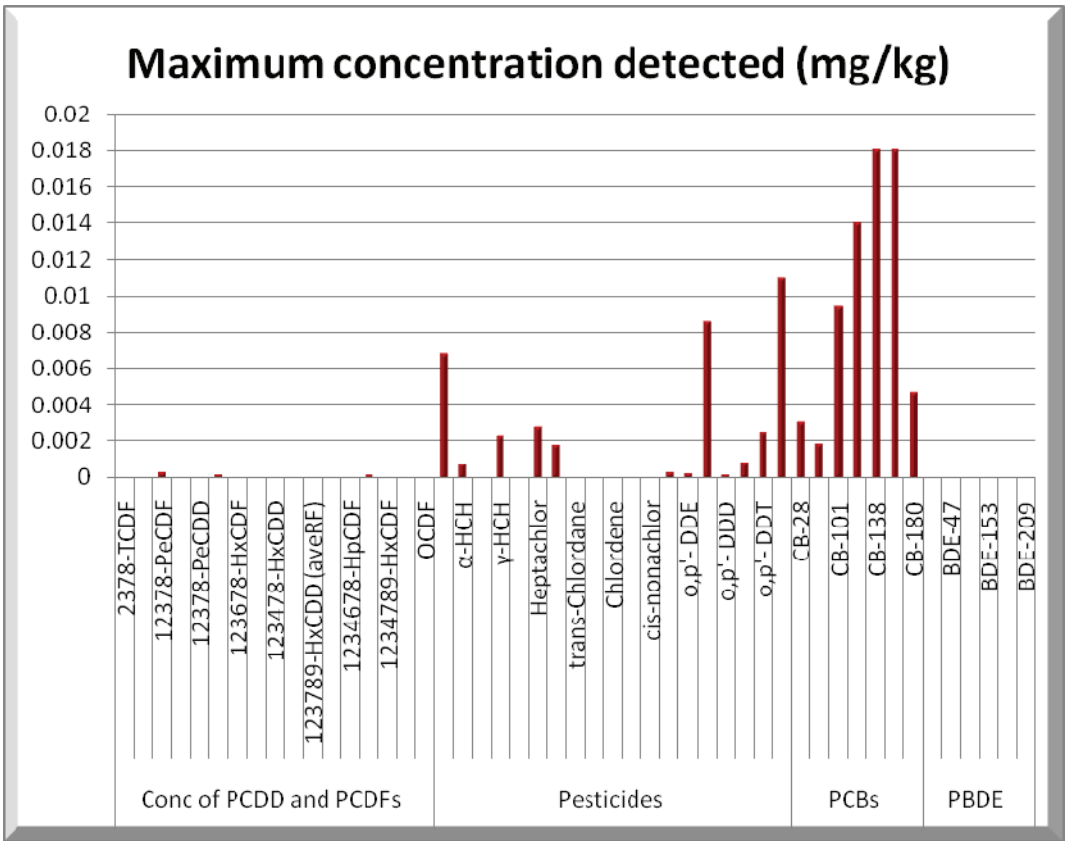


Figure 4.15 Maximum chemical concentrations (mg/kg) detected at the sites tested

4.3.2 Cross-media transfer equations for exposure estimates

The formulae used to generate the exposure concentrations based on sediment concentrations was that described by the US-EPA (1990) for sediment to fish concentrations.

$$C(w) = \frac{C(sd)}{[Koc \times OC \times DN]}. \quad \text{Equation 3}$$

$$BCF = [0.79 \times \log(Kow)]\text{-}0.40 \quad \text{Equation 4}$$

$$C(f) = BCF \times \left(\frac{\%fat}{3}\right) \times C(w) \quad \text{Equation 5}$$

Where:

- C(f) = Concentration in fish
- C(w) = Concentration in water
- C(sd) = Concentration in Sediment
- DN = Sediment Density (Relative to Water Density of 1.0 kg/l) (1.90)
- OC = Organic Carbon Fraction of Sediment (4.00%)
- Koc = Octanol-Carbon Partition Coefficient of the Compound
- Kow = Octanol – Water coefficient of the compound
- BCF = Bioconcentration factor

Table 4.21. Concentrations of chemicals calculated in fish as a result of sediment concentrations.

Chemical	Measured concentration mg/kg sediment	Calculated concentration in fish ug/kg fish
336-36-3 PCBs	0.0700	173.784
50-29-3 DDT	0.0135	15.620
72-54-8 DDD	0.0010	6.476
72-55-9 DDE	0.0090	1.693
86-73-7 Fluorenes	0.0934	195.804
85-01-8 Phenanthrene	0.7018	535.514
120-12-7 Anthracene	0.0890	49.400
129-00-0 Pyrene	0.7000	507.337
50-32-8 Benzo(A)Pyrene	0.8000	622.076
218-01-9 Chrysene	0.9000	519.151
207-08-9 Benzo(K)Fluoranthene	0.5000	154.757
205-99-2 Benzo(B)Fluoranthene	0.9000	1330.000
608-73-1 Hexachlorocyclohexane	0.0023	35.246
309-00-2 Aldrin	0	0
57-74-9 Chlordane	0.00012	1.580
385-85-5 Mirex	0.00033	-
76-44-8 Heptachlor	0.0028	101.25

Table 4.22. Exposure parameters used to generate exposure estimates

Exposure parameter	amount
Events per year	weekly (50 times per year)
kg per event	0.378
Body mass	70 kg
Exposure duration	30 years

The dose estimates in the following sections in this assessment, as well as the risk estimates derived from them, refer only to the specific exposures that have been described in Table 4.22.

The average daily dose was calculated taking into account the concentration of the chemicals in sediment, for a 70 kg adult, assuming an intake of 0.054 kg fish on a daily basis (equivalent to 378 g per week). A range of risks is presented making use of average and 95th percentile concentrations of chemicals detected in the sediment, calculated to represent concentrations expected in fish. The 95th percentile represents the "reasonable maximum" risk.

4.3.3 Results

The results of the exposure calculations are given in the Table 4.23 and are presented as both Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD) in mg/kg/d.

Based on the exposure assumptions (described in the section above), risks of developing cancer and toxic effects were calculated for the various persistent organic chemicals where sufficient data was available. Most of the chemicals were found at concentrations to be at below those where "unacceptable" risks, as defined by both the WHO and US-EPA, are anticipated. However, risks of developing cancer may be as high as 1 in a thousand resulting from exposure to benzo(a) pyrene, 4 in 10 thousand to maximum levels of PCBs, and 1 in 10 thousand at the maximum levels of heptachlor (Table 4.24 and Fig. 4.16). These chemicals were found at high concentrations at sites Croc 1B, CT7 for benzo(a)pyrene , site S/L 9 for PCBs and at site D7 for heptachlor.

Table 4.23. Average daily doses (ADD) and Lifetime average daily doses (LADD), based on maximum detected.

Chemical	ADD mg/kg/d	LADD mg/kg/d
336-36-3 PCBs	0.000129	0.000055
50-29-3 DDT	0.000012	0.000005
72-54-8 DDD	0.000005	0.000002
72-55-9 DDE	0.000001	5.4e-7
86-73-7 Fluorenes	0.000145	0.000062
85-01-8 Phenanthrene	0.000396	0.000170
120-12-7 Anthracene	0.000037	0.000016
129-00-0 Pyrene	0.000375	0.000161
50-32-8 Benzo(A)Pyrene	0.000460	0.000197
218-01-9 Chrysene	0.000384	0.000165
207-08-9 Benzo(K)Fluoranthene	0.000114	0.000049
205-99-2 Benzo(B)Fluoranthene	0.000984	0.000422
608-73-1 Hexachlorocyclohexane	0.000026	0.000011
309-00-2 Aldrin	0.0	0.0
57-74-9 Chlordane	0.000001	5e-7
385-85-5 Mirex	Missing data	Missing data
76-44-8 Heptachlor	0.000075	0.000032

Table 4.24 Cancer and non-cancer risks associated with the exposure assumptions.

Chemical	Toxic risk-hazard quotient	Risk of developing cancer[1]
336-36-3 PCBs	-	4e-4
50-29-3 DDT	0.023	2e-6
72-54-8 DDD	-	5e-7
72-55-9 DDE	-	2e-7
86-73-7 Fluorenes	0.0036	-
85-01-8 Phenanthrene	-	-
120-12-7 Anthracene	0.0000122	-
129-00-0 Pyrene	0.01251	-
50-32-8 Benzo(A)Pyrene	-	1e-3
218-01-9 Chrysene	-	B2[2]
207-08-9 Benzo(K)Fluoranthene	-	B2
205-99-2 Benzo(B)Fluoranthene	-	B2
608-73-1 Hexachlorocyclohexane	0.08691	B2
309-00-2 Aldrin	0	0 - B2 (17mg/kg/d)
57-74-9 Chlordane	0.01948	7e-7
385-85-5 Mirex	-	No slope
76-44-8 Heptachlor	0.15	1e-4
Totals	0.36	1.83 e-3

[1] A cancer risk of greater than 1 in 100 000 is considered to be unacceptable (WHO 2002)
[2] B2 refers to the strength of evidence that a substance causes cancer in humans as a “probable human carcinogen” (US EPA 1991)

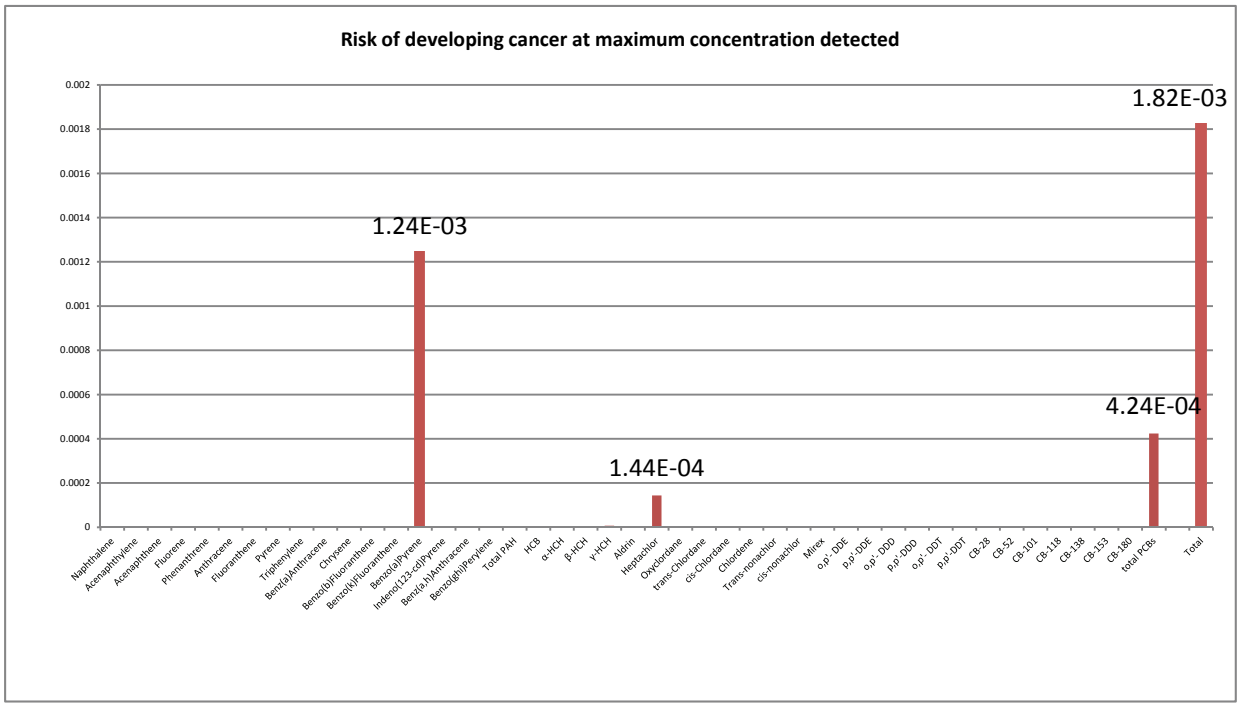


Figure 4.16. Risks of developing cancers at concentrations detected.

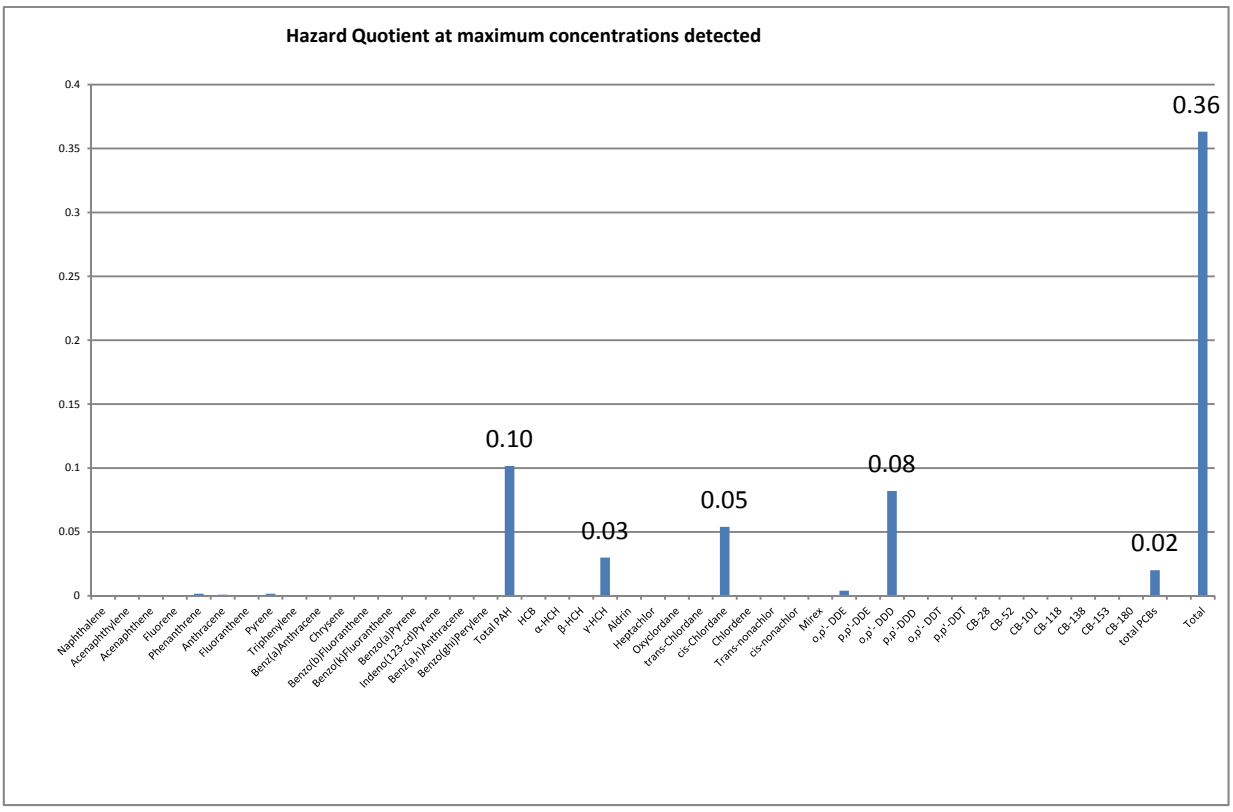


Figure 4.17 Hazard quotients at maximum concentrations detected.

The primary drivers of the human health risk were identified through this exercise. The chemicals responsible for the risks include benzo(a)pyrene and to a lesser extent PCBs and heptachlor (Fig. 4.16 and 4.17).

The highest potential risks were observed at sites CT7 and Croc1B for benzo(a)pyrene, S/L9, D14, and RW1 for PCBs and D14 for heptachlor.

Polycyclic aromatic hydrocarbons (PAHs) were combined into one profile since these chemicals often occur together in the environment and many have similar toxicological effects, environmental fate, etc. Instances in which it is known that the various PAHs differ with regard to toxicological effects or environmental fate will be pointed out. For example, PAHs can be classified as “alternant” (e.g., benzo[a]pyrene, benz]a]anthracene, chrysene, dibenz[a,h]anthracene) or “non-alternant” (e.g., fluoranthene, benzo[k]fluoranthene, benzol[j]fluoranthene, indeno[1,2,3-c,d]pyrene). This distinction is based on the electron density associated with the molecule. Alternant PAHs have an equally distributed electron density, whereas non-alternant PAHs behave almost as if they were two different molecules because of an uneven distribution of electron density from one portion of the molecule to another. The toxicological significance of this difference is that alternant and non-alternant PAHs appear to behave differently, for example, with regard to how they are metabolised to ultimate carcinogens.

There are several hundred PAHs, which usually occur as complex mixtures rather than as individual compounds. Benzo[a]pyrene (BaP) is the most potent. For the general public, the main route of exposure to PAHs is from inhalation of air or ingestion of food. Following chronic exposure in an occupational setting, a decrease in lung function has been reported, as well as chest pain, respiratory irritation, cough, dermatitis and depressed immune system, although in most cases it was not possible to evaluate the contribution of BaP to such effects. In animals, few adverse effects were observed in rats or hamsters exposed to BaP via inhalation. Following ingestion, myelotoxicity and hepatotoxicity was observed.

In mice, BaP has been shown to cross the placenta and cause adverse developmental and reproductive effects. Dietary administration during gestation reduced fertility and foetal abnormalities whereas administration by gavage caused an increase in foetal death and decreased fertility.

Polychlorinated biphenyls (PCBs) are mixtures of over 200 individual chlorinated compounds (known as congeners). There are no known natural sources of PCBs. Health effects that

have been associated with exposure to PCBs include neurobehavioral and immunological changes in children. PCBs are known to cause cancer in animals. Developmental delays were seen at all ages and were greater in children smaller in size. Both the EPA and the International Agency for Research on Cancer (IARC) have concluded that PCBs are probable human carcinogenic. WHO also classify PCBs as probable human carcinogens.

Heptachlor is one of several organochlorine pesticides that are persistent in the environment and accumulates in the food-chain (WHO, 2006). Lifetime exposure to heptachlor resulted in liver tumours in animals. The International Agency for Research on Cancer (IARC) and the US EPA have classified heptachlor as a possible human carcinogen (ATSDR 2007b). Animals ingesting heptachlor have shown liver damage, excitability, and decreases in fertility (ATSDR, 2007b).

In the USA, the Food and Drug Administration (FDA) controls the amount of heptachlor in edible seafood, with a limit of 0.3 mg/kg.

As benzo(a)pyrene was found to be the major contributor of risk of developing cancer in this screening health risk assessment its distribution was examined in more detail. The mean, maximum and minimum concentrations were used to model the uncertainty making use of @Risk. The uncertainty analysis comprised 1000 iterations, making use of Latin hypercube sampling, assuming a triangular distribution. Results can be seen in Figure 4.18.

Table 4.25. Summary statistics of concentrations and cancer risk for benzo(a)pyrene

	Benzo(a)pyrene concentrations in sediment	*Risk of cancer from Benzo (a)pyrene*
Maximum	800 ng/g	1 in 1 000
Mean	100 ng/g	6 in 10 000

The cumulative probability distributions are presented in the figures below (Figures 4.18 & 4.19). Figure 4.18 shows the cumulative probability of developing cancer resulting from exposure to benzo(a)pyrene and illustrates a 90% certainty that the probability of the risk of developing cancer as a result of exposure to fish contaminated with the organic pollutants can be expected to be between 0.181 and 0.859 in 1 000. This can be rounded off to between 2 and 9 in 10 000, respectively. This is much higher than what is considered as an acceptable risk (approximately 6 in 10 000 versus the acceptable risk of 1 in 100 000 of the WHO (2001)).

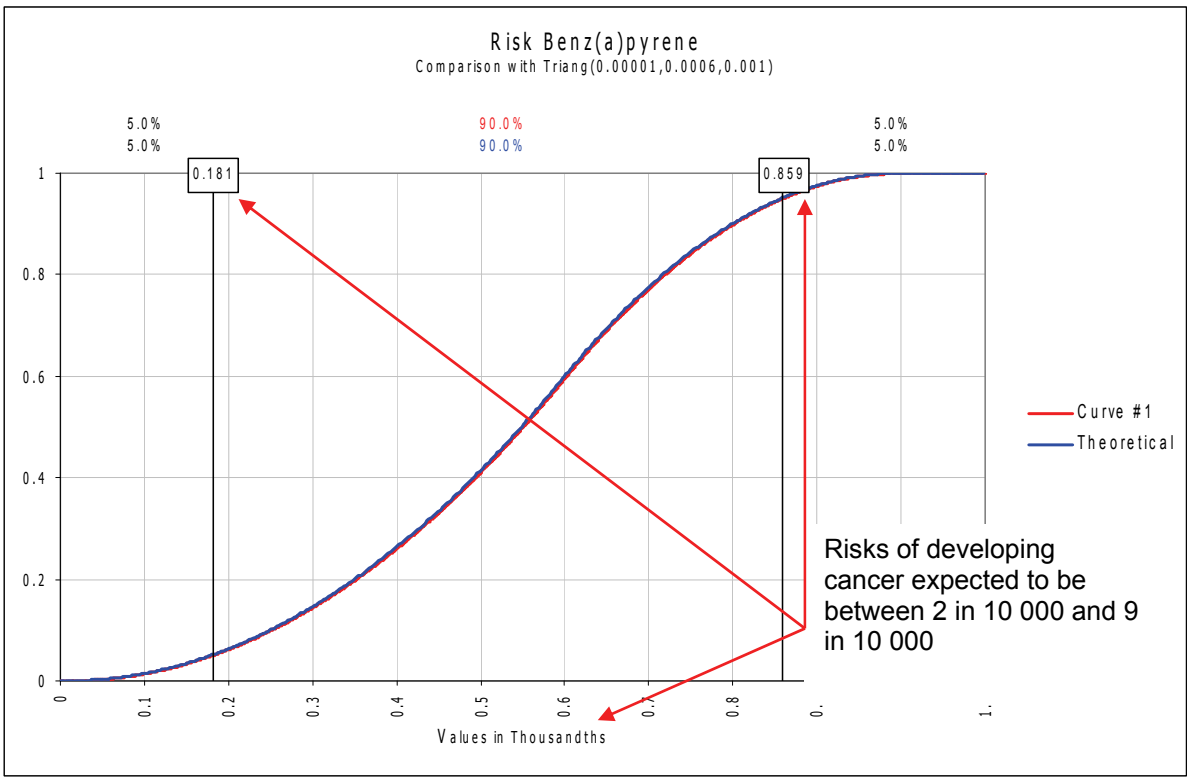


Figure 4.18. Cumulative probability of cancer risk. The probability distribution illustrates the percentage (y-axis where 1 represents 100%) where a probability (x-axis) can be expected. In this figure the risks are predicted to occur 90% of the time between 0.181 thousandths (rounded up to 2 ten thousandths) and 0.859 thousandths (rounded up to 9 ten thousandths). These are the corresponding x-values when y = 0.05 and 0.95.

The risk of toxic effects is low in comparison to the reference dose, shown by the cumulative probability distribution (Figure 4.19) with the hazard quotient being well below 1, with anticipated values ranging between 0.02 and 0.3, at 95% of the time.

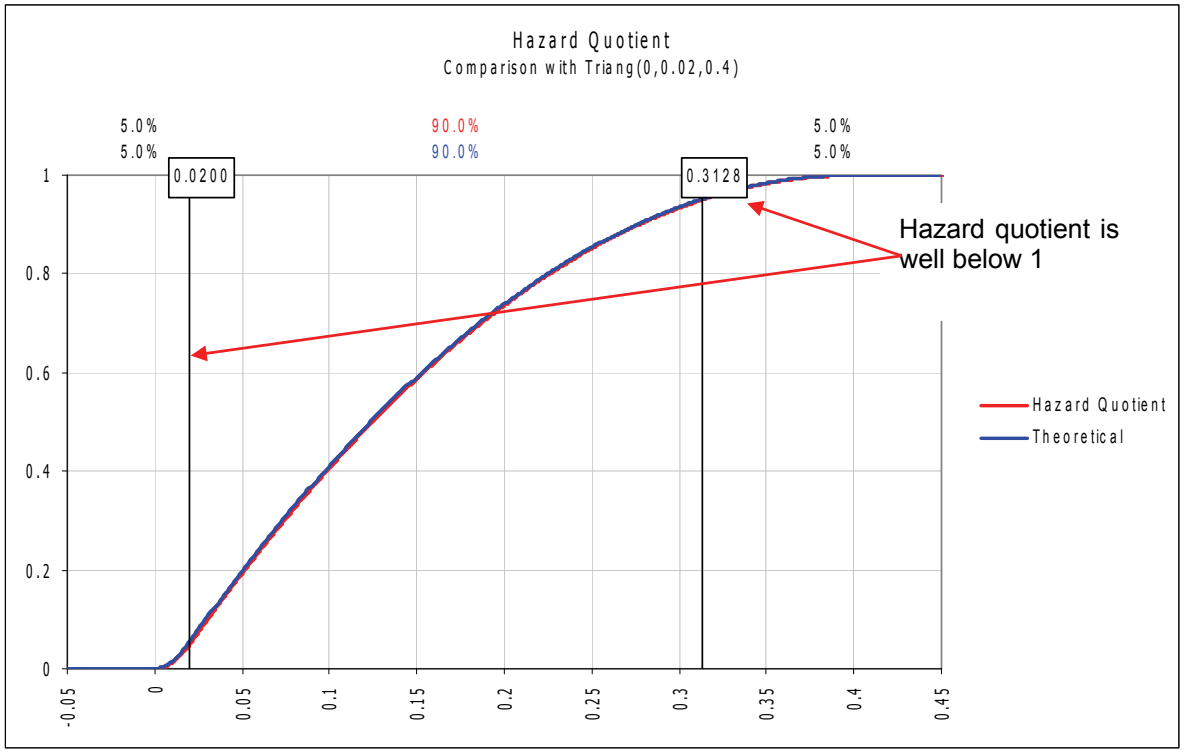


Figure 4.19. Cumulative probability of toxic effects risk.

4.3.4 Discussion

This chapter examined whether possible human health effects might be anticipated based on chemical contaminants detected in sediments in aquatic systems throughout South Africa. In order to determine whether this is possible, a human health risk assessment was conducted modelling the chemical contaminant concentrations expected in fish based on levels detected in sediments. Trans-media calculations (sediment to fish) were conducted based on individual chemical parameters described in the earlier sections.

The screening risk assessment identified the chemicals that could be responsible for adverse health effects if fish were to be eaten over a 30 year period. Although not present at the highest concentrations, the chemicals that were of principal concern were identified as benzo(a)pyrene, total PCBs, and heptachlor. The type of adverse effect that might result was also identified as predominantly carcinogenic, with no toxic effects being anticipated, as the predicted doses were well below those considered safe by the WHO and US EPA.

This screening risk assessment has highlighted that possible health risks can be anticipated resulting from ingestion of fish on a regular basis.

There are many uncertainties in any health risk assessment, and this study presents a screening or rapid human health risk assessment. Seasonal and spatial variations are not considered as the various sampling points were tested on only one occasion. This was used to provide an indication of potential health risks and should be investigated in more detail.

In addition to sample variation, dose calculations also represent uncertainty, based on the assumption of the number of times a year that people eat fish and the amount of fish eaten. This again illustrates that a more detailed study is needed to improve on the certainty of the result of the risk assessment.

5 CONCLUSIONS

All of the compounds tested for were found in South African sediments. However, they were not all present in all sediments, and the levels varied. The levels of DLCs in soils and sediments were generally low, with only 23 of the 96 sites eliciting quantifiable responses when screened with the H4IIE-*luc* bio-assay. Of the 23 sites, 77% was of industrial or semi-industrial origin, 15% was industrial-residential combinations, 6% was high-density low-income residential areas and 2% was residential-agricultural combinations. TOC content, seasonal and meteorological conditions (high temperatures, low precipitation and long summers), photodegradation, sedimentation shifts, effects of dilution, and degradation by micro-organisms were identified as the possible causes for the low levels of DLCs in the South African environment. A loss in cell viability caused by the cytotoxicity of some of the samples could also have contributed to reduced relative light/luminescence units (RLUs).

Chemical analyses results indicated that PAHs were the most abundant of all the groups of compounds investigated, and were present at the highest levels of all the compounds analysed. OCPs and PCBs were present in intermediate concentrations, while PBDEs were the least abundant and present in the lowest concentrations. Aldrin and chlordene were not detected at any of the sites, whereas nonachlor, chlordane and oxychlordane were present at only a few of the sites in minor concentrations. HCB, HCH and DDT were the predominant OCPs, while heptachlor and mirex were present in lower concentrations. This might be ascribed to the fact that HCB is still produced for industrial applications, and HCH and DDT are presently applied as pesticides in some parts of the country, while the use of heptachlor and mirex has been banned.

In general, 4-ringed PAHs were the most abundant, followed by 5-ringed congeners and finally either by 3- or 6-ringed congeners. Two-ringed PAHs were the least abundant. HMM-PAHs are less susceptible to biodegradation and loss from soil or sediment, which may explain the high prevalence of these congeners. CB-153, -138, -118 and -101 were the major PCB congeners in South African sediments. The lighter PCBs were less abundant, because they are rapidly biodegraded in the environment. PBDEs were generally present at low concentrations, with BDE-153 being the predominant PBDE.

Principle Component Analysis (PCA) distinguished between two main groups of chemicals – OCPs and industrially associated compounds. Separate PCAs for each group of compounds illustrated strong associations between:

- Lim 4 and *o,p'*-DDT indicating current use of the pesticide,
- D14 and the metabolites, DDE and DDD, indicating historic use of DDT,
- Some S/L sites and heptachlor,
- RB1, 3 and 4 and HCH and HCB,
- BO4, Lim4 and PBDEs,
- RB 1, 3 and 4, BF7, D14 and the lighter PCBs,
- CT8, S/L8 and the heavier PCBs,
- S/L 1, 4 and 8 and the lighter PAHs and
- Croc1b, RB2, Lim4 and the heavier PAHs.

Additionally, An(An+Ph) and Fl(Fl+Py) ratios indicated that the PAHs at the majority of the sites were of pyrogenic origin, or neither solely pyrogenic or petrogenic ("mixed origin").

The normalised concentrations (1% TOC) of the compounds of interest at a few of the sites exceeded the Canadian sediment quality guidelines. The concentrations of OCPs and PCBs were generally below the proposed sediment quality guidelines, while the concentrations of PAHs and DLCs at many of the sites exceeded the guidelines.

The concentration of pollutants measured in South African soils and sediments were intermediate when compared to the levels measured in some European, Asian and Scandinavian countries, with the exception of a few sites where exceptionally high levels of certain compounds were measured.

It should be noted however, that this study is only a snapshot of the POPs concentrations present in sediment as sampled at the time of sampling. Temporal and spatial dynamics have not been measured. However, these dynamics are expected, and may very well have changed with the floods in 2010/11. Follow-up analysis of compounds of interest at sites identified in this study will provide a much better picture of how pollutant concentrations enter the aquatic environment, whether there are still active sources, and if these pollutants are carried downstream or broken down close to source.

A screening risk assessment determined whether possible human health effects might be anticipated based on chemical contaminants detected in sediments. In order to determine whether this is possible, a human health risk assessment was conducted modelling the

chemical contaminant concentrations expected in fish based on levels detected in sediments. Trans-media calculations (sediment to fish) were conducted based on individual chemical parameters described in the earlier sections.

The screening risk assessment identified the chemicals that could be responsible for adverse health effects if fish were to be eaten over a 30 year period. Although not present at the highest concentrations, the chemicals that were of principal concern were identified as benzo(a)pyrene, total PCBs, and heptachlor. The type of adverse effect that might result was also identified as predominantly carcinogenic, with no toxic effects being anticipated, as the predicted doses were well below those considered safe by the WHO and US EPA. Therefore, this screening risk assessment highlighted that possible health risks can be anticipated resulting from ingestion of fish on a regular basis.

There are many uncertainties in any health risk assessment, and this study presents a screening or rapid human health risk assessment. Seasonal and spatial variations are not considered as the various sampling points were tested on only one occasion. This was used to provide an indication of potential health risks and should be investigated in more detail.

In addition to sample variation, dose calculations also represent uncertainty, based on the assumption of the number of times a year that people eat fish and the amount of fish eaten. This again illustrates that a more detailed study is needed to improve on the certainty of the result of the risk assessment, especially for the identified sites.

The aims and objectives of Phase II were:

- To assess the scale and significance of the occurrence of POPs and other OPs in the water environment in South Africa,
- To better identify and quantify the fate and effect of selected POPs and other OPs in the water environment, and
- To guide and inform the development of appropriate policy and regulatory measures that will support implementation of the requirements of the SCPOPs, and substantially contribute to the protection of water resources and water-linked ecosystem with regard to POPs, by:
    - Identifying and quantifying selected POPs and other OPs in the water environment,
    - Assessing the levels and distribution of these compounds,
    - Determining the possible sources and releases to the environment, and

- Assessing the effects on human health to identify communities possibly at risk.

The project has achieved all the aims and objectives. In addition it has also contributed towards the establishment of analytical capacity for these compounds in South Africa by NMISA, and outcome not originally anticipated.

Recommendations

Specific research recommendations

Further investigation is recommended into the sources and levels of certain POPs and organic pollutants at the following sites:

- D14 (Umgeni mouth – possibly receiving effluent from various industries up-stream), where the levels of heptachlor, DDE, DDT and DLCs were all above the ISQGs;
- RB1 and RB2 (Richards Bay industrial sites): At RB1 the ISQGs of several PAH-congeners and γ-HCH were exceeded, while the ISQG and PEL of DLCs were exceeded. At RB2, PAHs and PCBs were present at such concentrations that the ISQGs of these compounds were exceeded, and for γ-HCH and DLCs the PELs were exceeded as well;
- BF6, BF7 and BF8 (Bloemfontein industrial and low-income residential sites): The concentrations of some of the PAH-congeners at BF6 and BF7 where higher than the proposed ISQG, and the DLC concentrations at all three of the sites exceeded the ISQGs and the PELs;
- Lim4 (Low-income residential site in the Limpopo Province – a malaria-endemic area): The ISQGs of DDE, DDT and DLCs were exceeded at Lim4. Lim4 was identified as the only site where South Africa's contribution of POPs into a neighbouring country was substantial. This site should therefore be treated as a high priority.
- Croc1b (near the premises of a paper mill in Mpumalanga): This site had the largest amount of PAH-congeners exceeding the ISQGs, PELs and LELs. The concentration of DLCs at this site was above the ISQG. The sites situated down-stream of Croc1b and closer to the borders of neighbouring countries had less significant concentrations of organic pollutants.

Further investigations are also recommended for the following sites where the highest potential health risks were calculated:

- Soweto/Lenasia wetland sites, S/L1, 6 to 9, and 12;
- Cape Town industrial sites, CT2 and 7;
- Umgeni River mouth in Durban, D14;
- Richards Bay industrial sites, RB1, 2 and 3;
- Bloemfontein sites, BF6, 7 and 8,
- Botshabelo, BO4;
- The international rivers, Olif4 and Croc1b.

General recommendations

- It is recommended that other cell-based bio-assays are performed to determine if sediments are capable of eliciting estrogenic and androgenic responses. MVLN- and MDA bio-assays, where stably transfected human breast cancer cell lines are employed, are suggested.

- It is suggested that sediment samples should in the future be analysed by HRGC/HRMS for the presence of DLCs as well, to compare biologically and chemically derived results.

- The sites identified should be subjected a much closer scrutiny as to actual POPs sources and distribution. This will provide indications as to how management interventions may abate pollution. This study was only a snapshot of pollutant concentrations as sampled at the time of sediment sampling. Spatial and temporal changes are very likely and nothing is known about this. Time-based sampling of compounds of interest, as identified here, should be conducted in future studies.

- The workshop held on 26 January 2011 with stakeholders on the findings of this project suggested that there should be regular monitoring of problem sites as well as continued and extensive surveys to detect other relevant areas in need of closer monitoring. This monitoring scheme results should feed into the National Toxicity Monitoring Programme (NTMP) of the Department of Water Affairs (DWA). A fairly extensive list of the nature of the matrices to be monitored and/or surveyed was suggested by the workshop delegates. These matrices were selected on the grounds of their usefulness to identify point source and non-point source pollution as well as

to determine the levels of these compounds in wildlife and humans and to determine bio-accumulation.

- Attention should also be given to heavy metal levels at these sites as these may pose as significant co-stressors.

- The sites identified as being of concern should also be investigated as to actual levels of pollutants in aquatic organisms such as fish and birds, as well as to actual human fish consumption patterns, and thereby determine human and environmental health risk.

- Stress and effects due to POPs in fish and other aquatic biota at the identified sites should also be investigated.

- The results of this study should be published and, where possible, presented at appropriate fora. Note should also be taken of the appropriate comments on communication in Appendix 2.

- It is recommended that the results from this study be used to guide and inform the development of appropriate policy, development, scientific, and regulatory measures that will support the implementation of the requirements of the SCPOPs and thereby contribute to the protection of water resources and water-linked ecosystems with regard to POPs and similar organic chemicals. In this regard:
    - This report should be considered by the process that is completing the NIP for South Africa.
    - This report should also be considered by Provincial and National authorities as well as all other stakeholders.
    - Various other stakeholders will also find value from this report as well as the NIP.
    - As and when candidate POPs are considered by the SCPOPs, samples from all sites should be analysed so as to inform the SA position during negotiations prior to decisions. The present set of sites seems adequate (although more could be added) for this purpose. Hexabromocyclododecane (HBCD), endosulfane and short-chained chlorinated paraffins are currently on the agenda (in 2011). For some compounds, these are the only data available in Africa.

- Some compounds have almost no representation in data. PFOA (perfluoro-octanoic acid), also a 'new' POP, has almost no data for the whole of Africa. South Africa can play a major role as scientific leader for the continent in this regard.
- The presence of these compounds, albeit at relatively low general levels, shows that these compounds are present in the environment. However, almost no data, except for some organochlorine pesticides, are available about their levels in humans. Breast milk would make an ideal first matrix to assess the presence, levels, and possible health risks these compounds may pose. Again, South Africa could show scientific leadership.
- The analytical capacity in South Africa and Africa for these compounds is low. Except for the NMISA that developed the techniques to generate the data, few other laboratories seem to have the capacity, willingness, and scope to implement the high-level analytical procedures required. Although there are laboratories that can be capacitated to do so (although trained personnel and equipment seems to be available), the financial incentives are not in place for them to add these compounds to their accredited repertoires. The lack of a centralised, well equipped, well funded, and well managed national laboratory for POPs analysis in SA was discussed at length at the workshop (Appendix 2). Special mention was made of the need for well trained staff, and retaining of that staff in the laboratory and in the country (Appendix 2).
- The bio-analytical technique employed in this study was successful in determining those samples that should go for dedicated chemical analysis. The costs would otherwise be prohibitive. The capacity and trained personnel developed via this study should be nurtured and encouraged to fulfil a similar role in future projects. There is also good scope to add additional bio-analytical techniques. The workshop participants also suggested inclusion of testing representatives of various trophic levels (Appendix 2). It is also conceivable that the bio-analytical technique can also be considered as a technique within a regulatory context.
- As there is no centralised entity in South Africa dedicated towards following scientific and international developments regarding chemical pollution issues, the establishment of such a function should be considered. Such a function could *inter alia* pro-actively inform policy, determine knowledge gaps and, identify scientific needs.
- South Africa has no sediment quality guidelines. Sediment quality guidelines of the compounds considered here as well as others such as heavy metals

may be developed based on our data as well as others that are or may become available. However, the international SQG that we have used here may be used in the interim for comparative purposes.

- Although source reduction would be of prime importance in mitigation, there may be sites that would require remediation. However, this would entail an in-depth assessment of each site, as well as following international guidelines on remediation. These guidelines are currently in development by the Stockholm Convention and associated agencies.

Additional workshop recommendations

The recommendations below were suggested by the workshop:

- One of the needs identified is for a national scientific society/forum on POPs that might be affiliated with existing associations/societies. Another suggestion was the creation of a national committee consisting of representatives from DWA, WRC, DEA, DoH, DoA, RQS, Defence Force, and scientists that could inform Parliament on POPs, recent international research findings, and new developments regarding POPs in SA (Appendix 2).

  Another big gap that needs to be filled by such a committee would be to address communication of POPs related issues – from informing communities at risk of POPs exposure, to educating through various printed and electronic media.

  Delegates from the workshop were unanimous that the effectiveness of such a national committee would very much depend on a champion that could drive such an initiative.

- There is a need for a national database on all POPs related research in SA. The location of such a database needs to be determined.

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7 APPENDIX 1:

The results presented below were part of a project funded by the SA/Norway bilateral research agreement done in parallel with this study, and is for information purposes only.

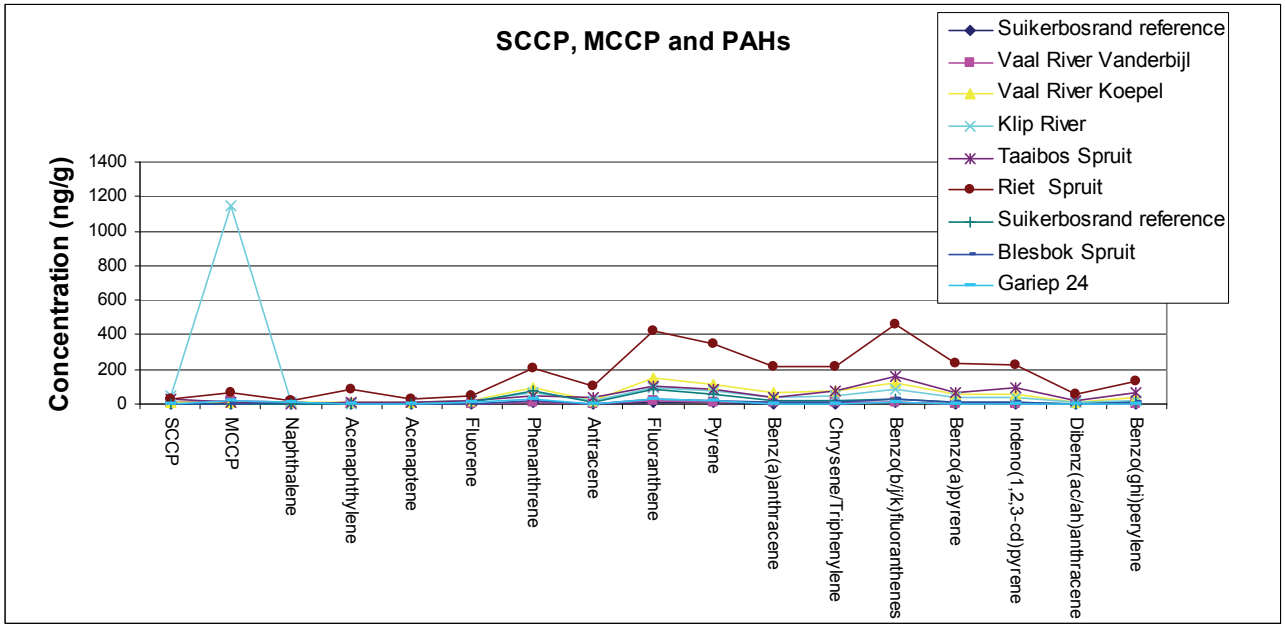

Figure 1. Short and medium chained chlorinated paraffins, and PAH's profile

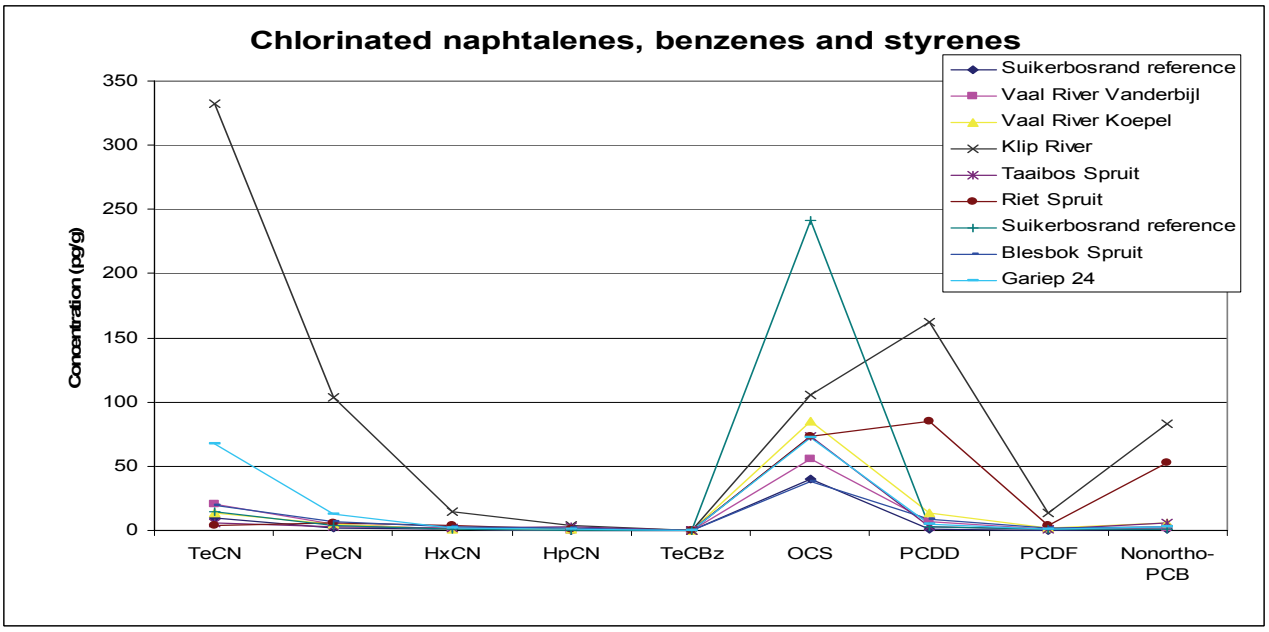

Figure 2. Chlorinated naphthalene, benzene and styrene profile

8 APPENDIX 2

Workshop report

(Please note independent page numbering)

WRC Project

K5/1561

PERSISTENT ORGANIC POLLUTANTS (POPS)

IN THE WATER ENVIRONMENT

Deliverable 12

Workshop Proceedings

H Bouwman & R Pieters

School for Environmental Sciences and Development

North-West University - Potchefstroom Campus

NORTH-WEST UNIVERSITY ®
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

REPORT

February 2011

WATER RESEARCH COMMISSION PROJECT K5/1561

Persistent Organic Pollutants in the Environment: Workshop Proceedings

Submitted to:
Prof Henk Bouwman and Dr Rialet Pieters
North-West University

Report Number. 13016-10354-1

Distribution:

1 x Copy - North West University
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Golder Associates

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APPENDICES

1.0 WELCOME AND INTRODUCTIONS

The workshop was held at Golder Associates Africa Offices in Hatfield, Pretoria. Dr Ralph Heath welcomed all and introductions were made. A copy of the attendance register is attached as Appendix A.

Apologies were received from Dr Martin Ginster (Sasol).

1.1 Objectives of the feedback workshop

The objectives of the workshop were discussed, which was as follows:

To guide and inform the development of appropriate policy and regulatory measures that will support implementation of the requirements of the Stockholm Convention, and substantially contribute to the protection of water resources and water–linked ecosystems with regard to POPs.

The outcomes from the workshop will be used in the final conclusions and way forward for the Water Research Commission project on persistent organic pollutants in the environment.

2.0 FEEDBACK SESSIONS

2.1 Feedback from Government on the Stockholm Convention and South Africa's National Implementation Plan

Gordon Khauoe from Department of Environmental Affairs (DEA) gave feedback on the progress of South Africa's National Implementation Plan (NIP) response to the Stockholm Convention. The following is a summary of the current status of the NIP:

- NIP is nearing completion and it is hoped that it will be finalised by end of February 2011;
- Working on action items; and
- 24 February 2011: Multi-stakeholder Committee on Chemicals Management (MCCM) meeting – first draft will be sent out prior to this;

Action: ***Gordon Khauoe to make sure that all workshop attendees are invited.***

Action: ***Dr R Heath to send Gordon Khauoe the list of the workshop attendees and contact details.***

- March 2011 – a follow up workshop will be held once feedback is received;
- April 2011 – circulation to the public at large;
- Challenges:
  - Status quo of POP's and usage in South Africa and NIP to fulfill Stockholm Convention commitments were drafted by consultant; DEA were not happy with results and it was reworked internally; only limited information used from the status quo document.
  - Loss of key staff members; new staff had to be trained. Often data was not given as requested; lengthy delays; no limits to assess exposure therefore there was a need to develop standards.
- Thanks to Prof Bouwman, Dr Jooste and Dr Pieters for providing data and support.

Questions

- Inventory: is it complete?
  - Polychlorinated biphenyls (PCBs) and dioxins completed;
- Only original Dirty Dozen 12 POPs?
  - No, included 9 new POPs, including polybrominated diphenyl ethers (PBDEs).
- Standards? Process going forward?
  - Still working on this. Hope to have a better understanding of what needs to be done by end Feb;
- Inventory (12 plus 9) – how was it compiled?
  - Requested companies to send their own inventories; market information was supplied however no verification was done;
- Percentage response?
  - Focused on companies from where you would expect the POPs to come from; got fairly good response.

2.2 Current status of the WRC POP's project: results, interpretation, health risk assessment (HRA), conclusions and recommendations

The following presentations (Table 1) were given and are included as Appendix C to the workshop notes.

Table 1: List of presentations and comments

Presentation	Presenter	Questions/comments
Persistent Organic Pollutants in the water environment	Claudine Roos (NWU)	High DDT in Canada? Please clarify in report
Technical difficulties in the extraction and analysis of dioxin-like chemicals	Laura Quinn National Metrology Institute of South Africa (NMISA)	■ Poor clean-up leads to masking of dioxins; ■ Very expensive to buy consumables. A great deal of money has been pumped into the NMISA lab buying equipment. There are problems related to: ■ finding suitably qualified people; ■ training; and ■ maintaining critical mass of skilled people and equipment. It is important to make sure that no duplication of such efforts occurs and that there is at least one well run central facility that can do the analysis for air, water, sediment and food.
Human health risk assessment of the organic chemicals detected in sediments	Bettina Genthe Council for Scientific and Industrial Research (CSIR)	■ Only based on sediment concentrations and assumed/extrapolated fish consumption; ■ An assumed model; ■ Based on the maximum detected levels at the various sites; ■ Not as bad as expected but concern that the model used sediment and not fish tissue so that there may be a huge underestimation based on this methodology; ■ Cumulative/synergistic effects – this model does not take

Presentation	Presenter	Questions/comments
		these into account.
Conclusions and recommendations	Henk Bouwman (NWU)	■ Are the results of these studies adequately communicated to Department of Agriculture that uses the water for irrigation? ■ Final report due at the WRC on 15 February 2011; ■ Steering Committee will review; ■ Results of this workshop will be fed into the report, changes made as necessary; ■ Dissemination of information: agreement that, as this project was funded with public money, the results should be distributed in the correct format.

2.3 Other research related activities

The following other POPs research related activities (Table 2) are being undertaken by various institutions.

Table 2: Other research activities

Institution	Research related activities
NMISA	Funded by Department of Trade and Industry (DTI) through the international trade agreement (not through any other national departments); need to get government sectors together to fund training and other laboratory requirements to build the capacity in the country. Need collaboration/coordination between relevant government departments and institutions, especially with Department of Science and Technology (DST) which must get more involved and be the driver; currently this is not a priority for them.
Department of Water Affairs (DWA)	Cannot do research themselves but have the following areas of interest: ■ Assessment of occurrence (national picture); ■ Assessment of risk; ■ Need to contextualize the human health aspects for Africa; ■ Method development for POPs; and ■ Measurement of POPs in water.
WRC	■ Agricultural point of view: non-point source pollution from agriculture (inorganics: nitrates, phosphates and potassium salts and organics (pesticides); ■ Toolbox of bio-assays for EDC activity; ■ Veterinary growth stimulants ending up in water; especially impacting on reproductive health; ■ DDT – University of Pretoria's Centre for malaria control has been established to look at various levels – efforts between different faculties such as Engineering and Agriculture; ■ Water related: packaging materials (bottled water and food packaging); ■ Agricultural chemicals (ecological and human health effects) – CSIR (WRC project); and ■ Nano-particle project

NWU	■ DDT in Limpopo Province (bird eggs, frogs, and snails);
	■ DDT (and others) in breast milk (Limpopo and KZN);
	■ Exposure pathways (malaria and DDT) together with UP;
	■ All POPs and all heavy metals in the Orange/Senqu catchment:
	  ▪ Sediment;
	  ▪ Fish; and

Institution	Research related activities
	▪ Bird eggs. ■ POPs in bird eggs (eg. penguins, gannets, terns) ■ Investigate passive sampling (in water) opportunities; ■ Passive sampling in air; ■ Crocodile mortalities in the Kruger National Park; and ■ Endocrine disruption (cell lab, snails, eggshell thinning, etc).
CSIR	■ Olifants River study including looking at international vegetable export (includes negative effects associated with irrigation of vegetables); ■ Waterberg area – coal mining: baseline and potential risks if the area is used; and ■ Participating in several Endocrine Disrupting Compounds projects.

2.4 Potential funding sources

The potential funding sources identified were as follows:

- WRC;
- Global Environmental Foundation (GEF);
- Medical Research Council (MRC);
- National Research Foundation (NRF);
- Cancer Association of South Africa (CANSA);
- Internationally funded projects, such as European Union (EU) funding;
- University's (NWU gave funds for infrastructure); and
- Parliamentary funds (CSIR).

It was noted with interest that the National Departments of Environmental Affairs (DEA), Water Affairs (DWA), Trade and Industry (DTI), Health (DoH) and Agriculture (DoA) were not identified as sources of research funding for POP's in South Africa.

3.0 WORKING GROUP SESSIONS

The following discussion items as on the agenda attached as appendix B were work-shopped in three groups and the findings presented to the workshop in a plenary session (Table 3).

Table 3: Working group sessions report back

Discussion theme	Questions	Report back
Identified sites	a) Implications of data and HRA i) Extent of pollution (Do we have enough detail? What about other pollutants?) ii) Health risk (Who is at risk? Do we have enough detail?) iii) Biotic risk (What is at risk? Do we have enough detail?) b) Should and can interventions be done immediately? (What would be the nature of the interventions?) c) How to identify sources (What should be done to identify specific sources?) d) Can interventions be based on the current information? e) Identified research needs from a-d.	■ Representative sites, not only hotspots; ■ Industry-type sources: Inventory; ■ Dioxins: Toolkit (United Nations Environment Programme, Excel based); ■ Information: historic vs. current agricultural use; ■ Human health levels; ▪ Environmental exposure routes (assumptions of people in the same 'classes' living in similar ways and means, such as: – High density urban; – Low density urban; – Rural etc.; and – Economic related. ▪ Human health exposure routes; ▪ Water use patterns; ■ Ecological regions (41 ECO-regions); ■ Routes of release need to be identified; ■ Needs to fit within the National Toxicity Monitoring Programme (NTMP) of DWA; i.e. it must inform monitoring;

- Biotic and abiotic matrices;
- Geomorphology is important for site selection; sediment dynamics;
- Consider land use that would also include identification of non-point sources;
- Consider bioaccumulation;
- Reasons to support type of sample needed;
- First order screening of the country; battery of tests to be able to identify (eg bio-assay, trophic system, etc);
- Are assumptions of where these expected hot spots are valid? (eg. assume high concentrations in high density areas; sewage treatment works; industrial areas, etc)
- Credible labs;
- Low cost technologies;
- Communication:
  - to all levels (public, government officials, industry etc) WRC suggests a simple policy brief for the Director General level; PRESENT PROBLEMS AND SOLUTIONS;
  - identify specific communities with high risk exposure;
  - risk communication - public awareness;
  - health promotion;
  - consider developing an exposure handbook based on a study including:
    - dietary data;

			– epidemiological info (blood/serum); and – start with a 'bio-marker' ■ Expected vs actual (human and environment); ■ Modelling of what happens between sites? ■ Correlation between metals and POPs; ■ Restrict use of pollutants; ■ Polluted soils; ■ Sewage works: ▪ sludge accumulation; ▪ which processes remove POPs? ■ Landfill sites and associated leachates; ■ Programme to address: ▪ technical problems; ▪ interventions for non-point sources; ▪ economic implications; ▪ cost benefits.
National Implementation Plan (NIP)	f) g)	How can the data inform the NIP? Uptake of data in current or future	■ How to inform the NIP before the final WRC report has been presented to the Steering Committee (SC)? Suggested that the draft WRC report to be sent to the DEA so that they can

	updated NIP h) Implications for SA as Party to the Stockholm Convention	anticipate what is coming. The WRC SC will meet and approve the draft report on 15th Feb. ■ Will take the lead from the NIP; ■ Will allow for action on certain priorities; ■ Annual audit report on effectiveness and efficiencies and actions taken; will support NIP; improvements as implementation occurs; ■ Water quality planning: need to address sediments; currently still no real data; ■ Communication: who, where, what, when – easy but honest digestible message; ■ Guidelines at very levels; various needs; ■ Priority is to get the NIP in and informed as soon as possible.; and ■ Must send list of workshop attendees to DEA.
Future requirements and arrangements	i) What about new/candidate POPs j) Regular surveys (Are they needed and how often? Who would be the driver/initiator?) k) New developments in POPs and chemical pollution in general (Who keeps tabs on it?) l) POPs research communication strategies. (Who tells what to whom and when?) m) Dissemination of data:	To be able to have a mechanism in place with which most of the points for discussion listed from a to g below can be facilitated, the group suggested the creation of a committee for POPs in SA, spearheaded by WRC and DEA (focal point for POPs in the SA government and responsible for the NIP). Members of such a committee should come from DWA: Resource Quality Service, DoA, DoH, armed forces, DTI, DST, and other applicable stake holders. One of the objectives would be to lobby for POPs associated needs at the Minister in The Presidency: National Planning Commission. l) **What about new/candidate POPs?** ■ Should also be analysed for, together with the old ones; ■ Appropriate standards should be obtained even though they are expensive; and ■ Current approach in SA to analyse for POPs, implemented by NMISA, to use a two dimensional gas chromatography coupled to a time-of-flight mass spectrometer (GCxGC TOFMS) allows for identifying and quantifying a broad range of compounds, even though it is slightly less sensitive than the gold standard of high resolution gas chromatography

- Who needs this information?
- Publication (WRC report, scientific and general articles, conference presentation, press release).

n) Research priorities (What should they be?)

o) Analytical infrastructure / arrangements / problems and issues.

high resolution mass spectrometry (HRGC/HRMS).

j) Regular surveys (Are they needed and how often? Who would be the driver/initiator?)

- A differentiated approach should be followed: areas already identified as priority sites ("hot spots") should be monitored more regularly than others;
- Surveys to scour for yet unidentified hot spots should continue as well;
- The driver/initiator should be RQS (to start with) assisted by of DST, CSIR, DoH, armed forces, DTI, DST, and other stakeholders;
- Reporting the findings of the monitoring and surveys should be done by an independent party, such as a university. The Persistent Organic Pollutant and Toxicant (POPT) research group under the leadership of Prof Henk Bouwman, was suggested.

k) New developments in POPs and chemical pollution in general (Who keeps tabs on it?)

Researchers in the field usually know which are the new, emerging pollutants, but, and this is where the group identified a big gap, communicating this knowledge to the decision makers on local, provincial and national levels is non-existent. Suggestions for correcting this are discussed in m) below.

l) POPS research communication strategies (Who tells what to whom and when?)

An association or society of professionals working in POPs was suggested. They could hold annual meetings/conferences to inform one-another of the status of POPs research in SA and also the decision makers. This association could link into existing societies, such as the Global Water Coalition (GWC) , Water Institue of Southern Africa (WISA) or Southern Africa Society of Aquatic Scientists (SASAqS).

m) Dissemination of data

Who needs this information?

Decision makers and communities directly involved by the results from the research BUT the information should be made available in a palatable, non-sensational manner, still conveying the importance of the findings.
For decision makers the bottom line should be highlighted and how the results will influence the person in the street.

		The need to have a maintained and open-access database was also expressed. Results from all the POPs related research should be available at a single (or two) domain(s). ***Publication (WRC report, scientific and general articles, conference presentation, press release)*** All of the above mentioned ways of publication should be used. Talking to communities and educating them might create awareness and an involvement that might influence decision makers. Good relations with scientific reporters should be cultivated in order to keep them updated with latest research. Programmes on radio and television on the environment could be approached as well. **n) Research priorities (What should they be?)** Continue with monitoring of the sediment, water, soil and air so that background levels and trends over periods of time can be determined, BUT find out what the impacts of these pollutants on SA's biota are. Using bio-assays is a practical approach to learn how these compounds might influence biota in the natural environment. **o) Analytical Infrastructure/arrangements/problems/issues** The lack of infrastructure or rather the random distribution of infrastructure is one of the many problems already discussed earlier. However, this group needed to emphasise the dire need of well trained technical staff and the importance of retaining them in the country (and at the lab of training).

4.0 GENERAL DISCUSSION AND RECOMMENDATIONS

There were some concerns that this WRC POP's project was initially hampered by a lack of focus (due to techniques not being readily available, costs of tests etc) but the final outcomes were useful and the recommendations should be implemented.

The general discussions resulted in the following recommendations:

- **The lack of a coherent and effective analytical infrastructure** is the major stumbling block to South Africa complying to its Stockholm Convention NIP requirements.
- Coupled to this is the need to **have well trained technical staff** and the importance of retaining (appropriate remuneration, keeping them in the country, and at the laboratory of training).
- Prof Henk Bouwman suggested the formation of a **Scientific Communications/Technical forum on POP's** to:
  - Inform findings and outcomes on current POP's projects;
  - Make/recommend research needs; and
  - Assist National Government with the role out of the NIP.
  - Access further funding for laboratory equipment, institutional arrangements, set research priorities, link to the NIP, international collaboration etc.
- **The researchers in the WRC EDC and POP's programmes** would make ideal candidates to be involved in this Scientific Communications/Technical forum.
  - The Director of the DWA RQS should be approached to discuss the establishment of such as committee.
  - The National Institute for Occupational Health should also be approached for collaboration and cooperation.
- Without a **Champion** this Scientific Communications/Technical forum will not be functional and it was recommend that a person is identified to champion this initiative.
- **Research priorities need to be determined to answer some of the following questions:**
  - Should we continue to monitor sediment, water, soil, air or organisms, both aquatic and terrestrial, that bio-accumulate?
  - Should we be using bio-assays as a practical screening approach?
  - Should we continue to monitor the background levels and trends in our rivers?
  - What are the human health risks at these levels?
  - What about new/candidate POPs?
- **Communication**, especially Risk Communication is a critical aspect of a project of this nature that needs to be addressed with the appropriate sensitivity. The WRC funded Endocrine Disruptive Compounds (EDC) programme is developing a Management Guide in which the methods of communication will be recommended for the different role players from regulators to the man in the street;
- **Repository of data** from these projects: the data generated on a WRC funded project is the Intellectual Property of the WRC and must be made available for public domain. There was no consensus of the

availability of data collected for other institutions. Should there be a central repository?; Does the Stockholm Convention have some requirement? DEA?

- It was suggested that WRC discuss this issue of databases with DWA as DWA has well established national databases which could possibly be expanded to include POP's (The NTMP being one of these).
- There was a call for the development of an equivalent of an Environmental Protection Agency (EPA) in South Africa, which could serve the whole of Africa (i.e. serves the people, not the government of the day).

5.0 CLOSURE

The workshop participants were asked to determine if the objectives of the workshop had been met. Those being :

"To guide and inform the development of appropriate policy and regulatory measures that will support implementation of the requirements of the Stockholm Convention, and substantially contribute to the protection of water resources and water–linked ecosystems with regard to POPs."

The participants and researchers agreed that the workshop had met the objectives.

The workshop closed at 15h10 and all the participants were thanked for their honest inputs and time. The project team members were complimented on "hanging tough" throughout this difficult project and making a useful contribution to the understandings of the difficulties of detecting and analysing POPs in the environment.

GOLDER ASSOCIATES AFRICA (PTY) LTD.

Lee Boyd
Water Specialist

Ralph Heath
Associate

LB/RH/aj

Reg. No. 2002/007104/07
Directors: FR Sutherland, AM van Niekerk, SAP Brown, L Greyling

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APPENDIX A

Attendance Register

13016

ATTENDANCE REGISTER

DATE: 26 January 2011 **VENUE:** Nkulu Boardroom Golder Associates Pretoria office

SUBJECT: WRC POPS II Workshop **PRESENTER:** Ralph Heath

Name and Surname	Client	Sub-Consultant	Sub-Contractor	Visitor	Golder Employee	Employee Number	Business Unit	Division	Signature
Bettina Genthe	x						CSIR	NRE	
Henk Bouwman	x						North West University	Env. Science	
Rialet Pieters	x						NWU	Env. Science	
Gordon Khauoe	x						DEA	Waste Stream mgt	
Sebastian Jooste	x						DWA	RQS	
Claudine Roos	x						North-West University	Centre for Environmental Management	
Odusanya David							Water Affairs	RQS	
Egmont Rohwer	x						Univ Pretoria	Chemistry	
Tiaan DeJager	x						School of Health Systems & Public Health Univ. of Pretoria		
Geert Grobler	x						DWA WRPS	Water Quality Planning	
James Dabrowski	x								
Albertus Randal	x								

ATTENDANCE REGISTER

Name and Surname	Client	Sub-Consultant	Sub-Contractor	Visitor	Golder Employee	Employee Number	Business Unit	Division	Signature
Charlie Reinhardt	x						Private consultant & UP		
Martin Ginster	x						Applyse		
Bonani Madikizela	x						WRC	KSA: 2	
Ralph Heath					x		Environmental Tech	Environmental Tech	
Lee-Ann Boyd					X		"	"	
Oliver Malete					x		Environmental Tech	Water Resources	
Jayne de Vos	X				x		NMISA ~~Environmental Tech~~	Organic chem Water Resources	
Laura Quinn	x						NMISA	Organic chem	

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APPENDIX B

Agenda

WATER RESEARCH COMMISSION

AGENDA: WRC POPS II WORKSHOP

PERSISTENT ORGANIC POLLUTANTS IN THE ENVIRONMENT
A WATER RESEARCH COMMISSION PROJECT

Date: **26 January 2011**

Venue: **Golder Nkulu Boardroom Pretoria office**

Time: **09H00 for 09h30**

Agenda items:

Registration and tea (09h00)

1. Welcome and introductions (09h30) — Dr Ralph Heath
2. Objectives of feedback workshop (09h40) — Dr Ralph Heath

"To guide and inform the development of appropriate policy and regulatory measures that will support implementation of the requirements of the Stockholm Convention, and substantially contribute to the protection of water resources and water–linked ecosystems with regard to POPs."

3. Feedback from Government on the Stockholm Convention and South Africa's National Implementation Plan (09h45) — GK
4. Current status of the project (results, interpretation, health risk assessment (HRA), conclusion and recommendations (10h05) — Project Team
5. Questions for clarification (11h15) — All
6. Feedback on other related research activities (NWU / CSIR / WRC / NMISA) (11h40) — All

Lunch (12h00 to 12h30)

Working Group session (12h30 - 15h00)

The following items 7 to 9 will be workshop in groups

7. Identified sites:
    a) Implications of data and HRA
        i) Extent of pollution (Do we have enough detail? What about other pollutants?)
        ii) Health risk (Who is at risk? Do we have enough detail?)
        iii) Biotic risk (What is at risk? Do we have enough detail?)
    b) Should and can interventions be done immediately? (What would be the nature of the interventions?)

c) How to identify sources (What should be done to identify specific sources?)

d) Can interventions be based on the current information?

e) Identified research needs from a-d.

8. National Implementation Plan (NIP)

a) How can the data inform the NIP?

b) Uptake of data in current or future updated NIP

c) Implications for SA as Party to the Stockholm Convention

9. Future requirements and arrangements

a) What about new/candidate POPs

b) Regular surveys (Are they needed and how often? Who would be the driver/initiator?)

c) New developments in POPs and chemical pollution in general (Who keeps tabs on it?)

d) POPs research communication strategies. (Who tells what to whom and when?)

e) Dissemination of data

- Who needs this information?
- Publication (WRC report, scientific and general articles, conference presentation, press release).

f) Research priorities (What should they be?)

g) Analytical infrastructure / arrangements / problems and issues.

10. Feedback from working groups (14h30) — All
11. General discussion (15h00) — All
12. Way forward and closure (15h30) — Dr Ralph Heath

R Heath

RH/ab

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