# Survey of certain Persistent Organic Pollutants in major South African waters

Report to the
WATER RESEARCH COMMISSION

BY

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EXECUTIVE SUMARY

## Introduction

In 1997 the United Nations Environment Programme (UNEP) initiated a process to develop a global, legally binding instrument to reduce the risks to human health and the environment caused by the release and long-range distribution of Persistent Organic Pollutants (POPs). This has resulted in the Stockholm Convention on Persistent Organic Pollutants, or SC for short.

POPs are considered organic compounds of natural or anthropogenic origin that resist photolytic, chemical and biological degradation, and also have toxic properties. They are compounds with low water solubility, readily soluble in lipid and can therefore accumulate in fatty tissue of biota. Because of the long persistence times and (low) volatility, they can be transported in the environment in low concentrations via water and air movements, as well as with migrating animals. This means that POPs can be transported to areas where they have never been used, and can therefore affect human and environmental health globally - consequently the need for international action on POPs.

The POPs convention is aimed at eliminating or reducing the production or releases of the first 12 identified POPs via an international convention. The initial group of 12 POPs includes a number of pesticides - aldrin, dieldrin, DDT, endrin, heptachlor, chlordane, hexachlorobenzene, mirex and toxaphene. The other three chemicals are actually classes of compounds that include the dioxins (PCDD), dibenzofurans (PCDF) and polychlorinated biphenyls (PCB).

There are a number of reasons why POPs is an international problem. Due to the cold conditions in the polar regions, POPs in the environment will tend to accumulate in these regions, especially via condensation from the atmosphere - a sort of a cold-trap. The climatic and environmental conditions of the polar regions are conducive to even longer residence times (less UV, lower temperature), and POPs will therefore stand a greater chance of being accumulated by biota, and entering the food web, eventually reaching man. This has been shown especially in the Arctic, where compounds such as PCB and DDT have been found in polar bears, seals and humans.

The main concern and part motivation for this project is that, although much is known about POPs in northern countries, far less is known about problems in other regions,

especially developing countries. If not enough is known about the situation in these regions, then the SC may be of less value to the developing countries, including South Africa (Bouwman, 2003). South Africa might in fact commit to a convention under which it will be obliged to act, while not knowing the full extent of its own contribution to global POPs (e.g. riverine transport to oceans). One of the problems facing South Africa, and referred to above, are the local releases of PCB and PCDD/PCDF. These two classes of compounds are of major concern, as not much is known about releases and environmental concentrations in the environment. PCDD/PCDF are unintended by-products of industrial processes and incineration. Briefly, these are variably chlorinated, polycyclic hydrocarbons, which have a flat planar structure. There are 75 PCDD (congeners) and 135 PCDF congeners. The recent food contamination scandal in Belgium was caused by contaminated oil that was inadvertently added to feed. It has caused massive damage to the agricultural sector in Belgium, and was the main contributor to the electoral defeat of the coalition government. If developed countries such as Belgium (and there are others) have serious POPs problems, developing countries are likely to have their own POPs problems, but that the nature and extent thereof is not known, as yet.

The major sources of PCDD/PCDF are combustion processes. This is especially the case with municipal, hospital and hazardous waste incineration. Incineration of organic and inorganic chlorinated compounds in which the flue gas does not reach a temperature of more then 400 °C, combined with a short residence time of the gas at that temperature, and gas turbulence in the incinerator, contributes towards the formation of PCDD/PCDF. These combustion conditions lead to incomplete conversion of the intermediate chlorinated compounds, and these contaminants are then fixed onto the fly ash. Such conditions also occur in automotive fuel combustion, and this process could be a significant source of PCDD/PCDF. It must also be recognised that PCDD/PCDF can be formed via vegetation fires and volcanic activities, in low concentrations.

PCB have gained notoriety as an ubiquitous contaminant, mainly due to its use as transformer fluid (insulating) and capacitors in electrical installations, but also as hydraulic oil, and in rubberised paints, glues and plastics. Theoretically there are 209 possible PCB congeners. Because some of the more symmetrical congeners are also

very stable (like PCDD/PCDF and DDT), they can cause biological effects at low levels, and can also bio-accumulate. A short overview of the possible biological effects of the POPs discussed above includes aspects such as impaired reproduction and development, immunosuppression, cancer, P-450 enzyme induction and adrenotoxicity. Endocrine disruption is a contemporary topic in the ecotoxicological community, and many of the identified POPs are known endocrine disruptors.

Although there exists some literature on the pesticide POPs in South Africa (Bouwman, Cooppan, Reinecke, & Becker. 1990; Bouwman, Becker, Cooppan & Reinecke. 1992. Evans & Bouwman, 1993; Evans & Bouwman 2000; Lötter & Bouwman, 1997; Lötter & Bouwman, 2001; van Wyk, Bouwman, Van der Bank, Verdoorn & Hofmann. 2001), very little is known about PCDD/PCDF and PCBs. On these, some work has been done on PCBs in the Isipingo Estuary (Grobler, Badenhorst & Kempster 1996), Wilderness Lakes (de Kock & Randall, 1984), and the Isipingo Estuary (Grobler, *et al*., 1996). Only one study could be traced that investigated dioxins and furans in SA (Schecter, Startin, Rose, Wright, Parker, Woods & Hansen, 1990). The presence of these classes of compounds was established in human breast milk, and showed that urban women had higher levels than rural women. The toxic equivalents (TEQs) for SA urban women (12.6) and rural women (8.3), are significantly lower than those from Tennessee (USA) (14.6) where dioxin containing herbicides were used, Karachi (Pakistan) (12.6), and with TEQ values ranging from 12.7 to 22.9 in Vietnam where Agent Orange was used. These studies show the presence (and in some cases significant concentrations) of POPs in various media in SA, but no overall survey has been attempted. This project was undertaken with the aim to contribute significantly towards the ability of South Africa to determine and interpret its commitments and obligations under the POPs convention. It was intended to establish for the first time a countrywide assessment of POPs in a selection of major water bodies, and would indicate geographical areas (such as industrial and or residential) where more concerted action, management or research needs to be focussed. It would also investigate cheaper means of analysis through bio-assays, since no accredited lab in SA, at the time of this project, could do routine PCDD/PCDF analysis (therefore the high analytical costs for this project). It would also establish a network of researchers that can form the nucleus of further collaboration and serve as a focus for levering international funding that will become

available to developing countries after the Stockholm Convention (SC) has been ratified by South Africa.

During the course of this project, linkages with other efforts, such as those initiated by GEF and UNEP have been established, as some of the researchers in this project have already been identified as potential collaborators by those organisations. The researchers also have close linkages with DEAT in South Africa, which is the lead agent for the SC. This project is therefore a strategic initiative for SA, not only with respect to the SC, but also for other current and future conventions that deal with pollutants.

**Aims of the project**

The aims of the project were:

1. To establish the presence and levels of 7 PCDD, 10 PCDF and 12 PCBs in fish from major South African rivers and estuaries.

2. To determine the possible implications and future obligations for South Africa, of the presence and levels of these POPs under the international, legally binding, Persistent Organic Pollutant Convention.

3. To establish the basis for further investigations, if levels found are deemed of concern.

4. To investigate alternative and cheaper means of analysis for PCDD/PCDF and PCBs in South Africa.

5. Through an initial risk assessment, based on analytical data from this project, establish the risk associated with the levels found.

6. To develop a short course on environmental sampling and Good Laboratory Practice.

Notes concerning addressing the aims:

1) The envisaged sampling of fish from rivers and estuaries proved to be impractical (see Chapters 2 and 3). It was subsequently decided to use sediments as integrating accumulation of POPs. Samples were taken at sites where high releases of POPs to the water environment were expected to occur. These samples were analysed by a German laboratory, as accredited facilities are not yet available in South Africa (Aim 1).

Potential implications for South Africa of these findings (Aim 2) are discussed under conclusions (Chapter 6).

2) The short course on environmental sampling and Good Laboratory Practice (Aim 6) is available on the accompanying CD.

3) The initial project proposal referred to ELISA as an alternative and cheaper means of analysis. During the inception of the project, and as a result of attending the DIOXIN 2002 conference in Barcelona by both authors, the project team became aware of a more advanced bio-assay technique. With permission from the WRC and with additional support from NRF (Thuthuka), it was decided to further investigate this technology.

**Materials and methods**

A total of 22 sites were selected to represent primarily areas with potentially high POPs concentrations in South Africa. Aquatic sites were selected on the grounds of being close to or down stream from areas regarded as possible sources of PCDD/PCDF and PCB.

Samples were collected from the surface sediment (0-5 cm deep) of the river/dam/harbour with a brass grab sampler. Equal volumes (120 mL) of individual sediment samples from five different locations at a particular site were pooled (combined).

The sediment samples were freeze-dried before 30g of each sample was sent to an international accredited laboratory in Germany. The sediment was extracted through a technique called Accelerated Solvent Extraction (ASE) and the extract analysed with high-resolution gas chromatography and mass spectrometry (GC/MS).

Total concentrations of TEQs (Toxic Equivalency Factor) based on this instrumental analysis were calculated as the sum of TEQs from individual compounds, assuming additive responses to chemicals in the mixture. The TEQs were obtained by multiplying the concentration by appropriate toxic equivalent factors (TEF).

The particle size distribution as well as the organic content of each of the samples was determined. This information was required since PCDD/PCDF and PCB are known to adsorb to the organic carbon of soils and sediments as is illustrated by their high $K_{OW}$

values (cf §2.2.) and more of these molecules would also adsorb to clay particles than to larger particles.

The samples were also extracted for bio-assay analysis. The rat hepatoma H4IIE cell line, stably transfected with luciferase reporter gene (H4IIE-*luc*) under the control of dioxin-responsive enhancers (pGudLuc1.1), was used to examine the extracts for their ability to elicit AhR-mediated activity. Sample responses were expressed as relative luminescence units (RLU). Due to unequal slopes and efficacies (maximal induction) of the response, probit analysis could not be used for dose-responses of the sediment extracts.

**Results**

In Figure 4.1 the TEQ contributions by the intentionally produced PCB are presented together with the TEQ concentrations of the unintentionally produced PCDD/PCDF. It is clear that dioxin-like substances are present in all 22 sites sampled in this investigation. Except for the Crocodile River site, the contribution of the dioxins and furans were higher than PCB at all the other sites.

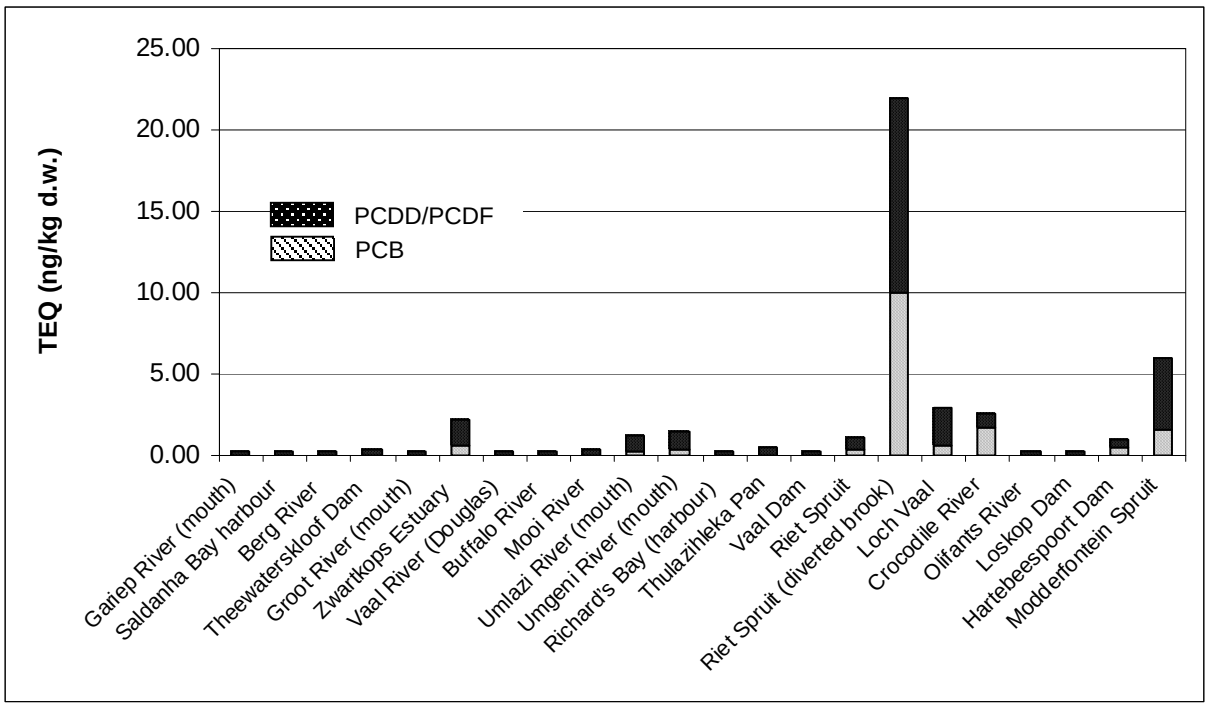


**Figure 4.1** A summary of the instrumentally determined (GC/MS) Toxic Equivalency Factors (TEQ) values for each site.

The highest TEQ value was determined for the Riet Spruit (diverted brook), which is close to an iron and steel refinery in Vanderbijl Park. A TEQ of almost 22 ng/kg was determined (Table 4.3). Another highly industrialised site, the Modderfontein Spruit, had a TEQ of almost 6 ng/kg. The lowest TEQ was found for the Loskop Dam (0.22 ng/kg) and not the Mooi River (0.34 ng/kg), which was selected because of its expected low impact status due to its location (§3.1).

**Conclusions**

- This research established the presence and levels of seven PCDD, ten PCDF and 12 PCB congeners in aquatic environments throughout SA (cf. Aim 1; § 1.3), using sediment.

  The change from sampling sediment instead of fish tissue proved to be fortuitous, although it was inevitable, due to the absence of available fish of the same species at all the sites; at some sites, fish of any kind was absent.

- Very little has been done on environmental concentrations of PCB in recent years, and even less on PCDD/PCDF. In this period, South Africa has signed and ratified the Stockholm Convention on Persistent Organic Pollutants and we need to comply with its provisions once it becomes internationally legally binding (on 17 May 2004). This study will therefore go a long way in contributing to South Africa's obligations towards the Stockholm Convention. (cf. Aim 2; § 1.3). In particular it will address the following articles of the Convention:

  - Contribute towards the development of the *National Implementation Plans* (Article 7, SC) where information needs to be assembled and considered.
  - Article 9 on *Information exchange* to the Secretariat regarding POPs
  - Article 10 on *Public information, awareness and education*, since the information and training course generated by this project is available through this report, the accompanying CD, and, in the future, also through publications.
  - Article 11 on *Research, development and monitoring*, which was the main motivation behind this project. (It should also be noted that this is one of the few projects on PCDD/PCDF entirely funded within any developing country.)

- Article 12 on *Technical assistance*, since the training and cooperation received from the Michigan State University falls within this category.

And

- Article 16 on *Effectiveness evaluation* on a global scale, since we have already been approached to participate in this regard, peripherally due to this project.

This project should therefore also be considered in the broader context of the Stockholm Convention, as South Africa is now one of the few developing countries, and the only African country, where such a survey has been conducted.

- The methods employed in collecting, transporting, storing and analysing of sediment were effective and efficient, including the choice of the accredited international laboratory that did the GC/MS analysis. They have been shown through independent testing to be reliable and efficient.
- In general, this study showed that South Africa have detectable levels of PCB and PCDD/PCDF which occur widespread in the entire country, in the aquatic sediment matrix, from freshwater rivers and dams to coastal harbours and river mouths (Tables 4.1 and 4.2). (cf. Aim 3; §1.3)

  The concentrations found, displayed the global trend of higher PCB concentrations vs lower PCDD/PCDF concentrations. The values did not however, exceed the level of 50 ng/kg sediment, which was the action level determined for the USA.

  Another global pattern repeated for South Africa was that higher concentrations were associated with the industrialised areas of the country.

  The values found for the Vaal and Gariep River systems, particularly at the Gariep River mouth (site 1), indicate the real possibility of long-range riverine transport of these pollutants, and therefore a possible contribution from South Africa, to the global burden. The extent of this contribution is however not known.
- Although the bio-assay results acquired up to now did not perform immediately as expected, much experience has been gained with this technology. This

technology is currently being established at Potchefstroom. Bio-assays are more cost effective than GC/MS, provides biologically relevant TEQ values, and more samples in less time can be screened. Almost any matrix can be analysed for their TEQ value by this reporter gene bio-assay. This project has therefore been instrumental (together with funding from the NRF through its Thuthuka programme) in developing South Africa's monitoring and research capacity for the analysis of toxic PCB and PCDD/PCDF. (cf. Aim 4; §1.3)

- The present study did not aim to address the issue of the risks of PCDD/PCDF and PCB to humans or wildlife directly. However, extrapolating the obtained data and drawing possible risk values from sediment/fish interactions from a Chinese study, gave the distinct indication that the PCDD/PCDF concentrations are possibly high enough to warrant concern about the risk of cancer development in humans consuming fish from aquatic environments, with sediment concentrations as measured for some of our sites. This conclusion is however, based on limited data, and needs verification before any substantiated inference can be drawn (cf. Aim 5; §1.3)

  Risk seems not to be restricted to the industrialised areas only. Places such as Alexander Bay and the Umgeni River mouth seem to be relatively polluted sites, if the organic carbon normalised concentrations are taken into consideration. The harbours sediments we sampled, seemed to have had rather low PCB and PCDD/PCDF concentrations.

- A short course on environmental sampling and Good Laboratory Practice has been developed and is available on the accompanying CD (cf. Aim 6; §1.3).

**Recommendations**

- To ensure comparability, extractions for GC/MS analyses and bio-analyses must be performed in exactly the same manner.

- The extraction and clean-up protocols needs to be followed carefully, as this determines which groups of pollutants *eg.* PAH and/or dioxin-like PCB and PCDD/PCDF will still be present in the extract.

- Other types of bio-assays can also be performed, such as EROD and AHH, using the same tissue culture as for the reporter gene bio-assay. This increases

the testing capacity of a monitoring laboratory, improving its applicability and scope of investigations.

- Since animal groups (birds, fishes mammals and humans) respond differently to the pollutants, the ideal would be to expose tissue cultures from the different animal groups to the same extract.

**Further research**

Although this study has gone a long way to provide first and urgently needed information on dioxin like PCB and PCDD/PCDF for South Africa, it is not enough. To truly understand the source characteristics, long-range transport of the compounds, their transfer between trophic levels and their distribution in matrixes at a particular site, sites must be sampled regularly and extensively, using multiple matrixes. In this way, bio-accumulation in the consecutive trophic levels can be traced. Especially the PCDD/PCDF concentrations at more than one site seemed to have concentrations of concern.

Research that is unique to SA will be to determine the exposure of people and environments exposed to the daily combustion of household wastes and fuel such as wood and coal.

The following specific areas of investigation are therefore proposed:

- More detailed investigations of polluted sites, as determined by this project, with the aim to better define the levels, and to investigate the possible sources.
- Determine the levels in fish and other aquatic biota to better determine the risks associated with the levels.
- A better characterisation of the possible long-range transport in some of the longer rivers in South Africa.
- A better characterisation of the estuarine and coastal distribution of these compounds.
- Investigations regarding the soil / aquatic interactions (eg. ground water or flood water) of POPs, especially in potential source areas such as industrial, waste and residential areas.

- A better characterisation of human exposure to POPs generated due to domestic heating and cooking that characterise the conditions of many South Africans.
- A better characterisation of human and wildlife exposure to POPs generated due to waste combustion and industrial activities that characterise many sites.

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**SURVEY OF CERTAIN PERSISTENT ORGANIC POLLUTANTS IN MAJOR SOUTH AFRICAN WATERS**

The steering committee responsible for this project consisted of the following persons:

| | |
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| Mr HM Du Plessis | Water Research Commission (Chairman) |
| Dr LA Sandham | North-West University (Potchefstroom Campus) |
| Ms M Hinsh | Department of Water Affairs and Forestry |
| Prof CF Reinhardt | University of Pretoria |
| Mr EB Mathebula | Department of Environmental Affairs and Tourism |
| Mr HR Meinhardt | ARC – Plant Protection Research Institute |

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| Dr Heidi Fiedler | UNEP Chemicals (Geneva) |

## ABREVIATIONS

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AHH | Aryl Hydrocarbon Hydroxylase |
| AhR | Aryl (aromatic) hydrocarbon Receptor |
| Arnt | AhR nuclear translocator protein |
| ASE | Accelerated Solvent Extraction |
| BAT | Best Available Techniques |
| BET | Best Environmental Techniques |
| BSAF | Biota-Sediment Accumulation Factor |
| CALUX | Chemically Activated Luciferase eXpression |
| CBR | Critical Body Residue |
| CCMS | Committee on Challenges of Modern Society |
| CITES | Convention on Trade in Endangered Species |
| COP | Conference of the Parties (SC) |
| DEAT | Department of Environmental Affairs and Tourism |
| DMEM | Dulbecco's Modified Eagle's Medium |
| DPBS | Dulbecco's Phosphate Buffered Saline |
| DRE | Dioxin-Responsive Element |
| EC20; EC50 etc | Concentration of test agent that causes 20%; 50% of maximal response |
| EDC | Endocrine Disrupting Chemical |
| ELISA | Enzyme Linked Immuno Sorbent Assay |
| EROD | EthoxyResorufin *O*-Deethylase |
| EU | European Union |
| $f_{OC}$ | Fraction of organic carbon |
| GC/MS | Gas Chromatography/Mass Spectrometry |
| GEF | Global Environment Facility |
| HCB | HexaChloroBenzene |
| HxCB | HexaChloroBiphenyl |
| IFCS | Intergovernmental Forum on Chemical Safety |
| IGO | Inter-Governmental Organisation |
| IOMC | Inter-Organisational programme for the sound Management of Chemicals |

| | |
|---|---|
| ISQG | Interim Sediment Quality Guideline |
| I-TEF | International Toxic Equivalency Factor |
| IUPAC | International Union of Pure and Applied Chemistry |
| $K_d$ | Partition coefficient: solid/water |
| $K_{OC}$ | Partition coefficient: octanol/carbon |
| $K_{OW}$ | Partition coefficient: octanol/water |
| LD50 | Dose at which 50% of exposed animals die |
| LOAEL | Lowest-Observe-Adverse-Effect Levels |
| MFO | Mixed-Function Oxidase |
| NATO | North Atlantic Treaty Organisation |
| ND | Non-Detectable |
| NEPAD | New Partnership for Africa's Development |
| NGO | Non-Government Organisation |
| NIP | National Implementation Plan (SC) |
| NOAEL | No-Observed-Adverse-Effect Levels |
| NTP | National Toxicology Programme |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PCB | PolyChlorinated Biphenyls |
| PCDD | PolyChlorinated Dibenzo-*p*-Dioxins |
| PCDF | PolyChlorinated DibenzoFurans |
| POP | Persistent Organic Pollutants |
| ppb | Parts per billion |
| ppm | Parts per million |
| ppt | Parts per trillion |
| PTS | Persistent Toxic Substances |
| RBA | Regional Based Assessment |
| REP | Relative Potency Values |
| RLU | Relative Luminescence Units |
| SAICM | Strategic Approach to International Chemicals Management |
| SC | Stockholm Convention |
| TCDD | TetraChloroDibenzo-*p*-Dioxin |
| TMDD | Trichloro-8-MethylDibenzo-p-Dioxin |
| TTF | Trophic Transfer Factor |

| | |
|---|---|
| TEF | Toxic Equivalency Factor |
| TEQ | Toxic Equivalent Quotient |
| UNDP | United Nations Development Programme |
| UNEP | United Nations Environmental Programme |
| USEPA | United States Environment Protection Agency |
| WHO | World Health Organisation |
| WRC | Water Research Commission |
| WWF | World Wide Fund for Nature |

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# 1 INTRODUCTION

## 1.1 The Stockholm Convention on Persistent Organic Pollutants

South Africa has, and continues to play, a major role in the negotiations and implementation of the Stockholm Convention (SC). The final text of the convention was successfully negotiated in Johannesburg in December 2000 (SC, 2003), and since then, 40 countries have ratified, including South Africa. Ninety days after the deposition of the 50$^{th}$ ratification, the convention will become international law. This is expected to occur late in 2004, with the first Conference of the Parties (COP) to be held in the first half of 2005. To understand the current and future implications and obligations for South Africa of this convention, a closer look needs to be taken at how it came about.

In May 1995, the United Nations Environment Programme initiated a process to prepare an internationally legally binding instrument for implementing international action, initially concentrating on 12 specific POPs (Persistent Organic Pollutants). These POPs included the chlorinated pesticides aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex and toxaphene, as well as hexachlorobenzene (an industrial chemical, industrial unintended by-product and pesticide), polychlorinated biphenyls (PCB – a class of industrial chemical used mainly in transformers), and the combustion by-products generally known as polychlorinated dibenzo-*p*-dioxins (chlorinated dioxins or PCDD), and polychlorinated dibenzo-furans (chlorinated furans or PCDF[1]).

The reason for this concerted international action was that enough scientific evidence have been accumulating, showing that the release of these chemicals into the environment, through whatever means, have actual and potential global effects, away from their sources of release. This is due to the physico-chemical as well as biological properties of these chemicals. They are all toxic, resist degradation, can be bio-accumulated, but can also be transported over long distances. The potential to be transported over continental distances provides the international context to this issue (and part motivation to this Water Research Commission (WRC) project). The combination of these characteristics therefore means that biological effects can

[1] Since PCDD and PCDF are formed together in the same combustion / high temperature or some chemical processes, they will from hereon be referred to as PCDD/PCDF, following generally accepted international convention (other documents also use a "PCDD/F" notation. See Chapter 2 for more information and structural formulae.

manifest itself far away from the source of release, even thousands of kilometres away. PCB and DDT have for instance been found in polar bears and Inuit Indians in the Arctic at significant levels (Whylie *et a*l., 2003), with the most likely sources industrial use (PCB) in Canada, USA and Russia, as well as malaria control in Mexico (DDT) and elsewhere in the northern hemisphere.

Although there exists some literature on the pesticide POPs in South Africa (Bouwman, Cooppan, Reinecke, & Becker. 1990; Bouwman, Becker, Cooppan & Reinecke. 1992. Evans & Bouwman, 1993; Evans & Bouwman 2000; Lötter & Bouwman, 1997; Lötter & Bouwman, 2001; van Wyk, Bouwman, Van der Bank, Verdoorn & Hofmann. 2001), very little is known about PCDD/PCDF and PCB. On these, some work has been done on PCBs in the Isipingo Estuary (Grobler, Badenhorst & Kempster 1996), Wilderness Lakes (de Kock & Randall, 1984), and the Isipingo Estuary (Grobler, *et al*., 1996). Only one study could be traced that investigated dioxins and furans in SA (Schecter, Startin,

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### 1.1.1 Long range transport

The long distance transport potential is realised in more than one way (Figure 1.1; Whylie *et al*., 2003). Since the overall context of this project also includes the potential contribution of SA to long range transport of these chemicals, a better understanding of the various means is in order.

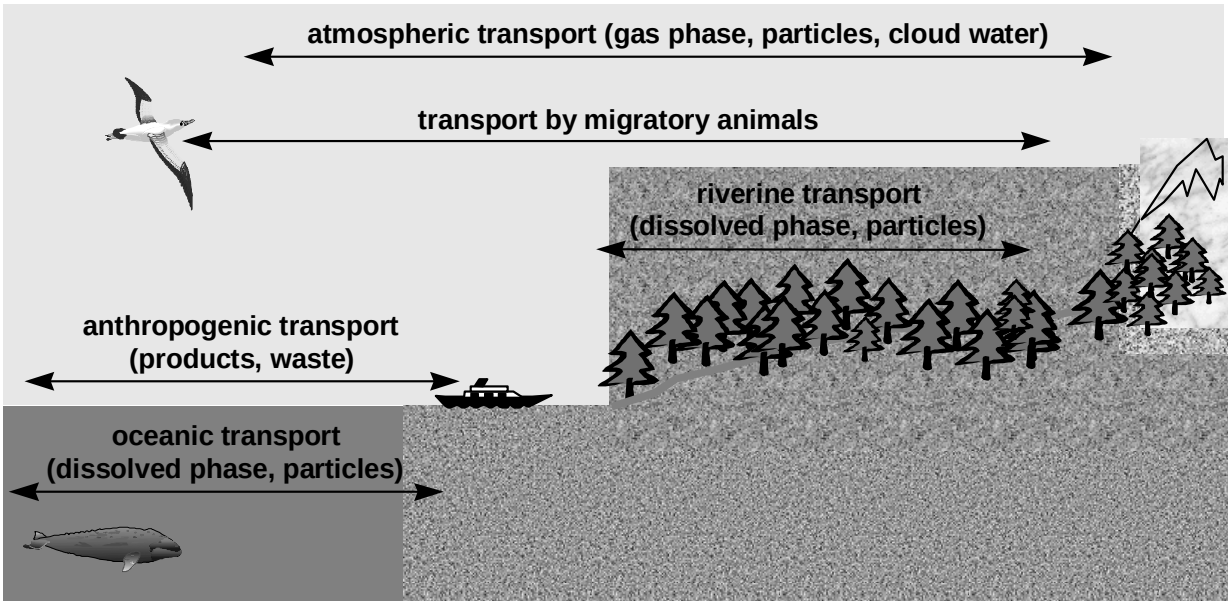


**Figure 1.1** Schematic representation of the various mechanisms through which a POP can be transported over long distances (from Whylie *et al*., 2003)

#### 1.1.1.1 Oceanic transport

Due to the volumes of water involved, the most obvious mode of long-range transport of POPs occurs via oceanic currents. The massive movement of water transport the compounds all around the globe. Water caught up in oceanic currents can therefore transport significant amounts of these chemicals, contaminating every bit of the ocean, its sediment and biota. The specific POPs chemicals have various solubilities in water, but do adsorb onto particles, or are taken up by biota, thereby adding to the environmental component available for bio-accumulation. Those components bound to organic particles will tend to settle out due to gravitation, and is therefore presumably more important for coastal, shelf and nutrient upwelling areas. The vertical transport tends to result in the ocean sediment as the major final sink for POPs on a global scale. This means that the rate of concentration decline through degradation of POPs in this compartment could lag significantly from other compartments, after phasing out or reducing the release of these components through various interventions (Whylie *et a*l., 2003).

#### 1.1.1.2 Riverine transport

The water solubility of PCDD/PCDF and PCB are so low that dissolved transport is not a significant vector for transport via rivers. Colloidal and suspended sediment is therefore the main method of transport in riverine systems. The hydrological regime and catchment characteristics therefore determine the load of transport to oceans (Figure 1.1). If rivers therefore flow through areas where contamination is present, there is a significant likelihood that the contamination will be transported further down stream, eventually contaminating coastal sediments. High-flow episodes are therefore likely to suspend sediments and contaminate downstream areas, while also possibly scouring recent deposits in the upstream areas (Whylie *et a*l., 2003).

#### 1.1.1.3 Air transport

The third method of transport is by air movements (Figure 1.1). These compounds do go into gas phase, and due to their resistance to break down, can be transported over long distances. Where such air currents meet colder conditions, they tend to condense out of the gas phase, and thereby again contribute towards the pollution of colder regions and their biota.

#### 1.1.1.4 Migratory animals

The next method of transport is via migratory animals (Figure 1.1), which, although important in polluting animals higher up in the food web (Lötter and Bouwman 1993), is not of immediate concern to this report. It should be noted however, it has been found that salmon migrating up rivers in huge numbers to spawn and then die, contribute significant amounts of POPs in the relative pristine headwaters where they reproduce, due to release from the decaying bodies (Krümmel, Macdonald, Kimpe, Gregory-Eaves, Demers, Smol, Finney, Blais, 2003). Calculations have also shown that the release of POPs from decaying whale carcasses, after decades of accumulation, can result in localised hot-spots of pollution. Such unexpected effects need to be kept in mind when dealing with the issue of global transport, bio-accumulation and the effect of toxic chemicals (Frank Wania, pers comm.).

#### 1.1.1.5 Trade as mode of transport

A fifth method of transport is anthropogenic (Figure 1.1). In many ways the best way to get an amount of POP from one place to another is via trade of POP products (such as

pesticides), or via POP contaminated products or waste. The Stockholm Convention does address the trade issue between Parties and non-member countries.

### 1.1.2 The Objective of the Stockholm Convention

The final negotiations for the text of the convention took place in December 2000 in Sandton, Gauteng. During the final session of 27 hours of marathon negotiations, the complete text was approved by all nations present (UNEP Convention Text). Although the complete text is available on the internet (http://www.pops.int/ documents/convtext/convtext_en.pdf and the accompanying CD), relevant sections will be presented to highlight specific aspects that have a bearing on this project (SC, 2000).

The objective of the Stockholm Convention is stated in Article 1 as:

> *'Mindful of the precautionary approach as set forth in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Convention is to protect human health and the environment from persistent organic pollutants.'*

It is interesting to note some of the concerns recognised in the preamble, the first two of which are provided below:

> *'The Parties to this Convention,*
>
> *Recognizing that persistent organic pollutants possess toxic properties, resist degradation, bio-accumulate and are transported, through air, water and migratory species, across international boundaries and deposited far from their place of release, where they accumulate in terrestrial and aquatic ecosystems,*
>
> *Aware of the health concerns, especially in developing countries, resulting from local exposure to persistent organic pollutants, in particular impacts upon women and, through them, upon future generations,'*

*Have agreed as follows:*

*Article 1*

*Objective*

*Mindful of the precautionary approach as set forth in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Convention is to protect human health and the environment from persistent organic pollutants.*

### 1.1.3 The Annexes of the Stockholm Convention

The Stockholm Convention follows a layout similar to previous environmental conventions, with a preamble stating the concerns and aims, a group of articles dealing with various aspects such as definitions, measures to prevent or minimise releases, exemptions, additions of new compounds, implementation, technical and financial assistance to developing countries, and procedural aspects dealing with the operation of the convention. The listing of specific compounds and more technical and compound-specific issues are placed in three Annexes (There are more annexes, but they deal with other issues). The reason to place this outside the main text of the convention is to prevent frequent amendments to the convention text itself, that, due to the nature of international conventions, amendments require a COP and a 75% majority vote for approval, followed by a subsequent 75% ratification by all members, and a further 90 days for the amendment to enter into force. At the time of going to press, it was announced that the Convention will enter into force on 17 May 2004.

To allow additional compounds to be placed in either of the annexes, a complicated procedure of nomination by parties based on specific physical and chemical criteria, followed by an evaluation procedure by a POPs Review Committee (colloquially already known as the POProck), submission of its recommendations to the Parties, invite comments on socio-economic considerations that such an action will entail, prepare a risk profile, followed by another round of comments, prepare a risk management evaluation, and, eventually, a vote by the COP (SC, 2000; Article 8, and various annexes).

For the purpose of this report, only those aspects relevant to PCDD/PCDF and PCB will be addressed further on, but it is obvious from the above, that many more issues form

part of the first international convention to focus on specific chemicals in the environment.

The Annexes, similar to those used by the CITES convention in dealing with various categories, deals with the individual chemicals in more technical detail. There are three annexes - Annex A - Elimination; Annex B - Restriction and Annex C - Unintentional production. For Annexes A and B, there is also a register of country (party) specific exemptions (Article 4). The intention is therefore that countries nominate themselves to be added to this register, stating the specific compound and the expiry date for each specific exemption. South Africa for instance has a specific exemption for DDT (the only compound in Annex B), while all countries have general exemption for PCB until 2025. Countries that wish to become a Party, should therefore state their specific production or use needs, and this information is public knowledge. Such exemptions expire automatically after five years, unless an extension is granted for an additional five years, taking the specific account of the circumstances of developing countries. Once no more exemptions are registered, no new exemptions may be registered (SC, 2000).

#### 1.1.3.1 Annex A

Annex A therefore deals with chemicals scheduled for elimination, where such elimination is already feasible, either by cessation of production (e.g. dieldrin and aldrin where no known production has been indicated by any Party), or where production could be terminated in the near future where alternative chemicals are available, but some specialised uses are still exempted. Chlordane (although registration has been terminated in South Africa) is one such chemical, where some countries applied for exemption for uses such as termite control and for use in underground cable boxes. Also included in this Annex is PCB (which will be dealt with in more detail in Chapter 2). All parties have agreed to its elimination, and no known production is taking place, but the phasing out of PCB in transformers, capacitors and other equipment, will take a rather long time. Complete elimination by all the parties, including South Africa, is planned for 2025.

Overall Party obligations (and therefore including South Africa) on chemicals in Annexes A and B are provided in Article 3 "Measures to reduce or eliminate releases from intentional production and use" of the Stockholm Convention (SC, 2000).

#### 1.1.3.2 Annex B

As already mentioned, Annex B currently deals only with DDT, and is not directly involved in this report, although specific measures are provided in Part II of Annex B (SC, 2000). Some aspects of the DDT issue have been (Sereda & Meinhardt, 2003), and are currently addressed in South Africa by WRC, GEF and WHO funded projects.

#### 1.1.3.3 Annex C

Annex C deals with compounds that are not intentionally produced, but do have the same characteristics as the others (toxic, persistent, organic and long range transport potential). Hexachlorobenzene also falls in this Annex, but is also not subject to this investigation. PCDD/PCDF are dealt with in this Annex, and, in many ways, represent probably the most difficult issue that the SC has taken up. Since these compounds are unintentional by-products, the reduction and or elimination of releases to the environment presents huge technical, organisational, financial and scientific challenges and obligations. These compounds will be dealt with in more detail in Chapter 2.

The specific Party obligations are the subject of perhaps the most far reaching of articles in the SC, which begins:

> *'Each Party shall at a minimum take the following measures to reduce the total releases derived from anthropogenic sources of each of the chemicals listed in Annex C, with the goal of their continuing minimization and, where feasible, ultimate elimination:'*

There then follows a long list of involved measures dealing with: inventory and evaluation of sources, regulatory arrangements, strategies, action plans, education and training, periodic review, promotion of application of available, feasible and practical measures that can expeditiously achieve a realistic and meaningful level of release reduction or source elimination, best available techniques (BAT) and best environmental practices (BEP).

### 1.1.4 Responsibilities of the Parties under the Stockholm Convention

Of particular note is the recognition of the common but differentiated responsibilities of the parties (only countries) to this convention, which is also taken up in the preamble (SC, 2000):

> *'Noting the respective capabilities of developed and developing countries, as well as the common but differentiated responsibilities of States as set forth in Principle 7 of the Rio Declaration on Environment and Development,'*

The countries that ratify the Stockholm Convention, therefore acknowledge that for global responsibility, local action and intervention needs to be taken (common responsibilities). In addition, there are provisions to provide technical and financial assistance (differentiated responsibilities) to developing countries to attain the objective of this convention, recognising that poor countries have less means to address the issues, and in many cases contribute less of an impact than developed countries (differentiated).

One of the common responsibilities is with malaria, where the continued use of DDT, is seen as essential in developing countries for the foreseeable future (Bouwman, 2003). During the negotiations it was therefore crucial that South Africa, together with other countries in a similar position, protected this requirement, as part of the convention. This was done successfully with support or understanding from many quarters, including the EU, USA, WWF and other NGOs, and especially the WHO.

The convention places responsibilities on both the developing and developed (or donor) countries. Again, because of the strong insistence of SA and other developing countries, a *quid pro quo* was reached, whereby the adherence of the developing countries to the Convention was made subject to the following (SC, 2000, Article 13, #4):

> *'The extent to which the developing country Parties will effectively implement their commitments under this Convention will depend on the effective implementation by developed country Parties of their commitments under this Convention relating to financial resources, technical assistance and technology transfer….'*

In the case of PCDD/PCDF specifically, it became obvious that the lack of knowledge concerning PCDD/PCDF in developing countries (sources and environmental levels),

hindered the effective negotiations. Whereas South Africa played a leading role regarding DDT, it was less effective concerning PCDD/PCDF, since so little was known. Extrapolations and conjecture are not enough to carry any advantage, and the need to find out more regarding the pollution levels of these compounds in SA - hence this initiative.

### 1.1.5 Interim activities in support of the Stockholm Convention

Even before the Convention becomes international law, interim actions and initiatives are being taken, including inventories of sources (factories, incinerators, etc), capacity building and assessments. One of these assessments included the now completed Regionally Based Assessments (RBA) of Persistent Toxic Substances (PTS). This is an effort funded mainly by the Global Environment Facility (GEF), to use existing data and information on PTS (including the identified POPs) for evaluation of threats and data gaps. Twelve regional reports and one global report were produced, that assessed the available knowledge and information regarding the impacts and threats of a number of compounds, including the twelve POPs. The report for Sub-Sahara Africa identified a number of priorities in the context of the current project. Some of them are listed below (Osibanjo, Bouwman, Bashir, Okond'Ahoka, Choong Kwet Yve, Onyoyo, 2002):

- **Survey of the current PTS contamination status of fresh, coastal and marine waters by PTS.**

  **Justification:**

  • Impact of pollution on export markets if PTS pollution is not managed from knowledge
  • These ecosystems are already incorporated in NEPAD as an important priority
  • Address the data gap indicated by this report, regarding lack of data
  • Pollution is closely linked to human health
  • Pollution affects the integrity of affected ecosystems
  • Polluted environments affect livelihood support of affected communities
  • Fresh and coastal water important to life and maintenance of ecosystems

- **Quantification and mapping of sources of PTS contaminants that release PTS to the environment.**

  **Justification**:

  • Address the data gap indicated by this report, regarding lack of data
  • Data gathered will facilitate appropriate intervention measures
  • Improved knowledge of pollution to support sustainable development and address poverty
  • To build trust and partnerships between all stakeholders

- **Awareness regarding PTS issues, amongst stakeholders, relevant government regulatory officers and civil society.**

  **Justification**:

  • Existing levels of ignorance of hazardous effects of PTS in most African countries needs to be urgently addressed

- **Inventory of PTS sources, contaminated sites, affected communities and others (other than PTS pesticides).**

  **Justification:**

  • Intensive use of PTS in Africa
  • Data gap on impacts of PTS use
  • Facilitate intervention measures such as remediation
  • Improved knowledge of pollution
  • To build trust and partnerships amongst all stakeholders
  • Protecting human health and environment

- **Support human resource development relevant to PTS programmes**

  **Justification**:

  • Capacity building/improvement needed to deal with various requirements
  • Implementing relevant international agreements (e.g. Stockholm, Basel and Rotterdam)

- **Inventory and strengthening of existing laboratories for PTS analysis**

  **Justification**:

  • Enable monitoring and research into residues, exposure, risk assessment and effectiveness of cleaner technologies and PTS management
  • Satisfy environmental and health standards
  • Compliance with international agreements

As can be seen from above, there are many elements common to both this project and the priorities identified in the Sub-Saharan Africa report. Together with the other 11 regional reports, a global synthesis was done in a Global Report (Whylie, Albaiges, Barra, Bouwman, Dyke, Wania, Wong, 2003). In this report, that will guide the direction of intervention and funding for the foreseeable future regarding POPs, some of the more conclusions pertinent to this study are repeated below:

- **Filling of data gaps –** Consistently throughout the regional reports, it was established that major data gaps existed that prevented the scientific acknowledgement of intuitive concerns for certain chemicals. These gaps varied from region to region and from chemical to chemical. The regional reports provide a useful measure of the areas prioritised for work to be undertaken to fill data gaps. With careful analysis, vital information could be obtained to make more informed decisions on the level of threats and effects that certain chemicals pose to the environment both regionally and globally. Unfortunately, it is difficult to prioritise the importance of these data gaps on a global scale given the differences between regions. The project has identified the wealth of measured data available in some regions and the large gaps that exist in many others. There is an urgent

need to begin to develop systematic, representative monitoring on a global basis. A strategy for this needs to be developed that takes account *inter alia* of the need for representative samples both for interpretation of results and validation of models, for harmonization of reporting procedures to ease the burden on those reporting, and for the additional resources to help initiate the reporting of data from many areas.

- **Conduct of a global assessment of dioxins/furans/PAHs emissions from open burning -** It is being shown from the RBA PTS that open burning is a major concern in all habitable regions under the project. Even the Arctic report expresses concern for open burning being a major local source for dioxins/furans and PAHs [polycyclic aromatic hydrocarbons] in that region. However, there is limited knowledge of the extent of the problem. The NIPs [National Implementation Plans] being developed by each signatory to the Stockholm Convention includes an assessment of the needs associated with the reduction of emissions of dioxins and furans.

There have been many references to the sources of PCB and PCDD/PCDF. Since an understanding of their sources are inherent in the planning, as well as during the interpretation of the results of this study, the origin and circumstances will now be discussed.

### 1.1.6 Project linkage to national priorities, action plans and programs

The following non-exhaustive list, are some of the pertinent linkages pertinent to this project:

1) South Africa ratified the Stockholm Convention. As one of the main obligations of this convention, parties to the convention undertake the limiting and final elimination of the releases of POPs into the environment.

2) Although South Africa does have a Draft National Chemical Management Profile, there is as yet no POPs profile. The draft does make mention of POPs, specifically relating the lack of analytical capacity (Section 9.2.4).

3) The POPs NIP and the POPs profile is in development through DEAT.

4) Pollutants are addressed in the Constitution of South Africa (Chapter 2, Article 24)

> 24. Everyone has the right
>
> a) to an environment that is not harmful to their health or well-being; and
>
> b) to have the environment protected, for the benefit of present and future generations, through reasonable legislative and other measures that

i) prevent pollution and ecological degradation;

ii) promote conservation; and

iii) secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development.

5) South African national priorities, action plans and profiles are also being addressed through NEPAD.

- POPs are mentioned in the Draft Action Plan for the Environment. They are mentioned in relation to" enable African countries to implement in a coordinated and comprehensive manner their commitments", "Public Education and Awareness Raising" and under "Health and the Environment".
- Under Chapter 8 (Annex III) in the context of sustainable development of the NEPAD Plan of Implementation, technology transfer is also addressed as follows:
    - "Promote technology development, transfer and diffusion to Africa and further develop technology and knowledge available in African centres of excellence"
    - "Support African countries in developing effective science and technology institutions and research activities capable of developing and adapting to world class technologies"
    - "Achieve sound management of chemicals, with particular focus on hazardous chemicals and wastes, *inter alia*, through initiatives to assist African countries in elaborating national chemical profiles and regional and national frameworks and strategies for chemical management and establishing chemical focal points"
    - "Enhance the industrial productivity, diversity and competitiveness of African countries through a combination of financial and technological support for the development of key infrastructure, access to technology, networking of research centres, adding value to export products, skills development and enhancing market access in support of sustainable development"
- In Annex II (Proposed Projects), the following are mentioned under cross-cutting issues:
    - Support for the implementation of the Stockholm Convention on POPs

- Development of national implementation plans for the management of POPs
- Preparation of national inventories of PCBs and PCB-containing equipment in the SADC sub-region
- African stockpiles project
- Survey of chlorinated Dioxins, Dibenzofurans and PCBs in the major waters of South Africa
- Establishment of improved capacity in: laboratory, scientific risk assessment
- Monitoring of environmental contaminants in environmental samples and marketable products

NEPAD is still under development, but it is obvious that this project falls within the scope of the intended activities. Technology transfer and research is directly addressed by this project, and the industrial and management issues could be addressed through the continuation of the current POPs initiative.

6) The South African progress report (July 2003) of the Implementation of the globally harmonised system of chemical classification and labelling (GHS) in South Africa mentions lack of capacity for testing as a barrier to implementation on three separate occasions.

There have been many references to the sources of PCB and PCDD/PCDF. Since an understanding of their sources are inherent in the planning, as well as during the interpretation of the results of this study, the origin and circumstances will now be discussed.

### 1.1.7 Project linkage to SAICM

The first session of the Preparatory Committee for the Development of a Strategic Approach to International Chemicals Management (SAICM PrepCom1) took place in Bangkok, Thailand, November 2003. PrepCom1 is the first substantive step in the SAICM process, which will culminate in a final "International Conference on Chemicals Management." Convened jointly by the United Nations Environment Programme (UNEP), the Intergovernmental Forum on Chemical Safety (IFCS), the Inter-Organization Programme for the Sound Management of Chemicals (IOMC), the World Bank, and the United Nations Development Programme (UNDP), PrepCom1 brought

together more than 400 participants representing over 120 countries, 14 UN bodies, four intergovernmental organizations (IGOs), 24 non-governmental organizations (NGOs) and other observers.

Although many issues were discussed at this meeting, the following list of action items have been agreed upon, and now awaits further action (list shortened to those relevant to this project):

- children and chemical safety;
- hazard data generation and availability;
- acutely toxic pesticides;
- capacity building;
- waste management and minimization;
- industry aspects;
- cleaner production;
- best available technologies and best environmental practices
- environment;
- pollutant release and transfer registers;
- international agreements;
- risk analysis;
- research and monitoring;
- education and training;
- endocrine disruptors, heavy metals and very persistent/bio-accumulative chemicals.

## 1.2 Source characterisation of PCB and PCDD/PCDF

Source characterization represents a crucial step in developing appropriate risk control strategies for PTS. This approach will present an informed and motivated basis from which to estimate sources in order of importance and thereby identify areas for sampling for this project (Whylie *et al*., 2003).

Source as a general term in the current context has to be defined in relation to the entry of a POP into the compartment of the environment under consideration. For a chemical that is manufactured (PCB in this case), the complete chemical life-cycle from production through use and any final disposal must be considered. Therefore, any

intentional release (such as dumping) or unintentional release (such as spillage) during transport, use, or from stockpiles and waste of a chemical to air, water, soil, sediment or biota is included as a source. For unintentionally produced POPs we are guided by where the chemical enters the environment. In both cases there is scope for some overlap, since PCB can also be formed as an unintentional by-product during combustion. It is also relevant to consider potential sources such as stockpiles, reservoirs and waste sites from which uncontrolled releases may occur.

Once released, the chemical can be transported through various means to other areas. Release to the environment can already start during production, but can also occur during storage, transport, use and disposal. In addition, unintentional by-product formation (mainly PCDD/PCDF, but also PCB) can also occur during production and disposal processes and, specifically for PCDD/PCDF, if thermal processes are used (Whylie *et al*., 2003).

PCDD/PCDF formation during combustion

The major source of PCDD/PCDF are combustion processes. This is especially the case with municipal, hospital and hazardous waste incineration. Incineration of organic and inorganic chlorinated compounds in which the flue gas does not reach a temperature of more then 400°C, a short residence time of the gas at that temperature, and gas turbulence in the incinerator, contributes towards the formation of dioxins and dibenzofurans. These combustion conditions lead to incomplete conversion of the intermediate chlorinated compounds, and these contaminants are then fixed on the fly ash. Such conditions also occur in automotive fuel combustion, and this process could be a significant source of PCDD/PCDF, as well as PAH, PCB and HCB, amongst others. It must also be recognised that dioxins and dibenzofurans can be formed via vegetation fires and volcanic activities, in low concentrations.

There are different types of sources of PTS as considered by the RBA (List 1.1). It includes both intentional and unintentional production, and can further be classified into point and diffuse (non-point) sources, as well as secondary sources. Point and diffuse sources include releases from industrial and domestic sites, traffic, waste disposal operations such as incinerators, and landfills. Secondary sources include the spreading of sludge on land and remobilisation of previously deposited compounds from soils and water-bodies. Some sources can be regulated (such as industrial point sources and agricultural application) while other diffuse emissions (in this regard, emissions are understood to include releases to all media) represent either unregulated or difficult to

control inputs (fugitive releases from landfills, domestic open burning of waste, forest fires, etc.).

PCB as a product and toxic pollutant

PCB have gained notoriety as an ubiquitous contaminant, mainly due to its use as transformer fluid (insulating) and capacitors in electrical installations, but also as hydraulic oil, and in rubberised paints, glues and plastics. There are 209 possible PCB congeners. Spillages, electric fires and old stocks are major sources of release to the enviroment. Because some of the more symmetrical congeners are also very stable, they can cause biological effects at low levels, and can also bio-accumulate.

The RBA Global Report has listed the major source categories of PTS. The following (List 1.1) is an extract from that list, which specifically deals with PCB and PCDD/PCDF (Whylie *et al*. 2003). This list does not mean that all these sources are present or important in South Africa, since an inventory has not yet been undertaken, but it can be used in aid of sample site selection.

**List 1.1** Indicative list of sources of PCB and PCDD/PCDF to the environment (adapted from Whylie *et al*., 2003)

**Manufacturing**

This sector includes chemical manufacturing of PTS, as well as the manufacturing of products that involves the use of materials that may be contaminated with PTS – for example:

- textile manufacturing
- chlorinated chemical production *i.e.* of chloro-aromatics (phenols, benzenes), oxy-chlorinators
- chlorine production using graphite electrodes
- oil refining and catalyst regeneration
- pulp and paper (elemental chlorine bleaching)
- pesticide production and formulation

**Thermal Processes**

Processes that involve high temperatures and usually combustion can lead to the formation and release of a suite of complex PTS, particularly PAH, PCDD/PCDF, PCB and HCB. Key variables to consider include the effectiveness of combustion, the addition of pollution controls and the nature of the materials being introduced. Also, in many parts of the world uncontrolled combustion processes and combustion process facilities not equipped with adequate air pollution control systems are likely to be a major source of these pollutants.

List 1.1 continued

*Thermal Manufacturing Processes*

- Metallurgical processes, primary processes, mainly copper, steel and aluminium, also zinc recovery from steel and other scrap recovery processes including aluminium, steel, copper, zinc, magnesium, lead and others (i.e. cable burning)
- coke production and carbo-chemical processes (especially using brown coal/lignite)
- mineral processing (especially cement kilns), asphalt mixing, production of lime, ceramic, glass,
- brick and other similar processes carried out at small-scale

*Controlled Combustion Processes*

- municipal (non-hazardous) waste incineration
- industrial waste combustion, including treated wood waste combustion
- hazardous waste incineration
- medical/clinical waste,
- sludge (non-hazardous) incineration
- coal combustion (large volumes)
- oil combustion (large quantities)
- wood/bio-mass (large and small scale quantities) combustion
- landfill gas/biogas
- crematoria and animal carcass burning

*Uncontrolled Combustion*

- bio-mass such as forest, bush, agricultural harvest residues (eg straw and sugar cane leaves)
- accidental fires, eg houses, industrial complexes etc
- landfill fires, unintentional and intentional
- combustion of other wastes, i.e. flaring of drilling mud, landfill gas etc., building waste and construction debris, domestic (backyard) waste burning
- plastic container/barrel burning
- hazardous waste/contraband CDs and DVDs/tires/rubber/cable
- e-waste - end-of-life electronic products
- general open burning

**Product application and use**

- transformers and electrical equipment

- PCB-paint use
- storage of products containing PTS, such as e-waste and contaminated feed

**Transport**

During the transport of PTS products and products containing PTS, accidents and spillages can and do occur.

**Recycling Processes (excluding thermal)**

- metals (incl. vehicle) recycling by-products such as shredder (mainly PCB), waste oil, scrap yards
- with stockpiles, refrigerator recycling, electronic scrap and circuit board recycling *etc*.
- paper recycling, especially de-inking sludges
- sewage sludge (including paper sludge) and effluent applications *i.e.* on land as agricultural
- solvent recovery processes and especially residue sludge from it
- waste oil recovery
- metal flyash recycling

**Waste Disposal (non-thermal waste disposal)**

- landfills (controlled and uncontrolled) of various waste types (municipal, hazardous *etc*.),
- contaminated incinerator ash, sludge, metal ash, also leaching from those landfills
- storage/stocks of transformers containing PCB-oil
- ocean dumping of solid/sludge/liquid wastes
- donations of equipment and contaminated oils to developing countries can result in toxic waste dumps.
- Reservoirs (potential for re-release subsequent to initial accumulation)
- soil and sediments
- waste and obsolete stockpiles
- PCP-treated wood i.e. telephone poles, railroad ties, etc.

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An assessment of the information above provides an indication of the direct and/or indirect involvement of water. In most cases manufacturing and processing plants are located close to, or are dependant on local water supply - with a concomitant potential for pollution by the POPs. This therefore provides one of the reasons for sampling PCB and PCDD/PCDF in the aquatic environment.

## 1.3 Aims of this project

With this project it was intended to contribute significantly towards the ability of South Africa to determine and interpret its commitments and obligations under the POPs

convention. It was intended to establish for the first time a countrywide assessment of POPs in a selection of major water bodies, and thereby indicating geographical areas where more action, management or research needs should be focussed. The project would also investigate cheaper means of analysis through bio-assays, since no laboratory in SA can currently perform routine PCDD/PCDF analysis (therefore the high analytical costs for this project). It would also establish a network of researchers that can form the nucleus of further collaboration and serve as a focus for levering international funding that will become available to developing countries after the convention has been ratified by South Africa.

With the above background in mind, the following aims were been identified:

1. To establish the presence and levels of 7 PCDD, 10 PCDF and 12 PCBs in fish from major South African rivers and estuaries (Chapter 4).

2. To determine the possible implications and future obligations for South Africa, of the presence and levels of these POPs under the international, legally binding, Persistent Organic Pollutant Convention (Chapters 5 and 6).

3. To establish the basis for further investigations, if levels found are deemed of concern (Chapter 6).

4. To investigate alternative and cheaper means of analysis for dioxins, PCDD/PCDF and PCBs in South Africa (Chapters 4, 5 and 6).

5. Through an initial risk assessment, based on analytical data from this project, establish the risk associated with the levels found (Chapter 5).

6. To develop a short course on environmental sampling and Good Laboratory Practice (accompanying CD).

Notes concerning addressing aims:

1) The envisaged sampling of fish from rivers and estuaries proved to be impractical (see Chapters 2 and 3). It was subsequently decided to use sediments as integrating accumulation of POPs. Samples were taken at sites where high releases of POPs to the water environment were expected to occur. These samples were analysed by a German laboratory, as accredited facilities are not yet available in South Africa (Aim 1).

Potential implications for South Africa of these findings (Aim 2) are discussed under conclusions (Chapter 6).

2) The short course on environmental sampling and Good Laboratory Practice (Aim 6) is available on the accompanying CD, as well as the text of the Stockholm Convention, and two recent UNEP/GEF reports on persistent toxic substances, that refer to PCB and PCDD/PCDF.

3) The initial project proposal referred to ELISA as an alternative and cheaper means of analysis. During the inception of the project, and because of attendance of the DIOXIN 2002 conference in Barcelona by both authors, the project team became aware of a more advanced bio-assay technique. With permission from the WRC and with additional support from NRF (Thuthuka), it was decided to investigate this technology (see 2.6).

# 2 LITERATURE REVIEW

## 2.1 Introduction

PCDD/PCDF are not produced intentionally. They are formed as by-products in industrial activities and all combustion processes (Fiedler, Hutzinger & Timms, 1990). Their general molecular structures are represented in Figure 2.1

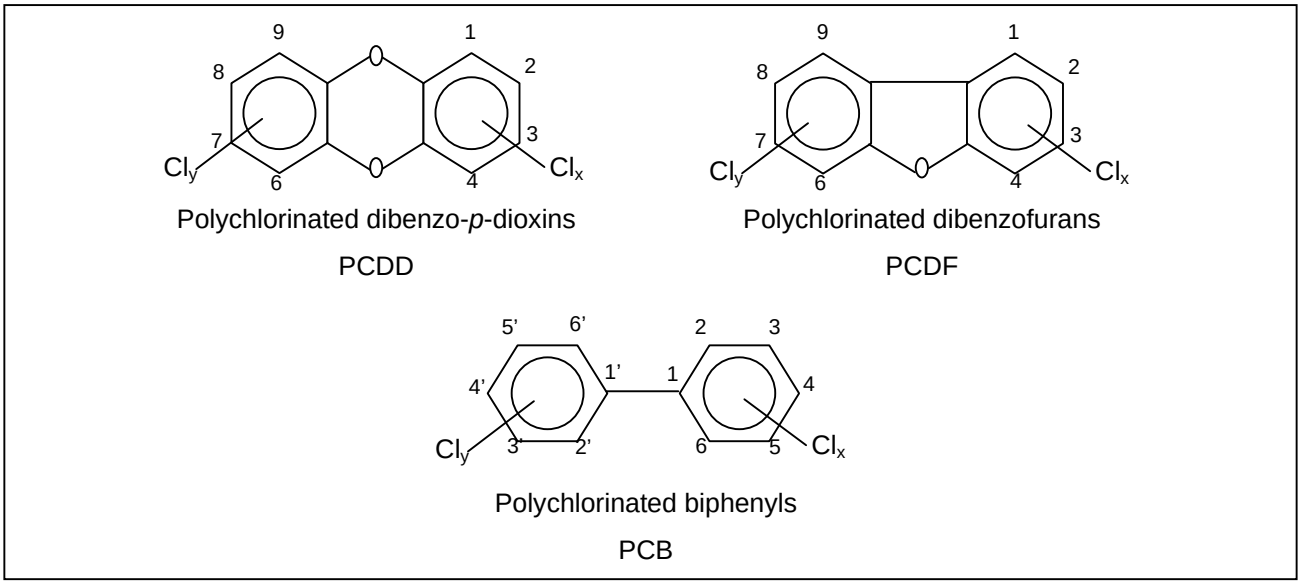


**Figure 2.1** Structural formula of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls.

Each of the generalised PCDD/PCDF-molecules can have chlorine atoms attached at the different numbered positions in a variety of combinations creating a possible 210 different molecules (75 PCDD- and 135 PCDF congeners). Only 17 of these molecules (Table 2.1) are significantly toxic to many laboratory animals and they all have chlorine atoms in the 2,3,7 and 8 positions of the parent molecule (Fiedler, 2003).

PCB are produced intentionally but also unintentionally as a result of incomplete combustion or other chemical processes. Depending on the number of chlorine substituents and their position at the two rings (Figure 2.1), there are 209 congeners theoretically possible. From a toxicological point of view, 12 non-*ortho*- and mono-*ortho*- PCB-congeners (Table 2.1) are of special interest because these show toxicological properties that are similar to dioxins. These congeners are often called “dioxin-like PCB” (Päpke & Fürst, 2003).

**Table 2.1** The PCB, PCDD/PCDF-congeners regarded as the most toxic of the possible congeners (WHO, 1997).

| PCB | PCDD | PCDF |
|---|---|---|
| 3,3',4,4'-$Cl_4$B | 2,3,7,8-$Cl_4$DD | 2,3,7,8-$Cl_4$DF |
| 3,4,4',5-$Cl_4$B | 1,2,3,7,8-$Cl_5$DD | 1,2,3,7,8-$Cl_5$DF |
| 3,3',4,4',5-$Cl_5$B | 1,2,3,4,7,8-$Cl_6$DD | 2,3,4,7,8-$Cl_5$DF |
| 3,3',4,4',5,5'-$Cl_6$B | 1,2,3,6,7,8-$Cl_6$DD | 1,2,3,4,7,8-$Cl_6$DF |
| 2,3,3',4,4'-$Cl_5$B | 1,2,3,7,8,9-$Cl_6$DD | 1,2,3,6,7,8-$Cl_6$DF |
| 2,3,4,4',5-$Cl_5$B | 1,2,3,4,6,7,8- $Cl_7$DD | 1,2,3,7,8,9-$Cl_6$DF |
| 2,3',4,4',5-$Cl_5$B | $Cl_8$DD | 2,3,4,6,7,8-$Cl_6$DF |
| 2',3,4,4',5-$Cl_5$B | | 1,2,3,4,6,7,8-$Cl_7$DF |
| 2,3,3',4,4',5-$Cl_6$B | | 1,2,3,4,7,8,9-$Cl_7$DF |
| 2,3,3',4,4',5'-$Cl_6$B | | $Cl_8$DF |
| 2,3',4,4',5,5'-$Cl_6$B | | |
| 2,3,3',4,4',5,5'-$Cl_7$B | | |

## 2.2 Properties

The water solubility of PCB decreases with increasing chlorination: 0.01 to 0.0001 µg/L at 25°C for one to ten chlorines. The vapour pressure varies between 2.1 x $10^{-4}$ and 4 x $10^{-3}$ Pa at 20°C and the log $K_{OW}$[2] from 4.3-8.26 (Whiley *et al*., 2003). PCB congeners lacking adjacent unsubstituted positions on the biphenyl rings such as 2,4,5- 2,3,5- or 2,3,6-substituted on both rings are extremely persistent in the environment. Their estimated half-lives range from three weeks to two years in air and, with the exception of mono- and di-chlorobiphenyls, more than six years in aerobic soils and sediments. PCB also have extremely long half-lives in adult fish: the half-life of PCB153 (the number refers to the IUPAC[3] number of the compound) was more than 10 years in eels (Whiley *et al*., 2003) and based on the assumption that the kinetics are first order, a half-life of 9 years were estimated for PCB in the anaerobic conditions at the bottom

[2] The lipophilicity of a molecule is measured by determining its relative solubility in water and octanol. If the molecule prefers water it is unlikely to concentrate in cell lipids, if it prefers the organic solvent it is likely to seek out the lipids. The concentrations of the test chemical in both the octanol and water are determined and calculated as $\log_{10}([X]_{octanol}/[X]_{water})$ where X represents the test chemical. Log $K_{OW}$ > 3 are regarded as bio-accumulative.

[3] International Union of Pure and Applied Chemistry

of Lake Ketelmeer, the Netherlands (Beurskens, Mol, Barreveld, Van Munster & Winkels, 1993). Su & Finley (in press) presented half-lives for the congeners under discussion in the surface sediment of the Passaic River (USA). The half-lives for the PCDD/PCDF varied from 8.5 years for 2,3,7,8 TCDD to 29 years for two HxCDD and one HxCDF congeners. The half-lives of the PCB-congeners varied from 16 years for PCB77 to 36 years for PCB189.

The range of water solubility for the PCDD/PCDF lies between 0.43 and 0.0002 ng/L at 25°C. The vapour pressure differs from 2 to 0.007 x $10^{-6}$ mm Hg at 20°C and the log $K_{OW}$ ranges from 6.60 to 8.20 for tetra- to octa-substituted congeners (Whiley *et al*., 2003). PCDD/PCDF are characterised by their lipophilicity, semi-volatility and resistance to degradation (half-life of TCDD in soil of 10 to 12 years) and to long-range transport. They are also known for their ability to bio-concentrate and bio-magnify under typical environmental conditions (Whiley *et al*., 2003). They prefer to bind to organic matter in soil and sediments (log $K_{OC}$[4] values for 2,3,7,8 TCDD are between 6.4 and 7.6) (Fiedler, 2003).

### 2.3 Environmental fate

PCDD/PCDF and PCB are multimedia pollutants, and once released into the environment, become distributed between environmental compartments. PCDD/PCDF and dioxin-like PCB are primarily bound to particulate and organic matter in soil and sediment. In biota, they are mainly concentrated in fatty tissues. Because these compounds are semi-volatile, they can exist in both the gaseous phase and bound to particles. Especially during warmer seasons the lower chlorinated congeners tend to be in the gaseous phase (fugacity). In the vapour phase they can undergo photochemical transformation, losing chlorine atoms. If this happens to octa- and heptachlorinated congeners, they degrade to the more toxic penta- and tetrachlorinated congeners, before finally degradation to non-toxic compounds with only three or less chlorine atoms (Carey, Cook, Giesy, Hodson, Muir, Owens & Solomon, 1998)

Because of their chemical characteristics and very low solubility these compounds accumulate in most soil types, with very little water leaching and negligible degradation of the 2,3,7,8-substituted PCDD/PCDF-congeners. (This finding, however, has not

[4] $K_{OC}$ is the sediment/water partition coefficient in terms of organic carbon content. It is known that the organic carbon in sediments has a strong capacity to sorb lipophilic compounds but does not have as strong a capacity as fat or lipid.

been substantiated for South African conditions.) They adsorb quickly to organic matter and so accumulate in sediments. They accumulate in aquatic fauna as a result of the ingestion of contaminated organic matter (Fiedler, 2003). The concentration of PCDD/PCDF and dioxin-like PCB in fish tissue is found to bio-magnify in the food web as a progressive ingestion of contaminated prey (EC, 1999).

In the terrestrial food chain (air→grass→cattle→milk/meat→man) these compounds are deposited on plant surfaces via wet deposition, via dry deposition of chemicals bound to atmospheric particles, or via diffusive transport of gaseous chemicals in the air to plant surfaces. Dry particle-bound deposition is mainly responsible for the uptake of the higher chlorinated compounds (six and more chlorines) while dry gaseous deposition plays the dominant role for the accumulation of the lower chlorinated compounds (Fiedler, 2003). The accumulation of the gas-phase concentrations by plant surfaces is affected by temperature, which influences gas-particle partitioning and air-leaf exchange (Paterson, *et al.* 1991, 1994). Grazing animals are exposed to dioxins by ingesting contaminated pasture crops.

Once absorbed by the gastrointestinal tract, the compounds are transported, mainly by binding to lipoproteins in the blood, to different tissues and organs in the body. Bio-accumulation in mammals is observed not only in adipose tissue or blubber but also in the liver, bone marrow and brain tissue (Carey *et al*., 1998).

In all species, chlorinated aromatic compounds accumulate in tissues in proportion to the percentage of lipid. Exceptions are the 2,3,7,8-substituted PCDD/PCDF, which also strongly accumulate in the livers of mammals and birds. This greater liver retention has, in part, been attributed to the presence of inducible binding proteins for these compounds, such as cytochrome P4501A2 (Poland, Teitelbaum & Glover, 1989). PCB, PCDD/PCDF are non-polar compounds that cannot be excreted or transformed to polar excretable compounds without the introduction of a polar functional group through metabolism. None of these compounds are metabolised at a significant rate by invertebrates, and therefore they bio-magnify through the diet in invertebrate food chains (Thomann, 1989; Gobas, 1993).

PCB that are easily metabolised by *meta*- and *para*-epoxidation and possess 2,5 or 2,3,6 chlorine substitution on the same ring, tend to form persistent methyl sulphone metabolites through a complex process involving substitution of glutathione at the 3 or

4 positions, excretion of the mercapturic acid, cleavage by gut microflora C-S lyase, resorption, methylation, and hepatic oxidation to 3- and 4-methyl sulphone PCB (Opperhuizen & Sijm, 1990). Many of these compounds are resistant to further metabolism and are sufficiently hydrophobic that they are not excreted.

PCDD/PCDF that do not possess chlorines at all four 2,3,7 and 8 positions do not bio-accumulate in vertebrates (except cetaceans). Cetaceans appear able to metabolise (or excrete) 2,3,7,8-substituted PCDD/PCDF and accumulate only the non-2,3,7,8-subsituted congeners (Norstrom, Simon & Muir, 1990).

Clearance rates of 2,3,7,8-substituted PCDD/PCDF are low enough (except in cetaceans) that bio-magnification occurs in food chains, in the order of 3- to 10- fold per trophic level (Endicott & Cook, 1994).

PCB bio-accumulate in invertebrates and most fish with little metabolic alteration of the compounds. Thus, excretion of less chlorinated compounds may occur across the gills in fish, but generally PCB are bio-accumulated by fish with little change in patterns (Norstrom, 1988). Vertebrates higher in order than fish, including reptiles, bird, and mammals (except marine mammals), possess hepatic cytochrome P450 mono-oxygenase enzyme systems that are efficient at degrading certain PCB-congeners. C4502B-enzymes are responsible for metabolism of PCB-congeners that are not substituted by chlorine at one or more *meta*- and *para*-positions. Thus, PCB-congeners with at least one unsubstituted *meta*- and *para*-positions are not bio-magnified in higher animals. As for PCDD/PCDF, cetaceans have a metabolic capability different from other mammals (Tanabe, Watanabe, Kan & Tatsukawa, 1988). They do not degrade *meta*- and *para*-unsubstituted PCB congeners to any extent.

## 2.4 Toxic effects

### 2.4.1 Non-specific toxicity (narcosis)

Narcosis occurs when the dose of compound in the organism reaches a threshold concentration, the critical body residue (CBR). Narcotic potency is related to molecular volume and partitioning properties. It is not a function of the structure of the chemical, nor has it anything to do with the substitution of chlorine or any other substituent group unless this changes the partitioning properties. Acute narcotic effects are similar in

most organisms, with an initial excitatory phase, followed by an activity depression phase and, with sufficient dose and duration, death (Carey *et al*., 1998).

Most organic chemicals can cause narcosis, and this mode of action can be found in all organisms when sufficient exposure occurs. Although well-described in laboratory toxicity testing, and important in some site-specific situations and spill events, organochlorine-induced narcosis does not appear to be a serious environmental problem (Carey *et al*., 1998)

### 2.4.2 Endocrine toxicology

Collectively, chemicals with the potential to interfere with the function of endocrine systems are called endocrine disrupting chemicals (EDCs) (Kavlock, 1999). EDCs have been defined as exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (Kavlock, Daston, DeRosa, Fenner-Crisp, Gray, Kaattari, Lucier, Luster, Mac, Maczka, Miller, Moore, Rolland, Scott, Sheenan, Sinks & Tilson, 1996).

Hormone action is mediated by hormone binding to specific hormone receptors in target tissues. Estradiol, for example, binds to its nuclear receptor in an estrogen target tissue such as the liver, causing a conformational change termed “activation” that enables the receptor to bind to specific acceptor sites on the DNA in the nucleus. This results in alteration of gene transcription, such as in the vitellogenin gene (Carey *et al*., 1998).

PCB-mixtures, particularly those with lower chlorine contents (up to 48%) have weak estrogenic activity (Bulger & Kupfer, 1985). PCB show weak binding affinity to estrogen receptors with a potency 10 000- to 100 000-fold less than estradiol (Nelson, 1974). PCB may alter reproductive endocrine function directly by altering the function of neurotransmitters involved in the regulation of gonadotropin hormone secretion (Thomas, 1989). Enhanced metabolic clearance of steroids and a decline in steroid levels, due to induction of cytochrome P450, has been shown for AhR-active PCB. However, compensatory mechanisms of the hypothalamus-pituitary-gonadal axis may eventually overcome this clearance, so that reproduction is delayed but not prevented (Thomas, 1989).

### 2.4.3 AhR-mediated toxic responses

PCDD/PCDF and planar PCB, act through the aryl hydrocarbon receptor (AhR) common to fish, birds, and mammals. AhR-mediated responses appear to include lethality, reproductive and developmental toxicity, immunotoxicity and cancer (Carey *et al*., 1998).

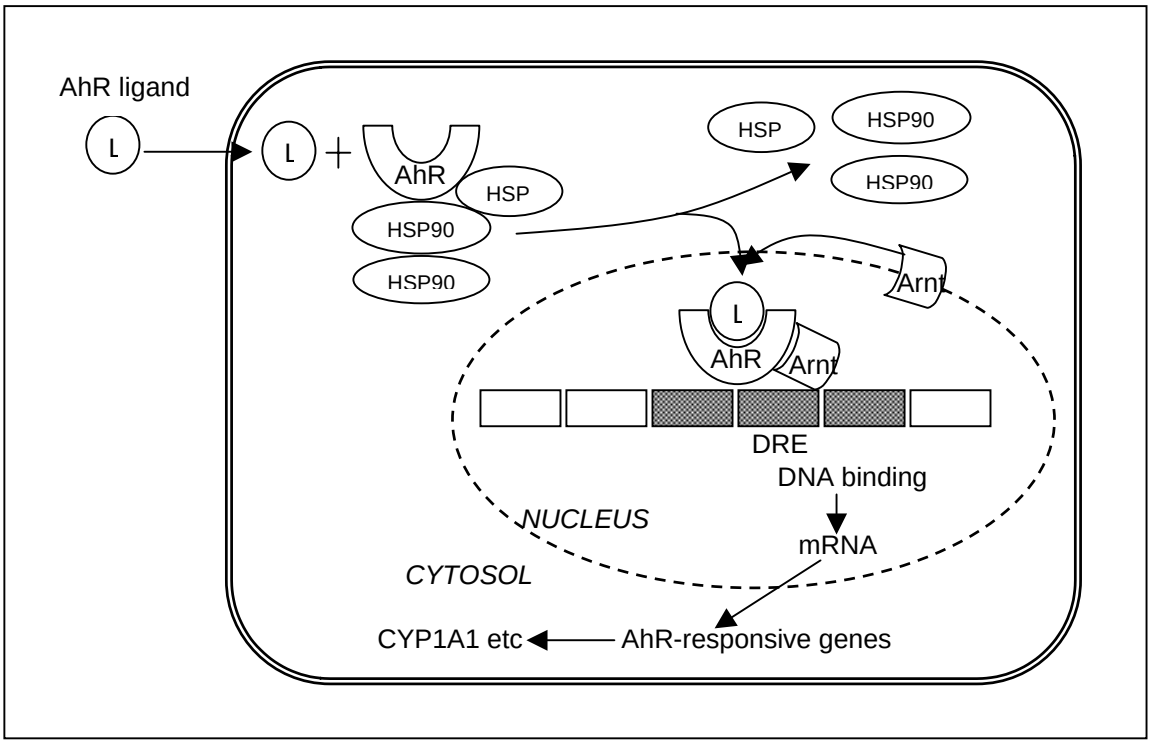


**Figure 2.2** Mechanism of aryl hydrocarbon receptor (AhR)-mediated response.

[HSP = heat shock protein; L = ligand; DRE = dioxin response element; Arnt = Ah receptor nuclear translocator] (Adapted from Hilscherova, Machala, Kannan, Blankenship & Giesy, 2000).

The initial step in the action of these compounds is stereospecific binding with greater affinity to the cytosolic AhR. The AhR, which belongs to the basic helix-loop-helix protein family (Zacharewski, Behane, Gilleby & Burnison, 1995) is a ligand-dependent transcription factor located in the cytosol, complexed with heat shock proteins (Figure 2.2). It has been shown that the strength with which congeners bind to the AhR is directly proportional to the (i) toxicity, (ii) enhanced gene transcription and (iii) enzyme activities mediated by the AhR mechanism (Safe, 1995).

After binding of ligand to cytosolic AhR, heat shock proteins dissociate from the complex, the receptor ligand complex is activated and translocated to the nucleus,

where it forms a dimer with the Ah receptor nuclear translocator (Arnt) protein and possibly other factors (Figure 2.2). The heteromeric ligand:AhR:Arnt complex binds with high affinity to specific DNA sequences, the dioxin-responsive element (DRE) (Denison & Heath-Pagliuso, 1998; Hankinson, 1995). The ligand-AhR-Arnt complex binds to dioxin-responsive elements on DNA causing the transcription of such genes in the AhR gene battery as cytochrome P450A 1A1 (*CYP1A1*) and cytochrome P450 1A2 (*CYP1A2*) (Figure 2.2) (Fiedler, 2003).

Binding with the AhR is a necessary step forward, but is not sufficient to produce, toxicity. The ligand-responsive genes that cause the various signs of toxicity in a highly tissue-specific and species-specific manner are currently unknown (Carey *et al.,* 1998).

Certain non-chlorinated environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs), bind to the AhR and elicit the prototypical, non-toxic, dioxin-like response: induction of cytochrome P450 1A1 (Eisen, Hannah, Legraverend, Okey & Nebert, 1983). In general, both the PAHs and the indolecarbinols have lower binding affinity for the AhR than TCDD and fail to produce a sustained effect on gene expression because they are rapidly bio-transformed to non-binding metabolites. Consequently, they fail to elicit the entire spectrum of dioxin-like toxic effects. TCDD and related coplanar chemicals cause, in addition to P450 1A1 induction, delayed-onset toxic effects that are the characteristic of sustained-action, polychlorinated AhR agonists (Carey *et al.*, 1998).

Functional AhR has been detected in teleost and elasmobranch fishes, birds, and mammals (including humans) but not in plants, 9 species of invertebrates representing 8 classes of 4 phyla, or in primitive fishes such as hagfish, lamprey, or fishes in the class Agnatha (USEPA, 1993). Depending on dose and species sensitivity, the symptoms of toxic response caused by TCDD and related compounds can include the following: lethality, body-weight loss, immuno-suppression, oedema, dermal toxicity, developmental toxicity, reproductive toxicity, neurobehavioural toxicity, liver toxicity, endocrine toxicity, alterations in lipid metabolism and gluconeogenesis, modulation of responsiveness to hormones and growth factors, alterations in circulation levels of hormones, thymic atrophy, cancer, tumour promotion and induction of various enzyme activities, including aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-

deethylase (EROD) that are associated with induction of cytochrome P450 1A1 (Poland & Knutson, 1982).

Because TCDD is the most potent substance that acts by an AhR mechanism, it is used as the prototype to describe four types of ecologically relevant effects: mortality, reproductive toxicity, developmental toxicity, and decreased growth. All of these effects are considered ecologically significant because they have the potential of ultimately reducing populations of fish, birds, and mammals exposed to TCDD and related compounds. In general, AhR-agonists, which produce sustained AhR-mediated responses, will cause the same profile of dose-related, specific effects as TCDD on mortality, reproduction, embryonic and foetal development, and growth. Thus, no-observed-adverse-effect levels (NOAELs) and lowest-observe-adverse-effect levels (LOAELs) for these endpoints in fish, birds and animals can be determined to assess ecotoxicological risk (Carey *et al*., 1998).

Following exposure to high concentrations of TCDD, delayed-onset mortality occurs in most species. Wide species differences in susceptibility to mortality are seen. The LD50 of TCDD in the hamster is three orders of magnitude greater than that in the guinea pig and mink (Geyer, Scheunert, Rappe, Kettrup, Korte, Greim & Rozman, 1990). The ultimate cause of death due to TCDD exposure has not been fully elucidated, but among mammalian species, it is invariably preceded by a wasting syndrome in which animals can lose up to 50% of their body weight (Seefeld, Corbett, Keesey & Peterson, 1984).

TCDD exposure causes female reproductive toxicity in various mammalian species (Peterson, Theobald & Kimmel, 1993), including reduced fertility, reduced litter size, effects on the gonads of females, alterations in menstrual or oestrus cycles, and alterations in responsiveness to estrogen. The most sensitive sign of reproduction toxicity, however, is embryo mortality (Peterson & Walker, 1992). In male animals, TCDD and related compounds decrease testis and accessory sex organ weights, cause abnormal testicular morphology, decrease spermatogenesis, and reduce fertility when given to adult animals in doses that are in the overtly toxic range (Carey *et al.*, 1998).

The most sensitive life-stages in an animal's life history, are the foetal and embryonic stages. In salmonic sac fry, the classical TCDD toxicity syndrome is characterised by

pericardial and yolk-sac oedema, subcutaneous haemorrhages, craniofacial malformations, and arrested growth and development, culminating in death (Spitsbergen, Walker, Olson & Peterson, 1991).

In birds, the LD50 of TCDD in the chicken embryo is less than 1/100 of that needed to cause mortality in the adult chicken. Birds exhibit developmental toxicity in different ways (Peterson *et al.*, 1993). In the chicken embryo, pericardial and subcutaneous oedema, liver lesions, lymphoid toxicity, structural malformations, and mortality occur while in other birds, many of these embryo toxic effects are not seen. Embryo mortality is the only toxic effect that appears common to all bird species (Carey *et al*. 1998).

In mammals TCDD and the likes of TCDD can adversely affect pregnancy maintenance, embryo or foetotoxicity, and postnatal development. Gestational exposure to dioxin-like substances produces a characteristic pattern of foetotoxic responses that consist of thymic hypoplasia, subcutaneous oedema, and decreased foetal growth (Peterson *et al*., 1993).

Decreased growth is generally observed in all classes of vertebrates that possess the AhR after exposure to overtly toxic doses of TCDD. This response tends to be more apparent in mammals, possibly because the presence of oedema in birds and fish masks the reduction in body tissue mass. The wasting syndrome is caused to a large extent, by dose-related decreases in feed intake (Seefeld *et al*., 1984), but alterations in intermediate metabolism are also involved.

TCDD has been demonstrated to be a potent animal carcinogen (Kociba, Keyes, Beyer, Carreon, Wade, Dittenber, Kalnins, Frauson, Park, Barnard, Hummel, Humiston, 1978) and a mixture of hexachlorinated dibenzo-*p*-dioxins has also been shown to be carcinogenic (National Toxicology Program [NTP], 1980). In addition, mixtures of higher-chlorinated PCB (Aroclor 1260) are carcinogenic in rodent assays. Subsequently, TCDD, several other PCDD/PCDF, and non- and *mono-ortho*-substituted PCB have been demonstrated to act as tumour promoters (reviewed by Carey *et al*., 1998). Although carcinogenicity is of human health relevance, its significance for assessing ecotoxicological risk of TCDD and related chemicals is less clear.

In several mammals, PCDD, PCDF, and PCB have interfered with the turnover of vitamin A and this effect is related to the toxic potency of these compounds (Brunström, Håkansson & Lundberg, 1991). Mammalian studies indicate that metabolites of the lower-chlorinated PCB and dioxins can interact with the thyroid-hormone transport protein (transthyretin). This protein is involved with vitamin A transport. As a result of this interaction, changes in thyroid hormone as well as vitamin A metabolism occur. Although reductions in thyroid hormone and vitamin A levels have been observed in marine mammals and fish-eating birds, the toxicological relevance of this mechanism of action should be validated. In addition, interference with thyroid-hormone metabolism can also be caused by induction of the thyroid-hormone glucuronidation. Laboratory studies with rodents indicate that increased glucuronidation might also be AhR-mediated (Carey *et al.*, 1998).

Several studies have shown that, after exposure to dioxins and PCB, mammals, birds, and fish can haemorrhage. Laboratory studies with rats show that TCDD as well as 2,2',4,4',5,5'-hexachlorobiphenyl (HxCB) interfere with several enzymes in the hepatic vitamin K-cycle. As a result, vitamin K-dependent, blood-coagulation factors are decreased in plasma. Dose-response studies with TCDD and 2,2',4,4',5,5'-HxCB indicate that a nonAhR mechanism might be involved, as the latter compound, an extremely weak AhR agonist, was more potent than TCDD (Bouwman, Seinen, Koppe & Van den Berg, 1992).

### 2.4.4 Immunotoxicity

Among organochlorine compounds tested to date, PCB and dioxins are the most potent immunotoxicants (active at low doses, causing a range of effects and multispecies toxicities). Immunotoxicity has been defined as the adverse effects on the immune system of foreign substances (Trizio, Basketter, Botham, Graepel, Lambre, Magda, Pal, Riley, Ronneberger, Van Sittert & Bontinck, 1988) and is classified in two main categories: (i) the alterations of normal immune responses through an immunodepression or an immunopotentiation that will consequently compromise the host's defence mechanisms and (ii) the induction of abnormal immune responses such as various types of hypersensitivity (allergy and autoimmunity) (Bennett and Mercier, 1987).

High PCB-exposure resulted in (i) structural alterations of immune system organs (loss of thymic cortical lymphocytes, reduction of germinal centre size, and reduction of leucocytes and T-lymphocyte counts in peripheral blood) and (ii) altered functional reactivity of the immune system, characterised by reduced antibody production against foreign antigens and reduced skin reactivity to specific antigens. These effects have been reproduced in several species: fishes, birds, rodents and non-human primates (Tryphonas, 1995). Functional effects were also observed in the mononuclear phagocytic cells and natural killer-cell activity and in increased susceptibility to normally tolerated doses of bacterial, viral, and parasitic infestations. *Eg*., monkeys chronically exposed to PCB, levels of antibodies to the T-dependent antigen sheep red blood cells were reduced by doses as low as 5 µg/kg body weight/d (Tryphonas, Hayward, O'Grady, Arnold, Bryce & Zawidzka, 1989).

In general, higher chlorinated PCB-mixtures are more immunotoxic than lower-chlorinated mixtures. Many of the immunotoxic effects of PCB-congener mixtures depend on the presence of the AhR and of the ability of the PCB to bind to this receptor. The molecular events following the binding of PCB to the receptor are believed to be similar to those of dioxin. Other nonAhR-interacting PCB-congeners also cause immunotoxicity, suggesting a different mechanism of action (Carey *et al*., 1998).

The immunotoxicity of TCDD depends on the AhR. In mice, the generation of a primary antibody response appears to be particularly sensitive to suppression when mature animals are exposed to TCDD. The effect on antibody production may result from effects on both T- and B- lymphocytes in rats (Birnbaum, 1994). These findings cannot be generalised to other species, since the rat is less sensitive. Although most macrophage functions appear to be resistant to TCDD-induced alterations, recent studies indicate that macrophage production of inflammatory cytokines is enhanced following TCDD exposure (Carey *et al*., 1998).

### 2.5 TCDD toxic equivalents

Because AhR-agonists act by a common mechanism, the effects of exposure to complex mixtures of these compounds are generally considered to be additive, on the basis of toxic equivalents (TEQs). Thus, although individual compounds in a mixture

may not be present at a toxic dose, the combined sub-toxic doses of individual AhR-agonists could result in toxicity.

The potential toxicity of mixtures of AhR-agonists in environmental samples has been evaluated using the concept of "TCDD equivalents", in which each compound is assigned a TEF (toxic equivalency factor), indicating its toxic potency relative to TCDD. The value of the TEF usually is determined based on short-term toxicity tests and sometimes even on *in vitro* tests. The concentration of each individual compound in a sample is then multiplied by its appropriate TEF to give its concentration as a TCDD TEQ. The TEQs of all substances are then added to give the total TEQ concentration of the mixture (Carey *et al*., 1998).

As TEFs are interim values and administrative tools, they are based on present state of knowledge and should be revised, as new data becomes available. Today's most commonly applied TEFs were established by NATO/CCMS Working Group on Dioxins and Related Compounds (1988) as International Toxicity Equivalency Factors (I-TEF). However, as can be observed from Table 2.2 the I-TEF list does not include values for the PCB congeners. However, in 1997, a WHO/IFCS (World Health Organisation / Intergovernmental Forum on Chemical Safety) working group re-evaluated the I-TEFs and established a scheme, which besides human mammalian TEFs also established TEFs for birds and fish (Table 2.2). The same expert group also assessed the dioxin-like toxicity of PCB and assigned TEF-values for 12 coplanar and mono-*ortho*-substituted PCB-congeners (WHO, 1997)

**Table 2.2** International Toxicity Equivalency Factors (I-TEFs) for PCDD/PCDF (NATO/CCMS, 1988) and WHO-TEFs for PCDD/PCDF and PCB (WHO, 1997).

| **Congener** | **I-TEF** | **WHO-TEF** | | |
|---|---|---|---|---|
| | | **Humans/Mammals** | **Fish** | **Birds** |
| 2,3,7,8-$Cl_4$DD | 1 | 1 | 1 | 1 |
| 1,2,3,7,8-$Cl_5$DD | 0.5 | 1 | 1 | 1 |
| 1,2,3,4,7,8-$Cl_6$DD | 0.1 | 0.1 | 0.5 | 0.05 |
| 1,2,3,6,7,8-$Cl_6$DD | 0.1 | 0.1 | 0.01 | 0.1 |
| 1,2,3,7,8,9-$Cl_6$DD | 0.1 | 0.1 | 0.01 | 0.01 |
| 1,2,3,4,6,7,8- $Cl_7$DD | 0.01 | 0.01 | 0.001 | <0.001 |
| $Cl_8$DD | 0.001 | 0.0001 | - | - |

| | | | | |
|---|---|---|---|---|
| 2,3,7,8-$Cl_4$DF | 0.1 | 0.1 | 0.05 | 1 |
| 1,2,3,7,8-$Cl_5$DF | 0.05 | 0.05 | 0.05 | 0.1 |
| 2,3,4,7,8-$Cl_5$DF | 0.5 | 0.5 | 0.5 | 1 |
| 1,2,3,4,7,8-$Cl_6$DF | 0.1 | 0.1 | 0.1 | 0.1 |
| 1,2,3,6,7,8-$Cl_6$DF | 0.1 | 0.1 | 0.1 | 0.1 |
| 1,2,3,7,8,9-$Cl_6$DF | 0.1 | 0.1 | 0.1 | 0.1 |
| 2,3,4,6,7,8-$Cl_6$DF | 0.1 | 0.1 | 0.1 | 0.1 |
| 1,2,3,4,6,7,8-$Cl_7$DF | 0.01 | 0.01 | 0.01 | 0.01 |
| 1,2,3,4,7,8,9-$Cl_7$DF | 0.01 | 0.01 | 0.01 | 0.01 |
| $Cl_8$DF | 0.001 | 0.0001 | 0.0001 | 0.0001 |
| 3,3',4,4'-$Cl_4$B (PCB77) | | 0.0001 | 0.0001 | 0.05 |
| 3,4,4',5-$Cl_4$B (PCB81) | | 0.0001 | 0.0005 | 0.1 |
| 3,3',4,4',5-$Cl_5$B (PCB126) | | 0.1 | 0.005 | 0.1 |
| 3,3',4,4',5,5'-$Cl_6$B (PCB169) | | 0.01 | 0.00005 | 0.001 |
| 2,3,3',4,4'-$Cl_5$B (PCB105) | | 0.0001 | <0.000005 | 0.0001 |
| 2,3,4,4',5-$Cl_5$B (PCB114) | | 0.0005 | <0.000005 | 0.0001 |
| 2,3',4,4',5-$Cl_5$B (PCB118) | | 0.0001 | <0.000005 | 0.00001 |
| 2',3,4,4',5-$Cl_5$B (PCB123) | | 0.0001 | <0.000005 | 0.00001 |
| 2,3,3',4,4',5-$Cl_6$B (PCB156) | | 0.0005 | <0.000005 | 0.0001 |
| 2,3,3',4,4',5'-$Cl_6$B (PCB157) | | 0.0005 | <0.000005 | 0.0001 |
| 2,3',4,4',5,5'-$Cl_6$B (PCB167) | | 0.00001 | <0.000005 | 0.00001 |
| 2,3,3',4,4',5,5'-$Cl_7$B (PCB189) | | 0.0001 | <0.000005 | 0.00001 |

## 2.6 Methods for determining environmental concentrations

Chemical analysis measured each individual congener and multiplies with the TEF-values to the resulting dioxin toxicity equivalents (TEQ) value. If the I-TEF is used in the calculation, I-TEQ is calculated, also implying that the contribution of the PCB-congeners was not taken into account. In most literature, when reference is made to TEF and TEQ, the TEF-values suggested by the WHO (Table 2.2) were used. Most bio-analytical detection methods measure the toxicity (e.g. AhR binding capacity and/or P450 (CYP1A1)-inducing potency) of the complex-mixture and are expressed in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents, including all compounds with

dioxin-like properties (Van den Berg, Peterson, & Schrenk 2000., Hilscherova *et al*., 2000).

### 2.6.1 Chemical analysis

The concentrations of these pollutants in environmental matrices can be determined by chemical analyses and / or employing any number of bio-assays. Chemical analyses are done by gas chromatography and mass spectrometry and the concentration for each congener can be determined very accurately. Quantitatively speaking, chemical analysis is the best method to determine the concentrations of these particular environmental pollutants, but the clean-up procedures can be time consuming as well as very costly (Hilscherova *et al*., 2000). Instrumentation analyses do not account for interactions among the chemicals in complex mixtures and provide little information on their biological effects. Thus, chemical analyses can underestimate the potential risks posed by these chemicals. (Behnisch, Hosoe & Sakai, 2001; Hilscherova *et al*., 2000).

In calculating the TEQ-value using concentrations determined by chemical analysis, one presumes that the effect caused by each congener is additive (WHO, 2000).

### 2.6.2 Bio-analysis

We have considered a variety of other tests such as typical acute toxicity tests, but from literature and advice from experts, we note that the danger of these chemicals is mainly expressed at a sub-lethal level. The toxicity should therefore be expressed on a sub-lethal manner. *In vitro* cell bio-assays offer a rapid, sensitive and relatively inexpensive solution to some of the limitations of instrumental analysis. They enable estimation of total biological activity of all compounds that act through the same mode of action present in extracts of any environmental media. Bio-assays also integrate possible interactions among chemicals. *In vitro* methods, while a useful adjunct to instrumental analyses are not a substitute for *in vivo* toxicological studies. The *in vitro* assay used in this research, is useful as a sensitive integrating method that provides quantitative estimates of the total activity of particular receptor-mediated responses. However, since it does not account for bio-availability and predict WHO-organism responses, the results of the *in vitro* bio-assays provide information that is similar to that provided by instrumental analyses. Just as with information on the concentration of residues obtained from instrumental analyses, the results of the bio-assay need to be compared

to appropriate toxicant reference doses, determined from *in vivo* studies (Hilscherova e*t al.*, 2000).

In § 2.4.3 the AhR-mediated response of PCB, PCDD/PCDF, was explained. Bio-assays measure different endpoints in this response. This response results in the induction of the cytochrome P450 enzyme(s) (Figure 2.2.). Cytochrome P450 (or simply P450) refers to a family of enzymes that transform the structure of organic chemicals. The reactions catalysed by these proteins are of a type generally referred to as mixed-function oxidase (MFO) or monooxygenase reactions. Some types of P450 can be induced (increased) in response to an organism's exposure to many types of chemicals. P450-induction can also serve as a highly sensitive indicator of an organism's toxic burden, or the extent to which it has been exposed to chemical inducers in the environment. The endpoints that can be measured in bio-assays, are: (i) increasing catalytic activity, (ii) detecting the protein immunochemically, (iii) employing DNA probes for P450 mRNA (Stegeman, Brouwer, Di Guilio, Förlin, Fowler, Sanders, & Van Veld, 1992).

(i) Catalytic activity

PAHs and halogenated hydrocarbons such as PCB, PCDD/PCDF cause increased MFO-activity of P450. Two activities catalysed by P450, ethoxyresorufin *O*-deethylase (EROD) activity and aryl hydrocarbon (benzo[a]pyrene) hydroxylase (AHH) activity are largely specific in their response to these compounds. The EROD bio-assay is either a fluorometric or a spectrophotometric assay and the AHH-assay either fluorometric or radiometric (Stegeman *et al.*, 1992).

AHH and EROD-activity can either be measured directly from the tissues and organs of exposed animals collected from the environment or tissue cultures can be exposed to extracts from the environment in the laboratory (Schramm, Klimm, Hofmaier & Kettrup, 2001).

(ii) Immunochemistry

Detecting the protein level of P450 is done by using monoclonal or polyclonal antibodies in Western blots, dot or slot blots, ELISA (enzyme-linked immunosorbent assay). Immunohistochemistry may be particularly useful for analysis of embryos or some organs for which other bio-chemical assays would be very difficult. Analysis of induction by immunohistochemical assay has the further advantage in that it can reveal

the cell-types where induction occurs. This could be important to evaluating the effects of induction in a given organ, and could show the route of exposure (Hahn, Woodin, Stegeman & Tillitt, 1998; Stegeman *et al*., 1992). As for catalytic activity, measurements of P450 can be done either *in vivo* (as described in the previous paragraph) or *in vitro* again, exposing environmental extracts to tissue cultures (Hahn, Woodward, Stegeman & Kennedy, 1996).

In competitive immunoassays, PCB in sample extracts compete with enzyme (horseradish peroxidase)-labelled PCB for a limited number of antibody binding sites on the inside surface of the test tube and, as with all competitive immuno-assays, sample concentration is inversely proportional to colour development. This type of assay is limited to the number and specificity of the antibodies that were created (EnviroLogix, 2003). In a study by Shan, Leeman, Gee, Sanborn, Jones, Chang & Hammock (2001) extracts from fish and egg samples were analysed by both GC/MS and ELISA and a good agreement between GC/MS and ELISA measured TMDD (2,3,7-trichloro-8-methyldibenzo-*p*-dioxin = surrogate for TCDD) equivalents was obtained. A fairly good correlation between ELISA and TEF-values was also observed with these samples.

ELISA is less sensitive for low PCDD/PCDF and PCB-concentrations in environmental matrices. The limit of detection for these pollutants in soil varies from 0.3 to 1.7 mg/kg (EnviroLogix, 2003) while the reporter gene assays (see § 2.6.2.(iii)) are more sensitive – concentrations in the range of ng/kg can be detected.

A recent development is the Ah-immunoassay method (Ah-I). This is a cell-free system that comprehensively analyses total toxicity potential contributed by dioxin and dioxin-like compounds. Analysis of toxicity is functionally performed by measuring their collective ability to bind to a cytosolic aryl-hydrocarbon receptor protein (AhR). This activated AhR-protein then binds an exogenous Arnt-protein to form an activated protein complex, which is able to bind an ELISA plate-bound oligonucleotide dioxin responsive element (DRE) and is then detected by an immuno-assay-based colour reaction. The Ah-I measures toxic dioxins and dioxin-like compounds, based on toxicity appearance mechanism without the need for live cell culture or radioactivity (Kobayashi, Hall, Hiraoka, Ashieda, Nakanishi, Yamada, Ogiwara, Uechi, Hughes & Inoue, in press).

(iii) DNA probes

Complimentary DNA for induced P450 can be cloned and sequenced and in this way determine whether a particular P450--gene was switched on and to which extent it was produced, giving an indication of the toxic load a particular organ in the organism was exposed to (Stegeman *et al*., 1992). Using gel retardation of AhR DNA binding as a probe can demonstrate how many AhR ligands are present in an environmental matrix (Denison, Rogers, Fair, Ziccardi, Clark, Murk & Brouwer, 1996).

Another, recently developed assay is the reporter gene assay. Different strains of tissue cultures such as the rainbow trout cell line RLT2.0, the rat hepatoma cell line H4IIE (Villeneuve, Richter, Blankenship & Giesy, 1999), human 101L cells and mouse hepatoma H1L1 cells (Behnisch *et al.*, 2001) are stably transfected with a luciferase reporter gene under control of dioxin-responsive elements (DREs). Binding of the AhR-ligand complex to the DREs results in an up-regulation of luciferase transcription (Figure 2.3), which upon addition of a substrate, luciferin, catalyses a light-producing reaction. The luminescent endpoint can be measured with a luminometer to provide a sensitive measure of AhR-mediated gene expression potency (Villeneuve *et al*., 1999). This assay is commercially available as CALUX® (Chemically Activated Luciferase eXpression) (Koppen, Covaci, Van Cleuvenbergen, Schepens, Winneke, Nelen & Schoeters, 2001).

The character of the dose-response curves for endogenous enzyme activities controlled by the AhR-mechanism is biphasic with a decrease in response at greater doses. Some chemicals inducing the cytochrome P4501A1 (CYP1A1) activity can also serve as substrates for this enzyme, so they cause competitive substrate inhibition and reduced activity at greater concentrations (Clemons, Dixon & Bols, 1997).

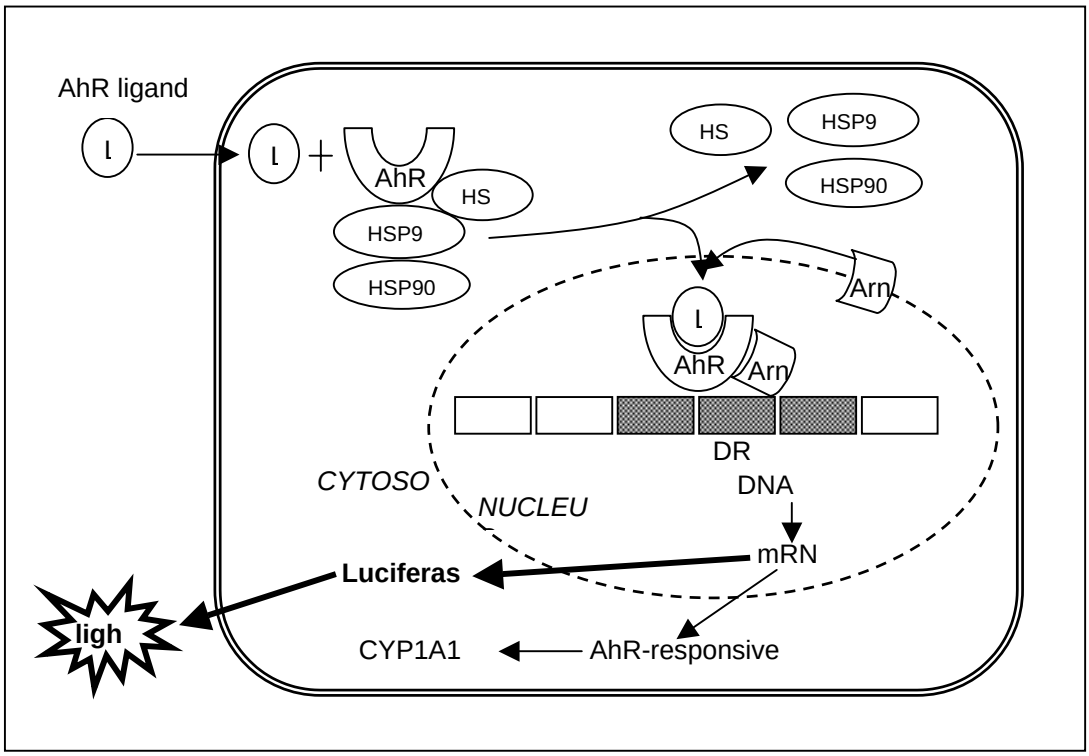


**Figure 2.3** The AhR response pathway (Figure 2.2) with the adoption for the bio-assay (parts in bold)

Greater sensitivity, selectivity, and dynamic range have been cited as potential advantages of using luciferase-transfected cell-lines rather than cytochrome P450 1A1 (*CYP1A1*) expression in wild-type cells as an endpoint (Sanderson, Aarts, Brouwer, Froese, Denison & Giesy, 1996).

## 2.7 Choice of environmental matrix to be analysed

In paragraph 1.3 the aims of this project were listed and according to aim 1 we were to collect fish and as explained on the same page the plans had to change to collecting sediment.

Aquatic sediments serve as a sink for a number of contaminants and thus as an integrative measure of exposure of the aquatic ecosystem. Sediment can contain mixtures of biologically active compounds with different mechanisms of action. The bottom-dwelling animals are directly exposed to these chemicals and through them the pollutants can enter aquatic food chains. In addition, contaminants in sediments can

directly affect micro- and meiobenthic communities (Hilscherova, Kannan, Holoubek & Giesy, 2002)

In the initial project application the matrix to be analysed was the muscle tissue of a bottom feeding fish *Clarias gariepinus* (sharptooth catfish). Fish has been used as a bio-indicator for the condition of the aquatic environment for many years now. Not just is this species a bottom feeder and therefore will accumulate these pollutants from the bottom sediments in freshwater sources, but determining the concentrations of the pollutants in its muscle tissue will provide an indication of the toxic loads consumers of this fish would be. For many rural South Africans, subsistence fishing is a major source of protein. This particular species also has a wide distribution through out South Africa (Skelton, 1993).

In order to gain comparable data from this type of matrix, the same fish species must be collected at the different sites. At least seven of the same age for all the sites must be collected in the same season. We encountered the following problems: Because some of the sites we selected were harbours and river mouths, *C. gariepinus* do not occur there. Also it is almost impossible to catch the required number of fish, all of the same age, all in the same season at sites spread across the whole of South Africa with handlines only. But the most significant reason for changing matrix is because some sites did not have any fish, possibly due to extensive pollution.

# 3 MATERIALS AND METHODS FOR COLLECTION AND ANALYSIS

## 3.1 Site selection and description

A total of twenty-two sites were selected to establish the presence and levels of PCDD, PCDF and PCB that are found in the aquatic environment of South Africa. Aquatic sites were selected on the grounds of being close to or down stream from areas regarded as possible sources of PCDD/PCDF and PCB (Table 3.1 and Figure 3.1).


**Figure 3.1** Map of South Africa indicating the location of the 22 sites that were sampled for this project (cf detailed table 3.1 for all sites).

The Vaal River system drains a large industrial area of South Africa (sites 7, 14, 17) before it joins the Gariep River. The motivation for choosing site 7 was to determine the total PCDD/PCDF and PCB load for the Vaal River and compare these values to that of the Gariep River, the farthest point down stream in the Gariep River (site 1). Site 16 is a trench running close to the iron and steel refinery before joining up with the Riet Spruit (site 15). The Riet Spruit enters the Vaal River downstream of site 14 but up-stream from site 7. The Hartbeespoort Dam (site 21) and Modderfontein Spruit (site 22) are more sites in highly industrialised areas. The Hartbeespoort Dam was

**Table 3.1** The 22 sites investigated in this study including coordinates and date of sampling.

| # | Name | Closest town/farm/resort | Date of collection | Coordinates | |
|---|---|---|---|---|---|
| | | | | S | E |
| 1 | Gariep River (mouth) | Alexander Bay | 31/03/2002 | 28°31.900’ | 16°36.338’ |
| 2 | Saldanha Bay harbour | Saldanha Bay | 01/04/2002 | 33°00.010’ | 17°59.805’ |
| 3 | Berg River | Hermon, Wellington, Paarl | 02/04/2002 | 33°26.011’ | 18°57.374’ |
| 4 | Theewaterskloof Dam | Villiersdorp | 02/04/2002 | 34°01.616’ | 19°15.795’ |
| 5 | Groot River (mouth) | Nature’s Valley | 04/04/2002 | 33°58.799’ | 23°33.913’ |
| 6 | Zwartkops Estuary | Port Elizabeth | 06/04/2002 | 33°52.030’ | 25°36.400’ |
| 7 | Vaal River (before confluence with Gariep River) | Douglas | 07/04/2002 | 29°03.132’ | 23°45.594’ |
| 8 | Buffalo River | Dundee | 21/04/2002 | 28°14.777’ | 30°30.599’ |
| 9 | Mooi River | Rosetta | 22/04/2002 | 29°18.315’ | 29°58.561’ |
| 10 | Umlazi River (mouth) | Durban | 23/04/2002 | 29°57.800’ | 30°58.210’ |
| 11 | Umgeni River (mouth) | Durban | 24/04/2002 | 29°48.480’ | 31°01.969’ |
| 12 | Richard’s Bay (harbour) | Richard’s Bay | 25/04/2002 | 28°47.496’ | 32°01.705’ |
| 13 | Thulazihleka Pan | Richard’s Bay | 25/04/2002 | 28°46.889’ | 32°02.430’ |
| 14 | Vaal Dam | Leboya Bay | 19/06/2002 | 26°48.566 | 28°08.607’ |
| 15 | Riet Spruit | Vanderbijl Park | 19/06/2002 | 26°44.205’ | 27°42.673’ |
| 16 | Riet Spruit (diverted brook) | Louisrus | 19/06/2002 | 26°39.825’ | 27°47.185' |
| 17 | Loch Vaal | Vanderbijl Park | 03/07/2002 | 26°45.039’ | 27°42.026’ |
| 18 | Crocodile River | Nelspruit | 08/07/2002 | 25°29.238’ | 31°09.535’ |
| 19 | Olifants River | Phalaborwa | 09/07/2002 | 24°03.085’ | 31°43.801’ |
| 20 | Loskop Dam | Groblersdal | 10/07/2002 | 25°25.088’ | 29°21.689’ |
| 21 | Hartbeespoort Dam | Oberon | 10/07/2002 | 25°44.250’ | 27°52.931’ |
| 22 | Modderfontein Spruit | Modderfontein | 11/07/2002 | 26°04.468’ | 28°08.167’ |

sampled at the inflow of the Crocodile River. Loskop Dam (site 20) is on the Olifants River and the Olifants River itself was also sampled just before it exits South Africa (site 19). The Olifants River and the Buffalo River were sampled because both drain that part of our country known for its coal mining and coal combustion electricity plants. Both the Crocodile River (site 18) and Groot River (site 5) flow through planted forests and plantations and the consequential wood treatment facilities. The Berg River (site 3) and the Theewaterskloof Dam (site 4) were sampled because they flow through and collect run-off from vineyards and orchards and could therefore contain possible PCDD/PCDF and PCB caused by the spraying of pesticides.

Saldanha Bay (site 2) and the Richard's Bay harbours (site 12) were selected because they are commercial harbours and the Thulazihleka Pan (site 13) was selected because of its position close to industries in Richards's Bay. It is an isolated freshwater pan. The Umlazi River mouth (site 10) was selected because the river runs through a huge informal settlement, Umlazi in Durban, before it passes a paper mill on its way to the Indian Ocean. The Umgeni River (site 11) is the other big river in the Durban area and supplies the drinking water for the city. The Mooi River (site 9) was selected to be the "reference site". The river has its origins in the Drakensberg Mountains and there is little PCDD/PCDF and PCB producing activities in the area.

As stated in Chapter 1, the approach of the study was to sample throughout the entire South Africa in order to derive an initial assessment of the concentrations of PCDD/PCDF and PCB in the aquatic environment, regardless of the influence of typical variables such as rainfall, season, temperature fluctuations and pH. A particular site was only sampled once during the sampling period (April to November 2002) and composite samples were made for each of the sites.

### 3.2 Sample collection

The protocol used here is similar to what is found in literature (cf Hilscherova, Kannan, Nakata, Hanari, Yamashita, Bradley, McCabe, Taylor, & Giesy, 2003). Samples were collected using the GLP-procedures developed under this project. Samples were collected from the surface sediment (0 to 5 cm deep; i.e. the biologically active zone) of the river/dam/harbour with a brass grab sampler. Equal volumes (120 mL) of individual sediment samples from five different locations at a particular site were pooled to obtain a composite sample. All other tools and containers that came into contact with sediment were stainless steel, avoiding plastic containers and utensils to prevent contamination of samples. The composite sample was stirred vigorously for one minute, before volumes of

sediment were transferred into four to six glass bottles, previously rinsed with hexane. The lid of each glass bottle was lined with an aluminium foil disk to protect the contents from coming into contact with the plastic lid. The aluminium foil was also rinsed with hexane. Each composite sample received its own unique number. The samples were transported and stored at -4°C.

### 3.3 Chemical analysis of sediments

The sediment samples were freeze-dried before 30g of each sample were sent to an internationally accredited laboratory in Germany[5]. The sediment was extracted through the technique called Accelerated Solvent Extraction (ASE) which means it was done under high pressure (138 atmospheres) and at high temperature (180°C). The solvent used was toluene. The extract was then analysed with high-resolution gas chromatography and mass spectrometry for each of the congeners listed in Table 2.1.

Total concentrations of TEQs based on this instrumental analysis were calculated as the sum of TEQs from individual compounds, assuming additive responses to chemicals in the mixture. The TEQs were obtained by multiplying the concentration by appropriate toxic equivalent factors (TEF). The WHO TEF values (Table 2.2) were used (Hilscherova, Kannan, Kang, Holoubek, Machala, Masunaga, Nakanishi & Giesy, 2001).

### 3.4 Sediment characterisation

The particle size distribution as well as the organic carbon content of each of the samples was determined by Viridus Technologies (Pty) Ltd. (Potchefstroom)[6]. The texture of a soil is extremely important in the sorption process. If a soil is made up of mostly clay and organic matter, a significant amount of sorption will take place. Clay, especially intermixed with organic particles, by far adsorbs the most out of the three main soil textures (clay, silt, and sand) because of its small particle size, high surface area, and high surface charge (Carey *et al.*, 1998) and this must be taken into consideration when comparing results from the different sites. The affinity of a hydrophobic organic chemical is symbolised by the solid-water partition coefficient $K_d$ and the value of $K_d$ is expressed by the following relationship: $K_d = K_{OC} \times f_{OC}$ where $f_{OC}$ is the fraction of organic carbon in soil. Thus, the higher the fraction of the organic carbon present in the soil, the more of the organic chemical will adsorb to the soil.

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[5] The Bayreuth Institute of Environmental Research, Ökometric GmbH, is accredited to the European standard EN 45001

[6] This laboratory participates in the following quality control schemes: (1) Agricultural Laboratory Association of Southern Africa. (2) International Soil-Analytical Exchange (ISE), Wageningen, The Netherlands.

## 3.5 *In vitro* bio-assay

### 3.5.1 Sediment extraction

Care was taken not to contaminate the samples by using properly cleaned apparatus that included rinsing three times with high-grade acetone and three times with high-grade hexane. After twigs and pebbles had been removed the freeze-dried sediment samples were homogenised with a mortar and pestle together with anhydrous sodium sulphate and sieved using a 2 mm sieve. Twenty grams of sediment were extracted with 400 mL high purity toluene[7] in a Soxhlet apparatus for 16h (Hilscherova *et al.*, 2001). The extracts were concentrated to almost dryness in a rotary evaporator before being transferred into test tubes with hexane and then evaporated to 1 mL under a gentle stream of nitrogen (Hilscherova *et al.,* 2001). The change from toluene to hexane was necessary because toluene is cytotoxic to the tissue culture used in the bio-assay (Hilscherova, Kannan, Nakata, Hanari, Yamashita, Bradley, McCabe, Taylor, & Giesy, 2003).

To remove PAH the extracts were diluted to 10 mL hexane and then acid-washed three times with concentrated sulphuric acid (Vondráček, Machala, Minsková, Bláha, Murk, Kozubík, Hovmanová, Hilscherová, Ulrich, Ciganek, Neča, Švrčková & Holoubek, 2001), each time discarding the acid phase. To remove all traces of acid from the hexane phase, the solution was washed with ultra-clean water before being filtered through anhydrous sodium sulphate and evaporated to 1 mL under the nitrogen stream (Hilscherova, personal comm., 2003). After the acid-wash treatment the only Ah-receptor ligands present in the extract would be PCDD/PCDF and PCB.

### 3.5.2 Reporter gene assay

The rat hepatoma H4IIE cell-line stably transfected with luciferase reporter gene (H4IIE-*luc*) under the control of dioxin-responsive enhancers (pGudLuc1.1) was used to examine the extracts for their ability to elicit AhR-mediated activity. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) without phenol red and with sodiumbicarbonate ($NaHCO_3$) and 10% foetal bovine serum. The foetal bovine serum used was charcoal dextran treated to remove any substances (such as natural occurring hormones) that might have an influence on the response of the cells. For the same reason DMEM without phenol red was used (Giesy, Jude, Tillitt, Gale, Meadows, Zajieck, Peterman, Verbrugge, Sanderson, Schwartz & Tuchman, 1997; Villeneuve *et al*., 1999).

[7] Burdick and Jackson, Muskegon, Michigan, USA

The assay was performed in 96-well cell culture plates. These plates had to comply with the following requirements: white opaque plates with flat bottomed wells; bottom of wells must be optically clear; wells must be tissue culture treated; plates must have clear lids and they must come individually wrapped and sterile. On day one of the assay, the wells were seeded with 0.25 mL cell suspension (70 000 cells.mL$^{-1}$). The outer wells of the plates were not seeded and received only 0.25 mL Dulbecco's phosphate buffered saline without $Mg^{2+}$ and $Ca^{2+}$ (DPBS) to act as buffer area. After a 24h-incubation period (37°C; 5% $CO_2$) the cells were dosed with 2.5 µL (3 wells per dose) of the sample extract or 2,3,7,8-TCDD standards. Cells were exposed to six different concentrations of the samples in a 33% dilution series, in triplicate. The TCDD standard was dosed at six concentrations (120.0, 30.0, 7.50, 1.88, 0.47, 0.12 pg/well) (Giesy *et al*., 1997).

After another 72h incubation period the plates were inspected microscopically for confluence and viability of cells before the culture medium was removed, the cells were washed three times with phosphate-buffered saline (DPBS with $Mg^{2+}$ and $Ca^{2+}$) and incubated for 10 min with LucLite$^{TM}$ reagent[8] at 37°C and 5% $CO_2$. The luciferase activity was measured as luminescence produced with a microplate-scanning luminometer (Hilscherova *et al.*, 2001).

Sample responses were expressed as relative luminescence units (RLU). Due to unequal slopes and efficacies (maximal induction) of the response, probit analysis could not be used for dose-responses of the sediment extracts. Calculation of multiple point estimates of the TCDD-EQs was used to characterise these responses when slopes and efficacies were not equal. Sample responses were converted to a percentage of the mean maximum response observed for the TCDD standard ($TCDD_{max}$) and plotted as a function of log µL-sample. Regression equations were derived for the linear portion of each dose-response curve. 2,3,7,8-TCDD equivalents or relative potency values (REP), based on bio-assay results (TCDD-EQs), were then calculated from the volume of sample producing a response equivalent to 20% of the maximal response (EC20) produced by the standard (TCDD). This means that the ratio between the EC20 for the standard and the EC20 for the sample was calculated. EC20-values were used because some of the samples reached 20% of the efficacy (Table 4.5). For most of the samples extrapolations were needed to calculate EC20-concentrations because the concentration of PCDD/PCDF and PCB in the sediments proved to be very low (cf. GC/MS results in Table 4.3). To account for the non-parallel slopes, (the slopes of the sample-dose response curve compared to

[8] Packard Instruments, Meriden, Connecticut, USA

the slope of the dose-response curve of the standard), the range of REP based on the level of response produced by EC20 and EC80 of TCDD were calculated and reported on. Since the REP-value is a relationship between the EC of the standard to the EC of the sample the value should be the same for EC20 and EC80 if the slopes of the two dose-response curves were the same.

# 4 RESULTS AND DISCUSSIONS

## 4.1 Results of the chemical analysis

In Table 4.1 and 4.2 the concentrations of the different congeners are represented, including the TEQ-concentrations as determined using the WHO-TEQ. In Figure 4.1 the TEQ-contributions by the intentionally produced PCB are presented separately from the TEQ-concentrations of the unintentionally produced PCDD/PCDF. It is clear that dioxin-like substances are present in all twenty-two sites sampled in this investigation. Except for the Crocodile River site, the contribution of the dioxins and furans were higher than PCB at all the sites. There is in fact, a statistically significant difference with a p-value of 0.0005. The data was not distributed normally and the Wilcoxon signed rank test was performed.

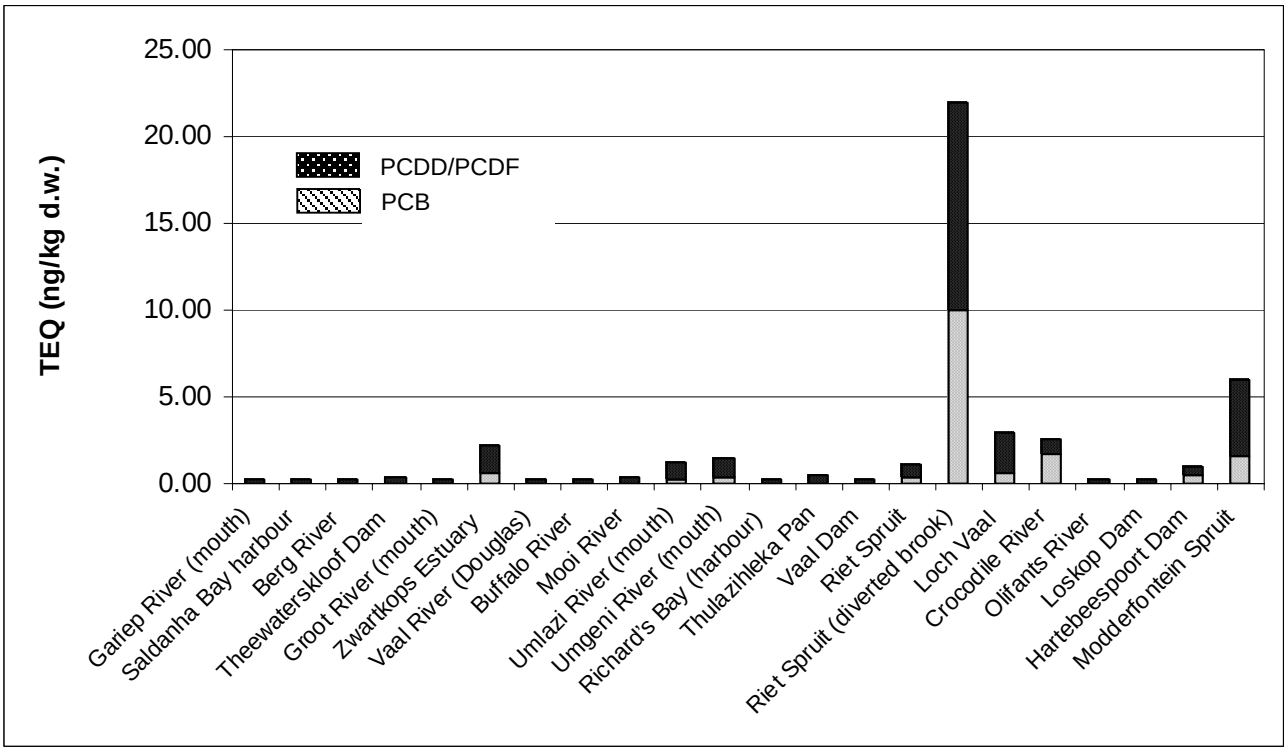


**Figure 4.1** A summary of the instrumentally determined TEQ values for each site.

**Table 4.1** Concentrations of instrumentally derived TEQs for the PCB at the different sites

| # | Site names | PCB congeners (ng/kg d.w.) | | | | | | | | | | | | TEQ* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PCB 77# | PCB 81# | PCB 126# | PCB 169# | PCB 105♦ | PCB 114♦ | PCB 118♦ | PCB 123♦ | PCB 156♦ | PCB 157♦ | PCB 167♦ | PCB 189♦ | |
| 1 | Gariep River (mouth) | 3.0 | 0.1 | 0.1 | 0.1 | 5 | 1 | 14 | 1 | 1 | 1 | 1 | 1 | 0.01 |
| 2 | Saldanha Bay harbour | 1.0 | 0.1 | 0.1 | 0.1 | 2 | 1 | 9 | 1 | 1 | 1 | 1 | 1 | 0.01 |
| 3 | Berg River | 6.3 | 0.1 | 0.1 | 0.1 | 10 | 1 | 24 | 1 | 4 | 1 | 2 | 1 | 0.02 |
| 4 | Theewaterskloof Dam | 3.2 | 0.1 | 0.1 | 0.1 | 4 | 1 | 13 | 1 | 4 | 1 | 2 | 1 | 0.02 |
| 5 | Groot River (mouth) | 5.0 | 0.1 | 0.1 | 0.1 | 5 | 1 | 18 | 1 | 2 | 1 | 1 | 1 | 0.02 |
| 6 | Zwartkops Estuary | 44.5 | 1.3 | 4.9 | 0.5 | 174 | 8 | 418 | 16 | 68 | 14 | 34 | 11 | 0.61 |
| 7 | Vaal River (Douglas) | 6.4 | 0.2 | 0.2 | 0.1 | 9 | 1 | 20 | 1 | 3 | 1 | 2 | 1 | 0.03 |
| 8 | Buffalo River | 2.7 | 0.1 | 0.1 | 0.1 | 4 | 1 | 10 | 1 | 1 | 1 | 1 | 1 | 0.01 |
| 9 | Mooi River | 3.6 | 0.1 | 0.1 | 0.1 | 7 | 1 | 17 | 1 | 3 | 1 | 1 | 1 | 0.02 |
| 10 | Umlazi River (mouth) | 32.5 | 0.6 | 2.0 | 0.2 | 139 | 10 | 339 | 13 | 61 | 11 | 24 | 5 | 0.30 |
| 11 | Umgeni River (mouth) | 52.7 | 1.8 | 2.4 | 0.1 | 129 | 10 | 266 | 7 | 47 | 9 | 20 | 6 | 0.32 |
| 12 | Richard's Bay (harbour) | 3.0 | 0.1 | 0.1 | 0.1 | 4 | 1 | 10 | 1 | 1 | 1 | 1 | 1 | 0.01 |
| 13 | Thulazihleka Pan | 16.0 | 0.6 | 0.2 | 0.2 | 22 | 2 | 44 | 2 | 7 | 1 | 3 | 1 | 0.04 |
| 14 | Vaal Dam | 2.3 | 0.1 | 0.1 | 0.1 | 3 | 1 | 10 | 1 | 1 | 1 | 1 | 1 | 0.01 |
| 15 | Riet Spruit | 78.5 | 1.6 | 2.3 | 0.1 | 123 | 12 | 227 | 9 | 44 | 5 | 19 | 5 | 0.31 |
| 16 | Riet Spruit (diverted brook) | 2500 | 104 | 62.1 | 7.1 | 6650 | 584 | 10600 | 590 | 2540 | 162 | 839 | 298 | 10.01 |
| 17 | Loch Vaal | 205 | 2.3 | 4.6 | 0.3 | 360 | 33.4 | 584 | 22.3 | 101 | 10.6 | 39.4 | 11.5 | 0.65 |
| 18 | Crocodile River | 20 | 0.2 | 14.8 | 2 | 53.7 | 4 | 337 | 62.7 | 339 | 15.2 | 153 | 100 | 1.74 |
| 19 | Olifants River | 4.9 | 0.1 | 0.1 | 0.1 | 8.1 | 1 | 20.1 | 1 | 2.5 | 1 | 1.2 | 1 | 0.02 |
| 20 | Loskop Dam | 2.3 | 0.1 | 0.1 | 0.1 | 3.7 | 1 | 12.6 | 1 | 1 | 1 | 1 | 1 | 0.01 |
| 21 | Hartbeespoort Dam | 19.4 | 0.3 | 3.4 | 0.4 | 152 | 7.4 | 417 | 23.6 | 102 | 15.2 | 55.8 | 17.9 | 0.47 |
| 22 | Modderfontein Spruit | 72.7 | 2 | 13.2 | 1.4 | 296 | 17.7 | 689 | 33.7 | 221 | 24.4 | 98.1 | 35.8 | 1.58 |

* Limit of quantification included and WHO-TEF (Table 2.2) used to calculate TEQ. # Non-*ortho*-substituted PCB ♦ Mono-*ortho*-substituted PCB

Values in shaded areas are lower than the detection limit of the system.

**Table 4.2** Concentrations of instrumentally derived TEQs for the PCDD/PCDF at the different sites

| # | Site names | PCDD/PCDF congeners | | | | | | | | | | | | | | | | | TEQ* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2,3,7,8-$Cl_4$DD | 1,2,3,7,8-$Cl_5$DD | 1,2,3,4,7,8-$Cl_6$DD | 1,2,3,6,7,8-$Cl_6$DD | 1,2,3,7,8,9-$Cl_6$DD | 1,2,3,4,6,7,8-$Cl_7$DD | $Cl_8$DD | 2,3,7,8-$Cl_8$DF | 1,2,3,7,8-$Cl_5$DF | 2,3,4,7,8-$Cl_5$DF | 1,2,3,4,7,8-$Cl_6$DF | 1,2,3,6,7,8-$Cl_6$DF | 1,2,3,7,8,9-$Cl_6$DF | 2,3,4,6,7,8-$Cl_6$DF | 1,2,3,4,6,7,8-$Cl_7$DF | 1,2,3,4,7,8,9-$Cl_7$DF | $Cl_8$DF | |
| 1 | Gariep River (mouth) | 0.05 | 0.05 | 0.05 | 0.15 | 0.08 | 0.26 | 0.87 | 0.05 | 0.05 | 0.05 | 0.2 | 0.16 | 0.05 | 0.09 | 0.76 | 0.15 | 0.73 | 0.22 |
| 2 | Saldanha Bay harbour | 0.05 | 0.05 | 0.07 | 0.2 | 0.07 | 0.25 | 0.61 | 0.05 | 0.1 | 0.14 | 0.16 | 0.18 | 0.05 | 0.11 | 0.31 | 0.15 | 0.5 | 0.27 |
| 3 | Berg River | 0.05 | 0.05 | 0.05 | 0.14 | 0.08 | 1.99 | 14.6 | 0.06 | 0.07 | 0.07 | 0.19 | 0.18 | 0.05 | 0.12 | 1.01 | 0.15 | 1.41 | 0.26 |
| 4 | Theewaterskloof Dam | 0.05 | 0.05 | 0.05 | 0.35 | 0.36 | 2.2 | 16.6 | 0.07 | 0.05 | 0.07 | 0.1 | 0.17 | 0.11 | 0.07 | 0.63 | 0.15 | 0.92 | 0.30 |
| 5 | Groot River (mouth) | 0.05 | 0.05 | 0.05 | 0.07 | 0.08 | 0.92 | 4.87 | 0.07 | 0.05 | 0.1 | 0.07 | 0.07 | 0.05 | 0.05 | 0.43 | 0.15 | 0.67 | 0.22 |
| 6 | Zwartkops Estuary | 0.06 | 0.33 | 0.49 | 1.78 | 1.02 | 30.9 | 184 | 0.57 | 0.45 | 0.46 | 0.52 | 0.58 | 0.05 | 0.49 | 5.47 | 0.17 | 9.86 | 1.58 |
| 7 | Vaal River (Douglas) | 0.05 | 0.05 | 0.05 | 0.12 | 0.2 | 0.75 | 5.06 | 0.06 | 0.07 | 0.06 | 0.07 | 0.08 | 0.06 | 0.05 | 0.29 | 0.15 | 0.5 | 0.21 |
| 8 | Buffalo River | 0.05 | 0.05 | 0.05 | 0.07 | 0.05 | 0.57 | 3.73 | 0.05 | 0.07 | 0.13 | 0.1 | 0.09 | 0.05 | 0.05 | 0.37 | 0.15 | 0.65 | 0.23 |
| 9 | Mooi River | 0.05 | 0.05 | 0.12 | 0.31 | 0.17 | 1.54 | 8.6 | 0.05 | 0.09 | 0.13 | 0.17 | 0.2 | 0.12 | 0.13 | 0.51 | 0.15 | 0.83 | 0.32 |
| 10 | Umlazi River (mouth) | 0.05 | 0.12 | 0.24 | 0.75 | 0.45 | 15.9 | 123 | 0.32 | 0.19 | 0.37 | 0.44 | 0.5 | 0.11 | 0.39 | 4.09 | 0.15 | 8.8 | 0.90 |
| 11 | Umgeni River (mouth) | 0.06 | 0.14 | 0.21 | 0.95 | 0.49 | 18.7 | 148 | 0.42 | 0.34 | 0.62 | 0.6 | 0.58 | 0.21 | 0.57 | 5.32 | 0.21 | 11.5 | 1.19 |
| 12 | Richard's Bay (harbour) | 0.05 | 0.05 | 0.05 | 0.18 | 0.06 | 0.33 | 1.45 | 0.05 | 0.06 | 0.11 | 0.13 | 0.15 | 0.05 | 0.11 | 0.29 | 0.15 | 0.5 | 0.24 |
| 13 | Thulazihleka Pan | 0.05 | 0.09 | 0.05 | 0.22 | 0.23 | 1.82 | 14.3 | 0.62 | 0.27 | 0.23 | 0.22 | 0.31 | 0.12 | 0.13 | 0.71 | 0.15 | 0.95 | 0.49 |
| 14 | Vaal Dam | 0.05 | 0.05 | 0.06 | 0.18 | 0.08 | 0.31 | 1.29 | 0.05 | 0.07 | 0.08 | 0.14 | 0.14 | 0.05 | 0.11 | 0.36 | 0.15 | 0.5 | 0.23 |
| 15 | Riet Spruit | 0.08 | 0.11 | 0.12 | 0.63 | 0.29 | 8.36 | 61 | 0.57 | 0.27 | 0.42 | 0.43 | 0.48 | 0.21 | 0.35 | 2.29 | 0.15 | 3.79 | 0.84 |
| 16 | Riet Spruit (diverted brook) | 1.02 | 0.94 | 1 | 6.39 | 2.45 | 104 | 906 | 13.6 | 6.72 | 7.57 | 7.39 | 7.06 | 0.81 | 5.28 | 26.1 | 2.17 | 38.4 | 11.90 |
| 17 | Loch Vaal | 0.09 | 0.23 | 0.34 | 1.71 | 1.02 | 26.2 | 191 | 1.83 | 0.9 | 1.15 | 1.31 | 1.48 | 0.7 | 1.07 | 7.24 | 0.52 | 10.7 | 2.25 |
| 18 | Crocodile River | 0.05 | 0.14 | 0.31 | 0.66 | 0.45 | 15.8 | 218 | 0.15 | 0.08 | 0.16 | 0.33 | 0.35 | 0.19 | 0.18 | 6.6 | 0.15 | 7.19 | 0.78 |
| 19 | Olifants River | 0.05 | 0.05 | 0.05 | 0.16 | 0.15 | 0.57 | 3.12 | 0.06 | 0.11 | 0.07 | 0.09 | 0.13 | 0.07 | 0.07 | 0.31 | 0.15 | 0.5 | 0.23 |
| 20 | Loskop Dam | 0.05 | 0.05 | 0.05 | 0.14 | 0.1 | 0.2 | 2.29 | 0.05 | 0.05 | 0.05 | 0.07 | 0.08 | 0.09 | 0.05 | 0.19 | 0.15 | 0.5 | 0.20 |
| 21 | Hartbeespoort Dam | 0.05 | 0.05 | 0.22 | 0.36 | 0.2 | 6.51 | 47.7 | 0.29 | 0.15 | 0.3 | 0.28 | 0.26 | 0.06 | 0.23 | 1.72 | 0.15 | 2.62 | 0.54 |
| 22 | Modderfontein Spruit | 0.14 | 0.73 | 0.69 | 3.13 | 2.03 | 40.2 | 421 | 3.16 | 1.62 | 2.05 | 3.03 | 3.26 | 0.7 | 1.9 | 18.4 | 1.06 | 32.6 | 4.41 |

*Limit of quantification included and WHO-TEF (Table 2.2) used to calculate TEQ. Shaded values are less than the detection limit of the system.

**Table 4.3** Summary of the PCB-TEQ, PCDD/PCDF-TEQ and the total TEQ of each site as determined by chemical analysis.

| # | Site name | PCB TEQ (ng/kg) | PCDD/F TEQ (ng/kg) | Total TEQ (ng/kg) | TEQ normalised (ng/kg) |
|---|---|---|---|---|---|
| 1 | Gariep River (mouth) | 0.01 | 0.22 | 0.23 | 370.70 |
| 2 | Saldanha Bay harbour | 0.01 | 0.27 | 0.28 | - |
| 3 | Berg River | 0.02 | 0.26 | 0.28 | - |
| 4 | Theewaterskloof Dam | 0.02 | 0.30 | 0.32 | 39.29 |
| 5 | Groot River (mouth) | 0.02 | 0.22 | 0.24 | 14.36 |
| 6 | Zwartkops Estuary | 0.61 | 1.58 | 2.19 | 192.53 |
| 7 | Vaal River (Douglas) | 0.03 | 0.21 | 0.24 | 20.30 |
| 8 | Buffalo River | 0.01 | 0.23 | 0.24 | 153.69 |
| 9 | Mooi River | 0.02 | 0.32 | 0.34 | 17.72 |
| 10 | Umlazi River (mouth) | 0.30 | 0.90 | 1.20 | 124.93 |
| 11 | Umgeni River (mouth) | 0.32 | 1.19 | 1.51 | 221.26 |
| 12 | Richard's Bay (harbour) | 0.01 | 0.24 | 0.25 | 147.29 |
| 13 | Thulazihleka Pan | 0.04 | 0.49 | 0.53 | 10.91 |
| 14 | Vaal Dam | 0.01 | 0.23 | 0.24 | - |
| 15 | Riet Spruit | 0.31 | 0.84 | 1.14 | 62.83 |
| 16 | Riet Spruit (diverted brook) | 10.01 | 11.90 | 21.90 | 302.54 |
| 17 | Loch Vaal | 0.65 | 2.25 | 2.90 | 134.11 |
| 18 | Crocodile River | 1.74 | 0.78 | 2.52 | 207.67 |
| 19 | Olifants River | 0.02 | 0.23 | 0.25 | 27.53 |
| 20 | Loskop Dam | 0.01 | 0.20 | 0.21 | 116.95 |
| 21 | Hartbeespoort Dam | 0.47 | 0.54 | 1.01 | 221.42 |
| 22 | Modderfontein Spruit | 1.58 | 4.41 | 5.99 | 241.06 |

The highest TEQ-value was determined for the Riet Spruit (diverted brook) that is close to an iron and steel refinery in Vanderbijl Park. A TEQ of almost 22 ng/kg was determined (Table 4.3). Another highly industrialised site, the Modderfontein Spruit, had a TEQ of almost 6 ng/kg. The lowest TEQ was for the Loskop Dam (0.22 ng/kg) and not the Mooi River (0.34 ng/kg), which was selected because of its expected low

impact (§3.1). According to the Agency for Toxic Substances and Disease Register in the USA, a screening level of 50 ng/kg for soils and sediments warrants further investigation into a site (Fiedler, 2003). None of the samples collected in this study came close to this value. In Germany the target concentration in soil is 5 I-TEQ ng/kg d.w. and products from soil with 5-40 I-TEQ ng/kg d.w. need to be controlled if the pollution is transferred into the product. In the Netherlands agricultural farming is allowed in soil with no more than 1 I-TEQ ng/kg d.w. and dairy farming on soil containing 10 I-TEQ ng/kg d.w. In Sweden there is a guideline concentration of 10 I-TEQ ng/kg d.w. for sensitive uses and of 250 I-TEQ ng/kg d.w. for less sensitive uses. In Japan the Law Concerning Special Measures against Dioxins sets an environmental standard of 1 000 ng WHO-TEQ/kg (Fiedler, 2003)[9].

However, the nature of each site's sediment has to be taken into account since a high organic carbon content and high clay content (small particles) increase the capacity of the sediment to adsorb PCB and PCDD/PCDF (cf. § 2.2) (Shimizu, Sato, Kim, Suzuki, Takamatu, Nakamura, Yabushita, Yamamoto & Murata, in press). Table 4.4 represents the composition of the sediment collected at each site as well as the organic carbon content of the samples. Figure 4.2 shows the correlation between the TEQ and the organic carbon content: the higher the carbon content, the higher the TEQ. A point below the regression line (site 13) may indicate a "clean" site, because a dioxin-like activity was measured that is low in relation to the adsorption capacity. Conversely a sample that plots above the line (site 16) may indicate a site of concern because relatively high dioxin-like activity was found in relation to the adsorption capacity.

[9] For explanation of difference between I-TEQ and WHO-TEQ see § 2.5.

**Table 4.4** The particle size distribution and organic carbon content of samples collected for the study.

| # | Site names | Particle size | | | | % C |
|---|---|---|---|---|---|---|
| | | % > 2mm | % < 2mm | | | |
| | | | Sand | Silt | Clay | |
| 1 | Gariep River (mouth) | 0.2 | 49.5 | 42.3 | 8.2 | 0.06 |
| 2 | Saldanha Bay harbour | 0.1 | 90.9 | 2.5 | 6.6 | 0.00 |
| 3 | Berg River | 0.0 | 94.6 | 0.6 | 4.8 | 0.00 |
| 4 | Theewaterskloof Dam | 11.9 | 63.3 | 21.6 | 15.1 | 0.81 |
| 5 | Groot River (mouth) | 1.2 | 89.9 | 0.7 | 9.4 | 1.66 |
| 6 | Zwartkops Estuary | 3.7 | 76.3 | 13.8 | 9.8 | 1.14 |
| 7 | Vaal River (Douglas) | 3.9 | 48.8 | 23.1 | 28.0 | 1.21 |
| 8 | Buffalo River | 2.3 | 75.3 | 12.2 | 12.5 | 0.16 |
| 9 | Mooi River | 4.3 | 66.6 | 16.5 | 16.9 | 1.92 |
| 10 | Umlazi River (mouth) | 0.2 | 85.3 | 5.0 | 9.7 | 0.96 |
| 11 | Umgeni River (mouth) | 1.9 | 78.5 | 8.6 | 12.9 | 0.68 |
| 12 | Richard’s Bay (harbour) | 1.6 | 86.5 | 4.6 | 8.9 | 0.17 |
| 13 | Thulazihleka Pan | 0.5 | 25.0 | 66.3 | 8.7 | 4.83 |
| 14 | Vaal Dam | 7.7 | 89.7 | 4.9 | 5.3 | 0.00 |
| 15 | Riet Spruit | 0.3 | 61.9 | 13.5 | 24.5 | 1.82 |
| 16 | Riet Spruit (diverted brook) | 0.3 | 22.0 | 68.5 | 9.5 | 7.24 |
| 17 | Loch Vaal | 0.0 | 5.4 | 31.7 | 63.0 | 2.16 |
| 18 | Crocodile River | 3.1 | 60.1 | 16.8 | 23.2 | 1.21 |
| 19 | Olifants River | 0.2 | 43.6 | 21.7 | 34.7 | 0.89 |
| 20 | Loskop Dam | 1.5 | 83.2 | 8.2 | 8.6 | 0.18 |
| 21 | Hartbeespoort Dam | 3.7 | 68.9 | 17.5 | 13.6 | 0.45 |
| 22 | Modderfontein Spruit | 4.6 | 64.5 | 17.6 | 17.9 | 2.48 |

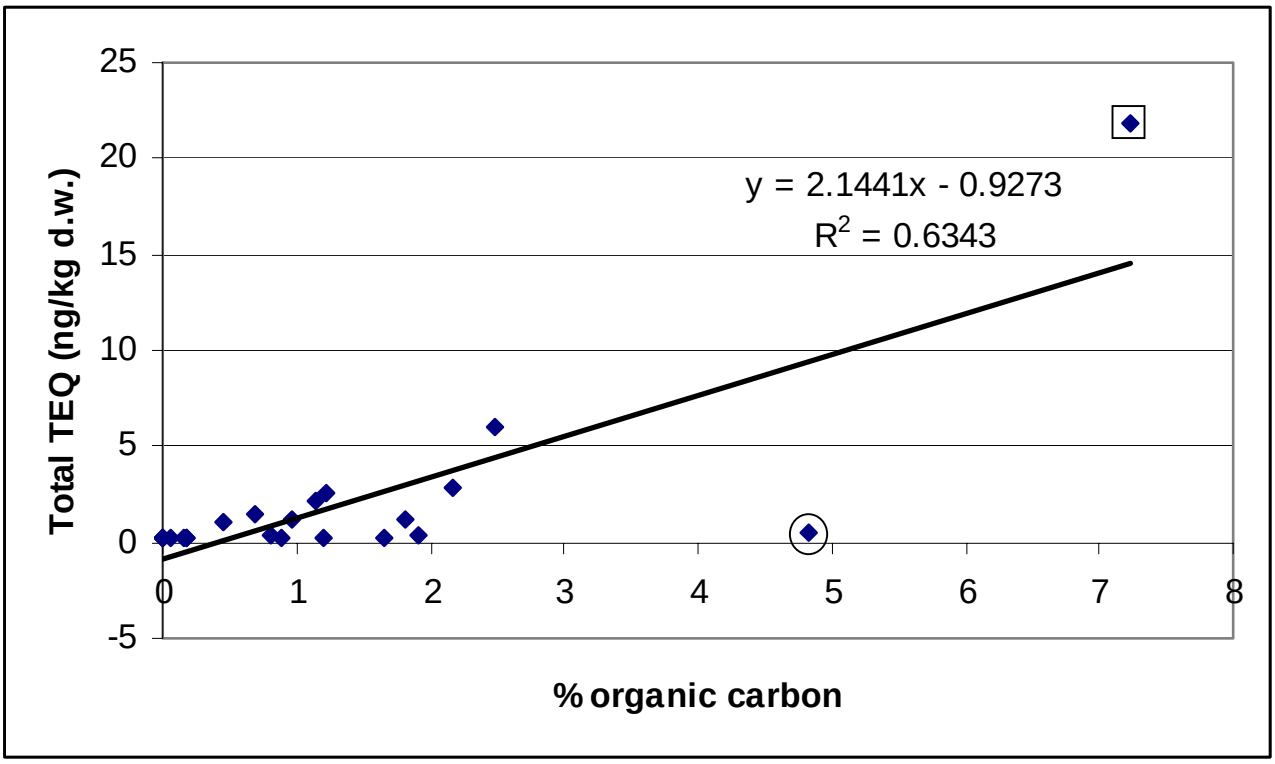


**Figure 4.2** The relationship between organic carbon content and the TEQ of each sample. Circle is Thulazihleka Pan (site 13 Richard's Bay). Square is Riet Spruit (site 16)

In Table 4.3 the last column contains the TEQ normalised for the organic carbon content of the sediment samples. The empty cells in the table are due to a TEQ-value for the site, but no detectable organic carbon was reported (Table 4.4). This means that even in the apparent absence of organic carbon, dioxin-like substances are still present. According to the normalised data, the mouth of the Gariep River had the highest TEQ, followed by the Riet Spruit (diverted brook) site. The lowest normalised TEQ is the Thulazihleka Pan.

These TEQ-values for the different sites selected to represent the whole of South Africa correspond to the lower TEQ-values in the range determined for the Detroit River (USA). Nine sites were selected and the TEQ-values ranged form 2.30 to 306 pg/g d.w. The average organic carbon between all nine sites was 0.81%; % sand 2.39, % silt 62.78% and % clay 34.78% (Marvin, Alaee, Painter, Charlton, Kauss, Kolic, MacPherson, Takeuchi, & Reiner, 2002). In another study on the Tittabawassee River (USA) TEQ-values ranging from 0.37 to 2 550 pg/g d.w. depending on the distance the site was upstream or downstream from the source of pollution. The average total

organic content of all the sites was 1.18% ranging from 0.11 to 6.5% (Hilscherova *et al.,* 2003).

### 4.2 Results of the reporter gene assay

One of the aims of this study was to evaluate the effectiveness of this particular bio-assay as a screening tool of environmental matrixes. What follows is a report on the bio-assay TEQ-values of nine sites.[10]

**Table 4.5** Bio-assay results compared to the TEQ as determined by the chemical analysis.

| # | Site name | TCDD-EQ(EC20)* (ng/kg d.w.) | TCDD-EQ(EC80)# (ng/kg d.w.) | GC/MS TEQ^ (ng/kg d.w.) |
|---|---|---|---|---|
| 12 | Richard's Bay (harbour) | $2.33 \times 10^{-8}$ | $9.42 \times 10^{-32}$ | $2.5 \times 10^{-1}$ |
| 13 | Thulazihleka Pan | $8.31 \times 10^{-6}$ | $1.02 \times 10^{-22}$ | $5.3 \times 10^{-1}$ |
| 15 | Riet Spruit | $1.02 \times 10^{-2}$ | $5.12 \times 10^{-8}$ | 1.14 |
| 16 | Riet Spruit (diverted brook) | $5.17 \times 10^{-3}$ | $8.01 \times 10^{-11}$ | 2.190 x 10 |
| 17 | Loch Vaal | $1.72 \times 10^{-1}$ | $2.46 \times 10^{-2}$ | 2.90 |
| 18 | Crocodile River | $2.59 \times 10^{-3}$ | $4.75 \times 10^{-8}$ | 2.52 |
| 20 | Loskop Dam | $2.32 \times 10^{-2}$ | $1.41 \times 10^{-3}$ | $2.1 \times 10^{-1}$ |
| 21 | Hartbeespoort Dam | $7.39 \times 10^{-2}$ | $3.15 \times 10^{-2}$ | 1.01 |
| 22 | Modderfontein Spruit | $2.02 \times 10^{-1}$ | $8.13 \times 10^{-2}$ | 5.99 |

* Bio-assay TEQ as determined by using the EC20

\# The EC80 is reported to give an indication of non-parallelism between sample and standard dose-response curves

^ Taken from Table 4.3.

According to the results summarised in Table 4.5 there is a great difference between the TEQ-values as determined by the bio-assay and that determined by GC/MS. However, the tendencies depicted by both techniques seem to correlate, i.e. when sites are compared to each other, sites with higher TEQ values than others for the one technique, show a similar tendency using the other technique (Figure 4.3).

[10] Due to a defective incubator in the host laboratory and consequential time constraints, no publishable results were available for the other sites at the publication time of this report.

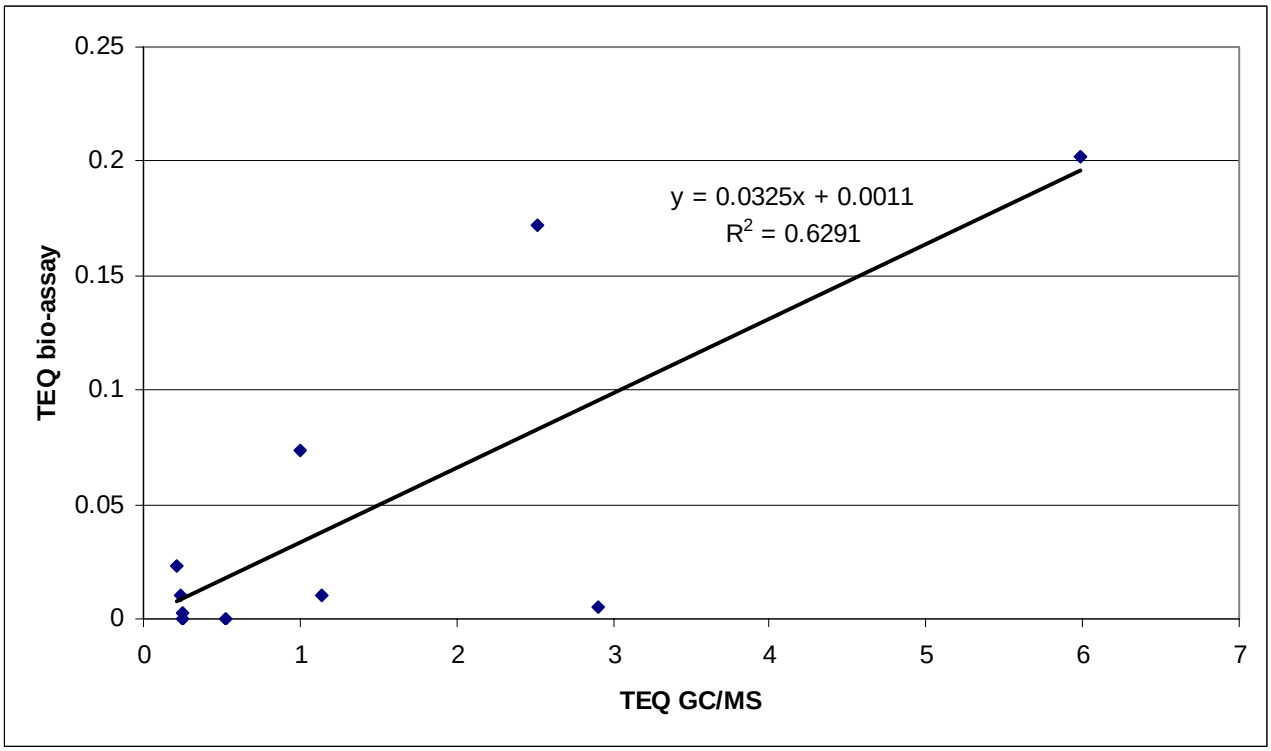


**Figure 4.3** This graph represents the correlation between the TEQ-values determined by the separate techniques.

Many reports on similar studies, i.e. comparing GC/MS results to bio-assay results in determining TEQ-values, can be found in recently published literature. Some reported on higher TEQ-values determined by the bio-assay (Anderson, Jones, McCoy, Fujita, Yamamoto & Iijima, in press) and others to lower TEQ-values (Engwall, Lindström & Van Bavel, in press) when compared to the GC/MS determinations. According to others there is little difference in the TEQ-values as determined by the different techniques (Hilscherova *et al*., 2003).

In a study where human serum samples were analysed by the same two techniques as employed in the present study, the chemically determined TEQ was twice as high as the TEQ determined by the reporter gene assay (Koppen *et al*., 2001). A significant correlation existed between concentrations of TCDD-EQs derived from H4IIE-*luc* bio-assay and TEQs estimated from instrumental analysis ($p < 0.05$) for sediment samples from the Tittabawassee River, Michigan (USA). In general the TEQ from the bio-assay were greater than those of the GC/MS results although the difference in magnitude of the concentrations between bio-assay TEQ and chemical TEQ was less than 2-fold

(Hilscherova *et al*., 2003). Because of the variability in bio-assay results, analytical errors within the assays, and variations in relative potency values, it has been suggested that a 2-fold difference is the minimum required to conclude that the concentrations of bio-assay and instrumental TEQs are not equivalent. In another study where dioxin-like activity of sediments from a Czech river basin was characterised, it was found that the TEQ derived from the bio-assay correlated significantly with the GC/MS determined TEQ ($R^2 > 0.8$; $p < 0.01$) (Hilscherova *et al*., 2001).

In a study by Anderson and co-authors (Anderson *et al*., in press) a variety of environmental matrices were analysed by the GC/MS analysis and also by a reporter-gene assay. The cell-line employed in their study was the human cell line: 101 litre and the following matrices were investigated: bottom fly ash, exhaust gas, soil and sediment and municipal dump ash. The correlation coefficient for all the matrices varied between 0.79 and 0.99 and for soil and sediment specifically it was 0.92. There was a tendency for the reporter-gene assay to have 1.5 to 3 times higher TEQ. . Engwall and co-authors (in press) reported at the latest Dioxin conference (Aug 2003) in Boston, USA, on an international inter-calibration of dioxin-like compounds in cod liver using bio-assays. The laboratories using the same reporter-gene assay as the one evaluated in this study, determined TEQ-values that varied from 0.1 to 28.2 pg TEQ/g fresh weight while the GC/MS had a TEQ-value of 27 pg/g wet weight. This was considered to be a relatively good agreement.

This bio-assay reflects synergistic, additive and/or antagonistic interaction of any compound (including ”non-dioxin-like” compounds (Koppen *et al*., 2001). Studies have reported that especially mono- or di-*ortho*-PCB can act as antagonists or partial agonists for the AhR, thus reducing the total potency of the mixture. The mono-*ortho*-PCB such as PCB118 may act as AhR antagonist (Sanderson & Van den Berg, 1999; Engwall *et al.*, in press), lowering TEQ-values for the bio-assays. The PCB-congener that was the most dominant congener in all the samples investigated in this study was PCB118 (Table 4.6). (Its corresponding TEQ-contribution for each site is low.) This, however, might explain a two to three times lower TEQ-value, but not the big difference found in this study. The big difference might be construed to different extraction methods that were followed for the GC/MS analyses and the bio-assay (cf.§ 3.3 and §

3.5.1). Not only were the extractions done by different people in different countries, but also different protocols were used, with different solvents.

**Table 4.6** The percentage contribution of PCB118 to the total concentration and TEQ of all the PCB, PCDD/PCDF congeners in this study

| # | Site name | Total concentration (ng/kg d.w.)* | % concentration contribution of PCB118 | % TEQ contribution of PCB118 |
|---|---|---|---|---|
| 12 | Richard's Bay (harbour) | 26.57 | 36.13 | 0.38 |
| 13 | Thulazihleka Pan | 118.17 | 37.07 | 0.83 |
| 15 | Riet Spruit | 606.05 | 37.46 | 1.99 |
| 16 | Riet Spruit (diverted brook) | 26073.10 | 40.656 | 4.84 |
| 17 | Loch Vaal | 1621.89 | 36.01 | 2.01 |
| 18 | Crocodile River | 1352.39 | 24.92 | 1.34 |
| 20 | Loskop Dam | 29.06 | 43.36 | 0.60 |
| 21 | Hartbeespoort Dam | 875.55 | 47.63 | 4.14 |
| 22 | Modderfontein Spruit | 2040.70 | 33.76 | 1.15 |

* Compare Tables 4.1 and 4.2

# 5 ASSESSMENT OF LEVELS

## 5.1 Introduction

In this chapter we will attempt to derive a statement of risk based on the data obtained. Risk is a combination of exposure and effects expressed as a probability. Exposure is a measure of the concentrations or persistence of a stressor (organic pollutant) within the defined system. Exposure can be expressed as a dose, but in environmental toxicology it is often possible to measure environmental concentrations (Landis & Yu, 1995). In this particular study, the closest to estimation of the environmental concentrations is the calculation of the TEQ.

At this stage, and with the limited knowledge on how PCDD/PCDF and PCB react in the environment, only a preliminary assessment can be attempted. No effect was measured in this study. This is in contrast with data-rich countries in the northern hemisphere, where, for instance Germany has a long history of research, and more than 10 000 data points (Whylie *et al*., 2003).

In § 2.3 background is given to the fate of PCB and PCDD/PCDF in the environment and keeping that in mind the reader will form an idea on what the fate of these compounds might be once released into the environment.

## 5.2 From the literature

The present study did not aim to address the issue of the risks of PCDD/PCDF and PCB to humans or wildlife directly. Risk is a factor of both exposure and hazard (toxicity). An assessment of the risks posed by PCDD/PCDF and PCB would need to consider the bio-availability of each congener as well as other factors that would affect potential exposure (Hilscherova *et al*., 2003). The Agency for Toxic Substances and Disease Registry in the United States has adopted interim policy guidelines for PCDD/PCDF and dioxin-like PCB in residential soils near or on hazardous waste sites (De Rosa, Brown, Dhara, Garrett, Hansen, Holler, Jones, Jodan-Izaguirre, O'Connor, Pohl & Xintaras, 1997). When concentrations of TEQ exceed 50 pg of TEQ/g dry weight, evaluation of site specific factors such as pathway analysis and soil cover are needed. Except for organic carbon content and particle size, other types of information also needed to predict bio-availability are organic carbon content, all the characteristics of the contaminant (eg water solubility, vapour pressure, $K_{OW}$, Henry's constant and $K_{OC}$; Sabljic, 2001), and information regarding organism physiology such as digestive

fluid composition, life-span, organism size and seasonal changes in biochemistry (Landrum, 2003). Although a steady state between the uptake and excretion rates may be established, the concentration in the organism will be greater than that in the diet. If, however, substances have a low or zero rate of metabolism and growth dilution, the excretion rate may be so low that steady state is not reached and the concentration builds op with age (Carey *et al.*, 1998).

The PCB-residue composition changes dramatically as PCB move from sediment, soil, or contamination source into biological systems, especially when they move by the process of bio-magnification (Oliver & Niimi, 1988). Recent investigations of the most toxic PCB-congeners in aquatic environments suggest that some of these compounds are preferentially accumulated in higher animals relative to the total mix of congeners (Oliver & Niimi, 1988). Consequently the total toxic potency of PCB-residues probably increases significantly as they move up the food chain (Hong, Xiao, Bush, Shaw, 1998). Maruya & Lee (1998) investigated the BSAF[11] and TTF (trophic transfer factor) of highly chlorinated PCB-congeners in the Turtle/Brunswick River estuary (USA) (Table 5.1). The conclusion these researchers came to was that field-derived BSAFs were highest for striped mullet and lowest for grass shrimp for a series of extremely hydrophobic PCB ($Cl_7$-$Cl_{10}$). BSAF were negatively correlated with log $K_{OW}$ for these species and also for spotted sea trout, which are animals of a simplified estuarine food chain. Lipid normalised TTF values decreased with increasing tropic level and were not correlated with $K_{OW}$. However, fish preferentially accumulated fully *ortho*-substituted $Cl_7$-$Cl_8$ homologs.

[11] Calculation of BSAF: organism concentration normalised to lipid divided by sediment concentration normalised to organioc carbon content.

**Table 5.1** BSAF and TTF values of highly chlorinated PCB congeners (Maruya & Lee, 1998)

| | **Total PCB concentration[#] (Mean ± SD µg/g)** | **BSAF (Mean ± SD)** | **TTF* (Mean ± SD)** |
|---|---|---|---|
| Marsh sediment | 132 ± 22 | | |
| Grass shrimp | 17 ± 2.2 | 0.28 ± 0.14 | 12 ± 6.9 |
| Striped mullet | 160 ± 31 | 3.1 ± 1.9 | 2.9 ± 1.1 |
| Sea trout muscle | 41 ± 6.7 | 0.18 ± 0.47 | 0.26 ± 0.08 |

[#] organic carbon or lipid normalised * lipid normalised

In a study that analysed only for PCDD/PCDF, it was shown that TEQ-values of larger fish were higher than that of smaller fish (14 vs 26 pg/g fat weight) for fish collected in a Finnish lake downstream of a possible pollution source. The TEQ for the sediment was 19 pg/g d.w. (Assmuth & Vartiainen, 1995). In another study on a tidal river-marsh on the Potomac River (USA) the median BSAF estimated in all finfish species for total PCB was 2.9. The mean sediment total-PCB concentration in the wetland was 50 ng/g dry weight, and mean concentrations in biota ranged from 150 ng/g to 450 ng/g w.w (Crimmins, Brown, Kelso & Foster, 2002).

Different congeners have different BSAF-values. Naito, Jin, Kang, Yamamuro, Masunaga & Nakanishi (2003) published a list of BSAF-values of all the dioxin-like PCB and PCDD/PCDF-congeners for sea bass and it varied from 0.01 to 283. The average was 59.76 (our calculation). For 2,3,7,8-substituted PCDD/PCDF, the BSAF-values were greater than 1 for the tetra- and penta- congeners, while they were less than 1 for higher chlorinated congeners. For non-2,3,7,8-substituted congeners, the BSAF-values were very small, ranging from 1/10 to 1/100 when compared to 2,3,7,8-substituted PCDD/PCDF. On the other hand, the BSAF-values for co-PCB were much higher than those for PCDD/PCDF. The $R^2$ of the correlation between log BSAF and log of the water solubility of these substances was 0.90 (Naito *et al*., 2003).

Wu, Xu, Schramm & Kettrup (2001) reported on concentrations in fish and sediment of a lake in China without apparently normalising the data. Below is a table compiled from their data (Table 5.2) to give an idea of the concentrations they determined:

**Table 5.2** A summary of PCDD/PCDF concentrations in sediment and fish from a lake in China (Wu *et al.*, 2001)

| Name of congener | Concentration in fish (ng/kg) | Concentration in sediment (ng/kg) |
|---|---|---|
| TCDD | 30.81 | 600 |
| PeCDD | 0.82 | 324 |
| HxCDD | 0.57 | 135 |
| HpCDD | 0.73 | 100 |
| OCDD | 7.92 | 615 |
| TCDF | 13.55 | 5 507 |
| PeCDF | 16.42 | 1 708 |
| HxCDF | 5.90 | 2 890 |
| HpCDF | 1.11 | 1 828 |
| OCDF | 10.08 | 138 |

## 5.3 Assessment of current data

As can be gathered from the above case studies, each situation is unique, and with its own parameters that must be taken into account when the true effect of the pollutants on the environment is to be understood.

**Table 5.3** A summary of the total concentrations and TEQ-values for the PCB-congeners and the PCDD/PCDF-congeners at each site

| # | Site name | PCB | | PCDD/PCDF | | Total TEQ (ng/kg d.w.) | Total TEQ normalised (ng/kg d.w.) |
|---|---|---|---|---|---|---|---|
| | | Concentration (ng/kg d.w.) | TEQ (ng/kg d.w.) | Concentration (ng/kg d.w.) | TEQ (ng/kg d.w.) | | |
| 1 | Gariep River (mouth) | 28.30 | 0.01 | 3.80 | 0.22 | 0.23 | 370.70 |
| 2 | Saldanha Bay harbour | 18.30 | 0.01 | 3.05 | 0.27 | 0.28 | - |
| 3 | Berg River | 50.60 | 0.02 | 20.27 | 0.26 | 0.28 | - |
| 4 | Theewaterskloof Dam | 30.50 | 0.02 | 22.00 | 0.30 | 0.32 | 39.29 |
| 5 | Groot River (mouth) | 35.30 | 0.02 | 7.80 | 0.22 | 0.24 | 14.36 |
| 6 | Zwartkops Estuary | 794.20 | 0.61 | 237.20 | 1.58 | 2.19 | 192.53 |
| 7 | Vaal River (Douglas) | 44.90 | 0.03 | 7.67 | 0.21 | 0.24 | 20.30 |
| 8 | Buffalo River | 22.30 | 0.01 | 6.28 | 0.23 | 0.24 | 153.69 |
| 9 | Mooi River | 36.60 | 0.02 | 13.22 | 0.32 | 0.34 | 17.72 |
| 10 | Umlazi River (mouth) | 637.40 | 0.30 | 155.87 | 0.90 | 1.20 | 124.93 |
| 11 | Umgeni River (mouth) | 549.90 | 0.32 | 188.92 | 1.19 | 1.51 | 221.26 |
| 12 | Richard's Bay (harbour) | 22.80 | 0.01 | 3.77 | 0.24 | 0.25 | 147.29 |
| 13 | Thulazihleka Pan | 97.70 | 0.04 | 20.47 | 0.49 | 0.53 | 10.91 |
| 14 | Vaal Dam | 21.80 | 0.01 | 3.67 | 0.23 | 0.24 | - |
| 15 | Riet Spruit | 526.50 | 0.31 | 79.55 | 0.84 | 1.14 | 62.83 |
| 16 | Riet Spruit (diverted brook) | 24936.20 | 10.01 | 1136.90 | 11.90 | 21.90 | 302.54 |
| 17 | Loch Vaal | 1374.40 | 0.65 | 247.49 | 2.25 | 2.90 | 134.11 |
| 18 | Crocodile River | 1101.60 | 1.74 | 250.79 | 0.78 | 2.52 | 207.67 |
| 19 | Olifants River | 41.10 | 0.02 | 5.71 | 0.23 | 0.25 | 27.53 |
| 20 | Loskop Dam | 24.90 | 0.01 | 4.16 | 0.20 | 0.21 | 116.95 |
| 21 | Hartbeespoort Dam | 814.40 | 0.47 | 61.15 | 0.54 | 1.01 | 221.42 |
| 22 | Modderfontein Spruit | 1505.00 | 1.58 | 535.70 | 4.41 | 5.99 | 241.06 |

The approach followed in the assessment of this data generated for SA (cf § 5.3.1), was to compare sites to each other regarding: concentrations of intentionally produced dioxin-like substances (PCB) to the unintentionally produced dioxins and furans; industrialised areas vs more rural areas and coastal sites vs. sites in the interior of the country. Where possible concentrations in the afore-mentioned South African "categories" were compared to international values. Where sites were within the same river system, such as sites 14,17, 7 and 1 in the Vaal and Gariep Rivers and site 15, 16 and 17 in the Riet Spruit, the difference in the downstream concentrations were discussed. Almost always the possible source of pollution was taken into consideration and if different sites had similar sources of pollution, such as coal mining as the possible source of pollution to sites 8, 19 and 20, then these sites were compared.

In § 5.3.2 international guidelines as to acceptable concentrations of these pollutants in aquatic sediment of two northern hemisphere countries are provided. This gives an indication of the importance of the findings of this study.

The last section of chapter 5 (§ 5.3.3) looks at the possible effects these concentrations may have on aquatic life, and specifically on fish, because fish are utilised as a protein source by humans. Knowing the possible concentrations in fish gives an indication of the levels of these substances humans are exposed to when consuming this fish.

### 5.3.1 Observed tendencies at SA sites

The total PCB-concentration of each site was higher than the total concentration measured for the PCDD/PCDF-congeners at each site. This is consistent with trends seen elsewhere in the world (Whylie, *et al*., 2003). But, when comparing the TEQ-values for the two groups of congeners, the contribution by the PCDD/PCDF-component was higher at all the sites, except the Crocodile River (site 18). This is because the PCDD/PCDF-congeners are regarded as more toxic than the PCB-congeners (cf their TEF values (Table 2.2).

It is also clear that the inland-industrialised areas such as sites 16, 17, 18 and 22 have higher concentrations than sites at less industrialised inland areas such as 3,4,7,8 and 9. Generally, the sites in estuaries or in harbours have lower concentrations and this could be because of the possible diluting effect of the ocean. At these sites there are also less organic carbon, and higher sand content, lowering the adsorption capacity of the sites. But even for these sites, the tendency for industrialised areas (sites 6, 10,

11) having concentrations higher than the less industrialised areas (site 1) is true. The two harbours that were sampled (Saldanha and Richard's Bay) seem to have little of these particular contaminants, but this could be explained by their high sand percentage and very low organic carbon content.

Both the sites chosen at Durban, the Umgeni (site 11) and Umlazi (site 10) Rivers, measured high concentrations, as can be expected for an industry-rich city. There is, however, no major difference between the concentrations measured for the single sites in the two rivers. The site in the Umlazi was selected for its close proximity to a paper mill and is also downstream of the Umlazi-township and industry. One would therefore expect a higher TEQ for this site when compared to the Umgeni River site.

The coastal site with the highest PCB and PCDD/PCDF-concentrations was the Zwartskops Estuary near Port-Elizabeth (another highly industrialised city). Its TEQ-value also was the highest of all the coastal sites.

To compare with data from other coastal sites: PCB-concentrations (Aroclor 1254) found in coastal sediments ranged from 50 to 24 500 ng/kg in Kuwait coastal sediments; from <7.8 to 190 ng/kg in Saudi Arabia; from 20 ng/kg in Quatar; varied between 13 to 130 ng/kg in UAE and between 4 to 139 ng/kg d.w. in Oman (Whiley *et al*., 2003). The coastal PCB-concentrations measured in this study, varied from 18.30 ng/kg d.w. (Saldanha Bay harbour, site 2) to 794.20 ng/kg d.w. at the Zwartkops Estuary (site 6). No SA site was higher than the value reported for Kuwait, and the values are similar to the other PCB-concentrations reported for the Middle East. In the east coast of India, PCB were found at levels from non-detectable (ND) to 1 400 ng/kg d.w. in sediments from river mouths. The South African river mouths in this study measured almost half of this maximum. PCB found in marine sediments around Australia in the 70s and 80s ranges from ND to 1 300 000 ng/kg d.w.

The concentrations of the sites in the Vaal River (14,17 & 7) and Gariep River (1) reflect the expected effect of industry on the aquatic sediment. The downstream order of the sites is 14, 17, 7 and 1. Site 14 (Vaal Dam) is upstream of the major industries and has lower concentrations (table 5.3) than at the Loch Vaal (site 17). This site is situated next to industries expected to contribute towards PCB and PCDD/PCDF-pollution and therefore the highest concentrations in this river system was measured at this site. Site 7 (Douglas) is downstream from the industry and reflects lower

concentrations again, probably due to the effect of dilution, but also indicates transport. The concentrations are still higher than those measured at site 14. Site 1 is at the mouth of the Gariep River and the concentrations here are lower than at site 7 as can be expected due to dilution, but still indicating long-range riverine transport.

What might be of some concern, is that the organic carbon normalised TEQ calculated for site 1 (table 5.3), is the highest for all the sites, indicating the possibility of other sources of these pollutants along the Gariep River, but due to its low organic carbon content (table 4.4), little of these pollutants adsorbed to the aquatic sediment. It would be a valuable contribution to determine the concentrations of PCB and PCDD/PCDF in other environmental matrices such as the water column, suspended particles and biota of the Gariep River to determine the existence of a threat to the environment, and to characterise more fully the extent of fate and transport.

Site 16 (Riet Spruit, diverted brook) is upstream of site 15 (Riet Spruit). Site 16 had the highest of all the measured concentrations, as was expected (cf. § 3.1). The heavily polluted Lake Ontario (USA) had a lake-wide average dioxin-like PCB-concentration of 9.4 pg/g TEQ in its sediment (Martin *et al.*, 2002). The corresponding value for site 16 is slightly higher at 10.01 ng/kg TEQ (table 5.3). PCB-concentrations in the sediments of Arctic lakes varied from 2 000 to 40 000 ng/kg d.w. (Muir, Omelchenko, Grift, Savoie, Lockhart, Wilkinson, & Brunskill, 1996). That is at least 400 times the highest value of the present survey. The total PCB-concentration for this site was 24 936.20 ng/kg d.w. which was the highest for the twenty-two sites and falls half-way in the range reported for the Arctic Lakes. Average sediment levels for PCB in South America is 9 100±7 700 ng/kg d.w. (Whylie *et al*., 2003) which is higher than all the sites measured in this study except for site 16.

The site in the Riet Spruit (site 15) had slightly lower concentrations, probably due to the dilution or chromatographic effect when moving down stream. The Riet Spruit flows into Loch Vaal (site17). The concentrations in Loch Vaal were higher than the Riet Spruit site, which could indicate other sources of PCB and PCDD/PCDF, or perhaps higher sedimentation rates of lakes.

Another site central to industry is the Modderfontein Spruit (site 22). Of the twenty-two sites of this study, it was the second-most polluted site. It had the second highest

concentration for both PCB and PCDD/PCDF (table 5.3). It also had the second highest TEQ-value and the third highest normalised TEQ value.

The Crocodile River (site 18) close to Nelspruit had high concentrations that may be explained by the presence of a paper mill upstream, at Ngodwana, or possibly other unknown sources. The Groot River (site 5) was selected for being inside a forestry area. This river was sampled at its mouth and it is possible that its high sand contents (it had the highest of all the sites, cf Table 4.4) limited the adsorption of the PCB and PCDD/PCDF-congeners that might have been derived from wood treatment plants upstream. This particular site also had no detectable organic carbon (Table 4.4).

Rivers that were selected to be sampled because of their association with coal mining and electricity producing areas, such as the Buffalo River (site 8) and the Olifants River (site 19) including the Loskop Dam (site 20), had low PCB and PCDD/PCDF-concentrations.

The site in the Hartbeespoort Dam did not measure a high concentration of PCB and PCDD/PCDF even though the site was at the inflow of the Crocodile River (draining a large part of Gauteng) and the expectation was for higher concentrations. Greichus, Greichus, Amman, Call, Hamman, & Pott (1977) reported a value of 70 000 ng/kg d.w. of PCB-congeners for the Hartbeespoort Dam in 1974.

Average PCDD/PCDF TEQ-values for sediment from Lake Ontario was 91 ng TEQ/kg (Marvin, Stern, Reiner, MacPherson, Kolic,, Braekevelt, & Painter, 2002) with four sites in excess of 200 ng TEQ/kg. None of the twenty-two sites in this study had PCDD/PCDF TEQ-values higher than 11.9 ng TEQ/kg (site 16). Values reported for surface sediments from Arctic lakes in Norway and Sweden showed concentrations ranging from 1.4 to 4.2 ng TEQ/kg d.w. (Whiley *et al*. 2003). The Zwartkops Estuary (site 6), the Umgeni River mouth (site 11), Loch Vaal (site 17) and Modderfontein (site 22) had values of 1.58, 1.19, 2.25 and 4.41 ng TEQ/kg d.w. respectively, all within the range reported for the Arctic lakes. PCDD/PCDF TEQ-values for Europe are generally in the same range as reported for the present study. For the Po River (Italy), values vary from 1 to 11 ng TEQ/kg d.w., 0.03 ng TEQ/kg for Lake Baikal (Russia), 0.05 ng TEQ/kg for the Selenga River (Russia), and for sediments close to a discharging point from a pulp and paper mill, 7.7 ng TEQ/kg (Whiley, 2003). Concentrations of

PCDD/PCDF in sediments from New Zeeland were in the range of 0.081 to 2.71 ng I-TEQ/kg (Whiley, 2003), which is similar to the ranges for this study (table 5.3).

The two sites in the Western Cape, the Theewaterskloof Dam (site 4) and Berg River (site 3) had low concentrations. The Berg River has little capacity to contain the congeners in its sediment because there was no detectable organic carbon present and it had a high sand content (table 4.4). Despite these conditions the presence of PCB and PCDD/PCDF were detected indicating a possible high-normalised TEQ. Further investigation is necessary to determine whether the concentrations are really this low, and how bio-available they are to the biota. The Theewaterskloof Dam seems to be a fairly “clean” site because low concentrations were measured even though the sand content was 63% and the organic carbon content 0.81%. The normalised TEQ-value for this site is very low (table 4.3).

The Thulazihleka Pan in Richard’s Bay is regarded as the “cleanest” site of the twenty-two investigated. It measured low concentrations PCB and PCDD/PCDF even though it had a high capacity for capturing these pollutants. Its organic carbon content was the highest of all the measured sites (table 4.4) and its sand content was the second lowest (cf Figure. 4.2).

### 5.3.2 International guidelines

The Canadian interim sediment quality guideline (ISQG) of PCB for freshwater is 34.1 µg/kg which translates to 34 100 ng/kg (Canadian Environmental Quality Guidelines, 2002). The target value used by the Dutch Ministry is 20 000 ng/kg (ERD-AG-003, 1999). The highest PCB concentration measured for the sites of this study was 24 936.20 ng/kg (site 16) which is the only one higher than the target acceptable by the Dutch Ministry but still lower than their intervention value of 1 mg/kg. The Canadian ISQG for the PCDD/PCDF is 0.85 ng TEQ/kg d.w. (Canadian Environmental Quality Guidelines, 2002) and five sites of the current study measured values higher than this guideline (site 6, 10, 11, 16 and 17; cf Table 5.3). Site 15 had a value of 0.84 ng TEQ/kg d.w. and site 18 came close with a value of 0.78 ng TEQ/kg d.w. In the light of how the South African sites compared to these guidelines, one realises that the levels in South Africa might not be as high as in fully industrialised countries, but we do have comparable levels thereof in our aquatic environment.

### 5.3.3 Extrapolation to fish

To form an idea of what the possible concentration of PCDD/PCDF in fish will be, the concentrations of PCDD/PCDF in Table 5.3 was transformed into normalised concentration (table 5.4) so that it can be compared to data on the relationship between sediment PCDD/PCDF-concentration and PCDD/PCDF-concentration in fish as reported by Wu, Schramm & Kettrup, 2001) (Figure 5.1) for Lake Ya-Er in China.

**Table 5.4** This table represents the concentrations of PCDD/PCDF in sediment

| # | Site name | Concentration (ng/kg d.w.) | % C | Concentration normalised (ng/kg d.w.) |
|---|---|---|---|---|
| 1 | Gariep River (mouth) | 3.80 | 0.06 | 6062.39 |
| 2 | Saldanha Bay harbour | 3.05 | 0.00 | - |
| 3 | Berg River | 20.27 | 0.00 | - |
| 4 | Theewaterskloof Dam | 22.00 | 0.81 | 2 726.06 |
| 5 | Groot River (mouth) | 7.80 | 1.66 | 468.47 |
| 6 | Zwartkops Estuary | 237.20 | 1.14 | 20 878.40 |
| 7 | Vaal River (Douglas) | 7.67 | 1.21 | 635.66 |
| 8 | Buffalo River | 6.28 | 0.16 | 4 007.56 |
| 9 | Mooi River | 13.22 | 1.92 | 690.09 |
| 10 | Umlazi River (mouth) | 155.87 | 0.96 | 16 239.66 |
| 11 | Umgeni River (mouth) | 188.92 | 0.68 | 27 714.64 |
| 12 | Richard’s Bay (harbour) | 3.77 | 0.17 | 2 187.10 |
| 13 | Thulazihleka Pan | 20.47 | 4.83 | 423.78 |
| 14 | Vaal Dam | 3.67 | 0.00 | - |
| 15 | Riet Spruit | 79.55 | 1.82 | 4 376.26 |
| 16 | Riet Spruit (diverted brook) | 1136.90 | 7.24 | 15 703.66 |
| 17 | Loch Vaal | 247.49 | 2.16 | 11 444.56 |
| 18 | Crocodile River | 250.79 | 1.21 | 20 650.43 |
| 19 | Olifants River | 5.71 | 0.89 | 639.27 |
| 20 | Loskop Dam | 4.16 | 0.18 | 2 308.43 |
| 21 | Hartbeespoort Dam | 61.15 | 0.45 | 13 456.10 |
| 22 | Modderfontein Spruit | 535.70 | 2.48 | 21 568.15 |

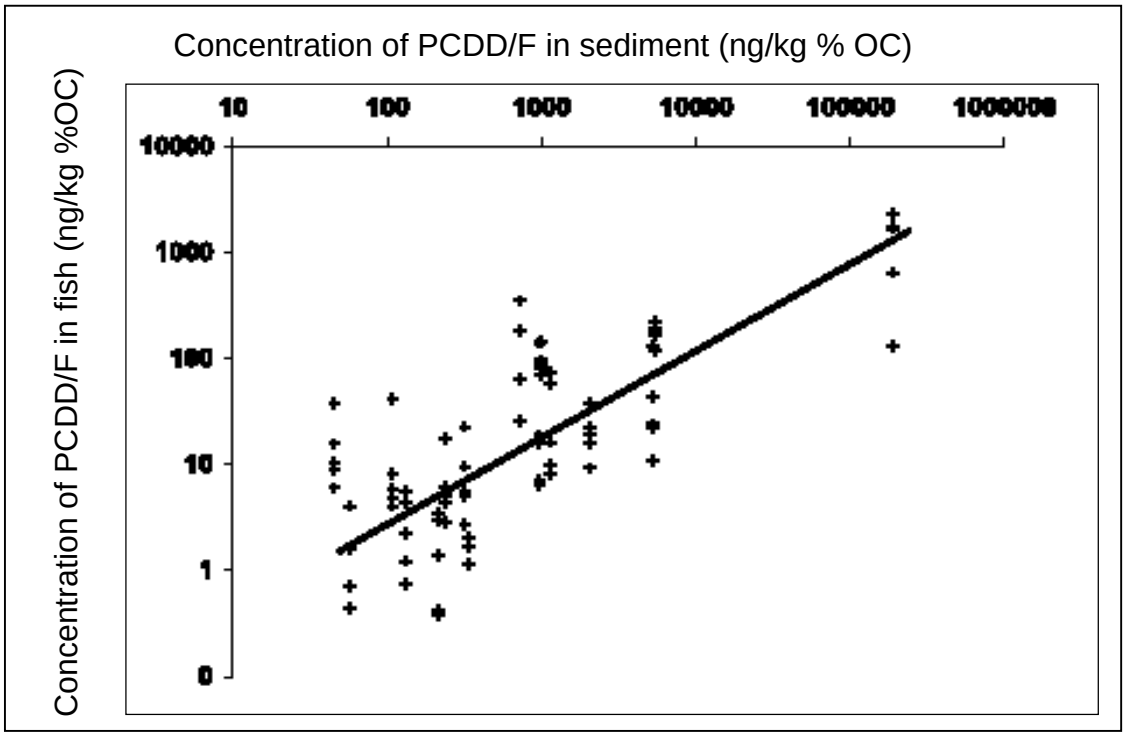


**Figure 5.1** Relationship between concentration of 2,3,7,8-substituted PCDD/PCDF in fish muscle and in sediment ($P < 0.05$) (as published by Wu *et al.* 2001)

The highest normalised PCDD/PCDF-concentration was 27 714.64 ng/kg for site11, the Umgeni River mouth. This could result in approximately 190 ng/kg w.w. in fish. The lowest normalised sediment concentration was at the Thulazihleka Pan (site 13) 423.78 ng/kg and this could result in approximately 3.8 ng PCDD/PCDF/kg w.w. in fish according to Figure 5.1.

Some states in the USA publish fish tissue action levels for screening evaluations based on EPA guidance. Action levels are concentrations of a contaminant in fish tissue below which there should be negligible risk of toxicity at a consumption rate of one meal a week. Action levels may be developed for several different toxicological endpoints (cancer, developmental, and other non-cancer effects). The state of Maine for *e.g.* has a cancer action level of 11 µg/kg in fish fillet wet weight. This means that if a person of 70kg body weight consumes 32.4 g of fish fillets containing 11 µg/kg PCB in its fillet every day, he/she is likely to develop cancer. The non-cancer action level is 43 µg/kg. The cancer action level for dioxins is 1.5 ng/kg and non-cancer action level 1.9 ng/kg (Bureau of Health Fish Tissue Action Levels, 2001). This implies that if the extrapolated fish concentrations for the sites in this study were valid, a recreational

fisherman consuming 32.4 g fish daily of any of the twenty-two sites in this study stands a risk of developing cancer!

The daily intake of dioxins and furans by the population of Western Europe through food is currently 1 pg I-TEQ/kg bodyweight. Based on new data, the Health Council of the Netherlands proposed lowering the accepted daily intake (ADI) to 1 pg I-TEQ. In Germany too, a long-term goal of 1 pg is proposed. The ADI laid down by the WHO is 10 pg 2,3,7,8-TCDD/kg bodyweight (Schriftenreihe Umwelt Nr 290, 1997). This would mean that a person with bodyweight of 70 kg stands the risk of cancer if he/she eats daily amounts of 0.368 g fish caught at the Umgeni mouth. According to the above extrapolation it is possible that the fish may have 190 ng PCDD/PCDF /kg wet weight.

The potential health risk identified by this preliminary hazard assessment based on twenty-two samples from across South Africa clearly needs further investigation, to confirm the route and extent of exposure to various sub-groups of fish consuming populations.

# 6 CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

- This research established the presence and levels of seven PCDD, ten PCDF and 12 PCB congeners in aquatic environments throughout SA (cf. Aim 1; § 1.3), using sediment.

  The change from sampling sediment instead of fish tissue proved to be fortuitous, although it was inevitable, due to the absence of available fish of the same species at all the sites; at some sites, fish of any kind was absent. Concentrations in fish gives an indication of the bio-accumulation factor and, in turn, an indication of possible risk to humans consuming the fish. However, without knowing the concentration of a bio-cumulative pollutant in an environmental matrix (*e.g.* water or sediment), no background concentrations are gained on these pollutants. Although sediment is a suitable matrix for regular monitoring because it is not influenced by breeding seasons and does not disappear due to ecologically limiting factors (such as inherent toxicity of waste discharges), it has other limiting factors; sedimentation rates may differ in time and locality, and floods can move sediment to localities downstream.

  Collecting the upper 5 cm sediment is especially useful because that is the layer that usually contains biota and will give an indication of the concentrations biota is exposed to.

- Very little has been done on environmental concentrations of PCB in recent years, and even less on PCDD/PCDF. In this period, South Africa has signed and ratified the Stockholm Convention on Persistent Organic Pollutants and we need to comply with its provisions once it becomes internationally legally binding. Not only does South Africa have to protect its own environment, wild life and human life, but must also take responsibility for its contribution to global levels of PCB and PCDD/PCDF, and its role in long range transport. This study will therefore go a long way in contributing to South Africa's obligations towards the Stockholm Convention. (cf. Aim 2; § 1.3). In particular it will address the following articles of the Convention:

- Contribute towards the development of the *National Implementation Plans* (Article 7, SC) where information needs to be assembled and considered.
- Article 9 on *Information exchange* to the Secretariat regarding POPs
- Article 10 on *Public information, awareness and education*, since the information and training course generated by this project is available through this report, the accompanying CD, and, in the future, also through publications.
- Article 11 on *Research, development and monitoring*, which was the main motivation behind this project. (It should also be noted that this is one of the few projects on PCDD/PCDF entirely funded within any developing country.)
- Article 12 on *Technical assistance*, since the training and cooperation received from the Michigan State University falls within this category.

And

- Article 16 on *Effectiveness evaluation* on a global scale, since we have already been approached to participate in this regard, peripherally due to this project.

This project should therefore also be considered in the broader context of the Stockholm Convention, as South Africa is now one of the few developing countries, and the only African country, where such a survey has been conducted.

- The methods employed in collecting, transporting, storing and analysing of sediment were effective and efficient, including the choice of the accredited international laboratory that did the GC/MS analysis. They have been shown through independent testing to be reliable and efficient.
- In general this study showed that South Africa have detectable levels of dioxin-like PCB and PCDD/PCDF which occur widespread in the entire country, in the aquatic sediment matrix, from freshwater rivers and dams to coastal harbours and river mouths (Tables 4.1 and 4.2). (cf. Aim 3; §1.3)

  The concentrations found, displayed the global trend of higher PCB concentrations vs lower PCDD/PCDF concentrations. The values did not

however, exceed the level of 50 ng/kg sediment, which was the action level determined for the USA.

Another global pattern repeated for South Africa was that higher concentrations were associated with the industrialised areas of the country.

The values found for the Vaal and Gariep River systems, particularly at the Gariep River mouth (site 1), indicate the real possibility of long-range riverine transport of these pollutants, and therefore a possible contribution from South Africa, to the global burden. The extent of this contribution is however not known. The role that the continuous degradation of organic materials, as it gets transported downriver, together with the persistence of the pollutants associated with this fraction, in possibly increasing the concentration, as found at the Gariep River mouth, is also unknown.

- Although the bio-assay results acquired up to now did not perform immediately as expected, much experience has been gained with this technology. This technology is currently being established at Potchefstroom. The acquisition of apparatus, equipment, consumables and tissue cultures is almost done. Many examples from the literature concerning bio-assay technique, as well as to the potential of this technology for general implementation in monitoring and research laboratories. It therefore provides solid motivation as to its applicability as a screening technique. Bio-assays are more cost effective than GC/MS, provides biologically relevant TEQ values, and more samples in less time can be screened. Almost any matrix can be analysed for their TEQ value by this reporter gene bio-assay. This project has therefore been instrumental (together with funding from the NRF through its Thuthuka programme) in developing South Africa's monitoring and research capacity for the analysis of toxic PCB and PCDD/PCDF. (cf. Aim 4; §1.3)
- The present study did not aim to address the issue of the risks of PCDD/PCDF and PCB to humans or wildlife directly. However, extrapolating the obtained data and drawing possible risk values from sediment/fish interactions from a Chinese study, gave the distinct indication that the PCDD/PCDF concentrations are possibly high enough to warrant concern about the risk of cancer development in humans consuming fish from aquatic environments, with

sediment concentrations as measured for some of our sites. This conclusion is however, based on limited data, and needs verification before any substantiated inference can be drawn (cf. Aim 5; §1.3)

Risk seems not to be restricted to the industrialised areas only. Places such as Alexander Bay and the Umgeni River mouth seem to be relatively polluted sites, if the organic carbon normalised concentrations are taken into consideration. The harbours sediments we sampled, seemed to have had rather low dioxin-like PCB and PCDD/PCDF concentrations.

- A short course on environmental sampling and Good Laboratory Practice has been developed and is available on the accompanying CD (cf. Aim 6; §1.3).

## 6.2 Technical recommendations

- To ensure comparability, extractions for GC/MS analyses and bio-analyses must be performed in exactly the same manner, selecting a solvent that is not cytotoxic. The best to use with this bio-assay technology would be hexane.
- The extraction and clean-up protocols followed, determine which groups of pollutants *eg.* PAH and/or dioxin-like PCB and PCDD/PCDF will still be present in the extract. This means that soil from the same sample can be treated differently in order to extract different groups of organic pollutants. If these pollutants are AhR ligands, the cells in the reporter gene assay will respond to their presence. Therefore, the bio-assay can be used to determine the TEQ of more than just those pollutants investigated in this study.
- Other types of bio-assays can also be performed, such as EROD and AHH, using the same tissue culture as for the reporter gene bio-assay. This increases the testing capacity of a monitoring laboratory, improving its applicability and scope of investigations.
- Since animal groups (birds, fishes mammals and humans) respond differently to the pollutants, the ideal would be to expose tissue cultures from the different animal groups to the same extract. In this way the effect of a pollutant on the different animal groups can be determined. A limited number of fish and bird tissue cultures are commercially available, and could be acquired.

## 6.3 Further research

Although this study has gone a long way to provide the the first and urgently needed information on dioxin-like PCB and PCDD/PCDF for South Africa, it is not enough. To truly understand the source characteristics, long-range transport of the compounds, their transfer between trophic levels and their distribution in matrixes at a particular site, sites must be sampled regularly and extensively, using multiple matrixes. In this way, bio-accumulation in the consecutive trophic levels can be traced. Especially the PCDD/PCDF concentrations at more than one site seemed to have concentrations of concern.

Research that is unique to SA will be to determine the exposure of people and environments exposed to the daily combustion of household wastes and fuel such as wood and coal.

The following specific areas of investigation are therefore proposed:

- More detailed investigations of polluted sites, as determined by this project, with the aim to better define the levels, and to investigate the possible sources.
- Determine the levels in fish and other aquatic biota to better determine the risks associated with the levels.
- A better characterisation of the possible long-range transport in some of the longer rivers in South Africa.
- A better characterisation of the estuarine and coastal distribution of these compounds.
- Investigations regarding the soil / aquatic interactions (eg. ground water or flood water) of POPs, especially in potential source areas such as industrial, waste and residential areas.
- A better characterisation of human exposure to POPs generated due to domestic heating and cooking that characterise the conditions of many South Africans.
- A better characterisation of human and wildlife exposure to POPs generated due to waste combustion and industrial activities that characterise many sites.

## ANNEX A Capacity building outcomes

i Short course on sampling and Good Laboratory Practice

A short course has been developed and will be published separately. It has been tested with students (including miss Lesejane) and junior faculty (total about 15) at the North-West University (Potchefstroom campus). Some of the students trained in this course were also involved with the sampling of this project. The short course is available on the accompanying CD.

ii Local centre on POPs analysis

During the course of this project, we were successful in obtaining additional and supportive funding from the National Research Foundation, via the Thuthuka programme, for Mrs Vosloo. With permission form the WRC, an extension was granted, to allow Mrs Vosloo to travel to the USA, to learn about bio-assay technology that can be used to analyse for PCDD/PCDF and PCB. This constitutes an additional capacity developed via this project.

Mrs Vosloo and Prof Bouwman have also attended a short course on Risk Assessment, presented at the North-West University (Potchefstroom campus).

In addition, we have initiated additional grant applications, in order to establish a strengthened centre for POPs analysis for SA.

iii Conference presentations

Mrs Vosloo and Prof Bouwman attended the DIOXIN 2002 conference in Barcelona (Spain) with funding from the University, and Mrs Vosloo has also attended the DIOXIN 2003 conference in Boston, USA, where she presented a paper on this project. This project was also presented at the ZSSA/SASAQS conference in Cape Town, 2003. A paper on this project will in all likelihood also be presented at the SETAC conference in Prague in 2004.

- Poster presentation: Dioxin 2002 (Barcelona): *An assessment of 30 years of environmental organochlorine residue data from southern Africa*.
- Poster presentation: Cape Town 21-23 Jan 2003: Joint European Southern African International Conference: Pesticides in non-target agricultural

environments. *An assessment of 30 years of environmental organochlorine residue data from southern Africa*

- Oral presentation: Cape Town 30 June - 4July 2003; Joint conference of SASAQS & ZSSA. *PCB's and PCDD/PCDF at selected aquatic sites of South Africa: an initial survey.*
- Oral presentation: Dioxin 2003, Boston. *PCBs and PCDD/PCDF in sediment in South Africa: an initial survey.*

iv Mrs Rialet Vosloo

This project has had, and still is, a major vector for the professional development of Mrs Rialet Vosloo. She has attended a three-month training course at the Michigan State University, and participated in two major international conferences. This project has also been a catalyst in obtaining additional funding from the NRF, through the Thuthuka programme for her career development. The skills, knowledge and experience she obtained as the principal scientist in this project, will stand her in good stead for the rest of her career, as well as through her leadership of younger students benefiting from the follow-up from this project.

v Miss Lesejane

Miss Lesejane has completed her practical work for her M.Sc, and is in the process of writing up. She has, during the course of this project, been identified by the CSIR and been offered an internship. After completing the internship, she has taken up a position at an environmental consulting firm.

vi Articles and papers published

No articles or papers have been published yet, but will be done.

vii Networks established

During the course of this project, the Sub-Saharan regional report on persistent toxic substances, executed by UNEP and funded by GEF, was completed (Osibanjo *et al.*, 2003). Prof Bouwman was part of the regional team. Following on this was an invitation from UNEP for Prof Bouwman to join the team to write the Global Report, which was finalised this year (Whylie *et al.*, 2003). Through these activities, more insight on the

issues involved with POPs and PTS, on a local and international basis, has been obtained. This has further strengthened the capacity of the team to address these issues in South Africa. The African and Global reports are available on the accompanying CD.

Contact with UNEP has further been strengthened, with Prof Bouwman having been invited on the steering committee of an initiative on Article 16 of the SC. Article 16 of the 2001 Stockholm Convention on Persistent Organic Pollutants (POPs) requires that the Conference of the Parties shall periodically evaluate the effectiveness of the Convention. This will include an evaluation of three elements: national reports; non-compliance information; and, an assessment of comparable environmental monitoring data on the chemicals listed in the action Annexes. Inherent in this process is a pilot project to be launched in two regions, one of which will be southern Africa. Although the final design still needs to be concluded, this two-year project will include POPs measurements in air, aquatic environments, breast milk and a biotic component still to be decided.

Another committee, on which Prof Bouwman now serves, is on research on alternatives for DDT in malaria control. This multi-country project, executed by the WHO, will take a number of years, with hopefully reduced pollution of the environment, as indicated by Sereda & Meinhardt (2003).

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