EXPERIMENTAL FIELD STUDIES AND PREDICTIVE MODELLING OF PCB AND PCDD/F LEVELS IN AUSTRALIAN FARMED SOUTHERN BLUEFIN TUNA

(Thunnus maccoyii)

by

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in

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CHAPTER ONE

INTRODUCTION
1.1 BACKGROUND

The gross value of production (GVP) from Australian fisheries alone has increased from an estimated $AUD 1.1 billion in 1990/91 (ABARE, 1991) to $AUD 2.1 billion in 2005/06 (ABARE, 2007). Rock lobster, prawn, abalone and tuna (*Thunnus*) are four of Australia’s major production species and account for approximately 55% of the GVP (ABARE, 2007). In 2005/06 the Australian Bureau of Statistics reported that 8806 tonnes of Southern Bluefin Tuna (*SBT, Thunnus maccopyii*) were produced from SBT farms centred offshore of Port Lincoln in South Australia, totalling a market value of approximately $AUD 156 million (ABARE, 2007). In order to obtain premium prices in export markets, farmers fatten wild-caught stock with a selection of baitfish. The SBT farming process occurs in sea-cages (floating pontoons each with a diameter of 32 m) anchored to the seabed, located approximately ten nautical miles offshore of Port Lincoln, South Australia.

Organic chemical residues of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are ubiquitous and can be found in foods. In recent years, consumers are increasingly aware of the presence of chemical residues including PCBs and PCDD/Fs that can be found in farmed products. From farm (farm managers and farmers) to fork (consumers) – all have an interest in managing chemical residues that have the potential to biomagnify\(^1\) in the fatty tissue(s) of foodstuff.

PCBs are man-made chemical mixtures that had widespread use in electrical transformers, in plastic manufacture and as heat exchange fluids. Although the production of PCB mixtures is now banned, these mixtures have been deposited in oceans because of industrial activity over the last century (Froescheis et al., 2000). PCDD/Fs are chemical compounds that are produced from unintentional events, for example, bush fires and incomplete combustion from industrial chemical processes (Schuhmacher et al., 2000; Wunderli et al., 2000). Both PCBs and PCDD/Fs are highly lipophilic and have long half-lives (in the order of decades) indicating that these chemical residues tend to biomagnify in predators at the higher levels of the food chain (Froescheis et al. 2000).

\(^1\) See Appendix A for definition of some important terms used in this research
Both PCBs and PCDD/Fs in farmed products are known to biomagnify from feed source. In Finland, Isosaari et al. (2002) compared dry fish feed and Baltic herring (a baitfish type) as a source of PCBs and PCDD/Fs fed to rainbow trout, and concluded that the PCDD/F congener profile closely represented that in the Baltic herring fed as feed. In Japan, Guruge et al. (2005) reported that a major source of PCBs and PCDD/Fs in farmed terrestrial and marine livestock came from feeds. In Australia, preliminary work indicated that both PCBs and PCDD/Fs in farmed SBT came from baitfish fed as feed (Padula et al. 2004a, 2004b).

To better manage levels of PCBs and PCDD/Fs in farmed SBT, the SBT industry would like a predictive tool (model) to help achieve targeted concentrations in the final fillet product by making scientific-based decisions on baitfish selection. The chemical residue research carried out with the industry also demonstrates to importing countries that Australia is actively managing levels of PCBs and PCDD/Fs in farmed SBT, to ensure that a high quality and safe product is delivered to the consumer.

To meet the research requirements for the SBT industry, the Australian Fisheries Research and Development Corporation (FRDC) together with the Cooperative Research Centre for Sustainable Aquaculture of Finfish (Aquafin CRC) have initiated a number of projects that include value-adding of farmed SBT. One of these larger projects – FRDC Project 2004/206 and Aquafin CRC Project 2.1(2) commenced in 2004 entitled “Management of Food Safety Hazards in Farmed Southern Bluefin Tuna to Exploit Market Opportunities” for which this research forms a part.

Against this landscape, a study of the temporal trends in the levels of PCBs and PCDD/Fs in farmed SBT and the use of a mechanistic model to predict these levels farmed SBT fillets are presented in this thesis.

1.2 OBJECTIVES

The principal objective of this research is to synthesise and experimentally validate a mechanistic model that can be used as an effective risk management tool (model) to predict the levels of PCB and PCDD/F in fillets of SBT at harvest. An additional industry-focused aim of this research is to determine if a Longer Term Holding (LTH) farming
period, with a duration of an extra 12 months after a typical farming period of approximately five months, could produce SBT with higher condition index (CI) and lipid content, while keeping levels of PCBs and PCDD/Fs low, compared to the typical farming period.

Farmed SBT from two commercial companies (Farm Delta Fishing Pty Ltd in 2005/06 and Farm Alpha Fishing Pty Ltd in 2006) were sampled with a view to applying the results to practical management of PCB and PCDD/F levels associated with commercial farming practices in the SBT industry.

As this is the first time chemical residue research and predictive modelling of PCBs and PCDD/Fs are carried out on farmed SBT, demonstrating a clear and transparent picture of the biomagnification (process) kinetics of PCB and PCDD/F levels in SBT from farming can be justified by increased confidence in both access (regulatory-based) and consumer markets.

1.3 THESIS OVERVIEW

A logical four-stage research approach was adopted.

The first stage encompasses literature review and the development of a new risk framework for predicting chemical residues in SBT (Chapters 2 and 3). The second stage covers the development of a set of Standard Operating Procedures (SOPs) for sampling of SBT for residue research, the experimental design, and the study of the temporal trends of the PCB and PCDD/F data in the fillets of farmed SBT in relation to Condition Index (CI) and lipid content in the fillets (Chapters 4 and 5). The third stage comprises predictive modelling of the PCB and PCDD/F data studied in Chapter 5. Here assimilation efficiencies of the WHO-PCB and WHO-PCDD/F congeners in farmed SBT are quantified for the first time (Chapter 6). The fourth stage includes the demonstration of the practical application of the predictive model, and the summary of findings from this research and further development (Chapters 7 and 8).
The relevant literature is surveyed in Chapter 2. Chemical residues of PCBs and PCDD/Fs that have been assigned a Toxic Equivalent Factor (TEF) by the World Health Organisation are highlighted. Appropriate theoretical models amenable to experimental testing are discussed and compared. Physiologically based pharmacokinetic modelling (PBPK) was discussed as a potential tool for assessing and possibly predicting chemical contaminants in foods. A book titled *Physiologically Based Pharmacokinetic Modelling* was reviewed (Phua, 2006) as part of the literature surveyed.

Chapter 3 presents a new risk framework developed as a first step to promote the systematic approach of chemical residue research – as this is the first time chemical residue research is carried out with the SBT industry. The developed framework is based on conventional principles of microbiological risk assessment highlighted in Codex Alimentarius (Codex, 1999). Elements of mathematics can now be developed and integrated in this risk framework of five governing principles. Criteria for an adequate model are established. These criteria are used to assess the goodness of fit of the predictive model. This preliminary work culminated in the publication of two refereed research papers, Phua et al. (2005) and Phua et al. (2007), respectively, in the Proceedings of the 33rd Australasian Chemical Engineering Conference (CHEMECA 2005) and in the international scientific journal *Chemical Engineering and Processing*.

Chapter 4 describes the experimental design, sampling methods and materials used in this investigation. The experiments for research on farmed Southern Bluefin Tuna (SBT) were designed as part of a larger inter-disciplinary study by the Aquafin CRC to primarily examine SBT health and product quality. SOPs for sampling of SBT for residue research were developed and implemented. Analytical methods based on USEPA 1668A and USEPA 1613B, used for determining PCB and PCDD/F concentrations respectively, are described. Because the concentrations of WHO-PCDD/F congeners (except 1,2,3,7,8-PeCDD, 2,3,7,8-TeCDF, 2,3,4,7,8-PeCDF and 2,3,7,8-TeCDD for \( t = 360 \) and 496 days) found in the fillets of SBT were reported at the Limit of Detection (LOD), a blank threshold concentration method was developed to ascertain true detects.

Chapter 5 presents the temporal trends of the PCB and PCDD/F data in the fillets of farmed SBT. Here, the net changes in PCB and PCDD/F levels during farming from wild-caught fish (as baseline) to harvested fillets (as final product) were determined, and the
relationships between these levels and the condition index (CI) and lipid content were investigated. The assessment for the levels of PCBs and PCDD/Fs in farmed SBT fillets has been published as Phua et al. (2008) in the international scientific journal *Chemosphere*.

Chapter 6 covers the synthesis and application of a mechanistic quantitative model to the PCB and PCDD/F (2,3,7,8-TeCDF) data detected in farmed SBT fillets obtained from Farm Delta Fishing Pty Ltd in 2005. Assimilation efficiencies for PCBs and 2,3,7,8-TeCDF in the fillets of SBT were obtained. The predictive model with the determined assimilation efficiencies for individual congeners were consequently validated against PCB and PCDD/F data from Farm Alpha Fishing Pty Ltd in 2006. The research carried out here has been published as Phua et al. (2006) in *Organohalogen Compounds* and will be submitted as Phua et al. (2008) to the *Journal of Food Engineering*.

Chapter 7 presents a practical application of the predictive model in dietary modelling on SBT consumers. Here, PCB and PCDD/F analyses were carried out on the three tissue-specific retailed fillets, namely, akami, chu-toro and o-toro for selected SBT (n = 7). Five hypothetical strategies for baitfish selection are presented in order to determine the intake of PCBs and PCDD/Fs from eating one of the three tissue-specific fillets. A manuscript to the *Journal of Food Protection* is being prepared.

A summary of findings and conclusions, together with proposed further development(s) are presented in Chapter 8.

The definition of some important terms and abbreviations used in this research are given in Appendices A and B respectively. SI units are used throughout. Additional significant analyses and raw data are presented in the appropriate Appendices to demonstrate the unambiguous management of information and transparency in this research.
Parts of this chapter have been prepared as:

2.1 INTRODUCTION

A wide range of chemical residues can be found in aquatic environments (Malins and Ostransder, 1994). Organic chemical residues of polychlorinated biphenyls (PCBs) and polychlorinated dibenz-p-dioxin and dibenzofurans (PCDD/Fs) are ubiquitous and available for uptake by aquatic organisms. Aquatic organisms higher up in the trophic food chain can bioaccumulate higher concentrations of organic residues that include PCBs and PCDD/Fs.

It is noted at this point that there are varied definitions of the term “bioaccumulation” in the literature over the past two decades. However, it is now widely accepted that bioaccumulation is the process that results in an appreciable increase in the concentration of chemical residues in an aquatic organism (e.g. fish) from the water (usually under laboratory conditions) and diet (Gobas and Morrison, 2000). The reader is directed to Appendix A “Definitions of Some Terms in This Research” in order to contrast the terms bioaccumulation, bioconcentration, biotransformation and biomagnification.

Applied to this research, biomagnification is the process resulting in an increase in the concentrations of PCBs and PCDD/Fs in farmed Southern Bluefin Tuna (Thunnus maccopyii) fish due to dietary absorption. Biomagnification may be regarded as a special case of bioaccumulation – where the concentration in the diet is usually higher than other routes of uptake, for example, from dermal absorption and diffusion through the gills (Mackay and Fraser, 2000).

There can be several incentives for quantifying the extent of biomagnification. Fish that freely swim in the wild and can biomagnify organic chemical residues can be used as biomonitors of environmental contamination. Wild-caught fish and fish that are farmed as food, can consequently result in consumers being exposed to the levels of chemical residues found in the fish. These persistent chemical residues can be transferred from one organism to another in a food chain, potentially increasing in concentrations at the high trophic levels. In the context of this research, quantifying the levels of PCBs and PCDD/Fs in farmed SBT can provide insight into feeding practices and subsequent
management of the feed fed to farmed SBT, and inform consumers of potential exposure to these levels.

The synthesis and application of a predictive model to describe the biomagnification of PCBs and PCDD/Fs in farmed SBT requires knowledge of the biology of SBT, toxicology of PCBs and PCDD/Fs, biotransformation of PCBs and PCDD/Fs in SBT, and practices of SBT farming. These multi-disciplinary aspects are normally studied separately and not integrated to provide a holistic understanding.

In this chapter, a thorough review of the synthesis and application of appropriate models for the biomagnification process of the levels of PCB and PCDD/F in fish are presented. This chapter concludes with the selection of a mechanistic biomagnification model for experimental field studies.

2.2 POLYCHLORINATED BIPHENYLS (PCBs) AND POLYCHLORINATED DIBENZO-P-DIOXINS / DIBENZOFURANS (PCDD/Fs)

Organic chemical residues such as the polycyclic halogenated aromatic compounds are a group of persistent chemical compounds that have been widely dispersed in the environment globally. These compounds biomagnify in food chains, particularly in tissues and organs rich in lipids. The two polycyclic halogenated aromatic compounds investigated in this work that have the potential to bioaccumulate and biomagnify in the fatty tissue of fish and other aquatic animals because of high lipophilicity and slow metabolism are PCBs and PCDD/Fs (Connell et al., 2002).

PCBs and PCDD/Fs (dioxins) are non-polar compounds with two connected benzene rings. The benzene rings of PCB are joined at the para position, PCDD are connected with two oxygen atoms and those of PCDF are connected with a single oxygen atom (Figures 1.1 through 1.3). Figures 1.1 though 1.3 also show the positions where chlorine atoms may be substituted. For example, 3,3’,4,4’,5 penta chlorobiphenyl (commonly known as PCB 126) has chlorine atoms substituted at the 3,3’,4,4’ and 5 positions, while 2,3,7,8-TeCDD represents tetra chlorinated dibenzo-p-dioxin substituted with chlorine congeners at the 2,3,7 and 8 positions. PCBs and dioxins can have chlorine atoms
substituted at various positions, resulting in several possible configurations for PCBs, PCDDs and PCDFs. The different configurations resulting in different compounds are termed congeners. There are 209 congeners of PCBs, 75 congeners of PCDDs and 135 of PCDFs. Highly chlorinated congeners are virtually insoluble and remain associated with soil and sediments. Lower-chlorinated congeners have a low solubility in water. Trace amounts of these substances migrate from soil into water and end up in the seas. Other potential pathways for both PCBs and PCDD/Fs entering the biosphere are described in the following section(s).

![Figure 1.1 Molecular structure of PCBs](image1)

**Figure 1.1 Molecular structure of PCBs**

![Figure 1.2 Molecular structure of PCDDs](image2)

**Figure 1.2 Molecular structure of PCDDs**

![Figure 1.3 Molecular structure of PCDFs](image3)

**Figure 1.3 Molecular structure of PCDFs**

### 2.2.1 Polychlorinated biphenyls (PCBs)

In 1929, the Monsanto Industrial Chemical Corporation began commercially producing PCBs as complex mixtures (Erickson, 1997). Because of the chemical and physical stability and dielectric properties, PCB mixtures were used in capacitor dielectric fluids, transformers, heat transfer fluids, pesticide additives, sealants and plastics. The properties
that made PCB mixtures ideal for such applications also resulted in PCBs becoming environmental contaminants that are very slow to degrade, and consequently biomagnifying through the food chain (Safe, 1993). Today, PCBs have been detected in almost every marine environment globally where analyses have been carried out (Martin et al., 2003).

Increased levels of PCBs in the environment and in wildlife and biota, including fish and humans, were first detected, reported and investigated in detail by Jensen (1966). Jensen (1966) postulated that PCBs have the potential to biomagnify through the food chain.

In 1969, the first widespread PCB contamination of the food chain in the United States was reported by Riseborough and Brodine (1971). The widespread news of accidental release and occupational exposure to PCBs resulted in bans from production, use and export. In 1977, Monsanto Industrial Chemical Corporation terminated production and export of PCBs (Belton et al., 1983; Anon. 1997; Grunwald, 2002).

The persistence of PCBs in the aquatic environment may be characterised by the solubility of PCBs, the partition coefficients (principally K\textsubscript{ow}) and half-life in water. The solubility of PCBs in water is low and decreases with increasing chlorination (Hutzinger et al., 1974). Hutzinger et al. (1974) reviewed studies on the water solubility of some PCB congeners and concluded that different congeners exhibited different solubilities (see Table 2-1), and that the partitioning behaviour of PCBs in water complicates the empirical determination of the solubility (in water).

In 1980, a first comprehensive analysis of PCBs by glass capillary gas chromatography was undertaken, with systematic numbering for all 209 PCBs (Ballschmiter and Zell, 1980) following the rules of substituent characterisation recommended by the International Union of Pure and Applied Chemistry (IUPAC).

### 2.2.2 Polychlorinated dibenzo-p-dioxin and dibenzofurans (PCDD/Fs)

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are collectively known as dioxins. During the pre-industrialisation period, dioxins were only present in trace levels because of natural combustion and geological processes (Czuczwa and Hites, 1984; Czuczwa et al., 1984; Schecter and Ryan, 1988). Because of widespread formation from numerous anthropogenic and industrial processes, today, dioxins are found in various
chemicals and technical mixtures, e.g. chlorophenols, agent orange (Schecter et al., 1992; Dwernychuk et al. 2002), as by-products of industrial waste, e.g. pulp bleaching (Whittle et al., 1993; Gilpin et al., 2003), or by-products of necessary combustion processes, e.g. production of iron, municipal solid waste (Olie, 1980; Hutzinger et al., 1985). PCDD/Fs have also been found in the environment because of accidental release during chlorophenol production (Cattabeni et al., 1978), application of pesticides (Baughman and Meselson, 1973) and improper disposal of waste (Carter et al., 1975).

The fate of PCDD/Fs has been investigated by several researchers (Czuczwa and Hites, 1984; Connell and Hawker, 1986; Eitzer, 1993; Segstro et al., 1995; Lohmann and Jones, 1998; Gaus, 2003). Briefly, PCDD/Fs discharged from combustion processes rely on air as carrier, thereby spreading contamination over a wide geographical area. The PCDD/Fs adsorb onto soil and sediment. Ground seepage and run-offs consequently serve as transport pathways for the presence of PCDD/Fs in the aquatic environment (i.e. convergence at the sea). The PCDD/F congeners in air may also be deposited via dry deposition processes (Shih et al., 2005; Lohmann and Jones, 1998).

Poland and Glover (1973, 1974) established that the most toxic congener among all dioxins was 2,3,7,8-tetra chlorinated dibenzo-p-dioxin (2,3,7,8-TeCDD). Experiments with guinea pigs showed mortality at orally ingested low doses of 0.6-2.5 μg.kg⁻¹-bodyweight for 2,3,7,8-TeCDD (Schwetz et al., 1973). The least toxic congeners are the dioxins without chlorine at the 2,3,7,8 positions and are unmeasured because these do not bioaccumulate in humans (Clement, 1991; Schecter and Piskac, 2001).

Of the 75 PCDD and 135 PCDF congeners, 7 PCDD congeners and 10 PCDF congeners are known to exhibit toxic effects. In the 1990s, the World Health Organisation formed an expert committee to quantify the toxicity of the 17 PCDD/F congeners and 12 PCB congeners in order to provide recommendations to national regulatory agencies worldwide.

In this research, the toxic 12 PCB and 17 PCDD/F congeners are referred as WHO-PCB and WHO-PCDD/F congeners.
Table 2-1. Solubilities of selected PCB congeners in water (after Hutzinger et al., 1974).

<table>
<thead>
<tr>
<th>PCB Congener</th>
<th>Solubility in Water (mg/L, or ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 chlorine atom</strong></td>
<td></td>
</tr>
<tr>
<td>2-mono chlorinated biphenyl (PCB 1)*</td>
<td>5.90</td>
</tr>
<tr>
<td>3-mono chlorinated biphenyl (PCB 2)</td>
<td>3.50</td>
</tr>
<tr>
<td>4-mono chlorinated biphenyl (PCB 3)</td>
<td>1.19</td>
</tr>
<tr>
<td><strong>2 chlorine atoms</strong></td>
<td></td>
</tr>
<tr>
<td>2,4-di chlorinated biphenyl (PCB 7)</td>
<td>1.40</td>
</tr>
<tr>
<td>2,2′-di chlorinated biphenyl (PCB 4)</td>
<td>1.50</td>
</tr>
<tr>
<td>2,4′-di chlorinated biphenyl (PCB 8)</td>
<td>1.88</td>
</tr>
<tr>
<td>4,4′-di chlorinated biphenyl (PCB 15)</td>
<td>8.00 x 10^{-2}</td>
</tr>
<tr>
<td><strong>3 chlorine atoms</strong></td>
<td></td>
</tr>
<tr>
<td>2,4,4′-tri chlorinated biphenyl (PCB 28)</td>
<td>8.50 x 10^{-2}</td>
</tr>
<tr>
<td>2′,3,4-tri chlorinated biphenyl (PCB 33)</td>
<td>7.80 x 10^{-2}</td>
</tr>
<tr>
<td><strong>4 chlorine atoms</strong></td>
<td></td>
</tr>
<tr>
<td>2,2′,5,5′-tetra chlorinated biphenyl (PCB 52)</td>
<td>4.60 x 10^{-2}</td>
</tr>
<tr>
<td>2,2′,3,3′-tetra chlorinated biphenyl (PCB 40)</td>
<td>3.40 x 10^{-2}</td>
</tr>
<tr>
<td>2,2′,3,5′-tetra chlorinated biphenyl (PCB 44)</td>
<td>1.70 x 10^{-1}</td>
</tr>
<tr>
<td>2,2′,4,4′-tetra chlorinated biphenyl (PCB 47)</td>
<td>6.80 x 10^{-2}</td>
</tr>
<tr>
<td>2,3′,4,4′-tetra chlorinated biphenyl (PCB 66)</td>
<td>5.80 x 10^{-2}</td>
</tr>
<tr>
<td>2,3′,4′,5-tetra chlorinated biphenyl (PCB 70)</td>
<td>4.10 x 10^{-2}</td>
</tr>
<tr>
<td>3,3′,4,4′-tetra chlorinated biphenyl (PCB 77)</td>
<td>1.75 x 10^{-1}</td>
</tr>
<tr>
<td><strong>5 chlorine atoms</strong></td>
<td></td>
</tr>
<tr>
<td>2,2′,3,4,5′-penta chlorinated biphenyl (PCB 87)</td>
<td>2.20 x 10^{-2}</td>
</tr>
<tr>
<td>2,2′,4,5,5′-penta chlorinated biphenyl (PCB 101)</td>
<td>3.10 x 10^{-2}</td>
</tr>
<tr>
<td><strong>6 chlorine atoms</strong></td>
<td></td>
</tr>
<tr>
<td>2,2′,4,4′,5,5′-hexa chlorinated biphenyl (PCB 153)</td>
<td>8.80 x 10^{-3}</td>
</tr>
<tr>
<td><strong>8 chlorine atoms</strong></td>
<td></td>
</tr>
<tr>
<td>2,2′,3,3′,4,4′,5,5′-octa chlorinated biphenyl (PCB 194)</td>
<td>7.00 x 10^{-3}</td>
</tr>
</tbody>
</table>

* PCB numbers in parentheses denote International Union of Pure and Applied Chemistry (IUPAC) assignment (Ballschmiter and Zell, 1980).
2.3 TOXIC EQUIVALENCY FACTOR (TEF) AND TOXIC EQUIVALENT (TEQ) HIGHLIGHTED BY THE WORLD HEALTH ORGANISATION (WHO)

Polycyclic halogenated aromatic compounds that include dioxins and PCBs are recognised as agonists of the aryl hydrocarbon (Ah) receptor in fish (Barron et al., 2004), because of the potential to induce the CYP1A enzyme (Billiard et al., 2002). The Ah receptor protein is present in most vertebrate tissues with high affinity for 2,3,7,8-substituted PCDD/Fs and some non-ortho substituted PCBs (Safe et al., 1985; Safe, 1986). Maximum affinity for the Ah receptor is achieved when four lateral positions in dioxin congeners are substituted with chlorine atoms. This results in the most toxic dioxin congener: 2,3,7,8 tetrachlorodibenzo-p-dioxin (2,3,7,8-TeCDD). The most toxic PCB congener (PCB 126) contains two para and fully substituted meta chlorine atoms. Chlorine atoms bound at the ortho position reduces affinity for the Ah receptor.

As the bioaccumulation and biomagnification of dioxins and PCBs in humans are a health concern, the World Health Organisation (WHO) together with a panel of experts developed consensus toxic equivalent factors (TEFs) for dioxins and PCBs for human, fish and wildlife risk assessment (Van den Berg et al., 1998). TEFs are assigned a numerical value relative to the value of unity for the toxicity of 2,3,7,8-TeCDD. The toxic equivalent (TEQ) for each congener can be calculated by multiplying its assigned TEF with concentration. An additive model of individual congener TEF dose will give total TEQ as shown in Equation 2-1, where \( n_1, n_2 \) and \( n_3 \) represent, respectively, PCDD, PCDF and PCB groups and \( i \) the \( i^{th} \) congener. Applied to this work the units of TEQ are picogram of residue per gram of fish fillet. This methodology applies only to Ah receptor-mediated responses.

\[
\text{TEQ} = \sum_{n_1} (\text{PCDD}_i \times \text{TEF}_i) + \sum_{n_2} (\text{PCDF}_j \times \text{TEF}_j) + \sum_{n_3} (\text{PCB}_k \times \text{TEF}_k)
\]

(2-1)

Tables 2-2 shows the congener TEFs for human risk assessment based on fish consumption and have been derived with the following four criteria (Van den Berg et al., 1998; Van den Berg et al., 2006):

- Compound shows structural relationship to PCDD and PCDF
- Compound binds to the Ah receptor
• Compound elicits Ah receptor-mediated biochemical and toxic responses
• Compound accumulates and is persistent in the food chain.

In June 2005, an expert meeting was held in Geneva to re-evaluate the 1998 TEF system leading to certain congeners assigned new TEFs. The PCDD/F and PCB congeners that have been assigned new weighted factors are, respectively: PCB 81, 169, 105, 114, 118, 123, 156, 157, 167 and 189; and 2,3,4,7,8-penta chlorinated dibenzofuran, 1,2,3,7,8-penta chlorinated dibenzofuran, octa chlorinated dibenzo-p-dioxin and octa chlorinated dibenzofuran (see Table 2-2). This expert panel has mentioned that some individual PCB congeners and other groups of compounds, presently not included in the TEF system, may be possibly included in the next re-evaluation of the WHO TEF system (Van den Berg et al., 2006). This re-evaluation may be conducted within the next two to five years (Prof. Martin Van den Berg, Dioxin 2006 Symposium Questions Session). The release of the WHO 2005 TEF system coupled with question-answer communication during the Dioxin 2006 Symposium indicated that research in this field is time-sensitive and may change very quickly depending on new scientific contribution to the toxicological field. However, for now, Van den Berg and co-workers (2006) concluded:

“In general it can be concluded that the changes in 2005 values have a limited impact on the total TEQ of these [biotic] samples with an overall decrease in TEQ ranging between 10 and 25 %.”

The research presented from this thesis is based on the WHO 1998 TEF system. This is because the 1998 TEF system will result in higher TEQ levels – exhibiting a possible worst-case scenario and also using 1998 TEF system will permit a direct comparison of the TEQ levels from the published scientific literature.
Table 2-2. Tabulated summary of the 12 PCB and 17 PCDD/F congeners ranked by TEF values for risk to human health (*after* Van den Berg et al., 1998; Van den Berg et al., 2006).

<table>
<thead>
<tr>
<th>Congener</th>
<th>1998 TEF</th>
<th>2005 TEF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-ortho Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,3',4,4',5-Penta chlorinated biphenyl (PCB 126)*</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>3,3',4,4',5,5'-Hexa chlorinated biphenyl (PCB 169)</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>3,3',4,4'-Tetra chlorinated biphenyl (PCB 77)</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>3,4,4',5-Tetra chlorinated biphenyl (PCB 81)</td>
<td>0.0001</td>
<td>0.0003</td>
</tr>
<tr>
<td><strong>Mono-ortho Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,4',5-Penta chlorinated biphenyl (PCB 114)</td>
<td>0.0005</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4',5-Hexa chlorinated biphenyl (PCB 156)</td>
<td>0.0005</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4',5'-Hexa chlorinated biphenyl (PCB 157)</td>
<td>0.0005</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4'-Penta chlorinated biphenyl (PCB 105)</td>
<td>0.0001</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3',4,4',5-Penta chlorinated biphenyl (PCB 118)</td>
<td>0.0001</td>
<td>0.00003</td>
</tr>
<tr>
<td>2',3,4,4',5-Penta chlorinated biphenyl (PCB 123)</td>
<td>0.0001</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4',5,5'-Hepta chlorinated biphenyl (PCB 189)</td>
<td>0.0001</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3',4,4',5,5'-Hexa chlorinated biphenyl (PCB 167)</td>
<td>0.00001</td>
<td>0.00003</td>
</tr>
<tr>
<td><strong>PCDD/F</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,7,8-Tetra chlorinated dibenzo-p-dioxin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1,2,3,7,8-Penta chlorinated dibenzo-p-dioxin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2,3,4,7,8-Penta chlorinated dibenzofuran</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>1,2,3,4,7,8-Hexa chlorinated dibenzo-p-dioxin</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1,2,3,6,7,8-Hexa chlorinated dibenzo-p-dioxin</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1,2,3,7,8,9-Hexa chlorinated dibenzo-p-dioxin</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>2,3,7,8-Tetra chlorinated dibenzofuran</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1,2,3,4,7,8-Hexa chlorinated dibenzofuran</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1,2,3,6,7,8-Hexa chlorinated dibenzofuran</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1,2,3,7,8,9-Hexa chlorinated dibenzofuran</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>2,3,4,6,7,8-Hexa chlorinated dibenzofuran</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1,2,3,7,8-Penta chlorinated dibenzofuran</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-Hepta chlorinated dibenzo-p-dioxin</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-Hepta chlorinated dibenzofuran</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8,9-Hepta chlorinated dibenzofuran</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Octa chlorinated dibenzo-p-dioxin</td>
<td>0.0001</td>
<td>0.0003</td>
</tr>
<tr>
<td>Octa chlorinated dibenzofuran</td>
<td>0.0001</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

* PCB numbers in parentheses denote International Union of Pure and Applied Chemistry (IUPAC) assignment.
2.4 SOUTHERN BLUEFIN TUNA (SBT)

Southern Bluefin Tuna (*Thunnus maccoyii*, SBT) are large pelagic fish found throughout the southern hemisphere mainly in waters between the latitudes of 30° to 50° south (CCSBT, 2008). Because SBT are a highly migratory species (spanning from the Indian Ocean to the Southern Ocean), they are equipped to cover long distances and swim very quickly with an average speed range of two to three km.h⁻¹. SBT are able to tolerate a wide range of water temperatures because of their advanced circulatory system, can have an expected lifespan of up to 40 years, and are known to dive to at least 500 m (CCSBT, 2008) for various reasons that include: the search for baithfish or to avoid larger predators such as sharks or seals. Sharks and seals are even known to have attacked SBT farmed in sea-cages in the open oceans (*pers. comm.* David Warland, SBT Farm Manager; David Ellis, Research Manager, Australian Southern Bluefin Tuna Industry Association Ltd). Juvenile SBT are found within the Great Australian Bight (Serventy, 1956; Leigh and Hearn, 2000; Farley et al., 2007).

The principle method employed to catch SBT (except for the Australian fishery) is longline fishing. Longline fishing involves using long lengths of fishing line with many hooks. The Australian fishery employs the purse seine method and captures SBT in the Great Australian Bight (Leigh and Hearn, 2000; Farley et al., 2007). Purse seine fishing involves using a net drawn by two or more fishing vessels to enclose a school of SBT. In contrast to longline fishing, purse seine fishing captures SBT live, with the intent to fatten SBT (via farming for approximately five to six months) prior to sale to premium sushi and sashimi markets in Japan (major market), Korea and the United States (emerging markets). SBT fetches premium prices because Bluefin is considered to be the “king of the tunas” (Hespe, 2004; Anon. 2005; Carter, 2006).

Because SBT farming is a niche Australian fishery, previous research projects were focussed on the nutrition and health of farmed SBT, and SBT production – ways to get SBT fattened quickly. One of the indicators for SBT production is the growth of farmed SBT. Carter and co-workers (1998) modelled growth in farmed juvenile SBT and various morphological, physical and biochemical indices. They concluded that defrosted pilchards produced the best growth, as it may have resembled feeding in the wild when compared
to the use of pellet feed (used in many aquaculture-based fisheries, e.g. farmed salmon and rainbow trout). As a result of this initial work, the Australian SBT industry presently practises using a mix of baitfish that included defrosted pilchards as feed to SBT.

SBT are valuable fish. In 2005/06 the Australian Bureau of Statistics reported that 8806 tonnes of SBT were produced from SBT farms centred offshore of Port Lincoln in South Australia, totalling a market value of approximately AUD$156 million (ABARE, 2007). In order to maintain market share and price, it is becoming necessary for aquaculture producers to consider factors such as sustainability of wild SBT stocks (CCSBT, 2008), environmental impact from farming and levels of chemical residues such as PCBs and PCDD/Fs in harvested product as well as product quality and the cost of production when managing farming operations.

In order to rigorously manage PCB and PCDD/F levels, a quantitative and through-chain (from farming) predictive model for PCBs and PCDD/Fs in SBT is needed. Currently, there is no predictive tool for estimating PCB and PCDD/F levels in SBT. Very little is published in the scientific literature on predictive modelling in farmed pelagic fish such as the SBT. A review of the literature is therefore carried out for quantitative studies in (general) fish.
2.5 MODELLING (BIOMAGNIFICATION) STUDIES IN FISH

The biomagnification of PCDD/Fs and PCBs in seafood is of significant importance with the rapid development of food engineering, processes and environmental concerns. Of the wide range of seafood, fish have received the most attention (Hochachka and Somero, 1984; Mottet and Landolt, 1987; Powers, 1989; Carter et al., 1998; Sweet and Zelikoff, 2001; Wu et al., 2001; Smith et al., 2002; Isosaari et al., 2002; Padula et al., 2004a, 2004b; Bell et al., 2005). Fish represents the majority class of vertebrates in terms of number of species and occupy virtually every aquatic habitat available (Miracle and Ankley, 2005). Because of the diversity of species and availability as seafood, fish is an important source of human exposure to PCBs and PCDD/Fs.

2.5.1 Chronological Development of a Biomagnification Model

The modelling of chemical residue levels in fish is not truly new. Some of the earliest proposals for using mechanistic models to estimate rate constants for the assimilation and clearance of chemical residues by fish include the work of Branson et al. (1975), Norstrom et al. (1976), Neely (1979), Bruggeman et al. (1981) and Spacie and Hamelink (1982). However, many of the early modelling work focussed on aqueous exposure, being the major source of PCB and PCDD/F exposure.

A two-compartmental kinetic model was applied to the data for 2,2’,4,4’-tetrachlorobiphenyl (PCB 47) in rainbow trout (*Salmo gairdneri*) by Branson et al. (1975). In this work, Branson and co-workers accounted for the uptake of PCB 47 solely from water contaminated with PCB 47 in the laboratory and observed that steady state conditions were achieved after five days of aqueous exposure.

Norstrom et al. (1976) extended the findings of Branson et al. (1975) to account for dietary exposure. Norstrom and co-workers proposed that the uptake of chemical residues could be controlled by the metabolism and growth of fish, as affected by environmental conditions such as temperature and food availability. An accumulation model based on fish bioenergetics, embedded with a series of equations was consequently developed. This resultant model was of a differential form and comprised a term for combining uptake of
the chemical from water and food and a term for depuration of the chemical based on bodyweight-dependent equations. Steady state conditions could not be achieved with this model because of the changes of the body weight of the fish through time – assuming that the fish gains an appreciable amount of weight over time. The obvious shortcoming of the model developed by Norstrom and co-workers was the difficulty in obtaining measurements for various metabolic rate constants needed within the series of equations.

In 1979, Neely highlighted that the rates of uptake and clearance of chemicals in fish are important and can provide a meaningful analysis when carrying out intra-fish species comparisons. Approaching only from an aspect of aqueous exposure, a model was proposed by combining the regression relationship between octanol-water partition coefficient ($K_{ow}$) and biconcentration in fish (Neely et al., 1974) and the uptake term established by Norstrom et al. (1976). After studying the data for 2,3,7,8-TeCDD in rainbow trout – that were kept in a 145 L aquarium, with the series of equations that make the model, Neely found that the proposed model over-predicted the uptake rate constant by approximately 30% and under-predicted the clearance rate constant by approximately 50%, compared to the observed data. Neely subsequently concluded that the proposed modelling method has the potential to generate “reasonable values for the rate constants”.

Further evaluation of the modelling method proposed by Neely was undertaken. Findings indicate that the application of a $K_{ow}$ regression equation could underestimate the bioaccumulation of chemical compounds that have log $K_{ow}$ values > 6 (Crosby, 1975). In addition, chemical compounds that are more polar and have low $K_{ow}$ values may be likely to biodegrade or excreted implying that bioaccumulation may be under-estimated (Forster and Goldstein, 1969). Studies that followed (Schuurmann and Klein, 1988; Chessells et al., 1991; Fisk et al., 1998) have indicated that $K_{ow}$ does not accurately reflect the behaviour of super-hydrophobic chemical compounds (i.e. log $K_{ow}$ > 6). Schuurmann and Klein (1988) highlighted also that for super-lipophilic compounds (i.e. log $K_{ow}$ > 6), uptake of these chemicals via aqueous exposure is greatly decreased because of the very low water solubilities and therefore reduced bioavailability. In essence, for the super-lipophilic compounds, biomagnification via the food chain by piscivorous fish appears to dominate the exposure route (Muir & Yarechewski, 1988; Thomann, 1989; Muir et al., 1990).
Oliver and Niimi (1985) compared the bioaccumulation factor (BAF) equation from their study with the bioconcentration factor (BCF) equation from Mackay (1982) and concluded that although both equations predicted the same bioaccumulation trends (i.e. parallel lines are obtained when plotted with log $K_{ow}$ as the abscissa), accounting for chemical uptake from water solely underestimates residue levels in fish by at least a factor of five. The comparison concluded that the consumption of contaminated food is a major source of residues in fish for the super-hydrophobic chemical compounds.

In a recent comprehensive examination of $K_{ow}$ values of PCBs (Hansen et al., 1999), it was established that the 12 WHO-PCB congeners were classed as super-lipophilic, i.e. super-hydrophobic, with log $K_{ow}$ values in the range of 6.14 to 7.30 (Table 2-3). According to Table 2-1, it appears that for the PCBs, solubility in water decreases as chlorination increases. The evidence therefore suggests that PCBs with log $K_{ow} > 6$, which include the 12 WHO-PCB congeners found in fish, come from dietary exposure.

Gobas et al. (1988) reported that assimilation efficiencies for chlorinated organic chemicals in fish exhibit a nonlinear relationship with $K_{ow}$. For chemicals with log $K_{ow}$ up to approximately 6, assimilation efficiencies may be constant. However for chemicals with log $K_{ow} > 6$, i.e. super-lipophilic chemicals, assimilation efficiencies declines as $K_{ow}$ increases.

In 1981, Bruggeman and co-workers extended the work of Branson et al. (1975) to include dietary exposure with aqueous exposure resulting in a first-order two compartmental kinetic model of a differential form. This kinetic model is based on a mass balance approach expressed in differential form and therefore did not require the use of $K_{ow}$ values (see Table 2-4) as contrasted with the model proposed by Neely (1979). The incentive of the mass balance approach overcame the shortcoming of the Norstrom et al. (1976) model. For the application of the first order two compartmental kinetic model, Bruggman et al. (1981) studied the bioaccumulation kinetics of five PCB congeners (PCBs 9, 18, 31, 52, 70) from water and food in goldfish (Carassius auratus). These researchers found that clearance rates of these PCBs in goldfish were very low, corresponding to biological half-lives of more than one week. Combined assimilation efficiencies from water and food were in excess of 40 %.
Table 2-3. Predicted Log $K_{ow}$ values (after Hansen et al., 1999) and chlorination for the 12 WHO-PCB congeners.

<table>
<thead>
<tr>
<th>PCB Congener</th>
<th>Number of Chlorine Atoms</th>
<th>Log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-ortho Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,3’,4,4’-Tetra chlorinated biphenyl (PCB 77)</td>
<td>4</td>
<td>6.14</td>
</tr>
<tr>
<td>3,4,4’,5-Tetra chlorinated biphenyl (PCB 81)</td>
<td>4</td>
<td>6.14</td>
</tr>
<tr>
<td>3,3’,4,4’,5-Penta chlorinated biphenyl (PCB 126)</td>
<td>5</td>
<td>6.60</td>
</tr>
<tr>
<td>3,3’,4,4’,5,5’-Hexa chlorinated biphenyl (PCB 169)</td>
<td>6</td>
<td>7.06</td>
</tr>
<tr>
<td><strong>Mono-ortho Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,3’,4,4’-Penta chlorinated biphenyl (PCB 105)</td>
<td>5</td>
<td>6.39</td>
</tr>
<tr>
<td>2,3,4,4’,5-Penta chlorinated biphenyl (PCB 114)</td>
<td>5</td>
<td>6.39</td>
</tr>
<tr>
<td>2,3’,4,4’,5-Penta chlorinated biphenyl (PCB 118)</td>
<td>5</td>
<td>6.46</td>
</tr>
<tr>
<td>2’,3,4,4’,5-Penta chlorinated biphenyl (PCB 123)</td>
<td>5</td>
<td>6.46</td>
</tr>
<tr>
<td>2,3,3’,4,4’,5-Hexa chlorinated biphenyl (PCB 156)</td>
<td>6</td>
<td>6.84</td>
</tr>
<tr>
<td>2,3,3’,4,4’,5’-Hexa chlorinated biphenyl (PCB 157)</td>
<td>6</td>
<td>6.84</td>
</tr>
<tr>
<td>2,3’,4,4’,5,5’-Hexa chlorinated biphenyl (PCB 167)</td>
<td>6</td>
<td>6.92</td>
</tr>
<tr>
<td>2,3,3’,4,4’,5,5’-Hepta chlorinated biphenyl (PCB 189)</td>
<td>7</td>
<td>7.30</td>
</tr>
</tbody>
</table>

In 1982, Spacie and Hamelink presented a series of alternative models to predict the chemicals that include 2,2’,4,4’-tetrachlorinated biphenyl, chlorinated paraffins, chlorobenzenes and atrazines, in fish via aqueous exposure. The models proposed were of the differential form. The models presented included: first order one compartmental and two compartmental models, Michaelis-Menten elimination through passive diffusion at the gills or membranes, second order elimination kinetics and a drug transport model that integrated $K_{ow}$. None of these models accounted for exposure via diet (biomagnification). In addition, it has been established previously that $K_{ow}$ is a poor predictor for super-hydrophobic chemical compounds.

Mackay (1979; 1981) introduced the fugacity concept to the field of environmental modelling. The fugacity concept is algebraic identical to the rate constant approach suggested by Neely (1979). However, fugacity is used as surrogate for concentration.
Details of using the fugacity approach are now available from Mackay (1991) and in this, Mackay covers the principle use of fugacity approach in environmental modelling. Mackay and Hughes (1984) described a three-parameter model based on the fugacity concept. The three parameters were a target-water partition coefficient, and two time constant coefficients, both as a function of $K_{ow}$. This model, akin to the models presented by Spacie and Hamelink (1982), focussed on aqueous exposure only.

It was noteworthy that many of the early published work was based on aqueous exposure – this was no doubt because of the attention given to PCBs discharged into water systems by manufacturing industries (termed as Ocean Dumping – Miller, 1975) between the time when PCBs were manufactured in 1929 and banned in 1977. It was only in 1973 when an ocean dumping control law was legislated (Miller, 1975). It was also noted that many of the early researchers (Branson et al., 1975; Norstrom et al., 1976; Neely, 1979) were from a specific geographical location – Canada and the United States, where the studies appear to elucidate the accumulation of PCBs to aquatic organisms through the process termed bioconcentration.

The Great Lakes straddle the border separating Canada and the United States. The Great Lakes hold 80% of drinking water for 24 million people (Environmental Defence and the Canadian Environmental Law Association, 2005). The high percentage of Canada’s manufacturing output from The Great Lakes basin – 75% of Canada’s manufacturing output and 16% of the gross domestic product of the North American Free Trade Agreement that includes over US$200 billion in trade between Canada and the United States annually (Environmental Defence and the Canadian Environmental Law Association, 2005), suggests that there are many industries within that region and chemical monitoring is essential (Delfino, 1976). Because The Great Lakes is the source of drinking water as well as a site for major manufacturing industries, consequently many of the early research and those carried out in recent years (Gobas et al., 1986; Gobas and Schrap, 1990) focussed on aqueous exposure to fish – with the intent of shedding light on water contamination and potential exposure levels to humans, who consume the water and eat fish caught from The Great Lakes region.

Models describing PCB and PCDD/F bioaccumulation in fish have been developed largely for use in limited environmental studies where the major uptake route of chemical
residues was aqueous exposure. Such models are useful to establish the link that fish can be a biomarker for monitoring PCBs and PCDD/Fs in the environment (i.e. research relating to The Great Lakes).

It was some ten years after the initial work done of Branson et al. (1975) that researchers found that dietary exposure is most likely the major route for the uptake of super-hydrophobic chemical residues in fish (Bruggeman et al., 1984; Thomann and Connolly, 1984; Oliver and Niimi, 1988; Gobas et al., 1988; Barber et al., 1991). Consequently, the engineering principles that underpin biomagnification models were the same principles that founded bioconcentration models. Following, a shift from modelling PCBs and PCDD/Fs due to the bioconcentration process to modelling PCBs and PCDD/Fs due to the biomagnification process is observed.

In 1984, Thomann and Conolly proposed a four-series food chain mass balance equation for modelling PCBs (Aroclor 1254) in Lake Michigan lake trout (top tier in the food chain). This food chain model could account for greater than 99% of PCB exposure in lake trout. The authors also investigated the data using Kow-BCF equation based on the work of Neely (1974) and found that the model under-predicted the actual PCB concentrations by four to five times. Because the Kow-BCF approach accounted only for aqueous exposure, the use of this approach elucidated that the dominant contributor of PCBs to the top predator lake trout was dietary exposure.

Connolly and Pedersen (1988) evaluated the use of a thermodynamic model based on the fugacity concept and a kinetic bioenergetic based model based on a mass balance approach to predict the biomagnification of organic chemicals in fish. The use of fugacity model to predict chemicals in fish, if dietary exposure was the major route of uptake, did not appear promising. The authors concluded that the ratio of fugacities in fish to water were generally above a value of 1 (up to a value of 14 was observed for lake trout) and increased with trophic level in the food chain, and the hydrophobicity of the chemical. The applied assumption for the fugacity model is that the maximum fish to water ratio = 1. When this ratio value > 1 – the assumption becomes no longer valid and implies that dietary exposure >>> aqueous exposure.
In 1988, Gobas and co-workers presented the dynamics of dietary exposure and elimination of hydrophobic organic chemicals in fish using data for dietary uptake of chemicals in fish. The authors presented a food uptake model expressed in fugacity form. An important conclusion drawn was that super-hydrophobic chemicals are “extremely slowly metabolised and eliminated by fish”. Gobas and co-workers also reported that for dietary exposure as the major exposure route, the fugacity in fish to water > 1. Again, the assumption for the fugacity model has been violated. It appears that a model in the differential form could overcome the drawback of the fugacity model assumption.

Muir and Yarechewski (1988) studied the dietary accumulation of four PCDD congeners. At this point in time, the WHO has not classed any PCDDs or PCDFs as potential risk to human health. The first such congener-based assessment was published by Ahlborg et al. (1989), and it was for Nordic countries. Muir and Yarechewski determined the assimilation efficiencies for each of the four PCDDs using a kinetic rate equation for constant dietary exposure based on the work of Bruggeman et al. (1981). The model was adequate and indicated that uptake from food was predicted to be the predominant source for PCDDs in aquatic food chains.

In 1991, Barber and co-workers developed and presented a model (termed Food and Gill Exchange of Toxic Substances, FGETS) describing both aqueous and dietary exposure of PCBs in Lake Ontario salmonids. The model extended the work of Norstrom et al. (1976) and included biological attributes of the fish and physicochemical properties of the chemicals. This model was presented in a differential form. The incentive for using a differential form based on mass balances is that the mass balance expression can readily predict the extent of bioaccumulation or biomagnification and the concentration changes through time (i) as the fish grows (especially important if the fish grows significantly in weight), (ii) as the diet of fish changes and, (iii) as the fish physiologically responds to changing environmental conditions (Mackay and Fraser, 2000). It is not surprising that Barber et al. (1991) concluded that their model simulated that aqueous exposure of PCBs to Lake Ontario salmonids as more significant than dietary exposure.

In 1992, Sijm and co-workers presented a life-cycle biomagnification kinetic model for fish. All the processes leading to biomagnification were assumed to follow first order one compartment kinetics except for reproduction. The strength of this model is that the terms
involved are additive (or subtractive) for any process leading to biomagnification, and the model is in the differential form. The flexibility of this model permits amendments to suit various experimental scenarios, including field experiments. The key parameters of the model are assimilation efficiencies and overall elimination rate constants. One of the key incentives of the life-cycle biomagnification model is that it can comprise a term \( C_0 \), see Table 2-4, to account for initial concentration of chemical(s) in the fish (additive property of the model), i.e. biomagnification in wild-caught fish. This is especially important for predatory fish that are farmed from wild-caught – as there already is an initial storage of chemicals in the lipid reservoir from feeding on smaller fish in the wild (open oceans). In addition, as the model does not contain a term comprising \( K_{ow} \), it appears that this model is not limited to hydrophobic chemicals but amenable even to super-hydrophobic chemical residues such as the 17 WHO-PCDD/F and 12 WHO-PCB congeners.

Up till now, the author has shown the purpose(s) and origination of predicting chemical residues in fish and the chronological progress (and approaches) that resulted in the evolution of the biomagnification model. By the mid-to-late 1990s, it became widely accepted that dietary exposure can be a major source of uptake of hydrophobic chemicals in fish (Niimi, 1996; Campfens and Mackay, 1997; Burkhard, 1998; Gobas et al., 1999).

2.5.2 Fugacity versus Differential Approaches for Modelling Biomagnification of PCBs and PCDD/Fs in Fish

The literature reviewed revealed two approaches to modelling biomagnification of super-hydrophobic chemical residues in fish. The two approaches are fugacity form and differential form. The fugacity form has been promoted by Mackay and co-workers and, Gobas and co-workers (Mackay, 1991; Clark et al., 1990; Gobas, 1993; Campfens and Mackay, 1997, Gobas et al., 1999; Gobas and Morrison, 2000). However, Mackay and Fraser (2000) have highlighted the limitation of the fugacity approach. In order to use the fugacity approach (model), concentrations in specific organs and, or tissues are required. Because fugacity parameters are determined based on phase and equilibrium changes within the fish, there is little merit in using the fugacity model if only a total concentration
of a chemical is measured in the fish, i.e. in whole fish or fillets of fish only (Mackay and Fraser, 2000).

The differential form overcomes the limitation of the fugacity approach. Where the chemical residue data is limited to a total concentration in fish, the differential form appears to be adequate for modelling. Extensions of the model proposed by Sijm et al. (1992) have received wide attention globally in recent years (Brown et al., 2002; Isosaari et al., 2002; Lundebye et al., 2004; Gewurtz et al., 2006; Phua et al., 2006b; Berntssen et al., 2007). It is clear that the model of Sijm et al. (1992) has gained general scientific acceptance and is now being used for theoretical extensions, and in several scientific and farm-based applications. Because laboratory analyses for experimental PCB and PCDD/F data are costly (AUD$2,000 per fish sample tested), often a total concentration in whole fish or fillets of fish is obtained. The ease of use of the model, adaptability to the data limitations and amenability to experimental scenarios and super-hydrophobic chemical residues directs this research to investigate the use of a (modified) life-cycle biomagnification kinetic model.

2.5.3 Laboratory Data versus Field Data

Hamelink and Spacie (1977) reviewed the work of Grezenda et al. (1970, 1971) and Lieb et al. (1974), and concluded that “the utility of laboratory data to predict dynamics under natural conditions may be highly arbitrary”. One of the plausible reasons for the limited and fragmented field data may be the costs and logistical aspects involved. It was only in recent years that scientific field data on the biomagnification of PCBs and PCDD/Fs in farmed fish (Rappe et al., 1998; Isosaari et al., 2002; Jacobs et al., 2002; Lundebye et al., 2004; Berntssen et al., 2007) emerged. Even so, there is very little information on wild-caught fisheries that carry out farming in the open seas (truly representative of natural conditions) – where the single controlled variable is the quantity of feed fed to fish.

The literature reviewed in this work indicates that there are a number of proposed models for both the bioconcentration and biomagnification processes in fish, but few have been evaluated against new experimental laboratory or field data. It is noteworthy that previously described models (Thomann and Connolly, 1984; Barber et al., 1991; Gobas, 1993; Madenjian et al., 1993; Burkhard et al., 1998; Morrison et al., 1999) were assessed
either against a single data set reported by Oliver and Niimi (1988) or assessed against data sets originating from The Great Lake s region. Mackay and Fraser (2000) highlights that proposed models published in the scientific literature should be tested against field data as an exercise in validation and that:

“more such [field] data are badly needed”.

In 2004, Hites and co-workers published a paper in the journal *Science* that received wide attention globally. In the paper, the authors found that worldwide, farmed salmon had higher levels of PCBs and PCDD/Fs than in wild-caught salmon. Consumers consequently became increasingly aware of the health risks (as well as the health benefits) of eating farmed fish. Hites and co-workers concluded in their study “further studies of contaminant sources, particularly in feeds used for farmed carnivorous species such as salmon, are needed”. It appears that there is a shortage of field data for PCBs and PCDD/Fs in farmed fish and field studies on biomagnification of these residues from feed.

### 2.5.4 Biomagnification Modelling Approach For This Research

Many of the published studies were carried out on small fish (see Table 2-4), with high levels of PCBs and PCDD/Fs introduced into either the diet (food) or environment (water), or a combination of both, with experiments under highly controlled conditions for example in a laboratory tank (Bruggeman et al., 1981; Muir and Yarechewski, 1988; Opperhuizen and Schrap, 1988; Loonen et al., 1991). In addition, these studies quantified few WHO-TEF assigned PCB and PCDD/F congeners and therefore were not relevant for human health risk assessment, and had not covered commercial-size predatory fish (e.g. 20 kg tuna fish).

A gap exists therefore in the literature for (i) PCB and PCDD/F field data for predatory (large) fish, (ii) PCB and PCDD/F data that covers all of the 12 PCB and 17 PCDD/F congeners that the WHO has classed as toxic, (iii) validation of a proposed model published in the literature against new experimental field data and, (iv) studies in fish of high commercial value, i.e. Bluefin tuna species.
2.5.4.1 The Life Cycle Biomagnification Kinetic (LCBK) Model (Sijm et al., 1992)

The life-cycle biomagnification model proposed by Sijm et al. (1992) is selected for theoretical extension and field data evaluation.

Assuming that all processes except reproduction follow first order kinetics, the life-cycle biomagnification model of Sijm et al. (1992) for a chemical entering and leaving a SBT can be described as:

\[
\frac{dm}{dt} = k_w W_{SBT} C_w + \alpha F C_{bait} - (k_c + k_b)m - R k_r C_{SBT}
\]  

(2-1)

where \(m\) is the mass of chemical in SBT (pg), \(t\) is time (day), \(W_{SBT}\) is the whole weight of an SBT (kg), \(C_w\) is the concentration of the chemical in the water (pg.L\(^{-1}\)), \(\alpha\) is the assimilation efficiency of chemical in the food (baitfish) by that SBT which varies between zero and unity, \(F\) is the mass of baitfish consumed by that SBT per day (kg baitfish.day\(^{-1}\)), \(C_{bait}\) is the PCB or PCDD/F concentration in baitfish (pg.g\(^{-1}\) baitfish), \(R\) is a trigger value which is either zero or unity, depending on whether reproduction occurs or not, \(C_{SBT}\) is the PCB or PCDD/F concentration in a farmed SBT (pg.g\(^{-1}\) SBT), \(k_w\), \(k_c\), \(k_b\) and \(k_r\) are rate constants for uptake via water, physicochemical elimination via gills and faeces, biotransformation and reproduction, respectively.

2.5.4.2 Modified LCBK Model

Equation (2-1) is consequently modified to suit the conditions for this research. Where terms in Equation (2-1) are assumed to be negligible, these assumptions are highlighted and consequently justified.

Reproduction

It has been shown that juvenile SBT do not reach sexual maturity until the ages of between six to eight years old (Farley et al., 2007). For juvenile SBT < 6 years old, reproduction is negligible. Literature surveyed specific to SBT in Australian waters
(Serventy, 1956; Gunn et al., 1996; Young et al., 1997; Leigh and Hearn, 2000; Farley et al., 2007) suggest that juvenile SBT dominate Australian waters. The data presented by Leigh and Hearn (2000) and Farley et al. (2007) further suggest that juvenile SBT between the ages of one and four dominate the waters of the Great Australian Bight, off South Australia – the region where industry purse seines wild SBT for farming.

**PCB and PCDD/F Concentrations in Waters of the Open Ocean and Marine Sediments Around Port Lincoln, South Australia**

Uptake of PCBs and PCDD/Fs from water to the SBT has been considered.

In a recycled pond system or polluted stagnated water body (i.e. lakes) the fish within receives chemical residues including PCBs and PCDD/Fs from both food intake and water uptake via gill transfer (Barber et al., 1991). However, for this research, SBT were farmed in sea-cages (approximately ten nautical miles offshore of Port Lincoln) where natural drift of ocean currents simulates dynamic water conditions.

Because the netting of the sea-cages were made from nylon, or an equivalent material depending on farm management, it is reasonable to assume that the sea-cage itself does not restrict the dynamics of sea water conditions. The assumption of the major uptake route of PCBs and PCDD/Fs for farmed SBT via food is logical and may therefore be inferred from the dynamic water conditions experienced by SBT in the sea-cages as compared to fish in a laboratory or recirculating tank.

In 2004, the Australian National Dioxin Program (NDP) study was conducted and sites of sampling for marine sediments included Coffin Bay and Spencer Gulf West (Mueller et al., 2004). Figure 2.1 shows the two sampling sites relative to the SBT farming zone.

Mueller et al. (2004) reported that for marine sediments sampled along the coastal region west of Spencer Gulf identified by Franklin Harbour, all PCB and PCDD/F congeners assigned a WHO-TEF value were reported with concentrations at the Limit of Detection (LOD), except for PCB 105 that had a concentration of 1.3 pg.g⁻¹ and 1,2,3,4,6,7,8-HpCDD that had a concentration of 0.58 pg.g⁻¹.
For the region of Coffin Bay, all PCB and PCDD/F congeners assigned a WHO-TEF value were reported with concentrations at the LOD, except for PCB 156 that had a concentration of 0.32 pg·g⁻¹ and OCDD that had a concentration of 5.3 pg·g⁻¹. Blank concentrations were not provided in NDP 2004 Report and therefore it was not possible to ascertain if concentrations in the blank were similar to LOD of individual congeners. In this instance, it was assumed that due to the very low LOD levels reported, congeners reported in sediment samples at the LOD may have had similar concentrations in the blank(s).

From the limited available data from the NDP study, it was concluded that overall there were no true detects in the marine sediments, and consequently it is inferred that the contribution of PCB and PCDD/F concentrations via water exchange can be assumed to be negligible.

NOTE: This figure is included on page 31 in the print copy of the thesis held in the University of Adelaide Library.

Figure 2.1 Franklin Harbour and Coffin Bay sampling sites relative to the SBT Farming Zone (Google Maps, 2008)
When elimination via reproduction (because wild-caught SBT are juvenile fish) and uptake from water are negligible, Equation (2-1) becomes:

\[
\frac{dm}{dt} = \alpha FC_{\text{bait}} - (k_e + k_b)m
\]  

(2-2)

Since calculations are based on the concentration of the chemical in SBT, the amount of chemical is divided by the weight of SBT giving:

\[
\frac{1}{W_{\text{SBT}}} \frac{dm}{dt} = \alpha \frac{F}{W_{\text{SBT}}} C_{\text{bait}} - (k_e + k_b) \frac{m}{W_{\text{SBT}}}
\]  

(2-3)

**Growth of SBT**

The growth of wild SBT has been previously studied (Leigh & Hearn, 2000; Polacheck et al., 2003), however the lack of published scientific data for growth of farmed SBT indicated the need to build an assumption to advance the predictive model for PCBs and PCDD/Fs.

Previous work done by Gunn et al. (2002) in the FRDC Project 1997/363 studied the Fabens form (Fabens, 1965) of the von Bertalanffy growth equation (von Bertalanffy, 1938) and concluded that there were major drawbacks that included: poor growth prediction and no physiological interpretation for the parameter estimates. Gunn et al. (2002) also examined a bioenergetics and regression model, and concluded that the bioenergetics model outperformed the regression and von Bertallanfy models. However the drawback with the bioenergetics model is that it is relatively complex and needed to be validated against a large experimental dataset with regular sub-sampling and analyses of the energy content of feed throughout the experimental program.

Hence the literature surveyed indicated that the challenge of predicting growth of farmed SBT has not been fulfilled. This is primarily because of the limited resources in conducting growth-specific experiments with regular sampling to build a large dataset (Gunn et al. 2002), also a consequence of working within a niche industry.
For this research, an industry assumption that the farmed SBT fed proportional to body weight and relative to seasonal changes was applied. Incorporating this assumption we apply a simplified logarithmic equation to determine the growth rate, \( \gamma \) (day\(^{-1}\)):

\[
\ln \frac{W_{SBT_i}}{W_{SBT_j}} = \frac{\gamma}{t_2 - t_1}
\]

(2-4)

Combining (2-3) and (2-4) and solving the differential form gives:

\[
C_{SBT,t} = \frac{\alpha F'}{(k_e + k_b + \gamma)} C_{baits} \left(1 - e^{-(k_e + k_b + \gamma)t}\right) + C_{SBT,0} e^{-(k_e + k_b + \gamma)t}
\]

(2-5)

where \( F' \) is now the feeding rate of SBT (kg baitfish.kg\(^{-1}\) SBT.day\(^{-1}\)) and \( C_{SBT,0} \) is the initial concentration of a chemical present in a SBT (pg.g\(^{-1}\) SBT).

The order of solution to Equation (2-5) is presented in Appendix C. Figure 2.2 represents a schematic of the biomagnification process in a SBT as described by Equation (2-5).

Figure 2.2. Schematic of a Southern Bluefin Tuna (after Ottolenghi et al. 2004) with representation of the biomagnification kinetics for chemicals from feed.
Elimination and Biotransformation

It has been highlighted that for super-hydrophobic chemicals, elimination occurs extremely slowly, i.e. $k_e$ is small (Gobas et al. 1988; Niimi, 1996). Buckman et al. (2006) found that super-hydrophobic chemicals either do not biotransform or if biotransformation occurred, $k_b$ occurred at an extremely slow rate. The negligible elimination and biotransformation conditions imply that the dilution of the chemical(s) by growth, $\gamma$, now therefore becomes a pseudo-elimination (i.e. chemical does not leave the fish but has been diluted as the fish becomes bigger). Sijm et al. (1992) highlighted that in the case of higher chlorinated PCBs, growth dilution was the only important process. The concentration within a SBT at time $= t$ can now be predicted with:

$$C_{SBT,t} = \frac{\alpha F'}{\gamma} C_{bait} \left( 1 - e^{-\gamma t} \right) + C_{SBT,0} e^{-\gamma t} \quad (2-6)$$

Specific Comments on Assimilation Efficiency, $\alpha$

When a SBT feeds on baitfish, only some of the chemical in the food is assimilated. Applied to SBT, assimilation efficiency refers to the percentage of the chemical that a SBT ingests that is assimilated rather than egested (after Chapman and Reiss, 1999).

Chapman and Reiss (1999) highlight that organisms differ greatly in their assimilation efficiencies depending on the type of food (and the chemicals within) they eat. Biomagnification through the food chain depends on feeding rate and $\alpha$. The feeding rate of a fish varies with food selectivity (e.g. high fat baitfish is more satiable to SBT, pers. comms. David Warland, SBT Farm Manager), and $\alpha$ varies with the type of chemical (residue) and food type, and also with chemical concentration in the food (Newman and Jagoe, 1996). Opperhuizen and Schrap (1988) determined the assimilation efficiencies of two PCBs, namely, 2,2′,3,3′,5,5′-hexa chlorinated biphenyl (PCB 133) and 2,2′,3,3′,4,4′,6,6′ octa chlorinated biphenyl (PCB 197) in guppies, Poecilia reticulata, and concluded that “the calculated $\alpha$ are not independent on the contaminant concentrations in the food” and that as chemical concentration in the food increases, $\alpha$ declines.
Selective feeding coupled with differential chemical partitioning to feed of differing composition (different ratios of baitfish fed every time), makes measurement of $\alpha$ difficult for organisms with selective feeding habits (Newman & Jagoe, 1996). For example, Bruner (1994) studied $\alpha$ of hexachlorobiphenyl (HxCB) in zebra mussels that feed on suspended sediments and those that feed on algae, and reported that $\alpha$ of HxCB in zebra mussels that feed on suspended sediments were 30 %, while $\alpha$ of HxCB in zebra mussels that feed on algae was nearly 90 %.

Several researchers (Klump et al., 1987; Opperhuizen & Schrap, 1988; Weston, 1990) found that an increased feeding rate, i.e. the actual feeding rate of a fish as contrasted to the perceived feeding rate if in a farming situation, will result in a decreased $\alpha$ because of a shorter residence time in the gut. Gobas et al. (1993) suggest that the mechanism from accumulation of the intestinal tract apparently increases fugacity resulting from assimilation of food materials. The accumulation mechanism would account for the effects of feeding rate and food composition on assimilation. An increased feeding rate would reduce the residence time in the gut as well as the fraction of food (and therefore the chemical in the food) assimilated. Gobas et al. (1993) further highlighted that the fugacity of the chemical would not be increased as much as when a more complete assimilation of food occurs with slower residence time in the gut.

Assimilation efficiency also depends on a multitude of abiotic and biotic factors that include: properties of the chemical, e.g. lipophilicity, hydrogen-bonding capacity, chemical reactivity, particle size, and pH, to the feeding physiology characteristics of the organism, e.g. feed selectivity, ingestion rate, gut passage time (that may be dependent on body size) and digestive chemistry (Mayer et al., 1997). Sijm et al. (1992) proposed also that $\alpha$ of a chemical in fish from ingested food change with the age of fish. Furthermore, several researchers (Weston and Mayer, 1998; Kukkonen and Landrum, 1995; Lee et al., 1990) have reported that $\alpha$ varies not only inter-species but also intra-species, primarily because of inherent biological variation.

Different $\alpha$ have been recorded even among fish species for the same group of chemicals, e.g. Atlantic salmon (Berntssen et al., 2007) and rainbow trout (Isosaari et al., 2002). It is noteworthy and important that assimilation efficiencies should always be reported with the chemical(s) studied.
The work done by Wang and Fisher (1997) for trace metal uptake in mussels highlighted that variance in $\alpha$ can be minimised by pre-selecting animals of uniform age, size and condition. While the process of pre-selection may be possible with mussels and may be applicable to small fish (e.g. guppies), it is not practical for the SBT industry – especially whilst the research is carried out alongside a typical farming period with commercial companies.

### 2.5.5 Other Mechanistic Models

A study was undertaken to determine other potential mechanistic models for use to predict chemical concentrations in SBT from farming. Overall, there was very little quantitative information on using Physiologically Based Pharmacokinetic (PBPK) models for chemical residue predictions in food (fish as food). The incentive for PBPK models is underscored by the ability to robustly describe the transport of chemicals via the blood to various organs within the fish, and consequently to the edible portion (fillets) of the fish. The shortcoming of PBPK models however is that several samples within a fish have to be analysed – blood, liver, kidney, fat, skin and tissues, in order to accurately synthesise a PBPK model for a fish. If SBT samples to build a PBPK model were taken in duplicates, the estimated costs to build this model would be in the order of AUD$24,000 (6 samples x 2 fish x AUD$2,000 for PCBs and PCDD/Fs laboratory analyses), not including the costs for obtaining the fish.

Nichols et al. (1996) presented a physiologically based toxicokinetic (PBTK) model to examine the absorption of chemical residues in fish from aqueous exposure. The model was based on mass balance and comprised six compartments, namely, liver, kidney, fat, richly perfused tissue, poorly perfused tissue and skin. The model also had a term to describe the countercurrent chemical flux at the gills. Consequently, Nichols et al. (1998) extended the PBTK model to account for maternal transfer of chemical(s) in fish. Due to the complexity of the model and requirements for sample sizes, the use of PBPK or PBTK models did not appear promising. There is therefore very little published (Hickie et al. 1999) on PBPK or PBTK modelling in fish of commercial value.

The author also reviewed a book on using PBPK for predicting chemical residues in food (Phua, 2006a).
Table 2-4. Chronological listing of modelling studies in fish and the experimental conditions.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>PCDD/F and/or PCB Congeners</th>
<th>Fish Species, initial weight and/or length</th>
<th>Fish tissue/whole</th>
<th>Model/Work</th>
<th>Condition(s) of Experiment(s)</th>
<th>Dietary Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neely</td>
<td>1979</td>
<td>2,3,7,8-TeCDD, 2,5-DiCB (PCB9), 2,2',5,5'-TCB (PCB18), 2,4',5-TCB (PCB31), 2,2',5,5'-TCB (PCB52)</td>
<td>Rainbow trout, average of 35g</td>
<td>Not stated – indications suggest whole fish</td>
<td>First order kinetic rate model incorporating Kow relationship with biocoentration factor</td>
<td>145L aquarium, experiments not carried out, instead data obtained from unpublished source</td>
<td>None</td>
</tr>
<tr>
<td>Bruggeman et al.</td>
<td>1981</td>
<td>PCB Aroclor 1254</td>
<td>Goldfish, 4.5-5.2cm</td>
<td>Not stated – indications suggest whole fish</td>
<td>First order kinetic rate model (dietary and water uptake, and clearance phases experiments)</td>
<td>30L glass aquarium, Average of 23°C</td>
<td>Fortified food</td>
</tr>
<tr>
<td>Pizza &amp; O’Connor</td>
<td>1983</td>
<td>PCB Aroclor 1254</td>
<td>Striped bass, 0.88 ± 0.04g (dry wt.)</td>
<td>Not stated – indications suggest whole fish</td>
<td>First order kinetic rate model</td>
<td>Aquaria (vol. not stated) ~20°C 2% salinity carbon-filtered water</td>
<td>Minced earthworms, <em>Daphnia</em> spp., <em>Gammarus tigrinus</em> From various literature</td>
</tr>
<tr>
<td>Mackay &amp; Hughes</td>
<td>1984</td>
<td>From various literature</td>
<td>From Bruggeman et al. (1981)</td>
<td>Not applicable</td>
<td>Fugacity-based model for uptake from water only</td>
<td>Not applicable From Bruggeman et al. (1981) Food web ecosystem From various literature</td>
<td></td>
</tr>
<tr>
<td>Connolly &amp; Pedersen</td>
<td>1988</td>
<td>From various literature</td>
<td>From various literature</td>
<td>Not applicable</td>
<td>Thermodynamic (fugacity based) model</td>
<td>Not applicable From Bruggeman et al. (1981) Food web ecosystem From various literature</td>
<td></td>
</tr>
<tr>
<td>Gobas et al.</td>
<td>1988</td>
<td>From various literature</td>
<td>From various literature</td>
<td>Not applicable</td>
<td>Fugacity-based model for uptake from food</td>
<td>Not applicable From Bruggeman et al. (1981) Food web ecosystem From various literature</td>
<td></td>
</tr>
<tr>
<td>Muir &amp; Yarechewski</td>
<td>1988</td>
<td>1,2,3,7-TeCDD, 1,2,3,4,7-PnCDD, 1,2,3,4,7-HxCDD, 1,2,3,4,6,7,8-HpCDD</td>
<td>Juvenile rainbow trout, 0.5-1.0g</td>
<td>Whole fish</td>
<td>First order kinetic rate model (dietary uptake and depuration phases experiments)</td>
<td>30L fibreglass aquaria UV-dechlorinated, carbon-filtered tap water (1L/min)</td>
<td>Spiked commercial pellets</td>
</tr>
<tr>
<td>Thomann</td>
<td>1989</td>
<td>From various literature</td>
<td>From various literature</td>
<td>Not applicable</td>
<td>Four-level food chain model</td>
<td>Not applicable From Bruggeman et al. (1981) Food web ecosystem From various literature</td>
<td></td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>PCDD/F and/or PCB Congeners</td>
<td>Fish Species, initial weight and/or length</td>
<td>Fish tissue/whole</td>
<td>Model/Work</td>
<td>Condition(s) of Experiment(s)</td>
<td>Dietary Source</td>
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<tr>
<td>Muir et al.</td>
<td>1990</td>
<td>2,3,4,7,8-PnCDD</td>
<td>Juvenile rainbow trout, 2-3g</td>
<td>Whole fish</td>
<td>First order kinetic rate model (dietary uptake and depuration phases experiments)</td>
<td>30L fibreglass aquaria UV-dechlorinated carbon-filtered tap water (1L/min)</td>
<td>Spiked pellets</td>
</tr>
<tr>
<td>Gobas &amp; Schrap</td>
<td>1990</td>
<td>2-Mono-CDD, 2,7-Di-CDD, 1,2,4-TriCDD, 1,2,3,4- TeCDD, OCDD, 2,4,5-triCB, deca-CC, OCDF</td>
<td>Guppies, average of 0.0967 ± 0.0103g, Lipid 5.0%</td>
<td>Not stated – indications suggest whole fish</td>
<td>Dietary accumulation model (molar basis)</td>
<td>40L fish tank Dechlorinated, carbon filtered tap water 22 ± 1°C</td>
<td>Contaminated food</td>
</tr>
<tr>
<td>Sijm et al.</td>
<td>1992</td>
<td>PCBs 52, 54, 80, 104, 136, 153, 194, 209</td>
<td>Guppies, no weight or length details provided</td>
<td>Whole fish</td>
<td>Life-cycle biomagnification kinetic (LCBK) model</td>
<td>30L aquarium, charcoal-filtered water, 20 2± 2°C, Time cycle of 12-hour day, 12-hour night</td>
<td>Contaminated food (Tetramin)</td>
</tr>
<tr>
<td>Gobas</td>
<td>1993</td>
<td>No congeners specified</td>
<td>Salmonids</td>
<td>None</td>
<td>Theoretical food web model for Lake Ontario</td>
<td>None</td>
<td>Food web ecosystem From Oliver and Niimi (1985)</td>
</tr>
<tr>
<td>Campfens &amp; Mackay</td>
<td>1997</td>
<td>From Oliver and Niimi (1985)</td>
<td>From Oliver and Niimi (1985)</td>
<td>Not applicable</td>
<td>Fugacity-based food web model for Lake Ontario</td>
<td>Not applicable</td>
<td>From Oliver and Niimi (1985)</td>
</tr>
<tr>
<td>Gobas et al.</td>
<td>1999</td>
<td>PCBs 52, 87, 101, 153, 138 and 180</td>
<td>Adult rainbow trout, 365 ± 66g, and Rock bass, 77.8 ± 14g</td>
<td>Several organs and GIT sections within a fish</td>
<td>Fugacity-based biomagnification model</td>
<td>3000L bottom-draining tank, 240L/h flow through system, uncontaminated water at 7°C for trout, Rock bass obtained from Detroit River</td>
<td>Spiked pellets</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>PCDD/F and/or PCB Congeners</td>
<td>Fish Species, initial weight and/or length</td>
<td>Fish tissue/whole</td>
<td>Model/Work</td>
<td>Condition(s) of Experiment(s)</td>
<td>Dietary Source</td>
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</tr>
<tr>
<td>Brown et al.</td>
<td>2002</td>
<td>PCB 126</td>
<td>Juvenile rainbow trout, 2-5g</td>
<td>Not stated – indications suggest whole fish</td>
<td>First order kinetic rate model for constant dietary exposure (based on Sijm et al (1992) LCBK)</td>
<td>Not stated</td>
<td>Spiked pellets</td>
</tr>
<tr>
<td>Isosaari et al.</td>
<td>2002</td>
<td>17 WHO-PCDD/F congener, PCBs 8, 18, 28, 55, 77, 101, 118, 105, 126, 138, 153, 157, 159, 169, 180, 181</td>
<td>2 year-old rainbow trout</td>
<td>Fillets and viscera</td>
<td>Dietary accumulation model (based on Gobas and Schrap, 1990)</td>
<td>Brackish water in Baltic sea (open ocean), 12-18°C</td>
<td>Pellets and Baltic herring</td>
</tr>
<tr>
<td>Gewurtz et al.</td>
<td>2006</td>
<td>PCBs 28, 52, 101, 118, 138, 153, 180</td>
<td>Salmonids from Lake Oyangen and lake trout from Trout Lake</td>
<td>Not stated – indications suggest whole fish</td>
<td>First order kinetic rate model for constant dietary exposure (based on Sijm et al (1992) LCBK)</td>
<td>Wild-caught salmonids, Lake Oyangen 3°C, wild-caught lake trout, Trout Lake, 10°C</td>
<td>Assumed natural diet because fish were wild-caught</td>
</tr>
<tr>
<td>Berntssen et al.</td>
<td>2007</td>
<td>17 WHO-PCDD/F and 12 WHO-PCB congener</td>
<td>Atlantic salmon, farmed, 2.5 ± 0.2 kg, kinetic trial; Atlantic salmon smolts, 115 ± 14g, commercial trial</td>
<td>Whole fish</td>
<td>First order kinetic rate model for constant dietary exposure (based on Sijm et al (1992) LCBK)</td>
<td>Kinetic trial: 125m³ seawater net pens, water temperature 7.1 ± 1.5°C, Commercial trial: 11,520m³ seawater net pens, water temperature followed natural conditions (12 ± 6.5°C)</td>
<td>Commercially formulated fish oil-based pellets</td>
</tr>
</tbody>
</table>

Note that in the early 1980s, very small fish (0.5 – 350 g) were used for scientific studies. 20 years later, larger fish such as salmon (1 – 3 kg) were studied. This suggested the slow progression of research on biomagnification in fish species. Presently, very little is known on biomagnification model studies on piscivorous fish (e.g. tuna) that are at a higher trophic level on the food web, and at the same time, these fish as food for humans.
2.6 SUMMARY AND CONCLUDING REMARKS

Conventional methods of estimating PCB and PCDD/F concentrations by modelling the bioaccumulation in fish are based on experiments conducted in laboratories under well-defined conditions (e.g. controlled water temperature, flow rates, spiked pellets with fixed PCB and PCDD/F concentrations into the tanked environment, simulated daylight and night time), but relationships developed under these conditions do not accurately represent those occurring in commercial farming environments (i.e. fluctuating natural seawater temperatures, selected baitfish fed as feed, oceanic currents, weather conditions).

A critical survey of the scientific literature yielded the following important points relevant to this research:

1. Dietary exposure is thought to be the major route for biomagnification of superlipophilic chemical residues of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), and polychlorinated biphenyls (PCBs) for piscivorous fish at higher trophic levels on the food chain.

2. At present, the literature surveyed revealed very little has been published on PCB and PCDD/F levels, both modelling work and qualitative, in tuna (Thunnus family) and, very little work has been done on SBT farmed from wild-caught stock. This is no doubt the consequence of a niche market. Extensive experimental work, however, has been carried out on small fish of varying species: salmon farmed from hatchlings under well-controlled environments (e.g. water temperature is controlled to ± 2°C) in Norway and the United States, on pet fish (guppies) in a highly controlled aquatic laboratory in the Netherlands and game fish (lake and rainbow trout) in The Great Lakes, Canada.

3. The literature surveyed indicated there is very little information on predictive modelling of PCDD/F and PCB congeners that are within the WHO TEF system (i.e. of public health and food safety interests). Also, all the quantitative papers reviewed indicated that experiments were performed in environments that were
highly controlled, e.g. aquarium, or analysis of fish samples taken from industrially contaminated waters. There is very little literature to-date that reflects commercial farming environments (e.g. open seas, natural conditions).

4. Because the Life Cycle Biomagnification Kinetic (LCBK) model of Sijm et al. (1992) is based on basic chemical engineering mass balances principles, is amenable to industry practices and experimental conditions, and can account for biological fish growth, it is selected for theoretical extension, modification and experimental testing.

5. Recent literature surveyed revealed that overall there were no true detects in the marine sediments, in regions surrounding Port Lincoln, and consequently it is inferred that the contribution of PCB and PCDD/F concentrations via water exchange can be assumed to be negligible.

6. Assimilation efficiency of a chemical in a predatory fish such as SBT varies with the food type, feed selectivity and concentration of that chemical in the food. It is noteworthy that intra-species variation in assimilation efficiency values is expected because of the inherent biological variation.

7. As chemical residue modelling in foods is a multi-disciplinary field integrating biology, toxicology and food engineering, there is at present, very limited guidelines on how to coalesce these disciplines for a successful modelling process. The development of a framework is therefore necessary to put predictive modelling of chemical residues in context of a risk assessment for foods consumed by humans.

The following chapter presents a new risk framework of five governing principles. This framework, based on the principles and guidelines for the conduct of microbiological risk assessment outlined by Codex Alimentarius (Codex, 1999), provides the context in which the synthesis of a new model can occur. In this, suitable and important criteria for an adequate model are established.
CHAPTER THREE

DEVELOPMENT OF A NEW RISK FRAMEWORK FOR PREDICTING CHEMICAL RESIDUES IN FISH

Parts of this chapter have been published as:


3.1 INTRODUCTION

An extensive search of the literature yielded limited and fragmented guidelines as to how modelling chemical residues in foods can be carried out. The objectives of this chapter are therefore to: lay the foundation and provide the context of this Ph.D. research for which the developed model will lie within and, establish suitable and important criteria for an adequate model.

This chapter presents the development of a new risk framework of five governing principles. The conventional four principles of risk assessment highlighted in Codex Alimentarius (Codex, 1999) were tailored to a framework for consumer risk from farmed SBT. The first principle identifies the chemical residue(s) of interest. The second characterises adverse health effects on humans (if any) through the food chain. The third, quantifies this risk to humans. The fourth characterises quantitatively the certainty in data. The fifth, new governing principle, is an experimental validation of the model with independent data.

This new framework of governing principles will underpin predictive model development for assessment of risk to human health, and consequently deliver a practical model to assist management of concentrations of residue(s) in SBT.

3.2 RISK FRAMEWORK DEVELOPMENT

It is proposed that the logical starting point for development of a risk framework for residue(s) in SBT is the governing principles outlined in Codex Alimentarius (Codex, 1999). This consists of four governing principles that have been widely applied in microbiological risk research of contaminants in foods. These four principles are:

- Hazard Identification
- Hazard Characterisation
- Exposure Assessment
- Risk Characterisation.
3.2.1 Hazard Identification

Applied to development of a framework for residue(s) in SBT flesh, *Hazard Identification* is defined as: “whether a particular chemical residue is associated with adverse public health, or trade implications of sufficient importance to warrant scientific study, or, action by management immediately”. For SBT, hazard identification means the identification of chemical residues of interest. These particular (but not exclusive) residues of interest are polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) (Padula et al., 2004a).

For PCBs there are 209 congeners\(^2\), and for PCDD/Fs there are 210 congeners. Of these, 12 PCB congeners and 17 PCDD/F congeners (7 PCDDs and 10 PCDFs) are considered to have adverse effects on human health (EC, 2006; Van den Berg et al., 1998). These 29 congeners have been assigned a Toxic Equivalency Factor (TEF) (Table 3-1). TEF is defined as “the toxic potency of a compound relative to the most toxic congener – 2,3,7,8-TeCDD, to cause a biological effect” (Van den Berg et al., 1998). A TEF value of unity is assigned to 2,3,7,8-TeCDD.

3.2.2 Hazard Characterisation

When applied to a framework for SBT, a working definition of *Hazard Characterisation* of the Codex Alimentarius can be taken as the: “qualitative and quantitative evaluation of the nature of the adverse health effects associated with a hazard”. In practice, this principle means addressing the effects of the toxic residue(s) on normal health in humans from consumption of SBT. At this point, it is noteworthy that an extensive search of the literature shows that little is published on the levels of PCBs and PCDD/Fs in humans from consumption of fatty fish such as SBT – in contrast to a whole range of other daily foodstuffs (FSANZ, 2004).

\(^2\) See Appendix A for definition of terms
3.2.3 Exposure Assessment

The Codex Alimentarius (Codex, 1999) defines Exposure Assessment as “an assessment of the extent of actual or anticipated human exposure”. We define this, for the purpose of a framework for SBT, however, as “the quantitative change in levels of PCBs and PCDD/Fs in SBT flesh to which humans are exposed”. Exposure assessment therefore must start with analyses of SBT flesh.

A schematic of the flesh of a SBT is presented in Figure 3.1. It shows six sections, labelled a through f. These sections are derived originally from a sampling method in Japan, where portions of each are excised from a number of randomly selected SBT for testing of residues. Sections a and d, b and e, and; c and f, are sections termed kami, naka and shimo (Douglas, 2004).

Because sections d and e contain (mostly) the essential organs of the SBT, there is less edible flesh and a greater concentration of fat than in the other sections. As the PCBs and PCDD/Fs preferentially bioaccumulate in fat, there is consequently a greater concentration in these sections (Padula et al., 2004b). This is important to our framework development.

The concentrations (pg g⁻¹) of the 29 congeners of interest are usually expressed as total congener-based Toxic EQuivalent (TEQ), with the following formula (Van den Berg et al., 1998):

\[
\text{TEQ}_{\text{total}} = \sum_{i=1}^{12} (\text{PCB}_i \times \text{TEF}_i) + \sum_{j=1}^{7} (\text{PCDD}_j \times \text{TEF}_j) + \sum_{k=1}^{10} (\text{PCDF}_k \times \text{TEF}_k)
\]  

(3-1)

where for the PCB, PCDD and PCDF groups, i, j and k are the congeners and TEF the Toxic Equivalency Factor.
3.2.4 Risk Characterisation

*Risk Characterisation* is defined as “a qualitative and/or quantitative estimation, including uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a population based on hazard identification, hazard characterisation and exposure assessment”.

For the purposes of the framework for SBT, this in practice means that the concentrations of residues established in the Exposure Assessment must be weighed against dietary guidelines for recommendations of consumption of fish. These guidelines vary from country to country. Various thresholds have been established for example for Tolerable Monthly Intake (TMI) of residues from long term consumption of total daily foodstuffs.

In Australia the TMI = 70 pg.kg\(^{-1}\) of body weight (bw) of the consumer per month (FSANZ, 2004), while in Japan the Tolerable Daily Intake (TDI) = 4 pg.kg\(^{-1}\) bw per day (Anon., 1999; Anon., 2003; Maitani, 2004). WHO has a target TDI = 1 – 4 pg.kg\(^{-1}\) bw per day (Van Leeuwen et al., 2000).

3.2.5 Experimental Validation

Although the four foregoing risk principles are a logical starting point for development of the framework for concentrations of residue(s) in farmed SBT, there is no implicit requirement for experimental validation in Codex Alimentarius. In engineering sciences, predictive mathematical models are widely used. Experimental validation is always carried out - and deemed essential - to provide an eventual, reliable predictive model.

An additional governing principle therefore is needed for a quantitative output from the framework for predicting chemical residues. This principle will encompass functional relationships between chemistry and engineering principles, toxicological science and, management of associated risks. This fifth principle is *Model Validation*. 
3.3 FINDINGS

The schematic of Figure 3.2 reveals the resulting model framework of five governing principles for predicting concentrations of residue(s) in farmed SBT. This figure is in the form of a flow diagram to provide clarity. A feedback loop is employed to permit ongoing model refinement as industry requirements change, or as new information becomes available.

As an illustrative TEQ calculation, consider the congeners of Table 3-2 – a speculative list of congeners and typical concentrations in SBT flesh. Column 1 of the table lists the congener groups of, respectively, PCBs, PCDDs and PCDFs. The concentration at which each congener might be reported is given in column 2. The toxic equivalent factor (TEF) is provided in column 3. If the values shown in column 3 are multiplied by the values in column 2, the result is the Conc. x TEF of column 4. The summation for each congener group is shown as a subtotal in column 4 also. Applying Equation (1), and summing these three subtotals (i.e. 0.424 pg.g\(^{-1}\) + 0.025 pg.g\(^{-1}\) + 0.019 pg.g\(^{-1}\)) gives a final TEQ = 0.468 pg.g\(^{-1}\). If only SBT were eaten and all eight recommended serves (120 g each) consumed in a month by a consumer of body weight of 67 kg, then the intake for that month (8 x 120 x 0.468/67) = 6.71 pg-TEQ. The reader should note however that in practice all other sources of residues must be taken into account against the monthly intake.

3.4 DISCUSSION

3.4.1 The New Risk Framework

The schematic flow diagram of Figure 3.2 shows the four principles of Codex Alimentarius augmented with the fifth for model validation. Each step of the flow diagram, <1> through <5>, corresponds to a governing principle, into which elements of mathematics can now be integrated and developed.

An advantage of the framework is that research can be carried out, in parallel, for each step, or steps, by a number of researchers working simultaneously. Information obtained throughout can now be assigned a logical position within steps <1> through <5> of the
framework. Information assigned in this way can be handled unambiguously. This flexible approach permits a re-visit to any position within the framework whenever new data is confirmed. The evolutionary development of the model can therefore be explicitly presented in a transparent manner.

The feedback loop shown highlights a continuous refinement through modification, additions, changes and data updates. The predictive route permits engineering researchers to by-pass the risk characterisation step until (i) the data has been quantitatively studied, and (ii) a validated predictive model is developed. The advantages of using the predictive route over the qualitative one is that (i) there is the possibility of theoretical interpretation (of the data), (ii) traceability to how the levels came to be, (iii) not taking the data as reported but interrogating whether the reported levels are “real” and not background noise (blank) generated by the instruments (and processing techniques) used in laboratory analyses, and (iv) quantification of uncertainty in the data which results in a safety factor applied during risk characterisation (e.g. dietary modeling of public health for consumers).

This Ph.D. research focuses at steps <2> through <5> of the framework as presented as Figure 3.2.

Toxicological information for Step <1> are already available from the published literature (Van den Berg et al. 1998). From Table 3-1, for example for PCDD/Fs, 2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD and 2,3,7,8-TeCDF are of particular concern to normal human health because of their high TEF values.
Table 3-1. Tabulated summary of the 12 PCB and 17 PCDD/F congeners ranked by TEF values for risk to human health (after Van den Berg et al., 1998).

NOTE: This table is included on page 49 in the print copy of the thesis held in the University of Adelaide Library.
Table 2. An illustrative example of a TEQ calculation for speculative congeners and typical concentrations in SBT flesh.

<table>
<thead>
<tr>
<th>PCB Congener</th>
<th>Conc. (pg.g(^{-1}))</th>
<th>TEF</th>
<th>Conc. x TEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,3’,4,4’,5-Penta chlorinated biphenyl (PCB 126)</td>
<td>3.82</td>
<td>0.1</td>
<td>3.82 x 10(^{-1})</td>
</tr>
<tr>
<td>3,3’,4,4’,5,5’-Hexa chlorinated biphenyl (PCB 169)</td>
<td>4.10</td>
<td>0.01</td>
<td>4.10 x 10(^{-2})</td>
</tr>
<tr>
<td>3,3’,4,4’-Tetra chlorinated biphenyl (PCB 77)</td>
<td>10.1</td>
<td>0.0001</td>
<td>1.01 x 10(^{-3})</td>
</tr>
<tr>
<td>2,3’,4,4’,5,5’-Hexa chlorinated biphenyl (PCB 167)</td>
<td>12.2</td>
<td>0.00001</td>
<td>1.22 x 10(^{-4})</td>
</tr>
</tbody>
</table>

\[ \sum_{i=1}^{4} (PCB_i \times TEF_i) = 4.24 \times 10^{-1} \]

<table>
<thead>
<tr>
<th>PCDD Congener</th>
<th>Conc. (pg.g(^{-1}))</th>
<th>TEF</th>
<th>Conc. x TEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-Teta chlorinated dibenzo-p-dioxin</td>
<td>0.0221</td>
<td>1</td>
<td>2.21 x 10(^{-2})</td>
</tr>
<tr>
<td>1,2,3,4,7,8-Hexa chlorinated dibenzo-p-dioxin</td>
<td>0.0267</td>
<td>0.1</td>
<td>2.67 x 10(^{-3})</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-Hepta chlorinated dibenzo-p-dioxin</td>
<td>0.0210</td>
<td>0.01</td>
<td>2.10 x 10(^{-4})</td>
</tr>
<tr>
<td>Octa chlorinated dibenzo-p-dioxin</td>
<td>0.113</td>
<td>0.0001</td>
<td>1.13 x 10(^{-5})</td>
</tr>
</tbody>
</table>

\[ \sum_{j=1}^{4} (PCDD_j \times TEF_j) = 2.50 \times 10^{-2} \]

<table>
<thead>
<tr>
<th>PCDF Congener</th>
<th>Conc. (pg.g(^{-1}))</th>
<th>TEF</th>
<th>Conc. x TEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,7,8-Peta chlorinated dibenzofuran</td>
<td>0.0317</td>
<td>0.5</td>
<td>1.59 x 10(^{-2})</td>
</tr>
<tr>
<td>2,3,7,8-Teta chlorinated dibenzofuran</td>
<td>0.0125</td>
<td>0.1</td>
<td>1.25 x 10(^{-3})</td>
</tr>
<tr>
<td>1,2,3,7,8-Penta chlorinated dibenzofuran</td>
<td>0.0338</td>
<td>0.05</td>
<td>1.69 x 10(^{-3})</td>
</tr>
<tr>
<td>Octa chlorinated dibenzofuran</td>
<td>0.0119</td>
<td>0.0001</td>
<td>1.19 x 10(^{-6})</td>
</tr>
</tbody>
</table>

\[ \sum_{k=1}^{4} (PCDF_k \times TEF_k) = 1.88 \times 10^{-2} \]

From Equation (3-1), TEQ\(_{total}\) = 0.424 + 0.025 + 0.019 = 0.468 pg.g\(^{-1}\)
Figure 3.1 Schematic of a Southern Bluefin Tuna (after Ottolenghi et al., 2004) showing the six sections a-f.

Figure 3.2. Schematic of a new risk framework of five governing principles for predictive modelling of chemical residue(s) in flesh of farmed Australian SBT.
3.4.2 TEF and TEQ Values

Although PCDD/Fs have higher TEF values than PCBs, recent surveys of PCBs and PCDD/Fs in farmed SBT (Padula et al. 2004a) highlighted that PCBs are in greater concentrations than the PCDD/Fs in flesh; and contribute on average, about 80 % of the total (combined PCB + PCDD/F) TEQ.

The PCBs and PCDD/Fs identified by the WHO, share two common biochemical effects. These are the binding affinity to the aryl hydrocarbon receptor (AhR), and/or the induction of cytochrome P4501A1 (CYP1A1)\(^3\).

For this Ph.D. research, TEQ levels were determined using TEF values reported in Van den Berg et al. (1998). It is noted that there has been a recent revision of the TEF values, presented in Van den Berg et al. (2006). Since most of the literature published was based on the 1998 TEF values, this work is therefore structured upon the 1998 TEF values so that a direct comparison of TEQ values can be made. At this point it is noteworthy that data published in the disciplines of toxicology and human health risk assessment change very quickly. For example, the Nordic model for TeCDD equivalents was used in 1989 for health risk assessment (Ahlborg, 1989). Five years later, the WHO established the I-TEQ calculation method in 1994 (Ahlborg et al. 1994). In 1998, the I-TEQ method was replaced with the new WHO-TEQ calculation method (Van den Berg et al. 1998). Consequently in 2006 van den Berg and co-workers published a recent revision to TEF values but commented at the Dioxin 2006 Symposium in Oslo Norway that “the revision would have limited impact on the total TEQ”. At the same Symposium, van den Berg also presented that other compounds (e.g. HCB, PBB) may be added to the WHO-TEF scheme, as soon as additional toxicological data on these compounds become published (conf. comm., Prof. Martin Van den Berg).

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\(^3\) The definition for Cytochrome P4501A1 (CYP1A1) is provided in Appendix A. For further reading, see Malins and Ostrander (1994) p. 87 ff.
3.5 IMPORTANT CRITERIA FOR AN ADEQUATE MODEL

To complement the developed framework, the criteria for an adequate model are established. Similar sets of criteria have been established in the field of predictive microbiology modelling (Davey and Phua, 2005; Phua and Davey, 2007). An adequate quantitative model is an essential step to robust management of chemical residue levels. For this research, the essential criteria include:

- accurate predictions versus observed data
- potential physiological interpretation of model coefficients and parameters
- model parameters have to be easily (and conveniently) measured by farmers
- parsimonious – the model should be as simple as possible (but no simpler)
- easy to use.

The test for accurate predictions versus observed data can be qualified with analyses of residual plots (Snedecor and Cochran, 1989; Weisberg, 1985). The residual plots (can be in the form of observed value – predicted value, Weisberg, 1985) are important in appraising the fit of a predictive model. Residuals provide insight into the parameterisation of the model – whether it is over-parameterised or under-parameterised and may highlight the effects of inherent assumptions used.

The potential for physiological interpretation of the model coefficient and parameters is important in the modelling of biological samples, e.g. fish. The advantage of a mechanistic model is that each of the model parameters can be subject to interpretation – traced back to an activity, for example, the feeding parameter in the model can be traced back to the quantity of baitfish fed and the number of fish fed. This criterion ensures that the model and model parameters synthesised do not become a mere curve fitting exercise (McMeekin et al., 1993).

It is important that the predictive model can be readily integrated with equations characterising each of the model inputs, such as the equations for the feeding and growth rates. The equations for the model inputs should be of the form where individual parameters (within the equations) can be easily and conveniently measured by farmers.
(e.g. whole weight and fork length of the SBT). This would consequently result in ease of use by farmers and farm management.

3.6 CONCLUDING REMARKS

The developed framework of five governing principles of risk presented in this chapter has been used to provide a foundation for the synthesis of a predictive model for concentrations of residue(s). The framework has been modified from conventional principles of microbiological risk assessment highlighted in Codex Alimentarius. Specific to this research, the residue(s) to be investigated are PCBs and PCDD/Fs. The criteria for an adequate model have been established.

The following chapter presents the experimental and sampling methods, materials and developed standard operating procedures (SOPs) for sampling, to obtain new and commercial field data for PCB and PCDD/F in farmed SBT. Consequently, the following Chapters 5 and 6 present, respectively, the qualitative and predictive modelling studies on the temporal changes of PCB and PCDD/F levels in farmed SBT (steps <2>, <3> and <5> of the developed risk framework). A dietary modelling study is presented in Chapter 7 using the predicted and validation values obtained in Chapter 6. This dietary modelling fits into step <4> of the developed framework.
CHAPTER FOUR

MATERIALS AND METHODS

Parts of this chapter have been published as:

4.1 INTRODUCTION

This chapter describes the commercial-scale field experiments carried out with live Southern Bluefin Tuna (SBT) for three experimental phases. Because this is the first time an experimental program for residue research on farmed SBT has been carried out, post-mortem Standard Operating Procedures (SOPs) for sampling were developed. At the onset, it is noteworthy that because this research forms a part of a larger project (FRDC 2004/206 and Aquafin CRC 2.1(2)) entitled “Management of Food Safety Hazards in Farmed Southern Bluefin Tuna to Exploit Market Opportunities”, the experimental program was therefore designed to accommodate the different research disciplines involved.

This chapter also highlights the analytical techniques used to determine the extent of PCB and PCDD/F biomagnification in SBT. The PCB and PCDD/F data, together with feeding histories (e.g. feeding rate) of the tuna in sea-cages and other relevant parameters, were studied to assess biomagnification kinetics in the edible flesh of farmed SBT.

4.2 THE EXPERIMENTAL PROGRAM

The experimental program commenced in April 2005 and concluded in August 2006.

The experimental program was designed to reflect the typical farming period of the individual companies. A typical SBT farming period span approximately five months after transfer from tow sea-cages (used to contain live SBT caught from the wild) to floating sea-cages offshore of Port Lincoln. These sea-cages that are anchored to the sea floor and suspended (from the sea floor) are typically 32 m in diameter (see Appendix D) and approximately 20 m deep.

The farming of live SBT from wild-caught resulted in three phases that comprised:

1. 2005 Typical Farming Period (March to August 2005)
2. Longer Term Holding (LTH) Farming Period (August 2005 to August 2006)
3. 2006 Typical Farming Period (March to June 2006)
Materials and Methods

At each of the three phases, different baitfish profiles, i.e. different baitfish types with varying protein and fat levels, were fed to SBT.

The SBT companies working alongside this research have expressed their desire to remain confidential. As such, surrogates for actual company names are used throughout this thesis.

4.2.1 2005 Typical Farming Period – Farm Delta Fishing Pty Ltd

Wild-caught SBT \((n = 879)\) were purse seined\(^4\) in March 2005 in the Great Australian Bight (as far as 32°S, 128°E) and towed slowly (between 0.5 to 2 knots) to offshore Port Lincoln (grid square of (top left) 34° 36’S, 135° 58’E by (bottom right) 34° 41’S, 136° 2’E), South Australia (see Figure 4.1). These SBT were tagged individually with identification numbers, measured for fork length and transferred into four commercial pontoons (with coloured tags of blue, green, pink and white). Of these 879-tagged SBT, only 108 SBT from the pink tag sea-cage were weighed due to rough sea conditions and strong gale winds.

During the 2005 typical farming period, SBT were fed three types (or type) of baitfish that included: United States (US, Californian) and Australian sardine\(^5\) \((Sardinops sagax)\), and Australian (Tasmanian) red bait \((Emmelichthys nitidus nitidus)\) to simulate a natural diet. No manufactured feed pellets or nutritional supplements were fed to the farmed SBT. Table 4-1 presents the proximate analyses of the baitfish. The US sardine has the highest fat percentage whereas the Australian sardine has the lowest as observed from column four of Table 4-1.

Table 4-2 shows the four diet treatments designed to achieve specific protein-fat levels: For example, row four shows that for a high fat/low protein (HF/LP) treatment, a mixture of 65 % US sardine with 35 % Tasmanian red bait was required. Table 4-3 shows the combination of diet treatments offered to the four sea-cages throughout the 2005 typical

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\(^4\) See Appendix A for definition of terms

\(^5\) The author is aware that there are two latin names for Australian sardines highlighted by the Australian FRDC. However, the SBT industry prefers using the latin name \textit{Sardinops sagax} over \textit{Sardinops neopilchardus}.  

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farming period. It is noteworthy that SBT in both the green and pink tag sea-cages experienced varying diet treatments throughout the 2005 typical farming period.

Figure 4.1. Location of the tuna farming zone identified by a square grid with coordinates of (top left) 34° 36'S, 135° 58'E by (bottom right) 34° 41'S, 136° 2'E, relative to Port Lincoln (Google Maps, 2007).
Table 4-1. Proximate analyses for the three types of baitfish fed to farmed SBT during 2005 typical farming period.

<table>
<thead>
<tr>
<th>Baitfish Type</th>
<th>Protein (%)</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US sardine (Sardinops sagax)</td>
<td>16.4</td>
<td>63.8</td>
<td>14.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Australian (Tasmanian) red bait (Emmelichthys nitidus nitidus)</td>
<td>19.8</td>
<td>72.3</td>
<td>2.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Australian sardine (Sardinops sagax)</td>
<td>20.4</td>
<td>73.8</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table 4-2. Percentage ratios of baitfish for each of the four diet treatments.

<table>
<thead>
<tr>
<th>Diet treatment</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>% feed contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>US sardine</td>
</tr>
<tr>
<td>Low fat / High protein (LF/HP)</td>
<td>19.5</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Medium fat/Medium protein (MF/MP)</td>
<td>18.5</td>
<td>7.3</td>
<td>40</td>
</tr>
<tr>
<td>High fat / Low protein (HF/LP)</td>
<td>17.5</td>
<td>10.5</td>
<td>65</td>
</tr>
<tr>
<td>Australian Sardine (AS)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4-3. Experimental design for the varying diet treatments for each of the four sea-cages throughout the 2005 typical farming period.

<table>
<thead>
<tr>
<th>Sea Cage</th>
<th>Initial SBT Stock</th>
<th>Days 0-55</th>
<th>Days 56-97</th>
<th>Days 98-139</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue tag</td>
<td>219</td>
<td>MF/MP</td>
<td>MF/MP</td>
<td>MF/MP</td>
</tr>
<tr>
<td>Green tag</td>
<td>219</td>
<td>HF/LP</td>
<td>MF/MP</td>
<td>LF/HP</td>
</tr>
<tr>
<td>Pink tag</td>
<td>220</td>
<td>LF/HP</td>
<td>MF/MP</td>
<td>HF/LP</td>
</tr>
<tr>
<td>White tag</td>
<td>221</td>
<td>AS</td>
<td>AS</td>
<td>AS</td>
</tr>
</tbody>
</table>
Materials and Methods

Table 4-4. Number of farmed SBT allocated for residue research from the total number of farmed SBT harvested for the 2005 typical farming period.

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Time of Farming (days)</th>
<th>Total SBT harvested from the pink and green sea-cages</th>
<th>SBT for residue research</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 April 2005</td>
<td>0</td>
<td>11*</td>
<td>5*</td>
</tr>
<tr>
<td>30 May 2005</td>
<td>55</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>11 July 2005</td>
<td>97</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>22 August 2005</td>
<td>139</td>
<td>60</td>
<td>10</td>
</tr>
</tbody>
</table>

*These SBT were considered baseline (wild-caught) and not from the pink or green sea-cages – therefore were harvested prior to the start of the experimental program.

Farmed SBT harvested for residue research were limited to the pink and green tag sea-cages for the 2005 typical farming period. Table 4-4 shows the number of farmed SBT allocated for residue research from the total number of farmed SBT harvested from both the pink and green tag sea-cages, at each individual harvest.

After the final harvest on 22 August 2005 for the 2005 typical farming period, the remaining live SBT from all four sea-cages were transferred into a single sea-cage. The single sea-cage formed the basis for the LTH farming period.

4.2.2 2005/06 Longer Term Holding Farming Period – Farm Delta Fishing Pty Ltd

This is the first time a Longer Term Holding (LTH) farming experiment was carried out for the Australian SBT industry. For this 2005/06 Longer Term Holding (LTH) farming experiment, farming and feeding were consolidated due to logistical and economic reasons (from the highly fluctuating Japanese yen). The remaining SBT from the blue, green, pink and white tag sea-cages from the 2005 single farming period of Farm Delta Fishing Pty Ltd were consolidated into a single sea-cage (referred to as the LTH sea-cage).
Farmed SBT fed varying diet treatments for the typical farming period (up to $t = 139$ days) were now fed another different diet treatment, with additional baitfish types, until the end of the experiment at $t = 496$ days. A selection of baitfish consolidated from freezer storage was used as feed and these included: US (Californian) and Australian sardine (*Sardinops sagax*), premixed batch of Australian blue mackerel (*Scomber australasicus*) and US sardine, and Indonesian herring (*Sardinella lemuru*). Table 4-5 presents the proximate analyses for the four batches (and four types) of baitfish fed during LTH farming season. The sum of the individual component percentages do not add to 100% due to rounding of figures from the laboratory, and from averaging these values from different batches.

Table 4-6 shows the number of farmed SBT allocated for residue research from the total number of farmed SBT harvested from the LTH sea cage, at each individual harvest. The periodic and corresponding farming time have been continued from the 2005 typical farming period as the LTH research was conducted with the same company (i.e. same husbandry practices).

<table>
<thead>
<tr>
<th>Baitfish Type</th>
<th>Protein (%)</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US sardine (<em>Sardinops sagax</em>)</td>
<td>17.3</td>
<td>61.4</td>
<td>16.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Australian sardine (<em>Sardinops sagax</em>)</td>
<td>20.0</td>
<td>72.9</td>
<td>3.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Mix of Australian mackerel (<em>Scomber australasicus</em>) and US sardine ^</td>
<td>19.1</td>
<td>69.5</td>
<td>6.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Indonesian herring (<em>Sardinella lemuru</em>)</td>
<td>18.7</td>
<td>69.3</td>
<td>6.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

^ This batch of mixed baitfish was not available for testing of PCB and PCDD/F levels.
### 4.2.3 2006 Single Farming Period – Farm Alpha Fishing Pty Ltd

For the 2006 typical farming period, 2664 wild-caught SBT were purse seined in March 2006 in the Great Australian Bight and towed slowly to offshore Port Lincoln, South Australia. These 2644 SBT were consequentely transferred to two sea-cages. SBT harvested for residue research came from a single sea-cage where the initial population was 1435. Of these 1435 SBT, 69 SBT were tagged individually with identification numbers, measured for fork length and initial whole weights.

During this period, five types of baitfish were fed to SBT and these were: US (Californian) and Australian sardine (*Sardinops sagax*), Australian (Tasmanian) red bait (*Emmelichthys nitidus nitidus*), Moroccan sardine (*Sardina pilchardus*) and Indonesian herring (*Sardinella lemuru*). Table 4-7 presents the proximate analyses of the types of baitfish fed. Data for two of the baitfish types were not available. Systematic designed treatments similar to Tables 4-2 and 4-3 were not available from this commercial company.

Table 4-8 shows the number of farmed SBT harvested for residue research from the total number of harvested SBT (from the one sea-cage), at each individual harvest. It is
noteworthy that financial constraints limited the number of SBT for residue research to only the third harvest, 30 June 2006. Consequently, no experimental data were available for the corresponding farming time of 126, 127, 128 and 131 days.

Table 4-7. Proximate analyses (indicative) for the five type of baitfish fed to farmed SBT during 2006 typical farming period.

<table>
<thead>
<tr>
<th>Baitfish Type</th>
<th>Protein (%)</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US sardine (Sardinops sagax)</td>
<td>16.4</td>
<td>63.8</td>
<td>14.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Australian sardine (Sardinops sagax)</td>
<td>20.4</td>
<td>73.8</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Moroccan sardine (Sardina pilchardus)</td>
<td>n.a.*</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Indonesian herring (Sardinella lemuru)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

*n.a. Analyses were not available

Table 4-8. Number of farmed SBT allocated for residue research from the total number of farmed SBT harvested for the 2006 harvest period from the one sea-cage.

<table>
<thead>
<tr>
<th>Harvest Date</th>
<th>Time of Farming (days)</th>
<th>Total SBT Harvested from the one sea-cage</th>
<th>SBT for Residue Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 March 2006</td>
<td>0</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>21 April 2006</td>
<td>38</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>30 June 2006</td>
<td>116</td>
<td>313</td>
<td>5</td>
</tr>
<tr>
<td>10 July 2006</td>
<td>126#</td>
<td>188</td>
<td>0</td>
</tr>
<tr>
<td>11 July 2006</td>
<td>127#</td>
<td>364</td>
<td>0</td>
</tr>
<tr>
<td>12 July 2006</td>
<td>128#</td>
<td>226</td>
<td>0</td>
</tr>
<tr>
<td>15 July 2006</td>
<td>131#</td>
<td>314</td>
<td>0</td>
</tr>
</tbody>
</table>

# No experimental data were available
4.3 POST-MORTEM STANDARD OPERATING PROCEDURES FOR SAMPLING

Southern Bluefin Tuna (SBT) were received bled, gilled, gutted and frozen at SARDI Glenside. These were stored at -20°C until one to two days prior to sampling. A composite sample was taken from the one half of an individual SBT, and analysed for residue levels of PCBs and PCDD/Fs.

4.3.1 Developed Standard Operating Procedures (SOPs)

As this is the first time an experimental program for residue research on farmed SBT has been carried out, Standard Operating Procedures (SOPs) for sampling were developed and implemented.

4.3.1.1 Whole Fish Procedures

Prior to processing, SBT were partially thawed at 6°C one to two days to prevent drip loss. Thawing time is proportional to specimen size, i.e. 10 – 20 kg fish were thawed for one day, SBT > 20 kg were thawed for two days. SBT were reweighed to account for blood losses resulting from freeze thawing.

The final whole fish procedure was removal of the head and tail at locations c and o as shown in Figure 4.2. The remaining carcase was divided into three sections (1, 2 and 3). Section 1 corresponding with cuts A and D, (see Figure 3.1) Section 2 with cuts B and E, and Section 3 with cuts C and F. Separation of the carcass into these sections permitted internal cavity measurements (measurements d and g as shown in Figure 4.3) to be recorded at the leading edge of each of these sections. Each of the three sections, including the tail and head were individually weighed. After weights of the individual sections were recorded, sections were halved according to the left or right side of the fish.

As part of the developed SOPs, physical measurements were collected for another study and will not be reported in this work. These included both external measurements of length, height and width along the length of fish (see Figure 4.2), and internal
and cavity) measurements (see Figure 4.3). Measurements were made with a tape measure and a large pair of callipers, and recorded in centimetres to the nearest two decimal places.

4.3.1.2 Composite Sampling

One half of the section (either left or right side) was selected at random to make up the composite sample. Cuts A through F were placed in corresponding stainless steel bowls. All non-edible parts (skin, bones and dark red meat) were removed from each cut and the edible portions (A through F) weighed. This was done to provide validation by mass if sampling for composite on the one side of the fish approximately equals the mass when sampling for tissue group from the other side of the fish. Also, a portion of flesh (average of 50 g) that remained inside the head section was removed and included as part of cut A.

Length, height, width and girth measurements: a – centreline of eye; b – cheek; c – head length; d – anterior of 1st dorsal; e – posterior of pectoral fin; f – reserved; g – anterior 2nd dorsal; h – vent; i – 6th finlet anterior of caudal keel; j – 5th finlet anterior of caudal keel; k – 4th finlet anterior of caudal keel; l – 3rd finlet anterior of caudal keel; m – 2nd finlet anterior of caudal keel; n – 1st finlet anterior of caudal keel; o – standard length; p – fork length; q – total length

Additional measurements: Pectoral fin – length and maximum width; Caudal keel – maximum width and distance from snout; 1st dorsal fin – leading edge length, location of point “f”; 2nd dorsal fin – length and maximum width; pelvic fin - length and maximum width; anal fin – length and maximum width

Figure 4.2. Locality of physical measurements collected from intact (whole) SBT.
Materials and Methods

Figure 4.3. Sites where internal (of the fish) measurements were recorded: a, b and c – belly widths, d – cavity height, e – height from top of section to top of cavity, f – height from top of section to spinal cord, g – cavity width between a and b).

Samples were homogenised in a stainless steel Hobart™ food processor, wrapped in aluminium foil, placed in polyethylene bags and stored at -80°C for later analysis.

4.3.1.3 Tissue Group Sampling

The remaining half section was divided into the six cuts. Each cut was further divided into tissue groups. For PCB and PCDD/F analyses, tissue group sampling was carried out for each final SBT of a farming season. Tissue groups were separated based on expert opinion studied from Japanese assigned fillets (see Figure 4.4), namely the akami (lean portion), chu-toro (medium fat portion) and o-toro (belly fat portion). All non-edible portions were removed.

The three tissue groups (akami, chu-toro, o-toro) collected from all six cuts were weighed (for validation of mass with composite sampling mass), homogenised and collected individually in aluminium foil placed in polyethylene bags and stored at -80°C analysis at a later date.
Materials and Methods

![Figure 4.4. Fillets of the three tissue groups, akami, chu-toro and otoro, found in a farmed SBT at retail outlets in Japan (Douglas, 2004).]

4.4 ANALYTICAL TECHNIQUES - AGRIQUALITY LIMITED, WELLINGTON, NEW ZEALAND

Samples collected as described in Section 4.3.1 were analysed at AgriQuality Limited. Samples were analysed for PCBs and PCDD/Fs, respectively, based on methods USEPA 1668 Revision A (USEPA, 1999) and USEPA 1613 (USEPA, 1994). Because these methods permitted users the choice of various analytical techniques, those employed by AgriQuality are described briefly.

4.4.1 Sample Preparation / Soxhlet Extraction

Approximately 2-3:1 granulated anhydrous sodium sulphate (w/w) was blended with 40-50g of the sample. The blended mixture was fortified with a 13C-PCDD/F labeled internal standard (13C12-1,2,3,4-TeCDD and 13C12-1,2,3,7,8,9-HxCDD) for PCDD/F analyses or with a 13C-PCB labeled internal standard (13C-PCBs 9, 52, 101, 138 and 194) for PCB analy...
analyses, prior to loading into a Soxhlet extractor. The mixture was extracted with organic solvent methylene chloride:hexane (1:1) for 16 hours. A single simultaneous extraction was carried out for PCBs and PCDD/F determination. The organic solvent was removed using nitrogen blow down in a water bath (temperature = 60°C) to concentrate the residue until a constant weight was obtained prior to lipid determination.

### 4.4.2 Lipid Determination

Post extraction, the percent lipid in all sample extracts was determined according to US EPA method 600/3-90/022 (USEPA, 1990). Percent lipid was calculated according to the following:

\[
\text{lipid (\%) = } \frac{\text{weight of residue (g)}}{\text{weight of sample (g)}} \times 100
\]  

(4-1)

Following lipid determination, the extract was partitioned with concentration sulphuric acid to remove (the majority of) remaining lipids. Further clean-up and purification of the extract using acid and base modified silica gel and activated alumina and carbon columns was carried out. The final extracts for PCB (100 µL) and PCDD/F (10 µL) determination was concentrated and fortified with recovery standards prior to analyses by High Resolution-Gas Chromatography Mass Spectroscopy (HR-GCMS).

### 4.4.3 PCB and PCDD/F determination

PCB congeners are identified by the IUPAC numbering system (Ballschmiter and Zell, 1980). The 45 congeners analysed were: 77, 81, 126, 169 (non-ortho PCBs); 105, 114, 118, 123, 156, 157, 167, 189 (mono-ortho PCBs); and 1, 3, 4, 15, 19, 28, 37, 44, 49, 52, 54, 70, 74, 99, 101, 104, 110, 138, 153, 155, 170, 180, 183, 187, 188, 194, 196, 199, 202, 205, 206, 208, 209 (indicator PCBs). Total summed and TEQ values were reported at the lower-bound and upper-bound ranges. For lower-bound and upper-bound determinations, respectively, a value of zero and a value at the detection limit is assigned to congeners not detected.
PCDD/F congeners are identified by the chemical structure with the position and number of the chlorine atoms. The 17 congeners analysed by isotopic dilution were: 2,3,7,8-TetraCDD, 1,2,3,7,8-PentaCDD, 1,2,3,4,7,8-HexaCDD, 1,2,3,6,7,8-HexaCDD, 1,2,3,7,8,9-HexaCDD, 1,2,3,4,6,7,8-HeptaCDD, OctaCDD, 2,3,4,7,8-PentaCDF, 2,3,7,8-TetraCDF, 1,2,3,4,7,8-HexaCDF, 1,2,3,6,7,8-HexaCDF, 1,2,3,7,8,9-HexaCDF, 2,3,4,6,7,8-HexaCDF, 1,2,3,7,8-PentaCDF, 1,2,3,4,6,7,8-HeptaCDF, 1,2,3,4,7,8,9-HeptaCDF and OctaCDF. Total summed and TEQ values were reported at the lower-bound and upper-bound ranges. For lower-bound and upper-bound determinations, respectively, a value of zero and a value at the detection limit is assigned to congeners not detected.

Sample extracts of 1µL were analysed on a Micromass Autospec Ultima High Resolution Mass Spectrometer (United Kingdom) interfaced to an HP/Agilent 6890 and 7683 Injector on a 6890 Gas Chromatograph system, operating in the splitless mode equipped with Zebron ZB-5 capillary column (60 m, 0.25 mm ID, 0.25 µm film thickness) for PCB and PCDD/PCDF determination. It is noted that PCBs are analysed in one vial and PCDD/Fs in another. For confirmation analysis of PCDD/Fs, DB225 capillary column (30 m, 0.25 mm ID 0.25µm film) was used.

HR-GCMS analyses were carried out in the electron impact mode (~30 – 35 eV (variable), ~0.80 mA (variable), 350 V). Native and labelled compounds were acquired by Selected Ion Monitoring (SIM) with the mass resolution being maintained at 10,000 (5% valley) throughout the analysis. A constant flow rate of 1.5 mL.min\(^{-1}\) Helium (carrier gas, > 99.99 % purity) was used for the PCB chromatographic run, while a ramped flow rate of 2.5 – 1.5 mL.min\(^{-1}\) was used for the PCDD/F run.

Chromatographic data were processed using QuanLynx, part of a Waters MassLynx™ (version 4.0) software package. Levels of target analytes were determined via the isotope dilution technique. All data were corrected in-house for recoveries. Data were reported on a fresh weight (f.w.) and lipid weight (l.w.) basis.
4.4.4 Laboratory Quality Assurance and Quality Control

A laboratory reagent blank and Ongoing Precision and Recovery (OPR\(^6\)) sample is run with each batch of samples in accordance with US EPA methodology. The maximum batch size is 20, including Quality Assurance (QA) samples. The results of the OPR sample together with internal standard recoveries are assessed against the criteria in methods 1613B and 1668A.

4.4.5 Verification and Integrity of Data from Laboratory

Results of PCB and PCDD/F levels were reviewed as received. Lipid content was verified with farming time. For example, the lipid contents of three samples of one type of baitfish should be within a 20 % equipment error (pers. comms. Phil Bridgen, AgriQuality Limited). Congener pattern for each sample of each experimental period was normalised to observe if there is any visible difference in pattern between one sample and another.

4.5 ADDRESSING METHOD BLANKS

Methods used for addressing the presence of detectable background levels are often not well described when results are presented (Ferrario et al., 1996). Here we use a technique (after Ferrario et al., 1997) in order to reliably determine the true detected levels above background (i.e. contribution from method blank).

Because samples were collected at different time intervals and analysed in different batches, sample masses varied in the range of 40.0-101.5 g, with the majority of samples (68 %) weighing 50.8 ± 0.8 g. The Limit of Detection (LOD) varied from one batch to another and background levels detected in different batches of blanks, provided on a concentration basis, could not be directly compared.

To overcome this, the target congener concentration in the blank was converted to a mass basis for each batch: the mean mass of the target congener in a series of blanks was increased by two standard deviations (i.e. mean + 2 s.d.) to produce a threshold level that

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\(^6\) An OPR sample is a matrix that has been fortified with a known amount of analyte.
ascertained true detects. If the mass of a target congener (e.g. 2,3,7,8-TeCDD) in the sample was greater than that of the averaged blank threshold level, that congener was considered a ‘real detect’ with a 95% confidence level. For conversion back to a concentration basis the averaged mass of the target congener (picogram) in the method blanks was divided by the average batch weight.

The importance of blanks becomes an issue specifically when confronted with samples contaminated with low levels (e.g. in the order of picogram per gram) – in this work, samples detected with PCDD/Fs fall into the category.

### 4.6 STATISTICAL & DATA ANALYSES AND DATA MODELLING

Regression analyses (Snedecor and Cochran, 1989), Welch’s t-test (Welch, 1947), analysis of variance (ANOVA), data modeling (that includes maximising the log likelihood of the data) and, graphical procedures on the data were carried out using the R statistical package, version 2.4.0 (R Development Core Team 2006).

A more accurate technique for describing the fit of regression analyses over the Pearson’s product moment correlation coefficient ($r^2$), as highlighted by Snedecor and Cochran (1989) is the variance percentage accounted for (\%V) and is given by:

\[
%V = \left[ 1 - \frac{(1-r^2)(n-1)}{n - N_T - 1} \right] \times 100
\]

where $n$ is the number of data points and $N_T$ is the number terms in the regression model.

To overcome the shortcoming of the Student’s t-test that assumes equal variances in datasets, the Welch’s t-test is used and is given by:

\[
Welch, t = \frac{x_1 - x_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}
\]
where $\bar{x}$ is the sample mean, $S^2$ is the sample variance and $n$ is the sample size. It is noteworthy that from Equation (4-2), the variances of the two datasets are accounted for, and this is important for biological datasets. In the case of Student’s $t$-test, the denominator of Equation (4-2) is based on a combined variance estimate (Welch, 1947).

### 4.6.1 P-value

The statistical significance of a result is the probability that the observed relationship in a sample occurred by pure chance, and that no relationship or differences exist in the population from which the sample was taken.

If the P-value is small ($< 0.05$), the correlation is significant and not dependent on coincidence. For $P = 0.05$, the confidence level is set at 95%. Hence in contrast, a P-value $> 0.05$ indicates that the modelled data cannot be concluded to have a significant correlation – this however does not suggest that there is no correlation, it merely states that the evidence suggests no real correlation or that the correlation (if observable) may be coincidental.

### 4.6.2 Condition Index, CI

The condition index, CI, (kg m$^{-3}$) for farmed fish was calculated as:

$$CI = \frac{W_{SBT}}{FL^3} \quad (4-3)$$

where $W_{SBT}$ (kg) and FL (m), respectively, represent whole fish weight and fork length.

A typical industry assumption is that the gills, guts and blood contributed 13% of the body weight. Consequently, where only gilled-and-gutted weight ($W_{gg}$) was made available, Equation (4-4) was applied:

$$W_{SBT} = \frac{W_{gg}}{0.87} \quad (4-4)$$
4.7 Concluding Remarks

From a food safety perspective, we report PCB and PCDD/F fresh weight concentrations and PCB and PCDD/F TEQ levels as upper-bound values. It is observed that there is inherent variability of the data at a single point in time due to biological variability.

A structured experimental program for residue research on farmed SBT has been carried out for the first time, resulting in the development and implementation of a set of standard operating procedures (SOPs) for sampling.

The methods of sampling and analyses described in this chapter provided valuable data that were used for studying and reporting levels of PCB and PCDD/F in SBT as affected by farming. Levels of PCB and PCDD/F in SBT fillets in relation to condition index and lipid content are presented as Chapter 5. Prior to modelling in Chapter 6, the data reported in Chapter 5 were transformed (i.e. addressed for levels in blanks using the method described in Section 4.5) to ascertain that modelling is carried out only on the data that is ‘real’ and not levels below the blank threshold that overshadow the ‘real’ data. Following, dietary modelling investigations from model predictions are presented.
Findings from this chapter have been submitted as:

5.1 INTRODUCTION

Findings from the critical review of the literature (Chapter 2) highlighted that although experimental work had been conducted to determine PCB and PCDD/F levels in (small) fish within controlled environments (e.g. laboratories), a gap in the literature exists as there is very little published on PCB and PCDD/F levels in large pelagic and predatory (farmed) fish such as the tuna, and in relation to the condition index (CI) and lipid.

This chapter presents the levels of PCBs and PCDD/Fs in farmed SBT fillets, as final products, obtained and analysed with methods described in Chapter 4. The aim of this chapter was to determine the net changes in these levels during farming from wild-caught fish (as baseline) to harvested “fillets”, which is the final product and to investigate the relationships between these levels and the CI and lipid content. Additional aims were to investigate if a LTH farming period for a commercial fishery company could produce SBT that had higher condition index and lipid content, while keeping the levels of PCBs and PCDD/Fs low, relative to a typical farming period.

The study presented in this chapter fits into step <3> of the developed risk framework presented in Chapter 3 (Phua et al., 2007).

5.2 MATERIALS AND METHODS

The data studied in this chapter was obtained from the one farm – Farm Delta Fishing Pty Ltd, whose experimental program has been described in sections 4.2.1 to 4.2.3 of Chapter 4. It was established that the overarching aim of this study was to determine the net changes in PCB and PCDD/F levels during farming from wild-caught fish to final product and, to investigate the relationships between these levels in the fillets and the CI and lipid content. As such, the data is presented as SBT from a farm (final product to be sold), and not SBT from a specific sea-cage within that farm. Sampling of SBT, laboratory analyses methods and statistical and data analyses methods have been described in sections 4.3 through 4.6 of Chapter 4.
5.2.1 Baitfish As Feed

Briefly, for the typical farming season, SBT were fed a composition of baitfish to increase their fat content. This was achieved by changing the ratios of three baitfish types (Australian and American sardines, *Sardinops sagax*; Australian red bait, *Emmelichthys nitidus nitidus*). It is noteworthy that the latin name for the Australian and American sardines are similar, however, Chapter 4 presented that these two types of baitfish have different proximate values. Additional work presented in Appendix E indicates that the American sardines have a different congener profile to the Australian sardines. For the longer term farming period, in addition to the three baitfish types above, Indonesian herring (*Sardinella lemuru*) and Australian blue mackerel (*Scomber australasicus*) were included in the diet. In both farming periods, SBT were fed to satiation, twice a day, typically six days a week. No manufactured feed pellets or nutritional supplements were fed to the farmed SBT.

5.3 RESULTS

5.3.1 Whole Weight

Figure 5.1 shows the trend in whole weight for the harvested SBT, both for the typical farming period and the LTH period. The mean weight of 16.98 ± 1.18 (s.e.) kg for wild-caught SBT at transfer (t = 0 days) increased to 21.31 ± 1.99 kg for SBT harvested at the end of the typical farming period (t = 139 days). Weights at harvest continued to increase through the LTH period. A mean weight of 38.25 ± 2.06 kg was achieved for SBT harvested at the end of the LTH period (t = 496 days). For t = 0, 246, 358 and 496 days, each data point was represented by the mean of five SBT. For t = 55, 97 and 139 days, each data point was represented by the mean of 10 SBT. Overall, whole weights of SBT increased with time of farming.

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7 All values reported with standard errors (s.e.) associated with the mean of the data.
5.3.2 Condition Index

Figure 5.2 shows the trend of the condition index of whole SBT with time of farming, both for the typical farming period and the LTH period. The mean CI of $16.5 \pm 0.3$ kg.m$^{-3}$ for wild-caught SBT at transfer ($t = 0$ days) increased to $23.7 \pm 0.35$ kg.m$^{-3}$ for SBT harvested at $t = 139$ days. However it was also observed that the CI decreased in the early part of the LTH period before increasing again to a CI of $22.9 \pm 0.5$ kg.m$^{-3}$ for the final harvest of the LTH period.

5.3.3 Lipid Content

Figure 5.3 shows the trend of the lipid content in the (edible) fillet of SBT. The mean lipid content of $0.72 \pm 0.16$ % in the fillet of wild-caught SBT at transfer increased to $15.7 \pm 1.1$ % in the fillet of SBT harvested at $t = 139$ days. It was also observed that the lipid content decreased in the early part of the LTH period before increasing again to a $13.4 \pm 0.5$ % for the final harvest of the LTH period.
Figure 5.2  Temporal trend of the condition index of whole SBT with time of farming, for both a typical farming period (up to 139 days) and the LTH period (up to 496 days).

Figure 5.3  Temporal trend of the lipid content (%) determined in the edible fillet of SBT, with time of farming, for both a typical farming period (up to 139 days) and the LTH period (up to 496 days).
5.3.4 Relationship Between Condition Index And Lipid Content

Similarities in trends observed between condition index and lipid content in the fillet prompted further investigation into whether a relationship existed. Figure 5.4 indicates that indeed, a significant linear correlation \( (p = 2.2 \times 10^{-16}, \text{df} = 46, %V = 0.796) \) exists between the CI and the lipid content in the fillet.

Figure 5.4  Linear correlation between the condition index of whole SBT and lipid content (%) in the fillet of SBT for the combined typical farming (up to 139 days) and LTH periods (up to 496 days).
5.3.5 PCB And PCDD/F TEQ LEVELS IN FILLETS

5.3.5.1 PCB TEQ Level in Fillets

Figure 5.5 presents the trend of PCB TEQ level in the fillet of SBT with time of farming. The mean PCB TEQ level of $0.11 \pm 0.01 \text{ pg-TEQ.g}^{-1}$ found in the fillets of wild-caught SBT at transfer ($t = 0$ days) increased to $0.67 \pm 0.03 \text{ pg-TEQ.g}^{-1}$ in fillets of SBT harvested at the end of the typical farming period ($t = 139$ days). After approximately 12 months of LTH, a mean PCB TEQ level of $0.92 \pm 0.10 \text{ pg-TEQ.g}^{-1}$ was found in fillets of SBT harvested at the end of the LTH period ($t = 496$ days). Welch’s t-test revealed that the mean PCB TEQ level in fillets of SBT determined in the final harvest of the typical farming period ($t = 139$ days) was not significantly different to the mean level obtained for the final harvest of the LTH period ($t = 496$ days) ($P = 0.05$, df = 5), but a significant difference was observed in the variances ($P < 0.05$).
5.3.5.2 PCDD/F TEQ Level in Fillets

Figure 5.6  Temporal trend in TEQ level for PCDD/Fs on a fresh weight basis, in the fillet of SBT with time of farming, for a typical farming period (up to 139 days) and the longer term farming period (up to 496 days).

Figure 5.6 presents the trend in PCDD/F TEQ level in the fillet of SBT with time of farming. The mean PCDD/F TEQ in fillets of wild-caught SBT at transfer (t = 0 days) was $0.055 \pm 0.006 \text{ pg-TEQ.g}^{-1}$ and increased to a mean of $0.15 \pm 0.01 \text{ pg-TEQ.g}^{-1}$ in fillets of SBT harvested at the end of the typical farming period (t = 139 days). After approximately 12 months of LTH, a mean PCDD/F TEQ of $0.285 \pm 0.029 \text{ pg-TEQ.g}^{-1}$ was found in fillets of SBT harvested at the end of the LTH period (t = 496 days). Welch’s t-test together with ANOVA studies revealed that the mean PCDD/F TEQ level in fillets of SBT determined in the final harvest of the typical farming period (t = 139 days) was significantly different to the mean level obtained for the final harvest of the LTH period (t = 496 days).
5.3.6 PCB And PCDD/F Congener Concentrations In Fillets

5.3.6.1 PCB Congener Concentrations in Fillets

Table 5-1 shows the 24 PCB congeners studied for temporal trends in the fillets of SBT, together with reasons for their selection and number of chlorine atoms for each selected PCB congener.

Figure 5.7 presents the fresh weight concentration for four selected lower chlorinated (mono-, di- and tri-chlorination) PCB congeners. Following the IUPAC numbering system (Ballschmiter and Zell, 1980) these are: (A) PCB 1, (B) PCB 3, (C) PCB 28, (D) PCB 37. All the data presented in Figures 5.7A through 5.7D are above the blank threshold concentration with the exception of a few SBT randomly detected below the blank threshold concentration (shown with open symbols). Even within the low chlorinated group, PCBs 1 and 3 exhibited biomagnification trends that appear to be different to PCBs 28 and 37.

Figure 5.8 shows the fresh weight concentration for four selected WHO-PCB congeners (tetra- through hepta-chlorination). These are: (A) PCB 77, (B) PCB 126, (C) PCB 169, (D) PCB 189. All data presented in Figures 5.8A through 5.8D are above blank threshold concentrations. All WHO-PCB congeners exhibit similar biomagnification trends.

Figure 5.9 shows the fresh weight concentration for four selected higher chlorinated (hexa- through deca-chlorination) PCB congeners. These are: (A) PCB 138, (B) PCB 153, (C) PCB 208, (D) PCB 209. All data presented in Figures 5.9A through 5.9D are above blank threshold concentrations. The two most abundant congeners PCBs 138 and 153 exhibit similar biomagnification trends, and likewise, the highest chlorinated PCB congeners, 208 and 209 exhibit similar biomagnification trends.
Table 5-1. The 24 PCB congeners studied for temporal trends in the fillets of SBT.

<table>
<thead>
<tr>
<th>PCB Number</th>
<th>Reasons for Selection</th>
<th>Number of Cl atoms</th>
<th>Position(s) of Cl atoms in the ortho position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lowest chlorination</td>
<td>1</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>3</td>
<td>Lowest chlorination</td>
<td>1</td>
<td>Non-ortho</td>
</tr>
<tr>
<td>4</td>
<td>Lowest chlorination</td>
<td>1</td>
<td>Di-ortho</td>
</tr>
<tr>
<td>15</td>
<td>Lower chlorination relative to 126</td>
<td>2</td>
<td>Non-ortho</td>
</tr>
<tr>
<td>19</td>
<td>Lower chlorination relative to 126</td>
<td>3</td>
<td>Di-ortho</td>
</tr>
<tr>
<td>28</td>
<td>Lower chlorination relative to 126</td>
<td>3</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>37</td>
<td>Lower chlorination relative to 126</td>
<td>3</td>
<td>Non-ortho</td>
</tr>
<tr>
<td>77</td>
<td>WHO, toxicity</td>
<td>4</td>
<td>Non-ortho</td>
</tr>
<tr>
<td>81</td>
<td>WHO, toxicity</td>
<td>4</td>
<td>Non-ortho</td>
</tr>
<tr>
<td>105</td>
<td>WHO, toxicity</td>
<td>5</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>114</td>
<td>WHO, toxicity</td>
<td>5</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>118</td>
<td>WHO, toxicity</td>
<td>5</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>123</td>
<td>WHO, toxicity</td>
<td>5</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>126</td>
<td>WHO, toxicity</td>
<td>5</td>
<td>Non-ortho</td>
</tr>
<tr>
<td>138</td>
<td>Relative abundance</td>
<td>6</td>
<td>Di-ortho</td>
</tr>
<tr>
<td>153</td>
<td>Relative abundance</td>
<td>6</td>
<td>Di-ortho</td>
</tr>
<tr>
<td>156</td>
<td>WHO, toxicity</td>
<td>6</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>157</td>
<td>WHO, toxicity</td>
<td>6</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>167</td>
<td>WHO, toxicity</td>
<td>6</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>169</td>
<td>WHO, toxicity</td>
<td>6</td>
<td>Non-ortho</td>
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<tr>
<td>189</td>
<td>WHO, toxicity</td>
<td>7</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>206</td>
<td>Highest chlorination</td>
<td>9</td>
<td>Tri-ortho</td>
</tr>
<tr>
<td>208</td>
<td>Highest chlorination</td>
<td>9</td>
<td>Tetra-ortho</td>
</tr>
<tr>
<td>209</td>
<td>Highest chlorination</td>
<td>10</td>
<td>Tetra-ortho</td>
</tr>
</tbody>
</table>
Figure 5.7  Temporal trends in the fresh weight concentration for (A) PCB 1, (B) PCB 3, (C) PCB 28, (D) PCB 37, in the fillet of SBT with time of farming, for both a typical farming period (up to 139 days) and the LTH period (up to 496 days). The dashed lines represent the average blank detection threshold for each congener.
Figure 5.8 Temporal trends in the fresh weight concentration for the WHO-PCB congeners (A) PCB 77, (B) PCB 126, (C) PCB 169, (D) PCB 189, in the fillet of SBT with time of farming, for both a typical farming period (up to 139 days) and the LTH period (up to 496 days). The dashed lines represent the average blank detection threshold for each congener.
Figure 5.9 Temporal trends in the fresh weight concentration for (A) PCB 138, (B) PCB 153, (C) PCB 208, (D) PCB 209, in the fillet of SBT with time of farming, for both a typical farming period (up to 139 days) and the LTH period (up to 496 days). The dashed lines represent the average blank detection threshold for each congener.
5.3.6.2 PCDD/F Congener Concentrations in Fillets

Figure 5.10 shows the fresh weight concentration for four selected PCDD congeners, (A) 2,3,7,8-TeCDD, (B) 1,2,3,4,7,8-PeCDD, (C) 1,2,3,4,6,7,8-HpCDD and (D) OCDD. The data indicates that for the typical and LTH farming periods, all PCDD congeners but two – 2,3,7,8-TeCDD and 1,2,3,7,8-PeCDD, were consistently detected below the blank threshold level, with the exception of a few random samples (e.g. t = 55 and 139 days).

For the congener 2,3,7,8-TeCDD, although concentrations were consistently detected below the blank threshold level for the typical farming period, it appears that extended farming time into the LTH period resulted in SBT having detected concentrations above the blank threshold level for the two final harvests (t = 358 and 496 days).

For the congener 1,2,3,7,8-PeCDD, wild-caught SBT had concentrations below the blank threshold level. With time of farming, the number of samples detected above the blank threshold level increased. It is clear that the LTH farming period resulted in SBT having detected concentrations above the blank threshold level.

Figure 5.11 shows the fresh weight concentration for four selected PCDF congeners, (A) 2,3,7,8-TeCDF, (B) 2,3,4,7,8-PeCDF, (C) 1,2,3,4,6,7,8-HpCDF and (D) OCDF. The data indicates that for the typical and LTH farming periods, all PCDF congeners but two – 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF, were consistently detected below the blank threshold level, with the exception of a few random samples (e.g. t = 55 and 97 days).

For the congener 2,3,7,8,TeCDF, wild-caught SBT had concentrations below the blank threshold level, with the exception of one SBT. It is clear that this congener was detected above the blank threshold level for the typical and LTH farming periods.

For the congener 2,3,4,7,8-PeCDF, wild-caught SBT had concentrations below the blank threshold level, with the exception of one SBT. Consistent detects above blank threshold level were observed with minor exceptions (t = 55, 97 and 358 days).
Figure 5.10 Temporal trends in the fresh weight concentration for four selected PCDD congeners (A) 2,3,7,8-TeCDD, (B) 1,2,3,4,7,8-PeCDD, (C) 1,2,3,4,6,7,8-HpCDD and (D) OCDD, in the fillet of SBT with time of farming, for both a typical farming period (up to 139 days) and the LTH period (up to 496 days). The dashed lines represent the average blank detection threshold for each congener.
Figure 5.11  Temporal trends in the fresh weight concentration for four selected PCDF congeners (A) 2,3,7,8-TeCDF, (B) 2,3,4,7,8-PeCDF, (C) 1,2,3,4,6,7,8-HpCDF and (D) OCDF, in the fillet of SBT with time of farming, for both a typical farming period (up to 139 days) and the LTH period (up to 496 days). The dashed lines represent the average blank detection threshold for each congener.
5.3.7 Biomagnification (BMF) Study

Figure 5.12 shows the ratio of the 45 PCB congener concentrations in SBT and baitfish for one of the sea-cages (sea-cage three) for the final harvest of the typical farming period \( t = 139 \) days. The first 12 PCB congeners in the figure belong to the class of toxic congeners (i.e. WHO-PCBs). Remaining PCB congeners were arranged according to number of chlorine atoms in the structure. It is clear that the lower chlorinated congeners PCBs 1, 3, 4, 15, 19 and 37 are distinctly different from the rest of the PCBs.

![Biomagnification factors for all 45 PCB congeners for one of the sea-cages determined in SBT for the final harvest of the typical farming period (\( t = 139 \) days). The first 12 PCB congeners belong to the class of toxic congeners, remaining congeners were arranged according to chlorination.](image)
5.4 DISCUSSION

Temporal trends in whole weight, CI, lipid content and levels of polychlorinated biphenyls (PCBs) and dioxins (PCDD/Fs) were determined in composite fillet samples from five wild-caught (in the Great Australian Bight) and 45 Australian-farmed (offshore of Port Lincoln) Southern Bluefin Tuna (SBT).

It is clear from inspection of Figures 5.1 through 5.3 that CI and lipid content in the fillet increased with increasing whole weights for the typical farming period. It is noteworthy however, that for \( t = 55 \) days, the mean whole weight of SBT was higher than subsequent harvests in the typical farming period.

The spike in mean whole weight for \( t = 55 \) days was not expected. Instead, whole weights were expected to gradually increase with time of farming, as observed by the overall trend in Figure 5.1. The spike in mean whole weight at \( t = 55 \) days for this sampled batch may be attributed to a combination of the two factors: Firstly it may have been attributed to selective harvesting by the commercial company (i.e. larger SBT were preferentially harvested for \( t = 55 \) days). Secondly for this harvest, the residue team (including the author) travelled to Port Lincoln to select SBT for residue testing and consequently selected the larger SBT from the harvest batch. Because it was not financially sustainable to travel to Port Lincoln for each harvest to select SBT for residue testing, we provided a range of fork length values to the company, which became a selection criterion for SBT subsequently harvested for residue research – this may have resulted in a more consistent selection for subsequent harvests.

The CI was observed to decrease as farming proceeded into the LTH period. The data indicates that SBT were not putting on much weight between \( t = 246 \) and 358 days. It is therefore inferred that there was a greater increase in fork length for this duration. According to Equation 4-3, a small increase in weight with a greater increase in fork length will result in a lower condition index, observed between harvests (\( t = 246 \) and 358 days). For the duration between \( t = 358 \) and 496 days, the data suggests that the increase in weight was greater than (any) increase in fork length, and therefore applying Equation 4-3 results in a higher CI for \( t = 496 \) days.
Figure 5.4 indicates that the lipid content in the fillet increases proportionally with the CI of whole SBT. Since CI is a parameter easily measured by farmers whereas determination of lipid content requires costly laboratory analyses, the finding of a linear relationship is a significant contribution to the SBT industry. It is inferred from this finding that if (significantly) more data on CI and lipid content from across various SBT farms with different husbandry techniques and feeding strategies were available, a chart may be produced to provide an estimate of the lipid content in a SBT given a calculated CI.

TEQ levels were determined using WHO-TEF values reported in van den Berg et al. (1998). An example of the WHO-TEQ calculation method has been presented in Chapter 3. Figures 5.5 and 5.6 suggest that the PCB TEQ level contributes a mean of up to 76% of the total (combined PCDD/F + PCB) TEQ level in farmed SBT. This finding is fairly consistent with limited data presented in a report to the SBT industry (Padula et al., 2004a), where the PCB TEQ was determined to be approximately 80% of the total TEQ. Other researchers have reported similar findings on the PCB TEQ relative to the total TEQ. Lundebye et al. (2004) found that for Atlantic salmon farmed in Norway, PCB TEQ ranged 63-78% of the total TEQ. For the data extracted from Isosaari et al. (2002) where rainbow trout were farmed in Finland, PCB TEQ ranged approximately 60–77% of the total TEQ. Falandyasz et al. (2000) studied the accumulation of PCDD/Fs and PCBs in biota from the Baltic Sea and found that the WHO-PCBs dominated, accounting for up to 90% of the total TEQ for wild-caught fish group that included eel and perch. Findings in the southern and northern hemispheres indicate a consistently high contribution of PCB TEQ to total TEQ in fish, regardless of geographical locations; and may be regardless of species.

When comparing TEQ levels reported in the literature, it is important to determine if similar TEQ calculation methodology has been applied. Different methodologies employed would make data less comparable. One such example of a different methodology employed is the study reported by Fiedler et al. (1998). Fiedler et al. (1998) reported that PCB TEQ in farmed catfish contributed 4–16% of the combined total TEQ. It was, however, noted that for their study, the I-TEQ method established by the WHO in
1994 was used to determine TEQ levels\(^8\). Today, this I-TEQ method has largely been superseded by the WHO-TEQ method described by van den Berg et al. (1998). Caution must therefore be exercised when comparing TEQ values reported in the literature.

It is known that there are several mechanisms that can influence the concentrations of PCB and PCDD/F in fish (Boon and Duinker, 1985; Sijm et al., 1992); these include: an exposure route due to feeding (Isosaari et al., 2002), aqueous exposure (Branson et al., 1975; Mackay and Hughes, 1984), metabolism that encompasses residue mobilisation during starvation periods and faecal elimination (Norstrom et al., 1976), growth dilution and biotransformation (Buckman et al., 2006). Investigations into each of these mechanisms are usually done separately. The contributions due to each of these mechanisms have not been investigated here. However, some of these mechanisms will be discussed briefly.

Figures 5.7 through 5.9 indicate that the trend of biomagnification may be related to chlorination and toxicity. It is clear from inspection of Figures 5.7A and 5.7B that the trends of PCBs 1 and 3 are different to the trends of PCBs 28 and 37, shown in Figures 5.7C and 5.7D. The other lower chlorinated congeners studied (mono-, di- and trichlorination, see Appendix F) resembled the trends observed for PCBs 28 and 37.

Overall, because the lower chlorinated congeners have two or less chlorine atoms located in the ortho positions, it is postulated that these lower chlorinated congeners are eliminated preferentially relative to congeners with more chlorine atoms (≥ 4) and whose chlorine atoms are in the non-ortho positions. Buckman et al. (2006) found that PCBs 4 (see Appendix F) and 28 were readily biotransformed in juvenile rainbow trout and suggested that the observed biotransformation for these congeners are consistent with degradation by the CYP2B-like isoforms, proposed by Boon et al. (1994), based on the parent compound chlorine substitution.

It is clear from inspection of Figures 5.8A through 5.8D that similar trends exist among the toxic WHO-PCB congeners (van den Berg et al., 1998) (see Appendix G for

\(^8\) See Ahlborg et al., (1994) and Van den Berg et al. (1998), for the calculation methodology for I-TEQ and WHO-TEQ, respectively.
additional work on other WHO-PCB congeners). Buckman et al. (2006) found that PCBs 167 (see Appendix G) and 189 are typically not easily biotransformed, while other WHO-PCBs with chlorine atoms in the mono-ortho and di-ortho positions, such as PCBs 105, 114, 118 and 156 may be biotransformed by the CYP1A enzyme. The investigation of Buckman et al. (2006) did not include the most toxic WHO-PCB congeners (PCBs 77, 81, 126 and 169). However, it may be inferred from their findings that PCBs 126 and 169 should have no significant biotransformation due to the maximum (five or six) chlorine substitution in the meta and para positions, with none in the ortho positions of the biphenyl rings whereas PCBs 77 and 81 may not be easily biotransformed due to the four chlorine atoms in the meta and para positions, with none in the ortho positions of the biphenyl rings.

The two most abundant congeners, PCBs 138 and 153 (Figures 5.9A and 5.9B) appear to have a similar trend to the WHO-PCB congeners (Figures 5.8). PCBs 138 and 153 are environmentally abundant because these used to be major components of technical PCB formulations (McFarland and Clarke, 1989). Boon et al. (1994, 1997) have grouped PCBs 138 and 153 together with the WHO-PCBs based on their structure. PCBs 138 and 153, however, are less toxic than the WHO-PCBs because each congener has two chlorine atoms substituted in the ortho positions (McFarland and Clarke, 1989). Consequently, Buckman et al. (2006) found that the biotransformation mechanism(s) for PCBs 138 and 153 follow the WHO-PCB congeners with mono-ortho chlorination.

Other non-toxic but highly chlorinated congeners (nona- and deca-chlorination) such as PCBs 206 (see Appendix F), 208 and 209 (Figures 5.9C and 5.9D) exhibit similar biomagnification trends. The concentrations of PCBs 206, 208 and 209 increased consistently throughout both farming periods. This may be because it is completely substituted with chlorine atoms, has the greatest log $K_{ow}$ value ($> 8$) among the PCBs indicating a high affinity for binding to lipids, and has been classified as having “no significant biotransformation with the slowest elimination of all the PCB congeners” (Niimi and Oliver, 1983; McFarland and Clarke, 1989; Muir et al. 1992; Buckman et al. 2006). The increase in biomagnification trends may be attributed to the exposure route by feed.
For most of the SBT samples, PCDD/F congeners were either detected at the limit of detection (LOD) or below the LOD for that congener in the method blank. As samples were analysed in various batches over the entire experimental study (t = 0 to 496 days), several LODs were obtained for a single congener (e.g. for the congener 2,3,7,8-TeCDD, LOD ranged 0.00564 pg.g$^{-1}$ fresh weight basis to 0.0212 pg.g$^{-1}$ fresh weight basis). It was noted that on some occasions, SBT samples were analysed after an analytical run where samples of other matrixes had high concentrations of PCDD/Fs (i.e. in the order of ng.g$^{-1}$ fresh weight) (pers. comm. Phil Bridgen, AgriQuality Limited). Hence, a blank threshold level/concentration technique was developed and applied to overcome the drawback associated with the analytical data. For this work, congeners with concentrations that fall below the blank threshold concentration will be attributed to the blank and treated as non-detects in the SBT.

Figures 5.10 and 5.11 indicated that all PCDD/F congeners (except 1,2,3,7,8-PeCDD, 2,3,7,8-TeCDF, 2,3,4,7,8-PeCDF and 2,3,7,8-TeCDD for the final two LTH harvests) were detected below the blank threshold concentrations throughout the entire farming study. The data also show that PCDD/Fs were quantified at the parts per quadrillion (ppq)$^9$ level in SBT (i.e. 1 – 320 ppq or 0.001 – 0.32 pg.g$^{-1}$ fresh weight). It is noted that the term ppq is seldom used in the literature. However, ppq is highlighted here to indicate the magnitude of PCDD/F detection in SBT.

Unlike the PCBs, trends for the PCDD/F congeners that were detected in SBT did not appear to be similar among PCDD/F congeners. Figure 5.10A shows that the congener 2,3,7,8-TeCDD was not detected for a typical farming season and the early LTH farming period. It was however detected for the final two harvests of the LTH period (t = 358 and 496 days) suggesting that 2,3,7,8-TeCDD may have biomagnified in SBT after an extended farming process or it may be due to a change in the diet (i.e. changes in baitfish species fed) during the LTH.

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$^9$ Parts per quadrillion (ppq) term exists in the field of chemistry (Mills et al., 1998; Quinn and Mills, 1998) but is seldom used in the literature due to possible ambiguity if the definition of ppq has not been provided with the abbreviation.
Figure 5.10B indicates that the congener 1,2,3,7,8-PeCDD was not detected in wild-caught SBT but biomagnified with an increasing trend over time, although CI and lipid decreased for the first two harvests of the LTH farming period (t = 246 and 358 days). Sijm et al. (1993) reported that biotransformation was not observed for congeners with substituted lateral positions (i.e. 2, 3, 7 and 8 positions all substituted with chlorine atoms). It is therefore inferred from the finding of Sijm et al. (1993) and observations of the whole weights, CI and lipid in addition to the increasing trend for the concentration of 1,2,3,7,8-PeCDD, that the dominant mechanisms affecting the concentration of the congener 1,2,3,7,8-PeCDD in SBT are not biotransformation, growth dilution or elimination but an exposure route from feed.

Figure 5.11A shows that the congener 2,3,7,8-TeCDF increased with time for a typical farming period but decreased over the LTH farming period. Sijm et al. (1993) reported that biotransformation was not observed for the congener 2,3,7,8-TeCDF in goldfish in addition to the noteworthy finding that congeners with substituted lateral positions (i.e. 2, 3, 7 and 8 positions all substituted with chlorine atoms) are not biotransformed. Building on the finding of Sijm et al. (1993), it is inferred that the decrease in concentrations may therefore be restricted to elimination or growth dilution. The decrease in concentration may also be a result of the change in feeding strategy; or a combination of elimination, growth dilution and change in feeding strategy.

Bruggeman et al. (1984) and Gobas et al. (1988) found that there was no significant biomagnification or bioconcentration of OCDD and OCDF in the guppy, suggesting that its molecular size (0.95 nm internal cross section) was too large to cross membranes. In contrast, Muir and Yarechewski (1988), and Muir et al. (1990) reported biomagnification of 1,2,3,4,6,7,8-HpCDD and OCDD in the rainbow trout and fathead minnows. For this work, the data indicated that 1,2,3,4,6,7,8-HpCDD and OCDD were not detected in SBT. The varied findings in the literature among species and contribution from this work suggest that the biomagnification of 1,2,3,4,6,7,8-HpCDD and OCDD may be dependent on species-specific fish physiology or levels in the diet, or a combination of both.

Overall, PCDD/F congeners behave differently, may have biomagnified at different rates, and no similar trends have been observed among the congeners detected in SBT. However for PCBs, different trends exist for the congeners with lower and higher extents
of chlorination relative to PCB 126, and a different trend exists for the WHO-PCB congeners together with the two most abundant congeners (PCBs 138 and 153). Findings suggested that the mechanism of biotransformation did not affect the WHO-PCB congeners and the three PCDD/F congeners that were consistently detected in farmed SBT.

5.4.1 Specific Remarks For The Typical Farming Period

The typical farming period \((0 \leq t \leq 139)\) for Farm Delta Fishing Pty Ltd occurred between austral-autumn and the end of the austral-winter. It was observed that the CI and lipid content increased proportionally to whole weight of harvested SBT during the austral-autumn. It is clear that the CI obtained at transfer \((t = 0\) days) is significantly different \((P < 0.05)\) to the CI obtained for the final harvest of the typical farming period \((t = 139\) days). Welch’s t-test and ANOVA studies for whole weight, CI and lipid content between \(t = 97\) and \(t = 139\) days revealed no significant differences in the mean values and the variances. This may be due to the already fattened SBT that are less inclined to feed, or to the decrease in SBT feeding rates that occur with decreases in natural water temperatures (Glencross et al., 2002), or a combination of both.

Generally, it appears that with increasing weight, CI and lipid content (up to \(t = 97\) days), PCB and PCDD/F TEQ levels (Figures 5.5 and 5.6) and fresh weight concentrations for all PCB congeners (Figures 5.7 through 5.9) increased.

All 12 WHO-PCB congeners (Figures 5.7 through 5.9, and Appendix G) were reported above the blank threshold level and showed increases in concentrations throughout the typical farming period. It is noteworthy that for WHO-PCBs, with levels an order of magnitude greater than WHO-PCDD/Fs (Figures 5.10 through 5.11), the issue of blanks is less pronounced. The results indicate that PCB 126 accounts for approximately 82% of the PCB TEQ level.

The PCDD/F TEQ is dominated by the third most toxic congener, the 2,3,4,7,8-PeCDF \((TEF = 0.5)\) since the two most toxic congeners, 2,3,7,8-TeCDD and 1,2,3,7,8-PeCDD,
have levels below the blank threshold levels. A similarity in trend between 2,3,4,7,8-
PeCDF and the PCDD/F TEQ is also observed.

5.4.2 Specific Remarks For The LTH Farming Period

Whole weights of SBT continued to increase as farming proceeded into the LTH period
(140 ≤ t ≤ 496), achieving a maximum average weight of approximately 41.0 ± 1.1 kg at
the final harvest of LTH. Welch’s t-test and ANOVA studies revealed that the mean CI
obtained for the final harvest of the typical farming season (23.7 ± 0.3 kg.m⁻³ at t = 139
days) was not significantly different to the mean CI obtained for the final harvest of the
LTH farming period (22.9 ± 0.5 kg.m⁻³ at t = 496 days) (P > 0.05). Similarly for the lipid
content in the fillets, no significant differences (P > 0.05) were observed between t = 139
and 496 days, respectively, with 15.7 ± 1.1 % and 13.4 ± 0.5 %. This indicated that even
after approximately an additional 12 months of farming, there were no observable
differences in the mean CI, the mean lipid content and, in the variances of the means.

ANOVA studies were carried out for whole fish CI for SBT from the three LTH harvests,
and also for lipid content in the fillet of SBT. The data indicated that the mean CI for SBT
from the second LTH harvest (t = 358 days) was significantly different to those of the
first and third LTH harvests (P < 0.05) and, the mean lipid content in the fillet of SBT
from the third harvest was significantly different to those obtained from the first and
second harvests (P < 0.05). A correlating trend, as previously expected, was not observed.
This may be due to the small sample sizes for each LTH harvest, the variability in the
lipid content (maximum s.e. of 1.78, obtained at the second LTH harvest) and the
variability in the CI (maximum s.e. of 0.87, obtained at the second LTH harvest).

It is noticed that the highest mean CI of 24.0 ± 0.5 kg.m⁻³ achieved for the typical farming
period at t = 97 days was not significantly different to the highest mean CI of 22.9 ± 0.5
kg.m⁻³ achieved for the LTH period at t = 496 days (P > 0.05), however, the highest mean
lipid content of 17.6 ± 0.5 % achieved for the typical farming period at t = 97 days was
significantly different to the highest mean lipid content of 13.4 ± 0.5% achieved for the
LTH period at t = 496 days (P < 0.05). Again, a correlating trend was not observed
possibly due to the small sample sizes and variability in the data.
Additional work (see Appendix H) revealed that SBT harvested within a short time period, \(336 \leq t \leq 358\) days (i.e. a one week interval), showed decreases in whole weight. This could be due to stress from the previous harvesting episode that consequently affected the feeding habits of the school within the cage or, it may be due to biological variation in the data due to the small sample sizes. Weight data was available for SBT harvested at \(t = 336, 343, 351\) days, but these fish were not analysed for PCBs and PCDD/Fs due to cost constraints.

### 5.4.3 Biomagnification Factors (BMF)

It was determined from Figures 5.7 through 5.9 and the above discussion that there were similar trends for PCBs within the lower chlorinated group, higher chlorinated group and the group for WHO-PCBs together with the two most abundant congeners. A BMF study was therefore conceived to confirm if PCBs behaved differently.

For the BMF study, data was grouped to be sea-cage specific. This is because the two sea-cages throughout the typical farming period had experienced different diets. Figure 5.12 indicates that indeed, all PCB congeners behaved differently although similar trends were observed within a group as previously determined. Also from Figure 5.12, it is observed that the lower chlorinated group (PCBs 1, 3, 4, 15, 19 and 37) are distinct from the rest of the PCB congeners analysed. It has been highlighted in the discussion above that the lower chlorinated congeners are preferentially eliminated relative to congeners with more chlorine atoms (\(\geq 4\)) and whose chlorine atoms are in the non-ortho positions. Carlson and Hites (2005) reported that in farmed Atlantic salmon, the lower molecular weight congeners PCBs 1, 2, 3, 4, 11, 12/13, 15, 36, 37, and 38 have “substantially different behaviour”.

It is iterated here that BMF is different to the bioconcentration factor (BCF) as highlighted by Mackay and Fraser (2000) and, Gobas and Morrison (2000). BMF relates to the concentration in fish from uptake of food whereas BCF relates to the concentration in fish from uptake via water. It has been highlighted in the literature reviewed in Chapter 2 that for SBT farming in the open ocean off Port Lincoln, contribution of the
concentrations of PCBs and PCDD/Fs via the water is negligible, compared with the contribution of the concentrations via baitfish as feed.

5.4.4 Limitations Of This Experimental Field Study And Data

There have been some limitations identified in this commercial field study. Firstly, as this study was conducted on a commercial farm, SBT obtained for research had to be synchronised with the company’s harvest plans (i.e. when to harvest the SBT to meet market demand and to maximise profits due to fluctuating currency such as the Japanese yen).

Secondly, due to the niche market and high costs for SBT products (approximately A$800 per SBT wholesale) and laboratory analyses (approximately A$2000 per sample analysed for PCBs and PCDD/Fs), sample sizes were limited to five SBT for all harvests. For t = 55, 97 and 139 days, it is noted that sample sizes comprised of 10 SBT for each harvest, however this was because SBT were harvested from two sea-cages (i.e. five SBT from each sea-cage).

Thirdly, the mean values obtained from five samples may not be representative for the population in a sea-cage because of small sample sizes and, observed inherent variation in biological data within these small sample sizes. For the typical farming and LTH farming periods, sample sizes represented, respectively, approximately 1 – 2 % of the population in a sea-cage and 0.5 – 1 % of the population in the LTH sea-cage.

Finally, as SBT were farmed in sea-cages in the open ocean with environmental conditions varying with seasons, variables such as water temperature and water flow rate, respectively, were subject to seasonal and tidal changes. It is noted that this work is contrasted with other studies published in the literature for PCBs and PCDD/Fs in fish, where these fish were farmed in controlled environments (e.g. tanks).
5.5 CONCLUDING REMARKS

1. Fillets from wild-caught and farmed SBT were analysed and temporal trends in concentration for polychlorinated biphenyls (PCBs) and dioxins (PCDD/Fs) were reported for the first time, and elucidated in relation to trends in whole weight, condition index (CI) and lipid content.

2. A (statistically significant) linear relationship was determined between the CI and lipid content in the fillet of SBT.

3. A maximum mean CI of $24.0 \pm 0.5 \text{ kg.m}^{-3}$ and a maximum mean lipid content of $17.6 \pm 0.5 \%$ was achieved at $t = 97$ days, for the baitfish species and ratios employed as feed by this company.

4. There were no significant differences in the CI and lipid between the final harvests of the typical farming and LTH periods, i.e. even after an additional 12 months of farming following the typical farming period. PCB and PCDD/F TEQ levels, however, increased between the final harvests of the typical farming and LTH periods.

5. It is clear that the LTH farming period, whilst carried out for the first time, did not compare favourably with the typical farming period. Although whole weights increased with time of farming, the CI and lipid decreased during LTH period. It appears that a typical farming period is sufficient to achieve a maximum mean CI and lipid content with lower PCB and PCDD/F levels compared to LTH farming period, for the diets employed and husbandry practices by Farm Delta Fishing Pty Ltd. This is a significant finding for the SBT industry and, on a larger scale to the literature on tuna farming.

6. Levels of $0.67 \text{ pg-TEQ.g}^{-1}$ and $0.15 \text{ pg-TEQ.g}^{-1}$, respectively, for the PCB and PCDD/F TEQs, were found in the fillets of SBT at the end of the typical farming period. These levels increased to $0.92 \text{ pg-TEQ.g}^{-1}$ (PCB) and $0.29 \text{ pg-TEQ.g}^{-1}$ (PCDD/F) at the end of the LTH period. Since the data was collected with a single
commercial company, caution must be exercised when extrapolating these results to the entire SBT farming industry since various companies employ different husbandry practices.

7. After correcting for concentrations in the blanks, only three PCDD/F congeners were consistently detected in the fillets SBT throughout the typical and LTH farming periods. The three congeners are: 1,2,3,7,8-PeCDD, 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF. Only two of the three congeners, 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF were detected throughout a typical farming period. The congener 2,3,7,8-TeCDD was detected only during the final two harvests of the LTH farming period. All 24 PCB congeners, including the 12 WHO-PCB congeners, were consistently detected above blank threshold concentrations, in the fillets of SBT throughout the typical farming and LTH farming period.

8. The lower chlorinated PCB congeners appear to be preferentially eliminated or biodegraded. The higher chlorinated PCB congeners showed no significant biotransformation. The WHO-PCB congeners and two most abundant PCB congeners either showed no significant biotransformation or were not easily biotransformed due to their chemical structures. Findings indicated that the three PCDD/F congeners that were consistently detected in farmed SBT (fillets) may not have undergone biotransformation.

9. A biomagnification factor study revealed that all PCB congeners behaved differently within the (fillets of) SBT. The congeners with the lowest BMF were PCBs 1, 3, 4, 15, 19 and 37.

The trends over the farming periods in PCB and PCDD/F levels (TEQ and congener concentrations), condition index and lipid content, together with other farming parameters such as the feeding and growth rates, are necessary to develop quantitative model(s) to predict PCB and PCDD/F levels in Australian farmed SBT.

The model(s) can consequently be used to provide advice to farmers when developing feeding strategies to manage PCB and PCDD/F levels and, to ensure compliance against
reference health standards (e.g. Tolerable Daily Intake\textsuperscript{10}, TDI) for consumers of farmed SBT.

This chapter provided insight into which PCB and PCDD/F congeners to model. Modelling will be carried out on congeners having concentration above the blank threshold concentrations – as these congeners have been ascertained to be ‘true detects’ in fillets of SBT. Modelling will also be carried out for a typical farming period as the results presented here indicated that a typical farming season was sufficient in achieving the maximum CI and lipid content.

The following chapter deals with the development of the predictive model for farmed SBT using the analysed data and observed trends (from Farm Delta Fishing Pty Ltd) presented in this chapter. Following, validation of the model(s) is carried out on the data obtained from a different SBT farm – Farm Alpha Fishing Pty Ltd. Both the Predictive Congener Model and Predictive TEQ Model will be presented.

\textsuperscript{10} This term has been assigned by the European Commission Scientific Committee on Food as regulatory equivalent for Acceptable Daily Intake (ADI). Duffus (1993) highlights that TDI is normally used for contaminants found in foods. It is noteworthy that different countries use different terminology. For example, Australia uses Tolerable Monthly Intake (TMI).