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

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by

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CHAPTER SIX

PREDICTIVE MODELLING OF THE LEVELS OF PCBs and PCDD/Fs IN SBT (FILLETS) FARMED IN OPEN AUSTRALIAN WATERS

“Modelling is often regarded as an effort to understand or design a complex system, and that attention must be paid to a variety of other concerns ranging from experimental design to budget. Models are not universally valid, but are designed for specific purposes.”

Law and Kelton (1991)

Findings from this chapter have been published as:


6.1 INTRODUCTION

Mathematical models may be applied to predict the bioaccumulation of chemical contaminants (residues) in complex biological systems. Applied to aquaculture, these models can be used to address concerns regarding levels of the residues at harvest from feeding (as the source). These concerns may include the type of feed (e.g. baitfish or pellets), time of farming, levels of residues in the feed, and which strategy will be significant in abating the residue(s). Therefore the use of mathematical models is one approach to trace and manage levels in farmed fish by employing suitable strategies for feed selection, while permitting an alternative to destructive testing whereby fish have to be sacrificed on a frequent basis to determine levels of residues in the edible flesh at harvest.

In this chapter, the Modified LCBK model (previously synthesised in Chapter 2, see Section 2.5.4.2) is assessed against the experimental PCB and PCDD/F data obtained from Farm Delta Fishing Pty Ltd, as reported in Chapter 5. The Modified LCBK predictive model is studied on two bases: congener basis (Congener Model) and TEQ basis (TEQ Model). Assimilation efficiencies of PCBs and PCDD/Fs in farmed SBT were determined from the Modified LCBK Model using the data presented in Chapter 5. Consequently, the uptake efficiencies obtained were validated with data from a different farm, Farm Alpha Fishing Pty Ltd.

It was concluded in Chapter 5 that the maximum CI and lipid content were achieved within a typical farming period. In addition, it was shown that PCB and PCDD/F levels increased further with longer time of farming (i.e. LTH period). Furthermore, communication with SBT management indicated that the LTH farming period was a one-off experimental trial and therefore principal interest lies only for a typical farming period. Consequently, predictive modelling would be carried out for a typical farming season.

Chapter 5 also highlighted that sample sizes of the harvested SBT for PCB and PCDD/F analyses were small. It would not be prudent to use physical measurement data (i.e. whole weight and fork length) from this small sample size to estimate the population mean whole weight and fork length. To overcome the shortcoming of small sample sizes,
harvest data\textsuperscript{11} was used. Consequently, parameters used in the predictive model that represent the average fish in the sea-cage, e.g. feeding and growth parameters, were also determined from harvest data.

The important criteria for an adequate model established in Chapter 3, together with the analyses of residual plots provide the stringent test for accurate model predictions against experimental PCB and PCDD/F data for farmed SBT.

The research presented in this chapter fits into steps <3> and <5> of the developed risk framework (Phua et al., 2007) presented in Chapter 3.

6.2 MATERIALS AND METHODS

The sample data obtained for model development and parameterisation came from Farm Delta Fishing Pty Ltd in 2005. Materials and methods for sampling, to determining PCB and PCDD/F concentrations in samples have been described in Chapter 4. Ideally, for model validation, a different data set should be obtained from the same farm (Farm Delta Fishing Pty Ltd) so that husbandry (farming) practices may be kept consistent throughout the modelling study process, and that all other variables (e.g. baitfish used as feed, feed quantity) could be kept constant. However due to management decisions and logistics, the validation data set was obtained from a different SBT farm, Farm Alpha Fishing Pty Ltd, in the subsequent year 2006.

The following section highlights the methods employed for model synthesis, parameterisation and validation.

6.2.1 The Modified LCBK Predictive Model

Using engineering principles and the assumptions highlighted in Chapter 2 (see Section 2.5.4.2), the mechanistic model based on the work of Sijm et al. (1992) for biomagnification of PCBs and PCDD/Fs has been modified to predict the assimilation

\textsuperscript{11} Harvest data is defined as data that contained weights and fork lengths of all harvested SBT (i.e. including SBT harvested for commercial sale) and not limited to SBT harvested for PCB and PCDD/F analyses.
efficiency in SBT fillets, and consequently the concentrations of PCBs and PCDD/Fs in SBT fillets. The Modified LCBK Model has been presented as Equation (2-6) and is presented here as Equation (6-1):

\[ C_{SBT,t_i} = \frac{\alpha F'}{\gamma} C_{bait} (1 - e^{-\gamma t}) + C_{SBT,t_{i-1}} e^{-\gamma t} \]  

(6-1)

where \( C_{SBT,t_i} \) is the PCB or PCDD/F concentration in the fillet of a farmed SBT (pg g\(^{-1}\) SBT fillet) and the subscript \( t_i \) represents farming interval where \( i = 1, 2 \) and 3, \( \alpha \) is the assimilation efficiency of the PCB or PCDD/F found in the food (baitfish) by that SBT (fillet basis), \( F' \) is the (fillet corrected) feeding rate (kg baitfish kg\(^{-1}\) SBT fillet day\(^{-1}\)), \( C_{bait} \) is the PCB or PCDD/F concentration in baitfish (pg g\(^{-1}\) baitfish) and \( \gamma \) is the rate constant for growth (time\(^{-1}\)), corrected for the basis of fillet. The following parameters in the model were measured: \( C_{SBT,t_i} \), \( C_{bait} \), \( F' \) and \( \gamma \); while \( \alpha \) is the variable in the model. The parameter \( C_{SBT,t_{i-1}} \) was measured in the wild-caught SBT and was treated as an input parameter for the first step in the non-linear regression. However for subsequent steps in the non-linear regression, e.g. farming interval 2 (\( t_i = 2 \)), \( C_{SBT,t_{i-1}} \) was permitted to regress as a variable, i.e. \( C_{SBT,t_{i-1}} \) became the concentration in farmed SBT fillet determined for farming interval 1.

6.2.2 Model Parameters

6.2.2.1 Modelling the Age of Juvenile Southern Bluefin Tuna

In Chapter 5 the data obtained from Farm Delta Fishing Pty Ltd, with levels of PCB and PCDD/F in the final product was presented. Here, this data is further grouped into the age classes of SBT in order to reflect the growth rate of the individual age classes.

A recent study suggested that fork length with the assumption of a common standard deviation, can be used to determine the age of SBT (Leigh & Hearn 2000). Applying the maximum log-likelihood method described in Venables and Ripley (1999), a Gaussian mixture model given by Equation (6-2) was formulated to permit age class identification. This model was consequently fitted with the `optim()` method in the R package.
Predictive Modelling of PCBs and PCDD/F Levels in Farmed SBT Fillets

\[ L(\pi_1, \pi_2, \mu_1, \sigma_1, \mu_2, \sigma_2, \mu_3, \sigma_3) = \sum_{i=1}^{3} \log \left[ \frac{\pi_i}{\sigma_i} \phi \left( \frac{y_i - \mu_i}{\sigma_i} \right) + \frac{\pi_2}{\sigma_2} \phi \left( \frac{y_i - \mu_2}{\sigma_2} \right) + \frac{1 - \pi_1 - \pi_2}{\sigma_3} \phi \left( \frac{y_i - \mu_3}{\sigma_3} \right) \right] \]  

where \( L \) is the log-likelihood, \( \pi_i, \mu_i \) and \( \sigma_i \) are respectively, the proportion, mean and standard deviation of the \( i^{th} \) mode, and \( y \) is the sample fork length. The subscripts \( i = 1, 2 \) and 3 represent three modes identified in the data set which were based on fork length. Assuming that a school of SBT comprised fork length that follow normal distribution, the age classes can be estimated by minimising \(-L\) (Venables and Ripley, 1999).

Figure 6.1 Histogram distribution from the optimisation model of 876 wild-caught SBT, separated into age classes by fork length. The first, second and third modes, respectively, represent two year old fish whose fork length is \( \leq 95.0 \) cm, three year old fish with fork length between 95.1 and 112.0 cm, and four year old fish whose fork length is \( > 112.1 \) cm.
Figure 6.1 shows the distribution of 879 wild-caught SBT optimised using Equation (6-2) to give the fork length that separates the age classes. The first, second and third modes, respectively, represent two year-old fish whose fork length is \( \leq 95.0 \) cm, three year-old fish with fork length between 95.1 and 112.0 cm, and four year-old fish whose fork length is \( > 112.1 \) cm. It can be observed that there are three SBT whose fork length is \( > 130 \) cm – this represents 0.3 % of the school.

The approach of fitting a Gaussian mixture model resulting in Figure 6.3 has been carried out successfully in the past (Hasselblad, 1966; MacDonald and Pitcher, 1979) and recently revisited by Leigh and Hearn (2000). The data in Figure 6.1 shows that this school of wild-caught SBT was dominated by the two (~ 44 %) and three (~ 52 %) year-olds, with a minority group of four and five year-olds (~ 4 %). It is inferred from this finding that: (i) the four and five year-old SBT clearly cannot be representative of this school, and (ii) there can be several age classes in a school of wild SBT, and these age classes can be dependent on the time (of the year) and location of catch (Leigh and Hearn, 2000). Leigh and Hearn (2000) also presented data that suggested juvenile SBT between the ages of one and four dominates the waters of the Great Australian Bight, off South Australia. Because the two and three year-old SBT were the majority of this school, the parameters for the predictive model were based on these two age classes.

### 6.2.2.2 Modelling the Feeding (for the juvenile SBT) Parameter

Because the predictive model, Equation (6-1), will be developed on the basis of SBT fillets, the feeding parameter, \( F' \) (kg baitfish.kg\(^{-1}\) SBT fillet.day\(^{-1}\)), was consequently quantified with Equation (6-3):

\[
F' = \frac{\text{total mass of baitfish fed}}{\text{total biomass of fillet in seacage} \times \text{days of farming}}
\]  
(6-3)

### 6.2.2.3 Biomass of Fillet in a Sea-cage

The total biomass of fillet in a sea-cage was obtained with Equations (6-4) and (6-5):

\[
\text{Total biomass of fillet} = n_{i,j}^L \times W_r
\]  
(6-4)
\[ n_{ij}^L = n_{i,j-1}^T - n_{i,j}^H - n_{i,j}^M \] (6-5)

Where the term \( n_{i,j}^L \) represents the number of live (L) SBT with age \( i \), at farming interval, \( j \), after accounting for mortalities (M) and harvested (H) SBT numbers and, \( W_f \) represents the population mean fillet weight (kg).

### 6.2.2.4 Population Mean Fillet Weight

The population mean fillet weight, \( W_f \) was quantified with Equations (6-6 through 6-7) and (6-9). Note that Equation (6-7) is the same as Equation (4-4) with the term \( W_{gg} \) expressed on the right-hand-side of the Equation:

\[ W_f = W_{gg} \times \text{edible fillet (\%)} \] (6-6)

\[ W_{gg} = W_{SBT} \times 0.87 \] (6-7)

Where \( W_{gg} \) represents the population mean gilled and gutted weight of an SBT. A working assumption derived from the industry is that the gills, guts and blood contribute 13% of the whole weight of a SBT. The term \( W_{SBT} \) represents the population mean whole weight of a SBT derived from the sample harvest data using the spline modelling technique (Venables and Ripley, 1999) in the R statistical package.

### 6.2.2.5 Modelling The Population Mean Whole Weight, Fork Length And Condition Index

Harvest data (see footnote 11) was used to determine the population mean whole weight and fork length of specific age classes of SBT. Using the `spline()` function in the R package, the population mean whole weight and fork length was modelled.

With the population mean whole weight and fork length of SBT specific to an age class and sea-cage, the population mean CI can be determined with Equation (4-3). Using the population mean CI, the population mean lipid content for a specific age class and for that
sea-cage was determined. It is noteworthy that the lipid content data is available only for SBT \((n = 35\) for a typical farming period) that were analysed for PCBs and PCDD/Fs. Therefore to determine the population mean lipid content, values obtained for population mean CI was substituted into Equation (6-8), obtained from the linear model shown in Figure 6.2, with a percent variance accounted for \((\% V)\) of 84.1 \% \(V\), with standard errors of 0.45 and 0.03 associated with the condition index \((\text{kg.m}^{-3})\) and lipid content \((\%)\) respectively of the harvest data (see footnote 11). The slope of Equation (6-8) was significant.

\[
\text{Condition index} = 0.416 \text{ (Lipid \%)} + 16.5 \quad (6-8)
\]

Consequently, the population mean edible fillet \(\text{(%)}\) specific to each age class and sea-cage was obtained with the following linear model calculated by Equation (6-9), obtained from Figure 6.3, with a percent variance accounted for \((\% V)\) of 68 \% \(V\), with standard errors of 0.72 and 0.05 associated with the edible fillet \(\text{(%)}\) and lipid content \(\text{(%)}\) respectively of the harvest data (see footnote 11). The slope of Equation (6-9) was significant.

\[
\text{Edible fillet (\%)} = 0.422 \text{ (Lipid \%)} + 52.4 \quad (6-9)
\]

6.2.2.6 Growth rate of Juvenile SBT

The growth rate, \(\gamma\), for farmed juvenile SBT was expressed in terms of the population mean fillet weight and is given by Equation (6-10):

\[
\gamma = \frac{\ln \frac{W_{t_2}}{W_{t_1}}}{t_2 - t_1} \quad (6-10)
\]
### Table 6-1  Corresponding number of days for each interval within a typical farming period.

<table>
<thead>
<tr>
<th>Term used</th>
<th>Farm Delta Fishing Pty Ltd (2005)</th>
<th>Farm Alpha Fishing Pty Ltd (2006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval 1</td>
<td>0 – 55</td>
<td>0 – 37</td>
</tr>
<tr>
<td>Interval 2</td>
<td>56 – 97</td>
<td>37 – 107</td>
</tr>
<tr>
<td>Interval 3</td>
<td>98 – 139</td>
<td>NA*</td>
</tr>
</tbody>
</table>

* Not Applicable

#### 6.2.3 Modelling Package

The model presented as Equation (6-1) was coded using the R statistical package, version 2.4.0 (R Development Core Team, 2006). Step-wise non-linear regression was applied to the data in order to determine the model parameters $\alpha$ and $C_0$ specific to the fillets of farmed SBT.

#### 6.2.4 Data Set for Model Validation

The model developed with the data presented in Chapter 5 from Farm Delta Fishing Pty Ltd, was consequently validated with data obtained from another SBT farm, Farm Alpha Fishing Pty Ltd. It was intended to validate the model with a different data set (but with other parameters (e.g. feeding pattern, kept constant) obtained from Farm Delta Fishing Pty Ltd. However due to unforeseen circumstances just prior to the 2006 typical farming period whereby the validation data set was to be obtained, Farm Delta Fishing Pty Ltd was not able to provide the validation data set and therefore an alternative plan was made with Farm Alpha Fishing Pty Ltd.

For the validation work, similar procedures to obtain model parameters for growth rates, population mean whole weights, fork length, condition index, edible fillet weight, biomass of SBT fillets and feeding parameter were carried out according to methods described in section 6.2.2.
Figure 6.2  Condition index and the corresponding lipid content (%) in the fillet for the sampled SBT ($n = 35$).

Figure 6.3  Edible fillet (%) and the corresponding lipid content (%) in the fillet for the sampled SBT ($n = 33$).
6.3 RESULTS AND DISCUSSION

6.3.1 Spline Modelling to Obtain the Biomass of SBT Fillet in a Sea-cage

Because SBT are a niche commodity and command a high price in the markets, sample sizes dedicated for scientific experiments are small. The mean measurement values, i.e. whole weight and fork length, obtained from a small sample size of five SBT harvested at t = 0, 55, 97 and 139 days from a sea-cage may not be representative of the actual population mean values from that sea-cage. In order to provide better estimates of the population mean values, the spline modelling technique (Venables and Ripley, 1999) in the R statistical package, together with total harvest data (see footnote 11) were applied.

Figures 6.4 and 6.5 present the predictions for both the population mean whole weight and population mean fork length for, respectively, the two year-old and three year-old SBT obtained from the pink tag sea-cage from Farm Delta Fishing Pty Ltd, versus time of farming modelled with the spline technique in the R package. It is observed from Figure 6.4 that at t = 55 days, there was only a single two year-old SBT that was harvested from the pink tag sea-cage. There was limited scope for selection at harvest, of farmed SBT in the preferred age class. This is an inevitable consequence of working alongside commercial harvests where selectivity of SBT is not practical due to time and logistical constraints.

It is also clear from Figures 6.4A and 6.5A that predicted weight increment for the two year-old SBT is different from the three year-old SBT. A change in slope can be observed for the data from t = 100 days for the three year-old SBT which is absent in the predicted trend for the two year-old SBT. Fork length predictions for both age classes as presented in Figures 6.4B and 6.5B, however, had similar trends and increased pseudo-linearly.

The predictions of whole weight and fork length for the two and three year-old SBT consequently resulted in population mean predictions for CI and edible fillet (%). Tables 6-2 and 6-3 present the results from Equations (6-8) and (6-9) respectively. It is observed from Tables 6-2 and 6-3 at t = 139 days, that the plateau predictions for the three year-old
SBT resulted in a lower population mean CI, edible fillet (%) and lipid content (%) for this age class compared to the two year-old SBT.

Table 6-2. Results of the relationship between population mean CI and lipid content for both the two year-old and three-year old SBT in the pink tag sea-cage.

<table>
<thead>
<tr>
<th>No. of Days of Farming</th>
<th>Two year-old SBT</th>
<th>Three year-old SBT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population Mean CI (kg.m(^{-3}))</td>
<td>Population Mean Lipid Content (%)</td>
</tr>
<tr>
<td>0</td>
<td>18.55</td>
<td>4.82</td>
</tr>
<tr>
<td>55</td>
<td>21.15</td>
<td>11.08</td>
</tr>
<tr>
<td>97</td>
<td>23.31</td>
<td>16.27</td>
</tr>
<tr>
<td>139</td>
<td>25.96</td>
<td>22.64</td>
</tr>
</tbody>
</table>

Table 6-3. Results of the relationship between population mean edible fillet (%) and fillet weight for both the two year-old and three-year old SBT in the pink tag sea-cage.

<table>
<thead>
<tr>
<th>No. of Days of Farming</th>
<th>Two year-old SBT</th>
<th>Three year-old SBT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population Mean Edible Fillet (%)</td>
<td>Population Mean Fillet Weight (kg)</td>
</tr>
<tr>
<td>0</td>
<td>54.46</td>
<td>5.70</td>
</tr>
<tr>
<td>55</td>
<td>57.10</td>
<td>7.41</td>
</tr>
<tr>
<td>97</td>
<td>59.30</td>
<td>9.07</td>
</tr>
<tr>
<td>139</td>
<td>61.98</td>
<td>11.18</td>
</tr>
</tbody>
</table>
Figure 6.4A  Whole weight of two year-old SBT versus time of farming modelled with the spline function in the R package. This technique permits the determination of the population mean whole weight at a particular time of farming.

Figure 6.4B  Fork length of two year-old SBT versus time of farming modelled with the spline function in the R package. This technique permits the determination of the population mean fork length at a particular time of farming.
Figure 6.5A  Whole weight of three year-old SBT versus time of farming modelled with the spline function in the R package. This technique permits the determination of the population mean whole weight at a particular time of farming.

Figure 6.5B  Fork length of three year-old SBT versus time of farming modelled with the spline function in the R package. This technique permits the determination of the population mean fork length at a particular time of farming.
6.3.2 Baitfish as Feed, Feeding and Growth Rates

Figure 6.6 presents mass of baitfish fed to SBT in the pink tag sea-cage, on a daily basis, for the typical farming period of Farm Delta Fishing Pty Ltd. It is clear that for this field study conducted with and on a commercial farm, day-to-day variability in the mass of baitfish fed to SBT was observed. In addition, there were 21 days where no feeding occurred due to (i) day-off for the farmers and deck-hands employed – usually on the weekends, (ii) weather conditions out at sea, (iii) engine problems with the boat specifically equipped for feeding sorties, and (iv) the day prior to harvest (see Appendix J for Event Log for Farm Delta Fishing Pty Ltd).

Because farmed SBT were harvested only at the three intervals, namely at t = 55, 97 and 139 days, sea-cage specific mean feeding rates were used for these three intervals. It is noteworthy that the sea-cage specific mean mass of baitfish changed through time of farming and therefore the feeding rate also changed through time of farming. Applying Equation (6-3), the resultant feeding rate for the pink tag sea-cage is presented as Figure 6.7A.

Figure 6.7A shows the three mean feeding rates specific to the pink tag sea-cage. It is observed that the feeding rate decreased with time of farming. The typical farming period for Farm Delta Fishing Pty Ltd occurred in the Austral-autumn months (March to May) through to the Austral-winter months (June to August). The feeding during Interval 3 occurred at a rate approximately one-third that of Interval 1, where water temperatures (Appendix K) were higher – also a likely consequence of declining water temperatures, throughout the typical farming period.

Figure 6.7B shows the growth rate based on SBT fillets. It is observed that the growth of the three year-old SBT (fillets) follows a similar trend to the feeding rate, whereas a slight increase is observed for the two year-old SBT (fillets). The difference in growth rate between the two and three year-old SBT (fillets) suggest that even between juvenile SBT, there are differences in the biological systems that effect growth. Also, the upward trend versus the plateau trend for the whole weights as shown in Figure 6.4A and 6.5A for, respectively, the two and three year-old SBT contributed to the different observations in growth rates.
Figure 6.6  Total mass of baitfish fed (as the sum of the masses of the individual baitfish types) on a daily basis to farmed SBT in the pink tag sea-cage of Farm Delta Fishing Pty Ltd, for a typical farming period. It is observed that there was the day-to-day variability in the mass of baitfish fed and also observed were 21 days where no feeding of SBT was carried out.
Figure 6-7  Plots for the (A) feeding rate based on SBT fillets obtained from the pink tag sea-cage of Farm Delta Fishing Pty Ltd and, (B) growth rates for two and three year-old SBT (fillets) obtained from the pink tag sea-cage of Farm Delta Fishing Pty Ltd; (C) feeding rate based on SBT fillets obtained from Farm Alpha Fishing Pty Ltd and, (D) growth rates for two and three year-old SBT (fillets) obtained from Farm Alpha Fishing Pty Ltd.
Figures 6.7C and 6.7D present the feeding and growth rates for the farmed SBT obtained from Farm Alpha Fishing Pty Ltd. These figures are presented alongside Figures 6.7A and 6.7B and discussed here to indicate similarities although the SBT from Farm Alpha Fishing Pty Ltd was caught a year later, i.e. in 2006.

Similar to Farm Delta Fishing Pty Ltd, feeding rates decreased with time of farming due to the decreased water temperatures – SBT feed less during the colder months, as shown in Figure 6.7C. The trends in growth rates for the two and three year-old SBT farmed by Farm Alpha Pty Ltd appear similar to the growth rates of the two and three year-old SBT farmed by Farm Delta Pty Ltd presented in Figure 6.7D. Figure 6.7D may indicate that the trends observed for the two and three year-old SBT are not random but may be attributed to a biological response specific to an age class of SBT.

### 6.3.2.1 Non-steady State Conditions Due to Variable Feeding

It is noteworthy that steady state conditions were not achieved during the typical farming period or during the LTH farming period (see the residue data presented in Chapter 5). Variable feeding (i.e. changing of diet treatments at each of the three intervals) employed by Farm Delta Fishing Pty Ltd (and other SBT farms, *pers. comm.* Dr Robert van Barnveld, Consultant, Barnveld Nutrition) together with the day-to-day variation in the total mass of baitfish fed (see Figure 6.6 and discussion above) could be the principal reason for the non-steady state conditions.

In 1995, a mass mortality of the Australian sardines occurred and may have been attributed to a marine viral outbreak of the herpesvirus (Fletcher et al., 1997; Griffin et al., 1997; Hyatt et al., 1997; Whittington et al., 1997). Again in 1998, a second mass mortality of the Australian sardines occurred. Because of these two historical incidents, farm management are aware of the risks involved if farmed SBT were fed on a single baitfish diet, e.g. Australian sardines diet. Consequently, variable feeding as a mixture of baitfish to farmed SBT was practised.

Communication with SBT farmers and managers indicated that from their experience, SBT can “get bored with a fixed diet” (*pers. comm.* David Warland, SBT Farm Manager) and require a mix of baitfish to “make the fish happy” (*pers. comm.* David Warland) and
to achieve a high fat content (of approximately 0.2% at the start to approximately 20% at the end) for a typical and short farming period. In addition, it is known throughout the SBT industry (pers. comm. Dr Robert van Barneveld, Consultant, Barneveld Nutrition; pers. comm. David Warland) and from previous nutritional work carried out on farmed SBT (Carter et al., 1998), that SBT are selective in the types of feed fed. The established facts within the SBT industry described above also contribute to the use of variable feeding technique.

The availability of baitfish and market price of baitfish are other contributing factors to variable feeding. For example, SBT management would want the maximum financial returns for the least costs for baitfish fed. The financial returns from farmed SBT are consequently dependent on the fluctuating Japanese yen. These decision-making processes form a cycle indicating the rapid change of feeding strategies employed by SBT farms.

6.3.3 Investigation into Pooling of Data

Figure 6.9 shows the box plot comparison for the SBT harvested from the pink and green tag sea-cages at each of the three intervals for the typical farming period. Welch’s t-test and the box plots were employed to investigate if the PCB concentration data for the two sea-cages could be pooled due to the small sample sizes.

It is clear from the box plot for Interval 2 that the PCB concentration data determined in SBT fillets was significantly different (P = 0.010) between the two sea-cages. Results from the Welch t-test indicated that the PCB concentration data was not significantly different for Intervals 1 (P = 0.068) and 3 (P = 0.051). It is noted that sample sizes are small to provide an accurate statistical comparison, however it is clear from the box plot for Interval 2 that overall, the PCB concentration data from the two sea-cages cannot be pooled for modelling.

6.3.4 The Modified LCBK Predictive Model – Congener Basis Model

Modelling is often regarded as an effort to understand or design a complex system, and that attention must be paid to a variety of other concerns ranging from experimental
design to budget. A mathematical model evaluated analytically is only an approximation to the complex nature of real-world systems. Models are not universally valid, but are designed for specific purposes (Law and Kelton, 1991). For this research, specific purposes included obtaining PCB and PCDD/F assimilation efficiencies for farmed SBT and, provide SBT management with a tool to predict SBT levels after a certain time of farming.

Because this research is based on a food safety point of view, a conservative approach was therefore taken – although data sets from both the pink and green tag sea-cages were obtained, further studies (e.g. sensitivity analyses) will be done on the data set that exhibits the higher assimilation efficiencies.

Table 6-4 presents the PCB congener-specific nett assimilation efficiencies, \( \alpha \), and the regressed \( C_0 \) values from the predictive model with the data obtained from Farm Delta Fishing Pty Ltd. Also shown (in brackets) is the range of \( \alpha \) and \( C_0 \) for each congener for the 95 % confidence level. For the 12-WHO-PCB congeners, \( \alpha \) ranged 19.1 – 46.3 % in the fillets of SBT from the pink tag sea-cage, and 15.2 – 93.4 % in the fillets of SBT from the green tag sea-cage. The data indicates that \( \alpha \) vary with PCB congeners.

Several researchers have reported that \( \alpha \) vary with PCB congeners. Gruger et al. (1976) studied the \( \alpha \) of PCBs 101 and 153 in whole Coho salmon, and reported that for a fixed concentration of 1 \( \mu \text{g g}^{-1} \) of both PCBs 101 and PCBs 153 in the food pellets, \( \alpha \) of 73 % was obtained for PCB 101 while \( \alpha \) of 81 % was obtained for PCB 153. Fisk et al. (1998) studied the dietary accumulation of 16 PCB congeners in whole juvenile rainbow trout and reported that \( \alpha \) for these 16 congeners ranged 21 – 75 %. Isosaari et al. (2002) investigated the \( \alpha \) of 16 PCB congeners in the fillets of rainbow trout fed with herring (as baitfish) and concluded that the \( \alpha \) for these 16 congeners ranged 19 – 55 %. Of the 16 congeners investigated by Isosaari et al. (2002), six were WHO-PCB congeners, namely, PCBs 77, 105, 118, 126, 157 and 169, with \( \alpha \) in the range of 40 – 54 % (except for PCB 169). It is noteworthy that \( \alpha \) was not available for PCB 169 because this congener was not detected in the herring fed to rainbow trout.

In this research, it is clear that the assimilation efficiencies for all WHO-PCB congeners (except PCB 169) and the dioxin congener 2,3,7,8-TeCDF obtained from farmed SBT of
the pink tag sea-cage appear to be higher than the green tag sea-cage. This implies that there is a greater uptake of PCBs and the congener 2,3,7,8-TeCDF in farmed SBT of the pink tag sea-cage.

The lowest and highest assimilation efficiencies came from PCBs 123 and 169, respectively, for both sea-cages. No clear trend was observed for the mono-ortho PCB congeners. However, within the non-ortho PCB group, assimilation efficiencies increased with increasing chlorination (PCBs 77 and 81 – tetra-chlorination, PCB 126 – penta-chlorination and PCB 169, hexa-chlorination). This is in contrast to the studies of Isosaari et al. (2002) and Berntssen et al. (2007) where PCBs 77 and 81 had slightly higher assimilation efficiencies than PCBs 126 and 169 in rainbow trout and farmed Atlantic salmon respectively. The difference in this assimilation ranking for the non-ortho PCB congeners may be fish species-specific, where the digestibility of feed is consequently dependent on fish species (Fisk et al., 1998). It is noteworthy to contrast that the tuna species are warm-blooded fish (Carey, 1973; Graham, 1975) while the rainbow trout and salmon species are cold-blooded fish. The temperature of blood plays a major role in determining the effectiveness of digestive enzymes (Smith, 1980) and therefore this unique fish physiology characteristic may be the pivotal factor attributed to differences observed between other fish species and the tuna species.
Figure 6.9  Box and whisker plots for the green and pink tagged SBT for each of the three intervals (Interval 1 – 0 to 55 days, Interval 2 – 56 to 97 days, Interval 3 – 98 to 139 days). Concentration is presented on a fresh weight basis.
Table 6.4. Predicted assimilation efficiencies ($\alpha$) and the respectively initial concentration ($C_0$) for the 12 WHO-TEF PCB congeners found in fillets of Australian farmed SBT.

<table>
<thead>
<tr>
<th>PCB Number</th>
<th>Pink Tag Sea-cage</th>
<th>Green Tag Sea-cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha$ (95% CL range)</td>
<td>$C_0$ (95% CL range)</td>
</tr>
<tr>
<td><strong>Non-ortho PCBs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>20.9 (18.3 – 23.5)</td>
<td>1.43 (0 – 2.94)*</td>
</tr>
<tr>
<td>81</td>
<td>24.9 (21.9 – 28.0)</td>
<td>0.733 (0.248 – 1.22)</td>
</tr>
<tr>
<td>126</td>
<td>35.3 (30.4 – 40.3)</td>
<td>0.866 (0.167 – 1.56)</td>
</tr>
<tr>
<td>169</td>
<td>46.3 (37.0 – 55.6)</td>
<td>0.938 (0.683 – 1.19)</td>
</tr>
<tr>
<td><strong>Mono-ortho PCBs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>27.0 (24.1 – 30.0)</td>
<td>18.4 (0 – 36.9)*</td>
</tr>
<tr>
<td>114</td>
<td>24.0 (21.4 – 26.6)</td>
<td>0.979 (0 – 1.99)*</td>
</tr>
<tr>
<td>118</td>
<td>25.4 (22.5 – 28.2)</td>
<td>56.9 (3.12 – 110)</td>
</tr>
<tr>
<td>123</td>
<td>19.1 (16.6 – 21.6)</td>
<td>2.96 (1.24 – 4.69)</td>
</tr>
<tr>
<td>156</td>
<td>25.8 (23.1 – 28.5)</td>
<td>9.00 (4.19 – 13.8)</td>
</tr>
<tr>
<td>157</td>
<td>24.8 (22.3 – 27.4)</td>
<td>2.18 (0.923 – 3.43)</td>
</tr>
<tr>
<td>167</td>
<td>35.3 (31.2 – 39.4)</td>
<td>7.97 (3.74 – 12.2)</td>
</tr>
<tr>
<td>189</td>
<td>23.1 (20.2 – 26.0)</td>
<td>1.24 (0.310 – 1.67)</td>
</tr>
</tbody>
</table>

* Lower values have been truncated at zero if regression returns $C_0$ as a negative value.
Table 6-5 presents the assimilation efficiency, $\alpha$, and the regressed $C_0$ value for the dioxin congener 2,3,7,8-TeCDF from the predictive model. Only one dioxin congener was modelled because 2,3,7,8-TeCDF was the only congener detected in baitfish feed above the 2,3,7,8-TeCDF blank threshold concentration. A nett assimilation efficiency of 39.2% for 2,3,7,8-TeCDF was determined in the fillets of farmed SBT from the pink tag sea-cage and 20.6% in the fillets of farmed SBT from the green tag sea-cage.

It is clear from Tables 6-4 and 6-5 that the assimilation efficiency of 2,3,7,8-TeCDF in farmed SBT fillets obtained from both the pink tag sea-cage is higher than all the WHO-PCB congeners, except for PCB 169. With the exception of PCB 169, Berntssen et al. (2007) reported a similar finding that 2,3,7,8-TeCDF was found to have higher assimilation efficiency compared to all 12 of the WHO-PCB congeners. It is unclear as to why the assimilation efficiency of the congener 2,3,7,8-TeCDF in farmed SBT fillets from the green tag sea-cage did not exhibit a similar trend. A possible explanation may be that the SBT harvested from the green tag sea-cage may not have been representative of the calculated population mean feeding rate, leading to an underestimate of the actual assimilation efficiency for the congener 2,3,7,8-TeCDF.

The congener PCB 169 was detected only in the Australian sardines. However to account for a ‘worst case scenario’, concentrations at the limit of detection (LOD) for PCB 169 were used for the other baitfish types (and it was ensured that the LOD concentrations were above the blank threshold concentration for PCB 169). This may explain the high assimilation efficiency for PCB 169 over other PCB congeners in farmed SBT of both sea-cages, and the performance of the model for PCB 169 (Figures 6.10D and 6-13D).

Figures 6.10 and 6.11 present the model predictions for selected WHO-PCB congeners and selected indicator PCB congeners, respectively, in the fillets of farmed SBT from the pink tag sea-cage. Overall figures 6.10 and 6.11 suggest that the model fitted the data well except for one SBT harvested at $t = 55$ days. Further investigation revealed that this SBT was infected with a parasite *Kudoa* and therefore was not representative of the population in the pink tag sea-cage.
Predictive Modelling of PCBs and PCDD/F Levels in Farmed SBT Fillets

Table 6.5. Predicted assimilation efficiency ($\alpha$) and the initial concentration ($C_0$) for the dioxin congener 2,3,7,8-TeCDF in fillets of Australian farmed SBT. This congener is the only dioxin congener found above the blank threshold concentration in baitfish.

<table>
<thead>
<tr>
<th>Dioxin</th>
<th>Pink Tag Sea-cage</th>
<th>Green Tag Sea-cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha$ (95% CL range)</td>
<td>$C_0$ (95% CL range)</td>
</tr>
<tr>
<td><strong>PCDF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,7,8-TeCDF</td>
<td>39.2 (31.1 – 48.6)</td>
<td>0.0718 (0.0295 – 0.111)</td>
</tr>
</tbody>
</table>

* Lower values have been truncated at zero if regression returns $C_0$ as a negative value.
Predictive Modelling of PCBs and PCDD/F Levels in Farmed SBT Fillets

Figure 6.10 Model predictions (solid line) for (A) PCB 77 (B) PCB 81 (C) PCB 126 (D) PCB 169 (E) PCB 157 and (F) PCB 189 in the fillets of Australian farmed SBT from the development data set. Dotted lines represent the upper and lower prediction boundaries determined from the degrees of freedom and residual standard error of the model. Concentration is presented on a fresh weight basis.
Figure 6.11  Model predictions (solid line) for (A) PCB 3 (B) PCB 28 (C) PCB 138 (D) PCB 153 (E) PCB 208 and (F) PCB 209 in the fillets of Australian farmed SBT from the development data set. Dotted lines represent the upper and lower prediction boundaries determined from the degrees of freedom and residual standard error of the model. Concentration is presented on a fresh weight basis.
As assimilation efficiencies of PCBs and 2,3,7,8-TeCDF in SBT fillets (and in the tuna, *Thunnus* species) are quantified for the first time, a comparison of values obtained in this work and the literature can only be done against those obtained for different fish species.

Isosaari et al. (2002) reported nett assimilation efficiencies in the range of 40 – 48 %, for PCB congeners 77, 105, 118 and 126 in (fillets of) rainbow trout (*Oncorhynchus mykiss*) fed with herring. For the dioxin congener 2,3,7,8-TeCDF, an assimilation efficiency of 40 % was obtained. Assimilation efficiencies obtained for the same PCB congeners in SBT fillets were lower than those in the rainbow trout fillets. This may be attributed to the different feeding rates employed, fish species studied and the model used to determine the assimilation efficiencies. However for 2,3,7,8-TeCDF, similar values were observed.

The recent work by Berntssen et al. (2007) covered all the 12 WHO-PCB congeners and 17 WHO-PCDD/F congeners detected in farmed Atlantic salmon (*Salmo salar*) and feed used. Assimilation efficiencies for the 12 WHO-PCBs ranged 73-83 % and a value of 88 % was obtained for 2,3,7,8-TeCDF in whole farmed Atlantic salmon. These assimilation efficiencies in farmed Atlantic salmon were approximately three times higher than assimilation efficiencies found in farmed SBT. The difference in assimilation efficiencies may be attributed to (i) the sampling method – for the work of Berntssen et al. (2007), composite sample was based on whole salmon whereas for this work, a composite sample was based on the fillet obtained from one half of an SBT, (ii) fish and farm husbandry practices, and (iii) the most important and likely contributing factor, fish species.

It is well-known that SBT are large fish with a sturdy bony structure (teleosts). If equipment capability permitted and whole SBT (with bones, body frame, etc.) were blended to make a composite sample, higher assimilation efficiencies would be expected. This may be due to the accounting of assimilation efficiencies in the organs of whole SBT.

Figure 6.12 highlights that the residuals (as predicted value versus observed value) of the predictive model for (A) the four non-ortho PCBs (77, 81, 126 and 169) and the two mono-ortho PCBs (157 and 189), (B) the lowest and highest chlorinated PCBs (3, 208, and 209), (C) the two most abundant PCBs (138 and 153) and PCB 28, in the fillets of Australian farmed SBT. The residuals appear uniformly distributed about the line of
symmetry. The predictive model therefore appears neither under- nor over-parameterised for the data obtained from Farm Delta Fishing Pty Ltd.

It was determined in Chapter 5 that as time of farming increased for a typical farming season, the concentration of PCBs and PCDD/Fs increased. Figure 6.12 revealed that the data tended to exhibit a fanning-out effect with increased concentration. This implied that as the concentration in farmed SBT increased, the variability in the data obtained increased. It is inferred from this finding that increased biological variability in farmed SBT may be a function of time of farming and attenuated at higher concentrations.

The mechanistic model is advantageous because it facilitates ease of use (by the management of SBT farms) and provides the interpretation of individual model parameters at the biological level. For example, the assimilation efficiency of approximately 35.3 % predicted for PCB 126 in the fillet of SBT indicated that 35.3 % of this PCB congener in the baitfish as feed has been deposited in the fillet of that SBT, while the remaining 64.7 % may be attributed to complex biological processes such as faecal elimination or passive diffusion and deposition in the organs of SBT, or a combination of these.

### 6.3.5 Sensitivity Analysis

One of the most useful tools in modelling is sensitivity analysis. This can be employed to determine if the simulation output changes significantly when the value of an input parameter is changed. Overall, if the output is sensitive to some aspect of the model, then that aspect must be modelled carefully. By showing how the model behavior responds to changes in parameter values, sensitivity analysis is a useful tool in model building as well as in model evaluation (Breierova and Choudhari, 2001).

In this work, the farming process, live SBT and behavior of PCBs and PCDD/Fs represents a dynamic system. Breierova and Choudhari (2001) highlighted that in reality, the parameters used in the model of a dynamic system represent quantities that are very difficult for an exact replication. Where the model is determined to be insensitive to
changes in parameter values, it may be therefore possible to use an estimate rather than a value with greater precision.

A sensitivity analysis (Appendix O) was carried out on the predictive model and findings indicated that the model was insensitive to the growth rate parameter, but sensitive to the feeding parameter, which in turn was dependent on the mass of baitfish fed to SBT and sensitive also to the concentration in the baitfish. Varying the growth rate parameter by up to 20% resulted in an overall decrease by only approximately 5% in final PCB and PCDD/F concentrations in SBT fillets (at t = 139 days). However, varying the mass of baitfish fed and the concentration in the baitfish by up to 20% resulted in an approximate 19% increase in the final PCB and PCDD/F concentrations in SBT fillets (at t = 139 days). Oppenhuizen and Schrap (1988) and Chapman and Reiss (1999) highlighted that the resultant chemical concentration in a fish with fish-specific assimilation efficiency as a limiting factor, is dependent on the concentration in the food (baitfish).

The sensitivity analysis revealed that because the model was insensitive to the growth rate, an estimate of the growth rate may be used instead. Since the (fillet corrected) growth rate was calculated from the weight of SBT fillets, it is therefore implied that an estimated weight of SBT may be sufficient. This finding may be of interest to farmers who expressed the difficulty of measuring precise weights of SBT whilst (harvesting SBT) out at sea.

Because the model was sensitive to the amount of baitfish fed (consequently the feeding rate) and the concentration in baitfish, it implied that these input parameters require greater precision of measurement.

### 6.3.6 Model Validation Work

Validation is concerned with determining whether the developed model or derived model parameter(s) is an accurate representation of the system under study. However, Law and Kelton (1991) stresses, “there is no such thing as an absolute valid model”. This is because a model is an approximation of a real-world system and this system is subject to biological variation. For example, one of the factors in biological variation was the year-
to-year variability in fork length of SBT, which consequently determined their age class. For more detail on year-to-year variability in fork length, see Gunn et al. (1996), Leigh and Hearn (2002) and Farley et al. (2007).

Figures 6.13 and 6.14 present the model predictions for selected WHO-PCB congeners and selected indicator PCB congeners in SBT fillets obtained from Farm Alpha Fishing Pty Ltd. It was observed that the model over-predicted for most of the PCBs studied except the lowest chlorinated (PCB 3) and highest chlorinated congeners (PCBs 208 and 209).

The over-prediction of the model at \( t = 37 \) and 107 days may be attributed to the varying husbandry practices employed. For example, for Farm Delta Fishing Pty Ltd, at the start of the time interval \( t = 97 \) days, it was observed that the mass of baitfish fed decreased by approximately 40 % relative to the start of the farming period, as the population in the sea-cage decreased to approximately 93 % of the original population, as a consequence of harvesting and mortalities. However for Farm Alpha Fishing Pty Ltd, at approximately the same time interval (\( t = 107 \) days), the mass of baitfish fed increased by approximately 40 % relative to the start, while the population decreased to approximately 98 % of the original population. The increase in the mass of baitfish fed for Farm Alpha Fishing Pty Ltd may be due to the farmers trying to fatten the fish in a shorter period of time in order to meet market demands.

According to Equations (6-1) and (6-3), if overfeeding occurs, the model would consequently over-predict. The model predictions as shown in Figures 6.13 and 6.14 together with an analysis of the mass of baitfish fed suggest that in reality, overfeeding may have occurred.

Another varying husbandry practice was that Farm Delta Fishing Pty Ltd stocked the sea-cage studied in this work with 221 SBT while Farm Alpha Fishing Pty Ltd had 1435 SBT in a sea-cage of similar size. The difference in stocking numbers may have an impact on the method of feeding. For example, for a sea-cage stocked with fewer SBT, farmers may easily observe when SBT are fed to satiation, whereas for a sea-cage of similar dimensions stocked with approximately seven times more SBT, observation for feeding to satiation may be more difficult, leading to preferential overfeeding of the SBT.
Figure 6.12  Plot of residuals (as observed value versus predicted value) for (A) the non-ortho PCBs (77, 81, 126 and 169) and the two mono-ortho PCBs (157 and 189), (B) the lowest and highest chlorinated PCBs (3, 208, and 209), (C) the two most abundant PCBs (138 and 153) and PCB 28, in the fillets of Australian farmed SBT.
Figure 6.13  Model predictions (solid line) for (A) PCB 77 (B) PCB 81 (C) PCB 126 (D) PCB 169 (E) PCB 157 and (F) PCB 189, in the fillets of Australian SBT from a different farm and farming year. Concentration is presented on a fresh weight basis.
Figure 6.14 Model predictions (solid line) for (A) PCB 3 (B) PCB 28 (C) PCB 138 (D) PCB 153 (E) PCB 208 and (F) PCB 209, in the fillets of Australian SBT from a different farm and farming year. Concentration is presented on a fresh weight basis.
6.3.6.1 Uncertainty in the Data for Model Validation

In order to determine the population mean whole weight and fork length at the start of the commercial field study, measurements from a total of only 69 SBT were obtained – as this was all the information available. This sample size represented 5% of the total number of SBT in the sea-cage. Consequently, the initial population mean fillet biomass in the Farm Alpha Pty Ltd sea-cage was determined based on derived parameters, i.e. condition index, lipid content (%) and edible fillet (%), using this sample size. It was noted that of the 69 SBT, 51% of the total were two year-old SBT with a weight range of 6 – 17 kg and the remaining 49% were three year-old SBT with a weight range of 18 – 25 kg (Appendix L).

It was established at the onset of this discussion section that there are different age classes in a school of wild-caught SBT. If the school of wild SBT caught by Farm Alpha Pty Ltd comprised of older SBT with greater mass, then the initial population mean fillet biomass would have been under-predicted.

Of the 10 SBT that were harvested at t = 37 days, it was found that five (not provided for residue research) of the 10 SBT had a weight range of 55 – 68 kg. It is therefore inferred that indeed there may have been a potential bias of heavier (and older) SBT that have not been accounted for in the initial sample size in determining the initial population mean fillet biomass – since there was no information available to account for the number of older and heavier SBT.

Further investigation into the possibility of the larger, heavier and older SBT not accounted for at the start of the commercial field study revealed that the larger and heavier SBT tend to swim at the lower section of the sea-cage whereas the smaller SBT, i.e. the two and three year-olds, tend to swim at the top section of the sea-cage (pers. comms. David Warland, SBT Farm Manager).

In order to better estimate the mean population whole weight and fork length for the final harvest t = 107 days and to compare the magnitude for over-prediction of the initial fillet biomass, weight and fork length measurements for all SBT, at all harvests as highlighted in Table 4-8, were requested from Farm Alpha Pty Ltd. However due to commercial in-
confidence, Farm Alpha Pty Ltd would not provide this information. Consequently, it was not possible to determine if the two and three year-old SBT were truly representative of the wild-caught school farmed by Farm Alpha Pty Ltd.

The uncertainty in the data for the fillet biomass discussed in this section may have been a major contributor to the resultant over-prediction of the model applied to the validation data set.

6.3.7 The Modified LCBK Predictive Model – TEQ Basis Model

Figures 6-15 presents the PCB TEQ model developed from the data obtained from Farm Delta Fishing Pty Ltd. In contrast to the Congener Basis Model, the concentrations for baitfish and SBT have been replaced with the calculated TEQ levels. The method to determine TEQ levels has been presented in Chapter 3. Figure 6-15 indicates that the model fits the data well. An assimilation efficiency of 30.1% was obtained for the PCB TEQ level in the fillets of SBT from the pink tag sea-cage.

Because there was only one PCDD/F congener detected in both baitfish and SBT, it was not necessary to build a PCDD/F TEQ model.

Figure 6-16 shows the model predictions on a TEQ basis for the validation data set obtained from Farm Alpha Pty Ltd. Similarly to the congener basis model, the TEQ basis model over-predicts the actual level for $t = 37$ and 107 days.

The predictive TEQ model has both an advantage and a shortcoming. The advantage of using a TEQ model is the convenience it may provide to SBT farm management. Presently, food regulatory bodies worldwide are interested in the TEQ levels determined in food products, because the congeners that sum to the TEQ have been identified as toxic congeners by the WHO. The TEQ model therefore serves as a quick method to predict the TEQ level(s) in farmed SBT to be exported.

The shortcoming is that the TEQ model does not have any physiological interpretation. The assimilation efficiency predicted by the TEQ model may be viewed as a weighed
average for the WHO-PCB congeners, i.e. only single assimilation efficiency was obtained for the 12 WHO-PCB congeners. If the model is to be extended beyond experimental conditions, the TEQ model should not be used since the pseudo assimilation efficiency does not have real meaning outside the experimental conditions.

Because most of the PCDD/F congeners in SBT were below the blank threshold concentrations, a true PCDD/F TEQ could not be ascertained. Therefore, a search was conducted to determine if a surrogate PCB congener may be used as an indicator to estimate a possible combined (PCB + PCDD/F) TEQ level.

Extensive analyses of the experimental field data for PCBs in SBT (fillets) revealed that the TEF concentration for the congener PCB 126 contributed to approximately 80% of the PCB TEQ. The literature surveyed indicated that PCB TEQ contributed to approximately 80% of the combined (PCB + PCDD/F) TEQ.

While PCB 126 may be adequate as an indicator to model TEQ in SBT specifically for the two locations of the SBT farms investigated in this research, caution should be exercised when using PCB 126 to model TEQ in SBT farmed at other locations. The influence of point sources, which may be present at other locations, may result in different overall findings compared with the findings quantified in this research.

To capitalise on the advantage and overcome the shortcoming of a TEQ model, it is proposed that the industry uses the congener model for PCB 126. The justification of this approach is that PCB 126 is the most toxic PCB congener (van den Berg et al., 1998), contributes to majority of the PCB TEQ and therefore to the total TEQ, and has been found to have the highest assimilation efficiency in farmed SBT fillets (except PCB 169 which was discussed previously) – inferring that the congener PCB 126 is preferentially taken up in SBT over other PCB congeners, and therefore can be treated as the worst-case scenario.

The industry could develop a database for PCB 126 in baitfish and together with easy-to-measure model inputs such as whole weight and fork length, predict the concentration of PCB 126 in farmed SBT. Consequently, the predicted PCB TEQ level and the total TEQ (combined PCB + PCDD/F) level can be calculated based on indicator factors of 0.8.
approach is an alternative to having single assimilation efficiency for a group of congeners, when it has been established that all (PCB) congeners behave differently (Chapter 5).

6.3.8 Congener Profile Study

Complementary to the predictive modelling work undertaken and presented in this chapter, a congener profile study was undertaken to determine if there were any observable differences in the feed profiles and profiles in the SBT (fillets).

The work presented in Chapter 5 revealed that all 12 of the WHO-PCB congeners and only three of the 17 PCDD/F congeners were detected in farmed SBT. As the major source of PCB and PCDD/F intake was from feed, baitfish concentrations were studied.

Baitfish concentrations in the baitfish types (i.e. US and Australian sardines, and Australian red bait) were corrected for threshold concentrations in the blanks. Investigations revealed that for the range of PCB congeners tested, all were detected above blank threshold concentrations with the exception of PCB 169 not detected in US sardines and Australian red bait. Of the 17 PCDD/F congeners, only 2,3,7,8-TeCDF was found above blank threshold concentrations in (only) US sardines.

Because only 2,3,7,8-TeCDF was found in the feed (and furthermore detected only in US sardines), congener profiling was done based on the PCBs. Figure 6.17 shows the averaged ($n = 3$ for each baitfish type) congener profiles of the US and Australian sardines and, Australian red bait over a typical farming period. Profiles for each of the baitfish samples are found in Appendix E. Overall, all three profiles for the three baitfish types appeared similar. However an observable difference exists between the US sardines and the Australian sardines and red bait – for the US sardines, the PCB congener dominating the profile was PCB 138, whereas PCB 153 dominated the profiles of the Australian sardines and red bait. This finding suggested that geographical regions may be the contribution factor to different profiles.

As mentioned previously, PCB 169 was detected only in Australian sardines. A further investigation into the geographical locations where the Australian sardines and Australian
red bait were caught revealed that the Australian sardines were caught in the waters off New South Wales, whereas the Australian red bait was caught in the waters off Tasmania. The finding of detectable concentrations of PCB 169 in the Southern Ocean suggested that even within specific sites of capture of the baitfish, slightly different PCB congener profile through the presence (or absence) of a specific congener could be observed.

Finally, although the US sardines had higher magnitudes of PCBs, the profiles of farmed SBT as shown in Figure 6-18 represented the profiles of the Australian sardines and red bait. This is likely because of the mass of baitfish fed – the Australian sardines and red bait comprised approximately 70% of the total mass of baitfish fed for the pink tag sea-cage and approximately 60% of the total mass of baitfish fed for the green tag sea-cage.
Figure 6.15  Model predictions (solid line) for the PCB TEQ level in the fillets of Australian farmed SBT from the development data set. Dotted lines represent the upper and lower prediction boundaries determined from the degrees of freedom and residual standard error of the model. Concentration is presented on a fresh weight basis.

Figure 6.16  Model predictions (solid line) for the PCB TEQ level in the fillets of Australian farmed SBT from the validation data set. Concentration is presented on a fresh weight basis.
Figure 6.17  Averaged PCB congener profiles for (A) US sardines (n = 3), (B) Australian sardines (n = 3) and (C) Australian (Tasmanian) red bait (n = 3) obtained from Farm Delta Fishing Pty Ltd. Congener bars in red represent the WHO-PCB congeners and bars in blue represent the indicator PCB congeners. Concentration is presented on a fresh weight basis.
Figure 6.18  Averaged PCB congener profiles for (A) wild-caught SBT (fillets, n = 5), (B) farmed SBT (fillets, n = 5) harvested at t = 139 days from the pink tag sea-cage of Farm Delta Fishing Pty Ltd, and (C) farmed SBT (fillets, n = 5) harvested at t = 139 days from the green tag sea-cage of Farm Delta Fishing Pty Ltd. Congener bars in red represent the WHO-PCB congeners and bars in blue represent the indicator PCB congeners. Concentration is presented on a fresh weight basis.
6.4 LIMITATIONS ON THIS WORK

1. The day-to-day variability in the mass of baitfish fed has been highlighted in this research. The field studies conducted in this research is in contrast with published experimental methods (Muir et al., 1988; Gobas et al., 1989; Niimi and Dookhran, 1989; Gobas and Schrap, 1990) that can be conducted in a controlled environment where fish may be fed on a routine schedule, the mass of feed may be kept relatively constant and where weather conditions do not affect the feeding process.

2. Early nutritional work carried out by the SBT industry (Carter et al., 1998) highlighted that farmed SBT preferred baitfish to manufactured pellets. It is inherently more difficult to obtain a consistent mass of baitfish fed to SBT on a daily basis over a feeding interval than if manufactured pellets (usually of a consistent mass within specific pelleting equipment error allowance) are used. The preference for baitfish as feed by SBT contributes also to the variability in mass of feed – as baitfish comes in varying size and mass, and is also dependent on species (type).

3. Non-steady state conditions could not be achieved because of industry practices on baitfish selection and dynamic feeding.

4. Ideally validation work should be carried out on the same farm, to ensure similar husbandry practices and model input parameters, e.g. type of baitfish fed, mass of baitfish used, frequency of feeding. However due to management decisions based on logistical and economical aspects of SBT research, it was not possible to obtain an ideal validation data set for the 2006 typical farming period.

5. Farm Alpha Pty Ltd would not provide information on whole weight and fork length of all SBT for all harvests due to commercial in-confidence. True distribution of the age classes in the wild-caught school could not be determined and consequently led to an under-prediction of the fillet biomass.
6.5 CONCLUDING REMARKS

1. Assimilation efficiencies in the fillet of SBT have been obtained for the 12 WHO-PCB congeners and one dioxin congener (2,3,7,8-TeCDF) detected above the blank threshold concentrations. For the PCBs, assimilation efficiencies based on SBT fillets range 19.1 – 35.3 % with the exception of PCB 169, for the pink tag sea-cage. An assimilation efficiency of 39.2 % was determined in SBT fillets (from the pink tag sea-cage) for the congener 2,3,7,8-TeCDF, which was higher than the assimilation efficiencies determined for the WHO-PCB congeners.

2. A residual plot as predicted value versus observed value indicated that the predictive model was neither under- or over-parameterised.

3. It is not robust to use the average values from a sample size of five SBT as it is clear, for example, that there is inherent biological variation in the PCB and PCDD/F concentrations obtained. Biological variability exhibited by the PCB concentration data in SBT fillets may be proportional to the time of farming and higher PCB concentrations.

4. When the predictive model was assessed against a different data set from another SBT farm, the model over-predicted the actual PCB and PCDD/F concentrations. From a food safety point of view, in the absence of ideal predictions because of a lack of ideal validation data sets, an over-prediction instead of under-prediction is preferred.

5. Overall, this work revealed that the model is sensitive to the feeding parameter, which in turn is dependent on the fillet biomass, both of which are a consequence of farm-specific husbandry practices.

6. It is proposed to use the congener model for PCB 126 with an assimilation efficiency of 35.3 % to predictively model TEQ level(s) in farmed SBT using a scale-up factor of 0.8 to the PCB TEQ, and 0.8 from the PCB TEQ to total TEQ (combination of PCB + PCDD/F).
7. Caution must be exercised when extrapolating the findings of this research to the SBT industry as different SBT farms have varying husbandry (and feeding, and baitfish selection) practices.

Law and Kelton (1991) highlight two key principles in predictive modelling: (i) A model should contain sufficient detail to capture the essence of the system for the purposes for which the model is intended, and (ii) predictions from the model will only be as good as the data supplied to develop and validate the model. The first key principle has been presented and discussed through the use of model predictions and residual plots. The second key principle has been discussed and elucidated based on communication with SBT farm management and observations on the data for validation.

The predictive model presented in this chapter can be married with dietary modelling studies to provide advice to consumers of farmed SBT regarding the number of servings permitted on a time-period (e.g. weekly) basis. This application permits consumers to monitor and estimate their intake of PCBs and dioxins based on the number of SBT fillet servings. Chapter 7 embodies a dietary modelling study and shows how the predictive model can be integrated to effect advisory statements for consumers. The predictive model can also be used as a first step to manage strategic feeding of farmed SBT to effect target PCB concentrations and PCB TEQ levels in final harvested product.
CHAPTER SEVEN

DIETARY EXPOSURE MODELLING: PRACTICAL APPLICATION OF THE PREDICTIVE MODEL – SBT
MANAGEMENT OF FEEDING STRATEGIES AND HYPOTHETICAL DIETARY ADVICE TO CONSUMERS OF FARMED SBT

Parts of this chapter have been published as:

7.1 INTRODUCTION

In this chapter, the application of the predictive model for the experimental field data quantified in Chapter 6 is demonstrated. The predicted PCB concentrations or PCB TEQ levels obtained from valuable experimental data for SBT (fillets) as affected by farming, together with dietary modelling, are presented to provide the SBT industry with a practical application of the model.

The predictive model can be used by the SBT industry to manage feeding strategies using a selection of baitfish to achieve target concentrations in harvested SBT (fillets) after a typical farming period. These target concentrations can consequently be assessed against a reference regulatory standard (e.g. Maximum Residue Level, MRL) or assessed against a reference health standard (e.g. Tolerable Dietary Intake, TDI). From a food safety and human health risk assessment point-of-view, TDI is the better indicator as it can provide information directly to consumers in contrast to the MRL, which is the indicator used by the import and export trading regulators.

For example, Farm Delta Fishing Pty Ltd may have access to a low cost baitfish type that has high lipid and PCB concentrations and a higher cost baitfish type that has lower lipid and PCB concentrations. Farm Delta Fishing Pty Ltd can now use the predictive PCB model to estimate the quantity of the low cost baitfish type that could be used together with the higher cost baitfish type to achieve a target PCB concentration in harvested SBT (fillets).

Because SBT fillets are retailed as one of the three specific tissue groups (see Figure 4.4), namely akami, c-toro and o-toro, PCB concentrations and lipid content (%) were determined for these groups, for SBT harvested at the end of the 2005 typical farming period, \( n = 5 \), at the end of the 2005/06 LTH farming period \( n = 1 \), and at the end of the 2006 \( n = 1 \) typical farming period.

The research presented in this chapter fits into step <4> via the predictive route of the developed risk framework presented in Chapter 3 (Phua et al., 2007).
7.2 MATERIALS AND METHODS

It was established in Chapter 6 that only one of the 17 PCDD/F congeners were found in both SBT fillets and baitfish as feed, whereas the 12 WHO-PCBs were found in both SBT fillets and baitfish. To demonstrate the application of the predictive model, calculations presented in this chapter are based on PCB concentration data in farmed SBT.

7.2.1 Dietary Exposure Model for Humans

A dietary exposure model can be defined as the product of the mass of food consumed and the residue concentration in that food (FSANZ, 2004) as is expressed as Equation (7-1), based on the mean bodyweight of a target population:

\[
\text{Dietary exposure} = \text{Mass of food consumed} \times \text{Residue concentration in the food} \quad (7-1)
\]

Applied to the PCB TEQ data for farmed SBT fillets to determine dietary exposure gives Equation (7-2):

\[
\text{PCB TEQ dietary exposure} = \frac{\text{serving size of SBT fillet} \times \text{PCB TEQ in SBT fillet}}{\text{bodyweight of consumer}} \quad (7-2)
\]

However since food safety agencies worldwide use combined PCB + PCDD/F TEQ as reference health standards, it was necessary to integrate the findings from Chapters 5 and 6, to give the combined (PCB + PCDD/F) TEQ. Equations (7-2) together with (7-3) and (7-4) give the dietary exposure from combined PCB + PCDD/F TEQ.

\[
\text{PCB TEQ in SBT fillet} = \frac{\text{PCB 126 TEF} \times \text{PCB 126 concentration}}{0.8} \quad (7-3)
\]

\[
\text{Combined PCB + PCDD/F TEQ in SBT fillet} = \frac{\text{PCB TEQ in SBT fillet}}{0.8} \quad (7-4)
\]

Table 7-1 shows the reference health standards for different countries and international regulatory bodies (FSANZ, 2004; SACN, 2004; COT, 2001). There is presently no
specific information on the serving size and body weight for the general population in Japan, therefore the only information available for pregnant women in Japan is presented. It is noted that the health standards provide guidelines for a combination of PCB + PCDD/F TEQ. It is clear from Table 7-1 that dietary exposure among countries is varied because of the different targeted population, differences in body weight, portion size of fish consumed and duration of exposure. For example, in Japan, the average weight of a pregnant woman is 50 kg, while in Australia the average weight of a ‘woman of child bearing age’ is 66 kg. In Japan, the portion of fish consumed is 80 g while in Australia the portion of fish consumed is 150 g for women and the general population, and 75 g for children six year-old and below.

Table 7-1. Dietary exposure based on the reference health standards for selected countries, with the targeted population, the mean body weight specific to that population and the portion of fish consumed.

<table>
<thead>
<tr>
<th>Country / Regulatory Body</th>
<th>Targeted Population</th>
<th>Body weight, b.w. (kg)</th>
<th>Portion size(g)</th>
<th>Health Standard (PCB + PCDD/F TEQ)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>General population</td>
<td>67</td>
<td>150</td>
<td>70 pg-TEQ.kg⁻¹ b.w./month</td>
<td>FSANZ (2004)</td>
</tr>
<tr>
<td></td>
<td>Children (2-6 year-old)</td>
<td>19</td>
<td>75</td>
<td>70 pg-TEQ.kg⁻¹ b.w./month</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Women of child-bearing age (16-44 year-old)</td>
<td>66</td>
<td>150</td>
<td>70 pg-TEQ.kg⁻¹ b.w./month</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Pregnant women</td>
<td>50</td>
<td>80</td>
<td>4 pg-TEQ.kg⁻¹ b.w./day</td>
<td>Anon. (1999)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>General population</td>
<td>60</td>
<td>140</td>
<td>2 pg-TEQ.kg⁻¹ b.w./day</td>
<td>SACN (2004)</td>
</tr>
<tr>
<td>EUSCF #</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>14 pg-TEQ.kg⁻¹ b.w./week</td>
<td>SCF (2001)</td>
</tr>
<tr>
<td>JECFA *</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>70 pg-TEQ.kg⁻¹ b.w./month</td>
<td>JECFA (2001)</td>
</tr>
<tr>
<td>WHO</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>1-4 pg-TEQ.kg⁻¹ b.w./day</td>
<td>Van Leeuwen et al. (2000)</td>
</tr>
</tbody>
</table>
7.2.2 Tissue Group Sampling and PCB Concentrations

In order to determine PCB concentrations in the akami, chu-toro and o-toro tissue groups, one half of each of the five SBT, obtained from the pink tag sea-cage from Farm Delta Fishing Pty Ltd, at harvest $t = 139$ days were sampled. Composite sample results were previously reported from the other one half of each of these same five SBT. Tissue groups from one SBT from Farm Alpha Pty Ltd, harvested at $t = 107$ days was sampled. Further methods are presented in Section 4.3.1.3 onwards.

7.3 RESULTS AND DISCUSSION

Table 7-2 presents the lipid content (%) for each of the three tissue groups and composite sample obtained from each of the five SBT (fillets) harvested from the pink tag sea-cage at $t = 139$ days. Table 7-2 indicates that the akami has the lowest lipid content (%) whilst the o-toro has the highest lipid content (%). While it appears that the lipid content (%) in the composite is lower than the lipid content (%) in the chu-toro, Welch’s t-test revealed that there was no statistically significant difference in the lipid content (%) for the composite samples and chu-toro tissue group ($P = 0.24, n = 5$).

Figure 7.1 presents the concentrations for PCB 126 against the lipid content (%) for each of the three tissue groups and composite sample obtained from each of the five SBT (fillets) harvested from the pink tag sea-cage at $t = 139$ days. It is noted that the concentrations for PCB 126 determined in the three tissue groups are above the blank threshold concentration for PCB 126. Figure 7.1 shows that concentrations for PCB 126 are lowest in the akami and highest in the o-toro. The other WHO-PCB congeners and PCDD/F congeners, specifically the congener 2,3,7,8-TeCDF that was detected in both baitfish and farmed SBT, exhibited similar trends. This finding is consistent with the hypothesis that PCBs and PCDD/Fs are lipophilic and biomagnify preferentially in the lipid partition(s).
To evaluate the validity of the linear trend among the tissue groups, one farmed SBT from the longer term farming period (obtained from Farm Delta Fishing Pty Ltd) and one farmed SBT from the typical farming period for Farm Alpha Fishing Pty Ltd were analysed for PCB concentrations in the tissue groups. Because of the high costs associated with laboratory analyses (two SBT x three tissue groups = six samples, six samples x A$1200 per sample = $7200), samples for validation in this study were limited.

Figures 7.2A and 7.2B presents the concentrations for PCB 126 against the lipid content (%) for each of the three tissue groups and composite sample obtained from one SBT (fillets) obtained from the LTH sea-cage of Farm Delta Fishing Pty Ltd at the end of the LTH farming period, i.e. t = 496 days; and one SBT obtained from Farm Alpha Fishing Pty Ltd at the end of the typical farming period, i.e. t = 107 days. Indeed, linear trends were observed between PCB 126 concentrations (and other PCB congeners) and the lipid content (%) for the two SBT shown in Figure 7.2. Similarly, a re-assessment of lipid content (%) in the composite samples and chu-toro tissue group with inclusion of these two SBT revealed no statistical significant difference in the lipid content (%) between composite and chu-toro (P = 0.29, n = 7).

The linear trend for PCB concentration against lipid content (%) in the tissue groups indicate that the PCB concentration determined in the composite sample may be used as a linear scale-up and linear scale-down to respectively, the PCB concentration in the o-toro and akami tissue groups. Since there is no statistical difference in the lipid content (%) between the composite sample and chu-toro tissue group, the PCB concentration in the chu-toro tissue group is assumed to be similar to the PCB concentration in the composite sample. It was determined that the mean linear scale-down factor from composite to the akami (n = 7) was 0.3 whilst the mean linear scale-up factor from the composite to the o-toro (n = 7) was 2.1.

A hypothetical scenario for applying the predictive model for PCB 126 together with Equations (7-3) through (7-4) was developed. In this basic scenario, it is assumed that for subsequent typical farming periods, the management from Farm Delta Fishing Pty Ltd exercises the option to feed two baitfish types: Australian and US sardines. An implied assumption is that all other parameters, i.e. mass of baitfish fed, stock of SBT in the sea-
cage and feeding rate, and husbandry practices remain the same as the 2005 typical farming period.

It was previously established from the baitfish congener profiles in Chapter 6 that the Australian sardines have a lower PCB 126 concentration (mean of 0.513 pg.g⁻¹) compared with the US sardines (mean of 2.50 pg.g⁻¹). It is therefore expected that the baitfish strategy of 100 % US sardines fed to farmed SBT would result in a higher dietary exposure to consumers relative to the baitfish strategy of 100 % Australian sardines.

In order to reflect the dynamic feeding conditions practised by the SBT industry, five baitfish strategies for feeding of the farmed SBT are presented using:

(i) 100 % US sardines
(ii) 70 % US sardines and 30 % Australian sardines
(iii) 50 % US sardines and 50 % Australian sardines
(iv) 30 % US sardines and 70 % Australian sardines
(v) 100 % Australian sardines.

Since the reference health standard for Australia is based on a monthly intake, i.e. Tolerable Monthly Intake (TMI), reference health standards for Japan and the United Kingdom have been normalised on a per month basis, assuming 30 days in one month. Based on this assumption, the TMIs for Japan and the United Kingdom are respectively, 120 pg-TEQ/b.w. per month and 60 pg-TEQ/b.w. per month. It is noted however, that the serving size and bodyweight of consumers from Australia, Japan and the United Kingdom are different due to geographical regions and preference for fish as part of the diet based on studies conducted by the various food standard agencies (FSANZ, 2004).

Table 7-3 presents the findings of the hypothetical scenario. It is observed that baitfish strategy for the feeding of farmed SBT affect the dietary exposure to SBT consumers. Because of the higher fat and higher PCB and PCDD/F concentrations in US sardines relative to Australian sardines, greater quantities of US sardines fed to SBT will result in a higher dietary exposure to SBT consumers.

Table 7-3 also shows that consumers of the akami tissue group fillets have a lower exposure to PCBs and PCDD/Fs compared to consumers of the otoro tissue group fillets.
However, it is clear that even consuming the otoro tissue group fillet at the serving size specified by the various food standard agencies, at a rate of one serving per week, did not exceed the TMI for Australia, Japan and the United Kingdom, for all target populations. This accounts only for consumption of SBT tissue group fillets and does not account for contribution from other foods and background exposure. More information on contribution from other foods and background exposure can be obtained from the technical report by FSANZ (2004).

It is observed that the dietary exposure for Japanese pregnant women across the three tissue group fillets and composite fillets are lower than the dietary exposure for target populations of Australia and the United Kingdom. This is because of the higher Allowable Daily Intake (ADI) issued by the Japanese Food Standard Agency of 4 pg-TEQ/b.w. per day compared to the ADI issued in the United Kingdom of 2 pg-TEQ/b.w. per day. One of the explanations for a higher ADI in Japan is because the Japanese population consume a higher percentage of fish as part of the diet compared to Australia and the United Kingdom. In fact Japan is among the top five countries for having the highest fish consumption per capita at > 60 kg per capita per year, among other countries such as Maldives and Iceland (FAO, 2007). Even if Japanese pregnant women consume twice as many serves, i.e. eight 80 g serves per month or, larger serves, i.e. four 150 g serves per month – similar serving size to the Australian women, the percentage of TMI from the three tissue groups and composite would still be less than the percentage of TMI determined in Australian women of child-bearing age.

Overall, this research presented an application for the predictive model developed in Chapter 6 with the data that was interrogated in Chapter 5, using materials and methods described in Chapter 4. Finally, the application in this chapter fits into step <4> of the risk framework developed and presented in Chapter 3.
Table 7-2. Lipid content (%) determined in each of the three tissue groups and the composite sample for the five SBT (fillets) harvested from the pink tag sea-cage of Farm Delta Fishing Pty Ltd at the end of the typical farming period.

<table>
<thead>
<tr>
<th>SBT number</th>
<th>Tissue group / Composite</th>
<th>Lipid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Akami</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Composite</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Chu-toro</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>O-toro</td>
<td>35</td>
</tr>
<tr>
<td>1</td>
<td>Akami</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>Composite</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Chu-toro</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>O-toro</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>Akami</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>Composite</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Chu-toro</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>O-toro</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>Akami</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Composite</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Chu-toro</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>O-toro</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>Akami</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Composite</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Chu-toro</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>O-toro</td>
<td>34</td>
</tr>
</tbody>
</table>
Figure 7.1  Concentration for PCB 126 in each of the three tissue groups: akami, chu-toro and o-toro, and in the composite sample, for each of the five farmed SBT harvested from the pink tag sea-cage of Farm Delta Fishing Pty Ltd.
Figure 7.2  Concentration for PCB 126 in each of the three tissue groups: akami, chu-toro and o-toro, and in the composite sample, for (A) one SBT harvested from the LTH sea-cage of Farm Delta Fishing Pty Ltd in 2005/06, and (B) one SBT harvested from the sea-cage of Farm Alpha Fishing Pty Ltd in 2006.
Table 7-3  Baitfish strategies for feeding farmed SBT and the resultant percentage of TMI from consuming fillets from the different tissue groups within a farmed SBT. The consumer is assumed to have eaten one serving of the portion size per week, i.e. four servings per month.

<table>
<thead>
<tr>
<th>Baitfish mix</th>
<th>Percentage of Tolerable Monthly Intake (TMI)</th>
<th>Country of reference health standard</th>
<th>Target population</th>
<th>Bodyweight of consumer (kg)</th>
<th>SBT fillet serving size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Composite</td>
<td>Akami</td>
<td>Chu-toro</td>
<td>O-toro</td>
<td></td>
</tr>
<tr>
<td>100% US sardine</td>
<td>23.3</td>
<td>7.0</td>
<td>23.3</td>
<td>49.0</td>
<td>Australia General population</td>
</tr>
<tr>
<td>70% US sardine, 30% Australian sardine</td>
<td>18.0</td>
<td>5.4</td>
<td>18.0</td>
<td>37.7</td>
<td>Australia Children (2-6 year-old)</td>
</tr>
<tr>
<td>50% US sardine, 50% Australian sardine</td>
<td>14.4</td>
<td>4.3</td>
<td>14.4</td>
<td>30.2</td>
<td></td>
</tr>
<tr>
<td>30% US sardine, 70% Australian sardine</td>
<td>10.8</td>
<td>3.2</td>
<td>10.8</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>100% Australian sardine</td>
<td>5.5</td>
<td>1.6</td>
<td>5.5</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>100% US sardine</td>
<td>41.1</td>
<td>12.3</td>
<td>41.1</td>
<td>86.3</td>
<td></td>
</tr>
<tr>
<td>70% US sardine, 30% Australian sardine</td>
<td>31.7</td>
<td>9.5</td>
<td>31.7</td>
<td>66.5</td>
<td>Australia Women (16-44 year-old)</td>
</tr>
<tr>
<td>50% US sardine, 50% Australian sardine</td>
<td>25.4</td>
<td>7.6</td>
<td>25.4</td>
<td>53.3</td>
<td></td>
</tr>
<tr>
<td>30% US sardine, 70% Australian sardine</td>
<td>19.1</td>
<td>5.7</td>
<td>19.1</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>100% Australian sardine</td>
<td>9.6</td>
<td>2.9</td>
<td>9.6</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td>100% US sardine</td>
<td>23.7</td>
<td>7.1</td>
<td>23.7</td>
<td>49.7</td>
<td></td>
</tr>
<tr>
<td>70% US sardine, 30% Australian sardine</td>
<td>18.2</td>
<td>5.5</td>
<td>18.2</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>50% US sardine, 50% Australian sardine</td>
<td>14.6</td>
<td>4.4</td>
<td>14.6</td>
<td>30.7</td>
<td></td>
</tr>
<tr>
<td>30% US sardine, 70% Australian sardine</td>
<td>11.0</td>
<td>3.3</td>
<td>11.0</td>
<td>23.1</td>
<td></td>
</tr>
<tr>
<td>100% Australian sardine</td>
<td>5.5</td>
<td>1.7</td>
<td>5.5</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>100% US sardine</td>
<td>9.7</td>
<td>2.9</td>
<td>9.7</td>
<td>20.4</td>
<td></td>
</tr>
<tr>
<td>70% US sardine, 30% Australian sardine</td>
<td>7.5</td>
<td>2.3</td>
<td>7.5</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>50% US sardine, 50% Australian sardine</td>
<td>6.0</td>
<td>1.8</td>
<td>6.0</td>
<td>12.6</td>
<td>Japan Women (pregnant)</td>
</tr>
<tr>
<td>30% US sardine, 70% Australian sardine</td>
<td>4.5</td>
<td>1.4</td>
<td>4.5</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>100% Australian sardine</td>
<td>2.3</td>
<td>0.7</td>
<td>2.3</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>
Table 7-3 (cont’d) Baitfish strategies for feeding farmed SBT and the resultant percentage of TMI from consuming fillets from the different tissue groups within a farmed SBT. The consumer is assumed to have eaten one serving of the portion size per week, i.e. four servings per month.

<table>
<thead>
<tr>
<th>Baitfish mix</th>
<th>Percentage of Tolerable Monthly Intake (TMI)</th>
<th>Country of reference health standard</th>
<th>Target population</th>
<th>Bodyweight of consumer (kg)</th>
<th>SBT fillet serving size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Composite Akami Chu-toro O-toro</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% US sardine</td>
<td>28.3 8.5 28.3 59.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% US sardine, 30% Australian sardine</td>
<td>21.8 6.6 21.8 45.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% US sardine, 50% Australian sardine</td>
<td>17.5 5.3 17.5 36.8</td>
<td>United Kingdom</td>
<td></td>
<td>General population</td>
<td></td>
</tr>
<tr>
<td>30% US sardine, 70% Australian sardine</td>
<td>13.2 3.9 13.2 27.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Australian sardine</td>
<td>6.6 2.0 6.6 13.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
7.4 CONCLUDING REMARKS

1. The baitfish strategy employed for the feeding of farmed SBT consequently affects dietary exposure to SBT consumers.

2. Baitfish with higher lipid (fat) and therefore higher PCB and PCDD/F concentrations fed to SBT result in higher dietary exposure to SBT consumers relative to using baitfish with lower lipid content and lower PCB and PCDD/F concentrations.

3. A linear relationship was obtained for the lipid content (%) and PCB concentration among the tissue groups and the composite sample.

4. There was no statistical significant difference in the lipid content (%) in the composite samples and in the samples of the chu-toro tissue group. It is inferred therefore that the PCB concentration is proportional to the lipid content (%) among the three tissue groups. It was determined that the mean linear scale-down factor for PCB concentration from composite to the akami \((n = 7)\) was 0.3 whilst the mean linear scale-up factor for PCB concentration from the composite to the o-toro \((n = 7)\) was 2.1.

5. Based on the rate of one serving of tissue-specific SBT fillet per week, i.e. four serves of tissue-specific SBT fillets per month, the contribution to the TMI from consuming the akami fillet is approximately seven times lower than from consuming the otoro fillet.

6. Consuming the otoro tissue group fillet at the serving size specified by the various food standard agencies, at a rate of one serving per week, did not exceed the TMI for Australia, Japan and the United Kingdom, for all target populations. This accounts only for consumption of SBT tissue group fillets and does not account for contribution from other foods and background exposure.
CHAPTER EIGHT

CONCLUSIONS AND RECOMMENDATIONS


8.1 CONCLUSIONS

The overall aims of this research were (i) to gain insight into the biomagnification of PCBs and PCDD/Fs in farmed SBT, (ii) to extend the life-cycle biomagnification model to account for super-hydrophobic chemical residues in large pelagic and piscivorous fish such as the SBT, (iii) 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, and (iv) to develop a predictive tool (model) to help the SBT industry achieve targeted concentrations in the final fillet product by making scientific-based decisions on baitfish selection.

Several important conclusions drawn from this research are:

1. The low chlorinated PCB congeners appear to be preferentially eliminated or biodegraded in farmed SBT. The highly chlorinated PCB congeners (PCBs 206, 208 and 209) showed no significant biotransformation. The twelve WHO-PCB congeners and two most abundant PCB congeners (PCBs 138 and 153) either showed no significant biotransformation or were not easily biotransformed due to their chemical structures. The three PCDD/F congeners (1,2,3,7,8-PeCDD, 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF) that were consistently detected in farmed SBT (fillets) may not have undergone biotransformation.

2. A maximum mean CI of $24.0 \pm 0.5$ (standard error) kg.m$^{-3}$ and a maximum mean lipid content of $17.6 \pm 0.5$ (standard error) % in the fillet was achieved after a farming time of 96 days (SBT were harvested on the 97th day), using a mixture of US and Australian sardines, and Australian red bait for the ratios employed as feed by Farm Delta Fishing Pty Ltd.

3. The LTH farming period did not compare favourably with the typical farming period. Levels of 0.67 pg-TEQ.g$^{-1}$ and 0.15 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 pg-TEQ.g\(^{-1}\) (PCB) and 0.29 pg-TEQ.g\(^{-1}\) (PCDD/F) at the end of the LTH period. Although whole weights increased with time of farming relative to the typical farming period (mean weight of 21.31 ± 1.99 (standard error) kg at the end of the typical farming period to 38.25 ± 2.06 kg at the end of the LTH farming period), both the CI and lipid decreased with values of 23.7 ± 0.35 kg.m\(^{-3}\) to 22.9 ± 0.5 kg.m\(^{-3}\) and 15.7 ± 1.1 % to 13.4 ± 0.5 %, respectively. The decrease in values for both the CI and lipid content in the fillet however, was not statistically significant.

4. The life-cycle biomagnification model was modified to suit the farming practices for SBT. This research closes the gap in the literature for a lack of field data on large predatory fish (as food). For the WHO-PCBs, the model predicted assimilation efficiencies based on SBT fillets in the range 19.1 – 35.3 % with the exception of PCB 169, for SBT fed with varying ratios of US and Australian sardines, and Australia red bait. An assimilation efficiency of 39.2 % in SBT fillets for the congener 2,3,7,8-TeCDF was obtained – this was the only PCDD/F congener detected in both SBT fillets and baitfish. The assimilation efficiency for 2,3,7,8-TeCDF in SBT fillets was higher than the assimilation efficiencies for all 12 WHO-PCB congeners in SBT fillets.

5. Among the 12 WHO-PCB congeners, PCB 126 had the highest assimilation efficiency of 35.3 %, with the exception of PCB 169. PCB 126 can be used as an indicator to determine PCB TEQ (factor of 0.8) and consequently the total (combined PCB + PCDD/F) TEQ (factor of 0.8).

6. A predictive tool based on PCB 126 is proposed. The use of this predictive model has been demonstrated through the use of dietary modelling for humans. The predictive model based on PCB 126 is capable of informing the industry on final chemical concentrations in the fillets on baitfish selection and ratios.

7. Baitfish selection and ratios (i.e. strategies) employed for feeding of farmed SBT consequently affects dietary exposure to SBT consumers. Baitfish with higher lipid content and therefore higher PCB and PCDD/F concentrations fed to SBT result in
higher dietary exposure to SBT consumers relative to using baitfish with lower lipid content and lower PCB and PCDD/F concentrations.

8. Application of the developed predictive tool indicated that based on the rate of one serving of tissue-specific SBT fillet per week\(^\text{12}\), i.e. four serves of tissue-specific SBT fillets per month, the contribution to the Tolerable Monthly Intake (TMI) from consuming the akami fillet is approximately seven times lower than from consuming the otoro fillet.

9. An assessment based on the criteria for an adequate model established in this research has shown that the modified life-cycle biomagnification model is adequate for predicting PCBs and the PCDD/F congener 2,3,7,8-TeCDF in farmed SBT. This is a useful first step to help industry manage residue levels in farmed SBT. Caution must be exercised when extrapolating the findings of this research to the SBT industry as different SBT farms have varying husbandry (and feeding, and baitfish selection) practices.

\(^{12}\) In Australia, the serving size for the general population and women (16-44 years old) is 150 g, whilst the serving size for children is 75 g. In the United Kingdom, the serving size for the general population is 140 g. In Japan, the serving size for pregnant women is 80 g.
8.2 RECOMMENDATIONS FOR FUTURE RESEARCH

Recommendations for future research are:

1. It is known that different SBT farms have varying husbandry practices. Findings from this research indicated that the model was conservative (over-predicts the actual chemical residue concentrations) when validated against a data set from another SBT farm. The availability of more data sets from different SBT farms can confirm if the model is indeed conservative or sufficiently robust so that the model can be generalised across the SBT industry.

2. This research was carried out in parallel with other Aquafin CRC projects for SBT. One of the Aquafin CRC projects investigated the metabolism of SBT. A future study on the effect on metabolism on the lipid in farmed SBT and the levels of super-hydrophobic chemicals in other organs of farmed SBT may elucidate if these super-hydrophobic chemicals are bioactivated to other organs from the lipid in the fillet during starvation. Such an effect could be exploited in a temporary starvation period prior to harvest, to achieve potentially lower levels of chemicals relative to the present farming practices.