Improving our Understanding of Bioaccumulation in Humans, Fish and Surrogate Lipid Systems

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
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Abstract

The accumulation of polychlorinated biphenyls (PCBs) into humans was described using CoZMoMAN, a mechanistic multimedia fate and transport model coupled to a human food chain model. Model results demonstrated that concentration-age relationships for population cross-sections and individuals over time are not equivalent and that, under steady-state conditions, the lipid-normalized concentration of PCBs in an individual does not monotonically increase with age. By considering the decades-long emission history of PCBs in the model simulations, it was shown that an individual’s concentration mostly depends upon when she/he was born relative to the peak in emissions. Similarly, the two most influential factors controlling the shape of cross-sectional concentration-age trends obtained in human biomonitoring studies are the time lapse between the peak in emissions and sample collection and chemical elimination half-life. As a result, it should be possible to deduce information on these two factors from the shape of cross-sectional concentration-age trend. Reproductive behaviours (parity, age at birth, breastfeeding) were shown to potentially have a significant impact on exposure (and can contribute substantially to the observed variability in biomonitoring studies) though the mother’s reproductive history has a greater influence on the prenatal and postnatal exposures of her children than it does on her own cumulative lifetime exposure. A case study of the influence of dietary transitions in a hypothetical Arctic community demonstrated that dietary transitions are an important factor underlying the variability in PCB body burdens within and between subpopulations in addition to partially explaining the observed temporal trends.
Comparison of PCB partitioning to various lipid materials suggested that 1) triolein is a good surrogate for human storage lipids; 2) liposomes are not an appropriate surrogate for human storage tissues; and 3) that partitioning into human MCF-7 cells is dominated by the storage lipids rather than by membrane lipids. Finally, a new bioenergetically-balanced bioaccumulation (3B) fish model is presented. Comparison of results from the 3B model with that of existing models revealed that feeding and growth rates used by previous fish bioaccumulation models were not bioenergetically consistent. Differences in biomagnification factors with fish size and temperature as a result of differing energetic requirements demonstrated the importance of the assumptions regarding growth rate and feeding rate.
Acknowledgments

Firstly, I would sincerely like to thank my supervisor Dr. Frank Wania for his patience, guidance and support throughout this thesis work. His scientific insight and approach continue to inspire me. I would like to thank Dr. Jon Arnot for taking me under his wing, sharing his knowledge, and providing encouragement. I am truly grateful to the many incredible opportunities provided to me by both Frank and Jon – you are both amazing supervisors, mentors, and scientists. I would like to thank my colleagues Dr. James Armitage and Dr. Trevor Brown for their assistance and support in various projects. I also thank my supervisory/examination committee members Drs. Jennifer Murphy, Derek Muir, and Miriam Diamond for their guidance during my PhD. A special thank you to Dr. Tom McKone (UC Berkeley) for acting as my external examiner.

Thank you also to my collaborators: Drs. Gertje Czub (Swedish Chemicals Agency, Sweden); Knut Breivik (Norwegian Institute for Air Research, Norway; University of Oslo, Norway); Dr. Chiel Jonker and Stephan van der Heijden (Utrecht University, The Netherlands).

I would like to extend my appreciation and gratitude to my parents, sister and husband (Pablo) for their unwavering support and encouragement. This thesis would not have been possible without your love and faith.
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<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AFT</td>
<td>Abdominal Fat Tissue</td>
</tr>
<tr>
<td>BAF</td>
<td>Bioaccumulation Factor</td>
</tr>
<tr>
<td>BCF</td>
<td>Bioconcentration Factor</td>
</tr>
<tr>
<td>BF</td>
<td>Breastfeeding</td>
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<tr>
<td>BM</td>
<td>Breastmilk</td>
</tr>
<tr>
<td>BMF</td>
<td>Biomagnification Factor</td>
</tr>
<tr>
<td>CBAT</td>
<td>Cross-sectional body-burden versus age trends</td>
</tr>
<tr>
<td>FM</td>
<td>Formula Milk</td>
</tr>
<tr>
<td>GCC</td>
<td>Global Climate Change</td>
</tr>
<tr>
<td>IGDE</td>
<td>Intergenerational Differences in Exposure</td>
</tr>
<tr>
<td>LBAT</td>
<td>Longitudinal body-burden versus age trends</td>
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<tr>
<td>NBF</td>
<td>Non-breastfeeding</td>
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<tr>
<td>PBDE</td>
<td>Polybrominated Diphenyl Ether</td>
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<tr>
<td>PCBs</td>
<td>Polychlorinated biphenyls</td>
</tr>
<tr>
<td>POM-SPE</td>
<td>Polyoxymethylene Solid Phase Extraction</td>
</tr>
<tr>
<td>POP</td>
<td>Persistent Organic Pollutant</td>
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<tr>
<td>PPLFER</td>
<td>Polyparameter linear free energy relationship</td>
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<tr>
<td>SPME</td>
<td>Solid-phase microextraction</td>
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Chapter 1
Bioaccumulation in Humans, Fish and Surrogate Lipid Systems: An Overview

This thesis examines bioaccumulation in three different systems - humans, fish and surrogate lipid systems. In Chapters 2, 3, and 4, a modelling approach is used to address bioaccumulation in humans. Chapter 5 compares experimentally measured partition-coefficients of real human lipids and surrogate lipids. Chapter 6 describes the development and evaluation of a new bioaccumulation model for fish. In the following section, I review several of the themes and concepts of importance to this thesis.

1.1 What is Bioaccumulation?

Bioaccumulation is the process that causes an increased chemical concentration in an organism relative to its surrounding environment (USEPA 2008). Typically, chemical exposure occurs from uptake of contaminated food, respiration of contaminated air or water and dermal absorption (Mackay and Fraser 2000). Upon exposure, the chemical partitions into the most energetically favorable phase. For organic compounds this is usually another organic phase such as lipids, proteins, polysaccharides, etc. For most persistent organic pollutants (POPs), lipids are the favorable phase (Endo et al. 2011). The distribution of a chemical between two phases is often quantified with a partition coefficient i.e. the ratio of contaminant concentrations in two phases at equilibrium. The partitioning of a chemical into the lipid phase is typically approximated by a chemical’s preference for n-octanol. Using octanol as a surrogate organic phase, three general equilibrium partition coefficients can be defined for each chemical – the octanol-water partition coefficient \( (K_{ow}) \), octanol-air partition coefficient \( (K_{oa}) \), and air-water partition coefficient \( (K_{aw}) \). These are defined as:

\[
\log K_{ow} = \log \left( \frac{C_{octanol}}{C_{water}} \right) \quad \text{(Equation 1.1)}
\]
\[
\log K_{oa} = \log \left( \frac{C_{octanol}}{C_{air}} \right) \quad \text{(Equation 1.2)}
\]
\[
\log K_{aw} = \log \left( \frac{C_{air}}{C_{water}} \right) \quad \text{(Equation 1.3)}
\]

where \( C_{octanol}, C_{water}, \) and \( C_{air} \) are the concentrations of the chemical in octanol, water, and air at equilibrium. Chiou et al. (1977) demonstrated that the logarithm of a compound’s aqueous solubility decreased with increasing \( \log K_{ow} \). Hence, the partitioning of chemicals into the lipid phase is considered to be driven by hydrophobic properties of the chemical rather than by its lipophilic
properties. Furthermore, this implies that bioaccumulation should increase with increasing $K_{OW}$. In order to confirm this hypothesis, metrics for measuring bioaccumulation need to be defined.

1.1.1 Metrics of Bioaccumulation

The amount of chemical that accumulates in an organism can be described by three different endpoints: the bioconcentration factor ($BCF$), the bioaccumulation factor ($BAF$) and the biomagnification factor ($BMF$), mathematically defined as:

$$BCF = \frac{C_{\text{Organism}}}{C_{\text{Water}}} \quad \text{(Equation 1.4)}$$

$$BAF = \frac{C_{\text{Organism}}}{C_{\text{Water}}} \quad \text{(Equation 1.5)}$$

$$BMF = \frac{C_{\text{Organism}}}{C_{\text{Diet}}} \quad \text{(Equation 1.6)}$$

where $C_{\text{Organism}}$ is the concentration of the chemical in the organism of interest at steady-state and $C_{\text{Diet}}$ is the concentration of the chemical in the organism’s diet (Gobas and Morrison 2000; Mackay and Fraser 2000). In the case of $BCF$, $C_{\text{Organism}}$ reflects the concentration in an aquatic organism established solely due to exposure via respiration. In contrast, in the equation for $BAF$ and $BMF$, $C_{\text{Organism}}$ reflects the concentration in an organism that is exposed to the chemical through both diet and respiration. Since hydrophobic chemicals are thought to partition predominantly into lipid tissues and partitioning to other biological components is negligible in comparison, the measured concentration of a chemical in a tissue or organism is typically reported on a lipid normalized basis. In this way, accumulation differences due to differing amounts of lipid within a sample are eliminated. Furthermore, because POPs are thought to predominantly bioaccumulate in the lipid compartments, and lipids can be approximated by octanol, the log $BCF$, log $BAF$ and log $BMF$ of a chemical are expected to increase as log $K_{OW}$ increases (Figure 1.1) (Mackay and Fraser 2000).

In addition to these three endpoint metrics, it is also common to refer to the half-life of a chemical in an organism, $t_{1/2}$, defined as the amount of time required for half of the chemical to be eliminated from the organism. Mathematically, this is defined as:

$$t_{1/2} = \ln 2/k_d \quad \text{(Equation 1.7)}$$

where $k_d$ (1/day) is the depuration rate constant. The slower a chemical is eliminated from the organism (by all elimination processes), the longer the chemical resides in the body (i.e. longer half-life) and
hence the greater the accumulation. Therefore, increased bioaccumulation is expected to be reflected by higher BCF, BAF, BMF and $t_{1/2}$.

In the case of a non- or slowly metabolizable chemical, greater bioaccumulation is expected to occur for chemicals with high log $K_{OW}$. Empirical relationships have been derived to directly correlate log BCF and log BAF to log $K_{OW}$ (Arnot and Gobas 2006; Bintein et al. 1993; Fox et al. 1994; Mackay, 1982; Meylan et al. 1999 Neely et al. 1974; Veith et al. 1979). Over time, these simplistic correlations have been increasingly replaced by mechanistic models (see Section 1.5) to determine the BCF, BAF, and BMF. Despite nearly 40 years of correlating bioaccumulation endpoints with log $K_{OW}$, these relationships are still being debated in the literature. A linear relationship between log BCF and log $K_{OW}$ was originally reported by Neely et al. (1974), Veith et al. (1979) and Mackay (1982) as demonstrated in Figure 1.1 (line A). Bintein et al. (1993) later proposed that the relationship between BAF and log $K_{OW}$ is actually non-linear, as demonstrated by line C in Figure 1.1, with a maximum around a log $K_{OW}$ of 6. Meylan et al. (1999) and Fox et al. (1994) also demonstrated a non-linear relationship for non-ionic compounds between log BCF and log $K_{OW}$, with a maximum around a log $K_{OW}$ of 7 and 7.4, respectively.

Figure 1.1: Literature reported relationship between the bioaccumulation endpoints log BAF, log BCF, and log $t_{1/2}$ with log $K_{OW}$.

Empirical relationships have correlated BMF and log $t_{1/2}$ with log $K_{OW}$. Again, it might be expected that a linear relationship (Figure 1.1 line A) between BMF and log $t_{1/2}$ with log $K_{OW}$ would exist. However, several studies have observed a decrease in log $t_{1/2}$ at higher log $K_{OW}$ (i.e. line C in Figure 1.1) (Buckman et al. 2004; Buckman et al. 2006; Fisk et al. 1998; Konwick et al. 2006). This curvilinear relationship of log half-life with log $K_{OW}$ was attributed by Fisk et al. (1998) to a) limited data points in the higher log $K_{OW}$ region; b) difficulty in accurately measuring log $K_{OW}$ for highly hydrophobic compounds; and c) potentially an experimental artifact due to insufficient time to reach steady-state.
In 2006 Arnot and Gobas critically reviewed BCF and BAF measurements. They evaluated existing datasets in the context of quality control criteria including whether water analysis was conducted and met quality control guidelines, if the aqueous concentrations of the chemical was below its aqueous solubility, whether the parent compound or radio-labeled compounds were measured, if exposure was long enough to achieve steady-state, and which tissues were analyzed and if lipid-normalized concentrations are reported. This evaluation considerably reduced the uncertainty in the reported BCF data and indicated that 45% of reported BCF data are subject to at least one major source of uncertainty which generally lead to underestimation of BCF. Once these data were removed, a strong and statistically significant positive correlation between log BCF and log $K_{ow}$ could be found for chemicals with a log $K_{ow}$ greater than zero (i.e. line A in Figure 1.1). For chemicals with a log $K_{ow}$ less than zero, no statistically significant relationship existed between log BCF and log $K_{ow}$.

Jonker and van der Heijden (2007) demonstrated that any curvilinear relationship or plateau of log BCF with log $K_{ow}$ is likely due to experimental artifacts. The authors conducted a series of six experiments using aquatic oligochaete worms in which the experimental protocol was varied (Jonker and van der Heijden 2007). Protocol 1 followed a batch-shake method with BCF measurements conducted periodically over 11 days. The water concentrations were measured using solvent extraction. This method yielded the curvilinear log BCF trend (i.e. line C in Figure 1.1) observed by so many previous studies with the maximum log BCF occurring around a log $K_{ow}$ of 5.5. Protocol 2 used the batch-shake method combined with solid phase micro-extraction (SPME) fibers. Using this method, BCFs were measured only on day 11. This protocol yielded an increasing log BCF trend with log $K_{ow}$ until a log $K_{ow}$ of 6, after which, a log BCF plateau was observed (i.e. line B in Figure 1.1). Since the major difference between protocol 1 and protocol 2 was the method used to measure the water concentration, the authors concluded that solvent extraction likely measures a higher water concentration than SPME. The authors further concluded that since SPME only measures the dissolved chemical concentration, at higher log $K_{ow}$ the dissolved concentration must be lower than the total concentration. Hence, previous studies, in which the water concentration was measured using solvent extraction, likely overestimated the truly dissolved fraction of chemicals with a log $K_{ow}$ above 5.5 and therefore underestimated BCF.

In protocol 3, Jonker and van der Heijden (2007) extended protocol 2 to four weeks. Protocol 4 was an in-situ study whereby worms and SPME fibers were installed in two different river systems for 4 weeks. Protocol 5 again implements the batch-shake method with SPME for 4 weeks this time using freeze-dried worm homogenates. Finally, protocol 6 used a batch-shake method with liposomes, SPME
and polyoxymethylene solid phase extraction (POM-SPE) for 4 weeks. The results from each of these 4 protocols demonstrated a linear log $BCF$-$log K_{OW}$ relationship (i.e. line A in Figure 1.1) for the log $K_{OW}$ range of 4.5 to 7. Since the commonality between protocols 3-6 is the equilibration time of 4 weeks rather than 11 days, the authors concluded that the plateau produced by protocol 2 was likely due to non-equilibrium conditions between the worms and water. Hence, eliminating experimental artifacts by measuring the truly dissolved fraction of chemical in the water phase and by implementing appropriate equilibration times, an increasing log $BCF$ with log $K_{OW}$ should be observed (i.e. line A in Figure 1.1).

One benefit of developing correlations between bioaccumulation endpoints and chemical properties is to establish predictive guidelines for regulatory purposes. In general, it is suggested that appreciable bioaccumulation will occur for chemicals with a log $K_{OW}$ of 5 or greater (Mackay and Fraser 2000). The bioaccumulation criteria used by the Canadian Environmental Protection Act are log $K_{OW}$ ≥ 5, log $BCF$ ≥ 3.7, or log $BAF$ ≥ 3.7 (Government of Canada 2000). The United States Environmental Protection Agency defines chemicals with a log $BCF$ between 3 and 3.7 as bioaccumulative and those with log $BCF$ above 3.7 as very bioaccumulative (USEPA, 1976). The European Union REACH program classifies chemicals with a log $BCF$ ≥3.3 and ≥3.7 as bioaccumulative and very bioaccumulative, respectively (European Commission 2001) while the United Nations Environmental Programme classifies chemicals with a log $K_{OW}$ ≥ 5 or log $BCF$ ≥3.7 as bioaccumulative (UNEP 2001).

These guidelines are usually based on observations in fish (Mackay and Fraser 2000). It has been noted however, that criteria developed for fish and other water-breathing organism may not be directly transferrable to air-breathing organisms (Czub and McLachlan 2004b; Kelly et al. 2001, 2007). Extensive analysis of lichen, caribou and wolf samples in the Canadian Arctic revealed that chemicals with a log $K_{OW}$ <5 may still biomagnify in terrestrial-animals if they have a high $K_{OA}$ (Kelly et al. 2001). This was further supported by modelling studies comparing biomagnification in various food webs including fish, marine mammals, terrestrial mammals, and humans (Czub and McLachlan 2004b; Kelly et al. 2007). These studies demonstrated that although only the log $K_{OW}$ of a chemical is required to assess the bioaccumulative behaviour of chemicals in water-breathing organisms, the log $K_{OA}$ is also necessary to assess the bioaccumulative behaviour of chemicals in air-breathing organisms. This is because while water-breathing organisms can eliminate chemicals only through partitioning to water (i.e. gill ventilation), air-breathing organisms can eliminate chemicals through partitioning to air (i.e. respiration) and water (i.e. urinary excretion). Therefore, for marine mammalian food webs, Kelly et al.
(2007) suggest that chemicals with log $K_{OW}$ 2 – 8 and log $K_{OA} \geq 6$ will biomagnify and, for terrestrial food webs, chemicals with log $K_{OW}$ 2 – 10 and log $K_{OA} \geq 6$ will biomagnify. Finally, in the human food chain, Kelly et al. (2007) suggest that chemicals with log $K_{OW}$ 2 – 9 and log $K_{OA} \geq 6$ will biomagnify which is similar to the predictions by Czub and McLachlan (2004b) that chemicals with log $K_{OW}$ 2 – 11 and log $K_{OA}$ 6 – 12 will biomagnify in humans.

1.1.2 Gastrointestinal Magnification

The experimental work by Gobas (1993a) was the first to experimentally demonstrate that bioaccumulation of chemicals in fish is a result of gastrointestinal magnification. Originally, biomagnification appeared to contradict thermo-dynamical principles in that chemicals were moving from a phase of lower fugacity (i.e. the prey) to that of higher fugacity (i.e. the predator). Fugacity is a measure of the partial pressure of a chemical and is related to concentration by:

$$f(Pa) = C(mol/m^3)/Z(mol/Pa/m^3) \text{ (Equation 1.8)}$$

where $f$ represents the chemical fugacity, $C$ represents the chemical concentration and $Z$ represents the fugacity capacity of the phase for the chemical. Chemicals move from a phase of high fugacity to a phase of low fugacity (Mackay and Paterson, 1982). As such, it was assumed that an active transport mechanism must be responsible for driving biomagnification although no mechanism had been experimentally demonstrated (Gobas 1993a). It was not until the work of Gobas in 1993 that a passive diffusion mechanism was demonstrated. Originally it was thought that the food and chemical were equally absorbed from the gut by the organism. Gobas (1993a) demonstrated that in reality, food is absorbed more readily across the gut membrane than the chemical and subsequently a reduction in the fugacity capacity of the GIT contents occurs. This results in a higher fugacity of the chemical in the gut than in the food. Furthermore, when food is absorbed at a faster rate than the chemical, the mass of chemical remaining in the gut is contained within a smaller phase volume and hence both the concentration and the fugacity of the chemical increase. Therefore, Gobas (1993a) demonstrated that, as a result of food digestion, the fugacity of the GIT contents is able to rise above that of the diet and the fish and hence passive diffusion of chemicals from the GIT to the organism could in fact occur by passive diffusion. While passive diffusion is generally thought to be the main mechanism for bioaccumulation of neutral organic chemicals, active transport of metals and ionogenic compounds remains a possibility.
Following this demonstration of gastrointestinal magnification in fish, several investigations of gastrointestinal magnification in humans were conducted (Moser and McLachlan, 1999, 2001; Schlummer et al. 1998). The study by Schlummer et al. (1998) demonstrated that net absorption of polychlorinated dibenzo-\(p\)-dioxins (PCDDs), dibenzofurans (PCDFs), hexachlorobenzene (HCB) and PCBs from food seemed to be occurring against a concentration gradient i.e. from a lower lipid-based concentration in food to a higher lipid-based concentration in blood. Accounting for the depletion of lipids in the GIT during digestion, as demonstrated for fish by Gobas (1993a), was insufficient to completely account for this discrepancy. As such, Schlummer et al. (1998) proposed a fat flush theory. Briefly, they hypothesized that the fat compartment of the absorbing tissue expands during digestion to accommodate the added lipids thus temporarily decreasing the overall concentration in the tissue to below that of the gut contents. Eventually, the lipid content of the absorbing tissue returns to normal as the absorbed lipids is transported to the lymph and blood. Therefore, the fat flush theory hypothesizes that the combined processes of lipid depletion in the gut contents and simultaneous temporary increased capacity of the absorbing tissue that amplify the diffusion gradient from the gut to the tissue results in human biomagnification. This two-step diffusion gradient hypothesis of biomagnification in humans was further supported by the demonstration that excretion of PCDDs, PCDFs, PCB and HCB could be increased by increasing the non-absorbable fat content of the food and by decreasing the chemical concentration in the food thereby decreasing the concentration gradient between the gut contents and absorbing tissue (Moser and McLachlan 1999, 2001).

1.1.3 The Role of Lipids in Bioaccumulation

As discussed previously, octanol is commonly used as a surrogate for lipids. Empirical measurements have sought to evaluate the validity of this assumption. Furthermore, partitioning to different types of lipids (i.e. neutral non-polar storage lipids and the amphophilic phosphopholipids found in membranes) as well as surrogate lipid materials has also been evaluated.

The triacylglyceride triolein has been studied several times as an alternative storage lipid surrogate to n-octanol (Platford 1983; Chiou 1985; Niimi 1991; Bahadur et al. 1999; Jabusch and Swackhamer 2005). Platford (1983) measured triolein/water partition coefficients (\(K_{\text{triolein/water}}\)) for 14 different hydrophobic organic compounds and found that, on average, \(K_{\text{triolein/water}}\) was 0.3 log units larger than \(K_{\text{OW}}\). Chiou (1985) also observed a generally larger \(K_{\text{triolein/water}}\) than \(K_{\text{OW}}\) for small organic compounds but that this difference decreased with increasing molecular size. Jabusch and Swackhammer (2005) measured \(K_{\text{triolein/water}}\) and also noted slightly higher \(K_{\text{triolein/water}}\) than \(K_{\text{OW}}\) for 12 polychlorinated
biphenyl (PCB) congeners for $K_{OW}$s ranging 3 orders of magnitude. Furthermore, comparison of the lipid/water partition coefficient for another type of triacylglyceride, tricaprylin, also showed generally higher values than $K_{OW}$ but similar values to $K_{triolein/water}$ (Bahadur et al. 1999). These observations are all in agreement with the work by Niimi (1991) who measured the solubility of 31 different organic compounds in triolein, octanol and cod liver oil at 4, 12, and 20°C and discovered that only 6 of these chemicals have similar solubilities in all three solvents. In all other cases, solubility was greater in triolein and cod liver oil than octanol – although to a different extent depending on the chemical. While all five studies have evaluated the partitioning properties of triacylglycerides as storage lipid surrogates for organic chemicals no clear uniform trend was observed between the studies.

Membrane lipids are the main component of biological membranes and therefore partitioning of chemicals to membrane lipids has also received considerable attention over the last several decades (Gobas et al. 1988; Dulfer and Govers 1995; Escher and Schwarzenbach 1996; van der Heijden and Jonker 2009). Membrane lipid molecules typically consist of a polar headgroup, a glycerol molecule, and two long-chain fatty acid tails. Phosphates are the most common headgroup in biological membranes (Yorek 1993) thus yielding a phospholipid (Figure 1.2). In aqueous solution, phospholipids will generally arrange themselves into a vesicle whereby the polar headgroups are on the outside and the non-polar fatty acid tails on the inside (Figure 1.2C). These vesicles are referred to as liposomes and are considered to be artificial membranes that can be used as a model for real cell membranes. Common fatty acids found in membrane lipids are listed in Table 1.1.

An extensive review by Endo et al. (2011) evaluated the liposome-water partition coefficients ($K_{Liposome/water}$) of 240 neutral organic compounds. They noted that most reported $K_{Liposome/water}$ measurements are for phosphatidylcholine (PC) liposomes. This is likely because it is a major lipid component of biological membranes (Cevc 1993). Other major lipid components of membranes include phosphatidylethanolamine, phosphatidylinerine, phosphatidylglycerol, phosphatidylinositol, sphingomyelin, cholesterol and cardiolipin (Gennis 1989; Endo et al. 2011). Furthermore, PC is a popular lipid for liposome studies due to its convenience as an experimental material, i.e., it spontaneously forms stable liposome vesicles in aqueous solutions over a wide pH range (Pauletti and Wunderli-Allenspach, 1994). This is not true of all membrane lipids.
Figure 1.2: A) The general molecular structure of a phospholipid molecule where $X$ is a polar group (i.e. choline, ethanolamine, etc.) and $R_1$ and $R_2$ are long chain fatty acids; B) Graphical representation of a phospholipid molecule; C) Graphical representation of liposome.

Table 1.1 Common fatty acids found in membrane lipids (Gennis, 1989).

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chain length: unsaturated bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric</td>
<td>12:0</td>
</tr>
<tr>
<td>Myristic</td>
<td>14:0</td>
</tr>
<tr>
<td>Palmitic</td>
<td>16:0</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>16:1 (9-cis)</td>
</tr>
<tr>
<td>Stearic</td>
<td>18:0</td>
</tr>
<tr>
<td>Oleic</td>
<td>18:1 (9-cis)</td>
</tr>
<tr>
<td>Vaccenic</td>
<td>18:1 (11-cis)</td>
</tr>
<tr>
<td>Linoleic</td>
<td>18:2 (9-cis, 12-cis)</td>
</tr>
<tr>
<td>γ-Linolenic</td>
<td>18:3 (6-cis, 9-cis, 12-cis)</td>
</tr>
<tr>
<td>α-Linolenic</td>
<td>18:3 (9-cis, 12-cis, 15-cis)</td>
</tr>
<tr>
<td>Arachidic</td>
<td>20:0</td>
</tr>
<tr>
<td>Behenic</td>
<td>22:0</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>20:4 (5-cis, 8-cis, 11-cis, 14-cis)</td>
</tr>
</tbody>
</table>

An additional consideration is the fluidity of the membrane at biological temperatures since the lipid bilayer undergoes a phase transition at different temperatures. At lower temperatures, bilayers are in the lamellar gel phase where the lipid molecules are packed tightly and the acyl chains of the fatty acids are highly ordered with unsaturated bonds in the trans conformation. Because of this, the bilayer thickness and density are greater in the lamellar gel phase than in the lamellar liquid crystalline phase, which
prevails at higher temperatures. When the bilayer is in the lamellar liquid crystalline phase the acyl chains are in considerable disorder and unsaturated bonds are usually in the cis conformation. This is generally thought of as the phase relevant for most membrane lipids. In both the lamellar gel phase and the lamellar liquid crystalline phase, the overall conformation of the liposome can be represented by Figure 1.2 C. The temperature at which the liposome changes from the gel phase to the liquid crystalline phase is referred to as the phase transition temperature ($T_C$). In general, as chain length increases, $T_C$ increases and as the number of unsaturated bonds increases, $T_C$ decreases. In terms of chemical partitioning to liposomes, below $T_C$, $K_{\text{Liposome/water}}$ decreases sharply by a factor up to 100 (Endo et al. 2011). However, above $T_C$, $K_{\text{Liposome/waters}}$ for different types of PC liposomes have been measured within ±0.2 log units across various determination methods and therefore measurements above $T_C$ are considered to have relatively minimal uncertainty (Endo et al. 2011).

One of the most common phospholipids used in liposome experiments is 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylchoine (i.e. POPC). Van der Heijden and Jonker (2009) compiled measured log $K_{\text{Liposome/water}}$ (POPC) values from five studies (Landrum 1988; Petersen and Kristensen 1998; Boese et al. 1999; Van Hattum and Montanes 1999; Muijs and Jonker 2009) and over a range of 5 log units observed a strong correlation ($r^2 = 0.92$) between log $K_{\text{Liposome/water}}$ (POPC) and the log BAFs reported for hydrophobic chemicals. They concluded that log $K_{\text{Liposome/water}}$ (POPC) is a good model for log BAF. Endo et al. (2011) compared log $K_{\text{Liposome/water}}$ for five different PC liposomes with the log $K_{\text{OW}}$ values for 181 neutral organic compounds. From this comparison, a strong 1:1 agreement was obtained with deviation from the 1:1 line being 0.4 log units on average and mostly smaller than 0.8 log units. Therefore, log $K_{\text{OW}}$ values are considered to yield reasonable estimates for log $K_{\text{Liposome/water}}$.

Expression of log $K_{\text{Liposome/water}}$ as a function of log $K_{\text{OW}}$ or expression of log BAF as a function of log $K_{\text{Liposome/water}}$ are both examples of a single parameter linear free energy relationship (or spLFER). Linear free energy relationships are used in environmental chemistry to predict partition coefficients. They are empirical regressions that relate the free energy of a chemical in one phase to that in a second phase (Schwarzenbach et al. 2003). The following expression is an example of an spLFER to predict log $K_{\text{Liposome/water}}$ from log $K_{\text{OW}}$:

$$\log K_{\text{Liposome/water}} = a \cdot \log K_{\text{OW}} + b \text{ (Equation 1.9)}$$

where $a$ and $b$ are regression constants (Endo et al. 2011). Polyparamater linear free energy relationships (or ppLFERs) on the other hand are linear regression models that consider multiple
interactions of the chemical with the two phases. To predict \( \log K_{\text{Liposome/water}} \), there are two different ppLFERs that have been proposed:

\[
\log K_{\text{Liposome/water}} = eE + sS + aA + bB + vV + c \quad (\text{Equation 1.10})
\]

\[
\log K_{\text{Liposome/water}} = lL + sS + aA + bB + vV + c \quad (\text{Equation 1.11})
\]

where the solute descriptors \((E, S, A, B, V, L)\) describe the molecular interactions between the chemical and the condensed phases and the phase descriptors \((e, s, a, b, v, l)\) describe the different capacities of the two condensed phases for interacting with the solute (Endo et al. 2011). Specifically, \(E\) describes the excess molar refraction, \(S\) the dipolarity/polarizability, \(A\) the solute H-bond acidity, \(B\) the solute H-bond basicity, \(V\) the molar volume and \(L\) the logarithm of the hexadecane-air partition coefficient. A more detailed explanation of the evolution of these ppLFERs is given in Chapter 5. Using these ppLFERs, an improved prediction of \( K_{\text{Liposome/water}} \) was achieved whereby the ppLFER was able to predict \( K_{\text{Liposome/water}} \) for 131 chemicals with a standard deviation of 0.28-0.31 compared to a standard deviation of 0.43 in the spLFER with \( \log K_{\text{OW}} \) (Endo et al. 2011). Interestingly, while there is relatively good correlation between \( \log K_{\text{Liposome/water}} \) and \( \log K_{\text{OW}} \), \( \log K_{\text{Liposome/water}} \) and \( \log K_{\text{Olive oil/water}} \) are not well correlated (Endo et al. 2011). Since olive oil should approximate storage lipids, this implies that liposomes and octanol are not good surrogates for storage lipids. This was later supported by the work of Geisler et al. (2012) where the storage lipid/water partition coefficients (i.e. \( K_{\text{Storage lipid/water}} \)) for 247 chemicals and 5 storage lipid types (i.e. olive oil, milk fat, fish oil, linseed oil and goose fat) were compiled or measured. Geisler et al. (2012) demonstrated that the \( K_{\text{Storage lipid/water}} \) was not significantly different between storage lipid types indicating that chemicals partition equally to all storage lipids. From this dataset, they were then able to derive a ppLFER to describe \( K_{\text{Storage lipid/water}} \). From this ppLFER, it became apparent that predicted membrane/water partitioning coefficients differ from storage lipid/water partitioning coefficients. Specifically, they identified that H-bond donor compounds have \( K_{\text{Liposome/water}} \) values that are 0.5 to 1.5 log units greater than their \( K_{\text{Storage lipid/water}} \), but alkanes, alkenes and cycloalkanes have \( K_{\text{Liposome/water}} \) 0.5 to 1.5 log units lower than their \( K_{\text{Storage lipid/water}} \). Since tissues consist of both membrane lipids and storage lipids, van der Heijden and Jonker (2011) measured partitioning to tissue homogenates from 8 different aquatic species. Lipid normalized concentration factors for these 8 species differed by up to 0.9 log units. This observation led the authors to conclude that differences in lipid composition can lead to substantial differences in bioaccumulation. Since the proportion of lipid membranes and storage lipids in these homogenates was not reported, the
contribution of each lipid type to the overall partitioning could not be determined. Clearly, the relevance of partitioning coefficients for octanol, liposomes, and storage lipid surrogates as models for bioaccumulation endpoints as well as the relative importance of membrane and storage lipids to total bioaccumulation is still uncertain and requires further investigation.

Chapter 5 of this thesis seeks to address some of the data gaps that remain in the bioaccumulation literature on the role of lipids in bioaccumulation by considering the following questions:

1. How well does octanol perform as a storage lipid surrogate when compared to actual human lipid tissues? How well does triolein perform as a storage lipid surrogate for real human tissue?
2. How well does octanol perform as a membrane lipid surrogate when compared to real human cells and artificial membranes (i.e. liposomes).
3. How well is partitioning of PCBs to real human cells represented by partitioning to artificial membranes (i.e. liposomes)?
4. How different is PCB partitioning to real cells from that of human lipid tissues?
5. How well do published ppLFER models predict PCB partitioning to actual human lipid tissues, cells and liposomes?

1.2 Polychlorinated Biphenyls

This thesis focuses on polychlorinated biphenyls (PCBs), and in particular on PCB congener 153, due to its abundance and persistence in the environment. Furthermore, because of its abundance and persistence, there is a wealth of experimental data available for model evaluation.

PCBs were first synthesized in 1881 (Schmidt and Schultz, 1881) with industrial manufacturing starting in the US in 1929 (Borlakoglu, 1991). PCBs are formed by the chlorination of biphenyl (Figure 1.3). As shown in Figure 1.3, there are 10 possible chlorination sites on a biphenyl molecule which leads to 209 possible combinations (or congeners) of chlorination. Each of these congeners is assigned a specific congener number according to systematic numbering scheme (Ballschmiter and Zell, 1980). The properties of PCBs are highly dependent upon this degree of chlorination. The commercial advantage of PCBs was based on the properties of high chemical stability, low aqueous solubility, miscibility with organic compounds, non-flammability, and electrical insulation (Borlakoglu, 1991; Safe, 1984). These led to their widespread use as hydraulic fluids, plasticizers, adhesives, heat transfer fluids, wax extenders, dedusting agents, organic dilutants/extenders, lubricants, flame retardants and
dielectric fluids (in capacitors and transformers) (Borlakoglu, 1991; Safe, 1984). According to literature reports, it is estimated that 1.3 million tonnes of PCBs were produced worldwide between 1930 and 1993 (Breivik et al. 2007). Breivik et al. also point out that this number does not include production from factories in Austria and Germany for which production amounts are unknown.

It is also because of their chemical stability that PCBs are so persistent when released into the environment. The first discovery of PCBs in fish was by Jensen in 1966 (Jensen, 1966). Shortly after they were reported in air (Bidleman and Olney 1974), water (Harvey et al. 1973), soil (Nimmo et al. 1971), and sediment (Duke et al. 1970) as well as globally in organisms at all levels of the food web (Risebrough et al. 1968; Holden 1973; Wassermann et al. 1979).

![Figure 1.3: Chemical structure of PCBs](image)

The first major documented case of human PCB poisoning occurred in 1968 in Japan when an apparent outbreak of a skin disease resembling chloracne occurred in over 1000 people (Fujiwara 1975). Other symptoms included increased eye discharge, swelling of the eyelids and respiratory distress (Aoki 2001; Masuda, 1996). The source of the outbreak was traced to the ingestion of Kanemi brand rice oil that had been inadvertently contaminated by PCBs. During the production of the oil, PCBs, being used as a heat transfer fluid in the production equipment, leaked into the rice oil (Aoki 2001). The contaminated rice oil was then shipped out for sale. This skin disease later became known as Yusho disease (where yu means oil and sho means disease in Japanese) (Aoki 2001). The symptoms gradually diminished over the next 10 years with 149 deaths being attributed to Yusho disease over the next 40 years (Masuda, 1996). Unfortunately, this was not the only case of direct PCB contamination. A similar incident occurred in Taiwan in 1979, this time with rice-bran oil, where contamination also occurred from PCB leakage during rice oil production (Guo et al. 2003). This is referred to as the Yucheng incident. More than 2000 people were affected including 39 children born to Yucheng women (Guo et al. 2003). It was later determined that, in both cases, it was not the PCBs which caused the adverse health effects but the PCDFs co-contaminant (a byproduct formed when PCBs are exposed to
extreme heat) (Kimbrough, 1995). Nevertheless, awareness of the potential for health effects from PCBs was created through these tragedies.

While human exposure to PCBs can occur through incidents like the ones described above as well as occupational exposure, most humans’ exposure to PCBs is via air, drinking water, and food. PCBs can be absorbed by the gastrointestinal tract and partition into storage lipids, membrane lipids and the liver. PCBs are capable of crossing the placenta into the fetus and can also be excreted in breast milk. Both of these are routes of exposure directly from the mother to the fetus/infant. Generally, PCB exposure is associated with lower birth rate, smaller head circumference, lower gestational age, neuromuscular immaturity, reduced neurological functioning of offspring (such as reduced memory capacity, lower IQ scores, weak reflexes, attention deficit, and impaired autonomic function) and reduced sperm motility (Faroon 2003). The implications of these effects over the lifetime of the affected individual are still unknown. According to the Comparative Toxicogenomic Database (ctdbase.org), PCBs have been shown to interact with 3640 genes, through 220 different pathways, and are related to 65 different diseases. Since exposure is typically not from a single congener but from a mixture of PCBs and likely co-contaminants such as PCDFs and PCDDs, specific cause and effect relationships can be complicated and hence, the human health effects of PCB exposure continues to be an active area of research.

Due to the demonstrated environmental and health effects of PCBs, the import, manufacture, and sale of PCBs was made illegal in Canada in 1977 but release to the environment was only made illegal in 1985 (Environment Canada). Manufacturing of PCBs in the United States was banned in 1979 (USEPA, 1976). In both countries, continued use of equipment containing PCBs is still permitted. In 2001, PCBs production was banned globally according to the Stockholm Convention although continued use of existing PCBs is permitted.

### 1.3 Bioaccumulation in Humans

#### 1.3.1 Human Biomonitoring

In general, human biomonitoring refers to the measurement of chemicals in a population to determine the amount of chemical or chemicals that are present in the body. Typically, concentrations are measured in urine, blood, or breast milk but hair, nails, saliva and semen have also been used (Health Canada 2007). This information is used as an indicator as to which populations experience the greatest exposure, whether exposure is changing with time, to identify priority chemicals, and to support better environmental and public health policy (Becker et al. 2002). Often, these biomonitoring measurements
are conducted concurrently with questionnaires in order to gather information about the individuals’ overall health, diet or socioeconomic status (COPHES, Porta et al. 2008; Tremblay et al. 2007). When combined, this information can provide insight into potential sources of exposure and health impacts of chemical exposure (COPHES; Health Canada 2007).

PCBs are among the most commonly reported chemicals in human biomonitoring studies (Porta et al. 2008). Multivariate statistical analysis is often applied to data from these studies to evaluate the contributions of age, reproductive behaviour, and diet on PCB concentrations in a population as well as the variability observed in a given population. Several studies have reported that PCB body burdens increase with age (Dewailly et al. 1996; Porta et al. 2008; Bergonzi et al. 2009). Three explanations for this observation have been proposed: 1) that bioaccumulation increases due to longer exposure duration (Fernandez et al. 2008; Hardell et al. 2010; James et al. 2002; Naert et al. 2006); 2) that the body’s ability to biotransform PCBs is age-dependent (Ahlborg et al. 1995; Fangstrom et al. 2005); and 3) that older individuals lived during periods of greater PCB exposure (Ahlborg et al. 1995; Patterson et al. 2008; Porta et al. 2008). This age trend is consistent with the observations that older mothers have higher PCB body burdens than younger mothers (Bergonzi et al. 2009; Hong et al. 1994; Lignell et al. 2009, 2011). Breastfeeding has been identified as a major elimination route for PCBs from the mother (Hong et al. 1994; Skaare and Polder 1990; Uemura et al. 2008) but obviously at the expense of the newborn baby consuming the contaminated milk. While childbirth has also been suggested as an elimination route (Hong et al. 1994; Schade and Heinzow 1998; Vaz et al. 1993; Wang et al. 2009), and therefore an increased number of children leading to lower PCB body burdens for the mother, statistical analysis methods do not always indicate a substantial contribution by parity to overall body burden (Bergonzi et al. 2009). Since diet is the main route of exposure to PCBs for humans (Duarte-Davidson and Jones 1994; Dewailly et al. 2004 (Nunavik report); AMAP 2009), the contributions of different food types on body burdens has also been studied. In general, food with a high lipid content (such as fish (Duarte-Davidson and Jones 1994; Schade and Heinzow 1998; Tee et al. 2003), milk (Duarte-Davidson and Jones 1994; Son et al. 2012), and seal and whale blubber (Cameron and Weis, 1993; AMAP 2009) are also high in PCBs. Reduction of these items from the diet has thus been suggested to lower human body burdens of PCBs (Deutch et al. 2006; Tee et al. 2003). Overall though, there is a general consensus that PCB concentrations in human populations have been declining (AMAP 2009; Dallaire et al. 2003; Schade and Heinzow 1998; Vaz et al. 1993).
Due to the importance of biomonitoring data, there are several large-scale surveys conducted worldwide at any time. Examples of some of these national surveys are listed in Table 1.2. This list serves to demonstrate the widespread application of biomonitoring surveys throughout the world. Additionally, there have been major multi-national surveys conducted such as the Demonstration of a study to Coordinate and Perform Human Biomonitoring on a European Scale (DEMONCOPHES) which was started by the European Union in December 2009, the Biomonitoring of Human Breastmilk for Persistent Organic Pollutants program by the World Health Organization (WHO) and the Arctic Monitoring and Assessment Program (AMAP) which is a collaborative effort by the eight Arctic countries of Canada, Denmark/Greenland, Finland, Iceland, Norway, Russia, Sweden and the United States. For each biomonitoring study 100s to 1000s of people are surveyed. Since chemical analysis for POPs costs several hundreds of dollars for each sample, these initiatives cost millions of dollars. Furthermore, completion of analysis can take several years. Clearly, biomonitoring surveys are an enormous undertaking, yet, as evidenced by their international prevalence, are a necessary and critical component to understanding human exposure and bioaccumulation.

Table 1.2: Examples of current Human Biomonitoring Initiatives by Country.

<table>
<thead>
<tr>
<th>Country</th>
<th>Programs</th>
<th>Program Websites (Accessed October 5, 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>German Environmental Specimen Bank</td>
<td><a href="http://www.umweltbundesamt.de/gesundheit-e/survey/index.htm">http://www.umweltbundesamt.de/gesundheit-e/survey/index.htm</a></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Avon Longitudinal Study of Parents and Children</td>
<td><a href="http://www.bristol.ac.uk/alspac/">http://www.bristol.ac.uk/alspac/</a></td>
</tr>
<tr>
<td>United States</td>
<td>National Health and Nutrition Examination Survey (NHANES)</td>
<td><a href="http://www.cdc.gov/nchs/nhanes.htm">http://www.cdc.gov/nchs/nhanes.htm</a></td>
</tr>
</tbody>
</table>
1.3.2 Human Exposure/Bioaccumulation Modelling

Chemical exposure can generally be defined as the amount of chemical available for uptake at the interface of a receptor organism (USEPA 2010). This differs from bioaccumulation in that bioaccumulation refers to the net amount of chemical residing in the organism at a specific time after chemical uptake and elimination have occurred whereas exposure refers to the total potential amount available for uptake. Therefore, chemical exposure is greater than (or equal to) bioaccumulation. Human exposure modelling relates the amount of chemical present in the environment to the amount of chemical a person is exposed to and possibly the amount of chemical that bioaccumulates in that person (USEPA 2010). There are several human exposure models for POPs available and in use by governments world-wide, including CalTOX (McKone, 1993a, b, c), Total Risk Integrated Methodology or TRIM (Efroymson and Murphy 2001), Stochastic Human Exposure and Dose Simulation or SHEDS (Burke et al. 2001; Zartarian et al. 2000, 2006, 2012), and Far-field Human Exposure or FHX model (Arnot et al. 2010).

SHEDS is a probabilistic model developed by the USEPA and is one of the more popular models currently in use. SHEDS models utilize mechanistic equations to describe exposure and dose in combination with Monte Carlo sampling. It was originally developed (SHEDS-residential) to predict dermal and non-dietary residential exposure of children to pesticides (Zartarian et al. 2000). This version requires five types of data: 1) sequential time-location-activity information; 2) specific activity data (i.e. skin-to-surface, hand-to-mouth, etc.); 3) probability distribution for surface residue loadings; 4) exposure factor probability distributions (i.e. surface area contacts); and 5) chemical specific pharmacokinetic rate constants. The model calculates the daily dermal loadings for individuals and populations, blood concentrations of the chemical, eliminated chemical mass, and relative exposure from different pathways. Later versions of the model include SHEDS-PM (population exposure model for particulate matter) (Burke et al. 2001), SHEDS-wood (developed to estimate children’s exposure and dose to wood preservatives) (Zartarian et al. 2006), and SHEDS-multimedia (which combines residential and dietary exposure for children) (Zartarian et al. 2012). Each of the SHEDS models was designed with specific exposure routes in mind and, with the exception of SHEDS-PM, the models were specifically developed for modelling children.

The Farfield Human Exposure (FHX) model developed by Arnot et al. is a mechanistic model to predict multimedia inhalation and ingestion of contaminants over several age classes (Arnot et al. 2010). Whereas the SHEDS models require empirical information for each exposure source (i.e.
surface residues, chemical concentrations in the diet, etc), the FHX model calculates these values based on unit emissions of the chemical to the environment, the subsequent fate and transport of the chemical in the environment, its bioaccumulation in the food web, and finally total intake by an individual from air, water, soil/dust, and diet. The FHX model calculates chemical intake fractions for seven age groups (nursling, baby, toddler, child, teen, adult, and retiree) whereas the SHEDS models does so only for children.

Neither the SHEDS FHX model have been developed to evaluate exposure over a period of variable environmental concentrations but rather according to steady-state exposure scenarios. However, the model developed by Ritter et al. estimates long-term time trends in human exposure under a variable environmental contamination scenario. The Ritter et al. (2011) model is a multi-individual pharmacokinetic model that uses empirical measurements of chemical concentrations in food at different time points to predict chemical concentrations in a person. As such, the focus of this model is on dietary exposure. The model is calculates both longitudinal body burdens for an individual but also the cross-sectional body burden of a population. Using this model, Ritter et al. (2009, 2011) extracted human elimination half-lives for \( p,p' \)-dichlorodiphenyltrichloroethane, and \( p,p' \)-dichlorodiphenyl-dichloroethylene, and nine PCB congeners from biomonitoring studies. However, this model was specifically developed to describe a post-ban scenario where intake of contaminated food is declining.

In this research, the bioaccumulation model CoZMoMAN was used to predict chemical concentrations in people. CoZMoMAN is a mechanistic non-steady-state environmental fate and human food-chain bioaccumulation model (Breivik et al. 2010) that was created by linking the physical environmental fate model CoZMo-POP 2 (Wania et al. 2006) with the human food-chain bioaccumulation model ACC-HUMAN (Czub and McLachlan 2004a). CoZMo-POP 2 is a coastal zone multimedia fate and transport model with up to 19 physical environmental compartments as illustrated in Figure 1.3 including forest (deciduous, coniferous), soils (forest soil, agricultural, uncultivated), fresh water (with 2 sediment compartments) and marine water (estuarine, coastal, open and deep) with 2 sediment compartments for each of the marine water compartments (Wania et al. 2006). Chemicals are typically introduced into the system by emission into the atmosphere but can also be directly emitted into the soils, forest canopies, fresh water or estuary system. Annual emissions can be steady-state or non-steady-state and can vary seasonally. Once emitted, the chemical distributes between the various environmental compartments. The bioaccumulation model ACC-Human contains both a marine and terrestrial food chain with the human being the top predator of both food chains (Czub and McLachlan
The marine food chain consists of zooplankton, planktivorous fish, piscivorous fish and seawater. The terrestrial food chain consists of grass, beef cows, and dairy cows. Beef and milk cattle are fed from the grass grown in either agricultural or uncultivated soil. The human fish diet consists of both herring and cod usually driven by the estuarine marine compartment but can also be driven by any of 3 other marine compartments. A schematic representation of the human food chains is given in Figure 4.1. CoZMoMAN is the combination of the CoZMo-POP 2 model and the ACC-Human model whereby the concentration of the environment is calculated by CoZMo-POP 2 and the food chains residing in the environment are calculated by ACC-Human. CoZMoMAN calculates the concentration in an individual over their lifetime according to consumption of contaminated food, water and air (Breivik et al. 2010). The physical fate processes have been previously evaluated at both the regional and global scale (Wania et al. 1999; Breivik and Wania 2002; Wania and Daly 2002; Armitage et al. 2009) with the bioaccumulation processes evaluated by Czub and McLachlan (2004a, b).

![Figure 1.4 Generalization of environmental compartments in the CoZMoMAN model.](image)

Like the Ritter et al. model, the CoZMoMAN model was developed for non-steady-state calculations. Since chemical emissions are highly variable on an interannual time scale and can continue for several decades, non-steady-state calculations are a useful tool for evaluating the influence of variable emissions on reported body burdens from biomonitoring studies. However, not all models advocated for exposure assessment consider variable emissions in their analysis (Verner et al. 2008). CoZMoMAN traces a chemical’s fate through the environment, the food chain, and the human population at any time point using only the emissions profile and physical properties of the chemical. In contrast, other human bioaccumulation models (Alcock et al. 2000; Ritter et al. 2009) require specific information on the chemical burden of each food type over time in order to first calculate the time-variant chemical intake for a specific population and then calculate the corresponding human
body burdens. Such information is often difficult to obtain. As CoZMoMAN calculates these values mechanistically over time according to the emissions profile, it can be advantageous to apply the CoZMoMAN model when detailed food contamination information is lacking. For example, CoZMoMAN can be used to predict human exposure in the future. On the other hand, the reliance of CoZMoMAN on the emissions inventory can also preclude the use of the CoZMoMAN model if accurate emissions data are not available. Luckily, emission estimates for PCBs have been thoroughly evaluated and recently updated on a global scale (Breivik et al. 2002a, b, 2007).

1.3.3 Data Gaps in Human Bioaccumulation

Human biomonitoring studies provide a wealth of data on chemical concentration in humans in conjunction with information on lifestyle characteristics, dietary characteristics, socioeconomic status, etc. Human biomonitoring data can be extremely complex to interpret however, due to the temporal dimension of the many contributing factors that determine the chemical concentration in humans. For example, emissions of the contaminant to the environment are variable over a historical time scale which leads to variability in all environmental compartments over time. Furthermore, the properties of the environment are also changing over the same time period (i.e. global climate change). Since people are continually being born, the contamination level of the environment into which they are born is continually changing. In addition, the characteristics of an individual also change over time. For example, when a person is born, they originally feed on breast milk and grow very quickly. Eventually the infant will switch to solid foods but will continue to grow at a relatively fast rate for several years still. Overall, the amount of food consumed changes as a function of age as does the growth rate and both continue to change throughout the lifetime of an individual. Discerning of temporal trends of individuals over time as related to their chemical concentration is further complicated by the fact that there are also historical changes in the behaviour of people including reproductive behaviours (age at birth, number of children per women, length of breastfeeding) and type of diet consumed. The use of models in evaluating human biomonitoring data can help to untangle this complexity as well as provide feedback on where additional biomonitoring data are required.

In this thesis, we seek to address a number of questions by applying a human bioaccumulation model to existing PCB human biomonitoring data sets. Some of these questions include:

1. What variables contribute the most to human biomonitoring variability? (Chapters 2 and 4)
2. How does the body burden of an individual change with age? (Chapter 2)

3. To what extent do historically changing reproductive behaviours impact the variability of female body burdens over time? (Chapter 2)

4. How is the life history of a contaminant reflected in human body burdens? (Chapters 2 and 3)

5. What do cross-sectional body burden age trends (CBATs) tell us about changes in bioaccumulation with age in an individual? (Chapter 3)

6. Can CBATs be used to retroactively tell us about the life history of a pollutant? (Chapter 3)

7. What controls observed declines in contaminant levels in human biomonitoring data? Is this due solely to declining emissions? (Chapter 3 and 4).

8. To what extent do historical changes in dietary preferences impact the extent of contaminant levels and body burdens variability over time? (Chapter 4)

1.4 Modelling Bioaccumulation in Fish

Modelling bioaccumulating contaminants in fish, like human bioaccumulation modelling, is used to evaluate the bioaccumulation potential of a chemical based on its properties. A recent review by Barber (2008) compared sixteen existing bioaccumulation models and identified two major approaches to bioaccumulation modelling in fish. The first modelling approach uses chemical assimilation efficiencies to describe the net chemical uptake to fish from the food (Norstrom et al. 1976; Thomann and Connolly 1984; Thomann 1989; Connolly 1991; Madenjian et al. 1993). Since assimilation efficiencies refer to net uptake, dietary uptake and fecal elimination processes are not calculated independently of each other. In other words, the fish assimilates a constant fraction of the ingested chemical. As Barber (2008) points out, the problem with this approach is that the net assimilation efficiency is not necessarily constant with time. For example, the net assimilation efficiency of a chemical can be directly measured by calculating the difference between the amount of chemical in the fish’s ingested food and the amount of chemical in the fish’s feces. Initially, if the fish had been uncontaminated at the onset of the measurements, all unassimilated chemical can be accounted for by analysis of the feces. Over time however, some of the chemical in the feces will be due to egestion of previously ingested chemical. Therefore, the apparent net assimilation efficiency would be expected to decrease with time.
Another approach to fish bioaccumulation modelling is to explicitly formulate each uptake and elimination process (Gobas et al. 1988; Connolly and Pedersen 1988; Barber et al. 1991; Borgmann and Whittle 1992; Gobas 1993b; Eby et al. 1997; Morrison et al. 1997; Barber 2001; Arnot and Gobas 2004). These models typically employ a mass balance of the chemical and are thermodynamically based, i.e., the chemical exchange between the diet, the fish, and the feces are driven by the fugacity difference between these phases. As mentioned earlier, mass-balance models explicitly formulate each uptake and elimination process of the chemical. Uptake processes for fish include gill ventilation (i.e. breathing in) and dietary uptake while elimination processes include gill ventilation (i.e. breathing out), fecal egestion, biotransformation, and growth. While growth is not an elimination route, it is often considered a pseudo-elimination process since growth of the fish serves to increase the fugacity capacity or “dilute” the concentration of the pollutant in the fish and therefore has the same effect as an elimination route. The fugacity or concentration of the chemical in a fish can then be determined by calculating the difference between the chemical uptake and elimination processes over time. This approach is typically preferred to that of the assimilation efficiency method as it allows for biotransformation considerations to be accounted for and therefore more accurate calculations of fish bioaccumulation to be made (Mackay and Fraser 2000).

The mathematical functions describing the chemical uptake and elimination rates used in these mechanistic mass-balance models describe the physiological rates of these processes. For example, the uptake of chemical by gill ventilation is a function of the respiration rate and chemical uptake from the diet is a function of the feeding rate. Ideally, the bioaccumulation model would be based on empirical physiological parameters specific to the situation being modeled. However, modelling is often used to obtain information for scenarios that cannot be measured or for which not all the information is available. In these situations, generalized empirical relationships are substituted. For example, it is difficult (if not impossible) to determine the exact feeding rate of a fish in its natural environment. As such, empirical relationships describing the feeding rate of a fish according to its mass and temperature have been developed (Weininger 1978). Allometric relationships have also been derived for respiration rates (Hewett and Johnson 1992) and growth rates (Thomann 1981; Thomann et al. 1992). The models developed by Hendriks et al. (2001) and Gewurtz et al. (2006) are examples of the utility of this approach. Hendriks et al. (2001) developed a bioaccumulation model that is based entirely on empirical relationships for absorption, assimilation, and elimination that are a function of the chemical’s $K_{ow}$ and the weight, lipid content, and trophic level of the organism. This approach demonstrated that a large
fraction of observed variability in organism bioaccumulation can be removed by accounting for chemical hydrophobicity and organism mass. Furthermore, both absorption and elimination rate constants were highly dependent on organism mass with absorption and elimination rates decreasing with increasing organism mass (Hendriks et al. 2001). Gewurtz et al. (2006) compared the bioaccumulation dynamics of a fish in the Arctic (Arctic char) with that of a temperate fish (lake trout). In their approach, empirical regressions based on fish mass and temperature were used to calculate the respiration, feeding, and growth rate for the two fish scenarios. The Arctic and temperate fish were also of differing mass (1.3 and 2.4 kg, respectively) and temperature (3 and 10 °C, respectively). From this analysis, they determined that temperate lake trout were able to bioaccumulate 6-60% more PCBs than Arctic Char and attributed this to differences in the lipid content of the food and the fish as well the temperature dependence of the growth rate (Gewurtz et al. 2006).

The limitation of these models is that there is no internal check for consistency among the empirically derived physiological rates. That is, just as there is a mass-balance for the amount of chemical within the organism, there must also be an energy-balance for the organism. Drouillard et al. (2009) attempted to address this issue by developing a combined toxicokinetic and species-specific bioenergetic model for yellow perch. Evaluation of this model against three fish sizes under variably seasonal temperatures indicated a large discrepancy between the model predictions and empirical measurements. The authors proposed that overestimation of the gill assimilation efficiency ($E_W$) was the only explanation for this discrepancy and hence further evaluation of $E_W$ is warranted.

As demonstrated above, bioaccumulation modelling of fish can take many forms and is continually evolving. In chapter 6, a bioenergetically-balanced bioaccumulation model (i.e. the 3B model) for fish is presented. This model goes beyond that of the Drouillard et al. (2009) model in that it is parameterized over a large range of fish masses, temperatures and species such that it may be generally applicable to any fish. The role of bioenergetics on bioaccumulation is then evaluated in order to address the following questions:

1. How do bioaccumulation predictions by the 3B model compare to previous fish bioaccumulation models that are not bioenergetically-balanced?
2. Are the empirical relationships for food consumption and fish growth used by previous bioaccumulation fish models bioenergetically consistent?
3. Can the 3B model, which is parameterized for a large range of fish sizes, species, and temperatures, perform as well as a species-specific bioenergetically-balanced fish model when evaluated against a non-steady-state dataset?

4. Although the $E_W$ recommended by Gobas and Mackay (1987) has performed well for bioaccumulation models that are not bioenergetically-balanced, does $E_W$ need to be re-evaluated in the context of a bioenergetic bioaccumulation model?

5. What is the role of body size on bioaccumulation in fish?

6. What is the role of temperature on bioaccumulation in fish?

7. Is there a specific combination of body size and temperature that produce a fish type particularly vulnerable to bioaccumulation?

1.5 Objective and Structure of Thesis

The overall objective of this thesis was to improve our understanding of bioaccumulation processes at the molecular, individual, and population level.

Chapters 2 to 4 explore ways of enhancing interpretations of human biomonitoring data through the application of mechanistic models. Specifically, Chapter 2 evaluates the role of variable PCB-153 emissions on intergenerational difference in human exposure. Three endpoints of exposure for a given individual are evaluated – prenatal, postnatal and lifetime. In addition, the role of varying reproductive behaviours, such as age at childbirth, number of children, and breastfeeding prevalence, on human exposure are evaluated. Chapter 3 explains the difference in body burden as a function of age as represented by the cross-section of a population compared to the longitudinal trend of an individual over time. Again the important role of variable PCB-153 emissions on these trends and therefore their interpretation is highlighted. Chapter 4 examines the influence of dietary transitions over time on PCB-153 body burdens. This chapter uses the example of an Inuit dietary transition from highly traditional food sources to predominantly imported food sources as a case study.

Chapter 5 explores bioaccumulation of PCBs to various lipid types at a molecular level. Specifically, passive sampling methods are used to measure PCB partitioning to artificial membranes (i.e. liposomes), human cells, human abdominal tissues, and triolein - a storage lipid surrogate.

Finally, Chapter 6 contributes to the continuing evolution of fish bioaccumulation models by presenting a bioenergetically-balanced bioaccumulation fish model. The model is then compared against existing
bioaccumulation fish models and a non-steady-state bioaccumulation dataset for 5 PCBs. It is used for hypothesis development with respect to biomagnification factors for fish of various sizes and living in tropical, temperate and Arctic water.
Chapter 2
Investigating Intergenerational Differences in Human PCB Exposure due to Variable Emissions and Reproductive Behaviours

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*Environmental Health Perspectives* 2011, 119: 641-646

Contributions: The model used for this research project was previously developed and published by G. Czub and K. Breivik. Modelling assistance was provided to C. Quinn by G. Czub. Additional emissions scenario data were provided by K. Breivik. C. Quinn ran the model, interpreted model output, wrote the manuscript, revised it and responded to reviewers’ comments under the guidance of F. Wania.

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2.1 Introduction

Polychlorinated biphenyls (PCBs) are a group of anthropogenic persistent organic pollutants that were manufactured for a variety of applications until production was banned beginning in the 1970s (Safe 1994). Human exposure to PCBs typically occurs through consumption of contaminated food (Moser and McLachlan 2002). One of the first incidents to indicate the toxicity of PCBs occurred in 1968 when over 1000 people in Japan became ill after ingesting PCB contaminated rice oil (Fujiwara 1975). A similar incident occurred in Taiwan in 1979 (Chen et al. 1980). The populations affected by these incidents, referred to as Yusho and Yucheng respectively, have been well-studied and serve as references for the hazardous effects of high PCB exposure (Toft et al. 2004). Yusho adults exposed to PCBs suffered from dermal abnormalities and neurological disorders (Aoki 2001), and Yusho infants exposed to PCBs in utero exhibited, amongst other symptoms, decreased motor skills, growth impairment and reduced IQ (Aoki 2001; Jacobson et al. 1990). A common symptom of both adult and in utero exposure was reduced fecundity (Guo et al. 2000, Hsu et al. 2003) and abnormal reproductive development (Toft et al. 2004, 2006).

Three different time periods of PCB exposure are considered in epidemiologic studies: prenatal exposure, postnatal exposure and lifetime exposure (Aoki 2001; Jacobson et al. 1990). Prenatal exposure, due to transplacental transfer of PCBs from the mother to the fetus, is usually estimated based on PCB concentrations measured in umbilical cord serum (Jacobson et al. 1990). Postnatal exposure occurs during breastfeeding when maternal milk is contaminated by lipid soluble PCBs that have accumulated in the mother (Jacobson et al. 1990). Lifetime exposure is the cumulative exposure to a given contaminant over the lifetime of the individual resulting from prenatal, postnatal, childhood and adult exposure (Alcock et al. 2000). As emissions of PCBs have varied greatly over time, lifetime exposure will depend on the year of birth (Alcock et al. 2000; Moser and McLachlan 2002; Ritter et al. 2009) therefore resulting in intergenerational differences in exposure (IGDE).

Changes in reproductive behaviours during the time period of PCB emissions, including changes in average numbers of children (parity), maternal age at birth, and breastfeeding versus not breastfeeding, may further enhance or reduce IGDE. Body burden variability within a population has been attributed to age (Bergonzi et al. 2009; Dewailly et al. 1996), and body burden declines in mothers have been attributed to the cumulative duration of breastfeeding (Dewailly et al. 1996; Tajimi et al. 2004). Parity
has been both implicated (Vaz et al. 1993; Wang et al. 2009) and disputed (Bergonzi et al. 2009) as a cause of body burden decline.

Using a time-variant model, Alcock et al. estimated that the body lipid PCB concentration of a woman born in 1950 would decrease by 25% after 6 months of breastfeeding (Alcock et al. 2000). Our objectives were to quantitatively determine the extent of prenatal, postnatal, and lifetime exposure relative to atmospheric emissions assuming both hypothetical constant and historical time-variant emissions; and to evaluate the impact of reproductive characteristics on IGDE. To accomplish this, we used the CoZMoMAN model to predict PCB concentrations according to a mechanistic model of their environmental fate and food chain bioaccumulation (Breivik et al. 2010). Our results identify generations of the population with the greatest possibility of exposure to PCBs, and suggest the extent to which reproductive behaviours contribute to IGDE.

2.2 Methods

All simulations were performed with CoZMoMAN (Breivik et al. 2010), a time-variant multimedia mechanistic model that was created by linking the CoZMo-POP2 model (Wania et al. 2006), which describes contaminant environmental fate, and the ACC-HUMAN model (Czub and McLachlan 2004), which describes human food chain bioaccumulation. CoZMoMAN previously has been used to simulate time trends in PCB concentrations in air, seawater, soils, sediment, herring, cod, beef, and human breastmilk based on historical time-variant PCB emissions for the Western Baltic Sea region, which were compared with reported measured values (Breivik et al. 2010). Using the same parameterisation as in the previous study, we estimated time trends for lipid normalized PCB concentrations in Swedish women born at different times and explored the impact of reproductive behaviours that have undergone changes (specifically, the number of children born to a mother, maternal age at childbirth, and breastfeeding or formula feeding) during the historical PCB emissions time period, first assuming constant emission and dietary intake scenarios, and then assuming time-variant PCB emissions based on historical data.

In this paper we focus on model estimates for PCB-153 according to a single PCB emissions scenario that was judged most realistic and produced estimates that were most consistent with the observed time trends (Breivik et al. 2010). Specifically, we assumed that 5% of annual imports during the time of PCB production and import were emitted to the atmosphere, and, for models of constant emissions, assumed emissions of 0.198 tonnes/year, the average rate of the time-variant emissions for PCB-153
from 1930-2100 according to this scenario.\(^1\) In addition, we assumed that the metabolic 
biotransformation rate of the PCBs was constant over time regardless of body burden or age (Czub and 
McLachlan 2004), and used default settings for all other model parameters (Breivik et al. 2010) as 
described in the Supporting Information (see text and Table S2.1 for details, including dietary and 
physiochemical parameters.) Calculations also were performed for six additional PCB congeners (28, 
53, 101, 118, and 180) and for an alternative scenario of historical emission time trends, as described in 
the Supporting Information.

The amount of prenatal transfer of each PCB congener was determined by assuming that the infant is in 
chemical equilibrium (equifugacity) with the mother at the time of birth. The amount of lactational 
transfer was estimated assuming that breastmilk is in chemical equilibrium with the mother’s body 
tissues and blood. We also assumed that breastfeeding occurs for six months, after which the infant 
receives a regular diet adjusted for age (Moser and McLachlan 2002). Estimates are presented for a 
default female who is defined as a woman who was breastfed for six months and was the only child of 
a 30 year old mother, and who at age 30 breastfed her first and only child for six months. Nulliparous 
women were also assumed to have been breastfed as the first and only child of a 30 year old mother. 
Estimates to assess the influence of breastfeeding assumed that non-breastfed children were fed 
formula milk derived from cow’s milk.

A time step of 1 hour was used in all simulations with results plotted with a 5 day resolution for the 
first year of an individual’s life and on a 1 year resolution for years 1-80. Prenatal exposure, \(E_{\text{pre}}\), was 
considered to be equal to the body burden at birth (indicated by the arrow in Figure 2.1). Postnatal 
exposure, \(E_{\text{post}}\), was the cumulative exposure over the six months of lactational transfer. Lifetime 
exposure, \(E\), was defined as the cumulative exposure during the entire 80 year lifespan of a woman. 
Exposures are reported as lipid normalized concentrations (ng/g lipid) which should not be confused 
with overall body burden (ng/g body weight or ng/person).

2.3 Results and Discussion

2.3.1 Human PCB exposure predictions under constant emissions

In order to interpret how lipid normalized PCB concentrations are affected by time-variant emissions, it 
is helpful to first understand the estimated concentration profile assuming constant emissions. Figure

\(^1\) For a detailed explanation of the various emissions scenarios, refer to the supporting information in Breivik et al. 2010
2.1 illustrates the age-dependence of the lipid normalized PCB-153 concentration for a default female (as defined above). During the six months the woman is breastfed as an infant, her estimated lipid normalized PCB-153 concentration continually increases because the rate of PCB uptake from breast milk is assumed to be greater than the rate of growth (Figure 2.1). However, after breastfeeding ceases, the PCB concentration rapidly decreases because the rate of growth exceeds the rate of contaminant uptake from food until the age of 12-15. Minor fluctuations in the concentration time profile during this time period are a reflection of the changing lipid content of the body (calculated according to Moser and McLachlan 2002, see Figure S2.1). By the time a woman reaches her late teens and early twenties her growth rate slows and hence, with continued consumption of contaminated food, her lipid normalized PCB-153 concentration increases (Figure 2.1). When a woman becomes pregnant her body lipid content increases, resulting in a decrease in the overall lipid normalized PCB-153 concentration (Figure S2.1). Following childbirth at age 30, the woman’s lipid normalized PCB-153 concentration is further reduced by elimination of lipid soluble contaminants through milk production (Figure 2.1). Upon weaning of her child, the mother’s predicted lipid normalized PCB-153 concentration increases until it regains its pre-pregnancy concentration at approximately age 40. We assume that after age 40 the relative proportion of lipid weight to body weight increases while the volume of food consumed decreases, resulting in declining PCB-153 concentrations (Figure 2.1). Several statistical studies suggest that the PCB body burden increases with age (Bergonzi et al. 2009; Dewailly et al. 1996). In fact, over the age ranges examined in these studies (16-42 years), our model also predicts increasing body burden with age. On the other hand, our prediction of decreased lipid concentration after age 40 is based on the assumption that body fat increases continually with age, which may not be true for all individuals.
Figure 2.1: PCB-153 lipid normalized concentration profile for a default female under constant emissions. Prenatal exposure (i.e., the estimated PCB-153 concentration in ng/g lipid at birth) is indicated by the black arrow, and postnatal exposure (due to breastfeeding for six months after birth) is indicated by the dark grey shading. The area under the curve (dark + light grey area) represents lifetime exposure.

2.3.2 Human PCB exposure predictions under time-variant emissions

Under non-steady emissions, a default female’s lipid normalized PCB-153 concentration profile will depend upon the age at which her exposure began, and will reflect the variability in PCB emissions over time. This is evident in Figure 2.2 where estimated lipid normalized PCB-153 concentrations predicted for default females born every ten years between 1920 and 2010 under time-variant emissions (Scenario 2, see Supporting Information) are displayed. Our model predicts that the largest peak concentration at any given age was experienced by six-month old girls born in 1980 (Figure 2.2), which suggests that maternal transfer during pregnancy and lactation was a significant source of PCB-153 contamination among women born after the PCB production phase-out in the 1970s (Figure 2.3). While estimated PCB-153 concentrations peaked at 6 months of age in women born after 1980, peak concentrations for women born in 1970 or earlier would have occurred in the 1980s, regardless of a woman’s age during the 1980s (Figure 2.2). In other words, our model predicts that a woman born in 1940 would have experienced a peak PCB-153 concentration in 1980 at approximately 40 years of age, whereas a woman born in 1960 also would have experienced her peak concentration in 1980 at
approximately 20 years of age. Although women born after maximum PCB-153 emissions would have experienced the highest maximum lipid-normalized PCB-153 concentrations, our model predicted that women born approximately 15 years before the phase-out, i.e. the generation born in 1960, would have experienced the greatest cumulative lifetime exposure to PCBs (Figure 2.3C). Model predictions for additional PCB congeners (28, 53, 101, 118, 138, 180) can be found in the Supporting Information (Figure S2.3)

![Graph showing predicted lifetime lipid normalized concentration profiles of PCB-153 according to year of birth for default females born between 1920 and 2010 under time-variant conditions.](image)

Figure 2.2: Predicted lifetime lipid normalized concentration profiles of PCB-153 according to year of birth for default females born between 1920 and 2010 under time-variant conditions.

The predictions generated by our model are consistent with those reported by Alcock et al. (2000) and Moser and McLachlan (2002). In particular, the results of this study and Alcock et al. (2000) suggest that the previously reported trend of increasing body burden with age was most likely an artefact of the year of birth relative to PCB emissions rather than increasing PCB exposure with age. The peak in dietary PCB contamination coincided with the peak in prenatal PCB exposure in the study by Moser and McLachlan (2002), while the results of this study predict that the peak in prenatal exposure occurred approximately 10 years after the maximum PCB emissions (Figure 2.3A). Therefore, this 10
year delay may represent the length of time required for PCBs to move from the air through the environment and into the food chain (Breivik et al. 2010). Simulations with different PCB emissions scenarios (Figure S2.2) suggest that the temporal delay between generations with the highest cumulative lifetime PCB exposures and those with the highest prenatal and postnatal exposures was approximately 20-30 years regardless of the emissions scenario.

The findings of this study imply that since the highest accumulated exposure occurs at reproductive age for women born in the 1960s (Figure 2.2), this group may be especially at risk for reproductive disturbances, as has been reported for both males and females with high organochlorine exposures (Toft et al. 2006). We estimated that the highest prenatal PCB-153 exposure occurred among children born in 1980 (Figure 2.3A), which suggests this population may have been at increased risk of health effects that have been associated with prenatal PCB exposure, including decreased motor skills, growth impairment, reduced IQ, reduced fecundity, and early onset menstruation (Wigle et al. 2008). Therefore, even though women born in the 1960s may have experienced a greater cumulative exposure to PCBs than their children, their children may have suffered more health effects due to prenatal exposure. In other words, due to IGDE, the health repercussions of PCB exposures will span several generations but may be manifested differently by each generation depending upon the timing of their exposure.
Figure 2.3: Comparison of A) prenatal; B) postnatal; and C) lifetime lipid normalized PCB-153 exposure predictions for default females according to year of birth (black line) based on estimated time-variant PCB-153 emissions (solid grey line).

### 2.3.3 Contributions of reproductive behaviours to IGDE under constant emissions.

In addition to changing emission time trends, a secondary source of IGDE may arise from changing trends in reproductive behaviours. For example, in Sweden, the average age at first birth was 24 years in 1970, compared with 28.9 years in 2008, and the average number of children per woman was 2.1 in 1960 compared with 1.6 in 2000 (Statistics Sweden). In order to differentiate reproductive trends from emissions variability, we first examined reproductive characteristics under a constant PCB emissions...
scenario in which, once steady-state has been reached, there would be no intergenerational differences in PCB exposures assuming that reproductive behaviours are also held constant (Figure 2.4).

Figure 2.4: Predicted lipid normalized PCB-153 profiles and estimated prenatal, postnatal and lifetime exposures assuming constant PCB-153 emissions over time for a woman who A) gives birth to her first and only child at age 20, 30 or 40; B) has 0 children or 2 or 5 children (with births at two-year intervals and the last birth at age 30); and C) consumed breastmilk (BM) or formula milk (FM) exclusively for six months after birth and breastfed (BF) or formula fed (NBF) a single infant born when the woman was 30 years old.

The predicted lifetime lipid normalized PCB-153 concentration profiles of primiparous mothers at different ages, and prenatal, postnatal, and cumulative lifetime exposure estimates under constant PCB emissions, are compared in Figure 2.4. The prenatal exposure of an infant born to a 20 year old mother is 79% and 73% that of an infant born to a 30 or 40 year old mother, respectively, with similar differences in postnatal exposure according to the mother’s age at birth (Figure 2.4A). This supports the hypothesis that children born to mothers with greater lifetime PCB exposure will receive more maternal transfer (Bergonzi et al. 2009; Lignell et al. 2009; Uemura et al. 2008). However, the age at which the mother gives birth does not substantially influence the mother’s own estimated cumulative lifetime exposure (Figure 2.4A).
Figure 2.4B compares the estimated lipid normalized PCB-153 concentration profiles of a 30 year old woman with five children (born at two-year intervals at 22, 24, 26, 28, and 30 years of maternal age, respectively) to that of a nulliparous 30 year old woman. Prenatal PCB-153 exposures of the second- and fifth-born children are predicted to be 80% and 55% of the prenatal exposure of the first-born child, respectively, with similar predictions for postnatal exposure. Since multiparous women were assumed to have breastfed each child, the estimated decrease in both prenatal and postnatal exposure of the fifth born child is a consequence of decreased body lipid burdens of PCB-153 in the mother due to transplacental transfer and breastfeeding of the previously born children. These results assume 6 months of breastfeeding and births at 2 year intervals; PCB transfer from mother to child would increase, and the prenatal and postnatal exposure of each successive child would decrease, if breastfeeding continued > 6 months. On the other hand, prenatal and postnatal transfer to successive children would decrease as the amount of time between successive childbirths decreases because the body burden of the mother would have less time to recover to pre-pregnancy levels between births. A decrease in maternal transfer with increased parity might be expected to indicate large differences in the lifetime exposure of the mother (Schade and Heinzow 1998; Vaz et al. 1993; Wang et al. 2009), however our model suggests that a mother who gives birth to 1, 2 and 5 children still experiences 96%, 91% and 82% of the PCB-153 lifetime exposure of a nulliparous woman (Figure 2.4B), respectively, supporting the hypothesis that parity has a minimal influence on the lifetime exposure of the mother (Bergonzi et al. 2009).

In order to differentiate the role of breastfeeding as a source of contamination during infancy and as a loss mechanism for the mother, four breastfeeding scenarios were considered (Figure 2.4C) depending on whether a woman was fed by breastmilk (BM) or formula milk (FM) as an infant, and whether she breastfeeds (BF) or does not breastfeed (NBF) one infant of her own. Alcock et al. (2000) estimated that the maternal PCB body burden would decrease by 25% over 6 months of breastfeeding, but our model only predicts a 5% decrease. The reason for the discrepancy may be that Alcock et al. assumed that 7.5 kg of a mother’s overall lipid content is transferred to a child during 6 months of breastfeeding compared with only 4.5 kg assumed by our model. Furthermore, it is unclear, from the Alcock et al. study, what the original lipid mass is for the mother who experiences a 25% decrease in body burden. The greater the proportion of lipid transferred to the infant the greater the transfer of contaminant.

According to our model, at the age of 6 months, formula-fed infant experiences 75% less postnatal PCB-153 exposure than a breast-fed infant (Figure 2.4C). However, this only translates into only a 15%
reduction in lifetime exposure (Figure 2.4C). Furthermore, we estimate that breastfeeding as a loss mechanism accounts for < 5% of the difference in the lifetime exposure of a breastfeeding mother relative to a non-breastfeeding mother (Figure 2.4C). Of course, these predictions are based on 6 months of breastfeeding the infant 100% of the time. In reality, a mother may breastfeed her infant for 1 year or longer, which would increase the amount of contaminant transferred to the infant, or use a combination of breast milk and formula milk, which would decrease the transfer of contaminant.

From these constant emissions calculations, we can estimate the exposure variability within a population and between generations that can be quantitatively attributed to maternal age, parity and breastfeeding prevalence. Our results suggest that parity and being breastfed as an infant are the main factors contributing to variability in the lifetime PCB-153 exposure of the maternal population. On the other hand, breastfeeding an infant and age at childbirth do not significantly influence a woman’s own lifetime exposure. The previous number of offspring born to a mother is the greatest determinant of their infant’s prenatal exposure. Consequently, our findings suggest that a shift in the number of children per family over time will have a larger impact on intergenerational differences in both prenatal and lifetime PCB-153 exposures than the other reproductive characteristics examined. Our findings also indicate that the type of milk fed to an infant has the greatest impact on postnatal exposure, and therefore the prevalence of breastfeeding also may have a strong influence on intergenerational differences in postnatal exposure. Under constant PCB-153 emissions, shifts in the age at which women give birth will also introduce intergenerational differences in both prenatal and postnatal exposures because as the average age of childbirth increases, prenatal and postnatal exposure will also increase (Bergonzi et al. 2009; Dewailly et al. 1996). However, changes in maternal age will have less of an impact on IGDE than changes in the number of offspring and the prevalence of breastfeeding. In terms of population impact, our results suggest that women born to older mothers and who have fewer older siblings may be the most likely to experience reduced fecundity and abnormal reproductive functioning due to their prenatal PCB-153 exposure. Interestingly, regardless of the reproductive characteristics of the mother, under constant emissions all offspring achieve equal lipid PCB-153 concentrations by age 17 (Ritter et al. 2009) and our model suggests that all women achieve approximately equal lipid PCB-153 concentrations by about 60 years of age (Figure 2.3A-C). This has potential implications for studies aiming to evaluate changing body burdens of a population over time. Specifically, researchers aiming to deduce trends in the PCB exposure experienced by a population
over time should consider sampling females at age 17 or over 60, because such sampling would reduce the variability caused by factors other than dietary contamination.

2.3.4 Contributions of reproductive characteristics to bioaccumulation variability under time-variant emissions.

Having evaluated the effects of time-variant PCB-153 emissions on IGDE and the impact of reproductive factors under constant PCB emissions on IGDE, we are now ready to assess the influence of reproductive characteristics in combination with emission variability, which is the scenario closest to reality. Average estimated prenatal, postnatal and lifetime PCB-153 exposures according to year of birth and reproductive characteristics are shown in Figure 2.5. Our findings suggest that the age at which a mother gives birth only influences the prenatal and postnatal exposure of individuals born after the PCB production phase-out (Figure 2.5A) and that a younger mother (age 20) born after the phase-out would transfer less contaminant to an infant than an older mother (age 30-40). With respect to lifetime exposure, the age of the mother at birth appears to introduce only minor variability in IGDE.

The extent of prenatal and postnatal exposure is greatest for the first-born child and less for each successive child but again, this effect is only observed for women born after the PCB production phase-out (Figure 2.5B). On the other hand, the number of children that a woman has would only affect her lifetime exposure if her children were born before the PCB production phase-out. In this case, a nulliparous woman would have a higher lipid PCB-153 concentration than a parous woman, and lifetime exposure would decrease with increasing parity. These results suggest that reproduction is only a significant loss mechanism for PCBs, with respect to lifetime exposure, for women born during the peak period of PCB use and production.
Figure 2.5: Predicted lipid normalized PCB-153 profiles and estimated prenatal, postnatal and lifetime exposures assuming estimated time variant PCB-153 emissions for a woman who A) gives birth to her first and only child at age 20, 30 or 40; B) has 0, 1, 2, 3, 4, or 5 children (with childbirth at two year intervals with the youngest child born at age 30 for multiparous women); and C) consumed breastmilk (BM) or formula milk (FM) exclusively for six months after birth and breastfed (BF) or formula fed (NBF) a single infant born when the woman was 30 years old.
With respect to postnatal exposure, our findings suggest that the breast-fed child is exposed to more PCB contamination than the formula-fed child regardless of their year of birth, with the greatest contrast between breast-fed and formula-fed infants born in 1980 (Figure 2.5C). This suggests that breast milk is a significant source of contamination for infants. On the other hand, the difference in lifetime exposure is more complicated. We estimate that a woman who is breastfed (BM) but does not breastfeed her own child (NBF) will have the highest lifetime PCB exposure regardless of year of birth, while a woman who was formula fed (FM) as an infant but breastfeeds (BF) her own child will have the lowest lifetime exposure (Figure 2.5C). For women born between 1950 and 1980, differences between women who were breast-fed and also breastfeed a child and women who were formula-fed and do not breastfeed a child depend upon her year of birth relative to the PCB emissions scenario. In general though, the overall difference between the 4 groups of women is relatively insignificant to their cumulative lifetime exposure.

Finally, as noted for the constant emissions scenario, by the time women reach 60 years of age, reproductive factors no longer contribute to population variability (for a population that is only reproductively active from 20 – 40 years old). Furthermore, a mother’s reproductive characteristics appear to have a greater impact on the lipid PCB-153 concentration of her infant than on her own exposure, but the mother’s characteristics only appear to be important to infants born after the PCB production phase-out. This is most likely a reflection of the amount of contaminant accumulated in the mother by the time they are of childbearing age. Most of the variation in the infant population can be attributed to feeding behaviour while the mothers’ age at birth is relatively unimportant.

2.4 Conclusions

The role of reproductive characteristics and time-variant emissions were investigated to improve our understanding of intergenerational differences in PCB lipid concentrations. While our results suggest that women born before the PCB production phase-out experienced the highest cumulative lifetime exposure to PCBs, those born after the phase-out experienced higher prenatal and postnatal exposure. Because prenatal and postnatal PCB exposures have been associated with health complications, our results suggest that even though measures to phase-out PCBs were initiated over 40 years ago, the health effects of PCBs are likely to persist over several generations.
2.5 Supporting Information

2.5.1 Exposure Calculations

Exposure was defined similar to the method by von Goetz et al. (2010). Postnatal exposure, $E_{\text{post}}$, was calculated by summation of the lipid normalized concentrations of the infant at 5 day intervals over the six month period of lactational transfer as follows:

$$E_{\text{post}} = \sum_{t=0}^{t=0.5} C_B \cdot 1.3799 \times 10^{-2} \quad (\text{Equation S2.1})$$

where $C_B$ is the concentration of PCB-153 (ng/g lipid) at a given time, $t$ (years), and $1.38 \times 10^{-2}$ is the conversion factor for the number of years per 5 day interval. $E_{\text{post}}$ is represented in Figure 2.1 by the area shaded in dark grey. The cumulative lifetime exposure was calculated by summation of the lipid normalized concentration over the 79 year lifetime by:

$$E_{\text{life}} = \sum_{t=0}^{t=1} C_B \cdot 1.38 \times 10^{-2} + \sum_{t=1}^{t=79} C_B \quad (\text{Equation S2.2})$$

$E_{\text{life}}$ is represented in Figure 2.1 by the addition of the dark grey and light grey shaded area.

2.5.2 Dietary Parameters

The dietary composition for time-variant conditions mimics that of the Swedish population for 25-year old individuals (Czub and McLachlan 2004). For the steady-state calculations, the dietary composition was constant over the individual’s lifetime. To represent a “worst case scenario”, food consumption was set equal to the maximum values that occurred between 1930 and 2005. For a female, this corresponds to 49.87 g lipid per day of dairy products, 11.58 g lipid per day of beef, and 75.57 g wet weight per day of fish.

2.5.3 Growth Curve

The growth curve for the default woman is illustrated in Figure S2.1 below and was calculated according to (Moser and McLachlan 2002). The default female is defined as the primiparous woman who gives birth at the age of 30 and breastfeeds the infant for 6 months.
2.5.4 Emissions Scenarios

Figure S2.1: Growth curve for the default female.

Figure S2.2: Prenatal (dotted line overlapping with dashed line), postnatal (dashed line overlapping with dotted line) and lifetime (solid black lines) exposure time trends relative to emissions (solid gray lines) of PCB-153 under A) scenario 1 and B) scenario 2.
Previous studies identified that the uncertainty in emission time trends may have led to the underestimation of emission inventories (Breivik et al. 2010). For this reason, three emission scenarios were used to predict PCB exposure in the Swedish Baltic region (Figure S2.2) (Breivik et al. 2010). Scenario 1 represents the recommended maximum historical atmospheric emissions inventory for the region (Breivik et al. 2007). Unfortunately, this scenario lacks empirical emissions data from the early stages of PCB production (Breivik et al. 2002). As an alternative but still valid emission scenario, it was assumed that 5% of the annual imports during the time of PCB production and import were emitted to the atmosphere. This situation is represented through emissions scenario 2. Constant emissions were taken to be 0.198 tonnes/year which is the average rate of the time-variant emissions of PCB-153 (Scenario 2) from 1930-2100.

Under Scenario 1, emissions peak from 1975-1978 which yields a broader time profile than Scenario 2 (Figure S2.2). Regardless of this change in profile shape, lifetime exposure is still at a maximum in 1960 and the prenatal and postnatal exposure peaks in 1990 (Figure S2.2). These maxima correspond to 15 years before and after the maximum in emissions, respectively.

2.5.5 Alternate PCB Congeners
The fates of seven PCB congeners (28, 52, 101, 118, 138, 153, and 180) were modelled according to emissions scenario 2. The physical properties used to model these congeners are outlined in Table S2.1.

Table S2.1: Partitioning properties of alternate PCB congeners (Breivik et al. 2010)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>MW (g/mol)</th>
<th># of Cl atoms</th>
<th>Log $K_{OW}$</th>
<th>Log $K_{OA}$</th>
<th>Biotransformation Rate Constant ($h^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB-28</td>
<td>257.54</td>
<td>3</td>
<td>5.66</td>
<td>7.85</td>
<td>5.48E-5</td>
</tr>
<tr>
<td>PCB-52</td>
<td>291.99</td>
<td>4</td>
<td>5.91</td>
<td>8.22</td>
<td>7.42E-4</td>
</tr>
<tr>
<td>PCB-101</td>
<td>326.43</td>
<td>5</td>
<td>6.33</td>
<td>8.73</td>
<td>2.28E-5</td>
</tr>
<tr>
<td>PCB-118</td>
<td>326.43</td>
<td>5</td>
<td>6.69</td>
<td>9.36</td>
<td>1.26E-5</td>
</tr>
<tr>
<td>PCB-138</td>
<td>360.88</td>
<td>6</td>
<td>7.22</td>
<td>9.66</td>
<td>9.13E-6</td>
</tr>
<tr>
<td>PCB-153</td>
<td>360.88</td>
<td>6</td>
<td>6.87</td>
<td>9.44</td>
<td>5.25E-6</td>
</tr>
<tr>
<td>PCB-180</td>
<td>395.32</td>
<td>7</td>
<td>7.16</td>
<td>10.16</td>
<td>3.42E-7</td>
</tr>
</tbody>
</table>
The IGDE trends predicted for PCB-153 are also applicable to other congeners (Figure S2.3B-D), however the exact length of the time lag between the peak in prenatal, postnatal, and lifetime exposure relative to the emissions profile decreases with decreasing chlorination (Figure S2.3B-D). Although the time trends for the different congeners were similar, the extent of bioaccumulation was very different. While the quantity emitted decreased in the order PCB 28, 153, 52, 138, 118, 101, 180 (Figure S2.3A), the predicted lipid normalized concentration decreased in the order 153, 138/180, 118, 28/52/101, with cumulative lifetime exposures to PCBs 28,52 and 101 being negligible compared to PCBs 118, 138, 153, and 180 (Figure S2.3B). These trends largely reflect the impact of the extent of chlorination on the rate of metabolism (Matthews and Dedrick 1984) and indicate that the parameters dependent upon partitioning behaviour are of relatively minor importance.

Figure S2.3: Time trends A) emissions; B) lifetime exposure; C) prenatal exposure; and D) postnatal exposure of PCB 28, 52, 101, 118, 138, 153, and 180.
### 2.5.6 Summary of Simulated Reproductive Characteristics

Table S2.2: Reference Chart for modelled reproductive characteristics of infant and mother under constant and time-variant emission scenarios.

<table>
<thead>
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<th>Emissions Scenario</th>
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Chapter 3
Understanding Differences in the Body Burden-Age Relationships of Bioaccumulation Contaminants based on Population Cross-sections versus Individuals

Cristina L. Quinn and Frank Wania

*Environmental Health Perspectives* 2012, 120: 554-559

Contributions: The model used for this research project was previously developed and published by G. Czub and K. Breivik. C. Quinn ran the model, interpreted model output, wrote the manuscript, revised it and responded to reviewers’ comments under the guidance of F. Wania.

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3.1 Introduction

Biomonitoring studies involve the measurement of chemical concentrations in blood, urine, or tissues of a large number of individuals to assess the exposure of a population to environmental contaminants. Such studies may provide information about the chemicals a population is exposed to, changes in exposure over time, and factors that influence exposure. Numerous cross-sectional biomonitoring studies (Becker et al. 2003; Jursa 2006; Uemura et al. 2008) have reported human body burdens\(^2\) of polychlorinated biphenyls (PCBs) that increase monotonically with age (Figure 3.1A-C), leading some authors to conclude that bioaccumulation increases with age due to longer exposure (Fernandez et al. 2008; Hardell et al. 2010; James et al. 2002; Naert et al. 2006) or age-dependent changes in biotransformation (Ahlborg et al. 1995; Fangstrom et al. 2005). An alternative hypothesis is that older individuals lived during periods of greater PCB exposure (Ahlborg et al. 1995; Patterson et al. 2008; Porta et al. 2008). A recent review by Porta et al. (2008) highlighted that the interplay between age effects and birth cohort effects has yet to be quantitatively evaluated.

Not all cross-sectional PCB body burden versus age trends (CBATs) consistently increase with age however (Figure 3.1D-H) (Duarte-Davidson et al. 1994; Finklea et al. 1972; Kiviranta et al. 2005; Kreiss et al. 1981; Naert et al. 2006; Thomas et al. 2006). Furthermore, in contrast with data for PCBs, cross sectional data from Australia for 2002 – 2007 suggest a decreasing trend in serum concentrations of polybrominated diphenyl ethers (PBDEs) with age (Figure 3.1I) (Müller and Toms 2010). Covaci et al. (2008) proposed that opposing age trends for PCBs and PBDEs may suggest that PCBs have reached steady-state levels in human tissues. They also suggest that different exposure routes and metabolic rates could explain this difference. Others have proposed that the body burdens of infants are greater than adults because under steady-state conditions, exposure from breast milk is greater than adult food sources (Johnson-Restrepo and Kannan 2009).

The mechanistic bioaccumulation model CoZMoMAN suggests that in individuals with constant exposure, lipid-normalized body burdens of persistent PCB congeners may actually decrease after age 50 (Chapter 2). We propose that the increase in PCB body burden with age observed in cross-sectional population studies is a result of the timing of sample collection relative to peak PCB emissions, which determines the peak in exposure. In the present study, we used the CoZMoMAN model to investigate factors that influence the shape of CBATs and relate these findings to observed CBATs reported in the

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\(^2\) The term “body burden” throughout this chapter refers to the lipid normalized concentration of a chemical in the body.
literature. Ritter et al. (2011) recently applied a pharmacokinetic model to cross-sectional biomonitoring data to estimate intrinsic elimination half-lives of PCBs in humans. They also derived CBATs from their model, which relies on contaminant measurements in food to empirically estimate time trends in exposure. Here we go beyond this approach by applying a mechanistic multimedia fate model to quantify time-variant exposure. Furthermore, our analyses include both pre and post-ban populations rather than focusing only on the post-ban situation.

Figure 3.1: Literature reported cross-sectional body burden age trends for (A) Germany (Becker et al 2003), (B) Slovakia (Jursa 2006), (C) Japan (Uemura et al. 2008), (D) South Carolina (USA) (Finklea et al. 1972), (E) Alabama, (Kreiss et al. 1981), (F) Southern Finland (Kiviranta et al. 2005), (G) Belgium (Naert et al. 2006), (H) United Kingdom (DuarteDavidson et al. 1994, Thomas et al. 2006) and (I) Australia (Müller and Toms 2010). Panels A to H are PCB body burdens and panel I contains both PCB and PBDE measurements.
3.2 Methods

The previously evaluated mechanistic time-variant multimedia model CoZMoMAN (Breivik et al. 2010) was used to estimate CBATs for several chemicals varying in partitioning and degradation properties. CoZMoMAN calculates the transfer of time-variant emissions of an organic chemical through atmospheric, aqueous and terrestrial compartments and its subsequent bioaccumulation in the food web and transfer to humans through the uptake of contaminated food, air and water. Each environmental phase, is modelled as one compartment and the human is modelled as two compartments – the digestive tract and the main body (Czub and McLachlan 2004). Internal kinetic distributions are not considered. The model requires information on the contaminant’s octanol-water and octanol-air partition coefficients (log $K_{OW}$ and log $K_{OA}$), its environmental and metabolic half-lives, and the historical time trend of its emissions. A detailed discussion of the model can be found in Breivik et al. (2010) and references therein.

Cross-sectional data generated through biomonitoring studies are based on groups of different individuals sampled at the same time, whereas the longitudinal estimates derived by CoZMoMAN are for a single individual over their entire lifetime. For the present analysis, cross-sectional trends were determined from model-derived longitudinal estimates of lipid normalized concentrations for individual women born at 5 year intervals starting several decades prior to emissions of the chemical to the environment and ending several decades after emissions ceased. Each simulated woman was an only child born to a 30 year old mother and breastfed for six months, each had one child of her own at age 30 that she breastfed for six months, and each died at age 80. The process of deriving CBATs from the longitudinal body burden age trends (LBATs) simulated for individual women is illustrated in Figure 3.2 for PCB-153 (log $K_{OW}$ 7 and log $K_{OA}$ 9.5) assuming the historical atmospheric emissions profile (Figure 3.2A, Breivik et al. 2010), dietary intakes for Southern Sweden (Figure 3.2C, as in Chapter 2), changes in body weight and lipid weight with age (Figure 3.2D, Czub and McLachlan 2004), and a constant age-independent metabolic rate. Unless otherwise noted, all PCB-153 emissions are assumed to have been released into the atmosphere.
Figure 3.2: Schematic overview of how the CoZMOMAN model estimates cross sectional body burden age trends for PCB-153: First time trends in the concentrations in air and water (B) are derived from
historical atmospheric emission estimates (A, adapted from Breivik et al. 2010) using a physical environmental fate model. Longitudinal body burden age trends for woman born every five years (E) are calculated with a human food chain bioaccumulation model using these concentrations in exposure relevant environmental media (B) as well as assumptions concerning the dietary composition (C) and growth curves for these women (D, adapted from Czub and McLachlan 2004). Finally, cross-sectional body burden age trends for 1968, 2000, and 2030 are obtained by “sampling” the women whose longitudinal body burdens had been estimated.

In this example, 17 different LBATs (for 17 individual women aged 0, 5, 10…80 years old) were used to estimate each CBAT, with each LBAT contributing one data point to a given CBAT. For example, to derive a CBAT for PCB-153 measured in a cross-section population in the year 2000, the body burden for a 30 year old is taken from the LBAT estimated for a woman born in 1970, and the body burden of an 80 year old is taken from the LBAT estimated for a woman born in 1920 (Figure 3.2E).

In all simulations, we estimated dietary intakes assuming a typical Swedish diet which corresponds to daily consumption of 50 g dairy lipid, 12 g beef lipid, and 75 g wet weight fish for 25 year old women (Czub and McLachlan 2004). We assumed that the dietary proportions of dairy, beef and fish were constant over time but that daily consumption varied with age (Figure 3.2C, Czub and McLachlan 2004).

Since the focus of the analyses was on the shape of the CBATs and not on absolute human body burdens, estimated body burdens for individual women (at different ages) were normalized to the average value of the population cross-section at that point in time. For each scenario, $C_{N(t)}$, the normalized concentration for a woman of age $t$, was calculated as

$$C_{N(t)} = C_t / C_{Avg} \text{ (Equation 3.1)}$$

where $C_t$ is the estimated concentration for a woman of age $t$ and $C_{Avg}$ is the average estimated concentration of the population cross-section for the given scenario. In other words, the CBATs reflect the body burden of a woman at a given age relative to the average of the entire population.

### 3.2.1 Sensitivity Analyses.

To identify and better understand the factors that may influence the shape of CBATs we performed additional hypothetical simulations in a series of sensitivity analyses. The influence of human
metabolic half-lives on CBAT shape was evaluated for a hypothetical chemical of \( \log K_{\text{OW}} \) 7 and \( \log K_{\text{OA}} \) 9.5 (corresponding to the partitioning properties of PCB-153) and five different half-lives of 1, 3, 5, 15, and 50 years.

To explore the influence of the length of the emission period on CBAT shapes, we performed additional simulations for a chemical with the partitioning properties of PCB-153 (\( \log K_{\text{OW}} \) 7 and \( \log K_{\text{OA}} \) 9.5) according to three different metabolic half-lives (1, 15 and 50 years) and three hypothetical emission scenarios represented by bell-shaped functions that increased and decreased over 30, 50 and 100 years, respectively (Figure 3.3). The rate of decline represents emission half-lives of 1.8, 8.5 and 10 years, respectively.

![Figure 3.3: Shape of three theoretical emission scenarios lasting 30, 50, and 100 years.](image)

To explore the influence of partitioning properties, we estimated CBATs for four hypothetical partitioning property combinations representing the range of potentially bioaccumulative chemicals in the human food chain (Kelly et al. 2007): \( \log K_{\text{OW}} \) 4 and \( \log K_{\text{OA}} \) 7.4; \( \log K_{\text{OW}} \) 5.6 and \( \log K_{\text{OA}} \) 7.2; \( \log K_{\text{OW}} \) 7 and \( \log K_{\text{OA}} \) 9.7; and \( \log K_{\text{OW}} \) 8.7 and \( \log K_{\text{OA}} \) 15.3, corresponding to the partitioning properties of \( \alpha \)-hexachlorocyclohexane, hexachlorobenzene, \( p,p' \)-dichlorodiphenyl dichloroethylene and decabrominated diphenyl ether, respectively. For these analyses we assumed a human metabolic half-life of 15 years and the 50-year emission scenario.

We evaluated the influence of model assumptions regarding age trends in lipid content and liver metabolic capacity assuming the partitioning properties of PCB-153 and the 50-year emission scenario. Specifically, we assumed a slower increase in body fat between ages 20 and 80 such that at the age of
80, the female body lipid content was only 20 kg (27% of body weight) rather than 30 kg (40% of body weight) (Figure 3.2D). Since the body burdens are lipid normalized, a lower body fat content would translate into greater body burdens. To determine if age-dependent metabolic degradation capacity as a function of liver volume could influence CBAT shape, we modified the metabolic degradation rate to be dependent on liver volume according to Alcock et al. (2000), assuming age-dependent changes in liver volume according to van der Molen et al. (1996).

3.3 Results and Discussion

PCB-153 CBATs calculated every 10 years from 1950 to 2050 for women in Sweden, derived as described above, are shown in Figure 3.4. The shape of CBATs for PCB-153 depends strongly on the year of sampling relative to the emissions profile, which peaked in 1974 in this scenario. These results highlight the gradual shifts among age groups over time and the influence of the time of sampling relative to the emission time trend, which is crucial to understanding the relation between age and PCB body burden based on cross-sectional data collected at different points in time.

A pattern common to all of the CBATs is a drop in estimated PCB-153 levels in women at age 30 when all of the women included in the simulations give birth to a child (Chapter 2). For an entirely nulliparous female population, the CBAT minimum would actually occur at age 15 (Figure S3.1). In a realistic population the effects of reproduction on PCB-153 concentrations in a cross-sectional sample would be obscured due to variation in the age of childbirth, the number of children, and breastfeeding practices.

3.3.1 CBATs During Times of Increasing Emissions.

When emissions are still increasing (i.e., 1950 – 1970), CoZMoMAN predicts the highest body burdens in females around age 5 (Figure 3.4). This reflects the combined effects of increasing prenatal exposure with each generation and the relatively low body lipid content at age 5 (Figure 3.2D). In other words, 5-year olds in a biomonitoring study conducted when emissions are increasing will have a greater body burden than 15-year olds because they were born into a more contaminated environment and because they have yet to go through a substantial growth dilution phase during their teens. The model predicts that PCB levels will decrease in women over age 40 sampled during times of increasing emissions, not because levels decrease in individuals with age, but because women who were over 40 when these cross-sectional samples were assembled were not exposed until later in life.
Figure 3.4: Comparison of CoZMoMAN-generated cross-sectional body burden age trends for PCB-153 for cross-sectional biomonitoring studies conducted every ten years between 1950 and 2050 assuming the emission scenario in Figure 3.2A.

3.3.2 CBATs During Times of Decreasing Emissions.

The age at which the maximum body burden occurs in a CBAT during times of decreasing emissions depends upon the amount of time elapsed since the emissions peaked. That is, the age at which the maximum body burden occurs in adults will increase as the time between the peak of emissions and cross-sectional sampling increases (Figure 3.4). In CBATs representing the period after emissions have completely ended there is a rapid increase in body burden with age because the contaminated population is older and exposure is relatively low in younger members of the population. The Michigan Fish-eater Cohort, which is one of the few longitudinal data sets reported in the literature (Tee et al. 2003), illustrates this effect. In 1980, serum PCB concentrations were greatest for individuals aged 50 to 59. Ten years later, serum PCB concentrations were highest for the same group of people, who were now 60 to 69 years old. This supports our interpretation that the trend of increasing body burden with age observed in cross-sectional PCB biomonitoring studies is due to an emissions-related cohort effect rather than an age-dependent decline in metabolism (Ahlborg et al. 1995; Fangstrom et al. 2005) or bioaccumulation (Fernandez et al. 2008; Hardell et al. 2010; James et al. 2002; Naert et al. 2006).
3.3.3 Comparison of Measured and Predicted CBATs for PCBs.

Our model results suggest that the CBAT reported by Finklea et al. (1972) based on a population from South Carolina, USA sampled in 1968 – which is the only CBAT that did not show a clear relationship between age and PCB body burden (Fig. 1D) – is consistent with expectations given that the population was sampled at a time close to the peak in PCB emissions. The CBAT from Finklea et al. (1972) is consistent with the CBAT estimated by CoZMoMAN for 1980. In contrast, almost all other CBATs shown in Figure 3.1 show increasing body burdens with age, which is consistent with the CoZMoMAN predicted CBATs for the time periods when these samples were collected, which in most cases were more than 30 years after the PCB emission peak in the early 1970s. Biomonitoring studies focused on adults would not resolve the peak in body burden that CoZMoMAN predicts for children. However, a survey of PCBs in children from the Faroe Islands in 1986-1987 revealed that the serum concentrations of PCBs in 7 year olds were 2-3 times greater than those in 14 year olds and 2-8 times greater than concentrations measured in newborn cord blood (Barr et al. 2006). Results of the GerES IV (2003-2006) study of German children indicated that children aged 7 to 8 had 20% greater PCB serum concentration than 12 to 14 year olds (Becker et al. 2008). These studies support the influence of growth dilution on the relation between age and PCB body burden.

3.3.4 Sensitivity Analyses.

Of the parameters evaluated using sensitivity analyses, the metabolic half-life in humans had the greatest influence on CBAT shape (Figure 3.5). For sampling times before the peak in emissions ($t_{\text{max}}$), the human metabolic half-life has little influence on CBAT shape. After the peak in emissions (years > $t_{\text{max}}$), the CBAT shape is similar for all sampling times when the half-life is 15 years or longer, but for chemicals with half-lives of 3-5 years, the transition between CBAT shapes over time is delayed by 10 to 20 years relative to substances with a half-life of 15 years. Over the long term, the same CBAT shapes are observed for chemicals with a half-life of 3 years as for chemicals with a half-life of 15 years. However, for a chemical with a half-life of 1 year, the CBAT shape is always the same regardless of sampling time, and only the relative intensity of the maxima changes over time (Figure S3.2).
Figure 3.5: Comparison of CoZMoMAN-generated cross-sectional body burden age trends for chemicals with log $K_{ow}$ 7 and log $K_{oa}$ 9.5 and a human metabolic half-life of 1 year, 3 years, 5 years, 15 years, and 50 years assuming a bell-shaped emission scenario lasting 50 years. Notice that the y-axis scale of the top panels is smaller than the scale in the bottom panels.

The recent work by Ritter et al. (2011) showed that CBATs could be used to derive information on the intrinsic human elimination half-life. Observations from the present analysis suggest that the method described by Ritter et al. to derive such half-lives from CBATs will work better late in the emissions cycle ($t > t_{max} + 20$ years) and when the half-life is between 1 and 10 years. The CBAT shapes for chemical half-lives outside of this regime will be very similar (Figure 3.5).

For all emission scenarios, the CBATs remained unchanged with time for the human metabolic half-life of 1 year (Figure S3.3). For the case of a 100-year emission scenario, the CBAT shape was also unchanged with time for a 15-year human metabolic half-life but showed time-dependent behaviour with a 50-year human metabolic half-life. When the metabolic half-life was 15 and 50 years, the 30- and 50-year emission periods yielded similar CBAT trends at each time point. From these calculations, we hypothesize that for chemicals with a human metabolic half-life similar to or shorter than the emissions half-life, the CBAT shape will be constant over all time points. For chemicals with human metabolic half-life greater than the emissions half-life, temporal transitions in CBAT shapes will be observed.

Within the investigated range of partitioning properties, virtually no impact on the shape of the CBATs was observed (Figure S3.4). This is because the longitudinal age-body burden curves for individuals do
not change significantly except for the magnitude of bioaccumulation. This finding is supported by the work of Arnot et al. (2010) who observed similar age patterns in human dietary intake of organic contaminants with a wide range of partitioning properties. Model assumptions regarding lipid content and liver metabolic capacity also had little impact on CBAT shapes (Figure S3.5).

3.3.5 CBATs of PCB Mixtures.

PCB concentrations in human tissues are often reported as the sum of several detected congeners (e.g. dioxin-like PCBs). Each congener will have a unique metabolic half-life, partitioning properties, and possibly also emissions profile. To determine how the combination of these traits for individual congeners influences the CBATs of PCB mixtures, we calculated a CBAT for the combination of PCBs 52, 101, 118, 138, 153, and 180 (Table S3.1 and Figure S3.6) for realistic Swedish emissions scenarios (Breivik et al. 2010). From this calculation, it is apparent that the CBAT shapes for the PCB mixture resemble closely the CBATs of the congeners with the longest metabolic half-life (Figure S3.6B), because those congeners contribute the most to the PCB body burden (Figure S3.6A).

3.3.6 How do the CBATs of PCBs and PBDEs compare?

We propose that the CBAT shapes in any given sampling year should look significantly different for PCBs and PBDEs based solely upon differences in emissions trends over time and human metabolic half-lives. Whereas the production of PCBs has been banned globally for several decades (Breivik et al. 2007), the production of some PBDE congeners is still increasing in some areas (Ward et al. 2008). As such, current cross-sectional biomonitoring efforts for PBDEs will yield CBATs with different shapes than CBATs for PCBs. In addition, the apparent elimination half-life of PBDEs is estimated to be several months (Thuresson et al. 2006) compared with elimination half-lives in excess of a decade for highly bioaccumulative PCBs (Brown et al. 1989). CBATs estimated for chemicals with human metabolic half-lives of 1 year and 15 years are therefore relevant to a comparison of CBATs for PBDEs versus PCBs (Figure 3.5). At $t_{\text{max}}$-20 years, CBATs for both compounds suggest that children at age 5 have greater body burdens than either infants or adult women, consistent with previously reported PBDE biomonitoring studies (Sjodin et al. 2008; Toms et al. 2008, 2009). At $t_{\text{max}}$+20 years, the CBAT for a chemical with a human metabolic half-life of 1 year still presents the same age trends, but the CBAT for a chemical with a 15 year metabolic half-life shows increasing cross-sectional body burdens from 10 to 50 years of age. Since PBDEs are better represented by a metabolic half-life of 1 year than
15 years (Thuresson et al. 2006), we propose that CBATs for PBDEs will exhibit the same CBAT shape regardless of the sampling year. In addition, the shape of CBATs for PCBs and PBDEs would only be similar if they were both based on population cross-sections sampled during periods of increasing emissions.

3.4 Conclusions

Our analysis of CBAT shapes and the influence of various model parameters suggests that the most important influences on the shape of cross-sectional time-trends are the temporal relationship between the emissions scenario time trend and the biomonitoring sample collection period, and the human metabolic half-life. Although the extent of bioaccumulation of a chemical is also influenced by its partitioning properties, the length of the emissions period, and lipid content and liver clearance rates in the population, the age trend in cross-sectional studies is largely unaffected by these factors. Our results suggest that the observed increase in PCB body burdens for elderly individuals is consistent with an emissions-related cohort effect, and that differences in CBAT shapes for PCBs and PBDEs are consistent with differences in human metabolic rates and emission scenarios.

3.5 Supporting Information

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3 We acknowledge that this discussion implies that all PBDE congeners have short biotransformation half-lives. We would like to amend this by clarifying that at this point the biotransformation half-lives for PBDEs are not well known and may actually be longer than one year. Furthermore, it is likely that not all congeners have similar biotransformation half-lives but rather that significant differences between congeners exist.
Figure S3.1: CoZMoMAN-generated cross-sectional body burden age trends for PCB-153 assuming sampling of *nulliparous* Swedish females in contrast to CBATs for women who give birth to a single child at age 30. \( t_{\text{max}} \) refers to year of maximum atmospheric emissions.

Figure S3.2: CoZMoMAN-generated cross-sectional body burden age trends for a hypothetical chemical with \( \log K_{\text{OW}} \) 7 and \( \log K_{\text{OA}} \) 9.5 emitted to the atmosphere over a 50 period with a human metabolic half-life of 1 year. \( t_{\text{max}} \) refers to year of maximum atmospheric emissions.
Human metabolic half-life of 1 year

Human metabolic half-life of 15 years
Figure S3.3: Comparison of CoZMoMAN-generated CBATs for a chemical with log $K_{OW}$ 7 and log $K_{OA}$ 9.5 and a human metabolic half-life of 1 year, 15 years or 50 years using bell-shaped emission scenarios lasting 30, 50, and 100 years. $t_{\text{max}}$ refers to year of maximum atmospheric emissions.
Figure S3.4: Comparison of CoZMoMAN-generated cross-sectional body burden age trends for four chemicals with differing partitioning property combinations and a human metabolic half-life of 15 years using a bell-shaped emission scenario lasting 50 years. $t_{\text{max}}$ refers to year of maximum atmospheric emissions.
Figure S3.5: Comparison of CoZMoMAN-generated cross-sectional body burden age trends for a chemical with log $K_{OW}$ 7 and log $K_{OA}$ 9.5 (i.e. those of PCB-153) and a human metabolic half-life of 15 years using a bell-shaped emission scenario lasting 50 years while making different model assumptions concerning the lipid content with age (Figure 3.2D) and the metabolizing capacity of the liver with age. $t_{max}$ refers to year of maximum atmospheric emissions.
Figure S3.6: CoZMoMAN-generated CBATS for 6 PCB congeners (52, 101, 118, 138, 153 and 180) and their sum using congener-specific partitioning and degradation properties (see Table S3.1 for details) and realistic emission scenarios for Sweden (Breivik et al. 2010). (A) CBATs are plotted as lipid-normalized body burdens. (B) CBATs are plotted as population-normalized body burdens.
Table S3.1: Summary of model parameters used in the various CoZMoMAN simulations

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<td>Figure S3.6</td>
</tr>
<tr>
<td></td>
<td>6.3 (PCB-101)</td>
<td>8.8 (PCB-101)</td>
<td>0.4 f</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.7 (PCB-118)</td>
<td>9.4 (PCB-118)</td>
<td>6.3 f</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>7.2 (PCB-138)</td>
<td>9.7 (PCB-138)</td>
<td>8.7 f</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 (PCB-180)</td>
<td>10.2 (PCB-180)</td>
<td>231 f</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Properties according to Li et al. 2003.
b Representative of α-hexachlorocyclohexane (Xiao et al. 2004).
c Representative of hexachlorobenzene (Shen and Wania 2005).
d Representative of p,p'-dichlorodiphenyl dichloroethylene (Shen and Wania 2005).
e Representative of decabrominated diphenyl ether (Wania and Dugani 2003).
Chapter 4
A methodology for evaluating the influence of diets and intergenerational dietary transitions of historic and future human exposure to persistent organic pollutants in the Arctic

Cristina L. Quinn, James M. Armitage, Knut Breivik, Frank Wania


Contributions: The models used for this research project were previously developed and published. Modelling calculations pertaining to global climate change and global fate and transport were performed by J. Armitage. Additional emissions scenario data was provided by K. Breivik. C. Quinn performed modelling pertaining to bioaccumulation and interpreted model output. C. Quinn wrote the sections of the manuscript pertaining to food chain bioaccumulation and J. Armitage wrote the sections of the manuscript pertaining to climate change and environmental fate and transport, both under the guidance of F. Wania. Revisions and responses to reviewers’ comments were performed by both C. Quinn and J. Armitage under the guidance of F. Wania.

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4.1 Introduction

It is well-established that despite being located far from most major emission sources of organic contaminants, wildlife and humans inhabiting the Arctic environment exhibit much higher body burdens of environmental contaminants of concern (e.g. organochlorine pesticides, polychlorinated biphenyls (PCBs)) than might otherwise be anticipated (AMAP 1998, 2004; Donaldson et al. 2010; NCP 1997, 2003). Human subpopulations harvesting extensively from the marine environment are particularly susceptible to exposure to many of these contaminants, collectively known as Persistent Organic Pollutants (POPs), especially those which tend to consume fatty tissues (e.g. blubber, skin) as part of their traditional diet (Donaldson et al. 2010; Dewailly et al. 1993, 1994; Undeman et al. 2010). Extensive research efforts, in the form of monitoring campaigns (e.g. AMAP 1998, 2004; Donaldson et al. 2010; NCP 1997, 2003) and modelling assessments (Czub et al. 2008; Brown and Wania 2008; Kelly et al. 2007) have been dedicated to more comprehensively understanding the chemical fate, long-range transport and bioaccumulation underlying these observations and to elucidating the spatial and temporal aspects of these source-receptor relationships. One clear consensus from this body of research is that exposure to organic contaminants via the diet is a key driver of body burdens and the associated health risks for people inhabiting the Arctic region.

More recently, a focus on the potential implications of global climate change (GCC) for ecological and human exposure to organic contaminants has emerged in the scientific literature (Armitage et al. 2011; Borgå et al. 2010; Kraemer et al. 2005; Macdonald et al. 2005; Noyes et al. 2009). Modelling studies focused on the potential implications of GCC on chemical fate on a global-scale (Lamon et al. 2009) indicate that the influence of temperature, precipitation and atmospheric/oceanic circulation patterns may have only a modest influence on the occurrence of POPs in the Arctic (e.g. modelled concentrations in air within a factor of 2 compared to baseline 20th century conditions, given the same emission scenario). An additional factor to consider with respect to human exposure to organic contaminants is that people living in the Arctic have experienced increasing access to and consumption of imported food items over the past several decades, a trend that is likely to continue in the future (Deutch et al. 2007; Kuhnlein et al. 2004). While the dietary transitions observed to date have been related to poorer overall nutritional status and increased obesity (e.g. Body Mass Index) in these communities (Deutch et al. 2007; Kuhnlein et al. 2004), they also have implications for both historic and future exposure to organic contaminants. In addition, dietary shifts within and between cohorts may also contribute to the substantial variability typically observed in human tissue residues. For
example, PCB-153 plasma concentrations in Inuit mothers aged 15-45 from the Nunavut-Baffin region ranged from 33 to 335 ng/g lipid in 1997 and from 6 to 276 ng/g lipid in 2005 but exhibited no strong age-dependence (Donaldson et al. 2010). Similarly, in 2002, Greenlandic Inuit women, aged 19-39, had PCB-153 serum concentrations of 11-1400 ng/g lipid depending on the community (Jönsson et al. 2005). With respect to GCC, the extent to which subpopulations shift away from the consumption of locally-harvested food represents the extent to which these people become decoupled from any potential changes in exposure to POPs related to the environmental changes already occurring (e.g. reduced sea-ice cover) in the Arctic and those projected to occur in the future. As discussed recently (Undeman et al. 2010; Armitage et al. 2011) the dietary transition may overwhelm any other potential effects of GCC on human exposure to organic contaminants in this region, especially if it involves a substantial reduction in the consumption of marine mammals (e.g. ringed seal).

The purpose of this study is threefold: 1) to demonstrate that models are a suitable tool for evaluating the relative importance of the various factors that potentially influence human body burdens (e.g. GCC, dietary transitions, variable emissions); 2) to assess the potential influence of intergenerational dietary transitions on historic human exposure to POPs in the Arctic and to predict future exposure trends; and 3) to determine the type of information required for quantitative evaluation of dietary transition effects. As such, modelled body burdens are used primarily to characterize the potential variability in human exposure due to different dietary consumption patterns (i.e. differences between individuals at any given time) and the relative change in exposure over time that can be attributed to emission patterns compared to shifts in dietary consumption patterns (at the community level).

4.2 Materials and Methods

4.2.1 Conceptual Approach.

Assessing the potential implications of intergenerational dietary transitions on human exposure to organic contaminants requires an integrated approach combining chemical fate and bioaccumulation modelling. Here we combine the fate/transport model GloboPOP (Wania and Mackay 1995, Wania and Su 2004) with two food web bioaccumulation models, ACC-Human (Czub and McLachlan 2004) and Arctic ACC-Human (Czub et al. 2008), which have been evaluated against empirical data for PCBs in the past (see Czub et al. 2008, Breivik et al. 2010 and the Supplementary Information for Armitage et al. 2011). For example, a similar approach was previously shown to be capable of reproducing monitoring data for 7 different PCB congeners to within a factor of 2 to 4 in most compartments of the
Baltic Sea Environment (Breivik et al. 2010). The following elements need to be developed and synthesized: i) estimated emission rates over time in areas of interest (e.g. regional to global-scale), ii) ambient environmental levels over time corresponding to the emission scenario, iii) human food web bioaccumulation models representative of the major dietary items of interest (e.g. air-grass-cow; water-phytoplankton-zooplankton-Arctic cod-seal), iv) scenarios defining the composition of different diet types (e.g. 100% traditional/locally-harvested vs. 100% imported food items), v) scenarios defining the timing and nature of intergenerational dietary transitions. Once the overall scenarios were defined (i.e. accounting for considerations outlined above), simulations were conducted to calculate human body burden as a function of time (i.e. longitudinal body burden age trends). Details on each component of the scenario development for PCB-153 are presented in the following sections.

4.2.2 Emission Scenario.

Total global emissions of 22 individual PCB congeners have been estimated for the period 1930–2100 and made available at a spatial resolution of 1° x 1° (Breivik et al. 2007). Modifications to these emission estimates have been made recently, based on a comparison of modelled and empirical human tissue residues in the western parts of the Baltic region which suggests that emissions prior to the 1980s may have been substantially underestimated (Breivik et al. 2010). The revised emission estimates for PCB-153 are presented in the Supplementary Information (Section 2).

4.2.3 Ambient Environmental Levels.

To generate ambient levels of PCB-153 in the global environment over time, the emission inventory presented in the Supplementary Information (Section 2) was used as input to GloboPOP assuming that these emissions occur 100% to the lower atmosphere. Physical-chemical property values and a brief summary of recent changes to the GloboPOP model are provided in the Supplementary Information (Section 3 and 4, respectively). As environmental degradation half-lives (HLs) in general and in soils in particular are highly uncertain, two sets of simulations were conducted, i) the default scenario with a degradation HL in soil (25 °C) of approximately 62 years (Wania and Su 2004) and ii) an alternate scenario with a degradation HL in soil ten times lower (Supplementary Information, Section 3). Imported food items were assumed to originate from either the N. Temperate zone (38–54 °N) or the Boreal zone (54–64 °N) of the model (Wania and Mackay 1995) and hence the food web bioaccumulation models were driven by ambient environmental levels calculated for these regions. The
Arctic food web bioaccumulation model was driven by the ambient environmental levels in the N. Polar zone (64–90 °N) (Figure S4.2).

4.2.4 Human Food Web Bioaccumulation Models.

Inuit women were chosen as the target receptor for these simulations. All women were the third born child to a 30-year old mother and are assumed to give birth themselves to single children at the age of 20, 25 and 30 in accordance with the 2001 estimate of 3.4 children per Inuit woman in Canada (Indian and Northern Affairs Canada). Each child was breastfed for 6 months each as recommended by Heath Canada (Health Canada 2004). All children are born on the mother’s birthday. The body burden of a 30-year old woman is represented by their body burden immediately after the birth of the third child. Children are assumed to be at equifugacity with the mother at time of birth. However, because the infant is assumed to have a higher lipid content (i.e. sorptive capacity), parturition represents a loss term with respect to the mass and concentration of PCBs in the mother. Breast milk is also assumed to be at equifugacity with the mother and the mass of chemical transferred from the mother to infant is a function of the flow rate and assumed lipid content of the breast milk.

In order to simulate intergenerational dietary transitions in the Inuit population, a modified version of the Arctic ACC-Human model was created. Specifically, imported dietary options of milk, beef and fish were added to the existing food basket consisting of Arctic cod, seal blubber, and seal meat. The contaminant concentrations for imported food items were calculated using the model ACC-Human and the ambient environmental levels derived as explained above. In this approach, an Inuit woman can potentially consume foods from three different food webs: the Arctic marine food web, an imported marine food web and an imported terrestrial food web. Note that marine food webs are primarily driven by water column contamination (i.e. freely-dissolved concentration) whereas the terrestrial food webs (agricultural) are primarily driven by levels in the atmosphere. Figure 4.1 illustrates the interrelationships within each of these three food webs. Realistically, Arctic terrestrial food webs (e.g. air-lichen-caribou) also contribute substantially to the diet of some Arctic communities. However, our model is not currently parameterized for such a food web as the application is focused on coastal communities harvesting predominantly from the marine environment. Future evaluation of intergenerational dietary transition including an Arctic terrestrial food web would be a valuable contribution.
Figure 4.1: Schematics of food web relationships in the dynamic bioaccumulation model. Lipid contents of each organism are indicated in brackets. *indicates that the lipid content of the organism is seasonally variable (Czub and McLachlan 2004). The percent composition of prey items in the diet are indicated over the arrows.\(^4\)

### 4.2.5 Composition of Different Diet Types.

Five possible diets were considered for these simulations, as summarized in Table 4.1. Two boundary cases are defined, the first being a 100% traditional diet (seal meat, seal blubber, Arctic cod) and the second a 100% imported diet (imported beef, milk and fish). For a 25 year-old woman, the traditional daily diet is comprised of 33 g seal blubber, 50 g of seal meat and 70 g of Arctic cod (in accordance with Cameron and Weis 1993\(^5\)) whereas the imported daily diet is comprised of 38 g beef, 100 g of

\(^4\) Arrows point from predator to prey.

\(^5\) The actual fish species reported by Cameron and Weis is Arctic Char. Here we use Arctic Cod in its place since they have been shown to have similar PCB concentrations (Hoekstra et al. 2003)
milk and 70 g of imported fish (cod) (in accordance with Delormier and Kuhnlein 1999). A modified blubber-free traditional diet consists of 83 g of seal meat and 70 g of Arctic cod. The overall amount of food consumed at any given age is scaled based on Moser and McLachlan (2002). Three hypothetical diets representing intermediaries between the traditional and imported diet are also defined: i) high traditional-low imported (HTLI), ii) medium traditional-medium imported (MTMI) and iii) low traditional-high imported (LTHI) (Table 4.1). All traditional and imported food items are consumed but the proportions sequentially shift from dominance of the traditional diet to dominance of the imported diet.

Table 4.1: Hypothetical dietary consumption patterns for Inuit populations with access to both traditional and imported food options.

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>Traditional only</th>
<th>High traditional-low imported (HTLI)</th>
<th>Medium traditional-medium imported (MTMI)</th>
<th>Low traditional-high imported (LTHI)</th>
<th>Imported only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of traditional items in diet relative to fully traditional diet</td>
<td>100%</td>
<td>75%</td>
<td>50%</td>
<td>25%</td>
<td>0%</td>
</tr>
<tr>
<td>Proportion of imported items in diet relative to fully imported diet</td>
<td>0%</td>
<td>25%</td>
<td>50%</td>
<td>75%</td>
<td>100%</td>
</tr>
</tbody>
</table>

4.2.6 Timing and Rate of Intergenerational Dietary Transitions.

As noted above, dietary consumption patterns in Arctic communities have changed significantly over the last few decades. A recent survey of Canadian First Nation, Dene/Métis, and Inuit by Kuhnlein et al. (2004) concluded that only ~22% of dietary energy is consumed from traditional food sources. This survey also demonstrated that individuals aged 60+ consume approximately twice as much traditional food as those in the 20-40 year old age group. Scenarios defining potential intergenerational dietary transitions are defined in Table 4.2. The transitions were calculated by assigning different diet types to each age group with the younger cohorts consuming a more imported diet, the older cohorts consuming a more traditional diet, and all cohorts in between consuming diets with decreasing amounts of traditional food. We assume that any given individual does not change their diet within their lifetime.
but rather that each new cohort, at age 0, consumes a less traditional diet than the previous cohort and continues this diet throughout their lifetime.

Table 4.2: Dietary consumption types by age group in the year 2010 for two dietary transition rates and five different starting years.

<table>
<thead>
<tr>
<th>Age</th>
<th>Diet Transition Start Year - Rate 1 (rapid)</th>
<th>Diet Transition Start Year - Rate 2 (slow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0⁶</td>
<td>Imported</td>
<td>Imported</td>
</tr>
<tr>
<td>10</td>
<td>LTHI</td>
<td>Imported</td>
</tr>
<tr>
<td>20</td>
<td>MTMI</td>
<td>LTHI</td>
</tr>
<tr>
<td>30</td>
<td>HTLI</td>
<td>MTMI</td>
</tr>
<tr>
<td>40</td>
<td>Traditional</td>
<td>HTLI</td>
</tr>
<tr>
<td>50</td>
<td>Traditional</td>
<td>Traditional</td>
</tr>
<tr>
<td>60</td>
<td>Traditional</td>
<td>Traditional</td>
</tr>
<tr>
<td>70</td>
<td>Traditional</td>
<td>Traditional</td>
</tr>
<tr>
<td>80</td>
<td>Traditional</td>
<td>Traditional</td>
</tr>
</tbody>
</table>

Because the dietary transition may have occurred at different historical time periods and at different rates in different Arctic communities, we define a ‘rapid’ and ‘slow’ dietary transition. The “rapid” dietary transition assumes that with each cohort (born 10 years apart) an additional 25% of their diet is imported (Table 4.2), with the transition from a completely traditional to a completely imported diet taking 40 years. For example, if the 50-year old person consumes a traditional diet, the 40-year old consumes an HTLI diet, the 30-year old consumes a MTMI diet, the 20-year old consumes a LTHI diet, and the 10-year old consumes an imported diet. In the “slower” dietary transition, the diet change

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6 A person at age 0 is breastfed for the first 6 months of life. After breastfeeding, the infant themselves consumes a diet of the type indicated.
occurs every 20 years instead of every 10 years, so the whole process takes 70 years to complete (Table 4.2). The effect of dietary transition timing was explored for five starting years (SY1940, SY1950, SY1960, SY1970, and SY1980) for both transition rates (Table 4.2). In all cases, the observation year is 2010. Thus, for a starting year of 1980 (SY1980), only 4 newborn cohorts would have undergone the transition to a less traditional diet by the year 2010. In the case of the rapid diet transition, 4 cohorts is sufficient for a full transition from traditional diet to imported diet because there are only 4 diet steps at a rate of one step per cohort. For the slow dietary transition of one diet step per 2 cohorts, a start year of 1980 would mean that the newborn cohort is still consuming an MTMI diet in 2010. Table 4.2 outlines the type of diet consumed for each age group as observed in the year 2010 for each of the modelled scenarios.

In reality, not every member of the same cohort will have the same dietary composition and the dietary consumption for a specific age group is better represented by a frequency distribution. In order to illustrate the effect of the dietary variability within an age group over time, we used two hypothetical frequency distributions for the dietary intake of Inuit women aged 30 from the years 1980 to 2020. The cohort distribution represents a population where every 30-year old women consumes the same diet type such that in 1980 they are consuming a traditional only diet and, by 2020, an imported only diet. This scenario is identical to the SY1960-rapid dietary transition. The mixed distribution is more realistic and assumes a more gradual transition with a range of dietary habits in each cohort. For example, among the 30-year old women of 1980, 20% were assumed to consume a traditional diet, 25% an HTLI diet, 30% an MTMI diet, 15% an LTHI diet and only 10% consume an imported diet. Since the amount of traditional food consumption decreases over time while imported food consumption increases, by 2020, no women consume a completely traditional diet, 10% consume an HTLI diet, 15% an MTMI diet, 35% an LTHI diet and 40% consume an imported diet. These distributions correspond to a population that consumes 0-100% and 0-75% of their fat from traditional food sources in 1980 and 2020 respectively. These ranges encompass the upper and lower limits of the food recall values reported by Delormier and Kuhnlein (2004) of 18±14% of fat to 53±28% of fat for Cree women in Quebec in 1994-1995 depending on the age of the individual and season surveyed. Using these frequency distributions and the calculated body burdens for individuals consuming each

7 Although the Cree diet does not consist of the marine mammals assumed here to make up the traditional diet, these values provide a general approximation of modern day traditional diet consumption in northern communities.
diet type, we were able to calculate an average body burden for 30-year old Inuit women at each of these time points as well as the expected variability resulting from the dietary distribution pattern.

Dietary transitions were evaluated by comparing the lipid-normalized body burden of a 30-year old female over time under various dietary transition scenarios. By focussing on a single age group, the influence of longitudinal changes in body burden of an individual (i.e. age specific food consumption volumes, time since childbearing, etc.) are eliminated (see Chapter 2).

4.3 Results and Discussion

4.3.1 Simulated Long-term Human Exposure to PCB-153 (1930–2050).

Using the integrated modelling approach, the outputs from the three different models (GloboPOP, ACC-Human, and Arctic ACC-Human) were combined to calculate the body burdens of women living in the Arctic. Integration of these three models is demonstrated schematically in Figure 4.2. In a dynamic simulation, the concentration profile of the environmental compartments and the food organisms living in these compartments (Figure 4.2) will closely reflect the variability in the emissions profile over the period when primary emissions dominate. Since food consumption is the main route of exposure to humans for PCBs, variable concentration levels in the diet results in variable body burdens in humans. In particular, under non-steady emissions, the contamination of an individual will depend upon the age at which exposure began and will reflect the variability in the emissions (Chapter 2). This is evident in Figure S4.1 where the PCB-153 lipid normalized concentrations predicted for Inuit woman born every twenty years between 1930 and 2030 under time-variant emissions are displayed (MTMI diet) using the default scenario for degradation half-lives in the environment. The modelled longitudinal body-burden age trends for all five diet types yield similar trends to the MTMI diet and differ only in the absolute concentrations.

In Figure 4.3, we compare the body burdens of 30-year old women over time according to each diet type without a diet transition (coloured lines in Figure 4.2B, 4.2C, 4.2E, 4.2F, 4.2H and 4.2I). Without dietary changes in time, the model predicts that the PCB-153 body burden of a 30-year old Inuit woman in 2020 would be a factor of 6-13 times lower than in 1980 (coloured lines) (e.g. 6000 ng/g lipid in 1980 compared to 900 ng/g lipid in 2020 for a woman consuming a traditional diet; 400 ng/g lipid in 1980 compared to 30 ng/g lipid in 2020 for a woman consuming an imported only diet). These declines in body burden are a result of decreased global emissions but also reflect different rates of response in ambient environmental levels in each model zone (i.e. the N. Polar zone is relatively more
contaminated in 2020 compared to 1980 than the N. Temperate zone) and response rate/exposure history of organisms in the food-web, particularly the long-lived seals. Regardless of the sampling year, body burdens for 30-year old women consuming a traditional diet are 1.5 and 2.0 orders of magnitude greater than an imported diet from the N. Temperate and Boreal zone, respectively. This is not surprising since the traditional diet includes seal blubber which is approximately 100 and 500 times more contaminated on a lipid-weight basis than beef originating in the N. Temperate and Boreal zone, respectively (Figure 4.2) and 3 and 5 times more contaminated than Temperate and Boreal cod muscle. The elevated concentrations in seal blubber reflect bioaccumulation along the relatively long aquatic food chain seals feed from (i.e. high trophic position) and the high dietary absorption efficiencies and biomagnification factor calculated for this marine mammal. In other words, the relatively low ambient environmental levels in the Arctic zone are counteracted by the properties of the organism and its food chain that favour contaminant amplification (Undeman et al. 2010; Czub et al. 2008). Seal meat and Arctic cod have contamination levels lower than imported cod and similar to imported temperate beef (Figure 4.2). Consequently, a traditional diet which replaces seal blubber with seal meat or Arctic cod would serve to reduce PCB uptake (but perhaps increase mercury and methyl mercury intake) (Figure S4.6) while still consisting of locally harvested items with high nutritional value (see Supporting Information Section 4.6 for additional information on seal blubber assumptions). The body burdens for 30-year old women consuming this modified blubber-free traditional diet, shown in Figure 4.4, are 150 (in 1980) to 350 (in 2020) times lower than in those eating the traditional diet. Imported food items from the Boreal zone are up to 6 times less contaminated than food items from the N. Temperate zone because the latter receives a much higher proportion (~ 60% vs. 5%) of the global emissions and therefore has substantially higher ambient environmental levels over most of the simulation period. This model output demonstrates that substantial variability in human exposure to organic contaminants can be introduced by the geographic origin of the imported food items. Such considerations could be applied to the analysis of biomonitoring studies, assuming data characterizing diet in this way are available.
Figure 4.2: Schematic overview of modelling approach employed to assess the potential influence of intergenerational dietary transitions on human exposure to PCB-153. Emission estimates for PCB-153 over the period 1930–2050 (A) are used as input to a global contaminant fate model (GloboPOP) in order to generate ambient environmental concentrations in exposure-relevant media in the N. Temperate, Boreal and N. Polar (Arctic) zones (B, C). The ambient environmental concentrations are then used as input to bioaccumulation models (ACC-Human/Arctic ACC-Human) representative of human food chains in these three regions. These calculations yield concentrations in various food items (‘imported’ vs. ‘traditional’) assumed to be available year-round to consumers in the Arctic (D, E).
Figure 4.3: The consumption frequency for each of the five diets for 30-year old Inuit women from 1980-2020 according to (A) cohort distribution SY1960-Rapid, (C) cohort distribution SY1960-Slow, and (G) a mixed distribution. The PCB-153 body burden of a population of 30-year old Inuit women from 1980-2020 according to cohort distributions SY1940-Rapid through SY1980-Rapid for an imported diet originating from (B) N. Temperate regions, and (C) Boreal regions, The PCB-153 body burden of a population of 30-year old Inuit women from 1980-2020 according to cohort distributions SY1940-Slow through SY1980-Slow for an imported diet originating from (E) N. Temperate regions,
and (F) Boreal regions, The average PCB-153 body burden of a population of 30-year old Inuit women from 1980-2020 according to the mixed distribution for an imported diet originating from (H) N. Temperate, and (I) Boreal regions. Note (1): The body burdens for a population of 30-year old Inuit women who all consume the same diet are overlaid for comparison. Note (2): In panel H and I, the median and lower quartile values for 2000, 2010 and 2020 are superimposed on each other.

Figure 4.4: The PCB-153 body burden predictions for a population of 30-year old Inuit women from 1980-2020 where each cohort in the population consumes an identical diet of traditional, imported temperate, imported boreal or traditional (blubber-free) food.

4.3.2 Potential Influence of Intergenerational Dietary Transitions.

Figures 4.3B and 4.3C illustrate the combined effects of changing emissions and the rapid cohort dietary transitions (Figure 4.3A) outlined in Table 4.2 on the body burden of 30-year old Inuit women over time. For the rapid dietary transition SY-1960, the body burden of the 30-year old female cohort declines by 2.3 and 2.9 orders of magnitude over the same 40 year period (1980-2020) assuming consumption of food imported from the N. Temperate and Boreal zone, respectively. In other words, the dietary transition could cause up to an additional 50-fold reduction over a forty year time period, in addition to the 6 to 13-fold reduction driven by declining emissions. The time period over which this body burden decline is observed in the population is dependent on the start year of the dietary transition. For a rapid transition that occurred early, i.e. SY1940, the 30-year old cohorts would be consuming a purely imported diet by the year 2000 and therefore exhibit much lower body burden. For a population that did not begin a dietary transition until 1970, body burdens representing the imported food chain would not be observed in the 30-year old cohort until 2030.
These predictions are based on the rather quick dietary transition of one diet step per cohort. If the dietary transition was more gradual, as in our slow dietary transition examples, then the body burden declines over time could be less obvious (Figure 4.3E and 4.3F). For SY1960-slow, the dietary transitions are only predicted to yield body burden declines for 30-year old females of 1 order of magnitude from 1980 to 2020 or up to a 2-fold decrease in addition to that expected from emissions alone. This is much less than the 150 fold reduction predicted for the rapid dietary transition for the same population over the same time period and highlights the importance of understanding both the timing and rate of dietary transitions for the accurate interpretation of biomonitoring studies.

Of course the assumption in the calculations for Figure 4.3B, 4.3C, 4.3E and 4.3F is that every person in a specific age group consumes the exact same diet. In reality, each cohort will be composed of individuals with a wide range of diets and it is the distribution of these diets which shifts increasingly towards more imported food items over time. Figures 4.3H and 4.3I illustrate the changing body burden of 30-year old Inuit women over time assuming the mixed dietary consumption distributions shown in Figure 4.3G. According to this more realistic dietary transition, the average body burdens for the 30-year old female cohort in 2020 are predicted to be 12 times lower than those in 1980 for a transition towards either imported food source, i.e. the dietary transition could cause an additional two-fold decrease. Figure 4.3H and 4.3I also demonstrate that there is likely to be more variability within an age group than between age groups unless a very fast dietary transition is occurring (as in Figure 4.3B and 4.3C). Therefore, the distribution of dietary habits is likely to be an important source of variability seen in measured body burdens.

To our knowledge, there are currently no studies that report both the dietary profiles and POPs levels in a single population (i.e. the same region) at more than one time point thus making it impossible to rigorously evaluate these predictions against historical measurements. However, reported values for Inuit populations tend to range over 2 orders of magnitude. For example, Tofflemire et al. (2000) measured PCB-153 body burdens of 6.5-105 μg/kg plasma lipid in Inuvialuit women aged 15 to 37 in 1998-1999. Armstrong et al. (2007) observed a slightly broader range for the Inuvialuit population at 0.59-152 μg/kg plasma lipid for women aged 17-38 in the 2005-2006 follow-up study. In Greenland, Jönnson et al. (2005) detected PCB-153 concentrations ranging from 11-1400 μg/kg, depending on the community, for women aged 19-38. Although there are many assumptions and uncertainties in the modelling exercise, we note that the variability in the biomonitoring data broadly corresponds to the variability in model output attributable to the different dietary consumption patterns assumed here (i.e.
2-orders of magnitude). Furthermore, the lower range of reported body burdens is similar to the calculations made assuming a traditional but blubber-free diet (Figure 4.4). As for body burden declines over time, Dallaire et al. (2003) reported mean PCB concentrations in umbilical cord blood from women giving birth in Nunavik in 1994 that were approximately 1.8 times higher than in 2000. This corresponds to an 8% decrease per year. For the scenario described in Figure 4.3H (i.e. shifts in mixed dietary consumption distribution), the average body burden for a population of 30-year old women consuming a mixed diet is 1.6 times greater in 1990 than in 2000. This decline corresponds to a 5% decrease per year. Clearly, the assumption of the mixed dietary distribution is more representative of actual biomonitoring data than the cohort distribution assumptions. In other words, despite the vast generalization of the model assumptions and compounding uncertainties in the various input factors, the model is still capable of yielding predictions within an order of magnitude of the empirical measurements as well as reproducing the variability range and rates of decline observed in the monitoring data.

These results highlight possible sources of the large degree of variability that is observed in human biomonitoring data from the Arctic. In particular, the geographical source of the imported diet, timing of the dietary transition, rate of the transition, and the dietary distribution over time are all paramount for estimating the contribution that dietary transitions make to the decline in human body burdens. In general, declining consumption of specific traditional food items (i.e. seal blubber) can contribute to declining body burdens in the Arctic for PCBs, although the relative influence of declining global PCB emissions and dietary changes is highly sensitive to the specific characteristics of the dietary transition for a given population. Although this study has focussed on the transition away from traditional food, it is also possible to define scenarios where a transition back to traditional food occurs. The results presented here serve to emphasize potential sources of uncertainty associated with analysing biomonitoring studies for populations undergoing a dietary transition such that future studies can attempt to address these issues.

4.3.3 Influence of Environmental Degradation Half-lives on Long-term Trends in Human Exposure.

All model output discussed in the previous sections was based on the default environmental degradation half-life scenario (Supplementary Information Section S3). The temporal trends in ambient environmental levels assuming the default and alternate degradation half-life scenarios are compared in the Supplementary Information (Section S7). The key finding is that ambient environmental levels are
only sensitive to the assumed degradation HLs once primary emissions are dramatically reduced from peak values and secondary sources (esp. revolatilization from soil) become dominant. For example, ambient atmospheric and marine levels in the North Polar zone do not begin to diverge substantially (i.e. by ≥ a factor of 2) between the two degradation half-life scenarios until after the year 2020. From 1990 to 2020, trends in the contaminant levels in the global environment shift from being determined by the temporal trend in primary emissions to being determined by the degradation half-life in soil. Accordingly, ambient levels in the global environment decline faster in the alternate HL scenario and the influence of the emission scenario on the temporal trends in human body burdens increases in relative terms in comparison to the dietary transition. The difference in model output continues to increase over time such that the global environment is more than 10 times less contaminated in the alternate HL scenario by the year 2050 (and 100 times less contaminated by 2100). Clearly, the absolute value selected for the degradation HL in soil is an important consideration for projecting future exposure to POPs if primary emission sources are exhausted during the simulation period. Additionally, it is clear that projecting trends in human exposure based on primary emission patterns alone is problematic (i.e. not valid if secondary sources dominate).

Interactions between GCC and contaminant fate, transport and bioaccumulation may also influence future exposure levels to some extent (Armitage et al. 2011; Macdonald et al. 2003, 2005). However, fate/transport model simulations published to date indicate that projected changes to temperature and precipitation result in only modest changes in output. Furthermore, projected changes under GCC scenarios often exert a competing influence, which tends to dampen the overall response. For example, increased long-range transport of hydrophobic contaminants to the Arctic environment may be countered by decreased bioavailability in the water column related to enhanced primary productivity (i.e. concentrations of suspended particulate and dissolved organic carbon) (Armitage et al. 2011; Borgå et al. 2010; Lamon et al. 2009). As illustrated here, changes in absolute level of primary emissions over time and the absolute values selected for degradation half-lives are likely to be more important with respect to simulating fate/transport in the global environment than any relative changes introduced to represent the GCC scenarios. Alterations to food web interactions have the potential to exert a stronger influence on human exposure to organic contaminants in the Arctic environment (Undeman et al. 2010; Armitage et al. 2011) but realistic scenarios (e.g. alterations to the ringed seal food web) have yet to be defined. With respect to PCBs, it seems unlikely that any such changes will
be sufficient to return exposure levels to the peak values experienced during the primary emission phase.

4.4 Conclusions

This study highlights the complex array of factors determining human exposure to organic contaminants in the Arctic environment and the ability of this modelling approach to facilitate data interpretation, hypothesis development and field study design. While many aspects can be probed through the application of chemical fate and bioaccumulation models, substantial uncertainties and data gaps remain which limit the opportunities to directly apply this tool to existing data. For example, few biomonitoring studies have been conducted on a single community at multiple points in time making it difficult to more accurately simulate dietary changes over time. Furthermore, preferably multiple surveys of a community need to be available over longer-term time intervals (i.e. over a 10-20 year period) such that meaningful differences in dietary trends can be measured. A recommendation for future biomonitoring studies is to increase the amount of data collected by questionnaires per person as well as the detail of the questions. For example, the geographic origin of consumed food should be included, as well as multi-season and multi-decade data collection for the same community.

With respect to the interpretation of biomonitoring data, the long-term PCB-153 simulations illustrate the key roles of emission history and dietary composition in determining absolute exposure levels over time and potential variability within and between different subpopulations. For chemicals which are more susceptible to biotransformation, uncertainties related to estimating metabolic rate constants in humans and the various organisms in the human food chain present an additional challenge to model parameterization as these parameter values can be highly influential (McLachlan et al. 2011). Potential improvements to the generic framework demonstrated here for site-specific assessments include i) compiling/obtaining more detailed information on diets and dietary transitions in the communities of interest, ii) expanding the Arctic food webs to include additional terrestrial and aquatic species (e.g. caribou, eider duck), and iii) applying more spatially-resolved fate/transport models to better distinguish between imported food items (e.g. North American vs. continental European origin). The calculations could also explicitly account for potential losses of contaminants during cooking/preparation (e.g. raw vs. fried fish).

The long-term simulations conducted here depend on the existence of a detailed historic and projected emission inventory (Breivik et al. 2007). Similar calculations could be repeated for other PCB
congeners as well as other POPs for which at least historic emission inventories are available (e.g. some organochlorine pesticides) (Li and Macdonald 2005). These simulations could provide additional insight into spatial and temporal trends evident in the available biomonitoring data and provide guidance for future sampling campaigns. The lack of emission estimates for current-use pesticides and other contaminants of emerging concern limit the scope of modelling studies to hypothetical scenario assessment. These activities can still be quite valuable, particularly in the context of hypothesis development and field study design. Some commonalities can be expected to apply to all organic contaminants, namely the importance of assumptions regarding the temporal evolution of emission rate over time, the importance of partitioning properties and biotransformation in determining bioaccumulation potential and the influence of diet in determining absolute levels and variability in human exposure levels. As demonstrated here, modelling approaches are well suited to assessing such considerations in a holistic and transparent manner.
4.5 Supporting Information

4.5.1 Longitudinal Body Burden-Age Trends

The longitudinal body burden-age trends generated by the human bioaccumulation model are illustrated in Figure S4.1 for women who consume an MTMI (Table 4.1) diet with the imported food items originating from the North Boreal region. The model predicts that the largest peak concentration at any given age was experienced by six-month old girls born around 1980–1990 (depending on the diet of the mother) (Figure S4.1). As previously described, the peak body burden at 6 months of age coincides with the end of breastfeeding and indicates that maternal transfer is a significant source of contamination for cohorts born after the PCB production phase-out (Chapter 2).

![Figure S4.1](image-url)

Figure S4.1. Longitudinal body burden-age trend for Inuit women consuming an MTMI diet with imported foods originating from the North Boreal region.

4.5.2 Additional details on the global emission scenario

The global emission inventory was initially developed with the primary aim to understand contemporary emissions and thus focused on quantifying emissions from use and consecutive stages of the chemical life-cycle, i.e. after global production of PCBs had ceased (Breivik et al. 2007). Comparison with human tissue residues clearly demonstrated that observed trends in slowly responding compartments (i.e. humans) could not be rationalized without also accounting for historical releases that occurred during the initial stages of the chemical life-cycle (Breivik et al. 2010). For the context of this work, the global emission inventory (Breivik et al. 2007) was therefore updated and revised in a similar fashion as the regional estimates for the Baltic region (Breivik et al. 2010).
Emissions peak in the 1970s at around 170 metric tonnes per year (t/a) and decline substantially in the following decades due to restrictions on production and use and subsequent global regulatory efforts. Primary emissions do not immediately drop to zero following cessation of production as they are sustained by releases from ‘in-use’ stockpiles (e.g. due to volatilization from applications with long lifespans and/or during disposal). For example, total emissions in 1990 are still relatively high (approximately 50 t/a) in comparison to peak emissions. Emissions are projected to decline more rapidly as stockpiles are exhausted, falling to approximately 7 t/a by 2010, 1 t/a by 2030 and then less than 0.01 t/a by 2050.

Table S4.1. Spatial distribution of emissions assumed for uptake period.

<table>
<thead>
<tr>
<th>Zone</th>
<th>% of total annual emissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Polar</td>
<td>0.26</td>
</tr>
<tr>
<td>Boreal</td>
<td>5.20</td>
</tr>
<tr>
<td>North Temperate</td>
<td>58.1</td>
</tr>
<tr>
<td>North Subtropic</td>
<td>23.2</td>
</tr>
<tr>
<td>North Tropic</td>
<td>8.02</td>
</tr>
<tr>
<td>South Tropic</td>
<td>2.82</td>
</tr>
<tr>
<td>South Subtropic</td>
<td>2.28</td>
</tr>
<tr>
<td>South Temperate</td>
<td>0.07</td>
</tr>
<tr>
<td>South Subpolar</td>
<td>0.01</td>
</tr>
<tr>
<td>South Polar</td>
<td>0.00</td>
</tr>
</tbody>
</table>

~ 95%

~ 5%

Figure S4.2: Zonal division of the earth into latitudinal bands in the GloboPOP model and approximate range of latitudes for each zone.
The average spatial distribution of primary PCB-153 emissions over the period 1930–2100 (Breivik et al. 2007) is presented in Table S4.1. The spatial distribution for each year of the simulation was calculated by spatially aggregating the 1°x1° emission estimates into the latitudinal bands indicated in Figure S4.2.

Figure S4.3: Revised\textsuperscript{8} and previously published (Breivik et al. 2007) total annual global emission estimates (metric tonnes) of PCB-153 over the period 1930–2100

Note that in this scenario, approximately 95% of the total annual emissions occur in the Northern hemisphere, the majority in the Northern Temperate zone (38–54 °N). While there are direct emissions to the Arctic zone in these scenarios, long-range transport from other zones represents the dominant source overall. Seasonal differences in emission strength were simulated using a sinusoidal function with maximum emissions in summer (June) and minimum emissions in January. Peak emissions were approximately 3 times higher in June compared to January.

\textsuperscript{8} For a detailed justification and explanation of the revised emissions scenario refer to the supporting information in Breivik et al. 2010
The revised and previously published (Breivik et al. 2007) total annual global emission estimates (tonnes) of PCB-153 are presented in Figure S4.3. The revised emission estimates are roughly 2–3 times higher over the period 1930–1990 and then consistent with the original estimates over the period 1990 – 2100.

4.5.3 Physical-chemical property values for PCB-153

Table S4.2. Physical-chemical properties of PCB-153 assumed for the dynamic simulations of chemical fate and bioaccumulation in food webs (Schenker et al. 2005; Abraham and Al-Hussaini 2005; Wania and Mackay 1995; Wania and Su 2004).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCB-153</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Partitioning Properties (25 °C)</strong></td>
<td></td>
</tr>
<tr>
<td>log $K_{OW}$*</td>
<td>6.86</td>
</tr>
<tr>
<td>log $K_{AO}$</td>
<td>9.45</td>
</tr>
<tr>
<td>log $K_{AW}$</td>
<td>-2.13</td>
</tr>
<tr>
<td><strong>Temperature-dependence (kJ mol$^{-1}$)</strong></td>
<td></td>
</tr>
<tr>
<td>$\Delta U_{OW}$</td>
<td>-26.6</td>
</tr>
<tr>
<td>$\Delta U_{OA}$</td>
<td>-94.8</td>
</tr>
<tr>
<td>$\Delta U_{AW}$</td>
<td>68.2</td>
</tr>
<tr>
<td><strong>Solvation parameters (sorption to snow)</strong></td>
<td></td>
</tr>
<tr>
<td>$\Sigma \alpha$</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Sigma \beta$</td>
<td>0.11</td>
</tr>
<tr>
<td>$K_{HXA}$</td>
<td>9.59</td>
</tr>
<tr>
<td><strong>Degradation half-lives (25 °C)</strong></td>
<td></td>
</tr>
<tr>
<td>Freshwater (h)</td>
<td>55000</td>
</tr>
<tr>
<td>Freshwater sediment (h)</td>
<td>170000</td>
</tr>
<tr>
<td>Seawater (h)</td>
<td>55000</td>
</tr>
<tr>
<td>Soils (h)</td>
<td>550000</td>
</tr>
<tr>
<td>Snowpack (h)</td>
<td>55000</td>
</tr>
<tr>
<td>Human (h)</td>
<td>130000</td>
</tr>
<tr>
<td><strong>Degradation rate constant (25 °C)</strong></td>
<td></td>
</tr>
<tr>
<td>Air (cm$^3$ molecules$^{-1}$ s$^{-1}$)**</td>
<td>1.6e-13</td>
</tr>
<tr>
<td>Herring (h$^{-1}$)</td>
<td>0</td>
</tr>
<tr>
<td>Cod (h$^{-1}$)</td>
<td>0</td>
</tr>
<tr>
<td>Arctic Cod (h$^{-1}$)</td>
<td>0</td>
</tr>
<tr>
<td>Seal (h$^{-1}$)</td>
<td>0</td>
</tr>
<tr>
<td>Grass (h$^{-1}$)</td>
<td>0</td>
</tr>
<tr>
<td>Beef Cattle (h$^{-1}$)</td>
<td>0</td>
</tr>
<tr>
<td>Milk Cattle (h$^{-1}$)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Activation Energies (kJ mol$^{-1}$)</strong></td>
<td></td>
</tr>
<tr>
<td>Reactions in air</td>
<td>10.0</td>
</tr>
<tr>
<td>Reactions in all other media</td>
<td>30.0</td>
</tr>
</tbody>
</table>
Default degradation rate constants/half-lives were selected so as to be consistent with a previous publication on the global fractionation of PCBs (Wania and Su 2004). Simulations were also conducted assuming the same degradation rate constant in air but 55000 h in all surface media (i.e. ~ 3 times shorter in sediment and 10 times shorter in soil).

4.5.4 Brief Description of the GloboPOP model

Compartmentalization and intermedia exchange. Each latitudinal zone is subdivided into bulk environmental compartments which represent the atmosphere (4 layers), ocean (surface layer), soil (cultivated, uncultivated, forest), forest canopy (deciduous, coniferous) and fresh water (water, active sediment layer) (Figure S4.4). The current version of GloboPOP also includes a seasonal snow pack in the latitudinal zones that experience cold enough temperatures in winter (below 0 °C). Bulk environmental compartments include different phases between which the chemical is assumed to distribute between according to physical-chemical properties (partitioning coefficients). For example, the atmosphere includes the gaseous and aerosol phase, surface waters include a freely dissolved and particulate phase and soils include air, water and solid (organic matter) phases. Exchange between the various bulk compartments can occur through diffusive (e.g. gaseous deposition/revolatilization) or non-diffusive transport processes (e.g. precipitation scavenging, particle settling/resuspension). As indicated in Figure S4.4, snow acts as a barrier to diffusive and non-diffusive exchange between the atmosphere and other bulk surface compartments throughout the winter period. During the melt period, chemical in the snow pack can be transferred to soils and freshwater in the melt water or revolatilized to the overlying atmosphere. Sea-ice in polar regions acts as a barrier to diffusive exchange of chemicals (i.e. gaseous deposition and revolatilization) over the fraction of total ocean surface area covered during the year. Ice covering the freshwater environment is treated similarly.

Sorption to falling snow (i.e. snowflake-air partitioning) and snow pack. In a model that includes falling snow and a seasonal snow pack, an additional partition coefficient between snow and air is required ($K_{SA}$). Adsorption to the surface of snowflakes ($K_{SA}, \text{m}^3 \text{air m}^{-2} \text{snow}$) can be estimated using polyparameter linear free energy relationships (ppLFER), such as the one provided in Roth et al. (2004):

$$\log K_{SA}(-6.8^\circ C) = 0.639 \cdot \log K_{HXA} + 3.53 \cdot \sum \alpha + 3.38 \cdot \sum \beta - 6.85 \text{ (Equation S4.1)}$$

* ‘wet’ octanol; ** 2nd-order rate constant for reaction with OH radicals
where $K_{HXA}$ is the hexadecane-air partition coefficient (at 25 °C) and $\Sigma \alpha$ and $\Sigma \beta$ characterize electron acceptor and donor ability, respectively. To convert the calculated $K_{SA}$ values to a volumetric basis, the following expression is used:

$$
\log K_{SA}^{VOL} = \log K_{SA} \cdot SSA_F \cdot \rho_F \quad \text{(Equation S4.2)}
$$

where $SSA_F$ is the specific surface area of the snowflake (m$^2$ kg$^{-1}$) and $\rho_F$ is the density of the snowflake (kg m$^{-3}$). Typical values for $SSA_F$ and $\rho_F$ are in the range of 100 m$^2$ kg$^{-1}$ and 1000 kg m$^{-3}$ respectively (Thibodeaux and Mackay 2011). A similar approach is used for the seasonal snow pack-air partition coefficient except the specific surface area is reduced to 15 m$^2$ kg$^{-1}$ and density to 433 kg m$^{-3}$. 

Figure S4.4: Generic compartmentalization of each model zone in the GloboPOP model and seasonal water fluxes (represented by $w_{G_{ij}}$) between the environmental compartments in the current version of GloboPOP.
4.5.5  Additional results for the food web bioaccumulation

Figure S4.5. Comparison of the lipid normalized body burdens of the food web organisms at different ages and from different regions.
4.5.6 Additional Information on Seal Blubber Assumptions

The study by Czub and McLachlan (2007) explored the potential role of the temperature gradient in the blubber of marine mammals on the bioaccumulation of organic contaminants. Specifically, the lower temperature of the outer blubber layer (≥ 1 °C; depending on ambient water temperature) results in a higher fugacity capacity than in the inner blubber and core (i.e. meat) layers of the seal (37 °C) leading to higher concentrations of contaminants in the outer blubber than the meat of the seal (based on assumption of equi-fugacity in all blubber layers). Hence, the representation of the temperature differential between these layers in the model is part of the reason behind the large difference in body-burdens for women consuming a traditional diet versus a traditional (blubber-free) diet. However, comparison of the modeled ratios of lipid-normalized concentrations in blubber versus blood or milk lipids (Czub and McLachlan 2007) were up to one order of magnitude higher than field observations implying that the vertical gradient in PCB concentrations from outer blubber to core may be overestimated by this modelling approach.

4.5.7 Comparison of GloboPOP model output for default and alternate scenario for environmental degradation half-lives (25 °C)

The temporal trends in concentrations of PCB-153 in the lower atmosphere of the North Polar zone (1930–2100) is shown in Figure S4.6 for the default degradation half-life (HL) and alternate degradation half-life scenario. The ratios of model output in the lower atmosphere and surface ocean water are presented for the North Polar zone in Figure S4.7 and for the North Temperate zone in Figure S4.8. Note that model output is shown for these two compartments because ambient environmental levels in these two compartments drive the food-web bioaccumulation models.

As can be seen in Figure S4.6, model output is basically identical over the period 1930–1990 and then begins to diverge after that point (see also Figure S4.7). By the year 2100, modelled concentrations in the lower atmosphere assuming the default HLs are approximately two orders of magnitude higher than in the alternate HL scenario (Figure S4.7). A similar pattern is seen in the model output for the lower atmosphere of the North Temperate zone (Figure S4.8) and also in surface ocean water in both zones (i.e. ocean tracks the air concentration) (Figure S4.7 and S4.8).

These model results can be explained as follows. From 1930–1990, primary emissions of PCB-153 to the lower atmosphere are the dominant sources of PCB-153 to the global environment. Accordingly, concentrations in the air track emissions closely. During this period, the fugacities in surface ocean
water, soil and other surface compartments are less than the fugacity in air meaning that net deposition is favoured. As emissions decline from the peak in the 1970s, the fugacity ratios between air and the surface compartments move towards unity; secondary sources, revolatilization from soils in particular, become increasingly important in determining the temporal trend in concentrations in the global environment. By around the year 2000, the fugacity in the lower atmosphere is consistently lower than in soil (favouring net revolatilization) and the trends in concentrations in the global environment switch from being determined by the pattern in emissions to being determined by the degradation half-life in soil. As the soil degradation HL in the default HL scenario is approximately 60 years compared to 6 years in the alternate scenario, the burden in global soils in the default HL scenario is higher in absolute terms at the end of the primary emission phase (i.e. more mass in soil compartments) and far more recalcitrant (i.e. decline in soil fugacity over time due to degradation is minimal in comparison to the alternate HL scenario).

Figure S4.6: Comparison of model output for the lower atmosphere of the North Polar zone assuming the default and alternate HL scenario. Solid black lines represent 12-point running average concentrations of PCB-153.
Figure S4.7: Ratio of concentrations in Arctic atmosphere (lower) and surface ocean water assuming default degradation half-lives and alternate half-lives. The dotted line indicates a factor of two difference.

Figure S4.8: Ratio of concentrations in the North Temperate atmosphere (lower) and surface ocean water assuming default degradation half-lives and alternate half-lives. The dotted line indicates a factor of two difference.
Chapter 5
Partitioning of polychlorinated biphenyls into human membrane and storage lipids: Evaluation of surrogates and prediction methods

Cristina L. Quinn, Stephan van der Heijden, Michiel T.O. Jonker, and Frank Wania

Contributions: Liposomes were prepared by C. Quinn under the guidance of S. van der Heijden and M.T.O. Jonker. Cells were prepared by C. Quinn. Human abdominal fat tissues were prepared by C. Quinn and M.T.O. Jonker. Dosing experiments were a collaborative effort between C. Quinn, S. van der Heijden, and M.T.O. Jonker. Sample analysis was performed by S. van der Heijden. C. Quinn performed data analysis and wrote the manuscript under the supervision of F. Wania.
5.1 Introduction

Lipids have been shown to have an enormous capacity to accumulate chemicals (Mackay and Fraser 2000). A quantitative understanding of the partitioning of chemicals to lipids is therefore critical. When estimating the bioaccumulation potential of organic contaminants, models typically use octanol-water partitioning coefficients ($K_{\text{octanol/water}}$) as a surrogate to represent the partitioning of chemicals from aqueous systems to the storage (triacylglyceride) ($K_{\text{storage lipid/water}}$) and membrane lipid components of organisms ($K_{\text{membrane lipid/water}}$) (Chiou 1985; Gobas et al. 1988; Escher et al. 2000; Endo et al. 2011). Recently, the suitability of this approach has been questioned (Jonker and van der Heijden 2007). Furthermore, it has been argued that the storage capacity of membrane lipids differs from that of storage lipids (Geisler et al. 2012; Endo et al. 2011; van Wezel and Opperhuizen 1995) and that not all lipid homogenates have the same partitioning properties (van der Heijden and Jonker 2011). As such, there is a need to evaluate the differences between $K_{\text{octanol/water}}$, $K_{\text{storage lipid/water}}$ and $K_{\text{membrane lipid/water}}$ for improving bioaccumulation and toxicity models. The goal of this study was to measure and compare partition coefficients of a range of polychlorinated biphenyls (PCBs) between various lipid systems (liposomes, cells and lipid tissue) and water, and to compare these measurements against existing partitioning models.

Over the last 30 years, several measurements of liposome-water partition coefficients ($K_{\text{liposome/water}}$) for both neutral (Dulfer and Govers 1995; Gobas et al. 1988; Jabusch and Swackhamer 2005; van der Heijden and Jonker 2009) and ionogenic (Escher and Schwarzenbach 1996; Escher et al. 2000) organic chemicals have been conducted for the purpose of better understanding partitioning to membrane lipids. Liposomes are vesicles composed of phospholipid molecules which self-arrange to form a bilayered vesicle. The bilayer arrangement is driven by the hydrophilic head group of the phospholipid molecule and the hydrophobic tail consisting of two fatty acids (Gennis 1989). There are dozens of combinations of head group and fatty acid tails but, to our knowledge, no systematic evaluation of the effect of phospholipid type on $K_{\text{liposome/water}}$ (for phospholipids in the liquid-crystalline phase) has been conducted. Here, we vary the liposome composition, using phospholipids relevant to humans, to distinguish potential differences in partitioning capacities. The two most common head groups of the phospholipids present in the membranes of female fat cells are phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Zeghari et al. 2000) while palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2) are some of the most commonly found fatty acids in human
phospholipids (Amaru and Field 2009). Each of these fatty acids differs in chain length (16 or 18 carbons) and saturation (0, 1, or 2 double bonds).

Estimates of $K_{\text{membrane lipid/water}}$ can be obtained using poly-parameter linear free energy relationships (ppLFERs) given by Endo et al. (2011). The authors presented two different versions of the ppLFER equation:

$$\log K = eE + sS + aA + bB + vV + c \quad \text{(Equation 5.1)}$$

$$\log K = lL + sS + aA + bB + vV + c \quad \text{(Equation 5.2)}$$

Equation 5.1 was proposed by Abraham et al. (1994) and describes the partitioning of neutral organic compounds between octanol and water. Equation 5.2 was proposed by Goss (2005) and can be used to predict the partitioning of neutral organic compounds between any two condensed phases. The solute descriptors ($E$, $S$, $A$, $B$, $V$, $L$) describe the molecular interactions between the chemical and the condensed phases, where $E$ describes the excess molar refraction, $S$ the dipolarity/polarizability, $A$ the solute H-bond acidity, $B$ the solute H-bond basicity, $V$ the molar volume and $L$ the logarithm of the hexadecane-air partition coefficient. Although both equations generally give the same quality of fit, the ppLFER of type (2) is usually recommended (Geisler et al. 2012; Endo et al. 2011). The phase descriptors ($e$, $s$, $a$, $b$, $v$, $l$) describe the different capacities of the two condensed phases for interacting with the solute. There exist two sets of solute descriptors for PCBs which are by Abraham and Al-Hussaini (2005) and by van Noort et al. (2010). The dataset by van Noort et al. updates the descriptor values for $E$, $S$, $B$ and $V$ and retains the values for $A$ and $L$ from Abraham and Al-Hussaini in an attempt to reduce the discrepancy between ppLFER predictions and $K_{\text{octanol/water}}$ observed for ortho-chlorinated PCBs. By combining the ppLFERs of type (1) or (2) with either solute descriptor dataset, the partitioning of PCBs from aqueous solution to membrane lipids can be predicted.

The triacylglyceride triolein has previously been evaluated as a surrogate to measure partitioning to storage lipids (Chiou, 1985; Jabusch and Swackhamer 2005). Jabusch and Swackhamer (2005) reported $K_{\text{tri olein/water}}$ up to 0.3 log units higher than $K_{\text{octanol/water}}$ which is in agreement with earlier work by Chiou et al. (1985) who found that $K_{\text{tri olein/water}}$ for PCBs was generally larger than the corresponding measured $K_{\text{octanol/water}}$. To our knowledge, no measurements have been conducted comparing $K_{\text{tri olein/water}}$ with real $K_{\text{storage lipid/water}}$ values, hence, the relevance of $K_{\text{tri olein/water}}$ as a surrogate for either $K_{\text{octanol/water}}$ or $K_{\text{storage lipid/water}}$ is yet to be determined. There are, however, ppLFERs of type (1) and (2) for
partitioning to other storage lipids including olive oil, milk fat, fish oil, linseed oil, and goose fat (Geisler. et al. 2012). These ppLFERs can be used to estimate $K_{\text{storage lipid/water}}$ with the solute descriptors by Abraham and Al-Hussaini (2005) and van Noort et al. (2010).

Despite an abundance of measurements reported for $K_{\text{octanol/water}}$, accurate and consistent measurements of $K_{\text{octanol/water}}$ are difficult to obtain (Li et al. 2003). In order to assure consistency between interrelated thermodynamic partitioning properties, Beyer et al. (2002) developed an adjustment technique whereby the consistency between empirically determined physical-chemical properties and potential systematic error in the measurement are considered. Li et al. (2003) applied this adjustment technique to a selection of PCB congeners and Schenker et al. (2005) later improved upon their final adjusted values (FAVs). As with $K_{\text{membrane lipid/water}}$ and $K_{\text{storage lipid/water}}$, a ppLFER for $K_{\text{octanol/water}}$ is also available (Goss 2005) which has the same form as equation 2.

The goal of this study was to evaluate the validity of using surrogate systems (i.e. octanol, liposomes and triolein) as representatives for realistic systems (i.e. cells and tissues) as well as the validity of the various models available for predicting partitioning. To this end, three different lipid systems were investigated: (i) commercially available triolein as surrogates of storage lipids, (ii) phospholipids (in the form of liposomes) which serve as surrogates for cell membranes, and (iii) human cells (cultured in the laboratory) and human abdominal fat tissues (AFT) as systems representing real human lipid tissues.

### 5.2 Materials and Methods

The partitioning of PCBs for each of the lipid systems ($K_{\text{lipid/water}}$) was measured using batch sorption experiments with polyoxymethylene (POM) passive samplers according to a method described previously (Jonker and van der Heijden 2007). Briefly, a lipid phase (4 - 10 or 32 mg) was weighed into a 50 or 100 mL amber-coloured glass bottle and filled with a known amount of aqueous solution (0.01 M CaCl$_2$ and 50 mg/L NaN$_3$) to yield solutions of 10 mg lipid/mL solution. Rectangular POM pieces (CS Hyde Company; Lake Villa, IL, USA) were cut to the appropriate mass (4 or 20 mg, depending on the system), washed (via shaking with Pestiscan™ grade hexane (30 min), acetonitrile (2 x 30 min), and methanol (2 x 30 min)) and added into each system. The PCBs were spiked into the systems via a cocktail solution of PCBs (approximately 3.5 mg/L each of PCBs 18, 28, 52, 66, 72, 77, 101, 103, 118, 126, 138, 153, 155, 156, 169, 170, 180, and 187) in acetone (50 μL) for a total PCB concentration of approximately 27 μg/L. For each lipid type, 4 or 5 replicate systems were prepared.
plus 2 or 3 blanks (i.e. no PCBs added). Previous experiments have demonstrated linear sorption behaviour with respect to PCB concentration therefore only one dosing concentration of PCBs was required.

The resulting systems were sealed and shaken on a reciprocal shaker at 150 rpm for 4 weeks at 37°C. Afterwards, the POM pieces were retrieved from each system, rinsed with milli-Q water, thoroughly wiped with a wet tissue, and transferred to GC vials with 0.45 mL of acetonitrile. Since POM retrieval and transfer was conducted at room temperature, each system was kept at 37°C until immediately prior to retrieval such that the equilibrium would not be disturbed. All vials are stored in the freezer until GC analysis at which time 50 μL of internal standard (PCB-209) was added to each vial.

Background concentrations of PCBs in each lipid type were determined from the blank samples which had received no spike with PCBs. These background concentrations were then subtracted from the final measured concentrations in the dosed samples.

5.2.1 Membrane lipids.

Liposome vesicles were prepared according to a method recently developed at the Institute for Risk Assessment Sciences. Briefly, a phospholipid/chloroform solution (Avanti Polar Lipids, Inc.) was transferred to a round-bottom flask and the solution evaporated to dryness. The dried phospholipids were resolubilized in aqueous solution (50 mg/L NaN₃ and 0.01 M CaCl₂). Throughout this process the suspension was kept under a nitrogen flow. The dispersion underwent 10 freeze-thaw cycles and was then extruded 10 times through a 0.20 μm filter. The exact concentration of the final phospholipid solution was gravimetrically determined. Liposome suspensions were stored at 4°C and used within one week.

Nine different liposomes were prepared in total in order to evaluate the influence of chain length, degree of unsaturation, and head group on the sorption of PCBs to liposome bilayers (Table 5.1 and Table 5.2). The phospholipid types were selected such that thermodynamically stable bilayers could be formed at 37 °C while still representing fatty acids of varying chain length, and degree saturation. For comparison purposes, it is preferable to prepare liposomes with only one type of fatty acid tail but, for all of the saturated fatty acids of interest, stable bilayers will not form at a human-relevant temperature (37 °C) if only these fatty acids are used in the liposomes (Taylor and Craig 2003). Furthermore, pure PE phospholipids are thermodynamically stable as reverse micelles (i.e. hexagonal II phase) in aqueous
solution rather than bilayered vesicles (Yeagle and Sen, 1986) and hence, the addition of at least 30% PC phospholipids is required to stabilize PE phospholipids in a bilayer (Cullis and Dekruijff, 1979). For comparison purposes, vesicles containing only PE phospholipids were prepared anyway (although they form a hexagonal phase rather than a lamellar phase) in addition to 3 liposome mixtures of varying contributions of PC and PE (Table 5.2).

5.2.2 Human Cells.

MCF-7 cells were selected as a representative human cell. This cell line was originally derived from breast cancer cells from a 69 year old Caucasian female. The MCF-7 cells were obtained from ATCC and cultured in Dulbecco’s modified Eagle’s Medium (Invitrogen 31053) supplemented with 5% fetal calf serum (Invitrogen 10270), 1% penicillin/streptomycin (Invitrogen 15140), 1% sodium pyruvate (Invitrogen 11360), 1 % glutamine (Invitrogen 25030), and 1 μg/ml insulin (sigma I-5500). The cells were cultured in a humidified atmosphere at 37°C/5% CO₂. Cells were subcultured over a period of 2.5 months in order to obtain a sufficient number of cells. Cells were stored at -20°C until enough cells had been produced. All cells were combined and freeze dried prior to the sorption experiments. Lipid analysis of the freeze dried cells using the Bligh and Dyer method (Bligh and Dyer, 1959) yielded 13% lipid by dry weight.

Table 5.1: Selected Phospholipids

<table>
<thead>
<tr>
<th>Liposome</th>
<th>Fatty Acid 1</th>
<th>Fatty Acid 2</th>
<th>Head Group</th>
<th>Tₘ (Avanti Polar Lipids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>16:0</td>
<td>18:1</td>
<td>Phosphatidylcholine</td>
<td>-2</td>
</tr>
<tr>
<td>POPE</td>
<td>16:0</td>
<td>18:1</td>
<td>Phosphatidylethanolamine</td>
<td>25</td>
</tr>
<tr>
<td>DPPC</td>
<td>16:1</td>
<td>16:1</td>
<td>Phosphatidylcholine</td>
<td>-36</td>
</tr>
<tr>
<td>SOPC</td>
<td>18:0</td>
<td>18:1</td>
<td>Phosphatidylcholine</td>
<td>6</td>
</tr>
<tr>
<td>DOPC</td>
<td>18:1</td>
<td>18:1</td>
<td>Phosphatidylcholine</td>
<td>-20</td>
</tr>
</tbody>
</table>

Table 5.2: Liposome mixtures of POPE and POPC Phospholipids

<table>
<thead>
<tr>
<th>Mixture</th>
<th>POPE Content (%)</th>
<th>POPC Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30/70</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>50/50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>70/30</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>
5.2.3 Abdominal Fat Tissues.

Seven abdominal fat tissues (AFT-A through AFT-G) were obtained from female patients with ages 40-70 who undertook elective abdominoplastic surgery at two clinics in the Netherlands. Tissue subsamples free of skin were freeze dried and subsequently homogenized. Lipid analysis using the Bligh and Dyer method (Bligh and Dyer, 1959) determined that all samples contained 95-98% total lipid. Background concentrations of the samples was measured in three blank samples for each tissue and subtracted from the total concentrations of the dosed samples. All handling of the abdominal tissues was performed under strict biohazard conditions and approved by the medical ethical commission of the University Medical Centre of Utrecht.

5.2.4 Instrumental Analysis.

PCB analyses were done on a TRACE GC Ultra, equipped with a Triplus autosampler and an electron capture detector (all Thermo Scientific, Waltham, MA, USA). Samples were injected on-column on a polar guard column, connected to a Zebron ZB-5Msi analytical column of 30 m x 0.25 mm with a 0.25 μm film thickness (Phenomenex, Torrance, CA, USA). After injection of a sample, the column temperature was kept at 80 °C for 1 min before increasing it first to 190 °C at 15.0 °C/min, then to 250 °C at 3.0 °C/min, and subsequently to 300 °C at 15.0 °C/min, where it was held for 5 min, before initiation of the cooling cycle. Chromatograms were processed manually using Chrom-Card 2.4.1 (Thermo Fisher Scientific Inc., Rodano Milan, Italy).

5.2.5 Poly-parameter Linear Free Energy Relationships.

We compared the measured $K_{\text{storage lipids/water}}$ and $K_{\text{membrane lipids/water}}$ values with the ppLFERs models recently published by Geisler et al. (2012) and Endo et al. (2011), respectively. These were used in combination with the solute descriptors from Abraham and Al-Hussaini (2005) and van Noort et al. (2010) to estimate the $K_{\text{storage lipids/water}}$ and $K_{\text{membrane lipids/water}}$ values for the PCBs of interest.

5.2.6 Selection of $K_{\text{octanol/water}}$.

Due to lack of consistency in published $K_{\text{octanol/water}}$ values, comparison of $K_{\text{lipid/water}}$ with $K_{\text{octanol/water}}$ was challenging. We used three approaches for obtaining $K_{\text{octanol/water}}$. The first of these was to use the published values by Hawker and Connell (1988). The second approach was to use the final adjusted values (FAV) for log $K_{\text{octanol/Water}}$ by Schenker et al. (2005). Some of the FAVs could be taken directly
from Schenker et al. (2005) but for those PCB congeners where FAVs did not exist, log $K_{octanol/water}$ was predicted using the regression:

$$\log K_{octanol/water} = 0.0125MM + 0.0208n_{O-Cl} + 2.41$$ (Equation 5.3)

where $MM$ represents the molar mass of the congener and $n_{O-Cl}$ represents the number of orth-chlorines (Schenker et al. 2005). Finally, the third set is predicted using the ppLFER for $K_{octanol/water}$ by Goss (2005) which has the same form as equation 5.2, and the solute descriptors for the PCBs by Abraham and Al-Hussaini (2005) and by van Noort et al. (2010).

The log $K_{octanol/water}$ values from all of the above mentioned methods were for 25°C and thus needed to be temperature adjusted to 37°C. This was done using (Li et al. 2003):

$$\log K_{octanol/water}(T) = \log K_{octanol/water}(25°C) - \Delta U_{OW}/(\ln(10) \cdot R) \cdot (1/310.15K - 1/298.15K)$$ (Equation 5.4)

and the internal energy of phase transfer between octanol and water ($\Delta U_{OW}$ in kJ/mol). FAVs for $\Delta U_{OW}$ where taken from Schenker et al. (2005) when available or else calculated using:

$$\Delta U_{OW} = -0.0405MM + 1.71n_{O-Cl} - 13.4$$ (Equation 5.5)

### 5.3 Results and Discussion

#### 5.3.1 Similarities and Differences in the Measured Lipid/Water Partition Coefficients.

Figure 5.1 plots the measured $K_{cell \ lipid/water}$ and $K_{AFT/water}$ against the measured $K_{triolein/water}$. Within the measurement uncertainty, the various partition coefficients between the seven abdominal fat tissues and water, $K_{AFT/water}$, are identical with each other (Figure 5.1B). This suggests that there are no differences in the partitioning properties of storage lipids from different human individuals. The near perfect agreement between (i) the $K_{cell \ lipid/water}$ and $K_{triolein/water}$ (Figure 5.1A) and (ii) $K_{AFT/water}$ and $K_{triolein/water}$ (Figure 5.1B) suggests that partitioning to MCF-7 cells and AFT on a lipid normalized basis are similar despite different lipid compositions. For example, the phospholipid content of MCF-7 cells is approximately 90% of total lipid content (Kourtidis et al. 2009). Furthermore, the AFT homogenates are not pure storage lipids in that they also contain proteins and lipids of membranes and plasma and possibly also structural tissues. Despite the presence of these other potential partitioning phases and different lipid composition, AFT, cells and triolein all exhibit the same partitioning properties for PCBs
(Figure 5.1A and 5.B). On the other hand, the measured $K_{\text{liposome/water}}$ (Figure 5.1C) are consistently lower by 0.48 log units than the measured $K_{\text{storage lipid/water}}$ (i.e. $K_{\text{triolein/water}}$, and $K_{\text{AFT/water}}$) and $K_{\text{cell lipid/water}}$ which reveals that liposomes do not exhibit similar partitioning behaviour to either storage lipids or cells. This is consistent with similar findings by Geisler et al. (2012) who found that partitioning of alkanes, alkenes, and cycloalkanes to membranes lipids is 0.5 to 1.5 log units lower than to storage lipids.

Within measurement uncertainty, the various $K_{\text{liposome/water}}$ are also identical with each other (Figure 5.1C) implying that neither the length, saturation of the carbon chains, head group, nor the composition of the liposome appears to have an impact on the partitioning properties of PCBs into liposomes. This has important implications for bioaccumulation modelling. For example, it justifies using only a single partition coefficient for all lipid membranes regardless of membrane composition differences between homeotherms and poikilotherms or even poikilotherms at different temperatures. It is particularly interesting that the POPE phospholipids have the same partitioning as the other liposomes since PE phospholipids are not expected to form a stable liposome in the absence of PC headgroups (Cullis and Dekruijff, 1979). Rather the POPE phospholipids are arranged as reverse micelles (Yeagle and Sen, 1986). This would suggest that the physical conformation of the phospholipids is not significant for partitioning to occur but simply the presence of phospholipids. Further investigation of this observation by future studies is warranted.

Figure 5.1: Comparing measured log $K_{\text{lipid/water}}$ values for (A) lipids extracted from a human breast cell culture, (B) abdominal fat tissues of seven human individuals (AFT-A to AFT-G), and (C) synthetic liposomes of variable composition (Table 1&2) with the measured log $K_{\text{triolein/water}}$ values for 18 PCB congeners. The black 1:1 line corresponds to perfect agreement between the partitioning coefficients.
To further investigate the discrepancies between the $K_{\text{liposome/water}}$ of different liposomes and that between the $K_{\text{AFT/water}}$ of different AFT samples, the deviations of the individual lipid types from the average were plotted as a function of the average (Figure 5.2). More specifically, the difference between the $K_{\text{liposome/water}}$ for a specific liposome and the average $K_{\text{liposome/water}}$ for all liposomes was plotted as a function of the average $K_{\text{liposome/water}}$ (Figure 5.2A) and the difference between $K_{\text{AFT/water}}$ for a specific AFT sample from the average $K_{\text{AFT/water}}$ for all AFT samples was plotted as a function of the average $K_{\text{AFT/water}}$ (Figure 5.2B).

From Figure 5.2A, it becomes apparent that the $K_{\text{liposome/water}}$ of all liposomes is within 0.1 log units of the average the $K_{\text{liposome/water}}$ with the exception of the measurement for PCB 170 for DPPC which is 0.12 log units greater than the average. PCB 18 has the most variable $K_{\text{liposome/water}}$, with deviations up to 0.09 log units above and 0.06 log units below the average depending on the liposome. SOPC was most representative of the average liposome (rmse=0.01) while DOPC and DPPC displayed the greatest deviation from the average (both have rmse=0.04) and typically were lower than the average $K_{\text{liposome/water}}$. On the other hand, POPC was consistently higher than the average $K_{\text{liposome/water}}$ (rmse=0.03). Overall, however, the observed deviations are remarkably small, especially in the context of the precision that can be expected of such experimental measurements. Any of these liposomes can be considered to accurately represent the average PC or PE membrane lipid.

The differences between the measured $K_{\text{AFT/water}}$ are illustrated in Figure 5.2B. For the AFTs, PCB-28 has the most variable $K_{\text{AFT/water}}$ with deviations between -0.11 and 0.17 log units deviation from the average. Otherwise, all $K_{\text{AFT/water}}$ measurements are within 0.1 log units of the average $K_{\text{AFT/water}}$. While the $K_{\text{AFT/water}}$ for AFT-A and AFT-G are almost all slightly higher than the average, AFT-C and AFT-D are generally slightly below the average. However, as with the $K_{\text{membrane lipid/water}}$ measurements, the $K_{\text{AFT/water}}$ are extremely similar, which highlights just how miniscule inter-individual differences in PCB partitioning to human abdominal fat tissues are.
Figure 5.2: Comparing the deviation of (A) log $K_{liposome/water}$ from the average log $K_{liposome/water}$ for each liposome as a function of the average log $K_{liposome/water}$ for all liposomes and of (B) log $K_{AFT/water}$ from the average log $K_{AFT/water}$ for each AFT as a function of the average log $K_{AFT/water}$ for all AFTs, for 18 PCB congeners.

5.3.2 Prediction of Partitioning into Lipids using Polyparameter Linear Free Energy Relationships

Figure 5.3 plots the measured log $K_{cell lipid/water}$ and log $K_{AFT/water}$ against the log $K_{storage lipid/water}$ as predicted by the ppLFERs from Endo et al. (2011) and Geisler et al. (2012), respectively, in combination with the solute descriptors for PCBs from Abraham and Al-Hussaini (2005) and Van Noort et al. (2010). The rmse values for the fit of these measured data by the various ppLFER combinations are listed in Table 3. As demonstrated by Figures 5.3E and 5.3I, equation 5.1 from Geisler et al. (2012) in combination with the solute descriptors for PCBs from Abraham and Al-
Hussaini (2005) does an excellent job of predicting the partitioning of PCBs into the AFT storage lipids (rmse of 0.09 to 0.15) (except for PCB 18 for which the model tends to overpredict the $K_{\text{storage lipid/water}}$). As demonstrated by Figures 5.3A, the measured log $K_{\text{liposome/water}}$ against the log $K_{\text{membrane lipid/water}}$ as predicted by the ppLFER equation type 5.1 from Endo et al. (2011) in combination with the solute descriptors for PCBs from Abraham and Al-Hussaini (2005) does an equally good job (rmse of 0.12 to 0.14) of predicting the partitioning of PCBs into liposomes (again except PCB 18 for which the model tends to overpredict the $K_{\text{liposome/water}}$). This may suggest that either the solute descriptor for PCB 18 may be slightly wrong or analytical issues affected the accuracy of our measured concentrations of PCB 18.

Figures 5.3C, G, and K show the same comparisons as those in Figures 5.3A, E, and I except that the solute descriptors for the PCBs by van Noort et al. (2010) are used instead of those by Abraham and Al-Hussaini (2005). In this case, the adjustments to the solute descriptors for PCBs by van Noort et al. (2010) does not lead to improved predictions of the partitioning behaviour into lipids since the rmse increases in both cases (rmse of 0.18 to 0.24 and 0.18 to 0.20 for storage lipids and membrane lipids, respectively). In particular, the overprediction for PCB 18 persists. Figures 5.3B, F, and J also show the same comparisons as those in Figures 5.3A, E, and I but this time equations of type 5.2 from Geisler et al. (2012) and Endo et al. (2011) are used instead of type 5.1. These plots demonstrate that replacing $V_i$ with $L_i$ in the ppLFER equations for storage lipids and membrane lipids (as advocated in Geisler et al. (2012) and in Endo et al. (2011)) leads to much poorer predictions of PCB partitioning into lipids (rmse of 0.52 to 0.65 and 0.28 to 0.30 for storage lipids and membrane lipids, respectively).

Finally, Figures 5.3D, H, and L compare the measured partition coefficients of the liposomes, cells and AFT, respectively, with the ppLFER predictions made using equations of type 5.2 from Geisler et al. (2012) and Endo et al. (2011) but with the updated solute descriptors from van Noort et al (2010). Compared with the Abraham and Al-Hussaini (2005) descriptors (rmse of 0.52 to 0.65 and 0.28 to 0.30 for storage lipids and membrane lipids, respectively), the van Noort et al (2010) descriptors yield less scatter around the 1:1 line (rmse of 0.32 to 0.43 and 0.25 to 0.26 for storage lipids and membrane lipids, respectively) when using equation 5.2 particularly for the cells and AFTs. Overall, we propose that the best ppLFER prediction for PCBs was achieved using equations of type 5.1 from Geisler et al. (2012) and Endo et al. (2011) using the descriptors from Abraham and Al-Hussaini (2005).
Figure 5.3: Comparison of measured $K_{\text{Lipid/Water}}$ for liposome, cell, and ATF lipid systems with ppLFER model predictions. The black 1:1 line corresponds to perfect agreement between the partitioning coefficients.

5.3.3 Prediction of Partitioning into Lipids using Single Parameter Linear Free Energy Relationships based on the $K_{\text{octanol/water}}$.

Figure 5.4 below plots the measured $K_{\text{liposome/water}}$, $K_{\text{cell lipid/water}}$ and $K_{\text{AFT/water}}$ against the four sets of $K_{\text{octanol/water}}$ values. The first column (Figures 5.4A, E, and I) compares the measured $K_{\text{octanol/water}}$ values by Hawker and Connell (1988) against the measured log $K_{\text{liposome/water}}$, log $K_{\text{cell lipid/water}}$ and log $K_{\text{AFT/water}}$ respectively. The second column (Figures 5.4B, F, and J) compares the FAV $K_{\text{octanol/water}}$ values against the $K_{\text{lipid/water}}$ values measured in this study and the third (Figures 5.4C, G, and K) and fourth column (Figures 5.4D, H, and L) compares the predictions of the log $K_{\text{octanol/water}}$ (ppLFER/Goss (2005)) model using the Abraham and Al-Hussaini (2005) descriptors (which we will now refer to as the Goss/Abraham ppLFER) and the updated van Noort et al. (2010) descriptors (which we will now refer
to as the Goss/van Noort ppLFER), respectively. The goodness of fit of each $K_{\text{octanol/water}}$ set as a model representative for each of the lipids is demonstrated by the rmse values listed in Table 4.

Figure 5.4: Comparing the predicted log $K_{\text{octanol/water}}$ values from Hawker and Connell (1988), FAV (Schenker et al. 2005), Goss/Abraham, and Goss/van Noort models against the measured log $K_{\text{lipid/water}}$ values of the liposomes (A-D), cells (E-H), and AFTs (I-L). The black 1:1 line corresponds to perfect agreement between the partitioning coefficients.

In terms of the general order of magnitude of $K_{\text{liposome/water}}$, octanol is a reasonably good surrogate for most liposomes (Figures 5.4A-D). However, for the FAV and Goss/Abraham ppLFER methods of predicting log $K_{\text{octanol/water}}$ (Figures 5.4A and 4B), there is considerable scatter (rmse of 0.29-0.33 and 0.37-0.39, respectively) around the 1:1 line. Endo et al (2011) has similar findings with deviations from experimentally measured log $K_{\text{octanol/water}}$ generally being less than 0.8 log units (Endo et al. 2011). By substituting the van Noort et al. (2010) descriptors, the scatter is considerably reduced (rmse of 0.29-0.31) but the measured values by Hawker and Connell most accurately reproduce the $K_{\text{liposome/water}}$.
measurements with an rmse of 0.23-0.25. For the Hawker and Connell values and both ppLFER methods, the largest outliers among the PCBs evaluated were PCB 155 and 187.

Figure 5.4 also shows that octanol is clearly a poor surrogate for storage lipids (see Figures 5.4E-L) as it underpredicts the partitioning potential of PCBs. On average, the predicted log $K_{\text{octanol/water}}$ is 0.73 to 0.92 log units lower than the measured $K_{\text{storage lipid/water}}$ (Figure 5.5). Furthermore, there is also considerable scatter in Figure 5.4 that was not observed in Figure 5.1 when comparing the measured partitioning coefficients against triolein. To explore the root of this scatter we calculated the difference between the measured $K_{\text{lipid/water}}$ and $K_{\text{octanol/water}}$ for all lipids and PCB congeners used in the experiments. Specifically, we define this difference as

$$\text{Deviation} = \text{measured log } K_{\text{lipid/water}} - \text{model predicted log } K_{\text{octanol/water}}$$ (Equation 5.6)

The averages of these deviations are plotted in Figure 5.5 as a function of both lipid sample (Figure 5.5A) and PCB congener (Figure 5.5B). For each storage lipid, the average deviation across all PCBs between measured log $K_{\text{lipid/water}}$ and log $K_{\text{octanol/water}}$ is greatest for the Goss/Abraham ppLFER and smallest for Hawker and Connell measured values. The average deviation by the FAVs and the Goss/van Noort ppLFER prediction is consistently 0.05 and 0.09 log units lower than the Goss/Abraham ppLFER prediction, respectively, while the Hawker and Connell values are consistently 0.23 log units lower. In other words, the Hawker and Connell measurements appear to perform much better than the FAV and ppLFER predictions when the deviation is averaged across all PCBs within a storage lipid (Figure 5.5A). However, when comparing the average deviation across the lipids as a function of PCB congener, there are substantial differences between congeners with deviations ranging from 0.32 to 1.54 log units (Figure 5.5B) (except for PCB 18 that has deviation as low as 0.17 log units) and no apparent trend with $K_{\text{octanol/water}}$ datasets.

To explore whether the deviations are random within each lipid type, the correlation coefficients between lipid types were calculated (Tables 5-7). The deviations with log $K_{\text{octanol/water}}$ between AFT samples, cell lipids, and triolein were highly correlated with each other using the Hawker and Connell (1988) values ($R^2>0.95$), FAV ($R^2>0.90$), Goss/Abraham ppLFER ($R^2>0.95$) and Goss/van Noort ppLFER ($R^2>0.93$) methods (See Tables S5.3-6). Similarly, the deviations for liposomes are also highly correlated with each other for all four approaches ($R^2>0.97$) (Table S5.3-6). Even the deviations for storage lipids and liposomes are highly correlated with each other for the Hawker and Connell (1988) values ($R^2>0.89$), the FAV method ($R^2>0.82$) and both ppLFER methods ($R^2>0.89$) (Table
S5.3-6). These high correlations indicate that the average deviation of log $K_{\text{octanol/water}}$ from the measured log $K_{\text{lipid/water}}$ is not dependent on lipid type.

![Figure 5.5: Comparing differences between measured log $K_{\text{storage lipid/water}}$ and predicted log $K_{\text{octanol/water}}$ by Hawker and Connell (1988) FAV (Schenker et al. 2005) Goss/Abraham ppLER and Goss/van Noort methods according to A) storage lipid and B) PCB congener. Deviations between $K_{\text{octanol/water}}$ datasets were also evaluated. That is, the deviations of $K_{\text{octanol/water}}$ (approach 1) from $K_{\text{lipid/water}}$ were correlated against the deviations of $K_{\text{octanol/water}}$ (approach 2) from $K_{\text{lipid/water}}$, where approach 1 and 2 were any combination of the 4 $K_{\text{octanol/water}}$ approaches being evaluated in this study. No significant correlations between these approach specific deviations were observed. This lack of a relationship is consistent with Figure 5.6 which compares the various $K_{\text{octanol/water}}$ approaches with $K_{\text{octanol/water}}$ values reported by Hawker and Connell (1988). Clearly, $K_{\text{octanol/water}}$ values are still highly uncertain and any comparison between lipid partitioning and octanol is highly dependent upon the source of $K_{\text{octanol/water}}$ values used.
Figure 5.6: Comparison of the ppLFER (Goss/Van Noort), ppLFER (Goss/Abraham) and FAV $K_{\text{octanol/water}}$ values of 18 PCBs with the corresponding $K_{\text{octanol/water}}$ values reported by Hawker and Connell (1988). The black 1:1 line corresponds to perfect agreement between the partitioning coefficients.

### 5.4 Conclusions

The goal of this study was to evaluate 1) the validity of using surrogate systems as representatives for realistic systems; and 2) the validity of various models available for predicting partitioning. Our results have demonstrated that, for PCBs, octanol is a reasonable surrogate for liposomes but not for storage lipids. Furthermore, partitioning into liposomes underestimated PCB partitioning into human abdominal fat tissues and human MCF-7 cells by approximately half a log unit. Triolein on the other hand, appears to be an excellent surrogate for lipids in both human abdominal fat tissues and human MCF-7 cells with respect to PCB partitioning. While the agreement in partition coefficients between the abdominal fat tissues and cells will likely apply also to other compounds with chemical properties similar to PCBs, this generalization may break down for ionogenic compounds. Published ppLFERs for both storage lipids and liposomes accurately predicted the PCB partitioning properties of the systems investigated in this study. As such, the application of ppLFERs to calculating partition coefficients of human storage lipids is appropriate.
5.5 Supporting Information

Table S5.1: RMSE of (A) measured $K_{\text{membrane lipid/water}}$ by ppLFER predictions by Endo et al.(2011) and of (B) $K_{\text{storage lipid/water}}$ by ppLFER predictions by Geisler et al.( 2012)

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Table S5.2: Statistical analysis of four $K_{\text{octanol/water}}$ datasets as surrogates for $K_{\text{liposome/water}}$, $K_{\text{cell lipid/water}}$ and $K_{\text{AFT/water}}$.

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Table S5.3: Correlation coefficients ($R^2$) for deviations by log $K_{octanol/water}$ (Hawker and Connell, 1988) between various lipid systems

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Table S5.4: Correlation coefficients ($R^2$) for deviations by log $K_{octanol/water}$ (FAV, Schenker et al. 2005) between various lipid systems.

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Chapter 6
Developing and evaluating a combined bioenergetics/mass-balance bioaccumulation model for fish

Cristina L. Quinn, Jon A Arnot, and Frank Wania.

Contributions: Under the guidance of J.A. Arnot, C. Quinn designed and programmed the model. Under the guidance of J.A. Arnot and F. Wania, C. Quinn interpreted the model output and wrote the manuscript.
6.1 Introduction

The bioaccumulation of persistent organic pollutants in aquatic organisms and other biota in various ecosystems is well documented (Oliver and Niimi, 1988; Rasmussen et al., 1990; Ramesh et al., 1992; Fisk et al., 2001; Weber and Goerke, 2003; Letcher et al., 2010; Galvao et al., 2012;). Numerous bioaccumulation models have been developed (see reviews by Mackay and Fraser (2000) and Barber et al. 2008) in order to better understand the underlying mechanisms, to predict chemical concentrations in organisms, and to address data gaps in chemical hazard, exposure and risk assessment. The mass balance bioaccumulation models used to quantify chemical uptake and elimination processes for fish and other aquatic species have evolved over the past few decades (Neely, 1974; Thomann, 1981; Thomann, 1984; Barber, 1988; Gobas, 1993b; Morrison and Gobas, 1997; Hendriks, 2001; Arnot and Gobas, 2004; Gewurtz et al., 2006; Arnot et al., 2008). The chemical uptake and elimination rates used in the models are a function of the organism’s feeding, ventilation, and growth rates. These physiological parameters are related to the biological energy balance of an organism, in which the energy derived from the organism’s diet is matched with the energy it expends on processes such as metabolism, egestion, excretion, activity, digestion, and growth (Karas and Thoressson 1992; Kitchell et al. 1977). Ideally, to maximize their predictive capacity, mass balance bioaccumulation models should be parameterized with physiological data that are species-specific and adhere to bioenergetic constraints; however, these data are not always available.

To facilitate the application of bioaccumulation models to a range of species under various environmental conditions, key bioenergetic parameters are often estimated using empirical allometric relationships (Hendriks et al. 2001; Arnot and Gobas 2004; Gewurtz et al. 2006; Arnot et al. 2008). While such relationships facilitate model parameterization in the absence of species- and system-specific information, they are often obtained from disparate sources and therefore may or may not be consistent with each other. For example, in the commonly applied AQUAWEB food web bioaccumulation model (Arnot and Gobas 2004) the respiration rate is derived from an allometric relationship based on data for approximately 200 fish species in the OXYREF database (Thurston and Gehrke, 1990), the feeding rate is an allometric relationship derived for trout by Weininger (1978), and the allometric growth equation is based on the size distribution of particles in the ocean (Sheldon et al. 1972; Thomann, 1981). Evaluations of various food web bioaccumulation models relying on this growth rate equation indicate that chemical concentrations are often predicted reasonably well (Arnot et al. 2004, 2008) despite the disparate sources of physiological data.

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An alternative method for approximating these physiological rates is by linking mass balance bioaccumulation and bioenergetics models (Norstrom et al. 1976). Recently, Drouillard et al. (2009) combined a modified version of the Wisconsin bioenergetics model (Hewett and Johnson 1992), which is specific to perch and walleye, with the AQUAWEB model (Arnot and Gobas 2004). Empirical bioenergetics models such as the Wisconsin model are often specific to a fish species or taxa. Given that bioaccumulation models may be applied to a broad range of fish species, conditions (laboratory, field) and ecosystems (tropical, temperate, polar), there is a need to examine the bioenergetic consistency of the assumptions and allometric relationships in typical fish bioaccumulation models and, if necessary, to develop easily scalable mass balance bioaccumulation models that are bioenergetically consistent.

In the present study we develop a bioaccumulation mass balance model using physiological data derived from a broad range of fish species and parameterize and evaluate it under various conditions. The new model uses this database of information to obtain bioenergetically consistent rates for growth, feeding and ventilation, i.e., it is a bioenergetically-balanced bioaccumulation model (3B model). The 3B model is parameterized for 1) comparison with three existing non-bioenergetically balanced bioaccumulation models (i.e. Arnot and Gobas 2004, Arnot et al. 2008, and Gewurtz et al. 2006); 2) evaluation against an empirical dataset of concentrations of five polychlorinated biphenyl (PCB) congeners in perch of three different sizes over a year with variable temperature; and 3) generation of hypotheses with respect to the relationship of fish size and temperature with biomagnification factors (BMFs).

6.2 Methods

6.2.1 Theory

Fish bioaccumulation is typically modelled using a chemical mass balance approach whereby the body burden of a chemical in a fish is a function of the difference between contaminant uptake and elimination processes (Gobas and Mackay, 1987). As illustrated in Figure 6.1, a fish can take up chemical across its gills \( D_V \) and intestines \( D_A \), while eliminating chemical through egestion \( D_E \), biotransformation \( D_M \), exhalation \( D_V \), and growth dilution. When described in fugacity notation (rather than rate constant notation), each process can be mathematically defined by a transport parameter or D-value (i.e \( D_V, D_A, D_E, D_M \)) with units of \( \frac{mol}{Pa \cdot day} \). For a detailed mathematical description of each D-value and the overall contaminant mass-balance model, see section 6.4.2.
In the same way that the fluxes of chemical in and out of the fish need to be balanced, the energy taken up and expended by the fish needs to match. In bioenergetics modelling, the amount of energy the fish derives from its diet is allocated to energy consuming processes such as ventilation, digestion, activity, excretion, and growth. Since the amount of energy from food is finite, energy is allocated to the processes of greatest importance first i.e. metabolism, food gathering, digestion, defense, etc. A fish can therefore only experience growth if the energy derived from its diet is greater than the energy required for these essential processes (Kitchell et al. 1977). As such, the energy expended on growth cannot be determined independently from the other energy consuming processes. Furthermore, many of the chemical fate processes described by D-values require energy. In order to ensure that the energetic processes are internally consistent with each other (red arrows in Figure 6.1B) as well as with the physiological parameters used to describe bioaccumulation processes (blue arrows in Figure 6.1B), we integrated a biological energy balance into a non-steady-state mechanistic bioaccumulation model.

Figure 6.1: A) Conceptual overview of a bioaccumulation model based on non-balanced bioenergetics – the bioaccumulation processes are balanced with each other (curved black arrows) but the energetic contributions to these processes are isolated from each other. B) Conceptual overview of a bioenergetically-balanced bioaccumulation model – the bioenergetic processes are balanced with each other (red arrows) and then feed into (blue arrows) the bioaccumulation processes.

The 3B model first solves a bioenergy balance on the fish, using either a feeding rate or a growth rate supplied by the user. Using terms from the energy balance, the model then derives three internally consistent physiological rates (ventilation rate, growth rate, feeding rate), that are used in the calculation of D-values describing contaminant uptake and loss processes. Finally, the contaminant mass balance over the fish is solved to yield concentrations in fish, and fluxes in and out of fish as a
function of time. The bioenergy and contaminant mass balance equations are given in Sections 6.5.1 and 6.5.2. The model uses fugacity notation and is programmed in Excel/Visual Basic.

6.2.2 Relationship between the biological energy balance and the contaminant mass balance

The 3B model weaves a complex web of interrelated bioenergetics and bioaccumulation parameters, which can be illustrated in a flow chart (Figure 6.2). Many of the required input parameters (i.e. oxycalorific coefficient \( D_{O_2} \); kJ/mg O\(_2\), density of lipid, protein and water \( \delta_L, \delta_P, \) and \( \delta_W, \) respectively; g/m\(^3\)) are empirically known and widely applicable. Fish specific parameters include the activity multiplier \( A \), the assimilation efficiency of lipid, protein and water from the diet \( E_{L,D}, E_{P,D}, \) and \( E_{W,D}, \) respectively), composition of lipid, protein and water in the diet \( X_{L,D}, X_{P,D}, \) and \( X_{W,D}, \) respectively), the lipid fraction \( X_{L,F} \), mass \( W_F; g \) and density \( \delta_F; \) g/m\(^3\) of the fish. Chemical specific parameters include the octanol-water partition coefficient \( K_{OW} \) and the biotransformation rate constant \( k_M \). The only environmental parameters required are temperature \( T; ^\circ C \) and the concentration of oxygen in the water \( \left[ O_2 \right]; \) g O\(_2\)/ m\(^3\).

Figure 6.2 illustrates the case in which the growth rate is known and is only a function of temperature and fish mass. The specific parameters of importance to each of the D-values are highlighted in Figure S6.1A-D. Figure 6.2 reveals that the D-values for ventilation, food consumption and fecal egestion \( D_V, D_A, \) and \( D_E, \) respectively) are all dependent upon the four bioenergetic parameters routine metabolic rate \( RMR \), specific dynamic action \( SDA \), activity multiplier \( A \) and urinary excretion \( U \). All D-values are dependent on temperature. As \( RMR \) and \( U \) are also functions of temperature, temperature is a significant factor in both the bioenergetic and bioaccumulation sub-models (Figure 6.2).
Figure 6.2: Flow chart illustrating the relationship between the various parameters in the bioenergetically-balanced bioaccumulation (3B) fish model. The parameter acronyms are defined in Table S6.1.
6.2.3 Comparison with other bioaccumulation models

To justify the necessity of an energy balance within a bioaccumulation model, we compared the contaminant uptake and elimination $D$-values of the new 3B model, which is bioenergetically-balanced, with expressions in three other bioaccumulation models (A&G model: Arnot and Gobas 2004, A&al model: Arnot et al. 2008, G&al model: Gewurtz et al. 2006), which utilize bioenergetics but are not bioenergetically-balanced. Since the three comparison models use rate constants rather than fugacity, rate constants were converted to $D$-values according to Powell et al. (2009) (see Table S6.3). The comparison was done for a hypothetical chemical with $\log K_{OW} 6.5$ and $\log K_{AW} -2$. Since the bioenergetics are highly dependent on fish weight and temperature, we compared five types of fish: a) a 1 g fish at 10°C; b) a 100 g fish at 10°C; c) a 10 kg fish at 10°C; d) a 100 g Arctic fish at 3°C; and e) a 100 g tropical fish at 27°C.

In all four models, the five types of fish are assumed to grow according to the following allometric growth rate equation (Thomann 1981; Thomann et al. 1992; Gewurtz et al. 2006):

$$dW_F/dt = W_F * 0.00586(1.113^{T-20}) * W_F^{-0.2} \text{ (Equation 6.1)}$$

In the three comparison models, fish feeding rates ($G_A$) are calculated using:

$$G_A(kg/d) = 0.022W_F^{0.85} \left(\frac{1}{kg}\right) \cdot \exp (0.06 T(\circ C)) \text{ (Equation 6.2)}$$

whereas in the 3B model, $G_A$ is derived from the bioenergy balance using the method outlined in Section S6.4.1, case 2. In other words, feeding rates in the comparison models are calculated independently of the growth rate, whereas the 3B model calculates food consumption according to the energetic requirements of the growth rate. During the comparison, the composition of the diet of all hypothetical fish was the same (15% lipids, 40% non-lipid organic matter, 45% water).

6.2.4 Model Evaluation.

The model was evaluated by comparing its results against published datasets. When the original publication did not report fish contamination levels in tabular format, datasets were extracted from graphs using DataThief III (www.datathief.org).

As temperature is a parameter used in the calculation of all D-values as well as the bioenergetic parameters $RMR$, $SDA$, and $U$ (Figure 6.2), temperature fluctuations are expected to affect most
bioenergetic processes and all bioaccumulative processes. Hence, in order to accurately predict bioaccumulation in environments with variable temperature, a non-steady-state model such as the 3B fish model is required. The datasets published by Paterson et al. (2007a, 2007b) and Drouillard et al. (2009), which record the depuration of PCBs from three different sizes of perch (*Perca flavesnecs*) (10 g, 45 g, and 87 g) over a period of one year during which the fish were exposed to seasonal temperature variations, provide an opportunity to evaluate the performance of the non-steady-state 3B fish model. Drouillard et al. (2009) reported lipid-normalized concentrations for PCBs 19 and 28/31 and Paterson et al. (2007a) reported data for congeners 74, 118, and 180. Since these were depuration studies, the chemical uptake processes were set to zero, and the fugacity in the fish at the beginning of the simulation was set to be in accordance with the reported dosed concentrations. In addition, as contaminant biotransformation of PCBs by perch was considered to be negligible relative to the other loss processes, $D_M$ was also set to zero.

The log $K_{OW}$ for the PCBs (congeners 19, 31, 74, 118, and 180) used for the model evaluation (Table 6.2) were either the values by Hawker and Connell (1988) or the final adjusted values (FAVs) by Schenker et al. (2005). For congeners for which no FAVs had been reported, log $K_{OW}$ was predicted using the regression:

$$\log K_{OW} = 0.0125MM + 0.0208n_{O-Cl} + 2.41 \text{ (Equation 6.3)}$$

where $MM$ and $n_{O-Cl}$ represent the molar mass and the number of ortho-chlorines of the congener (Schenker et al. 2005). All log $K_{OW}$ values were temperature adjusted according to (Li et al. 2003):

$$\log K_{OW}(T) = \log K_{OW}(298K) - \Delta U_{OW}/(\ln(10) \cdot R) \cdot (1/(T \ in \ K) - 1/298.15K) \text{ (Equation 6.4)}$$

where the internal energy of phase transfer between octanol and water ($\Delta U_{OW}$, kJ/mol) was taken directly from Schenker et al. (2005) when FAVs were reported or else calculated using:

$$\Delta U_{OW} = -0.0405MM + 1.71n_{O-Cl} - 13.4 \text{ (Equation 6.5)}.$$
Table 6.1: Physicochemical properties of selected PCB congeners at 25°C.

<table>
<thead>
<tr>
<th></th>
<th>PCB 19</th>
<th>PCB 31</th>
<th>PCB 74</th>
<th>PCB 118</th>
<th>PCB 180</th>
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<tr>
<td>log $K_{OW}$</td>
<td>5.02</td>
<td>5.67</td>
<td>6.20</td>
<td>6.74</td>
<td>7.36</td>
<td>Hawker and Connell (1988)</td>
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<tr>
<td></td>
<td>5.67</td>
<td>6.07</td>
<td>6.11</td>
<td>7.08</td>
<td>7.66</td>
<td>Schenker et al. (2005)</td>
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<tr>
<td>$H$ (Pa m$^3$/mol)</td>
<td>142.4</td>
<td>33.4</td>
<td>16.0</td>
<td>10.8</td>
<td>7.7</td>
<td>Schenker et al. (2005)</td>
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6.2.5 Model application: Biomagnification factors for fish of different size and at different temperatures

Contaminant concentrations in fish are used to estimate biomagnification factors (BMF) which are defined as the concentration in the fish at steady-state divided by the concentration in its diet. Both laboratory and field studies have been carried out to better understand the factors controlling BMF. The advantage of laboratory studies is the ability to empirically determine the feeding and growth rates. Typically, the feeding rate is known because the fish are fed a specific amount of food each day (e.g. 1-2% w/w/d (OECD, 1996)) and changes in fish mass over time are measured. In field studies, gut analysis may reveal the type and composition of food consumed; however, rates of food consumption and growth are based on assumptions rather than empirical measurements. Here we apply the 3B model to illustrate in a hypothetical context the dynamic nature of predator/prey concentration ratios for differently sized fish at different temperatures. To simulate a typical laboratory study, we utilize a constant feeding rate of 1% w/w/d and calculate bioenergetically the resulting growth (i.e. a diet-controlled growth). To simulate a field situation, we utilize the growth rate expression in Equation 6.1 and bioenergetically calculate the feeding rate required to sustain such growth (i.e. growth-controlled diet). However, this is not to say that all field conditions allow for a growth-controlled diet as this assumes that food is freely available. It is more realistic that there is a limited amount of food available to the fish.

For the purposes of this evaluation we define a pseudo-BMF (BMF*) as:

$$BMF^* = \frac{C_{Fish}(after \ 1000 \ days)}{C_{Food}}$$ (Equation 6.6)

to recognize that the fish may or may not have reached steady-state after 1000 days. Simulations assuming diet-controlled growth and a growth-controlled diet were again conducted for each of the following five types of fish: a) a 1 g fish at 10°C; b) a 100 g fish at 10°C; c) a 10 kg fish at 10°C; d) a
100 g Arctic fish at 3°C; and e) a 100 g tropical fish at 27°C using hypothetical chemicals of variable log $K_{OW}$ and with $k_M = 0$. The $BMF^*$ for each of these fish are plotted as a function of log $K_{OW}$. Because the calculated fish concentrations (ng/g lipid) are not growth corrected, we also defined $D_G^*$ as a pseudo-loss process for growth.

### 6.3 Results and Discussion

#### 6.3.1 Comparison with existing bioaccumulation models

The absolute $D$-values for dietary uptake ($D_A$), egestion ($D_E$) and gill-water exchange ($D_V$) calculated for each fish type by each of the four models are given in Figure S6.3. These D-values for the 3B model were divided by those from the comparison models and the ratios are shown in Figure 6.3A, 6.3B, and 6.3C, respectively.

Since each of the three comparison models rely on the feeding rate calculated with Equation 6.1, the ratios of the $D_A$-values predicted by each of the comparison models with that of the 3B model are also equivalent. For all five fish types, $D_A$ in the comparison models is higher by a factor of 7 to 17 than the 3B model. This implies that the comparison models overestimate the amount of food required to sustain the assumed growth rate by this factor. This overestimation increases with fish mass and decreases with temperature, implying that Equation 6.1 overestimates the feeding rate with respect to both fish mass and temperature. As a consequence of overestimating the feeding rate, the comparison models may be overestimating dietary uptake of contaminants and consequently, bioaccumulation levels relative to the 3B model. This is consistent with the review by Barber (2008) who also criticized feeding rates as being disproportionately large compared to growth rates.

Although the $D_A$-values were equivalent between the three comparison models, the rate of egestion ($D_E$) in the A&G model is higher by a factor of 3 to 9, in the A&al model by a factor of 1 to 3, and that in the G&al model lower by a factor of 2 to 7 compared to the 3B model (Figure 6.3B). The differences with the first two of these models arise as a result of different dietary assimilation efficiencies of lipids and non-lipid organic matter (see Table S6.4). The lower $D_E$ in the G&al model is partially due to the relatively large fraction of the ingested diet ($\beta=0.74$) that is absorbed by the organism (i.e. a greater assimilation efficiency) compared to the A&G ($\beta=0.51$), A&al ($\beta=0.36$), and 3B model ($\beta=0.49$). Of course, $D_E$ is also a function of the feeding rate so the cause of discrepancy discussed for $D_A$ also
carries forward to the analysis of $D_E$. Overall, the $D_E$-values are within one order of magnitude of each other.

Figure 6.3: $D$-values (after 28 days) for dietary uptake, $D_A$, egestion, $D_E$, and gill/water exchange, $D_V$, calculated for a chemical with a log $K_{OW}$ of 6.5 and five different fish (a: a 1 g fish at 10°C; b: a 100 g fish at 10°C; c: a 10 kg fish at 10°C; d: a 100 g Arctic fish at 3°C; and e: a 100 g tropical fish at 27°C) by the new 3B model divided by the corresponding $D$-values calculated by the models of Arnot and Gobas (2004), Arnot et al. (2008), and Gewurtz et al. (2006).

D-values for ventilation ($D_V$) in the different models are also all within one order of magnitude of each other (Figure 6.3C). The ventilation rate in the 3B model is based on a formulation adopted from the A&al model; and indeed the $D_V$-values of these two models are within 10% of each other for all
scenarios. The A&G and G&al models predict higher $D_V$ relative to the 3B model for the 1 g fish (by a factor of 2.4 and 1.4, respectively) but lower $D_V$ for the 10 kg fish (by a factor of 1.6 and 2.3, respectively) and tropical fish (by a factor of 1.5 and 1.2, respectively). It is interesting that the G&al model yields different predictions than the 3B model even though the ventilation rates are based on the same OXYREF dataset. The G&al derivation however is based on 2465 data points (the fit had coefficient of correlation of $r^2=0.73$) whereas the approach used in the 3B model (as derived by A&al) is based on 2957 data points ($r^2=0.83$). The difference between these two RMR expressions is particularly notable, on a mass basis, for smaller fish (see Figure S6.2) and explains the difference in $D_V$ value predictions between the G&al and 3B models. In all cases, the G&al model calculates a higher $D_V$ than the A&G model. Since ventilation rates for both the A&G and G&al models are based on the same OXYREF dataset, this difference is likely due to the inclusion of a temperature dependence for the ventilation rate in the G&al model that is not considered in the A&G model.

Clearly, the rate of these uptake and elimination processes is influenced not only by the bioenergetic expressions used in their parameterization, but also by ensuring consistency between these expressions through a bioenergy balance. Overall, the G&al model estimated higher contaminant uptake from the diet and lower fecal egestion relative to that in the 3B model. This implies that this model is likely to predict higher total biomagnification in fish, compared to the 3B model. Since the A&G model predicts both dietary uptake and fecal egestion to be higher than the 3B model by approximately one order of magnitude, $BMFs$ predicted by the A&G model are not expected to differ substantially from the 3B model for the five fish types evaluated here. On the other hand, the A&al model predicted much higher food uptake (by a factor of 7 to 17) but only slightly higher fecal egestion (by a factor of 1 to 3 depending on the fish type) relative to the 3B model. Therefore, it is also expected to predict higher overall biomagnification than the 3B model. The assumption in this analysis is that the growth equation 6.4 is accurate and therefore the feeding rate as derived in equation 6.1 must be inaccurate. It is also possible, however, that the feeding rate is accurate and hence the growth rate would be underestimated. Clearly additional research is required in order to clarify this issue. Furthermore, we do not suggest that the 3B model is “correct” and the comparison models are “wrong” but rather that the physiological parameters used in many commonly applied fish bioaccumulation models are irreconcilable with each other in a bioenergy balance and hence careful reconsideration of existing models is warranted.
6.3.2 Model Evaluation (Non-Steady-state)

The reported experimental values for the five PCBs (3 fish sizes at 9 to 11 time points - depending on the dataset - throughout the year, n=438) (Paterson et al. 2007a, 2007b, Drouillard et al. 2009) are compared with the predictions made by the 3B model, using the log $K_{OW}$ values by either Schenker et al. (2005) (Figure 6.4A) or Hawker and Connell (1988) (Figure 6.4B). All predictions are within 0.5 log units of the measured data with the exception of PCB-19. For this congener, predictions are within 1 log unit of the measured data, but are either consistently too high (above the 1:1 line, Schenker et al) or too low (below the 1:1 line, Hawker&Connell).

This is a much better fit than was achieved by a bioenergetically-based bioaccumulation model by Drouillard et al. (2009) (D&al model). The D&al model consistently overestimated elimination for PCB congeners with log $K_{OW}$ values less than 5.8 with overestimation increasing with decreasing log $K_{OW}$. Specifically, the model predicted the concentrations of congener 28/31 to within an order of magnitude but predicted concentrations of PCB-19 that were approximately 3 orders of magnitude lower than measured. Drouillard et al. (2009) concluded that their model’s performance could only be improved through adjustment of $E_W$, the parameter describing chemical exchange efficiency across the gills. They proposed that the gill transfer efficiency parameter in the bioaccumulation model was overestimating gill elimination and hence driving the model discrepancy. The expression describing chemical exchange efficiency across the gills, $E_W$, was originally developed by Gobas and Mackay (1987). It was assumed that transport of the contaminant to the fish and within the fish was controlled by diffusion through a series of aqueous and lipid phases which occur in series and that transport resistances across the lipid/water interface were negligible. Gill uptake efficiency could then be described as a function of the diffusion transport parameters and $K_{OW}$. Drouillard et al. (2009) suggested that the typical value of 0.54 for $E_W$ should be adjusted to 0.11 – 0.16, depending on the size of the fish, in order to calibrate their bioenergetically-based bioaccumulation model. Based on the derivation of $E_W$, such an adjustment implies that the diffusion transport parameters typically used to describe gill ventilation in fish are underestimated.

A fundamental difference between the D&al and 3B model is the calculation of the respiration rate. A comparison of these two approaches is illustrated in Figure S6.2 where the respiration rate is plotted as a function of fish weight and temperature. Drouillard et al. (2009) implemented the modified Wisconsin bioenergetics model developed by Enders et al. (2006) which was calibrated for yellow perch. Whereas the D&al model utilizes an expression for $RMR$ that has been specifically calibrated to
Walleye (Stizostedion vitreum vitreum) and yellow perch, the species used in the experimental data set, from 12 to 20°C (Enders et al. 2006), the 3B model utilizes a generic expression derived by Arnot et al. (2007) from a dataset of approximately 200 fish species over the temperature range of -1.5 to 40°C. The difference between the two approaches is particularly significant at higher temperatures; the D&al model predicts an RMR (kJ/g fish/day) that is twice as large as in the 3B model at 25°C and 5 times larger at 30°C (Figure S6.2B). Furthermore, it is during these warmer seasons that elimination of PCBs by fish was observed to be the greatest in the seasonal depuration experiments; an increased gill ventilation rate is necessary to meet the oxygen requirements of the increased RMR in warmer temperatures.

Figure 6.4: Evaluation of 3B model predictions (using log KOW values from Schenker et al. (2005), Hawker and Connell (1988), and the average of Schenker et al and Hawker and Connell) against the time-variant concentrations of PCB-19 (circles), and PCB congeners 28/31, 74, 118, and 180 (filled circles) in differently sized perch as reported by Paterson et al. (2007a, 2007b) and Drouillard et al. (2009).

The much better fit of the 3B model results with the measured data thus suggests an alternative hypothesis to that of Drouillard et al. (2009) which is that the overestimated elimination predicted by the D&al model was due to the Wisconsin model’s overestimation of the RMR rate at elevated temperatures rather than an overestimation of $E_w$. This hypothesis is also supported by Enders et al. (2006) who suggested that several of the published metabolic rate models overestimate the RMR by confounding the energetic requirements of activity and digestion with metabolism.
Figure 6.5: Comparison of the time variant concentrations of PCB congeners 19 and 31 in perch as measured by Drouillard et al. (2009) with the predictions of the new 3B model and the model by Drouillard et al (2009).

To further evaluate the hypothesis that overestimated elimination by the D&al model is due to an overestimation of the RMR expression, Figure 6.5 compares the measured PCB concentrations in perch as a function of time with values predicted by the D&al and 3B models. The curvature of the lines in Figure 6.5 can be attributed to the dependence of both the gill ventilation rate and fish lipid content on temperature. The temperature minimum of 2°C occurs around day 213 while the temperature maximum of 24°C occurs around day 19. The D&al model predictions diverges quickly from both the measured data and the 3B model predictions in the first 90 days of the experiment (Figure 6.5), when experimental temperatures were high (15 to 25°C, Figure S6.3) and the D&al model thus uses an RMR, and therefore also a $D_V$, that is twice as large as in the 3B model. As temperature decreased, food consumption decreased to a point below the minimum requirements for fish maintenance - despite a slower metabolic rate. To compensate, the lipid reserves in the fish were converted into energy and the lipid content decreased. As a result, the lipid normalized PCB concentrations increased. As temperature
increased in the spring and summer months, the fish began to consume more food than is energetically required allowing for the lipid reserve to be replenished. In addition, the gill ventilation rate increases at higher temperatures. The combined effects of increasing lipid content and increasing ventilation rate are reflected by the decreasing lipid normalized PCB concentration after day 300. Drouillard et al. (2009) stipulated that only size optimized empirical adjustments to the chemical exchange efficiency across the gills $(E_w)$ could account for the overestimated PCB elimination predicted by their model since the gill ventilation rates used in their simulations were consistent with previous measurements. However, this ventilation rate was derived for a relatively narrow temperature range of 12 to 20°C (Enders et al. 2006), yet applied in the D&al model to estimate ventilation at both lower and higher temperatures (2 to 24 °C). The gill ventilation rate in the 3B model, on the other hand, was parameterized using data obtained over a wide temperature range (from -1.5 to 40 °C). Our alternative hypothesis suggests that by implementing a non fish-specific gill ventilation rate which has been parameterized to encompass the temperature range of interest, adjustment of $E_w$ is not required as demonstrated by the improved fit of the 3B model to the measured data.

The role log $K_{OW}$ values on model performance becomes apparent in Figure 6.4 for PCB-19 where the value proposed by Schenker et al. (5.67) is 0.65 log units higher than that reported by Hawker and Connell (5.02). The model predictions are particularly sensitive to the value of the log $K_{OW}$ in this range, where the choice of log $K_{OW}$ can lead to concentrations that are up to 10 times greater or 10 times less than the measured ones. Since the log $K_{OW}$ value predicted by Schenker et al. is higher than that by Hawker and Connell, it always predicts higher PCB 19 concentrations in the fish. In this case, the Schenker et al. log $K_{OW}$ overpredicts the concentration while the Hawker and Connell log $K_{OW}$ underpredicts. According to the root mean squared errors (RMSE), the log $K_{OW}$ values by Hawker and Connell (1988) yield concentrations slightly closer to the measurements for PCB 19 and 31 than Schenker et al. (2005)’s log $K_{OW}$, but there is no substantial difference in the RMSE when using either source’s log $K_{OW}$ for PCBs 74, 118, and 180 (see Tables S6.5 for additional information). When the model predictions are evaluated as a function of fish size, the log $K_{OW}$ values by Hawker and Connell again yield a lower RMSE than those by Schenker et al. (see Tables S6.6). To further evaluate the uncertainty of log $K_{OW}$ values on model performance, additional calculations were performed using the average log $K_{OW}$ of the values proposed by Schenker et al. and Hawker and Connell. If the RMSE was due to flawed log $K_{OW}$ values, then it is expected that averaging the log $K_{OW}$ values may reduce the RMSE. However, when evaluated according to both PCB congener and fish size, no significant
reduction in RMSE is observed (Tables S6.3.5 and Tables S6.3.6). This indicates that the RMSE is likely being driven by the scatter in the measured data. Furthermore, since neither the Hawker and Connell nor the Schenker et al. value yield significantly better RMSE with the measured data, it is recommended that future evaluations consider both sources of log $K_{OW}$ in data analysis.

6.3.3 Biomagnification factors for fish of different size and at different temperatures

Figure 6.6 displays the $BMF^*$ - defined in Eq. 6.6 - for non-metabolized chemicals as calculated by the 3B model for five different fish as a function of the log $K_{OW}$, assuming either diet-controlled growth or growth-controlled diet. In all calculations, the $BMF^*$ peaked at around a log $K_{OW}$ of 6. This is consistent with previous studies which proposed that biomagnification of chemicals with log $K_{OW} < 6$ is limited by efficient loss through respiration (Gewurtz et al. 2006; Kelly et al. 2007), while above log $K_{OW} > 7$ it is limited by slow absorption rates (Kelly et al. 2007).

Figure 6.6A compares fish of different size (1 g, 100 g, 10 kg) at the same temperature (10 °C) assuming diet-controlled growth. Since these three fish feed at the same body mass-normalized rate, differences in dietary uptake and egestion are not expected to cause any differences in their $BMF^*$s. The bioenergetically-balanced growth rates for each of these fish was determined to be <0.1% w/w/day. As growth dilution can thus be considered negligible and dismissed as a significant contaminant loss process, ventilation is the loss process most likely controlling the differences in $BMF^*$ between these fish. Ventilation is determined by the $RMR$, with $RMR$ (on a mass basis) decreasing with increasing mass (See Figure S6.2A). Ventilation as a loss process will therefore be greatest for the 1 g fish and lowest for the 10 kg fish. This is indeed reflected in the $BMF^*$s where the 10 kg fish has the largest $BMF$, followed by the 100 g fish and finally by the 1 g fish (Figure 6.6A).

The case of diet-controlled growth for a 100 g fish at Arctic, temperate, and tropical temperatures is shown in Figure 6.6C. Again, these fish are assumed to be consuming the same amount of food, resulting in equivalent $D_A$, and the bioenergetically balanced growth rates are negligible over the 1000 day exposure period (<0.01% w/w/d). Therefore, the ventilation rates must also be driving the difference in the $BMF^*$s here. As shown in Figure S6.2, the $RMR$ for the tropical fish is the greatest, followed by the temperate fish, and finally the Arctic fish. In addition, the $O_2$ concentration is lower in warmer waters. Therefore, the ventilation rate increases with increasing temperature. Consequently, the $BMF^*$ increases with decreasing temperature, with the fish in Arctic waters having the highest $BMF^*$.
Figure 6.6: Comparing the relative contaminant concentration in fish to that in diet after 1000 days for A) a temperate fish of 1g, 100g, and 10 kg fed at 1%w/w/day; B) a temperate fish of 1g, 100g, and 10 kg fed to sustain growth according to Eq. 6.1; C) a 100g fish at Arctic (3°C), temperate (10°C), and tropical (27°C) temperatures fed at 1%w/w/day; D) a 100g fish at Arctic (3°C), temperate (10°C), and tropical (27°C) temperatures fed to sustain growth according to Eq. 6.1.

Figure 6.6B compares the same three fish but this time assuming a growth-controlled diet. In this case, there are substantial differences between the growth rates of the fish with the smaller fish growing faster than the big one (specifically, the 1 g fish grows 1.4 to 2.5 times faster than the 100 g fish (depending on the time point), which in turn grows 1.7 to 2.5 times faster than the 10 kg fish). Consequently, the smaller fish also consume accordingly more food (per body mass) than the bigger fish (see Figure S6.6). Since $D_A$ is typically 2 orders of magnitude greater than $D_V$ and 1 order of magnitude greater than $D_G^*$, it is expected that the differences in food uptake will dominate the overall $BMF$s*. As such, the $BMF^*$ of the 1 g fish is greater than that of the 100g fish which is greater than the 10 kg fish (Figure 6.6B).

Finally, Figure 6.6D compares the 100 g fish at different temperatures for the growth-controlled diet. Again, Eq. 6.1 results in substantial differences in growth rates for the different fish, with tropical fish
consuming and growing 1.6 to 6.2 times faster than the temperate fish, which in turn consumes and grows 1.5 to 2.1 times faster than the Arctic fish (depending on the time point) (see Figure S6.6). Again, since $D_A$ is typically 2 orders of magnitude greater than $D_V$ and 1 order of magnitude greater than $D_G^*$, it is expected that these elevated feeding rates will dominate the $BMFs^*$ rather than contaminant loss by growth or ventilation. As such, the $BMFs^*$ of the tropical fish are highest followed by the temperate and Arctic fish. Gewurtz et al. also predicted that temperate fish would have a higher $BMF$ than Arctic fish although their calculations were not tested for bioenergetics consistency. They attributed this difference to the higher lipid content of fish in colder water (and therefore higher fugacity capacity). Since the lipid content of all fish in this study is held constant, differences in fugacity capacity would not contribute to concentration differences calculated in these simulations.

These calculations demonstrate that $BMF^*$ results are highly dependent on the assumptions concerning feeding and growth rates. That is to say that the relationship between $BMF^*$ with body size and temperature is a function of the modelling assumptions rather than biological or bioenergetic factors. As such, the application of these modelling assumptions to laboratory and field studies could yield conflicting results. For example, under conditions where the feeding rate is so low that growth is negligible larger fish and fish at cold temperatures are expected to have higher BMFs but under conditions of unlimited food availability higher BMFs are found for smaller fish and fish at warm temperatures. For laboratory studies, fish are usually kept under conditions of a maintenance diet such that growth is minimal. However, for field studies, whether or not there is enough food to sustain the growth rate predicted by equation 6.1 would be site specific and likely seasonally variable. For example, the feeding rate required to sustain this growth rate for a 100 g fish at 27 °C ranges from 11 to 90 % w/w/d over the simulated 1000 day period (Table 6.2). Obviously, it may be difficult to really achieve such high feeding rates and sustained growth for a tropical fish may only occur in highly productive environments. Interestingly, even the Arctic fish growing according to equation 6.1 consumes between 5 and 7 % of its body weight per day (Table 6.2) which is much greater than that of the typical laboratory rate of 1% w/w/d (see Figure S6.6). It is thus possible that equation 6.1 overestimates growth for fish. For example, Table 6.3 gives the range of growth rates calculated for each of the five fish types according to the equation 6.1. In all cases, growth is greater than 0.2% w/w/d. In comparison, Hammar et al. (1993) reported growth rates of <0.07% w/w/d for landlocked Arctic Char (50-500 g) from northern Sweden. This is less than half of what is predicted by equation 6.1. However, sea run char have been shown to grow faster than landlocked char (Rikardsen et al.
A possible explanation for some of the discrepancy between measured and modeled growth rates is that the bioenergetic balance equation implemented in these calculations does not explicitly include energy costs for reproduction.

Overall, these calculations indicate that 1) further evaluation of the applicability of growth rates calculated by equation 6.1 is warranted as is further evaluation of the dietary uptake rate in equation 6.2; and 2) it is important to understand whether the bioenergetic balance is driven by the growth rate or the feeding rate such that the driving force for bioaccumulation in each specific environment can be better understood. This analysis also highlights the importance of bioenergetics considerations in biomagnification calculations. Typically, the biomagnification factor is thought to depend primarily on the physical properties of the chemical. While this is true, Figure 6.6 indicates that the extent of biomagnification may also differ between fish of different size and between fish under different temperature conditions as a direct result of differences in the bioenergy balance for different fish.

Table 6.2: Minimum and maximum feeding rates (w/w/d) that are required to sustain the growth rate predicted by equation 6.1, as calculated by the bioenergy balance in the 3B model.

<table>
<thead>
<tr>
<th>Fish Type</th>
<th>Feeding Rate</th>
<th>1 g, 10°C</th>
<th>100 g, 10°C</th>
<th>10 kg, 10°C</th>
<th>100g, 3°C</th>
<th>100 g, 27°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min (w/w/d)</td>
<td>9%</td>
<td>7%</td>
<td>4%</td>
<td>5%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Max (w/w/d)</td>
<td>37%</td>
<td>15%</td>
<td>6%</td>
<td>7%</td>
<td>90%</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.3: Minimum and maximum growth rates (w/w/d) predicted by equation 6.1 over a 1000 day time period.

<table>
<thead>
<tr>
<th>Fish Type</th>
<th>Growth Rate</th>
<th>1 g, 10°C</th>
<th>100 g, 10°C</th>
<th>10 kg, 10°C</th>
<th>100g, 3°C</th>
<th>100 g, 27°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min(w/w/d)</td>
<td>0.4%</td>
<td>0.3%</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td>Max(w/w/d)</td>
<td>1.4%</td>
<td>0.6%</td>
<td>0.2%</td>
<td>0.3%</td>
<td>3.6%</td>
<td></td>
</tr>
</tbody>
</table>
6.4 Conclusions

As with all models, there are both merits and limitations of the new 3B model. Previous food web models require minimal parameters, but may suffer from incompatible and/or unreasonable physiological rates. The 3B model, through solving the bioenergy balance of the fish, assures internal consistency between the metabolic and physiological processes that drive the mass balance bioaccumulation model; however, to do so it requires either the growth rate or the feeding rate, i.e. additional input parameters that, unfortunately, are often unknown. Comparisons of the new 3B model with previous models using disparate sources of bioenergetics data (including the OXYREF database) indicate that the feeding rates used by these models are not energetically consistent with the growth rates and potentially lead to overestimation of bioaccumulation. The extent of this overestimation is likely dependent on the size of the fish and the temperature of its environment. The 3B model was parameterized from bioenergetics data from a broad range of fish species, sizes and environments (temperatures) with the hope that it can be applied to many species in various settings and ecosystems. We have demonstrated that this more generalized approach seems to do as well or better at simulating chemical depuration than a previous “species-specific” coupled bioenergetics-mass balance model. Finally the merits of the 3B model were exploited to show how the biomagnification in different sizes of fish and in fish at different temperatures can vary greatly as a result of their differing energetic demands. Overall, this study has demonstrated that the 3B model can provide improved predictions for fish bioaccumulation and offers additional insight into bioaccumulation processes.

6.5 Supporting Information

6.5.1 The biological energy balance.

The equation balancing the energy taken up with food with the various energy expenditures of the fish is defined as:

\[ Q \cdot \varepsilon_D = RMR \cdot A + SDA + U + N \]  

(Equation S6.1)

where \( Q \) is the food consumption rate (g food/g fish/d), \( \varepsilon_D \) is the energy density of food (kJ/g food), \( RMR \) is the routine metabolic rate (kJ/g fish/d), the unitless multiplier \( A \) represents activity requirements, \( SDA \) is specific dynamic action (kJ/g fish/d), \( U \) represents energy lost through excretion (kJ/g fish /d), and \( N \) (kJ/g fish /d) is the energy used for growth. A requirement for solving equation
(S6.1) is that either \( N \) or \( Q \) is known. The energetic requirements for reproduction are assumed to be included in the energetic demands for growth and as such are not explicitly considered in this equation.

**Case 1: Food consumption rate is known.**

In the case where the food consumption rate, \( Q \), is known, the amount of energy available for expenditure on growth (\( N \); kJ/(g fish·day)) is calculated from the energy balance equation (equation S6.1). Since the generation of lipids and proteins require different amounts energy, the amount of lipid and protein mass produced are calculated according to:

\[
N_P \left( \frac{g \text{ protein}}{g \text{ fish·day}} \right) = N \left( \frac{kj}{g \text{ fish·day}} \right) / \left[ 39.3 \left( \frac{kj}{g \text{ lipid}} \right) \cdot \frac{X_{P,F}}{X_{L,F}} \left( \frac{g \text{ lipid}/g \text{ fish}}{g \text{ protein}/g \text{ fish}} \right) + 18 \left( \frac{kj}{g \text{ protein}} \right) \right] \quad \text{(Equation S6.2)}
\]

\[
N_L \left( \frac{g \text{ lipid}}{g \text{ fish·day}} \right) = N \left( \frac{kj}{g \text{ fish·day}} \right) / \left[ 18 \left( \frac{kj}{g \text{ protein}} \right) \cdot \frac{X_{P,F}}{X_{L,F}} \left( \frac{g \text{ protein}/g \text{ fish}}{g \text{ lipid}/g \text{ fish}} \right) + 39.3 \left( \frac{kj}{g \text{ lipid}} \right) \right] \quad \text{(Equation S6.3)}
\]

where the coefficients 39.3 kJ/g lipid and 18.0 kJ/g protein refer to the energy density of lipids and proteins, respectively (Drouillard et al. 2009). Note that the assumption in this calculation is that during growth the mass fractions of lipid and protein in the fish, \( X_{L,F} \) and \( X_{P,F} \), will remain constant.

The parameters \( \varepsilon_D \), \( RMR \), \( SDA \), and \( U \) are calculated from other known parameters. The dietary energy assimilation efficiency \( \varepsilon_D \) can be expressed as:

\[
\varepsilon_D = 39.3 \frac{kj}{g} \cdot X_{L,D} \cdot E_{L,D} + 18.0 \frac{kj}{g} \cdot X_{P,D} \cdot E_{P,D} \quad \text{(Equation S6.4)}
\]

where \( X_{L,D} \) and \( X_{P,D} \) are the mass fractions of lipid and protein in the food, respectively, and \( E_{L,D} \) and \( E_{P,D} \) are the assimilation efficiencies of lipid (0.92) and protein (0.60) from the diet, respectively (Arnot and Gobas 2004). \( RMR \) is calculated from

\[
RMR = \frac{M \cdot D_O}{W_f} \quad \text{(Equation S6.5)}
\]

where \( M \) is the respiration rate in units of mg O\(_2\)/d (Arnot et al. 2008), \( D_O \) is the oxycalorific coefficient (0.0143 kJ/mg O\(_2\)) and \( W_f \) is the mass of the fish (g). Arnot et al. (2008) used the OXYREF database (Thurston and Gehrke, 1990) representing approximately 200 fish species to derive the relationship

\[
log(M/\frac{mgO_2}{d}) = 0.786 log(W_f/g) + 0.017 T/°C + 5.158 \quad \text{(Equation S6.6)}
\]
where T is temperature (°C) (Arnot et al. 2008). The SDA is generally defined as a percentage of the energy in the diet and can be defined as (Kitchell et al. 1977)

\[ SDA = 0.172 \cdot Q \cdot \varepsilon_D \] (Equation S6.7)

and the energy excretion term can be defined as (Kitchell et al. 1977)

\[ U = 0.0253(T / K)^{0.58} \cdot e^{-0.299} \cdot Q \cdot \varepsilon_D \] (Equation S6.8)

Case 2: The growth rate is known

In the case where \( N \) is known, \( Q \) is calculated from Eq. S61. However, since \( SDA \) and \( U \) are also a function of \( Q \), equation S6.1 can be rearranged as:

\[ Q = \frac{RMR_A}{\theta \cdot \varepsilon_D} \] (Equation S6.9)

where \( \theta \) is a unitless multiplier

\[ \theta = 1.172 + 0.0253(T / K)^{0.58} \cdot e^{-0.299} \] (Equation S6.10)

6.5.2 The contaminant mass balance.

The mass balance equation for the contaminant within the 3B model is defined as

\[ \frac{d(V_F Z_F f_F p)}{dt} = f_W D_V + f_A D_A - f_F (D_V + D_E + D_M) \] (Equation S6.11)

where \( V_F \) is the volume of the fish (m\(^3\)), \( Z_F \) is the fugacity capacity of the fish (mol/Pa/m\(^3\)), \( f_W, f_A, \) and \( f_F \) represent the fugacity (Pa) of the water, diet and fish, respectively, \( D_V \) is the D-value for respiratory uptake and elimination (mol/Pa/day), \( D_A \) is the D-value for dietary uptake (mol/Pa/day), \( D_E \) is the D-value for fecal egestion (mol/Pa/day), and \( D_M \) is the D-value for metabolic biotransformation (mol/Pa/day) (Campfens and Mackay, 1997). The D-value for respiratory uptake and elimination can be further defined as

\[ D_V = E_W \cdot G_V \cdot Z_W \cdot W_F \] (Equation S6.12)

where \( E_W \) is the chemical exchange efficiency across the gills (unitless), \( G_V \) is the ventilation rate (m\(^3\)/g fish/d), \( Z_W \) is the fugacity capacity of water (mol/Pa/m\(^3\)). \( E_W \) can be estimated by from the octanol/water partition coefficient (\( K_{OW} \); unitless) of the chemical using (Arnot and Gobas 2004)

\[ E_W = (1.85 + \frac{155}{K_{OW}})^{-1} \] (Equation S6.13)
and is therefore only dependent on the chemical. The ventilation rate, $G_V$, is energetically determined by the amount of oxygen required to satisfy the routine metabolic rate, and can be expressed as (Norstrom et al. 1976)

$$G_V = \frac{RMR_A}{0.6 \cdot [O_2] \cdot D_O}$$  (Equation S6.14)

where $[O_2]$ is the concentration of oxygen dissolved in the water (g O₂/m³), and the constant 0.6 represents the oxygen extraction efficiency across the gills. Assuming oxygen saturation, the concentration of oxygen dissolved in the water can be estimated by (Norstrom et al. 1976)

$$[O_2] = (14.45 - 0.413 T/°C) + 5.56 \times 10^{-3} (T/°C)^2$$  (Equation S6.15)

The D-value for dietary uptake can be calculated from

$$D_A = \frac{E_G Q^W T^Z_A}{\delta_A}$$  (Equation S6.16)

where $E_G$ is the assimilation efficiency of the chemical from the food (unitless), $Z_A$ is the fugacity capacity of the diet (mol/Pa/m³), and $\delta_A$ is the density of the food (g/m³). $E_G$ also depends only on the chemical and can be estimated from (Arnot and Gobas 2004)

$$E_G = (3.0 \times 10^{-7} \cdot K_{OW} + 2)^{-1}$$  (Equation S6.17)

The density of the food is calculated based on the contribution of lipid and protein such that:

$$\delta_A = \delta_L X_{LD} + \delta_P X_{PD} + \delta_W (1 - X_{LD} - X_{PD})$$  (Equation S6.18)

where the densities of lipid ($\delta_L$) and protein ($\delta_P$) were assumed to be 8×10⁵ and 1.1 ×10⁶ g/m³, respectively (Drouillard et al. 2009), and the density of water ($\delta_w$) is 1×10⁶ g/m³. The D-value for egestion can be calculated from:

$$D_E = \frac{E_G G_E Z_E}{\delta_E}$$  (Equation S6.19)

where $G_E$ is the egestion rate (g feces /d), $Z_E$ is the fugacity capacity of the feces (mol/Pa/m³), and $\delta_E$ is the density of the excrement (g/m³). $G_E$ represents the unassimilated portion of the diet and is thus determined from (Arnot and Gobas 2004):

$$G_E = Q \cdot \left(1 - (X_{LD}(1 - E_{LD}) + X_{PD}(1 - E_{PD}) + (1 - X_{LD} - X_{PD})(1 - E_{WD}))\right)$$  (Equation S6.20)
where $E_{W,D}$ is the assimilation efficiency of water (0.25) and the fish diet is assumed to consist of only lipid, protein and water. Analogous to the density calculation for food, the density of the excrement was determined using:

$$
\delta_E = \delta_L X_{L,D} (1 - E_{L,D}) + \delta_P X_{P,D} (1 - E_{P,D}) + \delta_W \left(1 - X_{L,D} (1 - E_{L,D}) - X_{P,D} (1 - E_{P,D})\right)
$$

(Equation S6.21)

The D-value for biotransformation can be calculated from (Campfens and Mackay, 1997):

$$
D_M = \frac{k_M Z_F W_F}{\delta_F} (Equation \text{ S6.22})
$$

where $k_M$ is the biotransformation rate of the contaminant by a fish and $\delta_F$ is the density of the fish (g/m$^3$). The fish density can be calculated from

$$
\delta_F = \left(\frac{X_{L,B}}{\delta_L} + \frac{X_{P,B}}{\delta_P} + \frac{X_{W,B}}{\delta_W}\right)^{-1} (Equation \text{ S6.23})
$$

The fugacity capacities are dependent upon the physiochemical properties of the contaminant and were calculated according to Campfens and Mackay (1997) as:

$$
Z_O (mol/Pa/m^3) = K_{OW}/H \left(Pa \cdot m^3/mol\right) (Equation \text{ S6.24})
$$

$$
Z_W (mol/Pa/m^3) = 1/H \left(Pa \cdot m^3/mol\right) (Equation \text{ S6.25})
$$

$$
Z_F (mol/Pa/m^3) = X_{L,F} \cdot Z_O (mol/Pa/m^3) (Equation \text{ S6.26})
$$

$$
Z_A (mol/Pa/m^3) = X_{L,D} \cdot Z_O (mol/Pa/m^3) (Equation \text{ S6.27})
$$

$$
Z_E (mol/Pa/m^3) = X_{L,D} \cdot (1 - E_{L,D}) \cdot Z_O (mol/Pa/m^3) (Equation \text{ S6.28})
$$

where $H$ is Henry’s law constant and is calculated from the air-water partition coefficient by:

$$
H = K_{AW} \cdot 8.314(Pa \cdot m^3/mol) \cdot [T(\degree C) + 273.15] (Equation \text{ S6.29})
$$
6.5.3 Flow charts of 3B model

A

- Parameters influencing \( D_V \)
- Parameters influencing \( D_A \)
- Parameters influencing \( D_E \)
- Parameters influencing \( D_M \)
- Chemical input parameters
- Env. Input parameters
- Diet Input parameters
- Fish Input parameters
- Bioenergetic terms
- Assimilation Efficiencies
Figure S6.1: Flow chart illustrating the dependence of (A) $D_V$, (B) $D_A$, (C) $D_E$, (D) $D_M$, and (E) fish growth on various bioenergetics and bioaccumulation parameters assuming that the feeding rate is known.
Table S6.1: List of parameters used in the 3B model

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bioenergy Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMR</td>
<td>Routine metabolic rate</td>
<td>kJ/g fish/d</td>
</tr>
<tr>
<td>M</td>
<td>Respiration rate</td>
<td>mg O$_2$/day</td>
</tr>
<tr>
<td>U</td>
<td>Urinary excretion</td>
<td>kJ/g fish/d</td>
</tr>
<tr>
<td>N</td>
<td>Growth Energy</td>
<td>kJ/g fish/d</td>
</tr>
<tr>
<td>$N_P$</td>
<td>Protein growth</td>
<td>g protein/g fish/d</td>
</tr>
<tr>
<td>$N_L$</td>
<td>Lipid growth</td>
<td>g lipid/g fish/d</td>
</tr>
<tr>
<td>Q</td>
<td>Dietary consumption rate</td>
<td>g food/g fish/d</td>
</tr>
<tr>
<td>A</td>
<td>Activity multiplier</td>
<td>unitless</td>
</tr>
<tr>
<td>SDA</td>
<td>Specific dynamic action</td>
<td>kJ/g fish/d</td>
</tr>
<tr>
<td>$\epsilon_D$</td>
<td>Energy density of food</td>
<td>kJ/g food</td>
</tr>
<tr>
<td>$D_{O2}$</td>
<td>Oxycalorific coefficient</td>
<td>kJ/mg O$_2$</td>
</tr>
<tr>
<td><strong>Bioaccumulation Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$G_v$</td>
<td>Ventilation rate</td>
<td>m$^3$/g fish/d</td>
</tr>
<tr>
<td>$G_E$</td>
<td>Excretion rate</td>
<td>g feces /d</td>
</tr>
<tr>
<td>$G_B$</td>
<td>Growth rate</td>
<td>g fish /d</td>
</tr>
<tr>
<td>$D_V$</td>
<td>D-value for ventilation</td>
<td>mol/Pa/day</td>
</tr>
<tr>
<td>$D_A$</td>
<td>D-value for dietary consumption</td>
<td>mol/Pa/day</td>
</tr>
<tr>
<td>$D_E$</td>
<td>D-value for egestion</td>
<td>mol/Pa/day</td>
</tr>
<tr>
<td>$D_M$</td>
<td>D-value for biotranformation</td>
<td>mol/Pa/day</td>
</tr>
<tr>
<td>$f_A$</td>
<td>Fugacity of the diet</td>
<td>Pa</td>
</tr>
<tr>
<td>$f_W$</td>
<td>Fugacity of water</td>
<td>Pa</td>
</tr>
<tr>
<td>$f_F$</td>
<td>Fugacity of the fish</td>
<td>Pa</td>
</tr>
<tr>
<td>$\delta_L$</td>
<td>Density of lipid</td>
<td>g/m$^3$</td>
</tr>
<tr>
<td>$\delta_P$</td>
<td>Density of protein</td>
<td>g/m$^3$</td>
</tr>
<tr>
<td>$\delta_W$</td>
<td>Density of water</td>
<td>g/m$^3$</td>
</tr>
<tr>
<td>$\delta_A$</td>
<td>Density of diet</td>
<td>g/m$^3$</td>
</tr>
<tr>
<td>$\delta_E$</td>
<td>Density of excrement</td>
<td>g/m$^3$</td>
</tr>
<tr>
<td>$E_{L,D}$</td>
<td>Assimilation efficiency of lipid mass from the diet</td>
<td>unitless</td>
</tr>
<tr>
<td>$E_{P,D}$</td>
<td>Assimilation efficiency of protein mass from the diet</td>
<td>unitless</td>
</tr>
<tr>
<td>$E_{W,D}$</td>
<td>Assimilation efficiency of water mass from the diet</td>
<td>unitless</td>
</tr>
<tr>
<td><strong>Fish Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_F$</td>
<td>Volume of fish</td>
<td>m$^3$</td>
</tr>
<tr>
<td>$W_F$</td>
<td>Mass fish</td>
<td>g</td>
</tr>
<tr>
<td>$X_{L,F}$</td>
<td>Mass fraction of lipid in the fish</td>
<td>unitless</td>
</tr>
<tr>
<td>$X_{P,F}$</td>
<td>Mass fraction of protein in the fish</td>
<td>unitless</td>
</tr>
<tr>
<td>$X_{W,F}$</td>
<td>Mass fraction of water in the fish</td>
<td>unitless</td>
</tr>
<tr>
<td><strong>Environmental Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
<td>°C</td>
</tr>
<tr>
<td>[O$_2$]</td>
<td>Concentration of oxygen</td>
<td>g O$_2$/m$^3$</td>
</tr>
</tbody>
</table>
## Chemical Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{OW}$</td>
<td>Octanol-water partition co-efficient</td>
<td>unitless</td>
</tr>
<tr>
<td>$K_{AW}$</td>
<td>Air-water partition co-efficient</td>
<td>unitless</td>
</tr>
<tr>
<td>$Z_O$</td>
<td>Fugacity capacity of octanol</td>
<td>mol/Pa/m$^3$</td>
</tr>
<tr>
<td>$Z_W$</td>
<td>Fugacity capacity of water</td>
<td>mol/Pa/m$^3$</td>
</tr>
<tr>
<td>$Z_A$</td>
<td>Fugacity capacity of air</td>
<td>mol/Pa/m$^3$</td>
</tr>
<tr>
<td>$Z_E$</td>
<td>Fugacity capacity of excrement</td>
<td>mol/Pa/m$^3$</td>
</tr>
<tr>
<td>$Z_F$</td>
<td>Fugacity capacity of fish</td>
<td>mol/Pa/m$^3$</td>
</tr>
<tr>
<td>$k_M$</td>
<td>Biotransformation rate constant</td>
<td>/day</td>
</tr>
<tr>
<td>$E_W$</td>
<td>Assimilation efficiency of chemical across the gill</td>
<td>unitless</td>
</tr>
<tr>
<td>$E_G$</td>
<td>Assimilation efficiency of chemical from the diet</td>
<td>unitless</td>
</tr>
</tbody>
</table>

### 6.5.4 Comparison of respiration models from the literature

Several species-specific bioenergetic models have been developed and tested (Chizinski et al. 2008; Ciannelli et al. 1998; Jusup et al. 2011; Karas and Thoresson, 1992; Kitchell et al. 1977; Madenjian et al. 2004; Tarvainen et al. 2008; Zhou et al. 2005). Most are based on the Wisconsin model (Kitchell et al. 1977) using species-specific empirical constants (Ciannelli et al. 1998; Tarvainen et al. 2008). One of the goals of this study was to develop a model applicable to a wide number of species spanning masses of several orders of magnitude as well a greater temperature spectrum. For this reason, several available respiration models (e.g. equation S6.6) were compared. Both Gewurtz et al. (2006) and Arnot et al. (2008) derived equations relating the respiration rate with fish mass and water temperature using the fish oxygen requirements database (OXYREF) compiled by Thurston and Gehrke (1990). These respiration models yield $RMR$ as a function of fish mass (Figure S6.2A) and temperature (Figure S6.2B). For comparison, the models by Enders et al. (2006) and Kåras and Thoresson (1992), two modified versions of the Wisconsin model, are also included in Figure S6.2. In the 3B model, we used the $RMR$ model by Arnot et al. (2008).

The $RMR$ expressions were first compared on the basis of mass over 4 orders of magnitude (i.e. 1 g to 10 kg) at 15 °C (Figure S6.2A). On the basis of temperature dependent metabolism, all 4 models were compared for a fish weighing 500 g over the temperature range of -1 to 40°C (Figure S6.2B). Clearly, the $RMR$ expressions yield different estimates depending on both mass and temperature. This is likely a result of the different ranges of fish mass, temperature and potentially also the fish species used to  

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9 The model requires a $Z$-value for water $Z_W$ to calculate the D-value for respiration. Currently, the model calculates $Z_W$ from $K_{AW}$, even though air-water exchange is not a relevant process in fish bioaccumulation. Alternatively, $Z_W$ could also be calculated from $K_{OW}$, if information on the activity coefficient of the chemical in octanol is available, or from the chemical’s aqueous solubility in the liquid state.
derive the regressions (see Table S6.2) Although the Arnot et al. and Gewurtz et al. expressions were both based on OXYREF, they relied on slightly different subsets of the dataset, with the Gewurtz et al. using 2465 data points ($r^2=0.73$) and Arnot et al. using 2957 data points ($r^2=0.83$). Here we use the RMR expression presented by Arnot et al. as it widely applicable over a broad mass and temperature range and is also based on the largest dataset.

![Figure S6.2: Comparison of four published bioenergetic respiration models as a function of fish mass (A) and water temperature (B). The solid lines indicate values that fall within the range of experimental data used to derive the regressions. Dotted lines represent values that are extrapolated beyond the range of experimental data used to derive the regressions.](image)

Figure S6.2: Comparison of four published bioenergetic respiration models as a function of fish mass (A) and water temperature (B). The solid lines indicate values that fall within the range of experimental data used to derive the regressions. Dotted lines represent values that are extrapolated beyond the range of experimental data used to derive the regressions.
Table S6.2: Comparison of experimental data used for parameterization four RMR regressions.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (kg)</td>
<td>2x10^-3 to 8x10^-2</td>
<td>4x10^-3 to 2.5x10^-2</td>
<td>1x10^-4 to 10</td>
<td>1x10^-4 to 10</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>4 to 28</td>
<td>12 to 20</td>
<td>-1 to 40</td>
<td>-1 to 40</td>
</tr>
<tr>
<td>Species</td>
<td>Eurasian Perch</td>
<td>Juvenile yellow Perch</td>
<td>&gt;200 species</td>
<td>&gt;200 species</td>
</tr>
</tbody>
</table>

6.5.5  Comparison of the 3B model with existing bioaccumulation models

Table S6.3: Comparison of dietary assimilation efficiencies used by different models.

<table>
<thead>
<tr>
<th>Dietary assimilation efficiency</th>
<th>3B</th>
<th>Arnot &amp; Gobas 2004</th>
<th>Arnot et al. 2008</th>
<th>Gewurtz et al. 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipid (E_{L,D})</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>protein/non-lipid organic matter (E_{P,D})</td>
<td>0.60</td>
<td>0.60</td>
<td>0.70</td>
<td>0.60</td>
</tr>
<tr>
<td>water (E_{W,D})</td>
<td>0.25</td>
<td>0.25</td>
<td>0.50</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table S6.4: Conversion of rate constants to D-values.

<table>
<thead>
<tr>
<th>Rate Constant</th>
<th>D-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_2</td>
<td>D_V = k_2 V_F Z_F / \delta_F</td>
</tr>
<tr>
<td>k_A</td>
<td>D_A = k_A V_F Z_F</td>
</tr>
<tr>
<td>k_E</td>
<td>D_E = k_E V_F Z_F</td>
</tr>
</tbody>
</table>
Figure S6.3: Comparison of $D$-values predicted by the 3B model and the models by Gewurtz et al. (2006), Arnot et al. (2008), and Arnot and Gobas (2004) for five different fish: a) 1 g fish at 10°C; b) 100 g fish at 10°C; c) 10 kg fish at 10°C; d) 100 g Arctic fish at 3°C; and e) 100 g tropical fish at 27°C.

6.5.6 Model parameterization for validation against Paterson et al. (2007b) and Drouillard et al. (2009) data sets

Drouillard et al. (2009) report the growth rate and body composition for this dataset, with the small, medium, and large fish weighing on average 10.1, 45.9 and 86.7 g at the beginning of the study. Only the small fish exhibited growth which for the first 90 days followed a linear relationship:

$$W_F(g) = 0.069 \left( \frac{g}{day} \right) \cdot t(day) + 9.43 \text{ (g)} (\text{Equation S6.30})$$
where \( t \) is the experimental day (Drouillard et al. 2009). For the remainder of the study, growth continued at a rate of 0.014 g/d (Drouillard et al. 2009). Since the large and medium sized fish did not grow significantly during the period of the experiment, loss of contaminant from growth did not occur. The time-variant lipid contents of each size class as reported by Paterson et al. (2007b) were fitted with the following polynomial functions (Figure S6.2).

\[
X_{L,B}(\text{Large Fish}) = 5 \times 10^{-7}(t/d)^3 - 2 \times 10^{-4}(t/d)^2 - 1.14 \times 10^{-2}(t/d) + 10.66 \quad (\text{Equation S6.31})
\]

\[ R^2 = 0.94 \]

\[
X_{L,B}(\text{Medium Fish}) = 5 \times 10^{-7}(t/d)^3 - 2 \times 10^{-4}(t/d)^2 - 2.2 \times 10^{-3}(t/d) + 8.74 \quad (\text{Equation S6.32})
\]

\[ R^2 = 0.94 \]

\[
X_{L,B}(\text{Small Fish}) = 6 \times 10^{-10}(t/d)^4 + 9 \times 10^{-8}(t/d)^3 - 7 \times 10^{-5}(t/d)^2 + 1.93 \times 10^{-2}t + 12.17 \quad (\text{Equation S6.33})
\]

\[ R^2 = 0.93 \]

Figure S6.4: Polynomial fitting of the lipid content of each fish size over the course of the experiment (Paterson et al. 2007b).

The water content could be described for all sizes by (Drouillard et al. 2009)

\[
X_{W,B} = -0.68 \cdot X_{L,B} + 76.9 \quad (\text{Equation S6.34})
\]

The fish was assumed to consist of only lipid, water and protein such that the protein content could be determined by difference from the water and lipid contents (Drouillard et al. 2009).

The daily temperature over the year was fit (Figure S6.5) by:

\[
T/°C = -0.0016(t/d)^2 + 0.0913(t/d) + 22.749 \quad 0 \ d < t < 99 \ d \quad (\text{Equation S6.35})
\]
\[ R^2 = 0.67 \]
\[ T/°C = -2 \times 10^{-6}(t/d)^3 + 0.002(t/d)^2 - 0.5982(t/d) + 56.861 \quad 100 \, d < t < 365 \, d \] (Equation S6.36)

\[ R^2 = 0.91 \]

Figure S6.5: Polynomial fitting of temperature over the course of the experiment (Paterson et al. 2007a).

6.5.7 Model evaluation at non-steady-state

Table S6.5: The root mean squared error (RMSE) of the measured (Paterson et al. 2007b, Drouillard et al. 2009) and predicted (3B model) concentrations of five PCB congeners in perch (all fish sizes).

<table>
<thead>
<tr>
<th>PCB</th>
<th>RMSE (ng/g lipid)</th>
<th>RMSE (ng/g lipid)</th>
<th>RMSE (ng/g lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Using log ( K_{OW} ) from Schenker et al.(2005)</td>
<td>Using log ( K_{OW} ) from Hawker and Connell (1988)</td>
<td>Using log ( K_{OW} ) average from both Schenker et al.(2005) and Hawker and Connell (1988)</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>19</td>
<td>17</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>31</td>
<td>380</td>
<td>264</td>
<td>293</td>
</tr>
<tr>
<td>74</td>
<td>73</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>118</td>
<td>350</td>
<td>334</td>
<td>343</td>
</tr>
<tr>
<td>180</td>
<td>195</td>
<td>196</td>
<td>195</td>
</tr>
</tbody>
</table>
Table S6.6: The root mean squared error (RMSE) of the measured (Paterson et al. 2007b, Drouillard et al. 2009) and predicted (3B model) concentrations in three size classes of perch (all PCB congeners).

<table>
<thead>
<tr>
<th>Fish Size</th>
<th>Using log $K_{OW}$ from Schenker et al. (2005)</th>
<th>Using log $K_{OW}$ from Hawker and Connell (1988)</th>
<th>Using log $K_{OW}$ average from both Schenker et al. (2005) and Hawker and Connell (1988)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>small</td>
<td>132</td>
<td>89</td>
<td>108</td>
<td>49</td>
</tr>
<tr>
<td>medium</td>
<td>273</td>
<td>221</td>
<td>240</td>
<td>49</td>
</tr>
<tr>
<td>large</td>
<td>316</td>
<td>274</td>
<td>279</td>
<td>49</td>
</tr>
</tbody>
</table>

6.5.8 Implication of a bioenergetically-balanced bioaccumulation model on BMF*

Figure S6.6: Comparison of the food consumption rates calculated by the 3B model in order to be bioenergetically balanced with the growth rate equation 6.1 for 5 types of fish. For comparison, a constant food uptake rate of 1% w/w/d is also included.
Chapter 7
Summary and Outlook

7.1 Brief

The main motivation for this thesis was to improve our understanding of bioaccumulation particularly as it pertains to human exposure. In chapters 2-4, the impact on exposure of several time-variant factors (reproductive behaviors, diet, etc.) at both the individual and population level was evaluated in conjunction with temporal changes in environmental emissions of PCB 153. In chapters 2 and 3, the temporal variability of the emissions was shown to be the key determinant. However, in chapter 4, the impact of changes in dietary composition on exposure was shown to rival the impact of changing PCB emissions. Since the bioaccumulation model used in the calculations for chapters 2-4 is based on the assumption that PCB partitioning to human lipids can be approximated by PCB partition to octanol, the motivation for chapter 5 was to evaluate the validity of this modelling assumption. The results indicated that octanol underpredicts partitioning to human lipids. However, compared to the variability and uncertainty of the emissions scenario used in chapters 2-4, and the demonstrated impact of the emissions scenario, the error associated with using octanol as a substitute for real lipids is expected to be relatively minor. Finally, since dietary uptake of fish is one of the main pathways of human exposure to PCBs, chapter 6 seeks to improve the predictions of fish bioaccumulation models by incorporating a bioenergy balance.

7.2 Chapter summaries

Chapter 2 estimated prenatal, postnatal, and lifetime PCB exposure for women according to year of birth and evaluated the impact of reproductive characteristics on intergenerational differences in exposure assuming both hypothetical constant and realistic time-variant emissions. One of the key results of the model was the predicted body burden age trend of an individual under steady-state conditions. Specifically, the model predicts that body burden does not monotonically increase with age but rather that it increases during breastfeeding and during the late teen/early adult years when growth is minimal but dietary uptake is relatively large. Furthermore, body burdens actually decrease from the time that breastfeeding ceases until the individual stops growing. By considering the life history of the contaminant in the environment, it was demonstrated that an individual’s body burden mostly depends upon when an individual was born relative to the emission history of PCBs. Reproductive behaviours can have a significant impact (and can contribute substantially to the observed variability in
biomonitoring studies), though the mother’s reproductive history has a greater influence on the prenatal and postnatal exposures of her children than it does on her own cumulative lifetime exposure. In particular, a child’s birth order appeared to have a strong influence on their prenatal exposure, whereas postnatal exposure is determined by the type of milk (formula or breast milk) fed to the infant. Prenatal PCB exposure was estimated to be delayed relative to the time of PCB emissions, particularly among those born after the PCB production phase-out. Consequently, the health repercussions of environmental PCBs can be expected to persist for several decades, despite bans on their production for over 40 years.

One of the key implications of these results is that the health repercussions for each generation will likely be different since the type of exposure (i.e. prenatal, postnatal or lifetime) is different for each generation. Often, exposure differences between different generations as a result of variable emissions are not considered. The greatest example of this is the interpretation of population based body burden-age relationships as if they reflected the role of age in bioaccumulation for an individual.

In chapter 3, we further explored body burden age trends but this time at a population level. We used CoZMoMAN to reproduce concentration versus age relationships for hypothetical populations from a cross-sectional perspective and investigate the factors that influence the shape of the concentration versus age relationships. The model was also used to probe the influence of partitioning and degradation properties, length of emissions, and model assumptions regarding lipid content and liver metabolism on concentration-age trends of bioaccumulative and persistent contaminants. The key result of this study was the demonstration that body burden-age relationships for population cross-sections and individuals over time are not equivalent. Furthermore, the most influential factor controlling the shape of cross-sectional concentration-age trends (i.e. increasing or decreasing with age) is the time lapse between the peak in emissions and sample collection (for chemicals with human metabolic half-lives longer than one year). Sufficiently short human metabolic half-lives were also shown to yield decreasing CBATs. As such, this study was also able to explain that the differences observed in CBATs for PCBs and PBDEs are consistent with differences in the life history of the contaminant in the environment (i.e. production phase out in the 1970s versus only recently phased-out, respectively) and their human metabolic half-lives (i.e. 15 years versus 1 year, respectively). Additionally, it was proposed that the CBAT shape from a biomonitoring study could be used to either extrapolate the chemical half-life (if the emission scenario is well known with emissions decreasing for more than 20
years and the half-life is less than 10 years) or to extrapolate information about the historical emissions scenario (if the half-life is well known).

Chapter 4 continued to explore intergenerational differences in human exposure but this time due to dietary transitions in combination with variable emissions. The main objective of this study was to develop a methodology to quantify the potential influence of intergenerational dietary transitions on human exposure to organic contaminants in the Arctic environment using PCB-153 as a case study. Female body burdens were calculated over time assuming five diets with varying proportions of traditional and imported food items and then used to illustrate the potential variability at a community/population level. The results indicated that, at any given time, individuals consuming a 100% traditional diet (i.e. high intake of ringed seal blubber) have modeled body burdens approximately 10-150 times higher than individuals consuming a 100% imported food diet. Consumption of locally-harvested fish (e.g. Arctic char) and seal meat (other than the blubber) were also associated with comparatively low body burdens. Decreased emissions were predicted to decrease the PCB-153 body burden of 30-year old females by 6 to 13-fold from 1980 to 2020 with dietary transitions accounting for an additional factor of 2–50 (i.e. 12–650 times lower in total) depending on the type of dietary transition and the origin of the imported food items. Thus, dietary transitions are an important factor underlying the variability within and between subpopulations in addition to partially explaining the observed temporal trends and specific information on the nature and timing of dietary transitions is highly valuable when interpreting biomonitoring data.

Chapter 5 evaluated the assumption that chemical partitioning to octanol is an appropriate model for partitioning to biological lipids by measuring the partitioning of 18 polychlorinated biphenyls (PCBs) into two types human lipid materials and into 9 various lipid surrogates. In particular, we measured equilibrium partitioning coefficients at 37 °C (i) between triolein and water ($K_{\text{triolein/water}}$) as potential representative system for human storage lipids, (ii) between 8 types of liposomes and water ($K_{\text{liposome/water}}$) as potential representative systems for human membrane lipids, and (iii) between lipids of human cell cultures and water ($K_{\text{cell lipid/water}}$) and human abdominal fat tissues and water ($K_{\text{AF/\text{water}}}$) as systems representing real human lipid tissues. The data were compared (i) with each other, (ii) with predictions obtained using poly-parameter linear free energy relationships (ppLFER) for partitioning into storage and membrane lipids, and (iii) with the most traditional of lipid partitioning surrogates, $K_{\text{octanol/water}}$. The results show that there were no differences in $K_{\text{AF/\text{water}}}$ using tissues from different individuals and that triolein appears to be an excellent surrogate for human storage lipids since the
$K_{\text{triolein/water}}$ for PCBs was very similar to the $K_{\text{cell lipid/water}}$ and $K_{\text{AFT/water}}$. Within the measurement uncertainty, $K_{\text{liposome/water}}$s for PCBs were identical to each other regardless of the phospholipids’ chain length, saturation of the carbon chains, head group, and the composition of the liposomes. Partitioning into both AFT and the cells was represented well by partitioning into triolein but partitioning into liposomes was 0.48 log units lower than into AFT and the cell lipids. This suggests that, for PCBs, 1) triolein is a good surrogate for storage lipids; 2) liposomes are not an appropriate surrogate for storage tissues; and 3) that partitioning into MCF-7 cells is dominated by the storage lipids rather than by membrane lipids. Finally, it was demonstrated that ppLFER models do an excellent job of predicting PCB partitioning to liposomes and storage lipids, while $K_{\text{octanol/water}}$ provides a less accurate prediction for the AFT and cells but compares well to $K_{\text{liposome/water}}$ for PCBs.

As discussed in the introduction, one of the main reasons for biomonitoring studies and bioaccumulation modelling is to be able to predict the bioaccumulation potential of new chemicals. However, in terms of chemical regulation and hazard and risk assessment, the potential hazard of chemicals to human health is not actually evaluated from human bioaccumulation data (at least not in Canada). Rather, the bioaccumulative potential of a chemical in fish (mainly) and other organisms (both aquatic and mammalian) are used in the evaluation. Therefore, our understanding of bioaccumulation in fish is critical to the human health hazard and risk assessment process. Furthermore, understanding bioaccumulation in fish is directly important to human health as fish is an important dietary source. Chapter 6 describes the development of a combined bioenergetic/mass balance bioaccumulation model for fish. The 3B model was derived from a large dataset of bioenergetics (metabolic rate) information and was applied to examine the influence of bioenergetics processes such as metabolism, consumption, digestion and growth for fish ranging from 1 g to 10 kg and for temperatures ranging from 3 to 27 °C. The results from the new model were compared with results from existing models. In all cases, linking food uptake requirements to an empirical growth rate for fish revealed that the feeding and growth rates used by previous bioaccumulation models were not bioenergetically consistent and therefore substantial overestimation of food uptake may be estimated by existing bioaccumulation models. This overestimation of food uptake implies that there is also potential overestimation of bioaccumulation levels in fish. The 3B model was evaluated for 5 PCBs under non-steady conditions for 10 g, 45 g, and 87 g perch and a temperature range of 3-27 °C and was able to explain the measured concentrations to within 0.5 orders of magnitude in most cases. These model results were either better or as good as the predictions of the species specific Drouillard et al. (2009)
model but did not require re-calibration of $E_W$. Finally, differences in biomagnification between fish size and temperature as a result of differing energetic requirements demonstrated the importance of the model assumptions on growth rate and feeding rate. In particular, we demonstrated that if the feeding rate is the limiting factor, then larger fish in cold environments are more susceptible to bioaccumulation. However, if fish eat at a rate to sustain the Thomann (1981, 1992) growth rate, then the smaller fish in a warm climate are more susceptible to bioaccumulation. Overall, this chapter highlights the important role of bioenergetics consistency on bioaccumulation.

7.3 Future Research Needs and Recommendations

Chapters 2 to 4 have focused on the various factors that control human exposure to PCBs. In each study, the dominating factor that controls human exposure was emissions yet this is often the most uncertain factor. As such, a continued effort to collect accurate emission inventories of chemicals in production is recommended. A common concern with respect to the breastfeeding and dietary consumption results presented in Chapters 2 and 4 is whether dietary recommendations can be made based on these results. It is important to remember that the results presented in these chapters are based purely on calculations of the contaminant and does not directly calculate the uptake of co-occuring beneficial nutrients and fatty acids. As such, further research could involve modelling both contaminants and nutritional elements such that dietary consumption calculations would provide a more holistic view of both the beneficial and harmful components of different diets. In terms of additional intergenerational differences in human exposure, additional calculations on the role of increased obesity in current generations compared to past generations is warranted given that an increase in obesity indicates larger lipid reserves and therefore a larger fugacity capacity. On the other hand, increased lipid reserves could merely serve to dilute the contaminant. Further calculations in this area could highlight important differences in considering the lipid normalized concentration of a contaminant versus the total mass of chemical in the body. Also, evaluation of the increasing trend in obesity in combination with the dietary transition leading to this obesity could be beneficial in further explaining the variability observed in biomonitoring data.

The general applicability of the CoZMoMAN model used in these studies could be improved by inclusion of additional food items in the human food-chain. For example, the traditional Inuit diet according to the CoZMoMAN model consists only of an aquatic food-web. Inclusion of an Arctic terrestrial food-chain (i.e. caribou, duck, etc.) would be a valuable contribution. Expansion of the
terrestrial food-chain for temperate regions to include pigs and chickens as well as inclusion of
shellfish in the temperate aquatic food-webs would also increase the general applicability of the model.
The CoZMoMAN model could also be expanded to include a dermal exposure route. There would be
several challenges associated with including a dermal exposure route including: 1) deriving a
relationship between exposed skin surface area as a function of age; 2) determining the dermal uptake
rate as a function of the chemical properties; and 3) accounting for the various types of dermal
exposure i.e. direct application of a chemical to the skin versus secondary transfer from another object.
By including the dermal exposure route, the CoZMoMAN model could be used to evaluate chemicals
such as pesticides and those in personal care products which have increased potential for dermal
absorption due to the nature of their usage. Furthermore, the analysis in Chapters 2 to 4 has largely
focused on PCBs. Additional calculations for other chemicals and evaluation of these predictions
against biomonitoring data may also prove useful.

In addition, future modelling studies of the type conducted in chapters 2-4 could be applied to the
newly emerging concept of the “exposome” (Wild, 2005). The exposome relates conception-to-death
human exposure profiles to health outcomes. Specifically, the application of a CoZMoMAN-like model
could be used to 1) retroactively identify potential causes of exposure such that preventative measures
could be implemented in the future to reduce the corresponding health outcomes; 2) account for
lifestyle variability in the exposure data such that data uncertainty could be reduced; and 3) predict
future exposure profiles such that future health outcomes could be predicted.

The PCB partitioning comparison in Chapter 5 indicated that liposomes are not an appropriate
surrogate for either real human cells or real human abdominal fat tissue – at least not for PCBs. Rather,
it was demonstrated that triolein is an excellent surrogate for both and therefore utilizing triolein (or
any other storage lipid) instead of octanol would be suitable for bioaccumulation modelling purposes.
The work by Geisler et al. (2012) demonstrated that all storage lipids have more or less the same
partitioning properties yet the work by Jonker and van der Heijden (2011) suggested that not all lipid
homogenates have the same partitioning properties. Therefore, the partitioning differences observed by
Jonker and van der Heijden (2012) in various lipid normalized animal homogenates must be driven by
partitioning to other non-storage lipid materials. Since partitioning to the cells used in this study
appeared to be dominated by partitioning to the storage lipids, additional studies could be conducted to
determine 1) what proportion of membrane lipids to storage lipids is required to significantly affect the
overall partitioning of PCBs to cells; 2) if liposomes truly are representative of real cell membranes
with respect to their partitioning properties; and 3) what other materials are present in animal homogenates that can potentially contribute to its overall sorption capacity. Further experiments could also include additional persistent and bioaccumulative chemicals such as PBDEs and possibly perfluorinated chemicals.

Chapter 6 introduced the concept of a bioenergy balance in bioaccumulation modelling. An initial analysis of the impact that inconsistent physiological rates can have on $D_A$, $D_E$, and $D_V$ was presented. However, further research is required in order to evaluate the impact these inconsistencies have on overall bioaccumulation for fish of different sizes and at different temperatures. The key energetic inconsistency highlighted in Chapter 6 was that between the energy consumed from the diet and energy available for growth. Specifically, it was demonstrated that the empirical regressions used by the previous bioaccumulation models for growth and feeding are not energetically compatible. Therefore, it is suggested that additional studies are required to establish energetically feasible growth rates and/or feeding rates for a broad range of fish under field conditions. As a starting point, the fidelity of a bioenergetic bioaccumulation model should be demonstrated for fish under controlled laboratory settings such that the feeding, growth, and ventilation rates can be empirically determined. The feeding rate can be determined in a laboratory setting whereby fish are provided with an excess of food, allowed to feed to satiation, and then the remaining food is removed and quantified. However, even under this strict laboratory feeding regime, fish housed together will likely compete for food and hence individual specific (as opposed to population specific) feeding rates can only be obtained if fish are housed in isolation from each other. Concurrently, the corresponding growth rates and ventilation rates for these fish over time can be measured and compared to model predictions. Having demonstrated the reliability of this approach in a controlled environment, the development of regressions under field conditions could be conducted. Growth rates could be obtained by measuring fish mass as a function of age for numerous fish species, temperatures, and lakes. Compilation of a sufficiently sized database could then be used to derive an empirical relationship between fish mass, age and temperature. Ideally, such a regression would be parameterized over temperature and masses similar to that of the OXYREF database such that it would be generally applicable to any fish. Theoretically, application of such a regression would improve both the fidelity and reliability of a bioenergetic bioaccumulation modelling approach. Feeding rates would be much more difficult to determine in the field and likely could only be measured in a laboratory setting. Additional analysis is also recommended in order to determine the feasibility of fish achieving steady-state in different environments given that the assumption of steady-
state is key to current field-based bioaccumulation assessments. Finally, once the feasibility of steady-state under the various conditions has been assessed, calculation of biomagnification factors for fish with 1) different sizes; 2) different temperatures and 3) seasonal availability of food could be conducted.
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