Relationship between the Bioconcentration Factor (BCF), the Bioaccumulation Factor (BAF), and the Trophic Magnification Factor (TMF)

by

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Abstract

A degree of consensus exists among bioaccumulation scientists on the use of Trophic Magnification Factors (TMFs), which are calculated from patterns of observed tissue contaminant concentrations across a food web, as “conclusive” evidence of the bioaccumulative nature of chemicals in the environment. However, most regulatory criteria to determine whether a substance bioaccumulates rely on Bioconcentration Factors (BCFs), which are measured in single-species laboratory tests. BCFs do not account for chemical biomagnification via trophic transfer, nor do they reflect biodilution. I present the results from laboratory and field studies aimed at testing the hypothesis whether the BCF, or its field-based counterpart, the Bioaccumulation Factor (BAF) are adequate predictors of the TMF. I conclude that the BCF can be a useful predictor of the TMF for chemicals with certain characteristics (i.e., fat soluble substances), but that there are two major types of errors where the BCF does not provide accurate information about the bioaccumulative nature of chemicals in the environment.

Keywords: Bioaccumulation; biomagnification; bioconcentration; empirical; model; policy

For my family,
who offered me unconditional love
throughout the process of this project, and
to Chris McDonald,
who was with me every step of the way.

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List of Acronyms

BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BDE	Brominated diphenyl ether
BMF	Biomagnification Factor
CEPA	Canadian Environmental Protection Act
HBCD	Hexabromocyclodecane
K_{OW}	Octanol-water partition coefficient
K_{OA}	Octanol-air partition coefficient
OECD	Organization for Economic Cooperation and Development
PCB	Polychlorinated biphenyl
TMF	Trophic Magnification Factor

Glossary

Bioaccumulation	The combined increase in chemical concentrations in organisms compared to the surrounding environment as a result of bioconcentration and biomagnification (F. A. P. C. Gobas & Morrison, 2000)
Bioaccumulation Factor (BAF)	The ratio of a chemical concentration in an organism to the surrounding environment (F. A. P. C. Gobas & Morrison, 2000)
Bioconcentration	The process by which organisms' uptake chemicals through respiration and diffusion of hydrophobic chemicals from aqueous to organic media (F. A. P. C. Gobas & Morrison, 2000)
Biomagnification	The process by which chemical concentrations increase in predators relative to concentrations in diet items as a result of uptake from diet and transfer through the food web (F. A. P. C. Gobas & Morrison, 2000)
Biomagnification factor (BMF)	The ratio of a chemical concentration in an organism to their diet items (F. A. P. C. Gobas & Morrison, 2000)
Octanol-water partition co-efficient (K_{OW}):	The ratio of a chemical solubility in octanol to a chemical solubility in water at equilibrium. Used as a metric to describe chemical partitioning between lipid and water phases in aquatic biota. Generally expressed in logarithmic format (log K_{OW}) (MacKay, 1991)
Octanol-air partition co-efficient (K_{OA})	The ratio of a chemicals solubility in octanol to a chemical solubility in air. Used as a metric to describe chemical partitioning between lipids and air in terrestrial biota. Generally expressed in logarithmic format (log K_{OA}) (Mackay, 1991)
Persistent organic pollutant (POP)	Class of chemicals defined by their persistence in the environment, tendency to bioaccumulate and toxicity (Stockholm Convention on POPs, 2004)
Trophic Magnification Factor	Calculated as the slope of the logarithm of the lipid normalized chemical concentration versus the $\delta N^{15}/\delta N^{14}$ stable isotope ratio and represents the average increase or decrease in lipid normalized chemical concentrations for a unit increase in trophic position (e.g. Fisk, Hobson, & Norstrom, 2001)
Trophic position	A measure of an organism's trophic status and thus level in a food web which, by providing non-integer quantities, considers the effects of omnivory, cannibalism, feeding loops, and scavenging on food web structure (Vander Zanden & Rassmussen, 1996)

1. Introduction

The Stockholm Convention on Persistent Organic Pollutants was ratified by 131 countries in 2004, with the objective of eliminating the most persistent (P), bioaccumulative (B), and toxic (T) substances. Since this time, substances such as polychlorinated biphenyls (PCBs), dichlordiphenyltrichloroethanes (DDTs), brominated flame retardants, select perfluorinated carboxylic acids, and many others have emerged as new chemicals of concern (Giesy & Kannan, 2001; Ikonomou, Rayne, & Addison, 2002). To evaluate and categorize the approximately 100,000 existing substances and the 1,000 to 2,000 new substances developed each year, regulatory agencies in a number of countries have developed methods to assess chemicals for their environmental behaviour (Arnot & Gobas, 2006; Kelly, Ikonomou, Blair, Morin, & Gobas, 2007), building on the P/B/T framework of the Stockholm Convention. For example, the *Canadian Environmental Protection Act* of 1999 (CEPA 1999) required that all substances on the Domestic Substance List (DSL) be evaluated (Government of Canada, 1999, 2000). The first phase of the evaluation was a screening assessment of the persistence, bioaccumulation, and toxicity of 23,000 substances. Substances that were determined to be persistent or bioaccumulative and toxic were then subject to a comprehensive evaluation including risk assessment.

For many substances, the level of concern and regulatory attention was determined by whether the substance was determined to be bioaccumulative. Bioaccumulative potential is typically expressed in terms of the octanol-water partition coefficient (K_{OW}), the bioconcentration factor (BCF), or the bioaccumulation factor (BAF). Most often, the degree of bioaccumulation is based on the K_{OW}, which is a standardized, laboratory-based, physical-chemical measurement of the partitioning of a substance between an octanol phase (acting as a lipid surrogate) and a water phase. The K_{OW} is used to determine chemical bioaccumulation for the majority of substances under evaluation because empirical bioconcentration factors (BCFs) are available for only 4% of the chemicals on the Canadian DSL, and empirical bioaccumulation factors (BAFs) for

only 0.2%. Studies have shown that the K_{OW} is useful as an indicator of a chemical's potential to bioaccumulate in water-breathing organisms such as fish. However, the K_{OW} has important limitations: most notably, it does not consider the metabolic transformation rate of the chemical in organisms (Arnot & Gobas, 2006) and it is not particularly useful to estimate bioaccumulation in air breathing organisms (Kelly & Gobas, 2000).

If adequate information is available, the BCF and the BAF may also be used to assess the bioaccumulative behaviour of chemicals (Table 1, (Arnot & Gobas, 2006)). The BCF is a standardized, laboratory-based measure of the bioaccumulation of a substance in fish from water, calculated as the ratio of a chemical concentration in the organism to the chemical concentration of the water, ideally measured at steady state. The BCF does not account for dietary uptake, but it does reflect metabolic transformation and elimination processes that take place in the study organisms. The BAF is a field-based measure of the uptake and bioaccumulation in fish as a result of uptake from the water via gill, dermal, and dietary exposure via all pathways. However, the BAF is not determined under standardized conditions, resulting in variability among calculated BAFs due to the myriad sources of natural variability among wild organisms.

Table 1.1. An overview of regulatory bioaccumulation assessment endpoints (Arnot & Gobas, 2006)

Regulatory Agency	Bioaccumulation endpoint	Criteria (log values)	Program
Environment Canada	K_{OW}	≥ 100 000 (5)	CEPA (1999)*
Environment Canada	BCF	≥5 000 (3.7)	CEPA (1999)
Environment Canada	BAF	≥5 000 (3.7)	CEPA (1999)
European Union 'bioaccumulative'	BCF	≥2 000 (3.3)	REACh+
European Union 'very bioaccumulative'	BCF	≥5 000 (3.7)	REACh
United States 'bioaccumulative'	BCF	1 000 (3) – 5 000 (3.7)	TSCA, TRI+-
United States 'very bioaccumulative'	BCF	≥5 000 (3.7)	TSCA, TRI
United Nations Environment Programme	K_{OW}	≥ 100 000 (5)	Stockholm Convention #
United Nations Environment Programme	BCF	≥5 000 (3.7)	Stockholm Convention

* CEPA, Canadian Environmental Protection Act, 1999 (Government of Canada 2000).
+ REACh, Registration Evaluation, and Authorization of Chemicals. Anne XII (European Commission 2001).
+-TSCA, Toxic Substances Control Act; TRI, Toxic Release Inventory programs (USEPA, 1976)
Stockholm Convention on Persistent Organic Pollutants.

According to the United Nations Stockholm Convention on Persistent Organic Pollutants, chemicals are considered bioaccumulative if:

- The BCF or the BAF is greater than 5,000 L/kg wet weight or, in the absence of such data that log $K_{OW} \geq 5$;
- Evidence that a chemical presents other reasons for concern such as high bioaccumulation in other species, high toxicity or ecotoxicity; or
- Monitoring data in biota indicating that the bioaccumulation potential of the chemical is sufficient to justify its consideration within the scope of this convention.

(Kitano, 2007)

The first criterion has served as the basis for bioaccumulation regulations in the *Canadian Environmental Protection Act*, the *Toxic Substances Control Act* in the USA, and the Registration, Evaluation, and Assessment of Chemicals program in the EU. The other two criteria were not included Canadian, European Union, and American bioaccumulation regulations because they were deemed more difficult to implement and monitor, because of a lack of relevant criteria and methods for measurement (Kitano, 2007). In a review of the application of the bioaccumulation criteria in the Stockholm Convention, Kitano (2007) found five substances that fulfilled the requirement for bioaccumulation despite having BCF values below 5,000. Other studies have shown that K_{OW}, BCF and BAF may not fully characterize bioaccumulative potential – substances that do not meet these criteria have been shown to biomagnify, increasing in concentration through successive steps in wild food webs (F. A. Gobas, De Wolf, Burkhard, Verbruggen, & Plotzke, 2010; Kitano, 2007; van Wijk, Chénier, Henry, Hernando, & Schulte, 2010; Weisbrod et al., 2010).

Another review of bioaccumulation criteria for persistent organic pollutants and persistent, bioaccumulative, and toxic substances by a work group from a SETAC Pellston workshop suggested that a substance should be considered bioaccumulative if it biomagnifies in food chains (F. A. Gobas et al., 2010). The Trophic Magnification Factor (TMF) was proposed as an empirical indicator to support this definition. The TMF is calculated from measured contaminant concentrations in biota within a food web and the trophic position of the organisms, usually estimated using stable nitrogen isotope ratios ($\delta^{15}N$). A TMF greater than 1 indicates a substance that increases in normalized concentrations, against the thermodynamic gradient, with increasing trophic level (i.e., meets the definition of a biomagnifying substance). The TMF is similar to the

biomagnification factor (BMF), which is calculated from ratios of concentrations between a single predator-prey pair instead of over multiple trophic levels within a food web.

In this study, I used an empirical approach to explore the relationship between the current definition of a biaoccumulative substance (i.e., BAF or BCF ≥ 5,000) and the definition of biomagnifying substance identified with the TMF criteria (TMF > 1). I tested whether current criteria identifying bioaccumulative substances would also identify substances that biomagnify, as defined by a TMF > 1. I identified which substances may obtain false positive or false negative results in the current bioaccumulation screening process (Figure 5), defined as follows:

- A false positive result in this study occurred when a substance had a BCF or BAF above 5,000 (i.e., was screened as bioaccumulative) but a TMF below 1 (i.e., did not biomagnify in the studied food web). A false positive result could prompt regulatory attention and management that may not be warranted given the findings of the field study.
- A false negative result occurred when a substance had a BCF or BAF below 5,000 (i.e., was screened as not bioaccumulative) but a TMF above 1 (i.e., biomagnified in the study food web). A false negative result could identify a substance as being of low concern, when the findings of the field study indicate that biomagnification if occurring.

2. Methods

2.1. Overview

Pim Leonards and Heather Leslie at the Institute of Environmental Studies at the Free University, the Netherlands, completed the field sampling in the Western Scheldt Estuary in the Netherlands and analytical aspects of the project, including the stable nitrogen isotope calculations. The purpose of the field study was to test predictions of bioaccumulation based on laboratory BCF studies. The sampling was organized in cooperation with colleagues from the Dutch Institute Deltares and with local fisherman for the fieldwork itself.

All permits required by Dutch law for the sampling of biota were pre arranged in cooperation with Deltares, which also had the accredited personnel on board during sampling. The field work took place on Sept 17-18 2008. Both pelagic and benthic food webs were sampled ensuring that the food chains are as long as possible. The link to the terrestrial food chain is provided by the tern eggs from a colony feeding within the study area that were sampled in advance of the main sampling round. Water samples were taken in 1L glass and plastic bottles. Composite samples of sediment were taken at various locations using a core sampler. The freely dissolved chemical concentrations in the water phase and the total water were used to estimate the bioavailability of chemicals in the water phase and for calculating BAFs.

In the analytical design, target substances were identified in advance to ensure a span of log K_{OW}, K_{OA} , BCF, and biotransformation capacity. The selected substances also include high production volume chemicals, which have reported BCFs above and below the 2000 limit.

2.2. Study Area

The Western Scheldt, the estuary of the Scheldt River in southwestern Netherlands, contains many different substances above detection limits due to land-based industrial activity on shore and in nearby Antwerp (Baeyens, Van Eck, Lambert, Wollast, & Goeyens, 1997) (Figure 2.1). The sampling area was located near the Terneuzen harbor and the sandbar known as the *Middenplaat*. The Western Scheldt is well-studied in terms of national monitoring programs and surveys and extensive knowledge exists of the resident food web and the trophic positions of organisms in this region. The benthic-pelagic food web includes suspended particulate matter, phytoplankton, zooplankton, lugworms, rag worms, cockles, green crabs, goby, sole, flounder, plaice, sand eels, pouting, herring, and the common tern (Figure 2.2).

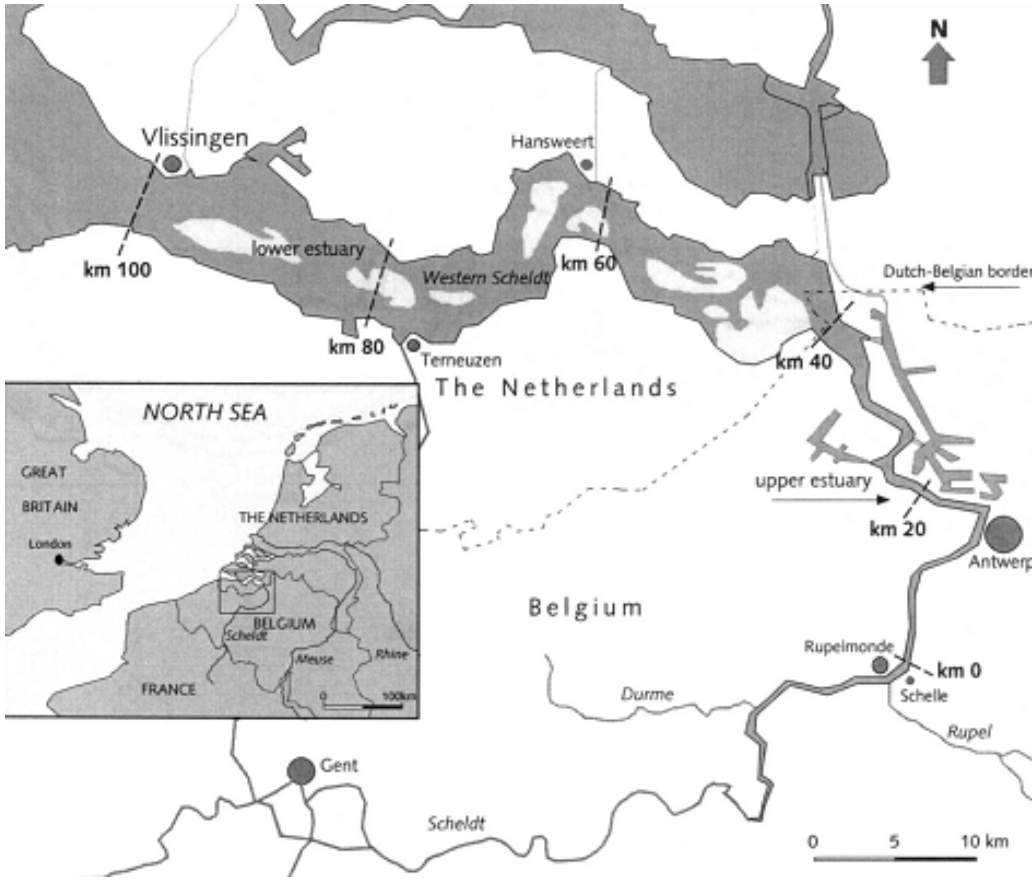

Figure 2.1. The Western Scheldt estuary (depicted by the arrow), the Netherlands and surrounding areas.

Table 2.1 lists species names and general dietary information for each species sampled in the food web.

Table 2.1. Scientific name, common name, and general diet of the species sampled in benthic-pelagic food web study in the Western Scheldt food-web (n/a indicates not applicable).

Species	Common name	General Diet
Suspended matter	(n/a)	(n/a)
Phytoplankton	Phytoplankton	The phytoplankton samples collected are a mixture of phytoplankton and suspended matter.
Mysis sp.	Mysids, (zooplankton)	Phytoplankton
Arenicola marina	Lugworm	Algae, small worms, detritus
Nepthys	Rag worm	Lugworms, nematodes, small shrimps, clams,
Cerastoderma edule	Cockle	Plankton, including suspended matter
Pleurobrachia sp.	Jellyfish	Zooplankton.
Clupea harengus	Herring	Phyto- and zooplankton
Ammodytes sp.	Sand eel	Zooplankton and some large diatoms
Carcinus maenas	Green crab	Young of bivalves and fish
Goby sp.	Goby	Molluscs, crustacean, insect larvae
Solea solea	Sole	Worms, molluscs and small crustaceans
Platichthys flesus	Flounder	Juveniles of less than a year old feed on plankton and larvae of insects, juveniles of more than a year and adults feed on benthic fauna, including small fishes and invertebrates (cockles)
Pleuronectes platessa	Plaice	Thin-shelled mollusks and polychaetes (lugworm, rag worm)
Trisopterus lucus	Pouting	Benthic crustaceans but also on small fish, mollusks and polychaetes
Myoxocephalus scorpius	Sculpin	Fish, and large crustaceans
Sterna hirundo	Common tern	Herring and flatfish

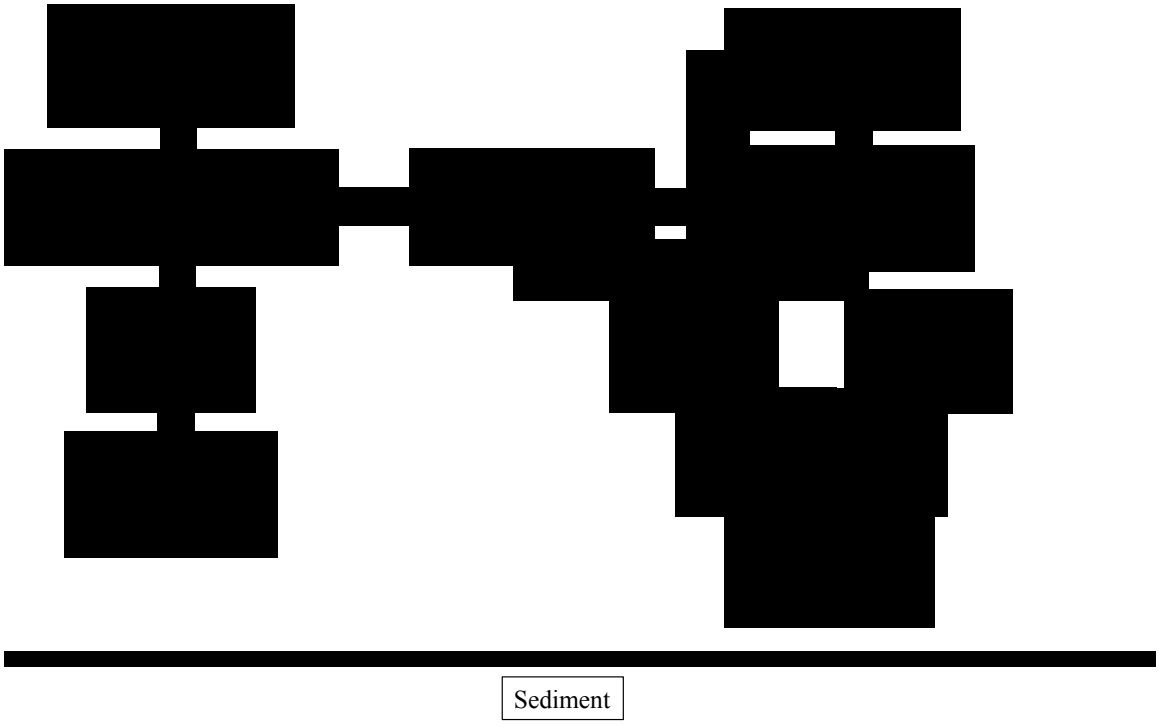

Figure 2.2. Illustrative representation of the benthic-pelagic water- and air-breathing food web in the Western Scheldt estuary

2.3. $\delta^{15}N$ and $\delta^{13}C$ Analysis

The nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic composition of tissues are integrated measures of diet that can be used to distinguish between food web positions and help interpret trophic transfer of chemicals that bioaccumulate (e.g., Fisk et al. 2001). Stable isotopes ratios ($^{15}N/^{14}N$, $^{13}C/^{12}C$) were determined in biotic samples using an elemental analyzer (NC2500, ThermoQuest Italia, Rodana, Italy) coupled with an Isotope Ratio Mass Spectrometer (Delta Plus, Thermo-Quest Finnigan, Bremen, Germany). Stable isotope values were expressed as a ratio (R) of the heavy to the lighter isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$ or) standardized with respect to internationally recognized reference materials (e.g. atmospheric air for nitrogen and Vienna Pee Dee Belemnite for carbon) as follows (Eqn 1):

$(‰) = [(R_{sample}/R_{reference}) - 1] \times 1000$ (1)

The standards used for $\delta^{13}C$ determination were USGS-24 graphite ($\delta^{13}C$ = -16.05 ‰), IAEA-601 benzoic acid ($\delta^{13}C$ = -28.81 ‰) and IAEA-CH7 PEF ($\delta^{13}C$ = -32.15 ‰). The standards used for $\delta^{15}N$ measurements were IAEA-N1 ($\delta^{15}N$ = 0.43 ‰) and IAEA-N2 ($\delta^{15}N$ = 20.41 ‰). Glutamic acid, with a known value of $\delta^{13}C$ = -24.08 ‰ and $\delta^{15}N$ = 3.20 ‰, was used as a quality control sample. Instrument precision was better than 0.15 ‰ for carbon and nitrogen based on replicate analysis of standard reference materials.

2.4. Chemical Properties

The 24 substances in this study exhibit a wide range of K_{OW} and octanol-air (K_{OA}) partition coefficients (Table 2.2) and chemical classes, including polychlorinated biphenyls, perfluorinated compounds, brominated compounds, hexachlorobenzene, pyrenes, and hexabromocyclodecanes. Appendix B contains more information about BCFs from the Arnot and Gobas database.

Table 2.2. Log KOW, log KOA, and select BCFs (Arnot and Gobas, 2006) for twenty-four substances measured in benthic and pelagic organisms in the Western Scheldt Estuary.

Chemical	Abbreviation	Log K_{OW}	Log K_{OA}	Geometric mean BCF (L/kg wet weight)
Benzo-a-pyrene	n/a	7.6	10.86	6,945
Brominated diphenyl ether 28	BDE 28	5.88	9.5	-
Brominated diphenyl ether 47	BDE 47	6	10.69	-
Brominated diphenyl ether 49	BDE 49	-	-	-
Brominated diphenyl ether 99	BDE 99	6.8	11.16	-
Brominated diphenyl ether 100	BDE 100	-	-	-
Brominated diphenyl ether 153	BDE 153	8.3	11.82	-
Brominated diphenyl ether 154 +BB153	BDE 154 +BB153	-	9.3	-
Brominated diphenyl ether 209	BDE 209	9.9	18.42	7
Diisopropylnaphtalene	n/a	5.68	7.37	9,208*
α-Hexabromocyclodecane	α-HBCD			899
γ- Hexabromocyclodecane	γ-HBCD			173
Hexachlorobenzene	HCB	5.86	7.38	4,780
Polychlorinated biphenyl-28	PCB 28	5.97	8.77	-
Polychlorinated biphenyl-52	PCB 52	6.04	8.4	12,133

Chemical	Abbreviation	Log K_{OW}	Log K_{OA}	Geometric mean BCF (L/kg wet weight)
Polychlorinated biphenyl-101	PCB 101	6.8	9.1	10,165
Polychlorinated biphenyl- 118	PCB 118	7.12	9.8	61,353
Polychlorinated biphenyl- 138	PCB 138	7.44	9.5	96,713
Polychlorinated biphenyl- 153	PCB 153	6.91	10.44	48,416
Polychlorinated biphenyl- 180	PCB 180	7.26	10.99	18,804
Perfluorooctanoic acid	PFOA	6.30**	5.73	1,258
Perfluorooctane sulfonic acid	PFOS	6.28**	6.63	3,981
Perfluorooctanesulfonamide	PFOSA	7.58**	-	-
Perfluoro-n-decanoic acid	PFDA	-	6.22	2,511
Pyrene	Pyrene	6.11	8.19	2,827
σ-Terphenyl	n/a	5.52	9.3	3,558*

- indicates data not available
* indicates only one BCF value was available and is not a geometric mean
** value predicted from KOWWIN v1.67

2.5. Calculation of the BAF, BCF and TMF

Field-based biota concentrations were calculated from whole-body samples. Concentrations of the substances found in biota and in water data are summarized in Appendix A. Field data were excluded from the BAF and TMF calculations if water or biota concentrations were unavailable or were below the detection limit.

2.5.1. BAFs

BAFs (L/kg ww) were calculated from observed concentrations of substances measured in organisms and in water for each organism in the food web (Eqn.2):

—

where C_{biota} is the geometric mean, wet-weight concentration of the observed chemical in a given organism (mg/kg ww) and C_{water} is the geometric mean total concentration of each substance observed in water (mg/L) in the Western Scheldt estuary. Chemical concentrations were measured in 11 components of the food web and 9 water samples.For each component of the food web (i.e. water, sediment, organisms etc…), multiple individual BAFs (calculated for individual samples) were summarized as

a geometric mean. The 5th and 95th percentiles of the distribution of BAFs for each component were calculated from the standard deviation of log BAF values.

2.5.2. Bioconcentration Factors (BCF)

Bioconcentration Factors (BCFs) are generally standardized, laboratory-based bioaccumulation indicators (OECD 305, Eqn. 3).

BCFs were compiled from the database in Arnot and Gobas (2006) (Appendix B). Only substances included in the Arnot and Gobas (2006) database were included in the BCF-TMF comparison. For substances with more than one BCF (i.e., PCB 52, PCB 101, PCB 118, PCB 153, PCB 180, HCB, pyrene, benzo(a)pyrene, and BDE 209), a geometric mean BCF and associated percentile values were calculated as described for BAFs.

2.5.3. Trophic Magnification Factors (TMFs)

Trophic magnification factors (TMFs), also sometimes termed Food Web Magnification Factors (Fisk et al., 2001; Hop, Borgå, Gabrielsen, Kleivane, & Skaare, 2002) were calculated for each substance using two different methods. The first method of calculating the TMF, referred to herein as the 'traditional method', is from the antilog of the slope of a regression between the log chemical concentration (normalized to lipid, or to nitrogen for perfluorinated substances) and trophic level for each organism of the tested food web (Eqn. 4) (Figure 2.3). Concentrations of perfluorinated substances were normalized to percent nitrogen as an index of the protein content, because these substances are known to associate with protein, rather than lipid. Phytoplankton were removed from the linear regression for perfluorinated substances because protein normalization of phytoplankton is not feasible due to the very low nitrogen content of algae.

The second method for calculating the TMF, referred to herein as the 'balanced method', was based on a regression of geometric mean concentrations and trophic levels, rather than concentrations and trophic levels of each individual organism. Calculating the geometric mean for each species reduces the influence of unbalanced sampling, i.e., a greater number of samples at certain trophic positions, in this study between trophic position 2.5 and 3.5, compared to other trophic positions. The balanced method was tested in response to concerns that the traditional method was unduly influenced by larger samples of organisms with higher trophic positions.

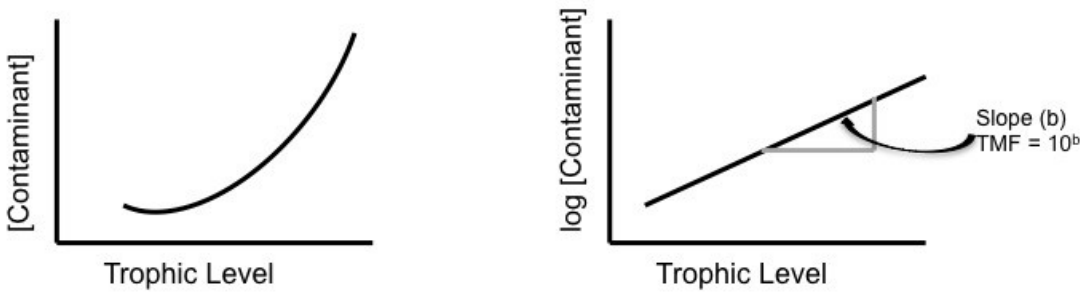

Figure 2.3. The relationship between normalized contaminant concentrations in biota and trophic level

2.6. Comparison between bioaccumulation indicators

The relationships between the BAF, BCF, and TMF were explored to test if the current regulatory criteria (i.e., BCF and BAF ≥ 5,000) adequately indicate a substance's biomagnification behaviour in the studied estuarine food web, as indicated by a TMF > 1. I assumed a finding of TMF > 1 to be a true indicator of a biomagnifying substance. The BAF and the BCF were compared to the TMF to identify one of four possible outcomes:

I. The contaminant was screened as bioaccumulative (BCF or BAF ≥ 5,000) but showed no biomagnification in the food web (TMF < 1). This outcome was referred to as a false positive.

II. The contaminant was screened to be not bioaccumulative (BCF and BAF < 5,000) but the chemical biomagnified (TMF > 1). This outcome was referred to as a false negative.

III. The contaminant was screened as bioaccumulative (BCF and BAF ≥ 5,000) and biomagnified (TMF > 1). This outcome was referred to as a true positive.

IV. The contaminant was screened to be not bioaccumulative (BCF and BAF < 5,000) and did not biomagnify (TMF < 1). This outcome was referred to as a true negative.

Figure 2.4 illustrates these four outcomes.

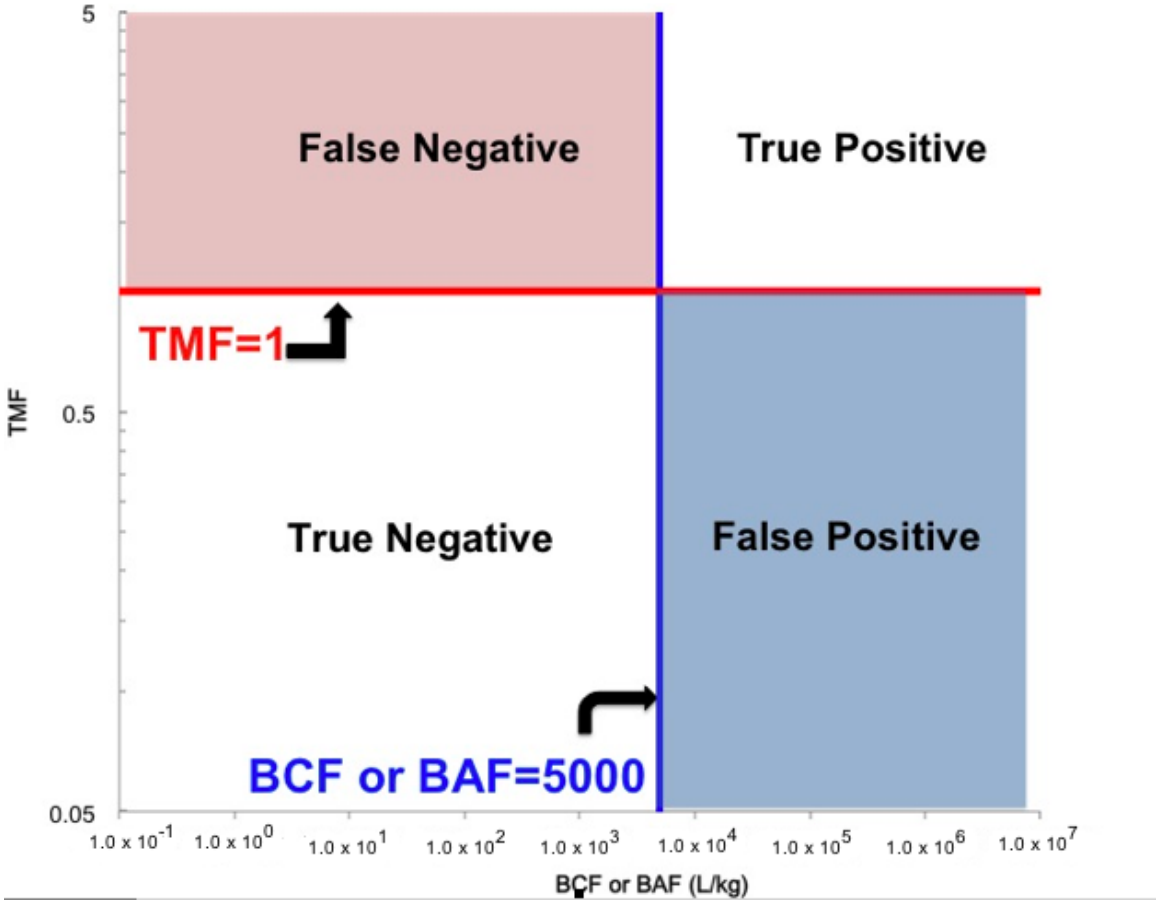

Figure 2.4. Illustration of four possible outcomes in the comparison of a BCF or BAF (relative to a screening criterion of 5,000) with a TMF (relative to a criterion value of 1).

3. Results and Discussion

3.1. Bioaccumulation Factors

Table 3.1 lists the empirical BAFs for each tested substance and for each sampled aquatic organism. The BAFs range from 2 L/kg ww for BDE 209 in mid to upper trophic level organisms such as pouting and flounder to 463 629 L/kg ww for PCB 118 in slimy sculpin. All tested substances with the exception of PFOA obtained at least one BAF above the current regulatory threshold of 5000 L/Kg. Among samples, i.e. the multiple BAFs calculated for each species, the BAFs generally remained consistent within a species. However, the zooplankton/jellyfish samples obtained one sample consistently higher than the other sample.

The variation of BAFs between species may be explained due to the diverse bioaccumulative nature of the studied substances, i.e. a substance with a high log K_{OW} or log K_{OA} will be more likely to bioaccumulate than a substances with a low log K_{OW} or log K_{OA}. the ability of the organism to metabolize the substance, and the trophic level of the organism. Specifically, among the perfluorinated substances, BAFs tend to be highest between pouting and herring with the exception of PFOA where lugworm obtained the highest BAF. The BAFs for the PCBs also have high BAFs among the pouting and herring. BAFs for the PCBs are also relatively higher than other species in sole, flounder, and goby. Pouting and herring also obtain the highest BAFs for HCB. Suspended particulate matter, lugworm, and cockle obtained the highest BAFs for pyrene and benzo-a-pyrene; the lowest BAFs for the same substances were found in upper trophic level benthic and pelagic organisms such as herring, pouting, sole, and flounder. The less brominated congeners of the brominated flame-retardants obtained low BAFs in suspended particulate matter, phytoplankton, lugworms, and cockle compared to upper trophic level organisms with higher BAFs. Herring and sole obtained the highest BAFs of all the species sampled for BDE 99. Phytoplankton and cockle obtained BAFs below the rest of the species sampled for BDE 100. The BAFs for BDE

154+BB153 and BDE 153 were similar in that highest BAFs were found in sole whereas the lowest were obtained for phytoplankton and zooplankton. Alpha-HBCD obtained low BAFs in phytoplankton, zooplankton and the common shore crab compared to Gamma-HBCD where low BAFs were obtained for the majority of species sampled with the exception of phytoplankton and suspended particulate matter. The variability may also be attributed to different biotransformation capabilities of the organisms for each substance.

The geometric means of the BAFs obtained for each substance studied generally agree with the prediction that a substance defined as bioaccumulative under current regulatory criteria, in this case BAF≥5000, also biomagnifies as defined by a TMF>1 (Figure 3.8).

The BAF for each substance studied varied depending on which organism was used for the calculation. For example, the BAFs were different between species i.e. one BAF for PFDA in herring was 1757 L/kg and one BAF for PFDA in pouting was 6003 L/kg. The 95% confidence intervals for BAFs range an order of magnitude and within that range many substances obtained BAFs both above and below the current regulatory criteria of 5000. For example, the substance PFDA may obtain a False Negative result or a True Positive result depending on which BAF was selected for evaluation because the 95% confidence intervals span across the line indicating the current regulatory criteria. In this study, we selected the geometric mean to represent the BAF for the substance to include as much field information as possible in the measurement. The inconsistency between BAF values may have implications when used as a bioaccumulation indicator in Canadian legislation (i.e. BAF ≥ 5000). The implications of using the BAF as a bioaccumulation indicator include the decision of which BAF is selected for evaluation may be a decision of the regulator or industry professional who may not be aware of the bioaccumulative nature of the substance or of the implications of selecting a BAF for a benthic fish compared to a pelagic fish.

Table 3.1. Bioaccumulation factors (L/kg) of various analytes in each organism of the study

3.2. Bioconcentration Factors

Appendix B lists the BCFs for the substances tested obtained from the Gobas and Arnot database (2007) for the available substances. The BCFs were highly variable within a species because the database contains BCFs from hundreds of different lab-based studies. The 95% confidence intervals for certain BCFs (i.e. pyrene, benzo-a-pyrene, and hexachlorobenzene) range above and below the current regulatory criteria in this case, BCF ≥ 5000 (i.e.Figure 3.9).

The differences in the BCFs can be explained by a potential inconsistency in the laboratory tests used to calculate the bioconcentration factor. The laboratory tests for many of the BCFs used in this study occurred prior to the OECD standardization protocol. However, with the OECD 305 standard protocol, the variability in BCFs for individual chemicals may decrease over time. The variability in BCFs for a given substance has consequences such as subjectivity in the selection of which BCF value to use for regulatory purposes. The database contained only one BCF for certain substances such as PFOS and PFDA and as a result no confidence intervals could be calculated for these substances.

3.3. Trophic Magnification Factors

3.3.1. Comparison between Trophic Magnification Factor Methods: Traditional vs. Balanced Method

Table 1-4 lists the trophic magnification factors obtained from two different methods used to calculate trophic magnification factors. Column (a) lists TMFs calculated from the use of individual concentrations of substances in organisms with increasing trophic level and column (b) lists TMS calculated from the geometric mean concentration of substances in a species with increasing mean trophic level. The 95 % confidence intervals calculated for the trophic magnification factor did not span the same order of magnitude as the confidence intervals calculated for the BAFs or BCFs (Figures

3.8 and 3.9). 95% confidence intervals for TMFs ranged less than 1 order of magnitude and few 95% confidence intervals overlapped the TMF threshold of 1.

The TMFs calculated for substances in this study using the Balanced Method were consistent with TMFs calculated traditionally with the exception of three substance, PFOSA, PCB 28, and BDE 49 (Table 3.2). Assuming the traditional method to be the true indicator of a substance's capacity to biomagnify, the Balanced Method over-estimates a substances' biomagnifying ability for PFOSA and BDE 49, and under-estimates a substances biomagnifying ability. A comparison between the two methods used to calculate a trophic magnification factor has not yet been documented to the best of this author's knowledge. The consistency of TMFs calculated from both methods to be above or below 1 for the majority of substances suggests there may not be a need to ensure a balance of trophic levels within the food web prior to determining the slope in the linear regression. The traditional method to calculating a TMF incorporates the available information of concentrations of substances in organisms.

Table 3.2. The trophic magnification factors calculated using two different methods, a) TMFs and the p-values from the linear regression of the concentration of substances in every organism and the trophic position of the organism and b) TMFs and the p-values from the linear regression of the geometric mean of substances in each species and the mean trophic position of the species.

Substance	Trophic Magnification Factor (a)	p-value (0.05)	Trophic Magnification Factor using the Balanced Method (b)	p-value (0.05)
PFOA	3.85	2.68×10^{-3}	3.30	1.8×10^{-1}
PFDA	6.69	1.87×10^{-8}	4.96	1.0×10^{-2}
PFOS	8.33	1.61×10^{-11}	7.15	7.2×10^{-4}
PFOSA	0.84	6.37×10^{-1}	1.04	9.5×10^{-1}
PCB 28	1.13	2.86×10^{-1}	0.95	8.6×10^{-1}
PCB 52	1.06	7.94×10^{-1}	1.38	2.2×10^{-1}
PCB 101	1.49	9.68×10^{-3}	1.48	1.9×10^{-1}
PCB 118	1.89	8.00×10^{-5}	1.70	1.1×10^{-1}
PCB 138	1.77	8.68×10^{-3}	1.81	7.0×10^{-2}
PCB 153	2.15	2.00×10^{-5}	1.95	5.0×10^{-2}
PCB 180	1.94	1.70×10^{-4}	1.67	1.3×10^{-1}
Hexacholorbenzene	1.24	2.08×10^{-1}	1.00	1.0×10^{0}
Pyrene	0.19	1.21×10^{-7}	0.17	1.8×10^{-4}
Benzo-a-pyrene	0.32	2.30×10^{-3}	0.12	9.2×10^{-4}
BDE 28	1.34	3.74×10^{-2}	1.27	1.3×10^{-1}

Substance	Trophic Magnification Factor (a)	p-value (0.05)	Trophic Magnification Factor using the Balanced Method (b)	p-value (0.05)
BDE 49	0.97	9.14×10^{-1}	1.38	3.0×10^{-1}
BDE 47	1.93	1.88×10^{-7}	1.87	5.0×10^{-2}
BDE 99	1.27	2.99×10^{-1}	1.11	7.5×10^{-1}
BDE 100	1.91	1.50×10^{-4}	1.80	2.0×10^{-2}
BDE 153	1.32	2.24×10^{-1}	1.06	7.6×10^{-1}
BDE 138	0.91	3.69×10^{-1}	0.89	3.3×10^{-1}
BDE 154+BB153	1.53	5.39×10^{-3}	1.22	2.9×10^{-1}
BDE 209	0.14	2.85×10^{-10}	0.13	3.3×10^{-4}
α-HBCD	1.51	4.57×10^{-3}	1.47	1.5×10^{-1}
γ-HBCD	0.27	1.13×10^{-3}	0.36	1.0×10^{-2}

The change of concentration for substances in organisms at different trophic levels varies depending on the analyte and on the method of TMF calculation, however the majority of substances studied increase in normalized, either protein-normalized or lipid normalized with increasing trophic position; select substances either no not increase in normalized concentration with respect to increasing trophic level or show no discernible pattern.(Figure 3.1 to Figure 3.7).

The fluorinated substances generally increase in protein-normalized concentrations in organisms with increasing trophic level, with the exception of PFOSA for which no discernible pattern of biomagnification is observed with increasing trophic level (3.1, (g) and (h)). The PCBs in this study also increase in lipid-normalized concentrations in organisms with increasing trophic level and is particularly evident in the higher-chlorinated congeners such as PCB 180 (Figures 3.2, 3.3 (e) (f)). Hexachlorobenzene also increases in lipid-normalized concentration in organisms with increasing trophic level (Figure 3.4, (e) and (f)). Pyrene and benzo-a-pyrene decrease in lipid-normalized concentration in organisms with increasing trophic level (Figure 3.4, (a), (b), (c), (d)) The BDEs in this study generally increase in lipid normalized concentrations with increasing trophic level with the exception of BDE 209 (Figure 3.5). BDE 209 decreases in lipid-normalized concentration in organisms with increasing trophic level. The HBCD isomers **α** and **γ** have opposite biomagnification patterns in the studied food web; α-HBCD increases in lipid normalized concentrations in organisms with increasing

trophic level and γ-HBCD decreases in lipid normalized concentration with increasing trophic level (Figure 3-7)

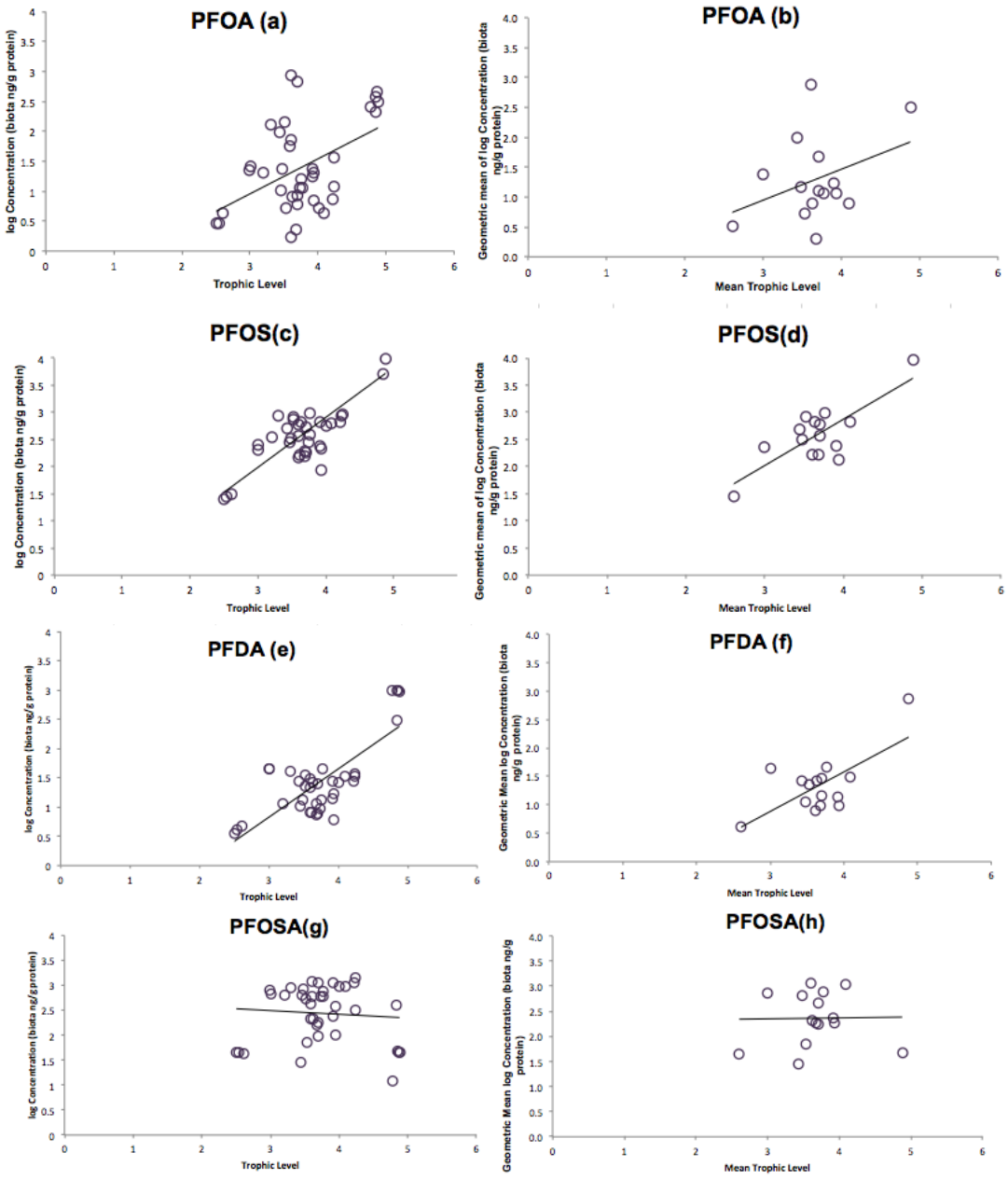

Figure 3.1. ***The log protein normalized concentration of PFOA, PFOS, and PFDA in organisms on the y-axis increases with respect to increasing tropic position on the x-axis for both methods of calculating the TMF; PFOSA neither increases or decreases in log protein normalized concentration with respect to trophic level. Method 1, using individual concentrations of substances in organisms, is depicted by (a), (c), (e), (g), and Method 2, using geometric mean concentrations of substances in a species and mean trophic level is depicted in graphs (b), (d), and (f).***

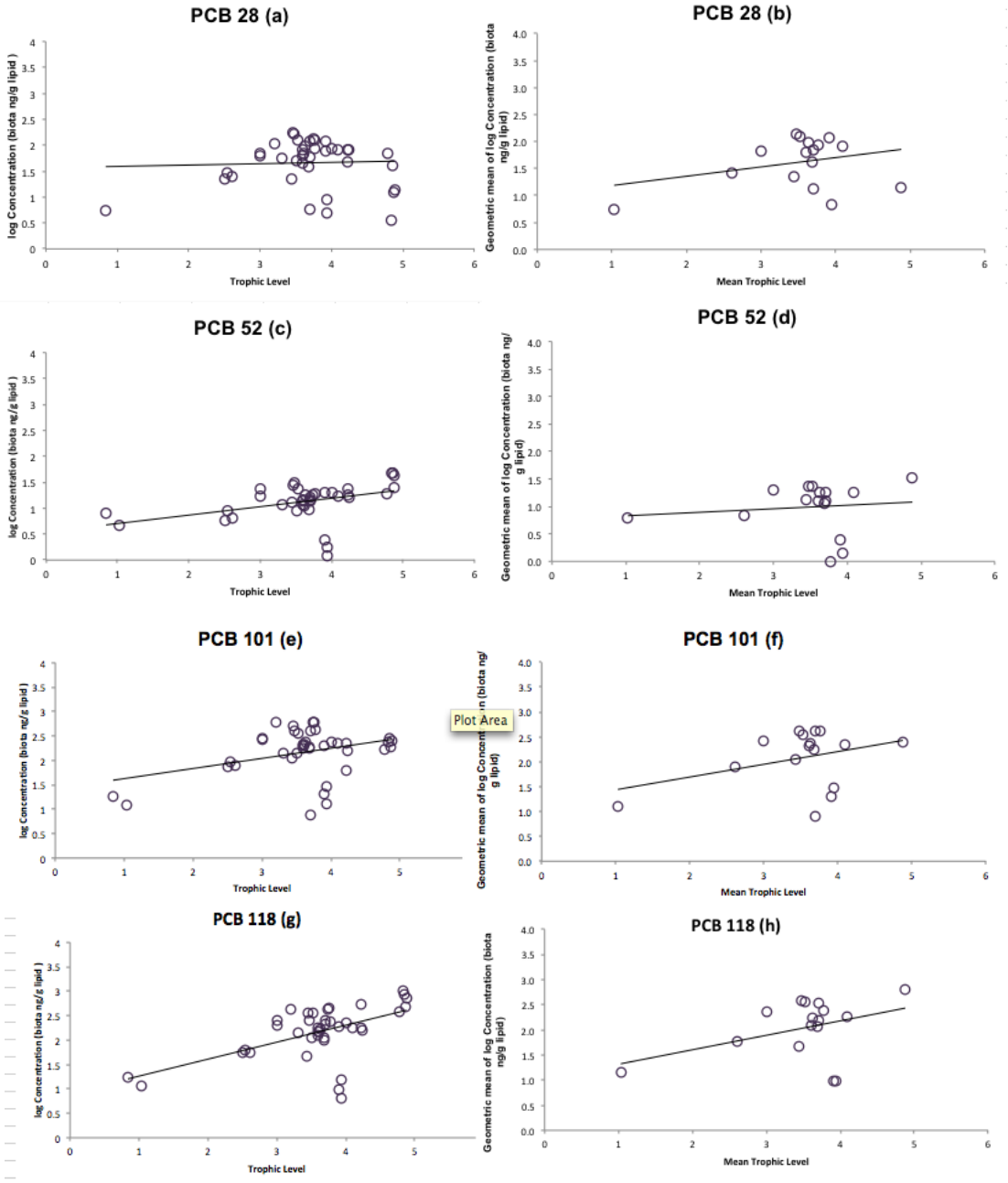

Figure 3.2. ***The log lipid normalized concentration of PCB 28, PCB 52, PCB 101, and PCB 118 in organisms on the y-axis increases with respect to increasing tropic position on the x-axis for both methods of calculating the TMF. Method 1, using individual concentrations of substances in organisms, is depicted by (a), (c), (e), and Method 2, using geometric mean concentrations of substances in a species and mean trophic level is depicted in graphs (b), (d), and (f).***

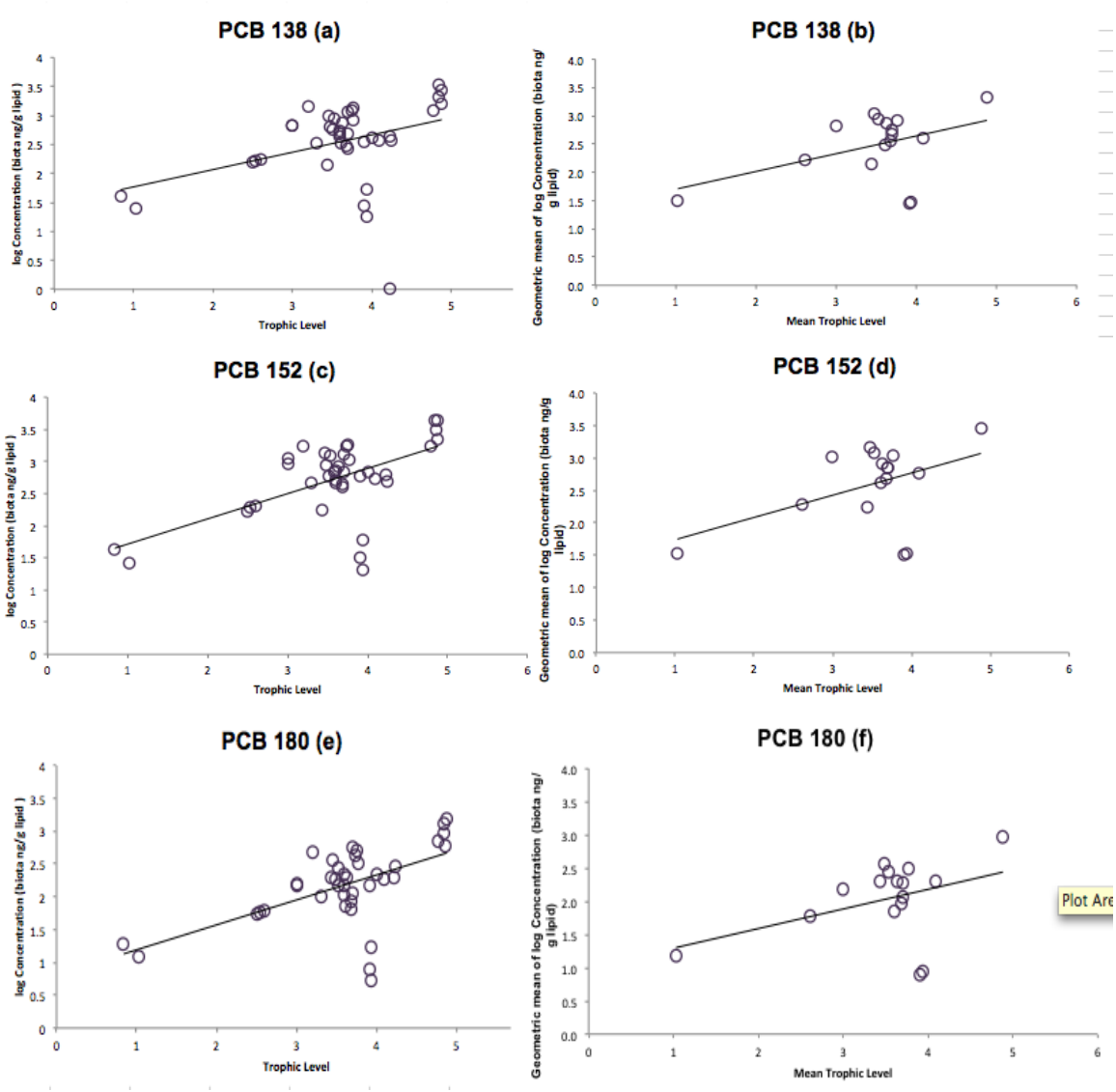

Figure 3.3. The log lipid normalized concentration of PCB 138, PCB 152, and PCB 180 in organisms on the y-axis increases with respect to increasing tropic position on the x-axis for both methods of calculating the TMF. Method 1, using individual concentrations of substances in organisms, is depicted by (a), (c), (e), and Method 2, using geometric mean concentrations of substances in a species and mean trophic level is depicted in graphs (b), (d), and (f).

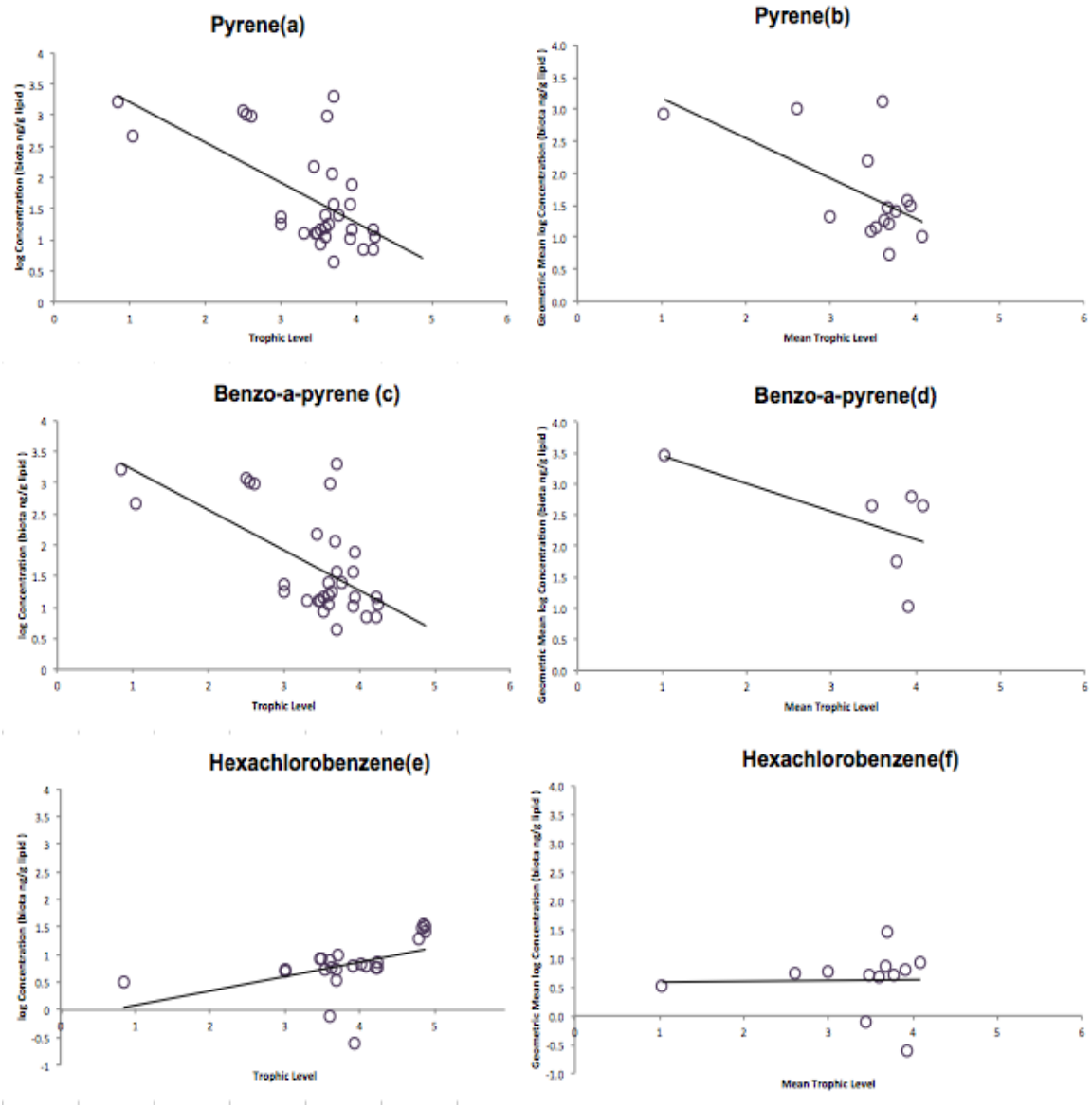

Figure 3.4. ***The log lipid normalized concentration of pyrene and benzo-a-pyrene, in organisms on the y-axis decreases with respect to increasing tropic position on the x-axis for both methods of calculating the TMF; HCB increases with Method 1 and neither increases nor decreases in normalized concentration with respect to trophic level in Method 2. Method 1, using individual concentrations of substances in organisms, is depicted by (a), (c), (e), and Method 2, using geometric mean concentrations of substances in a species and mean trophic level is depicted in graphs (b), (d), and (f)***

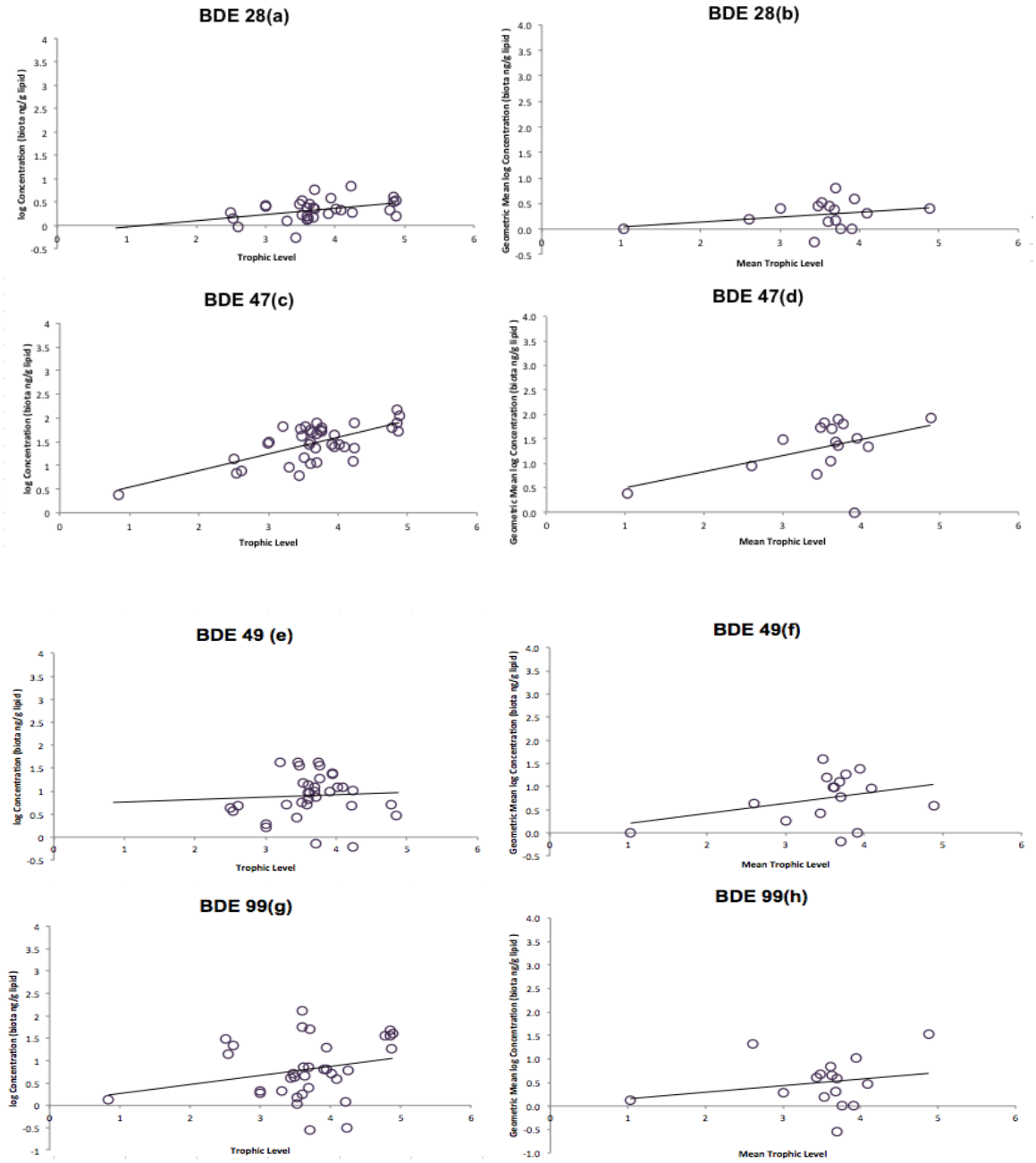

Figure 3.5. ***The log lipid normalized concentration of BDE 28, BDE 47, BDE 49, and BDE 99 in organisms on the y-axis increases with respect to increasing tropic position on the x-axis for both methods of calculating the TMF. Method 1, using individual concentrations of substances in organisms, is depicted by (a), (c), (e), (g) on the left and Method 2, using geometric mean concentrations of substances in a species and mean trophic level is depicted in graphs (b), (d), (f), (h) on the right.***

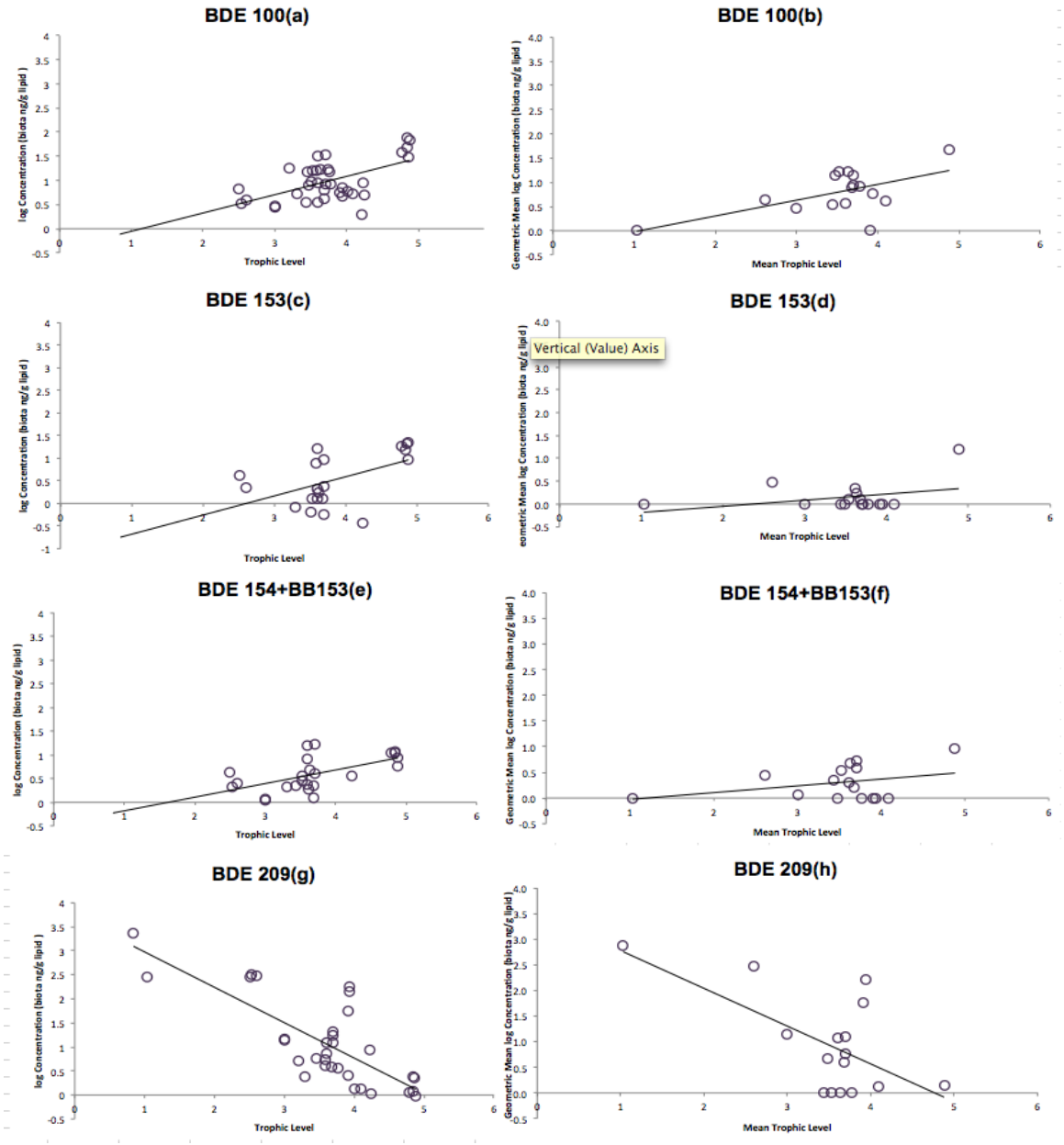

Figure 3.6. ***The log lipid normalized concentration of BDE 100, BDE 153, BDE 154+BB53 in organisms on the y-axis increases with respect to increasing tropic position on the x-axis for both methods of calculating the TMF; BDE 209 decreases in normalized concentration with respect to trophic level for both methods. Method 1, using individual concentrations of substances in organisms, is depicted by (a), (c), (e), (g) and Method 2, using geometric mean concentrations of substances in a species and mean trophic level is depicted in graphs (b), (d), (f), (h).***

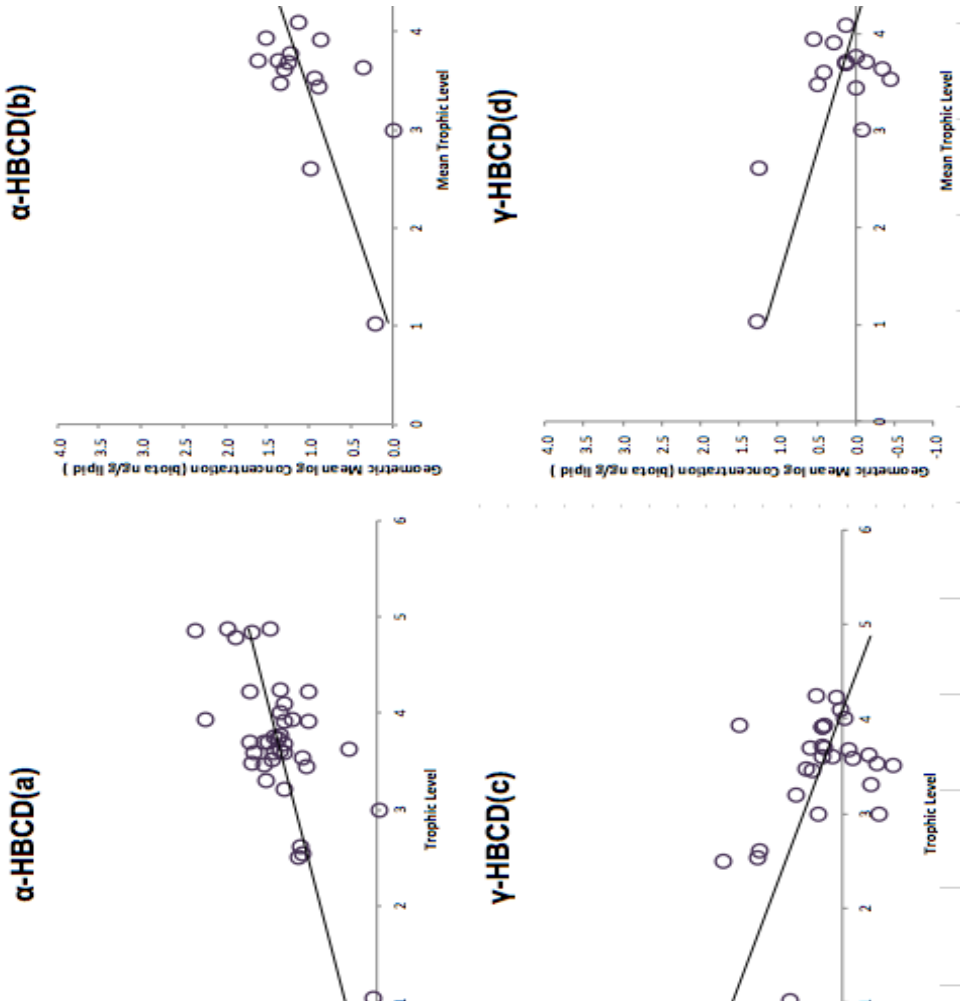

Figure 3.7. ***The log lipid normalized concentration alpha-HBCD in organisms on the y-axis increases with respect to increasing tropic position on the x-axis for both methods of calculating the TMF. Gamma-HBCD decreases in normalized concentration with respect to trophic position for both TMF methods. Method 1, using individual concentrations of substances in organisms, is depicted by (a) and (c), and Method 2, using geometric mean concentrations of substances in a species and mean trophic level is depicted in graphs (b) and (d).***

3.4. Comparison of Bioaccumulation Measures (BAF, BCF, and TMF)

In general, for both BCFs and BAFs, the current regulatory criteria for a bioaccumulative substance (the BCF and BAF ≥5000) adequately describes a biomagnifying substance as defined by a TMF>1(True Positive or True Negative results)

(Figure 3.8, Figure 3.9). Exceptions to the agreement between BCF-BAF and TMF do exist however, and certain groups of substances either biomagnify without being classified as bioaccumulative, or do not biomagnify despite being classified as bioaccumulative according to current regulatory criteria (False Positive or False Negative). The following sections describe the True Positive, True Negative, False Positive, and False Negative results and the corresponding substances.

3.4.1. True Positive and True Negative Results

For the substances included in this study the BAF and BCF regulatory criteria to identify a bioaccumulative substance (BAF or BCF ≥ 5000) adequately described the biomagnifying nature of most substances as defined by a TMF >1; the majority of substances studied obtained a True Positive or True Negative result.

The substances that obtained True Positive results in BAF-TMF relationship were PCB congeners 28, 52, 99, 101, 118, 138, 153, 180, BDE congeners 28, 47,49, 99,100, α-HBCD, PFOS, PFOSA. True Negative results in the food web containing aquatic organisms *and* air-breathing organisms were associated with the following substances: BDE 209, benzo-a-pyrene, pyrene, and γ-HBCD.

The substances that obtained True Positive results in the BCF-TMF comparison were: PCB congeners 52, 101, 118, 138, 153, 180, BDE congener 47, and α-HBCD. Substances with True Negative results were pyrene and benzo-a-pyrene. Certain substances were excluded from the BCF-TMF comparison because of unavailable BCF information from the database (Table 2).The following three sections contain a description of the different types of substances in this study with True Positive or True Negative results:

Polychlorinated Biphenyls

The biomagnification of lipophilic organochlorines (e.g. PCBs) is explained by the high K_{OW} and high K_{OA} properties, the general resistance to metabolic transformation, high gastrointestinal uptake rates, and low respiratory elimination rates in aquatic organisms (resulting in a BCF or BAF≥5000) and air-breathing organisms (resulting in a TMF>1)(Kelly et al., 2009). Current bioaccumulation understanding involves lipophilic

substances in an equilibrium environment where the higher the octanol-water partition coefficient (K_{OW}) the higher the tendency for a substance to accumulate in biota. This relationship generally holds true for lipophilic chemicals in an aquatic environment and explains why PCBs were adequately screened for bioaccumulation by the regulatory indicators that rely on the K_{OW} to measure a substance's bioaccumulative nature.

Polybrominated diphenyl ethers (congeners 47, 99, 100, 153, 209, γ –HBCD and α-HBCD)

BDE 47 and BDE 99 in the BCF-TMF relationship only, and PBDE 100, obtained true positive results and bioaccumulate and biomagnify due to chemical properties such as high lipophilicity (log K_{ow}: 5.9–10) (Braekevelt, Tittlemier, & Tomy, 2003; de Wit, 2002) and resistance to metabolism (Gustafsson, Björk, Burreau, & Gilek, 1999). The less brominated congeners (e.g. BDE 28 and 47) are known to be more bioaccumulative than their parent compounds due to metabolic debromination of higher congeners (i.e. BDE 209) into lower congener PBDEs resulting in higher concentrations of the lower substituted BDEs (i.e. BDE 47) in organisms relative to the aquatic environment and consequently results in BAF or BCF measurements above the current bioaccumulation screening standard of greater than or equal to 5000(Law et al., 2003; Shaw et al., 2008; Stapleton, Letcher, & Baker, 2004; Wan, Hu, Zhang, & An, 2008). The highly brominated congeners BDE 209 obtained True Negative results because of the metabolic debromination of these compounds into less brominated congeners (i.e. BDE 49) with increasing trophic level.

Benzo-a-pyrene and pyrene

Benzo-a-pyrene and pyrene had BAF and BCF values below 5000 and TMF values below 1. Polycyclic aromatic hydrocarbons are well known to metabolize by the inducible Cytochrome P450 pathway and aquatic organisms can metabolize these substances generally reducing the measured value in an organism resulting in a low BAF or BCF. PAHs are also known to biodilute, or decrease in concentration up a food-web, due to low assimilation efficiencies and efficient metabolic transformation at higher trophic levels, resulting in a TMF less than 1 (Wan, Jin, Hu, & Jin, 2007). Overall, benzo-a-pyrene is known to biodilute with increasing trophic level and the BCF was not deemed significantly different from the current screening criteria of 5000.

PFOS AND PFOSA

PFOS and PFOSA bioaccumulate and biomagnify in all BCF-BAF-TMF comparisons. Although lipid-based partitioning chemical characteristics are difficult to measure in a laboratory setting, predicted log K_{OW}s (log K_{OW}≥ 5) of the substances in this study may indicate the ability of PFOS and PFOSA to bioaccumulate in aquatic ecosystems. However, the difficulty in calculating the lipid-based partitioning constants in the lab result in increased reliance on empirical data to indicate the biomagnification of these substances. The relatively high amounts of PFOS in the seabirds in the food web may be due to an ability to biotransform and excrete other less persistent fluorinated substances (i.e. shorter chain fluorinated substances) (Haukås, Berger, Hop, Gulliksen, & Gabrielsen, 2007; Kelly et al., 2009; Walker & Livingstone, 1992) .The biotransformation of PFOSA and other PFOS precursors may also influence the increased concentration of PFOS in estuarine organisms by increasing the relative amount of PFOS (Tomy, Budakowski, et al., 2004; Tomy, Tittlemier, et al., 2004)

3.4.2. False Positive and False Negative Results

Exceptions to the general agreement between the current definition of a bioaccumulative substance (BCF or BAF≥5000) and the definition of a biomagnifying substance (TMF>1) do exist for the substances studied. Substances studied in both the BAF-TMF and BCF-TMF comparisons obtained False Negative and/or False Positive results.

False Positive and False Negative results have implications for the effectiveness of the current measures used to indicate a bioaccumulative substance as defined by the Canadian Environmental Protection Act. False Positive result may be costly from an environmental perspective because a non-biomagnifying substance is classified as biomagnifying and therefore removed from commerce due to its mis-classification. The mistake may result in lost revenues for the distributor or the chemical may be important to society yet it is banned from production. A False Negative result may also be costly to environmental and human health. Biomagnifying substances are classified as non-bioaccumulative and as a result are released into the environment potentially posing a threat to upper trophic level organisms (Fisk et al., 2005; Letcher, Norstrom, & Bergman,

1995). The PFCAs, however, remain near the boundaries between the True Positive and True Negative results and the False Negative and False Positive results. The proximity of these substances to the boundaries may suggest that even in aquatic food webs the BCF and BAF are inadequate or inappropriate bioaccumulation screening tools for the perfluorinated substances.

BAF-TMF Comparison

No substances tested in this study obtained False Positive results in the BAF-TMF comparison, however, some substances obtained False Negative results. Select fluorinated substances PFDA and PFOA, polybrominated diphenyl ethers congeners 153 and 154+BB153, and hexachlorobenzene were defined as not bioaccumulative according to current regulatory criteria (BAF <5000) yet show evidence of biomagnification (TMF>1). Two substances are classified as True Positive substances yet fall significantly close to the False Negative results; PFOS and BDE congener 28 are found near the BCF boundary of 5000 and have TMFs above one and despite the geometric mean of the BCFs remaining outside the False Negative result the substances may have potential to be inadequately characterized as bioaccumulative.

BCF-TMF Comparison

γ-HBCD and benzo-a-pyrene obtained False Positive results in the BCF-TMF comparison. γ-HBCD obtained a False Positive result. BDE 209, PFOA, PFDA, and hexachlorobenzene obtained False Negative results.

The following two sections describe the substances that generally obtained False Positive or False Negative results in the BAF-TMF and BCF-TMF relationships . Overall, substances in this study with False Positive or False Negative results may have other mechanisms influencing bioaccumulation not captured in current bioaccumulation screening criteria (e.g. BCF or BAF ≥5000). For example, these substances may not partition easily into the air phase (e.g. high log K_{OA} or protein partitioning) and/or ii) are ionizable.

Polybrominated diphenyl ethers (congener 28, 99, 153, 153 +BB54, 209, γ-HBCD)

The disparity between the results of the PBDE congeners, i.e. some with True Positive and True Negative results and others with False Negative results, may be due to the biotransformation of higher brominated congeners into lower brominated congeners over time and subsequently up the food chain. More specifically, when the higher brominated substances biotransform into the lower brominated substances, the lower brominated congeners are the available substance for uptake by organisms and over time accumulate in biota and upper trophic levels. When looking at concentrations of the higher congener PBDEs in organisms up a food- chain, high congener PBDEs actually decrease in concentration with increasing trophic level.

BDE 99 may obtain a True Positive result in the BAF-TMF comparison because air-breathing organisms are present in the tested food web; BDE 99 is known to debrominate in the intestines of fish (Mizukawa et al., 2013; Stapleton et al., 2004) however few studies have tested the debromination of BDE 99 in avian species such as the common tern in this study. As a result, BDE 99 may not be biotransformed in the common tern as it is in the upper trophic level aquatic species, resulting in an accumulation of BDE 99 with increasing trophic level producing a trophic magnification factor above 1.

PFDA and PFOA

Based on BCF or BAF values used in current bioaccumulation criteria, some fluorinated substances are classified as 'not biomagnifying', but in the field, specifically in air-breathing food webs, these chemicals have high potential for food web magnification obtaining a false negative result. The False Negative results may occur because bioaccumulation of fluorinated compounds may not be captured in aquatic-based indicators such as BCF or BAF. Fluorinated substances may require an indicator that captures bioaccumulation mechanisms in air-breathing food webs to adequately be assessed for biomagnification.

PFOA is also characterized as "low K_{OW} and high K_{OA}" substances and are known to biomagnify in air-breathing food webs (Kelly et al., 2007). Kelly *et al.* (2007) also reported that a large number of chemicals (ca. 4000) currently in use in Canada can

be classified as “low K_{OW} − high K_{OA}” and may also be at risk of improper screening for biomagnification.

Additionally, hydrophobic substances with lower K_{OW} values, e.g. from log K_{OW}~5 to 2, that are poorly metabolized and have high octanol-air partition coefficients (K_{OA}) may accumulate in upper trophic level organisms resulting in a TMF greater than 1(Kelly et al., 2007). As a result of low K_{OW}, high K_{OA} biomagnification occurs in food webs with air-breathing animals, but do not tend to magnify in water-respiring animals, although we do see evidence of biomagnification in aquatic organisms for certain substances such as PFOS. Air-breathing organisms have a low rate of respiratory elimination to air for these chemicals (high K_{OA}) resulting in a TMF greater than 1.

Further complicating the bioaccumulation of fluorinated substances is the ionizing capability of these compounds. Arnot and Gobas (Arnot & Gobas, 2006) called for further research on the bioaccumulation of ionizable substances due to their high production volume and likeliness to enter the environment. For example, 33% of ionizable substances registered under REACh ionize and dissociate at pH of 7 and 77% of pharmaceuticals are ionizing substances(Franco, Ferranti, Davidsen, & Trapp, 2010; Rendal, Kusk, & Trapp, 2011) . Bioaccumulation indicators of ionizing substances, such as the BCF, are sensitive to pH, log D (sum of log K_{OW} of neutral and ionic molecule), dissociation, the pH-dependent ion trap, and the electrical attraction of cations (Fu, Franco, & Trapp, 2009), none of which are incorporated in conventional bioaccumulation screening tests.

Current regulatory bioaccumulation criteria do not incorporate protein-water partitioning for aquatic organisms or protein-air partitioning for terrestrial organisms and these partition coefficients may be necessary to adequately identify fluorinated substances as bioaccumulative. Chemical-class specific bioaccumulation indicators may reduce the False Negative and False Negative results for ionizable and non-lipid partitioning substances such as the fluorinated substances in this study.

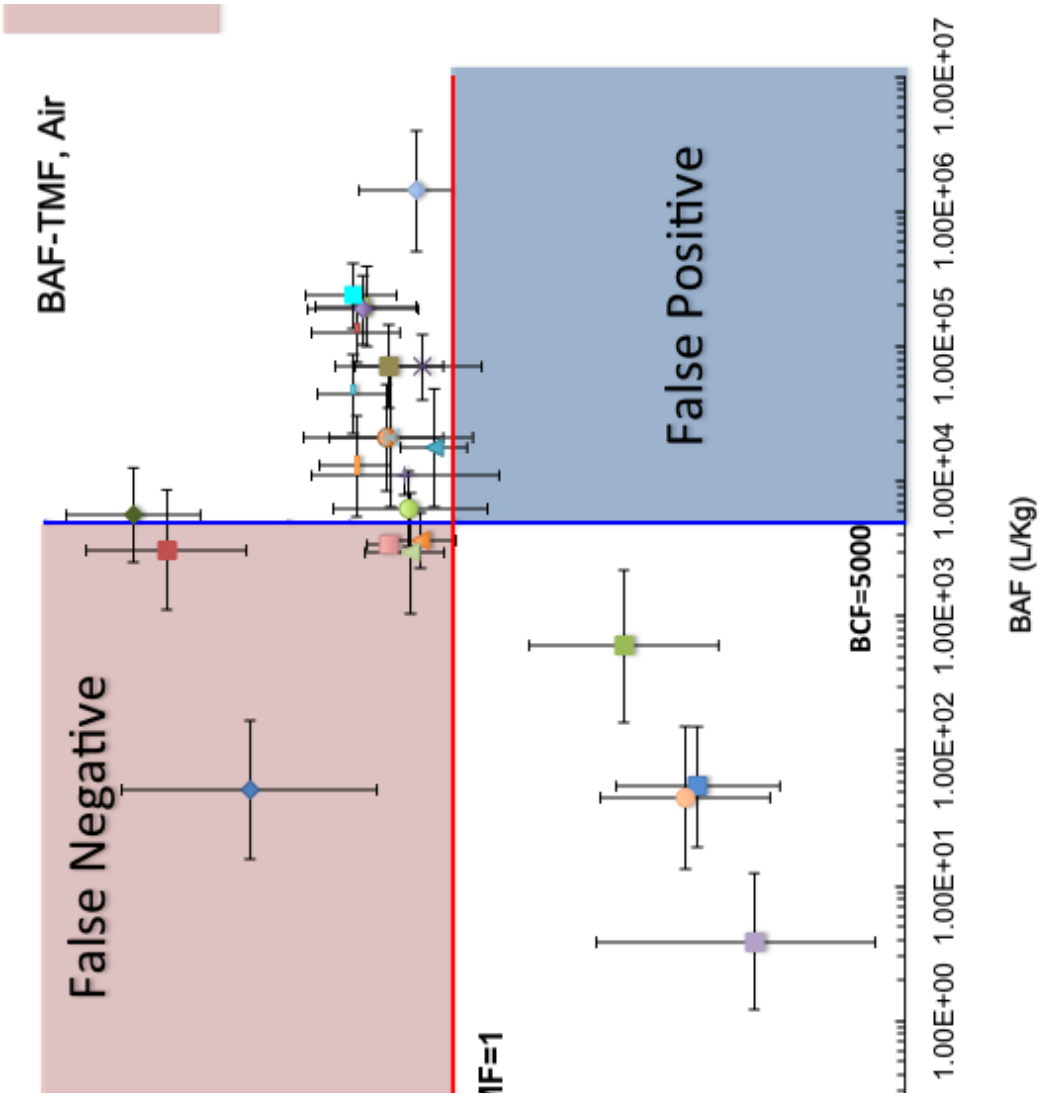

Figure 3.8. ***The Trophic Magnification Factor (TMF) as a function of the Bioaccumulation Factor (BAF), in a benthic-pelagic estuarine ecosystem including an air breathing organism. Most substances that are defined as bioaccumulative also biomagnify (i.e. BAF≥5000 and TMF>1). False Negative and False Positive results do occur with substances such as PFOS. Error bars are 95% confidence intervals***

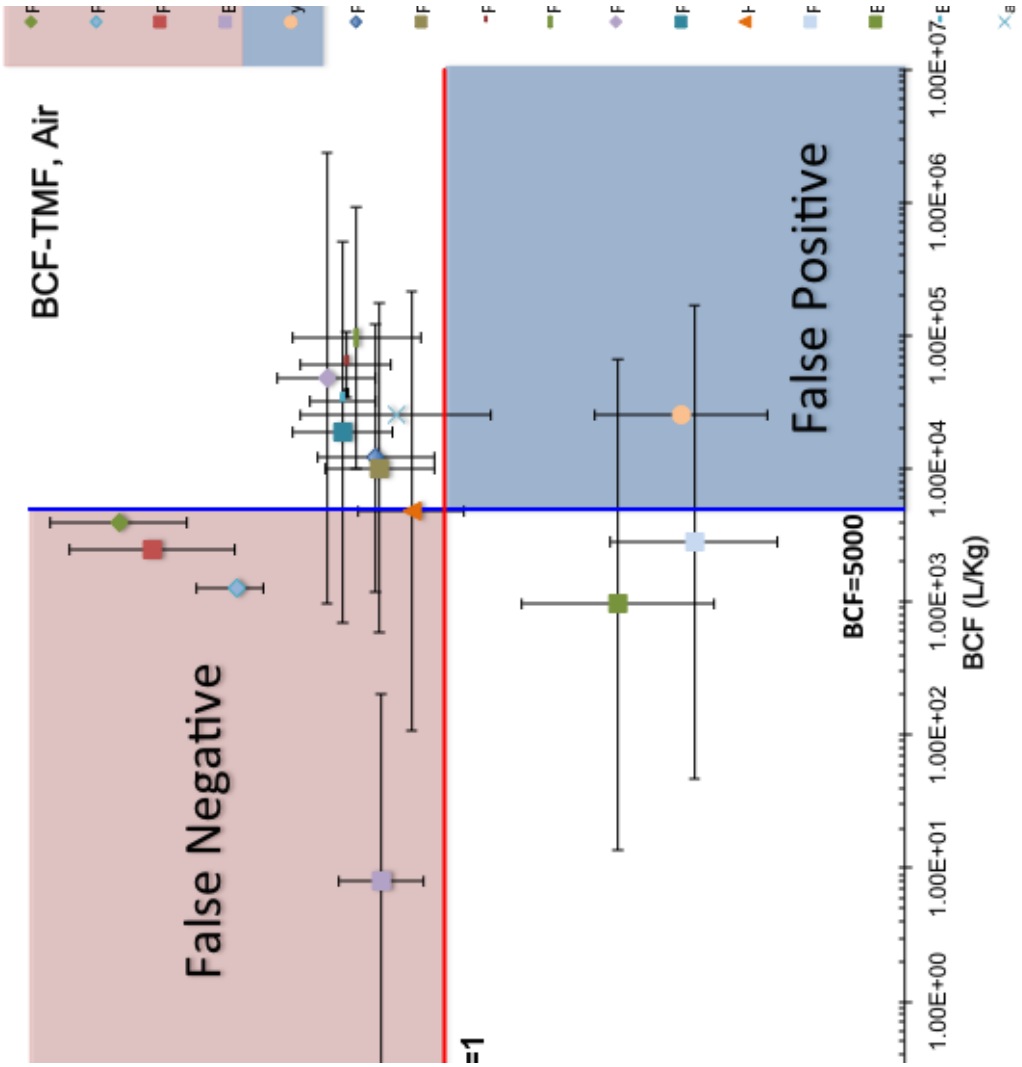

Figure 3.9. ***The Trophic Magnification Factor (TMF) as a function of the Bioaccumulation Factor (BAF), in a benthic-pelagic estuarine ecosystem including an air breathing organism. Most substances that are defined as bioaccumulative also biomagnify (i.e. BAF≥5000 and TMF>1). False Negative and False Positive results do occur with substances such as PFOS. Error bars are 95% confidence intervals.***

4. Conclusions and Recommendations

Overall, most hydrophobic, biotransformed substances measured in the Western Scheldt estuarine food web including air breathing organisms and aquatic breathing bioaccumulate according to the current definition of a bioaccumulative substance and also biomagnify. However some substances are listed as bioaccumulative because they meet the bioaccumulation criteria but do not show evidence of biomagnifying in a food web. In this study we explored the relationship between bioaccumulative and biomagnifying substances and find general agreement between the two, however exceptions to the agreement between a bioaccumulative and biomagnifying substance do exist.

The substances with the False Positive and False Negative results suggest that bioaccumulation may involve other mechanisms not included in current regulatory criteria of bioaccumulation such as protein-water partitioning, protein-air partitioning, and trophic transfer via diet. To reduce the False Positive and False Negative results and to include alternate bioaccumulation mechanisms while screening for a bioaccumulative substance, we suggest a change to the current definition of a bioaccumulative substance. Currently a bioaccumulative substance is one which obtains a BCF or BAF≥5000 or a log K_{OW}≥5, however we suggest a bioaccumulative substance is a substance that biomagnifies in a food chain. Biomagnification may be indicated by a trophic magnification factor or biomagnification factor greater than 1. Modifying the definition of a bioaccumulative substance would reduce False Positive and False Negative results without having to rely on the relationship between the bioconcentration, bioaccumulation factor, and trophic magnification factor.

Many bioaccumulation specialists already agree that a substance is bioaccumulative if it biomagnifies in a food web. The modified definition broadens the scope of the current definition of a bioaccumulative substance because it includes mechanisms of bioaccumulation such as trophic transfer and biotransformation and for certain substances reduces the reliance on the irrelevant criteria of a BCF or BAF. I also

suggest chemical-class specific bioaccumulation criteria where substances are classified into groups with appropriate bioaccumulation screening and evaluation methodologies for the respective chemical characteristic. Chemical-class appropriate screening may streamline the bioaccumulation screening process and reduce False Positive and False Negative results. An example of a chemical-class specific screening would be using protein partitioning for substances such as the perfluorinated compounds because the current lipid-partitioning indicators are irrelevant for the bioaccumulative nature of these substances.

The current study explores new ideas for further research in the study of bioaccumulation screening criteria. Field studies are required and are important to validate the laboratory and model-predicted bioaccumulation indicators used in many bioaccumulation screening decisions. Field studies can provide assurance that what is observed in the lab is also occurring in the field, or field studies can demonstrate differences between field results and lab results. Lab studies continue to be relevant and contribute information to the field studies and also help bioaccumulation scientists understand real-life issues such as the debromination of highly brominated BDEs in the intestines of carp. The field study identified a decrease of BDE 209 in upper trophic level organisms and lab studies identified the reason why. Increased research on the complex relationship between current bioaccumulation screening criteria and other indicators such as the TMF is important to support regulators in making informed decisions about the bioaccumulative nature of a substance in a regulatory setting. I also suggest more research further defining the difference between the two methods to determine the trophic magnification factor.

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Appendices

Appendix A. Concentrations of substances measured in organisms

Appendix B. Bioconcentration factors (L/Kg) from an Arnot and Gobas (2007) database