# A FINE KETTLE OF FISH: MARINE FISH CONSUMPTION, ENDOCRINE DISRUPTING CHEMICALS AND THYROID HORMONES IN RURAL NEWFOUNDLAND

by © Nicole Allen Babichuk (Thesis)

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## Abstract

# A FINE KETTLE OF FISH: MARINE FISH CONSUMPTION, ENDOCRINE DISRUPTING CHEMICALS AND THYROID HORMONES IN RURAL NEWFOUNDLAND

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Endocrine disrupting chemicals (EDCs), found in the environment, can cause hypothyroidism, infertility, and neurodevelopmental deficits. EDCs can be transported long distances on water currents and can bioaccumulate in marine food webs. Two water currents around Newfoundland are suspected of transporting EDCs to adjacent marine ecosystems. Newfoundlanders may be exposed to EDCs through consumption of local seafood contaminated with EDCs.

This study investigated EDC exposure in rural Newfoundland population by 1) testing local seafood species for the presence of EDCs, 2) determining the extent of local seafood consumption by surveying residents of two rural communities (Burin and New-Wes-Valley), 3) measuring serum thyroid hormones and plasma EDC concentrations in individuals who participated in the seafood consumption survey, and 4) exploring associations between seafood consumption, EDCs and thyroid hormones in participants.

Liver samples from cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) showed the presence of polybrominated diphenyl ethers (PBDEs), which were commonly used as flame retardants. There were higher levels of PBDEs in fish samples from the west coast compared to those from the northeast coast of Newfoundland. Residents from the study communities consumed local cod more than any other species, and seafood consumption was higher in males than in females; and higher in older (> 50 years) than in younger (<50 years) participants. Increasing frequency of local cod consumption was positively associated with increasing PCB (-105, -118, -138, -170, -180 and ∑PCBs) and p,p'-DDE concentrations in participants. Therefore local cod consumption may be a source of exposure to these EDCs for rural Newfoundlanders.

All participants had at least 11 EDCs present in their plasma; polychlorinated biphenyl (PCB), polybrominated biphenyl (PBB) and dichlorodiphenyldichloroethylene (p,p'-DDE) concentrations were higher in older participants, reflecting exposure to EDCs that have since been discontinued; while PBDEs were higher in younger participants which aligns with their recent production and use. Participants from Burin had higher levels of PCBs and p,p'-DDE (legacy contaminants from Labrador current) while NWV participants had higher levels of PBB-153 and PBDEs (novel contaminants from St. Lawrence River). In conclusion, there is evidence that the rural Newfoundland population is exposed to EDCs through local seafood consumption.

# Acknowledgments

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Finally, thank you to my parents (Lorna and Bill), my sister (Marina) and my husband (James) for your patience, support, and love.

## Co-authorship Statement

A portion of the work in this thesis was done in collaboration with Dr. Cora Young (currently in the Department of Chemistry, York University and with an adjunct position in the Department of Chemistry, Memorial University) and Dr. Joseph Bautista (former PhD student, Department of Chemistry, Memorial University). All aspects of the fish (cod and turbot) liver extraction and analysis were done in collaboration with Drs. Young and Bautista due to their expertise in analytical chemistry, and our shared interests in environmental contamination in Newfoundland marine species. The laboratory work was shared between myself and Dr. Bautista to facilitate my hands-on learning and training in analytical chemistry techniques. The statistical analysis of fish liver data was done by myself, independent of Drs. Young and Bautista, as we had different research goals for the data. Both Drs. Young and Bautista reviewed and approved the fish liver methodology and results sections of this thesis prior to submission. All intellectual property from the fish liver results rest with Drs. Young and Bautista, with the exception of presenting this work in my thesis. Dr. Bautista's thesis presents the same data with different analyses, results and conclusions based on his research goals, and his defence was completed in November 2019.

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# List of Abbreviations

EDC – Endocrine disrupting chemical

POP – Persistent organic pollutant

PBDE – Polybrominated diphenyl ether

PCB – Polychlorinated biphenyl

PBB – Polybrominated biphenyl

p,p'-DDE – Dichlorodiphenyldichloroethylene

TH – Thyroid hormone

TSH – Thyroid stimulating hormone

$FT_3$ – Free triiodothyronine

$FT_4$ – Free thyroxine

TTR – Transthyretin

TBG – Thyroid binding globulin

NL – Newfoundland

GSL – Gulf of St. Lawrence

SLR – St. Lawrence River

LC – Labrador current

# Chapter 1 Introduction

I will begin this thesis (chapter one), with a brief introduction and exploration of the topic leading up to the hypothesis and objectives of the study. The second chapter will delve into a more thorough literature review of the chemical contaminants, functioning of the thyroid system, methods and effects of exposure, environmental policy and management, and finally the history, geographical location, and social elements that make Newfoundland a unique place to study this exposure and the health outcomes. Next (chapter three) will be a detailed methodology of how this study was conducted, followed by the results (chapter four), and an interpretation of what they tell us in the context of environment contamination in Newfoundland (chapter five). Finally, there will be a conclusion to bring together all the findings of this study (chapter six).

This study focuses on persistent organic pollutants (POPs), organic compounds that require a long time to degrade and thus remain in the environment for lengthy periods of time. These carbon-based chemicals vary in structure; some POPs have aromatic carbon rings with varying degrees of molecular attachments, leading to many different congeners of the same compound that are classified based on their number and arrangement of aromatic moieties. All POPs are defined by 4 common, shared properties (Stockholm Convention, 2008b); 1) they have a long half-life (months or years depending on the congener) and persist in the environment for extensive periods of time, 2) they can undergo long-range transport, 3) they bioaccumulate and biomagnify in organisms, and 4) they have harmful health effects.

Many chemicals classified as POPs are manufactured for a specific use; they are released into the environment through manufacturing, application, use, and/or discarding processes, while others are produced as by-products of industrial practises or waste incineration (Gore et al.,

2015). From an environmental health perspective, POPs present a long-term public health hazard; their extensive distribution combined with slow degradation rates make them an ongoing threat to the health of humans and other organisms long after production and use of these chemicals has ceased. Some POPs can affect the endocrine system, and are thus also classified as endocrine disrupting chemicals (EDCs) (Gore et al., 2015). A small number are categorized as both POPs and EDCs, (Figure 1.1), namely, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyldichloroethylene (p,p'-DDE). These four chemicals are the main focus of this thesis, although there is a greater emphasis on PBDEs as it was possible to test for these contaminants in local fish; in human plasma, testing was possible on all four of these EDCs.

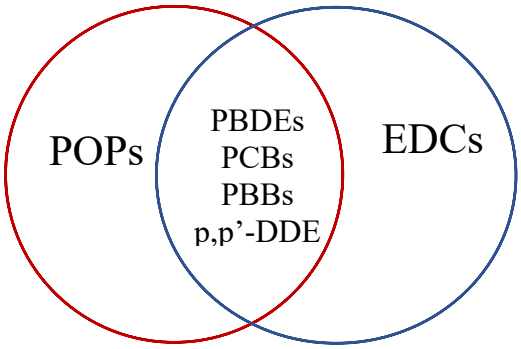



**Figure 1.1** The relationship between POPs and EDCs, with PBDEs, PCBs, PBBs and p,p'-DDE falling under both categories.

EDCs are exogenous substances that can cause adverse health effects through disruption to the body's endocrine systems. This can result in changes to circulating hormone concentrations, which act as signals to regulate growth, metabolism and reproduction. Hormones are maintained at very low concentrations in the blood stream; therefore, even low levels of EDC exposure can have large consequences on endocrine homeostasis (Crofton, 2008). The US

Environmental Protection Agency (USEPA) has identified potential adverse health effects resulting from exposure to EDCs, including developmental abnormalities, endocrine disruption, disorders of the reproductive systems in men and women, increased incidence of cancer, and alterations to both the immune and nervous systems (Kavlock et al., 1996). The bioaccumulative nature and slow degradation rates of EDCs means that organisms have continuous low-level exposure, and thus long-term endocrine disruption.

The thyroid system is arguably one of the most important systems in the body, and is responsible for maintaining growth, development, metabolism and temperature in humans. It is also a frequent target of EDCs. The most frequent consequences of thyroid disruption are changes in the serum concentrations of the major thyroid hormones including thyroid stimulating hormone (TSH), triiodothyronine ($T_3$), and thyroxine ($T_4$). The thyroid gland is regulated through a negative feedback system, therefore, changes in the circulating levels of thyroid hormones will cause a change in production of these hormones in the thyroid gland (Crofton, 2008).

PBDEs are most commonly used as flame retardants on indoor furnishings, electronics, textiles, and building materials (Rahman et al., 2001). PBDEs are classified as EDCs because they have been found to interact with the thyroid system, and can disrupt thyroid system homeostasis at multiple points along this hormone signaling pathway (Crofton, 2008). In addition, the metabolic products of PBDEs such as hydroxyl (OH)-PBDEs have also been shown to disrupt thyroid hormone signaling pathways (Li et al., 2010). PBDEs can be found throughout the marine food chain in species such as fish (Eljarrat and Barcelo, 2018), birds (Mo et al., 2018), and mammals (Bartalini et al., 2019), as seen in recent publications.

PBDEs, PCBs, PBBs and dichlorodiphenyltrichloroethane (DDT; the parent compound of p,p'-DDE) are all banned in Canada, however the legacy of their use can still be felt today. A study of Canadian women, sampled between 2007 and 2009, found that higher plasma concentrations of ∑PBDEs (20.5 ng/g) were associated with increased prevalence of hypothyroidism in the study group (Oulhote et al., 2016). While this does indicate that exposure to these chemicals could have affected the thyroid status of this sub-population during this time period, it is not possible to extrapolate this trend to the population level in Canada, as Canadians will have different levels of exposure depending on a variety of factors including age, sex, gender, location, diet, occupation, income, housing, etc. Another study that measured PBDE concentrations in Inuit children from Northern Quebec during the same time period (2006-2010), reported a downward trend (9.3-14.3% per year) in serum EDC concentrations (Turgeon O'Brien, 2019), indicating that exposure may be decreasing for some populations. Although there is evidence that PBDE concentrations are decreasing in the Canadian population, there is still a need to monitor these chemicals, to identify populations at risk and to pinpoint potential sources of exposure.

In Canada, PBDE contamination in the St. Lawrence River (SLR) has been studied by academic researchers (Houde et al., 2014; Gentes et al., 2012) and monitored by the Canadian government (Pelletier and Rondeau, 2013), as these chemicals can last for years in the environment after their production and use has ceased. The SLR basin is home to a large urban population as well as many industries, therefore there are many potential sources of EDC being released into the environment. Several types of PBDEs have been found in suspended particulates (∑PBDEs=50 ng/g) and sediments (∑PBDEs=26 ng/g) sampled along the river (Pelletier and Rondeau, 2013), and they are also found in organisms that are higher in the food

chain, such as birds (Champoux et al., 2017a; Champoux et al., 2017b) and belugas (Simond et al., 2017), indicating the bioaccumulation of these chemicals.

PBDEs can undergo long-range atmospheric transportation, meaning they are found throughout the world including places where there is minimal human habitation and no history of their production/use such as the Arctic. PBDE contamination has been observed in snow core samples from the Arctic (Meyer et al. 2011). When this contamination is released from the Arctic as ice and water, it may wash into the Labrador Current and travel down along the coast to the Island of Newfoundland. With an increasingly warming Arctic, EDCs that were trapped in ice may be released into the environment (Armitage et al., 2011).

The Island of Newfoundland has coastline that boarders the Atlantic Ocean and the Gulf of St. Lawrence (GSL). Contamination from upstream in the SLR and from the Labrador Current could make its way into the Newfoundland marine food chain and into species that humans would catch and consume on all three coasts of the island. Newfoundland has a long history of fishing and consuming seafood, and the province holds an annual recreational fishery for ground fish (i.e. cod) in the summer and fall, where Newfoundland residents do not require a license to catch fish for personal consumption.

Previous studies have shown residents prefer local over imported seafood, and that cod is the number one type of seafood consumed in the province (Lowitt, 2013). In high seafood-consuming countries like Norway, fish is the dominant source of PBDEs and other EDCs in the diet (Kiviranta et al., 2004). Additionally, data collected by Sarkar et al. (2015) on the number of people in Newfoundland hospitalized with hypothyroidism diagnosis per 100,000 population per year found that the south (96.3 per 100,000) and west (91.8 per 100,000) coasts of Newfoundland had much higher rates than the northeast coast (51.3 per 100,000). It is possible

that local fish consumption may be a source of EDC exposure in the Newfoundland population and that it could be contributing to higher hypothyroidism rates seen in some areas around the province, however no studies have yet explored these exposures or relationships in Newfoundland.

By taking a multipronged approach involving different methods to the study of environmental contamination, seafood consumption, and human health in Newfoundland, we were able to build a strong foundation for research on EDC exposure in the Newfoundland population via local seafood consumption. This is the first time this type of research approach has been taken in Newfoundland, therefore it is important to examine environmental sources, types and concentrations of EDCs in human samples, and thyroid effects in the population to truly expose the extent of this environmental health issue in the province.

The main objectives of this study are:

1. To explore contamination of PBDEs in fish from Newfoundland coastal waters.
2. To determine the species and frequency of local seafood consumption in rural Newfoundland residents (via seafood consumption questionnaire) from two coastal communities on different coasts of the island (Burin on the south coast, New-Wes-Valley on the northeast coast).
3. To test blood samples from rural residents who completed the seafood consumption questionnaire for thyroid hormone concentrations (TSH, free $T_4$, free $T_3$) and endocrine disruptors (PBDEs, PCBs, PBBs, p,p'-DDE).
4. To explore relationships between plasma EDC concentrations, thyroid hormones and local seafood consumption frequencies in rural Newfoundland residents.

It was important to explore PBDE contamination in fish to provide evidence of the presence of these contaminants in the local marine ecosystem, and to identify them as a potential source of PBDE exposure in rural Newfoundland residents. Finding PBDEs in rural resident's blood samples provides confirmation of human exposure to these chemicals, and conducting the seafood consumption questionnaire gives us a potential pathway for exploring the exposure in humans. Analyzing concentrations of EDCs and thyroid hormones in the same participant is an appropriate way to explore any association between their endocrine disrupting effects and thyroid hormone homeostasis.

The hypotheses of this study (corresponding to the objectives listed above) are:

a) There is contamination from PBDEs in marine fish from all coasts of Newfoundland, and there are variations in congener profiles by species and by location due to different sources of environmental pollution (St. Lawrence River versus Labrador Current versus open North Atlantic Ocean) to the four sampling locations.
b) Residents consume local seafood on regular basis and consumption frequencies are similar across study locations.
c) EDCs are present in blood samples from all participants, but geographical location affects their EDC profile.
d) There are higher EDC concentrations in participants that regularly consume local seafood, compared with those that do not regularly consume it, and there are relationships between EDCs and thyroid hormone concentrations.

In summary, this study explores the presence of environmental endocrine disruptors in marine species around the Island of Newfoundland, and the endocrine effects on the residents

that consume them. Environmental endocrine disruptors were examined in turbot (*Scophthalmus maximus*) and cod (*Gadus morhua*), which are top predators in the marine food chain and food species for humans. Human exposure to these environmental contaminants was explored by documenting self-reported frequency of local seafood consumption in rural residents of Newfoundland and analyzing blood samples for evidence of environmental endocrine exposure. Blood samples were also tested for thyroid hormone concentrations, to investigate the endocrine effects of exposure to these chemicals in rural Newfoundland residents.

# Chapter 2 Literature review

Human exposure to environmental EDCs and the resulting adverse health impacts are very complex topics, and therefore need to be addressed from a multidisciplinary perspective drawing upon the discourses of ecotoxicology, human behavior, and endocrinology. Many studies of EDCs extend across multiple subject fields, and thus collaboration between disciplines is essential to this research topic. EDCs, their persistence in the environment, human exposure and the resulting health effects have been reviewed in many books and articles from different fields, and this thesis draws upon many of those sources for information.

This literature review will begin a basic overview of the endocrine and thyroid systems, setting the context for the delicate homeostasis that this system maintains to preserve normal endocrine functioning. This will be followed by a discussion of EDCs, including defining characteristics, and then proceed into a more in-depth exploration of PBDEs, followed by the health effects and outcomes caused by PBDE, PCBs, PBBs and p,p'-DDE in humans, with a particular focus on thyroid disruption and hypothyroidism. Patterns of exposure will be discussed next, including the effects of age, sex, diet, ethnicity and lifestyle on exposure. Almost no human chemical exposure occurs in isolation from other types of chemicals, so we also discuss mixture effects and the difficulty this poses in determining specific health outcomes from exposures. Next, we discuss past and present legislation and policy of EDCs, and use this to look forward at what future EDC policies might entail. Finally, we discuss the history of Newfoundland and the socio-cultural factors that may put the population at risk of exposure to EDCs through seafood consumption. This literature review will set the context for this study, from analysis of fish liver PBDEs to human consumption patterns and corresponding plasma EDC and serum thyroid

hormone concentrations, and provide insight into how the results from this work can fill gaps in the knowledge about EDCs, particularly on the east coast of Canada.

## 2.1 The endocrine system

To understand the health effects that EDCs cause in humans, it is important to first understand the complexity of the endocrine system and the mechanisms by which it operates. The endocrine system evolved in vertebrates to control processes in the body via chemical messengers. The system itself is composed of a collection of organs situated throughout the body, each with different roles and in control of different biological processes. The chemicals secreted from endocrine glands are hormones, and they act as singaling molecules to initiate actions around the body (Hsiao and Gardner, 2011). There are many different types of hormones within the human body (e.g. proteins, steroids, amines, peptides). Peptides and proteins are the main types of hormones found througout the endocrine system, with the exception of the thyroid system which uses glycoproteins and amines (Molina, 2013). These hormones are stored as pre-hormones in granules in the cell, and are modified to form full hormones prior to being excreted from the cell; some hormones (such as thyroid stimulating hormone [TSH]) have additional attachments (e.g carbohydrate molecules on glygoproteins) that influence their actions and metabolism in the body (Molina, 2013).

Hormones can act locally or in distant parts of the body, and they travel through the blood stream or extracellular fluid to their target organ or tissue. The endocrine system has a very narrow set-point for regulation, and circulating levels of hormones are generally quite low ($10^{-7}$ to $10^{-12}$ mol/L; Molina, 2013). Hormones can travel freely in the circulation, or be bound by transport proteins for conveyance to a target site. Plasma hormone levels play an important role

in regulating biological actions, and this can be controlled through new hormone biosynthesis, metabolism, and binding to transport proteins in the plasma (Hsiao and Gardner, 2011). When hormone concentrations in the blood begin to fall, the appropriate endocrine gland adjusts the concentration by secreting more hormone, so the amount in circulation remains consistent and ready for function; these constant adjustments by the endocrine glands help maintain hormonal homeostasis in an organism (Molina, 2013).

Many hormones, such as those in the thyroid system, are only active and able to attach to tissue receptors when they are not bound by a carrier protein; the more tightly a carrier protein is bound to a hormone, the longer it will take for the protein to be cleared from the serum, therefore hormones that bind to transport proteins in blood (such as thyroxine), will last longer in the circulatory system than free hormones (Molina, 2013). Free hormone concentrations (such as free $T_3$ and free $T_4$) are unbound by transport proteins and therefore act as feedback signaling molecules regulating the endocrine system. Any changes in the concentrations of free hormones are indicative of disturbance taking place in the endocrine system, therefore measurements of free hormones in the serum can be useful in determining endocrine disruption.

Hormones exert their power through binding to specific high-affinity receptors on the cell surface or within the nucleus of the target cell (Hsiao and Gardner, 2011). When the hormone is bound to the receptor, it produces a biochemical change in the receptor that alters its structure and formation, activating the receptor. Once active, the receptor can cause a downstream signaling cascade that initiates the actions of the hormone on the target site. Hormones generally trigger one of two actions when they bind to a receptor (although some can act partially one way or another); agonists bind to and activate the receptor, initiating a biological response, while antagonists bind to the receptor, but do not initiate a downstream response therefore essentially

blocking the biological action of the receptor (Hsiao and Gardner, 2011). Agonists can alter signaling by initiating the transcription of specific genes, causing the production of mRNA (messenger RNA, the product of DNA transcription), which is translated into proteins, the end product of the signaling cascade.

### 2.2 The thyroid system

Many EDCs (including those that are the focus of this project) target the thyroid system, causing changes in circulating thyroid hormone levels. To enable a better understanding of how the thyroid system works and how EDCs disrupt this system, this literature review will include a thorough review of thyroid hormone biosynthesis.

The thyroid system is important for growth and development of vertebrates, and for maintaining normal biological functioning (heart rate, body temperature, blood pressure and metabolism) once the growth and development of an organism is complete. The human thyroid is located in the neck, with two joined lobes located on either side of the trachea. The lobes are comprised of follicles, made of cells that form a spherical shape filled with colloid fluid (Zoeller et al., 2007). The structure of the thyroid gland is richly vascularized, facilitating easy diffusion of thyroid hormones into the circulation. The thyroid gland begins formation early in human development (approximately 5-6 weeks' gestation), and becomes independently functional between 3-4 months of development in utero (Zoeller et al., 2007). Prior to this, the fetus is dependent on circulating concentrations of maternal thyroid hormones, therefore disruptions to these hormone levels (such as those caused by EDC exposure) could have potentially damaging effects on a developing fetus.

2.2.1 Thyroid hormone biosynthesis

There are two distinct thyroid hormones, thyroxine ($T_4$) and triiodothyronine ($T_3$). These amine hormones each have a slightly different configuration, but the basic structure of the molecules are similar. They consist of a base structure of two linked tyrosine molecules (amino acids) with three or four iodine molecules on their aromatic rings (Griffin and Ojeda, 2000). The position and number of the iodine molecules on the tyrosine determines its physiological activity and metabolism. $T_3$ has three iodines attached to its tyrosine rings, while $T_4$ has four iodines on its rings (Figure 2.1).

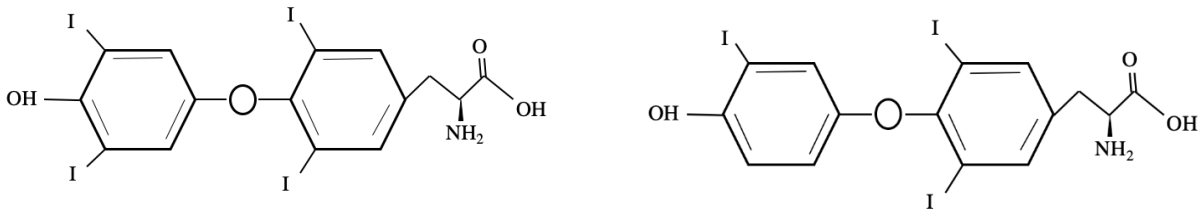



**Figure 2.1** Structure of thyroxine ($T_4$, left) and triiodothyronine ($T_3$, right).

Iodide is an essential component in the formation of $T_3$ and $T_4$ in the thyroid gland. The average human requires 150µg of iodide per day, and this intake can be acquired from many different food products including iodized salt, seafood, meat, dairy, and some vegetables and grain products (National Institutes of Health, 2019). The circulatory system and peripheral tissues contain portions of what is considered the body's iodide pool, with the largest amount (approximately 800µg) found in the thyroid gland (Griffin and Ojeda, 2000).

The main purpose of this pool of iodide is to act as a buffer, and to ensure normal thyroid hormone production and endocrine signaling continue should dietary sources of iodide decrease or cease for a short period of time (eventually this pool would be depleted). Iodide is taken up

into the follicular cell by the sodium/iodide ($Na^+$) symporter, and moves into the colloid of the follicle, where it is oxidized and covalently bound to thyroglobulin by thyroid peroxidase enzymes (see Figure 2.2). Iodination of iodine with thyroglobulin is followed by conjugation of the iodinated tyrosyl residues, before re-entering the cell by endocytosis (Griffin and Ojeda, 2000).

Thyroglobulin (Tg) is a protein produced in the thyroid gland, and used to form thyroxine ($T_4$) and triiodothyronine ($T_3$). In the colloid, Tg is iodinated at its tyrosine residues, and remains stored in the colloid until it is needed. Once needed, this pre-hormone is re-absorbed into the follicular cells in a colloid droplet. Enzymes fuse into the droplet to cleave the iodinated tyrosine residues from Tg, which are now $T_3$ and $T_4$, and the newly formed thyroid hormones diffuse out of the cell and into the circulation (Griffin and Ojeda, 2000). Once in the serum, the majority (75%) of $T_3$ and $T_4$ is bound by thyroid binding globulin (TBG), a serum transport protein, while 25% is bound to either transthyretin (TTR) or albumin (Refetoff, 2015). There is some free $T_3$ and $T_4$ in the serum, however it is a very small proportion (0.01%) of the total thyroid hormone concentration in circulation (Stockigt, 2001).

In a clinical setting, changes in free $T_3$ and $T_4$ are used to measure thyroid dysfunction as they are not affected by availability of binding proteins in the serum (Zoeller et al., 2007). Free $T_4$ has a half-life of 7 days in the serum, while the half-life of free $T_3$ is less than a day, but when these thyroid hormones are bound to transport proteins they have lower rates of metabolic clearance, and therefore a longer half-life in serum (Griffin and Ojeda, 2000).

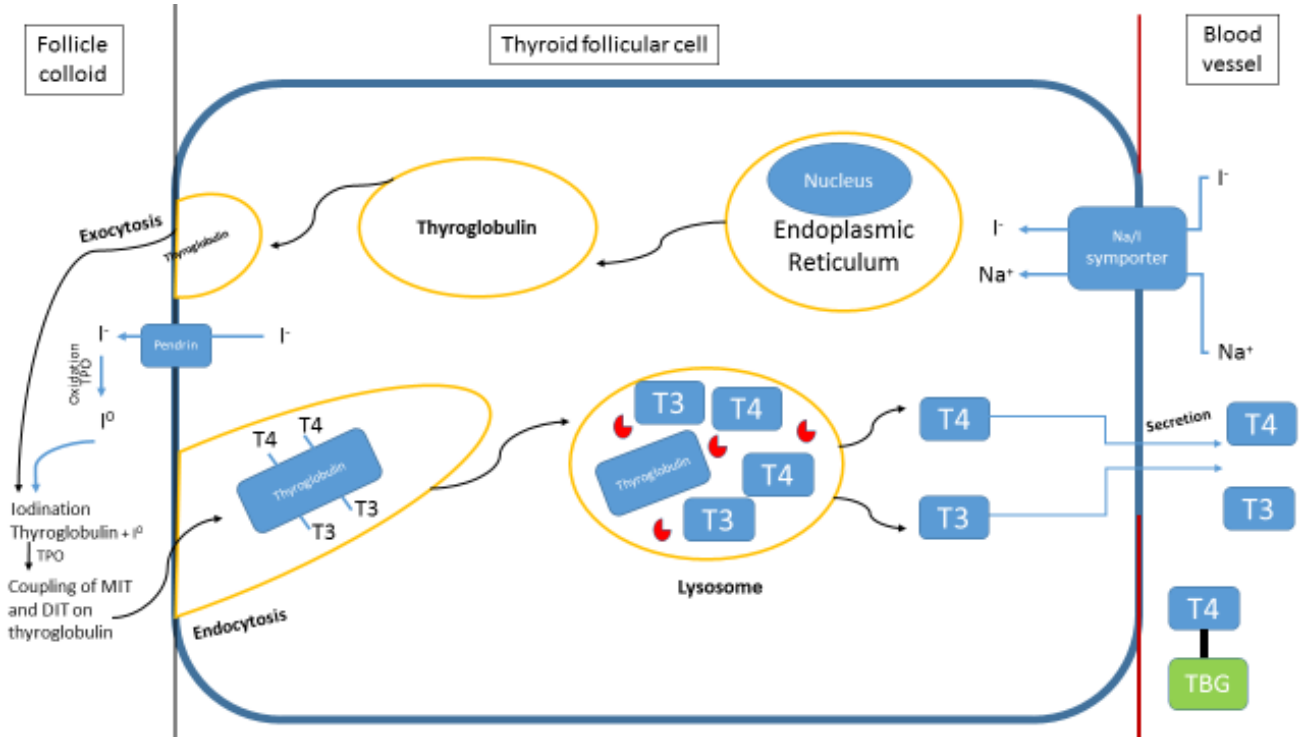



**Figure 2.2** Formation of thyroid hormones thyroxine ($T_3$) and triiodothyronine ($T_4$) in the thyroid follicular cell and follicle colloid, and the subsequent release into the circulation. TPO – thyroid peroxidase, MIT – monoiodotyrosine, DIT – diiodotyrosine.

### 2.2.2 Thyroid hormone release and circulation

The human thyroid gland is controlled by the hypothalamus-pituitary-thyroid (HPT) axis, and circulating thyroid hormones are kept within a very narrow range of 0.8-1.95 ng/dl (Griffin and Ojeda, 2000). The HPT axis controls the release of thyroid hormones based on an organism's physiological state, and depends on regular input signals from around the body to monitor the status of the organism. This input-response model is based on negative feedback; as thyroid hormone levels in the serum drop or as external signals indicate the need for more thyroid hormone, the system initiates increased hormone production and release from the thyroid gland, and if thyroid hormone concentrations in the serum are sufficient or too high, production and release from the thyroid gland is decreased (Greer et al., 1993).

When more thyroid hormone is needed, the HPT axis signals the release of thyrotropin-releasing hormone (TRH) from the hypothalamus. TRH secretion from the paraventricular nucleus of the hypothalamus acts on thyrotroph cells of the anterior pituitary, causing them to release thyroid stimulating hormone (TSH) into the circulation (Molina, 2013). Once TSH travels to the thyroid, it binds to receptors on the basolateral membrane of the thyroid follicular cells, and stimulates the formation of thyroid hormones in the follicle and their release into the circulation (Molina, 2013).

There are two loops in the negative feedback system controlling thyroid hormone release at the level of the pituitary and the hypothalamus (Figure 2.3); both loops are mediated by $T_3$ and $T_4$ binding to thyroid hormone receptors (TRs), which has been found to reduce transcription of TRH in the hypothalamus (Lechan and Kakucska, 2007) and TSH in the pituitary, as well as having other non-genomic effects (Storey at al., 2006). Because of this negative feedback loop, serum $FT_4$ concentrations are negatively related to serum TSH levels (Zoeller et al., 2007).

External stimuli such as cold exposure are also believed to influence this negative feedback system through increased production of phosphorylated cAMP response element-binding protein (CREB), which can bind to the promoter of TRH, causing upregulated TRH transcription and downstream thyroid hormone production (Lechan et al., 1986).

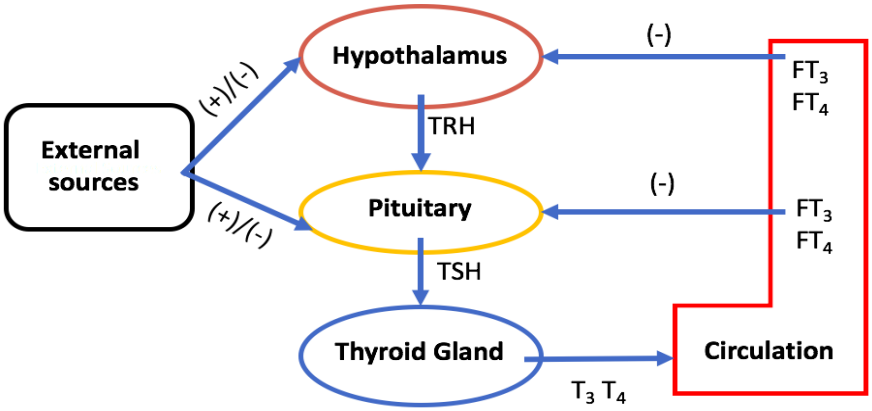



**Figure 2.3** Negative feedback loops of the thyroid system.

There is more $T_4$ than $T_3$ released by the thyroid gland; $T_4$ concentrations in the circulation are approximately 90nM while $T_3$ concentrations are several times lower at only 2nM (Molina, 2013). Although $T_4$ is the primary thyroid hormone produced by the thyroid gland, $T_3$ is 10 times more potent than $T_4$, and is the main active form of the hormone (Griffin and Ojeda, 2000). Approximately 80% of $T_4$ is eventually converted to $T_3$ by outer ring deiodination in peripheral tissues, where it acts on thyroid hormone receptors (Molina, 2013). When thyroid hormones reach their target tissue, they are released from their transport protein and brought into the cell by passive or active selective transporters (Zoeller et al., 2007). Once transported into the cell, $T_4$ is converted to $T_3$ by type I (D1) or type II (D2) deiodinase, before moving to the nucleus and binding to thyroid hormone receptors (Griffin and Ojeda, 2000). $T_3$ forms a complex with thyroid receptors and co-regulators, and attaches to the thyroid hormone response element on the

target DNA, leading to transcription and downstream physiological effects (Griffin and Ojeda, 2000).

### 2.2.3 Thyroid hormone metabolism

Thyroid hormone metabolism can occur via three different pathways; deiodination, glucuronidation, and sulfation (Kuiper et al., 2005). Deiodination of the inner rings of $T_3$ and $T_4$ occurs via types 1, 2 or 3 deiodinases (D1, D2 & D3 respectively). D1 (found mostly in liver and kidney) can convert $T_4$ to $T_3$, and $T_3$ to reverse $T_3$ ($rT_3$), a hormone with very little metabolic activity (Kuiper et al., 2005). The $rT_3$ can be further deiodinated to 3,5-diiodo-L-thyronine ($T_2$) by D1 and D2, where it becomes biologically inactive and is excreted from the body; D3 can also convert thyroid hormones to $rT_3$ and $T_2$, for excretion (Molina, 2013).

A second pathways to metabolize thyroid hormones is by conjugation of the phenolic hydroxyl group, which is done by sulfate or glucuronic acid (Peeters and Visser, 2000). This is considered a phase II detoxification reaction, and it is important for increasing the water-solubility of thyroid hormones so that they can be excreted in bile or urine (Peeters et al., 2001). Glucuronidation is catalyzed by UDP-glucoronyl transferases (UGTs), and sulfation is catalyzed by sulfotransferases (SULTs); these enzymes are important for the metabolism of thyroid hormones, and for removing the phenolic hydroxyl group from thyroid hormones (Peeters et al., 2001). This can facilitate the deiodination of thyroid hormones by D1, marking the beginning of an irreversible degradation pathway (van de Spek et al., 2017). Thyroid hormones can be metabolized in many tissues in the body including heart, muscle, brain, and kidney, with the liver being the most important organ for metabolizing thyroid hormone from the body and for recovering iodine from the breakdown products (van der Spek et al., 2017). Thyroid hormone

metabolism is susceptible to change from outside influences (such as EDCs), as will be discussed later.

2.2.4 Hypothyroidism and other thyroid disorders

Thyroid system disruption can result in negative health effects, due to over or under-secretion of thyroid hormones. Hypothyroidism (low thyroid hormone and/or underactive thyroid gland), can be caused by two different circumstances (Table 2.1).

**Table 2.1** Conditions affecting the thyroid system (Molina, 2013).

| Thyroid condition | Cause | Thyroid hormones |
|---|---|---|
| Primary hypothyroidism | Injury/disease affecting thyroid gland, overproduction of TSH | TSH levels ↑<br>$T_4$ and $T_3$ levels ↓ |
| Secondary hypothyroidism | Injury/disease to hypothalamus/pituitary, insufficient TRH from hypothalamus or insufficient TSH from pituitary | TSH levels ↓<br>$T_4$ and $T_3$ levels ↓ |
| Hyperthyroidism | Autoimmune (Graves') disease | TSH levels ↓<br>$T_4$ and $T_3$ levels ↑ |

Overt primary hypothyroidism is due to disease or damage of the thyroid gland and is characterized by low $FT_4$ and high TSH in serum (both relative to reference ranges of the population and testing lab). Subclinical primary hypothyroidism occurs if TSH is higher than the reference range, but $FT_4$ is within normal limits (Chakera et al., 2012), and this can be caused by

autoimmune disease, surgery, or radiation damage (Molina, 2013), or as research now suggests, by exposure to EDCs. Secondary or central hypothyroidism is associated with damage to the pituitary (secondary hypothyroidism), or the hypothalamus (tertiary hypothyroidism) and can present as lower $FT_4$ and low or normal TSH (Kostoglou-Athanassiou and Ntalles, 2010).

Hyperthyroidism is the opposite condition, and is characterized by excess levels of thyroid hormone secreted by the thyroid gland (De Leo et al., 2016). This condition can be determined through measuring thyroid hormone concentrations; overt hyperthyroidism is presented by low serum TSH levels and high serum $FT_4$, while the subclinical classification of hyperthyroidism is determined by lower than reference serum TSH, but in this case $FT_4$ can fall within normal range (De Leo et al., 2016).

Both hypo and hyperthyroidism cause a range of symptoms which can often be difficult to diagnose, which is why serum $FT_4$ and TSH testing is the definitive method for determining prognosis and treatment. The normal TSH concentration in the population varies from 0.4-4.5mlU/L, however, TSH in the general population has a right-skewed distribution, as more than 95% of people have a TSH concentration of less than 2.5mlU/L (Griffin and Ojeda, 2000). There is some debate in the medical community over whether the normal TSH concentration limit should be lowered from 4.5 to 2.5mlU/L (see Wartofsky and Dickey, 2005 and Surks et al., 2005 for opposing views on the topic). For this thesis, we used the lab reference range to determine normal/abnormal thyroid hormone levels.

The majority of the population displays a simple log-linear relationship between TSH and $FT_4$ concentrations in the serum (Rothacker et al., 2016). As small changes occur in serum $FT_4$ concentration, they elicit proportionally larger changes in TSH concentration. Individuals with hypothyroidism can still have TSH values that fall within normal range, therefore TSH must be

measured in conjunction with $FT_4$. Individuals exhibiting symptoms of hypothyroidism frequently have higher than normal TSH levels, and serum $FT_4$ concentration below normal (Vaidya and Pearce, 2008). Subclinical hypothyroidism is found at a much higher rate (3-12%) than clinical hypothyroidism (1-6%) in the adult population (Kim and Park, 2014). While definitions of subclinical hypothyroidism are less definite than clinical hypothyroidism, this condition typically presents as asymptomatic with abnormal serum TSH concentration, but normal $FT_4$ concentration (Kim and Park, 2014). EDCs are also linked to subclinical hypothyroidism; the odds of developing subclinical hypothyroidism were found to be elevated in pregnant females with serum PBDE concentrations in the highest quartiles (Chevrier et al., 2010).

Hypothyroidism can have many health effects, and depending on life stage of an individual there can be different health outcomes. Cretinism, also known as congenital iodine deficiency, is usually seen in infants exposed to low thyroid hormones, and can result in mental and physiological delays in development, and slow the progress of growth and age-appropriate milestones (Griffin and Ojeda, 2000). Hypothyroidism in adults has many symptoms that develop slowly; symptoms can range from common complaints such as exhaustion, depression, weight gain, and constipation, to more serious complications including bradycardia, pericardial effusion, and impaired consciousness (Vaidya and Pearce, 2008). EDC exposure may also directly or indirectly contribute to the disruption of normal thyroid hormone functioning, including the production, release, transport, signaling and metabolism of thyroid hormones, leading to negative health consequences.

## 2.3 Endocrine disrupting chemicals (EDCs)

EDCs are chemicals that disrupt normal endocrine functioning, including altering hormone concentrations. Often EDCs act on more than one endocrine system (estrogenic, androgenic, and/or thyroid); this can include mimicking or antagonizing existing hormones, altering hormone metabolism, competing for binding to transport proteins or receptors, and/or modifying hormone synthesis (Table 2.2).

**Table 2.2** List of chemicals which can be classified as both EDCs and POPs which are under investigation in this study. TH – thyroid hormone; ER – estrogen receptor

| Chemical | Source | Endocrine disrupting effects |
|---|---|---|
| Polybrominated diphenyl ethers (PBDEs) | Flame retardant, industrial processes, consumer goods | TH disruption (Legler and Brouwer, 2003), anti-androgenic and anti-estrogenic effects (Hamers at al., 2006). |
| Polychlorinated biphenyls (PCBs) | Transformers, electrical equipment | TH disruption (Brouwer et al., 1989), estrogenic and androgenic disruption (Bonefeld-Jørgensen et al., 2001) |
| Dichlorodiphenyltrichloroethane (DDT) Dichlorodiphenyldichloroethylene (p,p'-DDE) | Insecticide | Estrogen receptor (ER) agonist (Thomas and Dong, 2006), TH disruption (Meeker et al., 2007) |
| Polybrominated biphenyls (PBBs) | Flame retardant, consumer goods | TH disruption (Bahn et al., 1980) |

EDCs can cause disruption of endocrine system functioning and can result in adverse health outcomes; because of the narrow set-point for hormones in the endocrine system, even the smallest disruption in endocrine homeostasis can have negative health consequences. The most recent scientific statement from the Endocrine Society lists health outcomes that medical research has now attributed to EDC exposure to include (but not limited to): obesity, diabetes, impaired reproductive function, cancer, thyroid disruption and neurodevelopmental/neuroendocrine effects (Gore et al., 2015).

Most EDCs are manufactured intentionally or as byproducts of human industrial activities, and escape into the environment where they can disperse over long distances. They can be found in the household environment, shedding from consumer products into dust, and are also present in many personal care products, food items, and water. EDCs have been in use for many decades, however they began to garner widespread attention from the scientific community in the late 1950's. The extensive use of the chemical dichlorodiphenyltrichloroethane (DDT) on crops from the 1940s onwards culminated in the publication of Rachel Carson's "Silent Spring" in 1962, bringing EDCs into the public eye. This continued with "Our Stolen Future" by Colborn et al., which projected about the future reproductive capability and intelligence of our population in the face of environmental EDC contamination (Colborn et al., 1996). Since this time, EDCs have been a controversial issue in environmental policy and health, and their widespread use has led to worldwide environmental contamination. Global efforts are now being made to curb the production and use of these types of chemicals, however the long-lasting legacy of EDCs that are now banned will continue to be an environmental health issue for many years to come.

Some EDCs can also be classified as persistent organic pollutants (POPs). However, they must fit four main criteria to also receive this categorization (Stockholm Convention, 2008b):

- Persistent in the environment for long periods of time
- Can travel long distances in air and water from their point of entry
- Accumulate in the tissues of organisms, building to higher concentrations
- Have harmful or toxic effects on organisms

Not all EDCs are POPs, however those that do fall under this additional category are particularly troublesome because of their long half-lives in the environment, making them a potential source of chronic exposure. Though much progress has been made to identify and describe EDCs and their health effects, the Endocrine Society is pushing for more studies on genetic diversity and population differences in response to EDC exposure.

Studies of EDCs and their effects on thyroid hormones in human's can vary from exploratory (small sample size) to population level (large sample size). Large cohorts such as the National Health and Nutrition Examination Survey (NHANES) in the United States (see chapter 2 page 30, Sjödin et al., 2013), where blood samples are collected along with a multitude of demographic and health data, can be incredibly useful for examining population-level trends in EDC prevalence and association with thyroid hormones.

However, many smaller–scale research projects also contribute valuable information on EDCs and thyroid hormones, as they often explore these trends and associations in distinct sub-groups which may have unique exposures. For example, Byrne et al. (2018), who had a sample size of n=85, explored PBDEs and thyroid hormones in a remote Indigenous population in coastal Alaska, where they found PBDEs in this isolated population and an association with thyroid hormones (TSH, $FT_3$ and $T_3$). Other examples of successful studies with small sample sizes includes English et al. (2017), who looked at PBDEs in feces from Australian children (n=61 but only n=46 were testable), Li et al. (2017) who conducted a pilot study of PCBs in

breast milk of mothers from a recycling site (n=46), Lin et al. (2011) who looked at PBDEs and thyroid hormones in cord blood (n=54), Zota et al., (2011) who measured PBDEs and thyroid function in pregnant women (n=25), and Zhang et al. (2010) who investigated PCBs and PBDEs and thyroid hormone homeostasis in e-waste recycling workers (n=50). Of note is that most of these projects were considered pilot studies and were exploratory in nature.

## 2.4 Polybrominated diphenyl ethers (PBDEs)

### 2.4.1 Physical properties

PBDEs are human-made chemicals that were produced to be applied to consumer products to act as fire suppressants and flame retardants. Structurally, they are comprised of two connected halogenated rings with varying numbers of bromine moieties. There are a total of 209 different PBDE congeners, and the number and positions of bromines attached to the rings determines the chemical properties of the congener including partitioning coefficients and solubility. Because of the larger number of congeners, PBDEs are often grouped together based on their number of bromine atoms. The main groupings (which also correspond to the most popular commercial mixtures) are Penta-bromodiphenyl Ether (5 bromines, Penta-BDE), Octa-bromodiphenyl Ether (8 bromines, Octa-BDE) and Deca-bromodiphenyl Ether (10 bromines, Deca-BDE). Commercial/industrial flame retardant mixtures contained these groupings in varying proportions depending on their purpose and use (Environment and Climate Change Canada, 2006). Because PBDEs are non-polar and only applied superficially to consumer products, they are more susceptible to shedding, often as a result of use and breakdown of the products. The type and number of products that contain PBDEs is quite varied; polyurethane foams (furniture),

insulation, imitation wood, electronics, appliances, and automotive parts and upholstery often contain different mixtures of PBDEs.

The octanol-water partitioning coefficient (log $K_{OW}$) of a substance is a ratio of the concentrations of the substance in octanol to the concentration of the substance in water at equilibrium; octanol is used as a proxy for organic matter (such as an organisms' tissues) in this instance due to its hydrophobic properties. The size and shape of a substance has a strong influence on its $K_{OW}$ value, and the log $K_{OW}$ of a substance can be used to predict the bioaccumulation or bioconcentration of a substance in organisms (Li et al., 2008). Individual PBDE congeners have high octanol-water partitioning coefficients (log $K_{OW}$), which increase as the degree of bromination increases. However, commercial PBDE mixtures have decreasing partitioning coefficients in the more brominated mixtures: Penta-BDE log $K_{OW}$=6.57, Octa-BDE log $K_{OW}$=6.29, Deca-BDE log $K_{OW}$=6.27 (Environment and Climate Change Canada, 2006). Of note is that Watanabe and Tatsukawa (1990) found a much higher partitioning coefficient (log $K_{OW}$=9.97) for Deca-BDE, indicating that there may still be a degree of uncertainty to these analyses. This higher log $K_{OW}$ makes PBDEs more likely to partition to organic matter in the environment and to lipids in organisms. The octanol-air partitioning coefficient (log $K_{OA}$) of PBDEs follows a similar pattern, with lower-brominated congeners having smaller log $K_{OA}$ values and higher brominated PBDEs having greater log $K_{OA}$ values, however this makes more brominated PBDEs more likely to volatize and undergo atmospheric transport, an issue for the global fate of the environment (Environment and Climate Change Canada, 2006).

PBDEs are slow to degrade in the environment, and are also very resistant to metabolism in organisms. The rate of degradation in surface waters, soils and sediments can vary, and is most likely through microbial biodegradation (Gouin and Harner, 2003). The half-life of PBDEs

in the environment can range from 20 days (PBDE-15) to over 9 years (PBDE-153), however this is dependent on the medium; half-lives in air < water < soil < sediment (Gouin and Harner, 2003). The composition of bacteria in soils and sediments can also impact degradation rates of PBDEs, as can the congener and degree of bromination of the molecule (Yang et al., 2017). Photodegradation is another method by which PBDEs degrade, resulting in reductive debromination of the congeners, and this occurs most often in airborne PBDEs (Pan et al., 2016). In photodegradation, the more bromines that are attached to the molecular the faster the degradation rates, however the products formed from photodegradation can also be harmful to the environment and to organisms (Niu et al., 2006).

In humans, the half-life of PBDEs (whole body) is estimated to range from 1.8 years for the lower brominated PBDE-47, up to 11.7 years for the higher brominated congener PBDE-153 (Geyer et al., 2004). Lower brominated congeners are metabolized by hydroxylation and methylation, while higher brominated congeners are debrominated to lower congeners before metabolism and excretion (Lyche et al., 2015). While PBDEs may clear from the serum in a matter of days, their solubility makes them likely to sequester with lipids and into adipose tissue, where they are significantly harder to eliminate.

### 2.4.2 Historical and current usage

PBDEs have been in use as flame retardants since the 1960's, and this demand continued to rise until the late 1990's/early 2000's (Siddiqi et al., 2003), until global production was upwards of 67,125 metric tons per year (Bromine Science and Environment Forum, 2000). North Americans were the predominant producers and users of PBDEs, accounting for 95% of the market demand of Penta-BDE in 2001 (Bromine Sciences and the Environment Forum, 2003).

Canada was also involved in PBDE use; a report from 2000 indicated that while Canadians did not manufacture any PBDEs, they did import over 1300 tones that year for use in finished products (Environment and Climate Change Canada, 2006). In all, from 1970-2020 the North American consumption of PBDEs was estimated at ~46 000 (Penta-BDE), ~25 000 (Octa-BDE), and ~380 000 tonnes (Deca-BDE) (Environment and Climate Change Canada, 2013c).

Much has changed since the early 2000's; Penta and Octa-PBDE production and use have been virtually eliminated worldwide, starting with the banning of Penta-BDE and Octa-BDE by the European Union in 2004. This was followed by the Stockholm Convention adding several PBDEs to its manifest in 2009; the Stockholm Convention has now listed all PBDEs under its list of substances to be eliminated from production, use, and import. Canada banned PBDEs under the Canadian Environmental Protection Act in 2006; the US has banned Penta and Octa-BDE, and has stopped the largest producers and importers of Deca-BDE from continuing to use this product, however the import and use may still be ongoing in small companies (USEPA, 2017). Due to the widespread use of these chemicals in North America, approximately 60% of PBDE stock was estimated to remain in use in consumer products from 2014-2020 (Abbasi et al., 2015). This presents an ongoing public and environmental health issue, as these chemicals will continue to enter the environment for many years to come.

### 2.4.3 Metabolites and transformation products

EDCs can be transformed through interactions with micro and multicellular organisms, and can be biodegraded through chemical reactions, forming metabolites of their parent compound. Some EDC degradation products are more potent endocrine disruptors than their parent compounds, especially if their molecular structure is preserved throughout the process

(Van Zelm et al., 2010). Functional groups such as hydroxyls and ketones play an important role in the biotransformation and biodegradation of EDCs (Khetan, 2014).

PBDEs undergo breakdown (debromination) in the environment through a variety of mechanisms, producing less brominated end products. This poses a challenge, as some of these new less brominated PBDE are potent human endocrine disruptors. Microbes, such as the anaerobic bacteria *Sulfurospirillum multivorans* and *Dehalococcoides* species, have been shown to debrominate Deca-BDE and Octa-BDE to some of the more thyrotoxic PBDEs-47, 99, and 154 (He et al., 2006). Even in anaerobic soils and sediments, bacteria have still been shown to debrominate Octa-BDE into lower brominated PBDEs (Lee and He, 2010). This presents a huge concern for environmental and public health; as PBDEs degrade in the environment, more toxic products may be formed creating ongoing exposure and health issues for years to come.

Recent research has pointed to hydroxylated PBDEs (OH-PBDEs), the metabolites of PBDEs, as having greater binding affinity for transport proteins than thyroid hormones (Ren and Guo, 2012). The number of bromines located on OH-PBDEs change its binding affinity for, and effects on, the thyroid hormone receptor (TR), so that higher brominated OH-PBDEs exhibit agonist activities on the TR, while less brominated OH-PBDEs exert antagonist activities on the TR (Ren at al., 2013). OH-PBDEs bind to TR in the same location as $T_3$, and it is possible that smaller, lower-brominated OH-PBDEs can fit into the TR binding pocket unlike larger higher brominated OH-PBDEs (Ren et al., 2013). Therefore, it is important to consider that not all metabolites of a parent compound have the same actions, and that a small difference such as the degree of bromination of a molecule can have large impacts on their effects on the receptor ligand.

## 2.5 Health effects of EDC exposure

The health effects of EDC exposure in humans stem from disruption of the endocrine systems, but have much larger and further-reaching impacts due to the important nature of these systems in maintaining human health. Endemic, population-level health issues are now being associated with EDC exposure, and with no end to environmental exposure in sight it is as important as ever to continue to study EDCs and the effects they have on the health of human populations.

### 2.5.1 Neurodevelopmental deficits

Neurodevelopmental deficits linked to EDC exposure are becoming more common in research literature. PBDE exposure in utero and during childhood has been associated with poorer attention, fine motor coordination, cognition and poor brain development in children (Eskenazi et al., 2013; Park et al., 2009). A cohort of children in Mexico followed from prenatal exposure to p,p'-DDE showed reduced cognition, verbal skills and memory at 42-60 months of age (Hernández-Mariano et al., 2017).

The US National Health and Nutrition Examination Survey (NHANES) found thyroid disruptors such as PBDEs in the serum of almost all 2062 participants of the study, indicating the widespread prevalence of these EDCs (Sjödin et al., 2013). This is especially concerning because of the role thyroid hormones play in fetal neurodevelopment, resulting in pregnant women and fetuses being especially vulnerable to thyroid disruption. Development of the nervous and endocrine systems is a delicate process from the fetal stage onwards; these EDCs can interfere with hormones and receptor signaling, resulting in altered neural development and hormonal homeostasis (Bernal, 2007).

While the exact biological mechanisms by which EDCs disrupt neurodevelopment are not well understood, the role of thyroid hormone is incredibly important for normal growth and development in utero and onwards, therefore disruptions to this homeostasis can have profound neurodevelopmental effects.

### 2.5.2 Diabetes and obesity

The Endocrine Society has indicated that PBDEs, PCBs, PBBs and p,p'-DDE have been linked to diabetes and obesity (Gore et al., 2015). Studies of obese individuals have shown that PBDE concentrations in adipose tissue are associated with increased insulin resistance (Helaleh et al., 2018). Additionally, exposure to environmental PBDEs is associated with a greater prevalence of diabetes (Zhang et al., 2016).

Type 2 diabetes mellitus (T2DM) is caused by insulin resistance and/or disruption of insulin production in pancreatic beta cells. PBDEs have been linked to T2DM and metabolic syndrome through evidence from epidemiological studies, as have several other EDCs including PCBs and PBBs (Wang et al., 2008; Lim et al., 2008; Turyk et al., 2015). The exact role of PBDEs the development of T2DM is uncertain, as evidence suggests that multiple EDCs are involved in the association with diabetes. For example, exposure to p,p'-DDE and PBDE-153 is associated with a 1.6-2.2 times greater risk of Type 2 diabetes (Airaksinen et al., 2011).

This has led to some EDCs (including PBDEs, PCBs, PBBs and p,p'-DDE) being labelled as obesogens - molecules that increase obesity through alterations in metabolism and lipid storage (Tang-Peronard et al., 2011). Some EDCs may alter adipogenesis and lipid metabolism through binding to peroxisome proliferator activated receptors (PPARs), which regulate lipid cell differentiation and maturation (Evans et al., 2004). Exposure in utero can be

particularly important for altering lipid storage and weight gain later in life. The relationship between obesity, diabetes, and EDCs (PBDEs, PCBs, PBBs and p,p'-DDE) is only starting to emerge, and may present another global health concern linked to exposure to these environmental pollutants.

### 2.5.3 Thyroid-disrupting effects (PCBs, PBBs and p,p'-DDE)

Research has pointed to PCBs, PBBs and p,p'-DDEs as being thyroid disruptors, and their ubiquity in the environment makes them an ideal candidate to study population-level exposure and health effects. PCBs have been shown to decrease total $T_4$ levels in rats and mice (Hallgren et al., 2001). This trend persists at the population level in humans, where PCB concentrations were negatively associated with total $T_4$ in women from the US NHANES (Turyk et al., 2007). PBB levels have been found to be associated with higher $FT_3$ but lower $FT_4$ (Curtis et al., 2019), and at the population level PBB-153 is associated with thyroid disease (Jacobson et al., 2017). In men, a positive association has been found between p,p′-DDE and both $FT_4$ and total $T_3$, while a negative association has been established between p,p′-DDE and TSH (Meeker et al., 2007).

The exact mechanisms by which EDCs cause thyroid disruption are not well understood. Some EDCs alter metabolism; PCBs have been shown to upregulate microsomal enzymes (Hallgren and Darnerud, 2002), while PBB-153 has been associated with metabolic alterations (Walker et al., 2019). EDCs can also affect serum transport proteins; PCBs and their hydroxylated metabolites (OH-PCBs) can bind TTR, competing with thyroid hormones (Purkey et al., 2004). Many EDCs have multiple effects that may impact thyroid hormone homeostasis; in rat models, exposure to p,p'-DDE is associated with a decline in serum TTR, as well as with

elevated thyroid hormone receptor mRNA expression and upregulated hepatic enzymes (Liu et al., 2011). Evidence shows that these EDCs act on many different levels to affect thyroid hormone homeostasis, and that they pose a risk to human health.

### 2.5.4 Thyroid-disrupting effects (PBDEs)

PBDEs are most commonly known for their thyrotoxic effects, causing endocrine disruption of the thyroid system. The official position of the Endocrine Society, the leading authority in endocrine disruptors composed of researchers and clinicians who support endocrine-related research and education, is that PBDEs are endocrine disruptors, and specifically, that they can reduce circulating levels of thyroid hormones (Gore et al., 2015). PBDES can disrupt more than just thyroid hormones; anti-androgenic and anti-estrogenic effects of PBDEs have been demonstrated at the receptor level (Hamers at al., 2006).

There are 5 main PBDE congeners that are most commonly found in the environment and tissues (PBDE-47, -99, -100, -153, -154), which reflects their ability to bioaccumulate as well as their use in commercial products. PBDE-209 is also often found in tissues, though its ability to bioaccumulate is less due to its high molecular mass, thus it is more commonly excreted or metabolized to lower brominated products (O'Driscoll et al., 2016). Across human sampling studies, PBDE-47, -99, and -153 predominate in serum and tissues (Costa et al., 2008; Linares et al., 2015). All three of these chemicals have been associated with thryrotoxic effects in animals and/or humans.

Evidence indicates that PBDEs and OH-PBDEs may compete with thyroid hormones for binding to TTR in the serum (Yang et al., 2011). Because thyroid hormone release is controlled by a negative feedback system, if free (unbound) hormone serum levels increase because of

competition for binding to transport proteins, less hormone is released by the thyroid gland and serum levels of thyroid hormones decrease. Certain PBDE and OH-PBDE congeners have also been shown to suppress thyroid hormone receptor transcription in tissues (Lema et al., 2008; Macaulay et al., 2015).

Many studies on thyroid disrupting effects of individual PBDE congeners have been conducted in rodents, where they were shown to decrease circulating thyroid hormone levels (Hallgren et al., 2001; Lee et al., 2010a; Zhou et al., 2001). Figure 2.4 illustrates the various ways PBDEs and their hydroxylated metabolites (OH-PBDEs) interact with the thyroid system to cause endocrine disruption.

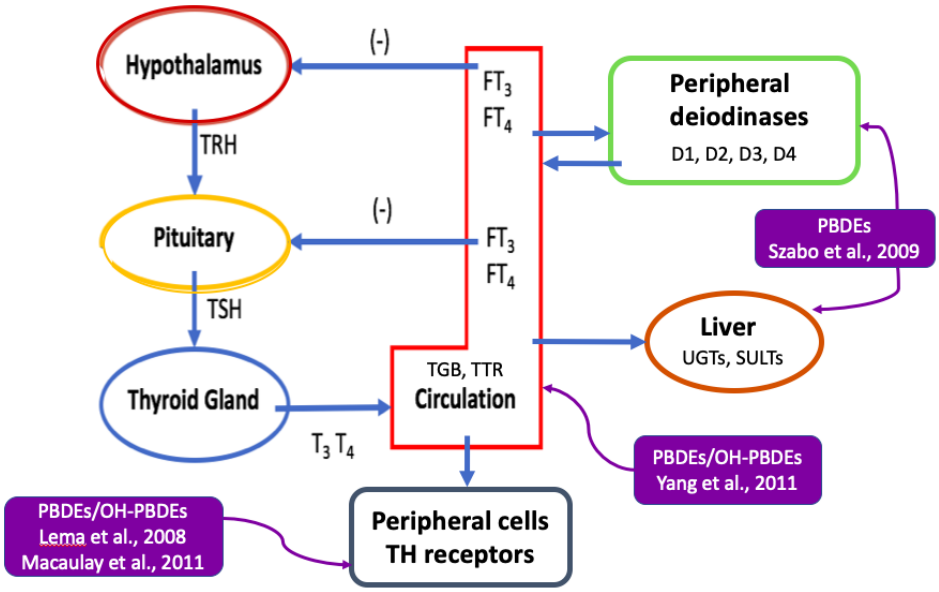



**Figure 2.4** Depiction of PBDEs targeting the thyroid system at multiple sites with cited sources. Of note is that metabolites of PBDEs also disrupt thyroid hormone signaling and function.

PBDE exposure has been shown to alter hepatic and deiodinase gene expression in rats, which may contribute towards increased thyroid hormone metabolism and lower serum

concentrations (Szabo et al., 2009). In humans, the documented effects of PBDEs on thyroid hormones are more varied. Children exposed to PBDEs had higher associated TSH concentrations, but lower total $T_4$ (Jacobson et al., 2016); a native Alaskan population showed a positive association of PBDEs with TSH but no association with $T_4$ (Byrne et al., 2018); and $T_4$ was positively associated with serum PBDE concentrations in a cohort of pregnant women (Stapleton et al., 2011).

Zhao et al. (2015) analyzed multiple studies of PBDEs and thyroid hormones together using regression analyses. There was a statistically significant effect of PBDE concentration on the results that were seen: when blood PBDE concentrations were <30 ng/g lipid a negative correlation with $T_4$ and TSH was seen, when PBDE concentrations were 30-100 ng/g lipids no correlation was observed, and when PBDEs were > 100 ng/g lipids a positive correlation was seen with $T_4$ and TSH. This multitude of endocrine disrupting effects from PBDEs is concerning, as humans are often exposed to PBDEs and other EDCs as a mixture, and low-dose long-term exposure is the most likely type of exposure for most of the population.

### 2.6 Dose-response relationships

EDCs do not always exhibit typical dose-response relationships with the endocrine system. This relationship of dose and health effect is categorized based on the shape of the curve (the health effect). In a non-monotonic dose-response curve, the slope of the relationship changes direction (positive and negative associations) over increasing dosage of the EDC (Figure 2.5). This can result in low doses causing different or more adverse health effects than high doses (Lagarde et al., 2015). For researchers, this has made establishing low-dose thresholds, below

which there are no observable physiological effects, very difficult for EDCs; often the lowest tested doses still disrupt normal endocrine signaling (Figure 2.6; Khetan, 2014).

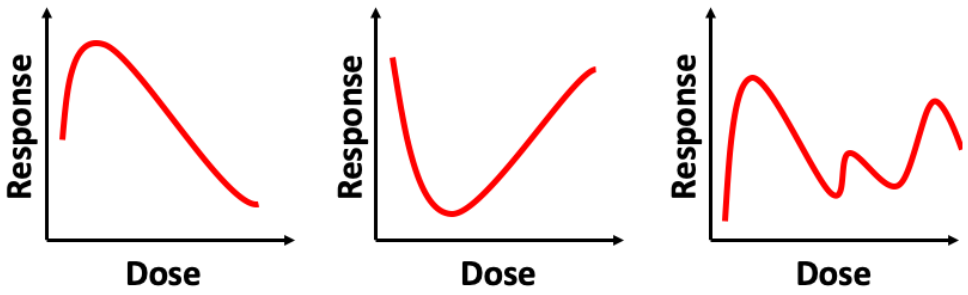



**Figure 2.5** A variety of different non-monotonic dose-response curves that show how disruption of endocrine hormones does not follow a linear response to increasing EDC exposure.

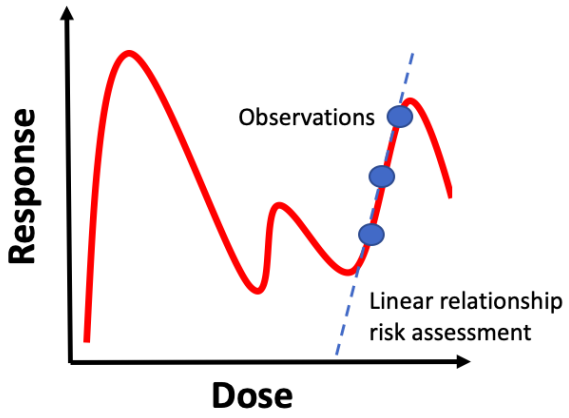



**Figure 2.6** Visual representation of how the non-monotonic dose-response curve of endocrine response to EDC exposure can lead to inaccurate assumed threshold of low-dose exposure below which no adverse health effects occur.

### 2.6.1 Lowest and no adverse level effects

In toxicology, most chemicals are tested on animals to determine the lowest adverse effect level (LOAEL) or no observed adverse effect level (NOAEL). Concentrations of chemicals that fall below the NOAEL are considered "safe" for human exposure, however in this section we will explore how this theory does not hold true for mixtures of EDCs. LOAELs are the lowest amount of a substance an organism can be exposed to without adverse effects occurring (Darnerud et al., 2001). Currently, the LOAEL of PBDEs has been suggested at 1mg/kg/day, based on a meta-analysis of available literature by Darnerud et al. (2001). While this may seem quite high, it is very difficult to estimate total exposure to PBDEs from all sources. Many exposure studies estimate exposure from a single source such as diet, however it is important to include other sources of exposure such as dust ingestion, which has been shown to contribute up to 77% of PBDE exposure (Johnson-Restrepo and Kannan, 2009).

### 2.6.2 Reference dose

Because of the endocrine system response to some EDCs, determining the no observed adverse effect level (NOAEL) can be very difficult, as low doses of a chemical may have different, similar, or worse health effects than high doses. One issue that arises from EDC research is that results that are derived from animal studies don't always manifest the same response in human exposure (Gore et al., 2015).

Reference dose (RfD) is an alternate measure of toxicity, and is the recommended maximum daily oral intake of a substance (Barnes et al., 1988). RfDs are estimations of the maximum daily exposure of a chemical that does not likely cause negative health effects during a lifetime (Barnes et al., 1988). The US Environmental Protection Agency (USEPA) has set

national RfD guidelines for the four most commonly found PBDEs in human tissues (Table 2.3), however health effects of exposure to multiple PBDEs below or above the RfDs are not known. Of note is that breastfeeding infants in the US are estimated to regularly surpass RfDs through ingestion of breastmilk (300 ng/kg/day; Schecter et al., 2006a).

**Table 2.3** Daily intake reference doses, recommended by the USEPA (adopted from Lyche et al., 2015).

| PBDE Congener | Reference Dose |
|---|---|
| BDE 47 | 100 ng/kg/day |
| BDE 99 | 100 ng/kg/day |
| BDE 153 | 200 ng/kg/day |
| BDE 209 | 7000 ng/kg/day |

An important point to note about the RfDs in Table 2.3 is that the recommended daily intake concentration changes based on the congener; this is because lower brominated PBDEs are more hazardous to health than higher brominated congeners. RfD is calculated by dividing the NOAEL by the product of an uncertainty factor (UF) and a modifying factor (MF), so that RfD = NOAEL/(UFxMF). The UF and MF are applied based on data used to conduct the RfD estimate, and reflect the degree of uncertainty by order of magnitude of the calculated RfD (Barnes et al., 1988).

Of note is that the detailed information used to determine UFs are not always made publically available by expert committees, however factors such as biological variability (animal-to-human extrapolation of results, human sensitivity), exposure length, and database

insufficiency are incorporated into the assessment of UFs, with any residual uncertainties addressed by MFs (Dankovic et al., 2015).

### 2.7 Effects of exposure to mixtures of EDCs

A cocktail is normally referred to as a mixed drink, however in today's environment it can also refer to the combination of EDCs to which an organism can be exposed to. Marine ecosystems typically contain a mixture of EDCs, and aquatic organisms would be exposed to a cocktail of chemicals; however, risk assessments are commonly based on individual effect assessments and no observed effect concentration (NOECs) levels (Walter et al., 2002). Studies of chemical mixture effects done on algae have found that exposure to mixtures of chemicals which all fall below their NOEC can still cause adverse effects on an organism (Walter et al., 2002). Fish can be useful environmental biomonitors and can serve as toxicity indicators for mixture effects of EDCs, as they often live in contaminated water and consume contaminated food from the marine food web.

There are several theories that are used to predict the effects of exposure to a mixture of chemicals. Dose additivity (DA) is used to calculate the total effects of multiple chemicals in a mixture with similar modes of action (Kortenkamp, 2007). Independent action (IA) is similar to DA in that it is used to predict the effects of a combination of chemicals, however it is primarily used for chemicals with different modes of action (Kortenkamp, 2007). Both IA and DA presume that the chemicals in the mixture do not interact with or influence each other at the biological target, and that they do not change each other's toxicity (Khetan, 2014). However, new evidence of the mixture effects of multiple low-dose chemicals (mixtures of similarly and dissimilarly acting agents) below their NOAELs may contradict these theories. For example, rats

exposed to mixtures of EDCs, including PCBs, had decreased $T_4$ concentrations 2-3-fold greater than that predicted by DA (Crofton et al., 2005).

The effects of exposure to a combination of chemicals may be higher than any individually tested compound, and may be more accurately predicted by IA then DA. This is in accordance with previous findings of IA being appropriate for predicting effect of mixtures of dissimilar chemicals, and reaffirms that a mixture of chemicals at NOECs can still produce toxic effects (Walter et al., 2002). A more recent study, where fish were fed a cocktail of PBDEs and PCBs at concentrations similar to what might be found in the marine environment, reported that the fish had slower body growth and delayed spawning (Horri et al., 2018). There are complex interaction effects of chemical mixtures with different modes of action, which may produce multiple responses to EDCs. Most organisms are likely exposed to a changing assortment of EDCs throughout their lifetime, as new chemicals are discovered and older ones fall out of use or are banned, therefore it can be difficult to determine the specific health effects elicited by individual chemicals. There is a clear need for more studies on mixture effects of EDCs, as modeling of mixture effects may not always represent biological response.

The use of precision-cut liver slices (PCLS) to test toxicity effects of EDCs on cell-tissue metabolism allows researchers a more *in vivo* look at metabolic processes (Bizarro et al., 2016). When mixtures of EDCs were applied to PCLS, genes regulating vitamin absorption were down regulated, and genes related to lipid/cholesterol metabolism were up regulated, whereas the application of single EDCs did not elicit a response from the PCLS (Bizarro et al., 2016).

In rats, exposure to commercial PBDE mixtures DE-71 and DE-79, the congener PBDE-209 and hexabromocyclododecane (a novel flame retardant) increased liver activity including transcription and catabolism of the liver's drug-metabolizing enzymes; other effects included a

statistically significant (73%) reduction in serum total $T_4$ levels, without causing a change in TSH levels, and an associated thickening of the epithelium of the thyroid gland (Ernest et al., 2012). This demonstrates just how important it is to consider that the NOAELs of some chemicals are not as straightforward as they seem, and that exposure to a single EDC at its NOAEL might not exert adverse health effects but when organisms are exposed to a mixture of EDCs below their NOAEL there may be more significant consequences.

While investigating individual mechanistic effects of known and newly emerging flame retardants is still very important, there should be increased focus on studies of the mixture and additive effects of EDCs. Animals models provide most of the information for EDC mixture exposure studies, and this presents an additional challenge of extrapolating the health outcomes observed in test animals to humans. Observational studies of human exposure to EDC mixtures can add to this information, and are important as a marker for current exposure levels in the population. Experimental (dosing) studies of EDC exposure in humans are not possible due to ethical reasons, therefore observational and epidemiological studies give us the best understanding of exposure patterns and health outcomes in the human population.

### 2.8 Environmental contamination and bioaccumulation

Many EDCs (especially those that also are classified as POPs) can bioaccumulate in tissues, magnifying in higher trophic level organisms. These EDCs have high octanol-water partitioning coefficients ($K_{OW}$), which is a measure of the ability of a chemical to move from an aqueous medium (such as water) into tissues (represented by octanol; Voutsas, 2007). For a chemical to bioaccumulate in an organism, it must have a log $K_{OW}$>5 (Environment and Climate Change Canada, 2006); PBDEs have log $K_{OW}$s ranging from 5.08 – 8.70 (Wania and Dugani,

2003). Chemicals with high bioaccumulation potential also have a greater potential of being toxic to organisms (Geyer et al., 2004).

Another method used to determine bioaccumulation up the food chain is the bioconcentration factor (BCF) of a chemical, which is the ratio of the concentration of an EDC in a specific species group to the exposure concentration. BCF is used to classify a chemicals ability to bioaccumulate; a BCF >100 is used by the European Union to classify a chemical that has the potential to bioaccumulate, although the USEPA uses a BCF>1000 (Geyer et al., 2004). PBDEs have been found to be highly bioaccumulative, with BCFs greater than 5000 for aquatic organisms (Environment and Climate Change Canada, 2006). This makes PBDEs more likely to accumulate in greater concentrations up the food chain, resulting in the higher concentrations seen in top predators such as fish, birds and marine mammals.

An example of environmental bioaccumulation is seen in the beluga whale population in the St. Lawrence River. Monitoring over the past twenty-one years by Lebeuf et al. (2014) has revealed the presence of EDCs in beluga blubber. Most beluga blubber samples in their study contained PCBs and DDTs, which declined in concentration over the 21-year sampling period. PBDEs were also detected in blubber samples, and were the only EDCs that increased during the sampling period, although their rates have leveled off in the past decade (Lebeuf et al., 2014). This beluga population has reached dangerously low numbers, some which is being attributed to the high concentrations of EDCs seen in their tissue samples (Lebeuf et al., 2014). The persistence of these chemicals in large marine mammals such as belugas is concerning, as they consume a wide array of seafood, some of which is also consumed by humans. This information may provide insight into the EDCs that humans are also consuming in their seafood.

## 2.9 Sources of EDCs to the environment

Most EDCs are anthropogenic (human-made), whose sources include industrial chemicals, agricultural chemicals (insecticides, herbicides, fungicides), animal waste effluents, personal care products, and pharmaceuticals (human and animal; Khetan, 2014). The particular source of an EDC also determines how it enters the environment, its fate there, and routes of human exposure.

### 2.9.1Waste water treatment plants and biosolids

Urban and industrial waste water is increasingly directed to waste-water treatment plants (WWTPs), where contaminants and solids can be removed before effluents are released back into the environment. WWTP effluents are major sources of EDCs into the environment, where they eventually end up in the local water system and can enter the food chain. Houde et al. (2014) found that WWTP effluent and urban runoff from the city of Montreal were major sources of PBDE contamination to the local river ecosystem. PBDE congener concentrations detected in perch, pike and muskellunge from around Montreal were among the highest reported levels in top predatory fish from North America, and PBDE-209 has been found in suspended matters downstream from this area (Houde et al., 2014). This could pose a hazard to populations living downstream from this pollution source, in the Estuary and Gulf of St. Lawrence.

Upstream sources of chemicals found in WWTP effluent and urban runoff include EDCs excreted by humans (i.e. pharmaceuticals, natural hormones), personal care products, cleaning supplies, and household waste, all of which is washed down into the urban water effluent (Khetan, 2014). Also of note is that untreated effluents from groundwater and runoff samples close to dairy farms and aquaculture facilities have contained EDCs for many years (Kolodziej et

al., 2004). Biosolids like sludge that are derived from wastewater treatment plants are often used in agriculture, and fields treated with WWTP biosolids have higher average concentrations of some EDCs (e.g. PBDEs) in their soils (Andrade et al., 2010).

WWTPs were not initially designed to remove EDCs from waste water, therefore new technologies have been adapted to help eliminate these chemicals from both effluent and sludge, before they are returned to the environment. Many EDCs can be removed through proper processing of waste water, using methods such as activated carbon, oxidation, nanofiltration, reverse osmosis membranes, as well as more traditional methods of sewage treatment including activated sludge and biotrickling filters (Bolong et al., 2009). Some EDCs are more difficult to remove from waste-water than others, however since they are usually found in combination with other EDCs, multiple treatment methods may be required before all chemicals have been removed. PBDEs tend to separate into sludge over effluent due to their partitioning into organic matter, which can make them easier to remove from the water portion of effluent (Kim et al., 2013). Various EDCs removed through these processes end up in biosolids, however there are very few methods to address EDCs in biosolids (Vassos, 2018).

Many Canadian WWTPs are quite effective at removing EDCs, and have been shown to remove up to 90% of PBDEs in the effluent of wastewater, however plants which perform only primary treatment had much lower rates at only 70% PBDE removal from effluent (Kim et al., 2013). In Canada, approximately 18% of the population is served by primary WWTP and 3% of the population receives no waste-water treatment (Canadian Water Network, 2018). Sludge containing PBDEs can also be removed through aerobic and anaerobic degradation, however levels of effectiveness vary (47-78%) and require long durations of time (11+ months), according to studies (Stiborova et al., 2015a; Stiborova et al., 2015b). As a result of these long or expensive

removal processes, many EDCs are not removed from wastewater, and are put back into the environment via wastewater effluent.

### 2.9.2 Landfills and consumer products

Landfill leachate is increasingly becoming another source of EDCs to the environment. Li et al., (2012) looked at concentrations of PBDEs in landfill leachates from across Canada; after sampling 24 sites across northern and southern Canada, the authors found BDE-209 to be the dominant PBDE congener in the samples, and estimated the amount of ∑PBDE leaching from urban landfills to be 3.5 tons/year. Southern landfills had greater PBDE concentration in their leachate than northern landfills, which was attributed to higher population densities in these areas, depositing more electronics and furniture into their local landfills. This is concerning as many landfills in southern Canada are located near water sources, including several located near or on the St. Lawrence River. Gulls living in close proximity to the Lachenaie dump (the third largest landfill site in North America, located northeast of Montreal), have high flame retardant concentrations in their eggs, and are considered by scientists to be the most contaminated colony in Canada (Gentes et al., 2012). Landfills can contain a wide variety of chemicals, and can affect the EDC congener profiles in the surrounding environment (Danon-Schaffer et al., 2014). Landfill leachate needs to be regularly monitored for environmental contamination and leaching of EDCs.

Cathode ray tube televisions (CRT TVs) contain high amounts of Deca-BDEs (Abbasi et al., 2015). In many developed countries CRT TVs are now obsolete electronics, and have transitioned into the waste phase (i.e. landfills), where they contribute significantly to the flow of Deca-BDEs into the environment (Abbasi et al., 2015). It is not just CRT TVs that contribute to

environmental PBDE contamination in landfills; many products including polyurethane foam in furniture, electrical and electronic equipment, and vehicles are also large contributors to the flow of PBDEs to the waste phase (Abbasi et al., 2015). It is projected that even if there is no new use of any Deca-BDE after 2013, approximately 120,000 tons of PBDEs (95% Deca-BDE) will have still been in the use phase in 2014, and approximately 60% (mainly Deca-BDE) will remain in use until 2020, mostly in vehicles (Abbasi et al., 2015).

Environmental contamination of PBDEs and other EDCs is especially prominent at e-waste recycling sites in developing countries, where improper recycling techniques can enable their release into the environment. Incorrect recycling and disposal of e-waste is a large source of PBDEs into the environment around these sites (Li et al., 2014). Those living near e-waste sites have higher body-burdens of EDCs commonly found in electronics, including PBDEs and PCBs (Zhang et al., 2010). This could be partially attributed to the ingestion of contaminated food products, as local residents of e-waste dismantling and recycling plants have been shown to be exposed through consumption of contaminated local foods. Chan et al. (2013) found that human ingestion of PBDEs from local foods from around e-waste recycling sites was higher than rates recommended by the USEPA, with PBDE-47 intakes of 584 ng/kg bw/day (USEPA reference dose is 100 ng/kg bw/day). This presents both a local and international environmental health issue, as contamination from these sites can travel long distances exposing populations around the world to these EDCs.

### 2.10 Patterns of exposure to EDCs

Due to the widespread use of EDCs as pesticides, flame-retardants, pharmaceuticals, and industrial chemicals, many people in developed countries, such as Europe, Canada and the US,

have high levels of exposure. Meta-analysis (Hites et al., 2004) of EDC exposure by region has shown that North Americans have much higher exposure to PBDEs (factor of >10) than Europeans, while the lowest exposure was seen in the Japanese, who have even lower levels of exposure than Europeans (factor of 5). As such, North Americans have higher body burdens than most other places in the world, although this may be changing as electronic waste recycling grows in developing countries.

The main exposure routes in humans for all environmental contaminants are through ingestion, inhalation, dermal absorption, and through the placenta in utero. Humans are most commonly exposed to EDCs through ingestion and inhalation, with very few EDCs being absorbed to any significant extent through the skin (Frederiksen et al., 2009). Ingestion mostly occurs through consumption of contaminated food products or water, and by dust ingested through hand-to-mouth activity or settling on food preparation surfaces. Food products contaminated with EDCs can occur through acute (direct) exposure, or by bioaccumulation in the food chain. The dominant exposure pathway of the EDC depends on the physical properties of the chemical, its source, and its location (air, water, indoor household etc.). Lifestyle, age, and diet of the local population are also important in determining exposure pathways, as will be discussed further in this section.

### 2.10.1 Dermal and inhalation exposure

Some EDCs have been shown to be absorbed dermally. For this pathway to contribute to overall body burden, there must be a high permeability coefficient from skin surface lipids to blood (Gong et al., 2014). PCBs have been shown to have very little dermal permeability, with only 6% absorption compared with an orally ingested dose of the same concentration (Schmid et

al., 1992). PBDEs can be absorbed dermally, with lower brominated congeners penetrating the derma at a faster rate and higher brominated PBDEs having greater buildup in epidermal tissue (Abdallah et al., 2015). In one study, estimated ∑PBDE exposure from palms, back-of-hands, and forearms was 25.9 ng/day (Liu et al., 2017). Thus, dermal absorption of PBDEs contributes to overall body burden, and PBDEs in epidermal tissue are a potential source of contaminants which may be gradually discharged to the circulatory system over time.

Inhalation is another exposure pathway for EDCs which are released from household products and found in the indoor environment (Schreder et al., 2016). Flame retardants found in indoor dust, like PBDEs, can be inhaled however the estimated exposure via inhalation is four times lower than exposure to the same dust via ingestion (Hou et al., 2018). While exposure to indoor airborne EDCs is lower than through other exposure pathways, they are still important to consider in overall exposure estimates as humans in developed countries spend the majority of their time in indoor environments (i.e. home, work, school, day care). Additionally, many of these airborne chemicals are able to settle on surfaces and be ingested.

### 2.10.2 Ingestion and dietary exposure

Ingestion is the predominant EDC exposure pathway in humans. The majority of PBDE exposure occurs through ingestion of contaminated food products or dust (Jones-Otazo et al., 2005). Currently there is some debate in the scientific community over which ingested PBDE source (dust or food) has a greater contribution to exposure; some believe that dust is greatest (Jones-Otazo et al., 2005), while others argue that food products contribute more to exposure (Fromme et al., 2009). There are even some studies that have found that they both contribute equally to PBDE intake in adults (Lee et al., 2013). Given that measuring EDC exposure is a

difficult and developing field, it is important to consider both diet and dust as ingestion sources of PBDEs when examining exposure in humans. Humans can also be exposed to other EDCs through ingestion. Duarte-Davidson and Jones (1994) found that food consumption accounted for 97% of PCB ingestion in the UK population.

Not all EDCs are absorbed equally through ingestion, therefore the bioaccessability of individual EDCs must be considered when examining human exposure. Bioaccessability of PBDEs can depend on the congener; PBDE-209 had much lower bioaccessability (14-23%) than other PBDE congeners (15-66%) based on ingestion of house dust using a simulated human gastrointestinal tract (Yu et al., 2012). Fat content of animal-based foods appears to play a role in bioaccessability of PBDEs from food products, with higher fat foods having greater bioaccessability of PBDEs (Yu et al., 2011).

**2.11 EDCs in food products**

Various food products have different contributions to EDC exposure, and this is dependent on the concentration of the EDC in the food product and the amount (frequency and quantity) of consumption of that product. Seafood products frequently have the highest concentrations of PBDEs as seen in numerous studies, followed by meat, eggs, dairy and oils (Bocio et al., 2003; Schecter et al., 2006a; Voorspoels et al., 2007; Domingo et al., 2008). Vegetables and fruits have some of the lowest PBDE concentrations of all food products (Kiviranta et al., 2004; Jacobs et al., 2004). Dairy and eggs have variable contributions to dietary PBDE exposure, with some studies finding high levels of PBDEs in these products (Schecter et al., 2006a), while others did not (Bocio et al., 2003). PCBs and DDT have also been shown to be present in food products, with fish having greater concentrations of these contaminants than

meat, eggs, dairy or fats/oils (Darnerud et al., 2006).

Cooking can play a role in concentrations of EDCs in food, especially in meats. Many EDCs are lipophilic, and are stored in adipose cells. Schecter et al. (2006b) broiled several types of red meat and fish while allowing the fat to drip off, and tested these effects on the meat's lipid percentage and PBDE wet weight. Most of their samples had a decrease in both lipid and PBDE concentrations following broiling, with the exception of trout, a relatively low fat fish (Schecter et al., 2006b). Raw red meats are infrequently consumed, therefor this information is important to consider when estimating daily intakes of EDCs, as some of these chemical concentrations can be lowered depending on the cooking method.

### 2.11.1 Exposure by diet

Research has shown that North Americans have very different dietary exposure patterns to EDCs than other regions of the world. Europeans are known for lower exposure to EDCs via diet then North Americans, with one study estimating European dietary PBDE intake to be 0.0022 ug/kg body weight/day, and North American PBDE dietary intake to be higher at 0.0036 ug/kg body weight/day (FAO and WHO, 2006).

In US adults, eggs and dairy (Schecter et al., 2010) and red meat and poultry (Fraser et al., 2009) were found to contribute the greatest proportion to PBDE exposure through diet. This trend holds strong for children as well; 2-5 year olds from California were found to have greatest dietary exposure to PBDEs through pork and poultry consumption (Rose et al., 2010). Meat consumption is much higher in the US than in both European and developing countries, and it is continuing to rise with red meat making up the majority (58%) of consumed meat products (Daniel et al., 2011).

In Canada, PBDE exposure patterns through diet are very similar to those in the US. The average Canadian exposure to PBDEs in 2002 was highest through consumption of meat (12.5 ng/day), followed by fish (8.6 ng/day) and dairy (5.9 ng/day) consumption (Health Canada, 2016). Health Canada sampling of foods from supermarkets in St. John's (the capital city of the province of Newfoundland) in 2001 revealed that freshwater fish had the highest PCB concentrations (16,201.5 ppt), followed by butter (1,513.5 ppt), and marine fish (1,307.9 ppt; Health Canada, 2004). No testing of food products for PBDEs was done in St. John's by the Canadian government.

European countries traditionally have lower environmental concentrations of EDCs due to their restrictive policies governing the import, manufacture, and use of chemicals. However, this does not necessarily mean less exposure; long-range transport means that even restrictive countries can be contaminated from nearby or far away neighbors. In Greenland, serum PBDEs and PBBs have been detected at 2.7 to 15 times higher concentrations than in Ukrainian and Polish serum samples, and this has been partially attributed to the close proximity of Greenland to North America (Lenters et al., 2013). Additionally, many European countries have diets rich in seafood which generally contain higher levels of EDCs. Norwegians have some of the highest dietary exposure to ∑PBDEs (1.5 ng/kg body weight/day) in Europe due to their consumption of oily fish (Knutsen et al., 2008).

Research into PBDE and other EDC exposure via seafood consumption in Asia is only starting to gain traction in the environmental health community, but there is a growing body of evidence that these populations may be exposed through consumption of seafood products. Exposure to PBDEs from seafood consumption is estimated at 40.6 ng/day in Japan (Ashizuka et al., 2008), 308 ng/day in Hong Kong (Wang et al., 2012) and 34.2-66.2 ng/g in China (Miyake et

al., 2008). Many Asian countries have diets rich in seafood (Miyake et al., 2008; Wang et al., 2012), therefore it is no coincidence that they have dietary exposure to PBDEs and other EDCs through seafood consumption.

Populations that traditionally consume higher amounts of seafood may be at a higher risk of exposure to these chemicals through food. In Norway, a country that regularly harvests and consumes local seafood, oily fish species dominate dietary source of BDEs (47, 99, 100, 153, 154 and 209) when compared with vegetables, diary, and other types of meat (Knutsen et al., 2008). A market-basket study on Finnish EDC exposure demonstrated that fish consumption was the highest contributors to PBDE and PCB exposure in the adult population (Kiviranta et al., 2004). Similar studies in Spain (Bocio et al., 2003), Belgium (Voorspoels et al., 2007), South Korea (Na et al., 2013), and Japan (Akutsu et al., 2008) all found seafood to be the greatest contributor to PBDE exposure in the diet. Overall, trends indicate that fish and shellfish are major contributors to EDC intake in many European and Asian countries, while meats, poultry and meat-derived products are major contributors in Canada and the US.

There is expansive evidence of EDC exposure through seafood consumption in many populations, however consumption of seafood also confers numerous health benefits, as many fish and seafood species are high in protein and omga-3 fatty acids. Studies of the benefit-to-risk ratio of exposure to PBDEs from consuming fish weigh in favor of fish consumption, with the exception of some species for pregnant women (Mozaffarian and Rimm, 2006).

### 2.11.2 Traditional and subsistence diets

General population trends in North America indicate meat, eggs and dairy as main dietary sources of EDC exposure, but there are small pockets of the population that may consume

subsidence foods harvested from the local environment, and this may alter or increase their risk of EDC exposure. Many First Nations populations (in both the US and Canada) have traditions of hunting and fishing. This may put these populations at a greater risk of exposure to EDCs as was found in a study in a Norther Ontario First Nations community, where consumption of native species of fish like walleye and pike were positively correlated with serum PCB levels (Philibert et al., 2009). In Greenland, consumption of traditional foods including local fish, seal and whale are sources of PBDEs and PCBs in the diet (Carlsson et al., 2014). Consumption of marine mammals including seal and whale blubber, have also been found to be dominant contributors to EDC exposure in people who consume traditional hunted foods (Johansen et al., 2004).

It is not just seafood that presents a risk of exposure; herring gulls (HGs) are top predators in the food chain, and their eggs have been traditionally consumed by First Nations populations in North America. Studies have shown that Canadian HG eggs from marine colonies have high BDE-47 in their eggs, indicating they may have exposure to tetra- or penta-BDE sources (Chen et al., 2012). HG eggs sampled from locations close to large urban centers have the highest sum PBDEs, which may be due to large and dense populations have greater use and waste of flame-retardant containing products (Chen et al., 2012). These species serve as potential biomarkers for environmental contamination, however they can also pose a hazard for the people who traditionally harvest and consume their eggs. Populations on the Faroe Islands consume seabird eggs, and their PBDE and PCB concentrations (measured via breast milk) were high compared with other European populations (Fängström et al., 2005).

Not all populations who consume wild foods are equally exposed. Exposure via subsidence diets in First Nations communities of Northern Canada was found to be within US

reference dose limits, and PBDE body burdens in these communities were more likely related to access to material goods (exposure via household dust) than to consumption of wild meats and fish (Liberda et al., 2011). This evidence demonstrates that exposure EDC exposure via subsidence foods is not equal across all populations and dietary patterns, and that further investigations of these eating patterns is warranted.

### 2.11.3 Exposure through breastfeeding

Breastfeeding can also be a source of EDC exposure for infants. Lipophilic EDCs can passively diffuse from the mothers' circulation into their milk, dependent on the mother's plasma concentration (Shaw, 2009). There are multiple factors that can influence the transmission of EDCs into milk, including the size of the molecule, its lipid/water solubility, and the binding to transport proteins in the plasma (Shaw, 2009).

Researchers now use human milk sampling as a marker of community-wide EDC contamination because it is a rapid, sensitive and less invasive method for testing for EDC exposure, and normally contains high concentrations of EDCs (especially lipophilic compounds). Breast milk is easily obtained (less invasive to collect than blood or tissue samples) and partitioning between human milk and adipose is usually close to 1:1, but can be higher for some EDCs (i.e. PCBs; Wittsiepe et al., 2007), making it a reliable method of measuring exposure.

In the US, breastmilk is the greatest contributor (91%) to dietary PBDE exposure in infants (Johnson-Restrepo and Kannan, 2009). As expected, PBDE concentrations in breastmilk were 20 times higher in North America than in Europe or Asia (Zhang et al., 2017). In New Zealand, there has been a 70% decline in PCBs in breast milk in a period from 1987-1999 (Bates

et al., 2002). However, levels of PBDEs in milk were increasing during this time period, as PBDEs became more commonly used in consumer products (Fängström et al., 2005).

## 2.12 EDC exposure and lifestyle

### 2.12.1 Effect of life-stage on exposure and outcomes

Exposure can occur at any stage of life, from in utero to adulthood; with the exception of high dose or occupational exposure, early life exposure to EDCs can be the most damaging to human health. Compounds that are more hydrophobic are more rapidly transferred across the fetal placental barrier (Takahashi and Oishi, 2000). Many maternal hormones can cross the placenta and enter the fetal blood supply, however some EDCs can also permeate this barrier.

The first trimester of pregnancy is an incredibly sensitive time for the developing fetus as it is dependent on maternal thyroid hormone for growth and development (Forhead and Fowden, 2014). Changes in maternal thyroid hormone levels during this period may contribute to lower birth weights, as seen in babies born to mothers with detectable levels of PBDEs (Lignell et al., 2013). Additionally, a maternal body burden of PBDE-153 is shown to be inversely associated with $T_3$ during their first trimester of pregnancy (Lignell et al., 2016).

EDCs can also have effects on the developing fetus later in life. PBDE exposure in utero and during childhood is associated with decreased cognition, motor skills, and attention span (Eskenazi et al., 2013), and with higher childhood body mass index (BMI) in males (Erkin-Cakmak et al., 2015). In utero exposure to DDT/ p,p'-DDE has also been linked to adolescent obesity rates (Smink et al, 2008; Verhulst et al., 2009).

In adolescent females, the age of menarche has declined considerably over the past century from 16-17 years to <13 years of age (Buttke et al., 2012). Some of this may be

attributed to EDC exposure in early stages of growth, however meta-analysis of current research indicates that some EDCs may accelerate age of menarche, while others may delay it (Greenspan and Lee, 2018). PBDEs have been associated with later age of menarche and onset of puberty in girls, and they are also associated with earlier onset of puberty in boys (Harley et al., 2017). This interference with puberty and menarche onset in adolescents by EDCs has been attributed to multiple alterations in the endocrine system, including activation of the aryl hydrocarbon receptor (altering metabolism of hormones), disruptions to thyroid hormones, and epigenetic changes (Leijs et al., 2014). Since EDC exposure contributes to both childhood obesity and changes in the onset of puberty and menarche, it is very plausible that obesity, type II diabetes, puberty and EDC exposure are all interconnected on this issue.

### 2.12.2 EDC exposure: socioeconomic status and ethnicity

There are many social and physical factors that can contribute to EDC exposure, including socioeconomic status (SES), educational attainment, gender, and ethnicity. Populations with low SES and/or minority ethnicities are often subject to greater exposures to environmental pollutants through diverse sources, including consumer product use, food consumption, and housing/work environments (James-Todd et al., 2016). In a study in Spain, adult participants categorized into the least affluent social class had higher serum PBDE-47 concentrations than the rest of the participants (Garí and Grimalt, 2013). In California, serum PBDE levels were inversely associated with household income (Zota et al., 2008). Research also indicates that children born to parents with low educational attainment have higher serum PBDEs than those born to more educated parents (Rose et al., 2010; Stapleton et al., 2012).

However, these trends in EDC exposure are not always consistent. Populations with lower SES generally have a higher disease burden and greater exposure to environmental EDCs, however, this trend may be reversing for some EDCs as these chemicals are now found in a wide array of consumables associated with higher SES including electronics, furniture, textiles and vehicles. As an example, PBDE-153 was found to be higher in the serum of toddlers born to more highly educated parents (Stapleton et al., 2012). These results were different from all other PBDEs tested in the serum of toddlers in this study, and was explained by the high rate of partitioning of BDE-153 into breastmilk; mothers with higher education are more likely to breastfeed, therefore their children are more likely to be exposed to PBDE-153 (Stapleton et al., 2012).

The US NHANES found 16 EDCs associated with low SES and 12 EDCs associated with high SES (Tyrrell et al., 2013). Fish/shellfish consumption, sunscreen use, and dental amalgams were all implicated as factors influencing the greater concentrations of chemicals in high SES groups. These factors represent patterns of behavior or advantages which higher SES populations may have, including more access to health care services (higher incidence of dental amalgams), sunscreen use (expensive skin protectant), and fish/shellfish consumption (more expensive in some regions, and may also reflect cultural background).

Exposure disparities still exist between racial/ethnic groups, and may be contributing towards adverse health outcomes for those populations. African American populations are more likely to be exposed to environmental pollution from landfill leachate (which often contains PBDEs), as they are more likely to live close to these sites (Ranjit et al., 2010). Racial disparities can be seen in concentrations of serum PBDEs, which are higher in African American and Hispanic adolescent girls than in Caucasian girls (Windham et al., 2010; Chen et al., 2011), and

in black and Hispanic Californians who were found to have higher serum PBDE-47 and PBDE-99 levels than non-Hispanic whites (Fraser et al., 2009). Explanations for racial disparities in EDC exposure may come from differences in housing stock, furniture quality, the presence of air exchange in residence, and diet (Zota et al., 2011). Lower quality housing materials and furniture may weather and break down sooner, exposing the residents and owners to EDCs; air exchange can help filter out airborne EDCs in the indoor environment; and consumption of contaminated foods (i.e. canned foods, high fat foods) can also increase EDC exposure in certain SES or ethnic groups.

### 2.12.3 EDC exposure: age, sex and gender

Age, sex and gender also contribute to EDC exposure. Nursing infants have the highest recorded estimates for PBDE intake, seconded by children (Schecter et al., 2006a). Serum PBDEs have also been found to be higher in children than in adults (Toms et al., 2009). This has been suggested to be a result of hand to mouth activity as seen in toddlers and young children (Stapleton et al., 2012). Even within adult age groups exposure can vary; younger persons (<30 years) have been observed to have higher serum concentrations of PBDE congeners than older persons (Garí and Grimalt, 2013). A possible explanation for this trend is that younger individuals would have been exposed to low levels of PBDEs throughout their life (and especially during childhood through methods mentioned above), resulting in higher body burdens than older participants.

Gender can also make a difference in EDC exposure, though the results are not consistent if males or females are more highly exposed. Higher PBDE concentrations have been found in hair samples taken from males than those from females (Król et al., 2014). However, women

have been found to have higher serum PBDE levels than men in one study of the US population (Schecter et al., 2006a). These mixed results can make it incredibly hard to create gendered risk assessments, as there are a multitude of differences between males and females that impact exposure, including biological, occupational, residential, behavioral, socioeconomic, cultural, and psychosocial characteristics (Vahter et al., 2007).

### 2.12.4 Latency of exposure

One of the greatest barriers to establishing EDC exposure as the cause of a health condition is the long latency period between exposure and health effects. The health outcomes from EDC exposure may not be apparent until long after the exposure period has begun, or occur long after it has passed. With the exception of a known occupational or large dose exposure, most humans are exposed to EDCs through long-term low-dose exposure, which makes it difficult to determine the length of exposure and to monitor the dosage. Some researchers are getting around this by tracking human exposure starting in utero, measuring a mother's serum levels at pre-partum or testing cord blood levels for EDCs post-partum, and following the growth and development of the infant after birth.

Prospective cohort studies have revealed a positive correlation between ADHD, social competence and postnatal exposure to PBDE-47 in children 4 years of age (Gascon et al., 2011), and an inverse relationship between maternal serum PBDE concentrations and cognitive and motor functions of their offspring (Eskenazi et al., 2013; Chen et al., 2014). This longitudinal cohort method is one of the few ways to study long-term exposure to EDCs, and since most of the participants in these studies have detectable levels of EDCs in their serum, it is possible to extrapolate these data to exposure on a global scale. EDC exposure in utero is a pertinent health

issue, as the health effects from this exposure are seen in later in life in children and adults due to the long latency between exposure and outcome.

## 2.13 Legislation and policies of EDCs

### 2.13.1 EDC conventions and treaties

On a global level, control and regulation of EDCs started with the Stockholm Convention on Persistent Organic Pollutants, an international treaty aimed to protect the environment and human health from chemical pollution (Stockholm Convention, 2008a). The treaty was put into force in 2004, however its inception began in 1995 when the United Nations Environment Program requested an international assessment of 12 chemicals that were of concern (now called the "dirty dozen"). The Stockholm Convention (SC) has several classifications for chemicals, including Annex A -chemicals that must be eliminated from production and use, Annex B - chemicals which must be restricted, and Annex C - chemicals which are banned but for which use is allowed under very specific conditions. The SC is partnered with the Basel Convention on the Control of Transboundary Movements of Hazardous Wasted and their Disposal, which aims to limit and control the production of hazardous wastes in signatory countries, and restrict the export of this waste to other countries (Basel Convention, 2011). Both treaties are signed by 180 states (and the European Union), and there are currently 30 chemical substances listed for regulation under the SC (Stockholm Convention, 2008a). Countries that are signatories of these treaties must adhere to the conditions of each treaty, including banning the use, application, and sale of chemicals listed under the convention.

Management and regulation of EDCs can be a very political issue. In North America, there are very strict flammability standards for consumer products, therefore the application of

brominated flame retardants to consumer goods has historically been widespread. In 1999, over half the global demand for PBDEs came from North America, and 97% of that was for Penta-BDE (Hale et al., 2003). As a result of this high usage of PBDEs in the past, North Americans are more likely to be exposed to PBDEs than Europeans (Trudel et al., 2011). Between 1989 and 1999, the majority of brominated flame retardants were produced in Asia (Alaee et al., 2003) however, as discussed above, the major demand for the product was in the US. The European Commission was the first international body to advocate for banning PBDEs, and succeeded in having the European Union accept the ban of Penta and Octa-BDEs in 2004, and Deca-BDEs in 2008.

### 2.13.2 EDC regulation in Canada

The Canadian Environmental Protection Act (CEPA) of 1999 is a policy founded to protect the Canadian environment from pollution. The act is controlled through the Chemicals Management Plan (CMP), a legislative body responsible for conducting risk assessments and managing previously unassessed chemicals in Canada. Under CEPA legislation, the federal government is responsible for investigating chemicals and designing policies to prevent the misuse and environmental contamination from said chemicals. The CMP is not without flaws, and recently has come under scrutiny due to the lack of consideration of how sex and gender affect exposure and health outcomes, given that some research indicates the risk of exposure is higher in women due to social, economic and cultural factors (Scott and Lewis, 2015). In 2006, both Health Canada and Environment and Climate Change Canada (ECCC) completed screening assessments of the risks of PBDEs to the environment and to human health, and concluded that PBDEs may cause negative effects on the environment and organisms, and thus should be

categorized as “toxic” (Environment and Climate Change Canada, 2013a).

Canada added PBDEs to the List of Toxic Substances in 2006, and proposed regulations on the use of PBDEs, including elimination of the use of Penta and Octa-BDEs in commercial mixtures (Ward et al., 2008). The Government of Canada published official PBDE regulations in 2008, detailing a ban on the manufacture of any PBDEs in Canada, and limiting the use, sale or import of all PBDEs with the exception of Deca-BDE; this final PBDE was banned from sale and import in Canada in 2010 (Environment and Climate Change Canada, 2013a).

The Great Lakes of Ontario, Michigan, Erie, Huron and Superior occupy a large section of Canada and the US. Together they contain 84% of North America’s fresh surface water, and 30 million people live within their drainage basin (United States Environmental Protection Agency, 2019). Analysis of core sediment samples from the Great Lakes indicates that there are approximately 100 metric tons of PBDEs in the Great Lakes sediments (Ward et al., 2008). ECCC proposed a ‘Risk Management Strategy for PBDEs’ in 2006, barring the use of all Tetra, Penta, and Hexa-BDEs, and limiting environmental emissions of PBDEs to prevent further contamination to this ecosystem (Environment and Climate Change Canada, 2013a). Later, The Great Lakes Water Quality Agreement Act (GLWQA; initiated in 1972 but modernized in 2012) was signed by the US and Canadian governments, committing them to working together to maintain and improve the environmental quality of these bodies of water. More recently, a Great Lakes Binational Strategy for Risk Management was launched by Canadian and US governments as part of the GLWQA, to reduce the anthropogenic release of EDCs and other chemicals into the environment of the Great Lakes. Risk management strategies for PCBs (Environment and Climate Change Canada and US Environmental Protection Agency, 2018), PBDEs (Environment

and Climate Change Canada and US Environmental Protection Agency, 2019) and other EDCs have been published.

### 2.13.3 EDC exposure monitoring in Canada

In an attempt to monitor EDCs in Canadian food products, Health Canada commissioned the Bureau of Chemical Safety to conduct a Total Diet Study (TDS). This project, which has been ongoing since 1969 with the most recent cycle started in 2005, takes place in major cities across Canada (Health Canada, 2009). The TDS was designed as a market-basket style study, a method recommended by the World Health Organization as a way for health authorities to monitor the population exposure to EDCs through food products. In the TDS, different types of food products were purchased in each city and evaluated for their EDC content. This information was combined with consumption data to calculate EDC intake values based on age/sex groupings. This study was major progress for Canadian risk assessment of EDCs and there were a wide range of EDCs tested, however very few chemicals were tested in all cities, and there are large time differences between first and last cities tested in each cycle. For example, PBDEs were tested only in two Canadian cities (Vancouver and Whitehorse), which does not equate to a comprehensive picture of PBDE contamination in food products across Canada (Health Canada, 2016). Further testing was done in conjunction with the TDS in 2002, when Health Canada conducted the Fish and Seafood survey to test wild and farmed seafood products for PBDEs and PCBs; both EDCs were below guideline levels set by Health Canada in the tested products (Health Canada, 2009).

2.13.4 Europe, EDCs, and the precautionary principle

Europe has been ahead of other developed countries in their use of the precautionary principle to direct their legislation and chemical management policies, and the European Union (EU) has a fairly comprehensive chemical management system in place. The precautionary principle is based on the tenant that a chemical or substance does need to be proven to be risky or toxic to warrant restrictions and banning (Godduhn and Duffy, 2003), and the environmental and health effects of new substances are considered uncertain and as such are treated with caution until proven otherwise. In the EU, legislation and control of industrial chemicals falls under the jurisdiction of the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation. This organizational body (REACH) is responsible for approximately 100,000 industrial substances classified as industrial chemicals used in the EU (Shaw and Kannan, 2009). Monitoring and investigation of environmental pollutants in food falls under the European Food Safety Authority (EFSA). Pharmaceutical products undergo safety assessments by the European Medicines Agency before being approved for marketing and use. This comprehensive management system, in which food, industrial, and pharmaceutical use of chemicals fall under different regulatory bodies, ensures that no product or chemical does not undergo some form of assessment before being used in the EU.

Europe has some of the tightest chemical regulation legislation in the world, and has earned a reputation of being overly cautious compared with other nations. As a result, they often have lower exposure to environmental contaminants than other countries. However, there are still issues with developing solid guidelines and policies for risk assessments and regulation of EDCs in the EU. For most chemicals in use (minus pharmaceuticals, industrial chemicals and pesticides) risk assessments are conducted as a response to evidence of negative health effects

from exposure, rather than as a preventative measure, usually only after concerns about the health and safety of the chemical have already been raised by the public or research community (Shaw and Kannan, 2009).

### 2.13.5 Pollution management and monitoring

Canada and Europe represent some of the most progressive governments in monitoring and regulation of EDCs. However, there are still many countries that are not signatory or do not abide by international pollution treaties. China is still far behind most western countries in their pollutant management and monitoring programs, although peer-reviewed research and monitoring studies out of China on EDCs in the environment and human exposure have exponentially increased since the early 2000s (Lu et al., 2015). China is a growing destination for international electronic waste, in direct defiance of the Basel Convention to reduce e-waste production and movement (Lau et al., 2012). Illegal e-waste recycling sites are a major source of environmental EDC contamination due to lack of proper recycling equipment and material recovery techniques, and workers at e-waste dismantling sites often have high occupational exposure to EDCs (Lu et al., 2015).

The Stockholm Convention has a well-established Global Monitoring Plan (GMP). This plan provides a framework to monitor and report on POPs listed under the convention for restriction or elimination, and tracks the effectiveness of the Stockholm and Basel Conventions in preventing further global contamination from POPs listed under the conventions (Stockholm Convention, 2019). This database contains monitoring information on 23 POPs that are part of the convention, and focuses on the presence and quantity of these chemicals in surface water, ambient air, and human blood and milk samples.

Another global monitoring effort, run by the World Health Organization (WHO) and several European countries including the United Kingdom, Hungary, Spain, France, Netherlands, Poland and the European Commission, is the Human Reproductive Health and General Environmental Network (HURGENT; Le Moal et al, 2015). This network aims to increase information exchanged between countries about EDC contamination and human health outcomes, and to collaborate on public health problems and developing precautionary management strategies and policies (Le Moal et al., 2015). Although they have yet to release any results from their collaboration, it is a step in the right direction for global environmental health monitoring.

### 2.14 Economic costs of EDC exposure, and newly emerging contaminants

There is an increased health burden on national and global economies as a result of human exposure to EDCs. In light of this, the European Union (EU) requested an impact assessment to gather information and to help shape EDC policy and management strategies (Trasande et al., 2016). The assessment estimated the cost of health effects from exposure to EDCs in the EU at €157 billion per year, which amounts to 1.23% of EU's the total GDP (Trasande et al., 2016). The highest costs were associated with prenatal organophosphate exposure, leading to decreased IQ and intellectual disability, and totaling €146 billion in health care costs (Trasande et al, 2016).

At the global level, humans will be subject to EDC exposure for decades to come, so continual monitoring of exposures are important to appreciate the economic consequences of poor health caused by these pollutants. New evidence points to EDCs having a role in the obesity

epidemic sweeping the world; if the costs of diseases related to obesity are factored into this cost-assessment, the impact of EDCs would be even higher than previous estimates.

New EDCs are produced every year, and it is important for governments to mandate testing and monitoring of these chemicals before they enter the environment and cause damage to the environment and human health. Many places, such as California, have very strict flammability standards for consumer products (Cooper et al., 2016). Bans on PBDEs have led to the emergence of novel flame retardants such as Tetrabromobisphenol A (TBBPA). Novel flame retardants are now being found at urban and industrial locations, with high detection frequencies and concentrations comparable to PBDE levels (Anh et al., 2018).

The safety of these novel flame retardants, which have been labeled by the governments as benign, are now being questioned as independent research groups test their toxicity and environmental health effects (Gramatica et al., 2016). Modeling systems comparing chemical structures of novel flame retardants have predicted that some of the novel FRs introduced as alternatives for banned substances would be persistent, bioaccumulative, and toxic (PBT) in humans and the environment (Gramatica et al., 2016). While biological testing is needed to verify the modeling predictions about the PBT properties of these chemicals, precautionary measures should be taken when mandating legislation of their use.

## 2.15 Newfoundland

### 2.15.1 Currents and EDCs around Newfoundland

The St. Lawrence River has been under federal government management since 1988 in an effort to curb the ecological impact of pollution and conserve the environment (Environment and Climate Change Canada, 2017). This waterway and the associated drainage basin (including the

Great Lakes) is home to over 45 million Canadians and Americans, and drains more than 25% of the Earths freshwater reserves (Environment and Climate Change Canada, 2017). It is home to many urban centers along its banks, as well as large swaths of industrial areas, all of which contribute to pollution in the river. Not all urban waste water treatments plants (WWTPs), such as that located in the Lake Saint Pierre area of the river, have processes for cleaning EDCs such as PBDEs from the waste water, and thus represent a major input of contaminants into the river ecosystem (Pelletier and Rondeau, 2013).

Numerous studies have documented contamination in the St. Lawrence River over the years, at different trophic levels of the food chain. Researchers have documented contamination in fish (Houde et al., 2014), seabirds (Champoux et al., 2017a), and large marine mammal's such as the resident beluga whales (Simond et al., 2017) in the St. Lawrence River and the Gulf of St. Lawrence. Over time, more EDCs and other contaminants have been discovered in the waters along the river, leading the Canadian Government to create a St. Lawrence River Action Plan, to address PBDEs accumulating in the food chain (Armellin et al., 2018). While remediation efforts are being made along the river, including the 2012 Great Lakes Water Quality Agreement, PBDE concentrations in suspended matter and sediments in the river are still on the rise, as they have been since the 1990s (Pelletier and Rondeau, 2013).

The Canadian Arctic and coastal Greenland are the main sources of the Labrador Current (Fratantoni and McCartney, 2010). These are commonly thought of as pristine environments, largely untouched by human urban and industrial activities and therefore relatively unexposed to environmental contamination including EDCs. However, more research focus is being put on contamination in the Arctic, as scientists understand more about water and atmospheric currents. Many EDCs including PBDEs, PCBs, PBBs, and DDT/p,p'-DDE are semi-volatile organic

compounds. These compounds can volatilize in warmer temperature regions (where there is often more human habitation and pollution), and travel long distances on wind currents until they reach cooler climates where they become deposited in the local environment (Xiao et al., 2012). In Alert, Canada (the most northern research station in the country), atmospheric flame retardants have been detected throughout the entire year (Xiao et al., 2012). High trophic-level predators in the Canadian Arctic have tested positive for EDCs, including PBDEs in the glaucous gull (Verreault et al., 2018), caribou, and wolves (Morris et al., 2018), and PCBs in polar bears (Letcher et al., 2018).

Global climate change in the Arctic also affects environmental contamination from EDCs, particularly through the reduction in sea-ice cover and changes in precipitation which may cause the release of more EDCs locked in ice and snow back into the ocean currents and marine ecosystem (Armitage et al., 2011). The Arctic also acts as a sink for many chemicals transported on atmospheric currents, so as climate change continues in these areas, older banned or legacy chemical may undergo a resurgence in the global environment.

Both the Gulf of St. Lawrence and the Labrador Current circle the coasts of Newfoundland, transporting contaminants to this coastal ecosystem (Figure 2.7). The west coast of the Island of Newfoundland receives three main current sources; the Labrador Current, the Gulf of St. Lawrence Current and the North Atlantic Ocean Current. At the northern end of the west coast, the Labrador Current is pushed through a narrow section of water called the Straight of Belle Isle, and follows the Labrador/Quebec coast into the Gulf of St. Lawrence. Meanwhile, the southern part of the west coast receives currents from the Gulf of St. Lawrence, which push up into the Straight of Belle Isle along the western coast of Newfoundland in a counter-clockwise fashion (Nalcor Energy, 2012).

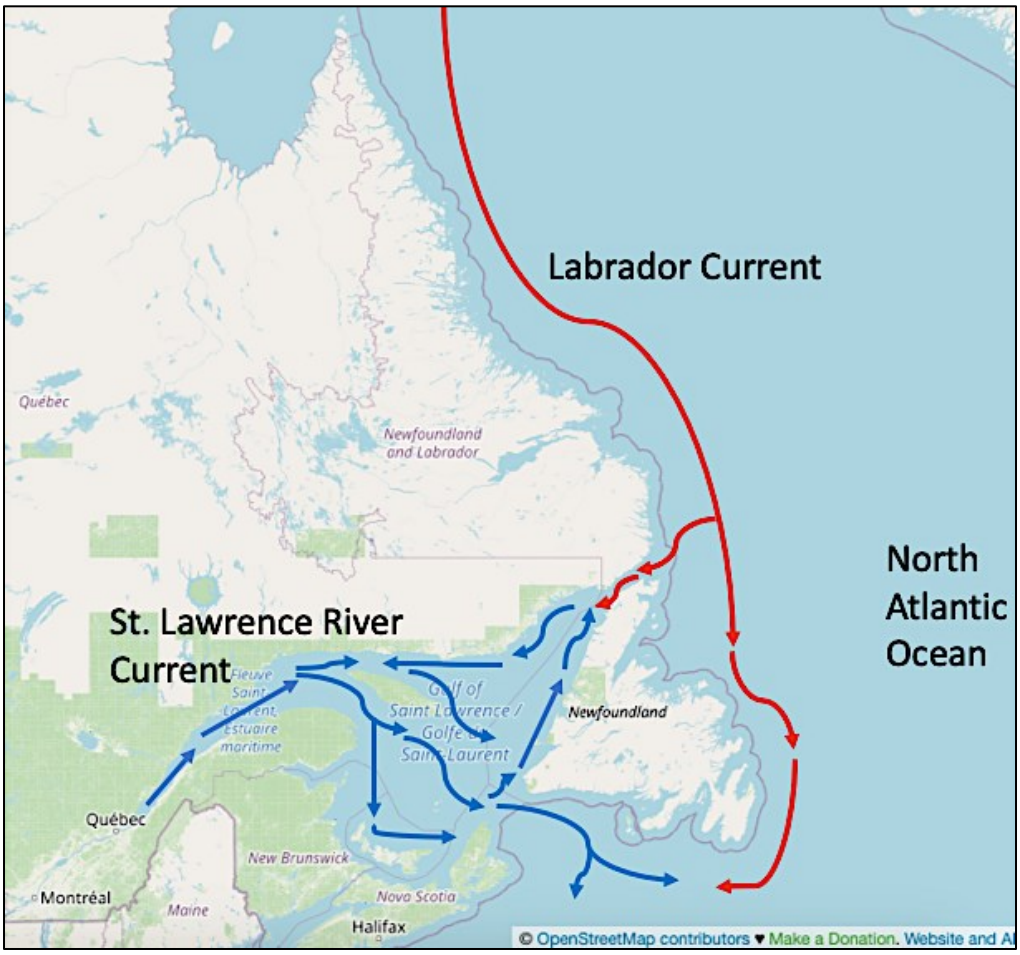



**Figure 2.7** Diagram of the currents around the Island of Newfoundland. Map base © OpenStreetMap contributors.

The south coast of the Island of Newfoundland receives currents from the Gulf of St. Lawrence and the open North Atlantic Ocean. In the Gulf of St. Lawrence lies the Laurentian Channel, which is an underwater valley (ancient St. Lawrence River channel) and a Marine Protected Area due to its importance to a variety of marine species. PCBs, DDT and other EDCs have been detected in sediment core samples from the upper part of the channel located in the St. Lawrence Estuary, indicating the potential presence of contaminants in the marine ecosystem (Lebeuf and Nunes, 2005). Additionally, layering in the estuaries sediments indicated that the highest levels of environmental contamination were years after the peak production and use of

these chemicals, and that the estuary acts as a sink for environmental chemicals, prolonging their presence and release in the environment of the Gulf (Lebeuf and Nunes, 2005).

The northeast coast of the Island of Newfoundland receives the Labrador Current and the open North Atlantic Ocean currents. There is evidence of EDC contamination at the source of the Labrador Current in Greenland (Vorkamp et al., 2008) and Baffin Island (McKinney et al., 2011), and in hooded seals caught in Nain, Labrador (Houde et al., 2017). The Gulf Stream current and the Labrador Current meet off the Grand Banks of Newfoundland, and the Gulf Stream has also been suspected of bringing EDCs such as PCBs up into the North Atlantic Ocean by researchers (Lohmann et al., 2012).

An additional source of contamination in marine species (particularly fish) may come from the ingestion of microplastics caught in the ocean. Research has demonstrated that these small pieces of plastics contain EDCs, and are distributed worldwide on ocean currents (Hirai et al., 2011). In Newfoundland, surveys of cod digestive tracts have revealed very little evidence of microplastics ingestion (2.4% of 205 sampled fish), however this study was conducted solely around the Northeast Avalon peninsula of Newfoundland, an area of high population density projecting out into the North Atlantic Ocean (Liboiron et al., 2016). No studies have been done on microplastics in the Gulf of St. Lawrence, but the St. Lawrence River has sections with very high densities of microplastics in sediments (Castañeda et al., 2014). Additionally, studies conducted in the open North Atlantic Ocean continuously turn up microplastics (detected in 94% of samples), indicating the global presence of these contaminants (Lusher et al., 2014).

2.15.2. History of Newfoundland fishing

Newfoundland is a small island that is part of the easternmost province in Canada. It has with a very low population density, at just 4.39 people per $km^2$, and a total population of approximately 480,000 people. This low population density is partially due Newfoundland's history; the first two hundred years of settler occupation in Newfoundland (from the 16th century onwards) was based on the seasonal fishery, with workers arriving throughout the spring and summer to fish, before returning to Europe or elsewhere to sell their catch in the fall and winter (Heritage Newfoundland and Labrador, 2015). Cod was the main seafood species caught in the fishery, and Newfoundland became known for its salt cod around the world. By the 19th century, more and more fishery workers and their families were choosing to stay in Newfoundland over the winter, and these settlers began to form the towns and cities that exist in the province today (Heritage Newfoundland and Labrador, 2015). Many towns (including the capital city of St. John's) are located on the water because of the persistence of the fishing industry well into the 21st century. By the peak of the fishing industry in 1880, over 90% of the male population of Newfoundland was working in some capacity in the fishery (Heritage Newfoundland and Labrador, 2015). However, a moratorium was placed on cod in 1992, which decimated the provincial fishing industry and forced many Newfoundlanders out of work. The restrictions on the commercial fishery are still in place today, but the tradition of fishing remains embedded in the social fabric of the province, and many small out port communities continue to run small fishing businesses and/or processing plants.

2.15.3 Newfoundland diet

Traditional food ways in Newfoundland involved hunting, trapping, fishing and gardening, though the majority of the local diet is comprised of western style foods purchased through stores (Lowitt, 2013). Many early residents of Newfoundland came for the fishery, and in additional to selling cod they also consumed it. Salt cod (the traditional preserving methods for cod caught in the Newfoundland fishery) was used in dishes such as *fish and brewis*, which remains a staple of Newfoundland cooking today (Lowitt, 2013). Cod tongues are another example of a traditional Newfoundland food, and show the extent to which the different parts of the fish were used for sustenance.

While the modern Newfoundland diet has been studied (Liu et al., 2013; Chen et al., 2015a, Chen et al., 2015b), current seafood consumption rates are not widely documented. Food security has been an issue for households in Newfoundland in the past (Loopstra et al., 2015), and research has shown that fisheries still play an important role in community food security in rural Newfoundland (Lowitt, 2014). A study in Bonne Bay, located on the west coast of Newfoundland, by Lowitt (2014) revealed that most households in the area preferred local over imported seafood, with a strong seasonal pattern of consumption (highest seafood consumption in the summer, lowest in the winter). In this survey, cod predominated (97%) over other seafood for favorite type of local seafood, and was also the most commonly consumed species.

Access to local seafood can occur through a variety of channels in Newfoundland; it can be obtained directly from harvesters, bought from the fish plant or supermarket, or caught directly by consumers themselves in the recreational food fishery. The recreational food fishery allows anglers to fish for ground fish (including cod) without purchasing a license. Fishers are

allowed to catch a maximum of 5 fish per day, and the most recent season of the recreational food fishery lasted 39 days.

### 2.15.4 EDCs in the Newfoundland environment

There have been several studies documenting various EDCs in the marine environment around the island of Newfoundland. Khan (2003) found flatfish from Placentia Bay (on the south coast of Newfoundland) were contaminated with PCBs, and that the health of these bottom-dwelling fish was being negatively impacted through chronic exposure to these chemicals. Khan suggested three local sources of contamination, including a local oil refinery (with a documented history of oil spills) and a PCB-contaminated naval dockyard, but did not explore other large-scale or upstream sources of contamination, such as the wastewater treatment plants in the St. Lawrence River, which have been shown to release PCBs into the river (Pham et al., 1997). Harbor porpoises caught off the coast of Newfoundland have been shown to have EDCs (including PCBs and DDT) in their blubber, indicating the movement of these contaminant up the food chain in the marine ecosystem off the coast of Newfoundland (Westgate et al., 1997). However, much of the literature on EDC contamination around Newfoundland is outdated, and new information is needed to assess and monitor pollution in these waters.

### 2.15.5. Hypothyroidism in Newfoundland

Thyroid disruption can be one health issue resulting from EDC exposure. Recently, Oulhote et al. (2015) documented PBDE exposure in Canadian women, and found higher serum levels of some PBDES (BDE-47, BDE-100 and ΣPBDEs) were associated with increased prevalence of hypothyroidism. In Newfoundland, Sarkar et al. (2015) found an unusual pattern of

hypothyroidism around the Island of Newfoundland, with higher rates of hypothyroidism along the south and west coasts, compared with the northeast coast. The south and west coasts of Newfoundland receive currents predominantly from the St. Lawrence River. Further upstream in the St. Lawrence River, youth from the Akwesasne Mohawk Nation (located by Cornwall, Ontario) showed reduced thyroid function (positive association with TSH, negative association with $FT_4$) correlated with serum PCB levels (Schell et al., 2008). Fish from this section of the St. Lawrence River were documented to have high contaminant concentrations (PCBs, p,'p-DDEs), and therefore may have been a source of exposure to this population.

This information has led to the current research project, to explore possible connections between thyroid disruption and EDC exposure from Newfoundland seafood consumption in the province. There is an array of evidence pointing to EDC pollution being present in the marine ecosystem off the coast of Newfoundland, however it has not been investigated whether consumption of marine species is an EDC exposure source for the population, and what (if any) impact this is having on thyroid function.

## 2.16 Conclusion

EDCs are human-made chemicals causing environmental damage and human health effects throughout the world. Though many of these chemicals have been around for decades, their high levels of use and environmental persistence make them an ongoing environmental and health issue for future populations. The generations being exposed to EDCs now may not experience the outcomes for years to come; therefore, more research needs to be done to prevent exposure, ban harmful or toxic chemicals, and develop sustainable alternatives to act as flame retardants, pesticides and other uses. Many developed countries have acknowledged the health

and environmental effects of EDC exposure, and have policies in place to curb environmental pollution and restrict highly toxic chemicals from use. This has led to the development of novel EDCs, which research shows may be just as, if not more damaging than the chemicals they were designed to replace. Not all countries are as proactive about regulating EDCs, leading to continuing exposure through WWTP effluents, landfill leachate, importation and dismantling of e-waste, and improper recycling of products containing EDCs. Now it is important to examine the effects of EDCs from an ecological, biomedical, public policy, gender, ethnic, and/or a socioeconomic perspective, and to include minority and marginalized populations in EDC research.

In conclusion, the mechanisms of EDC exposure are complicated, and lead to a diverse array of effects as explored in this review. Though much progress has been made in the monitoring and regulation of EDCs in Canada, there is still much to be done. Research from academic institutions such as universities provide a wealth of knowledge on this topic, and will continue to be an important source of information in the future.

# Chapter 3 Methodology

This thesis consists of four distinct sections that correspond to the objectives described in the introduction (Figure 3.1). The main objectives are to: 1) Explore contamination of PBDEs in fish from Newfoundland coastal waters. 2) Determine the species and frequency of local seafood consumption in rural Newfoundland residents from two coastal communities on different coasts of the island. 3) Test blood samples from rural residents who completed the seafood consumption questionnaire for thyroid hormone concentrations (TSH, free $T_4$, free $T_3$) and endocrine disruptors (PBDEs, PCBs, PBBs, p,p'-DDE). 4) Explore relationships between plasma EDC concentrations, thyroid hormones and local seafood consumption frequencies in rural Newfoundland residents.

Briefly, the methods to achieve the objectives were to measure PBDE concentrations in seafood samples (cod and turbot) from different coastal waters around the Island of Newfoundland (Objective 1). The seafood consumption questionnaire was used to explore the different types of local seafood consumed by residents of two different areas of Newfoundland (Objective 2). The third component was to measure thyroid hormones ($FT_3$, $FT_4$, TSH) and EDCs (PBDEs, PCBs, PBBs and p,p'-DDE) in blood samples of the same population that answered the food frequency questionnaire, and to use non-parametric tests to explore effects of age, sex and location (Objective 3). The fourth component was to use multivariate regression analysis to examine whether there were associations between EDC concentrations (predictor) and thyroid hormones (outcome), and also between seafood consumption (predictor) and EDC concentrations (outcome) (Objective 4).

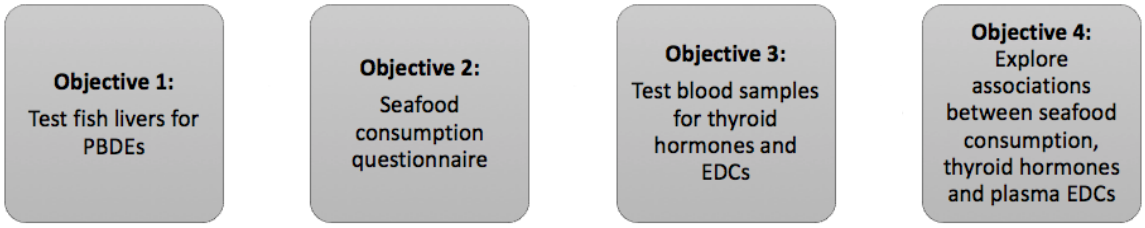



**Figure 3.1** Objectives of the study and corresponding methods

## 3.1 Objective 1: Fish Liver PBDE extraction and analysis

### 3.1.1 Liver Sample Acquisition

Whole liver samples from Atlantic Cod (*Gadus morhua*) and Turbot (*Scophthalmus maximus*) were used for the analysis of PBDE contamination in fish from the Newfoundland marine ecosystem. These species were chosen due to the availability of liver tissues from sampling done by the Marine Institute (Memorial University), and the Department of Fisheries and Oceans (collected May 2014), and from participants of the Newfoundland Recreational Food Fishery (collected Summer 2014). Additionally, there were four cod liver samples from 1993 obtained from the Department of Fisheries and Oceans. Atlantic cod consume food from both benthic and pelagic channels (Silberberger et al., 2018), while turbot are solely benthic predators (Martínez et al., 2016). Cod is the most frequently consumed local seafood species on the west coast of Newfoundland (Lowitt, 2014), which makes the concentrations of PBDEs in fish liver particularly relevant to human health.

Muscle from the fillet of cod and turbot would have been desirable to test for the presence of PBDEs, as this is the type of tissue most commonly consumed by humans, however liver was the only tissue available to us through the Department of Fisheries and Oceans and the Marine

Institute for this study. A benefit of using liver samples is their role in metabolizing and removing PBDEs from an organism making it a site of high tissue PBDE concentrations.

Samples were collected from the open ocean from latitude 47-50°N, and longitude 52 to -45°W, located around the three coasts of Newfoundland (northeast, west and south). Livers were collected directly after harvesting the fish, and stored whole in individually sealed sterile airtight plastic bags at -10°C until analysis. Cod liver tissues from the Gulf of St. Lawrence were obtained as homogenized samples (the tissue has been mechanically separated until it formed a homogenous substance) from two separate dates (1993 and 2014) from fish collected close to the St. Lawrence River section of the Gulf (latitude 48.372 to 50.377°N and longitude 57.318 to 59.387°W). There were 101 cod samples and 24 turbot samples used in the analysis of PBDEs and EDCs. Collection season varied amongst the fish liver samples, and included livers collected in spring, summer, winter and fall months. Cod liver POP concentrations have been shown to increase throughout the winter, due to a corresponding starvation-related decrease in liver fat content (Julshamn et al., 2013). Therefore seasonal variations in POPs may be the result of lipid level changes due to feeding and starvation.

### 3.1.2 Liver Extraction Preparation

Frozen fish livers were defrosted in warm water (approximately 10-15 minutes) until they reached room temperature (~20°C). Approximately ~2g (wet weight) of liver tissue was removed from the liver sample; no specific region of the liver was consistently selected for extraction because there was no consistency in the segments available from the Department of Fisheries and Oceans/the Marine Institute /volunteers (Figure 3.2). The samples were homogenized with a Tissue Tearor homogenizer (Biospec) until they formed a homogenous mixture. The Tissue

Tearor was thoroughly cleaned between each extraction with soapy water and solvent (hexane), and allowed to air dry.

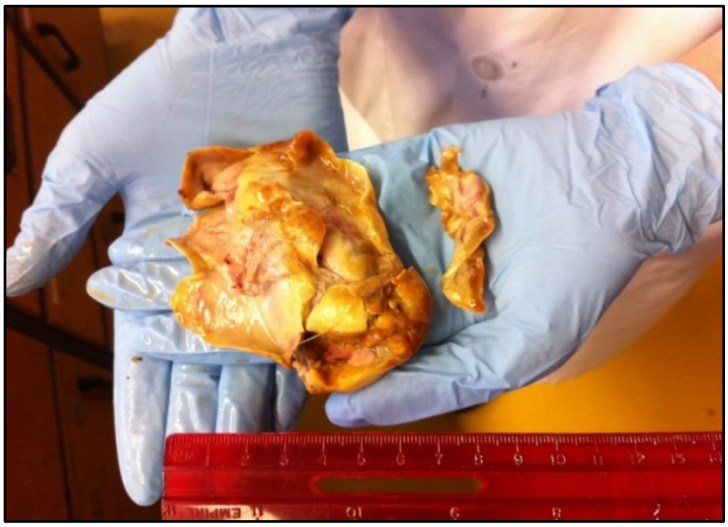


**Figure 3.2** Fish liver samples obtained for use in the study. Samples from the liver were extracted to test for the presence of PBDEs.

A limited number of samples were initially processed and extracted manually; later an Accelerated Solvent Extractor (ASE) was obtained by the supervising faculty member (Dr. Young, Department of Chemistry) and used to process the majority of the remaining samples (Figure 3.3). Both methods were similar in their extraction efficiency of PBDEs from fish livers, as validated by comparing recovery standard concentrations between methods.

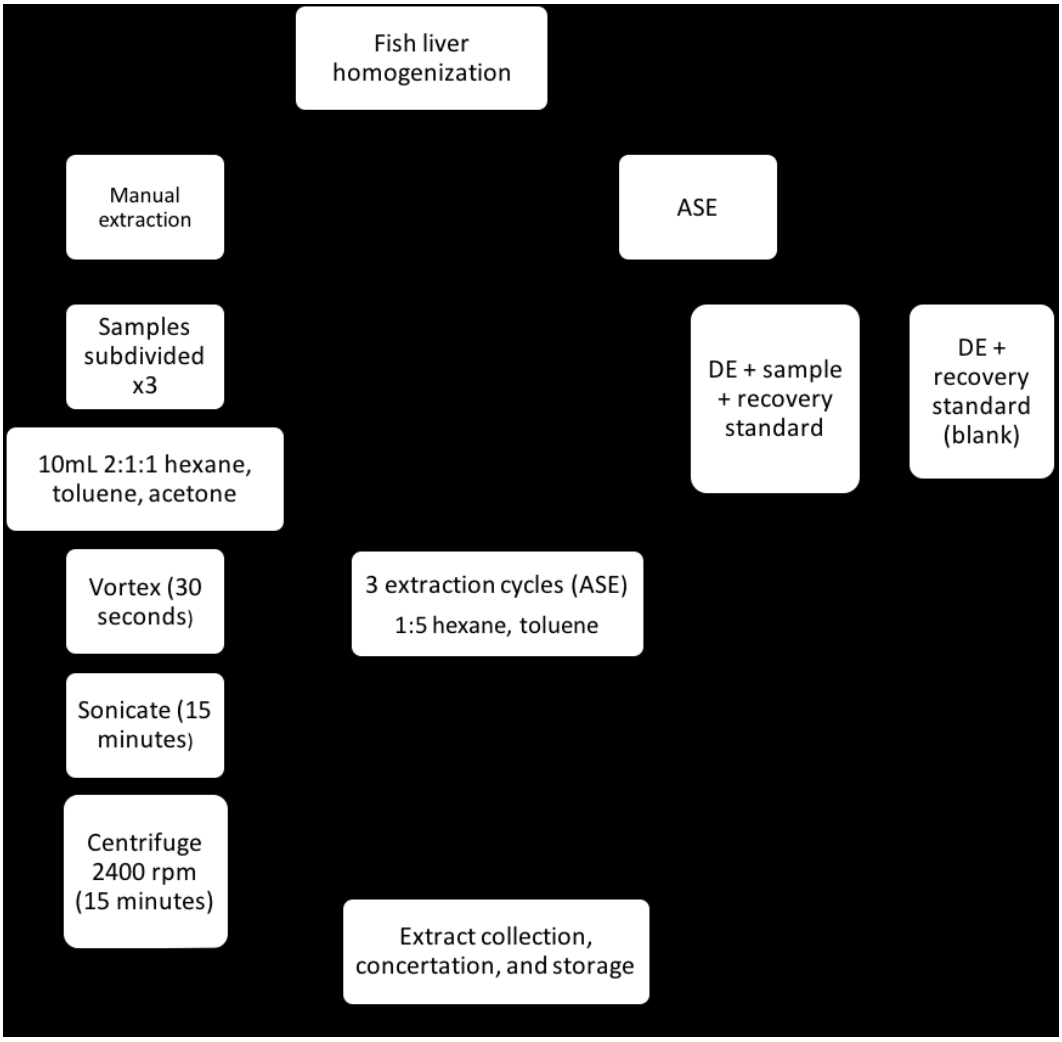



**Figure 3.3** Flow chart of the two extraction methods for fish liver PBDE determination; liquid-liquid extraction (left) and accelerated solvent extraction (ASE, right).

3.1.3 Analyte Extraction Method 1: Manual Liquid-Liquid Extraction

From the homogenized sample, three 0.5 g aliquots of each individual liver were transferred to clean, solvent-rinsed glass centrifuge tubes. 10mL of the extraction solvent mixture (a 9:1 ratio of hexane, and toluene) was added to the centrifuge tubes. Samples were then vortexed (approximately 30 seconds), sonicated (15 minutes), and centrifuged at 2400 rpm (15 minutes).

Solvent extract supernatant was collected by Pasteur pipet and transferred into a clean, solvent-rinsed centrifuge tube. Remaining tissue was subjected to extraction two more times, for a total of 3 solvent-based extractions. The pooled solvent extracts were concentrated to 1mL under a gentle stream of nitrogen, before further clean-up procedure by Solid Phase Extraction (SPE; see method in section 3.1.5 of methods).

### 3.1.4 Analyte Extraction Method 2: Accelerated Solvent Extraction (ASE)

The majority of fish liver samples were processed using automated extraction on a Dionex 350 ASE (Thermo-Fisher Scientific), obtained later in the study. The ASE is an excellent tool to reduce the length of time required to extract samples, as well as to improve precision, provide consistent experimental parameters, and process numerous extractions at one time. Specially-designed ASE cells were used for sample extraction. The ASE cells consisted of a stainless-steel body with screw tops at each end. Each cell and the corresponding glass collection vial was pre-cleaned with soapy water and rinsed first with acetone, followed by a rinse with extraction solvent mixture prior to use. Hexane and toluene were used at 5:1 solvent ratio mixture for the ASE extraction methods, based on method optimization performed prior to extracting the fish liver samples. Filters (solvent-rinsed) were positioned at the bottom of each ASE extraction vial, and new septa (solvent rinsed) were used for each collection vial (Figure 3.4).

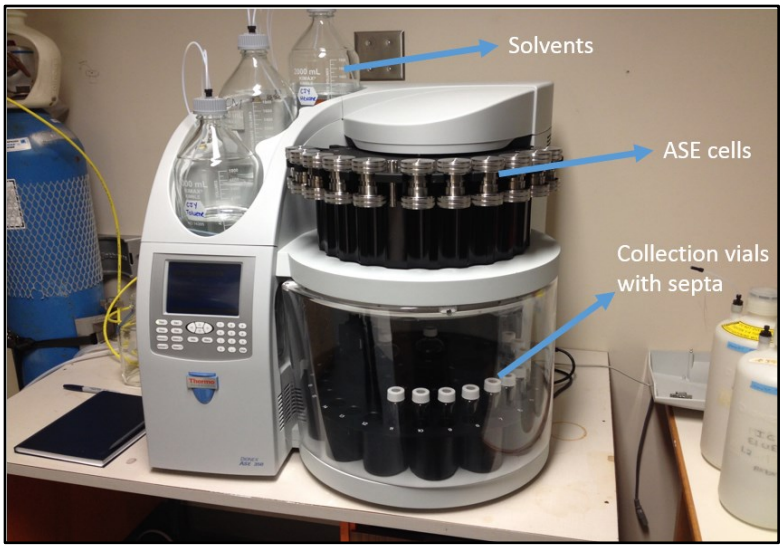



**Figure 3.4** Dionex 350 ASE (Thermo-Fisher Scientific) used in extraction method #2. Samples are held in stainless steel cells (top) and extracted into glass vials (bottom).

Diatomaceous earth (DE) was baked at 500°C for minimum 4 hours prior to use as a bulking material to fill the extraction cells. ASE cell preparations included loading the cells with DE approximately half way up the tube. This was followed by 0.5g homogenized fish liver (room temperature), and 2.5µL BDE-103 (4ng/g) recovery standard. This was topped with more DE until the tube of the ASE extraction cell was completely full. Extraction parameters for the ASE were based on methods determined by Ghosh et al. (2011), to maximize PBDE extraction from fish. Identical conditions were used for: temperature (100°C), pressure (1500 psi), nitrogen gas purge (100 seconds), and heat-up time (5 minutes), and each sample was extracted three times in a 5-minute static mode. After ASE samples were collected, they were concentrated to approximately 1mL under nitrogen gas, and stored at 10°C until SPE cleanup.

3.1.5 Sample Cleanup with Solid Phase Extraction (SPE)

All samples from both methods underwent cleanup using SPE prior to gas chromatography mass spectrometry (GC-MS) analysis (Figure 3.5). SPE was a necessary step to maximize analyte extraction and minimize analyte loss due to retention in the fish liver matrix or extraction column.

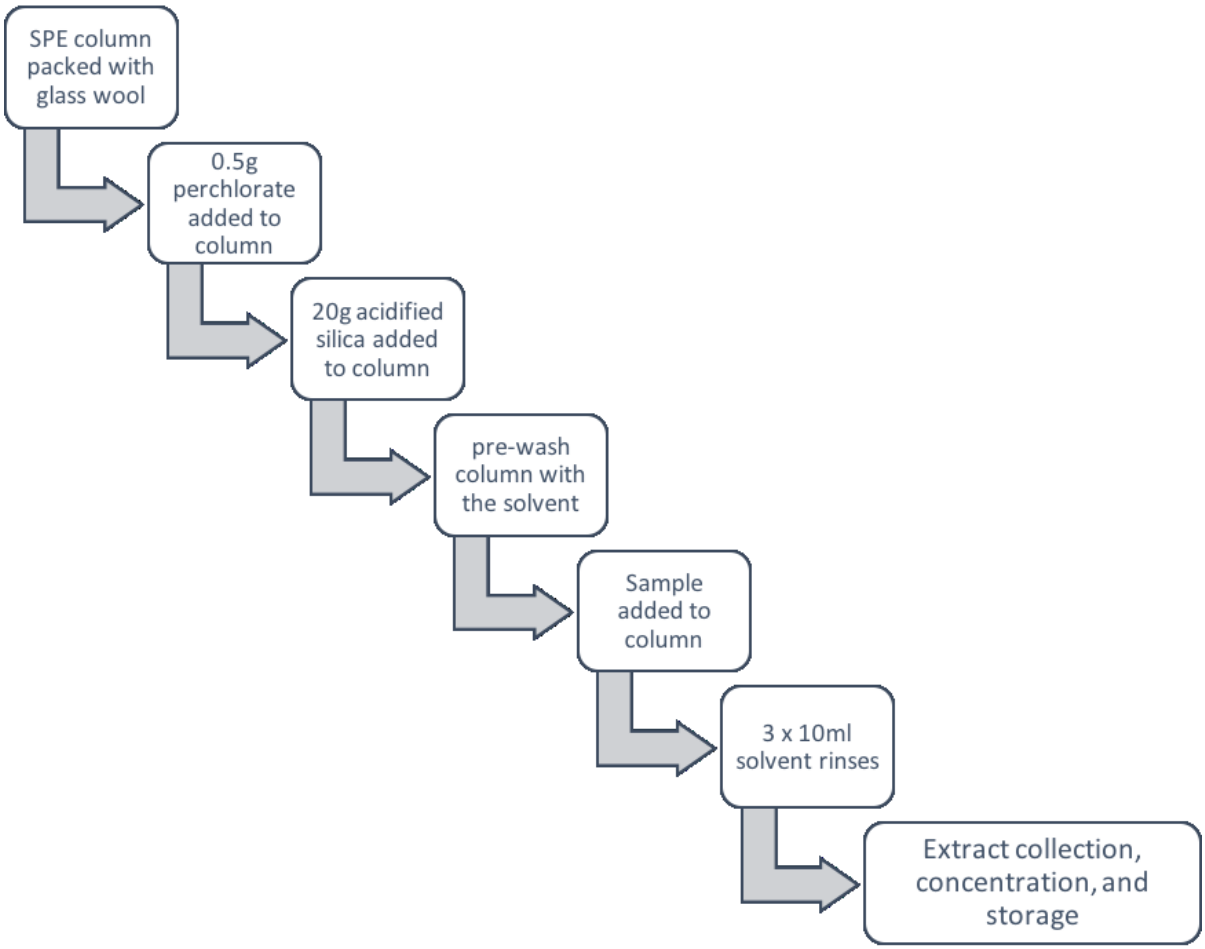
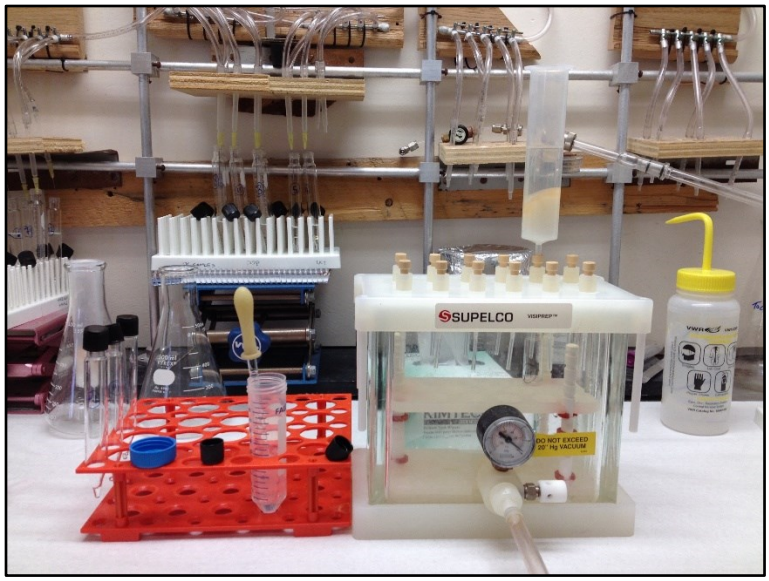



**Figure 3.5** Flow chart of SPE cleanup methods. After setup is complete (steps 1-5), columns are washed with three solvent rinses, while extracts are collected and concentrated to 0.5mL.

The SPE column consisted of silica gel (60 - 120 mesh, or 60 ECO 40 - 63 μm) as the inert, polar sorbent. Preparation for the SPE cleanup stage involved 1) baking silica and glass wool (column packing agent) at 500 °C for approximately 4 hours, then allowing them to cool to room temperature before use, and 2) cleaning the SPE columns (both 10mL and 30 mL columns were used at different times) and manifold with soapy water, followed by a rinse with extraction solvent. After cleaning, SPE columns were packed with glass wool at the bottom of the tube to hold back the silica and allow the solvent and extract to pass through.

To pack the column, 0.5g of sodium perchlorate was layered on top of glass wool to act as a drying agent. The silica gel was first treated with sulfuric acid (~40% w/w) to help break down the lipids in the liver samples. After acidification, the SPE column was then packed with approximately 20g of acidified silica gel. Prior to adding the sample, columns were pre-washed with 10mL of extraction solvent. Sample extracts (obtained from extraction methods 1 or 2) were added to the top of the column, followed by three 10mL solvent washes to rinse the analyte residue from the SPE column into a collection vial. Lipids and other impurities from the sample extract were retained in the column (stationary phase), as indicated by the emergence of a brown color after the extract was added to the column, while the analyte was eluted along with the solvent into collection vials (Figure 3.6). Following initial testing of the cleanup procedure, we increased the column size from 10 mL SPE columns to 30 mL SPE columns and used a greater quantity of acidified silica gel (300% increase from the initial amount), to ensure appropriate active exposure of the stationary phase during analyte extraction.

a)

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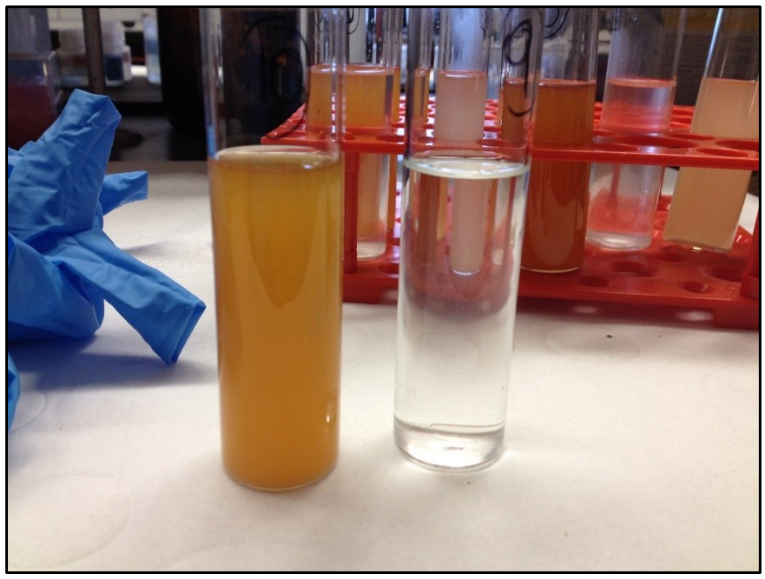


**Figure 3.6** a) Solid-phase extractions (SPE) of fish liver samples, and b) visual representation of extracts before (left vial) and after (right vial) SPE cleanup.

After the analyte was eluted and SPE cleanup was complete, the samples were concentrated under a stream of nitrogen until the final volume of samples were approximately 0.5mL. Post SPE cleanup, samples were transferred to 1.5ml glass gas chromatography (GC) vials and stored in the lab fridge (10 °C) until further analysis.

3.1.6 Gas chromatography mass spectrometry (GC-MS): analytical method used to determine fish liver PBDE concentrations

Fish liver extracts were analyzed by Agilent 7890A gas chromatograph (GC) coupled to a 5976C mass spectrometer (MS). This was operated in electron chemical negative ionization (ECNI) mode (70eV). Calibration of the MS was done with 1,2-bis(2,4,6-tribromophenoxy) ethane standard, under auto-tune parameters. Several PBDE standards (1,5-Dibromo-3-[2,4-dibromophenoxy]-2-methoxybenzene, 1,5-Dibromo-2-[2,4-dibromophenoxy]-3-methoxybenzene, 4'-2,3',4,6-tetrabromodipheyl ether, BDE-62, BDE-118, 2,2',4,5',6-Pentabromo[$^{13}C_{12}$]diphenyl ether, and 2,2',3,3',4,5,5',6,6'- Nonabromo[$^{13}C_{12}$]diphenyl ether) and Wellington Laboratories BDE-MX (pre-mixed and purchased standard) were resolved by GC-MS temperature program, to identify and measure PBDEs in the fish liver samples.

Prior to injecting samples, the GC-MS autosampler went through solvent washes with acetone and hexane. Fish liver extracts were then injected into the injection port by the autosampler (1µL/sample). Samples were run through a DB-5HT capillary column (15 m column length, 0.250 mm, 0.10µm, Agilent Technologies Inc.). A splitless injection mode (4.0 min splitless-time at 320 °C injection temperature), and helium gas as the carrier agent (flow rate of1.5 L/min) were chosen as they are more suitable for trace analysis and highly diluted samples, and do not allow gas (and sample) to vent through the split valve. After samples were added, the injection port remained at 260 °C and the MS transfer line was held at 280 °C. The following oven temperature program was followed for the remainder of the run (total run time 26.4 minutes):

- Initial hold (5 minutes) at 100 °C
- First temperature ramp up of 25 °C/min to 250 °C

- Second temperature ramp up of 5 °C/min to 265 °C
- Third temperature ramp up of 25 °C ramp to 325 °C

Elution results from the GC-MS of PBDEs were analyzed using GC-ECNI-MS, and concentrations were quantified using bromide anions (79 and 81 m/z). PBDE congeners were confirmed using 161 and 247 m/z, in selective ionization monitoring (SIM) mode.

### 3.1.7 Quality assurance and quality control

Quality assurance (to measure and assure the quality of the samples) and quality control (ensuring samples meet scientific standards) were determined through the use of an internal calibration standard (PBDE-118) implemented with every batch of samples that were extracted. Prior to injection, cleaned and concentrated samples had 1.5 μL volume of PBDE-118 (2 ppm) added as an internal standard. Variability in individual sample results (as a result of the GC-MS) was further reduced by running triplicate GC-MS injections of each sample. Analytical blanks were used to determine method detection limits, based on Wellington Laboratories Inc. (Ontario, Canada) PBDE standards.

## 3.2 Objective 2: Seafood Consumption Questionnaire (SCQ)

Two different towns were chosen as sites for the food frequency survey based on their coastwise locations (from data of higher hypothyroidism rates on the south versus the northeast coast of Newfoundland by Sarkar et al., 2015) and their proximity to a healthcare centre with blood collection services. The Burin data collection (south coast) was performed in February 2018, and New-West-Valley (NWV) data collection (northeast coast) was conducted in April 2018.

These population sampling sites were both chosen as the study locations due to their small population size (~2000-3000 residents), and their location (rural and located on different coasts of the island); these criteria were important as the aim of this project was to study exposure in locations where access to imported seafood would be less (smaller towns with fewer grocery stores) and access to local seafood was high (towns with fish plants and fish harvesters).

Participants were recruited by the Health Research Unit (HRU), a part of the Division of Community Health and Humanities at Memorial University that provides support and services to researchers in the area of health research. The HRU purchased telephone numbers for the chosen communities from InfoCanada©; participants were called and asked if they would be willing to take part in a seafood survey and give a blood sample. Inclusion criteria for participants was that they were over 18 years of age, not pregnant at the time of the study, and capable of giving consent to participate. We originally excluded participants who had a known thyroid condition or had partial of full removal of the thyroid gland, but further evaluation led to recruitment of a small number of participants (n=6) with diagnosed hypothyroidism. This was done to evaluate the environmental pollutant concentrations in individuals with hypothyroidism, to explore if they were different from the non-hypothyroid-diagnosed population. Participants were also recruited through advertisements on social media (town council Facebook page), and emails circulated through the local healthcare centre and YMCA centre by staff members. Several participants who had come for regular blood collection at the hospital lab were also recruited into the study by clinical staff at the hospital.

Initially, 130 participants agreed to participate in the study, however, the final number of study participants was 80. There were 6 participants recruited through social media, 2 recruited through advertising at the local YMCA (Burin), 16 recruited from the Burin blood collection

clinic at the Burin Peninsula Health Care Centre, 4 recruited through the NWV blood collection clinic at the Kittiwake Health Centre (NWV) and the remaining 52 were recruited through phone calls using purchased phone numbers. This sample size aligns with other peer-reviewed exploratory/pilot studies done in Canada (e.g. Sandanger et al., 2007 had a sample size of n=110). Additionally, studies in other countries have also used similar sample sizes; Byrne et al. (2018, US) had a sample size of n=84 participants, and Zhang et al. (2010, China), who had a sample size of n=50. Both studies examined relationships between blood EDCs and thyroid hormones.

Participants were booked into 30-minute time slot appointments to complete the Seafood Consumption Questionnaire (SCQ) and blood sample collection, with an average of 3-6 participants per slot. There were 50 participants who dropped out of the study between recruitment and sample collection due to a variety of factors including non-attendance (unable to be contacted before or after missed appointment), bad weather, lack of transportation to the collection site, and forgetting their appointment. This may be a possible point of bias in our study, as participants who had independent transportation, good memory skills, and an easy commute to the study site would have had a greater ability to attend the study collection location and participate.

All participants filled out the SCQ, designed and modified from the seafood consumption survey conducted on the west coast of Newfoundland by Lowitt (2013). The survey from Lowitt (2013) was an investigation of local versus imported seafood preference in the Bonne Bay region on the west coast of Newfoundland. The survey was obtained through correspondence with the author (Lowitt). The adapted SCQ was field tested on 6 non-participants of different ages, genders, education levels, and seafood consumption patterns to check the validity of the survey

(no changes were made to the survey after the field testing). The survey consisted of two pages of quantitative questions, and included questions on consumption of 15 types of local seafood over the past month (see Appendix A). Local seafood was defined in the SCQ as "seafood harvested close to the community (harvested from the same coast), whereas as non-local seafood refers to seafood from other coasts (Newfoundland), countries or provinces, or seafood of which the participant is unsure of the source". Other questions included frequency of local seafood consumption at different times of the year (fall, winter, spring, summer), and demographic questions about age, postal code, sex, and years of residence in the community. Surveys were coded and entered into SPSS for analysis.

### 3.3 Objective 3: Blood sample collection

All participants who completed the SCQ gave a blood sample to be tested for thyroid hormones and EDCs. Blood samples for EDC analysis were collected in 4 x 4mL BD Vacutainer ethylene-diamine-tetra-acetic acid (EDTA)-coated tubes by a medical lab technician. Samples were drawn into tubes and inverted 7-8 times, to mix thoroughly. Samples were centrifuged for 10 minutes at 2500 revolutions per minute (rpm) to separate out the plasma from blood cells. Plasma was transferred to a pre-cleaned 7 mL glass vial, with Teflon disc screw-top (Supelco #23741) (Figure 3.7). Plasma samples were frozen and stored at -20˚C, until shipment on dry ice to the Institute National de Santé Publique (INSPQ) in Quebec City (Quebec) for analysis of EDC concentrations.

Blood samples for thyroid hormone analysis were collected in 1 x 3.5ml BD Vacutainer gold top serum-separator tube. Briefly, blood was allowed to clot for 10 minutes, then centrifuged at 2500 rpm for 10 minutes. Serum was collected and stored at -20˚C until transportation back to

the Health Sciences Centre (St. John's). Samples were securely packed and shipped with frozen cold packs, before being stored at -20˚C upon arrival in the clinical biochemistry laboratories at the Health Sciences Centre.

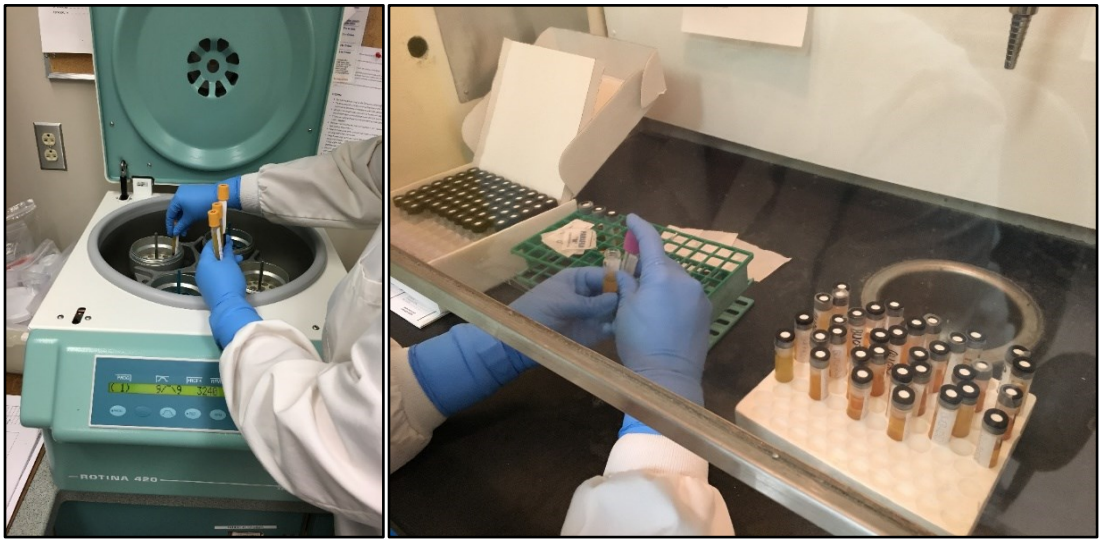



**Figure 3.7** Medical lab technician preparing plasma samples for centrifuging at Burin Peninsula Health Care Centre.

### 3.3.1 EDC extraction and analysis

Serum EDC extraction and analysis was completed by the Institute National de Santé Publique du Quebec (INSPQ) in Quebec City. Multiple congeners of PBDE, PCB, PBB and p,p'-DDE/DDT were measured in serum samples. Originally, the intent was to measure only PDBEs in serum to correlate to information from the fish live results. However, collaborating with INSPQ presented the unique opportunity to also test for PCBs, DDT/p,p'-DDE and PBBs in serum since they could be measured in the same run used for measuring PBDEs. Incorporation of these chemicals into the study was appropriate, as the literature shows that the fate of these EDCs in environment are similar; they bio-accumulate in the marine food chain, have been

shown to affect thyroid hormones, and can be detected in serum (reviewed in Boas et al., 2012). Additionally, correlating seafood consumption with serum concentrations of multiple EDCs and thyroid hormones may lend more strength to the results of this study, as humans are more likely exposed to a multitude of chemicals through seafood consumption.

Guidelines for the Study of the Dietary Intakes of Chemical Contaminants by the World Health Organization recommends monitoring for health effects caused by exposure to environmental pollutants in special population groups, whose diets may differ from general population (Food and Agriculture Organization and World Health Organization, 1985). This corresponds to the work that was done in this study, with assessing thyroid hormone and serum EDC concentrations in relation to seafood consumption. It should be noted that while we were able to measure PBDEs in both fish and human serum samples, it was not possible to measure the other EDCs (PCBs, PBBs, p,p'-DDE) from the serum samples in our fish liver samples, due to lack of laboratory capabilities.

EDCs were extracted from participant plasma samples, using the methods described by Fisher et al. (2016). Briefly, internal standards were added to the plasma samples; EDCs were extracted using liquid-liquid extraction with hexane and a saturated ammonium sulfate solution. The hexane layer containing the extracts was removed and concentrated, then purified on activated florisil columns using a solution of dichloromethane: hexane (9 mL; 25:75). Internal labeled standards including hexachlorobenzene- $^{13}C_6$ , α-HCH-$^{13}C_6$ , oxychlordane-$^{13}C_{10}$, trans-nonachlore-$^{13}C_{10}$, PCB 141-$^{13}C_{12}$, PCB 153-$^{13}C_{12}$, PCB 180-$^{13}C_{12}$, PCB-194-$^{13}C_{12}$, 3,6-F2-PBDE 99, Parlar 26-$^{13}C_{10}$, Parlar 50-$^{13}C_{10}$, p,p'-DDE-$^{13}C_{12}$, PBDE-101 and PBDE-77-$^{13}C^{12}$) were used to determine percent recovery of analytes after extraction. Solvent extracts were run through GC-MS (Agilent 6890 Network/7890A coupled to an Agilent 5973 Network/5975C, Agilent

Technologies; Mississauga, Ontario, Canada). Column used was an Agilent 60 m DB-XLB column (0.25 mm i.d., 0.25 μm film thickness), with helium as a carrier gas and 3μL injections (splitless mode). Methane was used as the reagent gas for mass spectrometry, which was operated in select ion monitoring mode, with negative chemical ionization. Final concentrations of the EDCs measured in serum were given in μg/L, and the limits of detection (LOD) fell between 0.005 and 0.3μg/L, depending on the compound.

Total serum lipid level (g/L) was a combination of total cholesterol (TC), free cholesterol (FC), triglyceride (TG) and phospholipid (PL) levels measured by enzymatic methods and colorimetry. EDC concentrations were lipid-normalized prior to statistical analysis to reduce variability due to lipid profile; this can be done with chemicals that vary in direct proportion to lipid concentration, and allow for corrections in variable serum lipid content. PBDEs, as an example, have lipophilic characteristics and because they are associated with lipid levels, their concentrations should be normalized to the lipid content of the serum sample (Vorkamp et al., 2014).

### 3.3.2 Thyroid hormone testing

Thyroid hormones were tested at the Clinical Biochemistry Laboratory, located at the Eastern Health Authority's Health Sciences Centre (St. John's). After defrosting, samples were briefly mixed and centrifuged at 2000 rpm. Each sample was tested for thyroid stimulating hormone (TSH), free thyroxine ($FT_4$) and free triiodothyronine ($FT_3$) using an Architect Chemiluminescent Microparticle Immunoassay kit (Abbott Laboratories). Serum samples were run on an Architect i2000SR immunoassay analyzer (Abbott Laboratories). Concentrations were given in mlU/L (TSH) and pmol/L ($FT_4$, $FT_3$).

### 3.4 Objective 4: Analysis of the association between EDCs and thyroid hormones

All statistical analyses (Mann-Whitney U-test, Shapiro-Wilkes tests, Kruskal-Wallis H-test, and multiple linear regressions) were conducted using SPSS© software (version 26, IBM). There were 5 PBDE congeners (PBDE-28, -47, -99, -156, -209) and 2 methoxy-BDE congeners (MeO-BDE-47 and MeO-BDE-68) detected in fish liver samples. We did not test for any other EDCs, as it was only possible to identify and quantify these congeners; therefore, they were the focus of this analysis. EDC concentrations at the four different collection locations (selected by DFO and Marine Institute to sample fish for other non-related research work) were compared separately for cod and turbot using a non-parametric Kruskal-Wallis H-test, as PBDE concentrations between locations were right-skewed (McDonald, 2009). The Mann-Whitney U-test was used to test for differences in EDC concentrations between species, as again the cod and turbot congener concentrations were right-skewed. The Kruskal-Wallis H-test differs from the Mann-Whitney U-test in that the former can accommodate three or more groups in the analysis of differences between medians.

For the seafood consumption questionnaire (SCQ), a difference in consumption frequencies by species and by season between demographic groups (age, sex, community, and hypothyroidism) were analyzed by chi square test. Some of the species had a limited number of responses for seafood consumption frequency more than once per month, and therefore violated the chi square test assumptions. The responses for these species were amalgamated into fewer response categories and analyzed by Fisher's exact test.

Serum lipid concentrations are highly predictive of PBDE (and other lipophilic EDC) concentrations, therefore it is important to control for lipid levels in serum EDC analysis as serum lipid levels vary in the population and may affect serum EDC concentrations (Makey et

al., 2014). As such, results from the EDC testing were adjusted for plasma lipid concentration prior to statistical analysis. SI units (pmol/g) were used for serum EDC analysis, as they take into account the molecular weight of the molecule. The number of halogens (i.e. bromines or chlorines) on EDCs such as PBDEs and PCBs can vary along with the molecular weight.

EDCs that had more than 5% of their total number of samples fall below the limit of detection (LOD), had their <LOD concentrations re-set to LOD/2 (Lubin et al., 2004). Median and interquartile range were calculated for all EDCs and THs with ≥70% detection frequency (more than 70% of our participant samples has concentrations above the LOD). Age, sex and community were coded as categorical variables, with age divided into two groups (<50 years and >50 years of age) based on changes in thyroid hormone concentrations with age (Surks and Hollowell, 2007). The Mann-Whitney U-test was used to compare the difference between EDC and thyroid hormone concentrations between sexes, age groups and communities, with a significance level of $p<0.05$.

We tested for normality in the thyroid hormone and EDC data by constructing histograms to look for skew and running Shapiro-Wilkes tests for normality; this has been recommended as one of the best statistical tests for assessing normality of a data set (Thode, 2002). All the EDC data were right skewed, as was the TSH data, so these values were $\log_{10}$-transformed to satisfy conditions of normality for subsequent regression analysis (Curran-Everett, 2018). $FT_4$ and $FT_3$ were not skewed, and therefore not transformed. Outliers were then identified by determining the inter-quartile range (IQR) and creating lower (Q1 –[1.5xIQR]) and upper (Q3 + [1.5xIQR]) fences (methods from Sjödin et al., 2018). There was one outlier outside of the IQR range ± 3 standard deviations (PBB-153), and this value was winsorized to the next highest value within 3 standard deviations of the range of the mean (Verner et al., 2015).

Relationships between local seafood consumption and plasma EDC concentrations were assessed using a Kruskal-Wallis H-test, as the plasma EDC concentrations did not satisfy conditions of normality (McDonald, 2009). Univariate and multivariate linear regression was used to test association between EDCs, thyroid hormones, and seafood consumption, and to test the effects of the population covariates (age, sex, community) on the outcome variable. There were a number of missing responses in the SCQ, and these values were coded as "No Answer" and excluded from further analysis.

Univariate linear regression was run first, to test for association between EDCs and thyroid hormones in the absence of any other co-variates. Afterwards, multivariate linear regression was run to test for relationships between serum EDCs and thyroid hormones with age and sex as covariates, and community as an exposure variable.

# Chapter 4 Results

This chapter presents the results and analysis of all aspects of this study, including levels of PBDEs in fish livers, seafood consumption frequency, plasma EDC concentrations [PBDEs, PCBs, PBBs and p,p'-DDE] and serum thyroid hormone concentrations in study participants. This study began with an analysis of the concentrations of PBDEs and methoxylated (MeO)-BDEs in fish liver samples from two fish species (cod and turbot) from different collection locations around the Island of Newfoundland (Objective 1). Local seafood consumption by rural residents was explored by fillable surveys, to determine the extent of local seafood consumption in different parts of rural Newfoundland (Objective 2). Blood samples were collected and tested for the presence of PBDEs, PCBs, PBBs and p,p'-DDE in the Newfoundland population, and thyroid hormone concentrations were measured in the same blood samples (Objective 3). The association between thyroid hormones and EDCs was assessed, and the effects of seafood consumption on plasma PBDE, PCB, PBB and p,p'-DDE concentrations were also investigated (Objective 4).

## 4.1 Presence of PBDEs in Fish Liver

Objective 1 of this thesis was to test samples of liver from cod (n=101) and turbot (n=24) from different parts of Newfoundland for the presence of PBDEs and MeO-BDEs.

### 4.1.1 PBDEs in fish liver

Fish liver analysis was limited to PBDEs -28, -47, -99, -156, -209, 6-MeO-BDE-47 and 2'-MeO-BDE-68 (expressed as ng/g of liver), as these congeners were reliably identified during method development of fish liver extraction in collaboration with Dr. Bautista in Dr. Young's

research laboratory. All fish had at least 2 of the 7 examined PBDEs present in their liver tissue, and the majority (63%) of fish liver samples had 5-6 of the PBDEs present. Fish were caught at a range of latitudes (45.082 - 50.9835 N) and longitudes (48.014 – 59.354 W), from boats in deep ocean (see Figure 4.1).

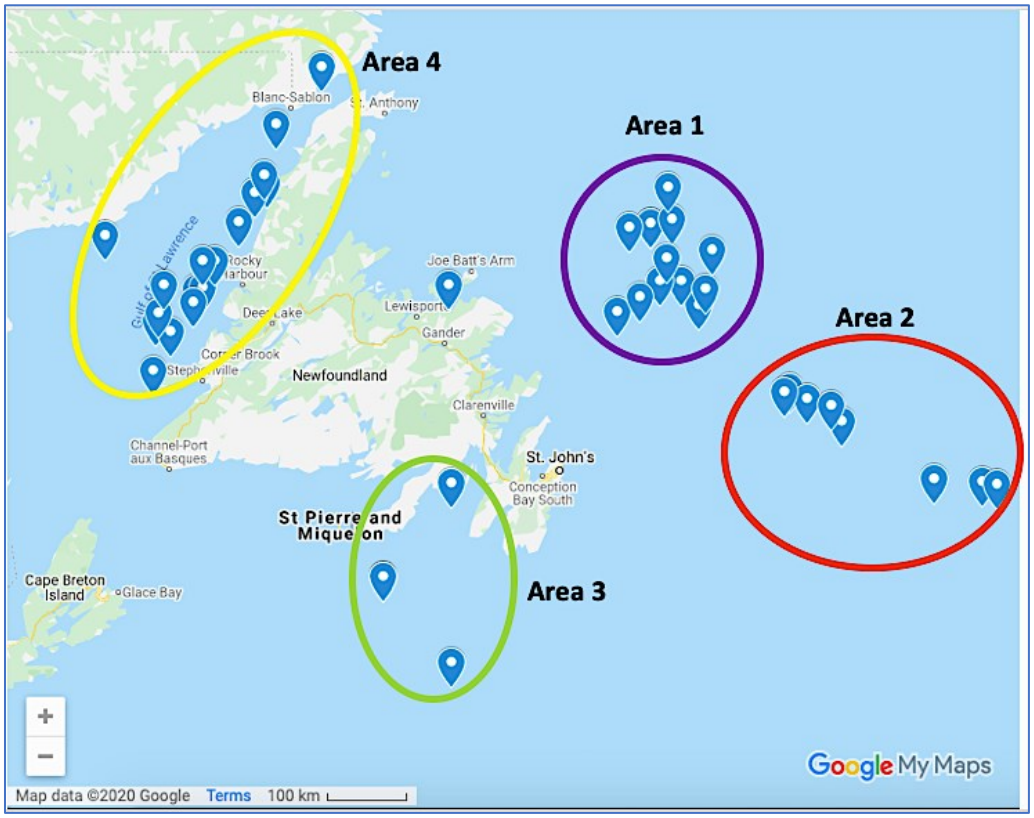



**Figure 4.1** Location of fish caught around Newfoundland. Fish sampling sites were sub-divided into 4 main areas based on their location coast-wise. Map data ©2020 Google.

The cod liver samples consisted of fish collect on different dates (1993 [n=4] and 2014 [n=97]) and from different locations (1993 samples from Area 4; 2014 samples from Areas 1 to 4). For cod, the number of samples per area (all collection dates) was: Area 1 n=38, Area 2 n=17,

Area 3 n=6, Area 4 n=40. For turbot, the number of samples per area (all collection dates) was: Area 1 n=15, Area 2 n=7, Area 3 n=1, Area 4 n=1.

Histograms were constructed for each EDC to test for skewness of the data (see Appendix B). PBDE concentrations were compared between locations (Areas 1 to 4, both species, all 2014 samples), between species (all areas, 2014 samples), and between dates (all areas and species) using non-parametric tests, as the concentrations were not normally distributed (right-skewed). Table 4.1 presents median concentration of different PBDE congeners using combined data from 4 areas (2014) and all fish species. Analyzing PBDE concentrations of both fish species together in Table 4.1 provides information on trends in congener levels across species from similar trophic positions in the food chain.

**Table 4.1** Descriptive statistics of PBDE and MeO-BDE concentrations (ng/g) (combined from cod and turbot) from 4 different locations around Newfoundland (all samples from 2014).

| PBDE congener | Range (ng/g) | Median (IQR) |
|---|---|---|
| PBDE-28 | <LOD-32.88 | 0.93 (0.00-3.10) |
| PBDE-47 | <LOD-93.94 | 9.17 (2.24-23.19) |
| PBDE-99 | <LOD-296.94 | 0.03 (0.00-1.78) |
| PBDE-156 | <LOD-128.58 | 0.00 (0.00-3.14) |
| PBDE-209 | <LOD-31.53 | 0.00 (0.00-0.00) |
| ∑PBDE | <LOD-368.27 | 15.46 (6.43-43.65) |
| 6-MeO-BDE-47 | <LOD-162.68 | 7.68 (0.00-20.33) |
| 2'-MeO-BDE-68 | <LOD-158.44 | 11.10 (3.09-21.92) |

IQR=interquartile range, LOD=limit of detection, n=124 fish for all PBDE congeners.

Only 15% of all recent (2014) fish liver samples were found to have detectable levels of PBDE-209. Other studies have also had trouble detecting the presence of this congener in fish samples (de Boer et al., 2003; Pulkrabova et al., 2007), possibly due to matrix effects within the fish sample, but our results could also be indicative of poor biomagnification of PBDE-209 in higher trophic level organisms in this ecosystem (Hardy et al., 2009). PBDE-209 is very hydrophobic with a log $K_{ow}$ of approximately 10 (note: the octanol | water partitioning ratio values are inversely related to a chemical's solubility in water and proportional to the molecular weight of the chemical [Voutsas, 2007]), which may affect its ability to bioaccumulate. PBDE-47 had the highest average concentration of all 2014 liver sample PBDE congeners, although PBDE-99 had the highest maximum congener concentration. Both 6-MeO-BDE-47 and 2'-MeO-BDE-68 had very similar concentrations, although 2'-MeO-BDE-68 was found at slightly higher concentrations in liver samples.

4.1.2 PBDE concentrations by date

We used a Mann-Whitney U-test to analyze samples from different dates (1993 and 2014). These results must be interpreted with caution, due to the small number of samples from 1993 (n=4) versus 2014 (n=120). Overall, there was a trend was towards higher PBDEs in the 1993 samples (median [IQR]):

- PBDE-28 was higher in 1993 (4.57 [4.04-25.87] ng/g) than in 2014 (0.86 [0.00-2.72] ng/g, U=429, p=0.007).
- PBDE-47 was greater in 1993 (71.62 [60.48-83.18] ng/g) than in 2014 (8.48 [2.16-19.23] ng/g; U=472, p=0.001).

- ∑PBDE concentrations were also higher in 1993 (95.46 [68.70-164.58] ng/g) than in 2014 (14.72 [6.34-40.23] ng/g; U=447, p=0.003).

This trend aligns with the timeline of peak PBDE production and use from the1990s-2000s in North America (Abbasi et al., 2014). Of note is that PBDE-156 or -209 were not detected in the older samples, however they were detected in the newer samples.

4.1.3 PBDE concentrations by species

A Mann-Whitney U test was used to compare PBDE and MeO-BDE concentrations between species using combined data from all locations around the Island of Newfoundland (note: the 1993 samples were excluded from all remaining analysis as they do not reflect more current levels of environmental contamination). There was a statistically significant difference between some cod and turbot liver samples (Table 4.2).

**Table 4.2** Differences in contaminant concentrations (ng/g) between species.

| PBDE Congener | Median (IQR) | | Mann-Whitney U | P-value |
|---|---|---|---|---|
| | Cod | Turbot | | |
| PBDE-28 | 0.99 (0.00-3.02) | 0.59 (0.10-3.80) | 1000.5 | p=0.314 |
| PBDE-47 | 11.54 (5.05-24.69)* | 0.96 (0.00-7.41) | 361.0 | p<0.0001 |
| PBDE-99 | 0.36 (0.00-1.89)* | 0.00 (0.00-0.17) | 703.5 | p=0.002 |
| PBDE-156 | 0.11 (0.00-3.65) | 0.00 (0.00-2.63) | 1032.5 | p=0.402 |
| PBDE-209 | 0.00 (0.00-0.00) | 0.00 (0.00-0.00) | 1161.0 | p=0.924 |
| ∑PBDEs | 17.40 (8.73-45.48)* | 6.36 (0.52-21.94) | 540.0 | p<0.0001 |
| 6-MeO-BDE-47 | 11.61 (0.37-31.96)* | 0.00 (0.00-4.82) | 602.5 | p<0.0001 |
| 2'-MeO-BDE-68 | 13.57 (4.88-22.95)* | 3.29 (0.07-17.41) | 531.0 | p<0.0001 |

Statistically significant results (p<0.05) between species denoted by *, IQR=interquartile range, cod n=96, turbot n=28.

There was a trend towards consistently higher PBDE and MeO-BDE concentrations in cod liver samples than in turbot. PBDE-47 and -99 as well as 2'-MeO-BDE-68, 6-MeO-BDE-47 and ∑PBDEs were statistically significant, with higher concentrations of these EDCs in cod than turbot. This trend has been seen in other marine ecosystems, where PBDEs have been show to accumulate up the food chain into higher trophic level organisms (i.e. Mizukawa et al., 2009).

4.1.4 PBDE concentrations by location

A Kruskal-Wallis H Test (H=test statistic, variance of the rank among groups) was used to look for difference in PBDE concentrations by species (cod and turbot) and by location, as this non-parametric test does not require equal variances between outcome variables and can be used for these data despite skewed PBDE concentration values. For turbot, there was no difference between EDC concentrations from different locations around Newfoundland.

In the cod livers, the Kruskal-Wallis H Test demonstrated that there was a statistically significant difference in the medians of PBDE-47 ($H=22.16$, $p<0.0001$), PBDE-99 ($H=21.85$, $p<0.0001$), and ∑PBDEs ($H=18.90$, $p<0.0001$) between Areas 2 and 4. Table 4.3 presents the results from the Dunn-Bonferroni post-hoc test was that was used for pairwise comparisons of the statistically significant EDC concentrations by area (Dinno, 2015).

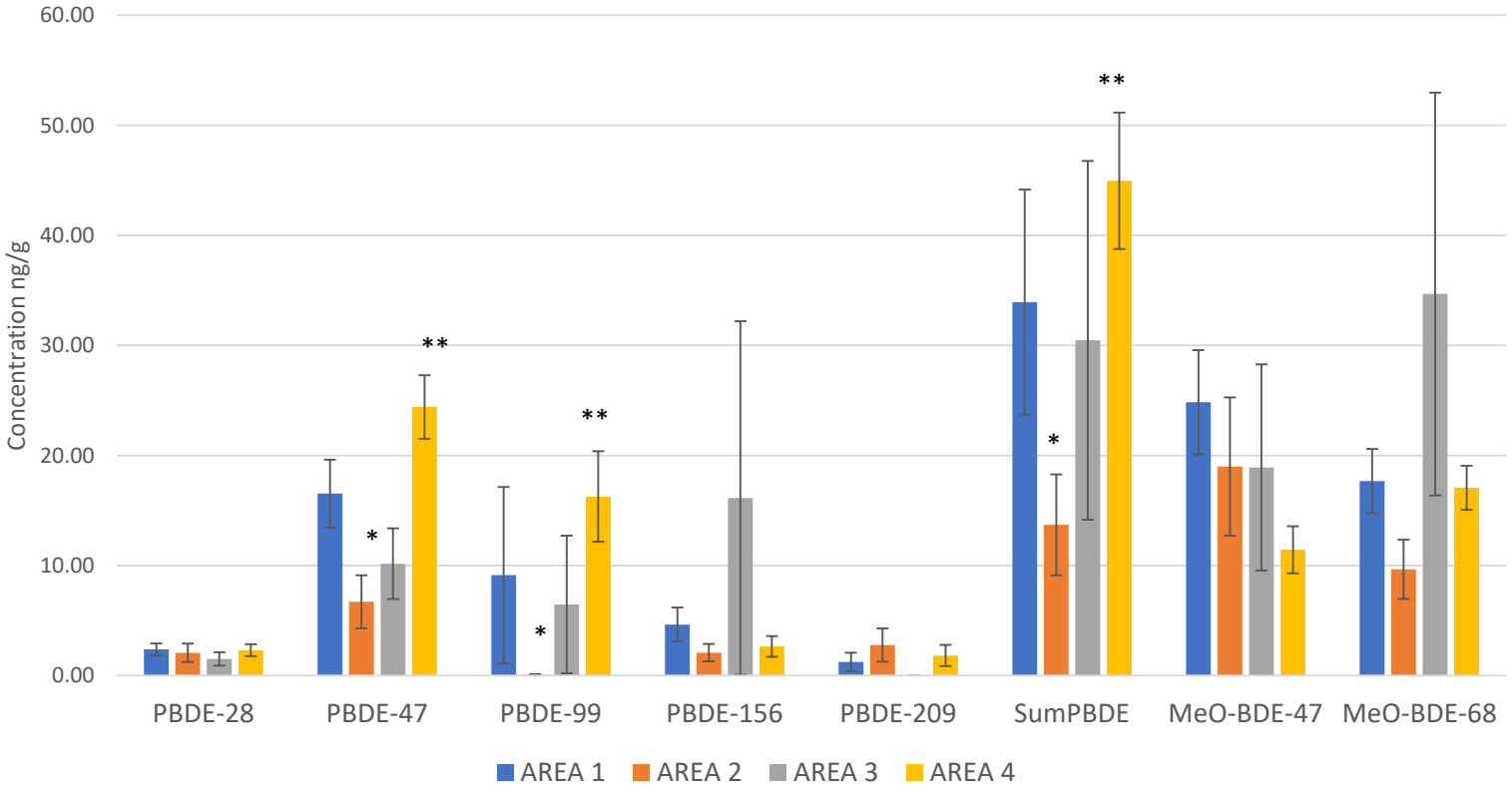
**Table 4.3** Pairwise comparison of cod liver PBDE concentrations (ng/g) by location.

| Contaminant | Area 2 Median (IQR) | Area 4 Median (IQR) | Significance level |
|---|---|---|---|
| PBDE-47 | 1.58 (0.00-6.85) | 23.51 (12-82-43.64) | $p<0.0001$ |
| PBDE-99 | 0.00 (0.00-0.03) | 2.03 (0.00-36.78) | $p<0.0001$ |
| ∑PBDEs | 6.11 (0.37-12.76) | 43.72 (17.49-84.08) | $p<0.0001$ |

Only statistically significant results ($p<0.05$) reported, IQR=interquartile range, Area 2 n=24, Area 4 n=41.

PBDE -47, -99 and ∑PBDEs concentrations were statistically significantly higher in cod from Area 4 cod compared with cod from Area 2 (Figure 4.2). No methoxylated (MeO) BDE concentrations were found to be different by location in cod. Overall, Area 4 cod liver PBDE concentrations were consistently higher than Area 2 for several PBDEs, although MeO-BDEs did not exhibit the same trend. Area 4 collection sites were located off the west coast of Newfoundland receiving contaminated water from St. Lawrence river, while Area 2 sites were located offshore around the Grand Banks (Figure 4.2).

**Figure 4.2** Concentrations (ng/g lipid) of PBDEs and MeO-BDEs in cod fish liver at different locations around Newfoundland (error bars represent standard error). Concentrations (by area) that are statistically significantly different from each other for a specific EDC at a $p<0.05$ significance level are indicated by * (significantly lower) or ** (significantly higher).



## 4.2 Seafood consumption questionnaire (SCQ)

Objective 2 of the study was to explore local seafood consumption in residents of two coastal communities in Newfoundland.

### 4.2.1 Demographics of the study population

Participants were randomly selected for the study, and volunteers who were less than 18 years old, were pregnant, and/or who had a known thyroid condition were excluded. A later decision was made to include participants with hypothyroidism (n=6), to investigate whether there was a relationship between plasma EDC concentrations and hypothyroidism in these Newfoundland residents. This was considered a useful addition to the study as they could be compared with non-hypothyroid participants to explore if plasma EDC concentrations were different between hypothyroid and non-hypothyroid resident of Newfoundland. Participants selected for the study lived in or adjacent to the communities of New-Wes-Valley (NWV, northeast coast) and Burin (South coast). Most participants lived within an 80km or 45-minute driving distance from the blood collection site, and provided their postal code as a verification of their residence (Figure 4.3).

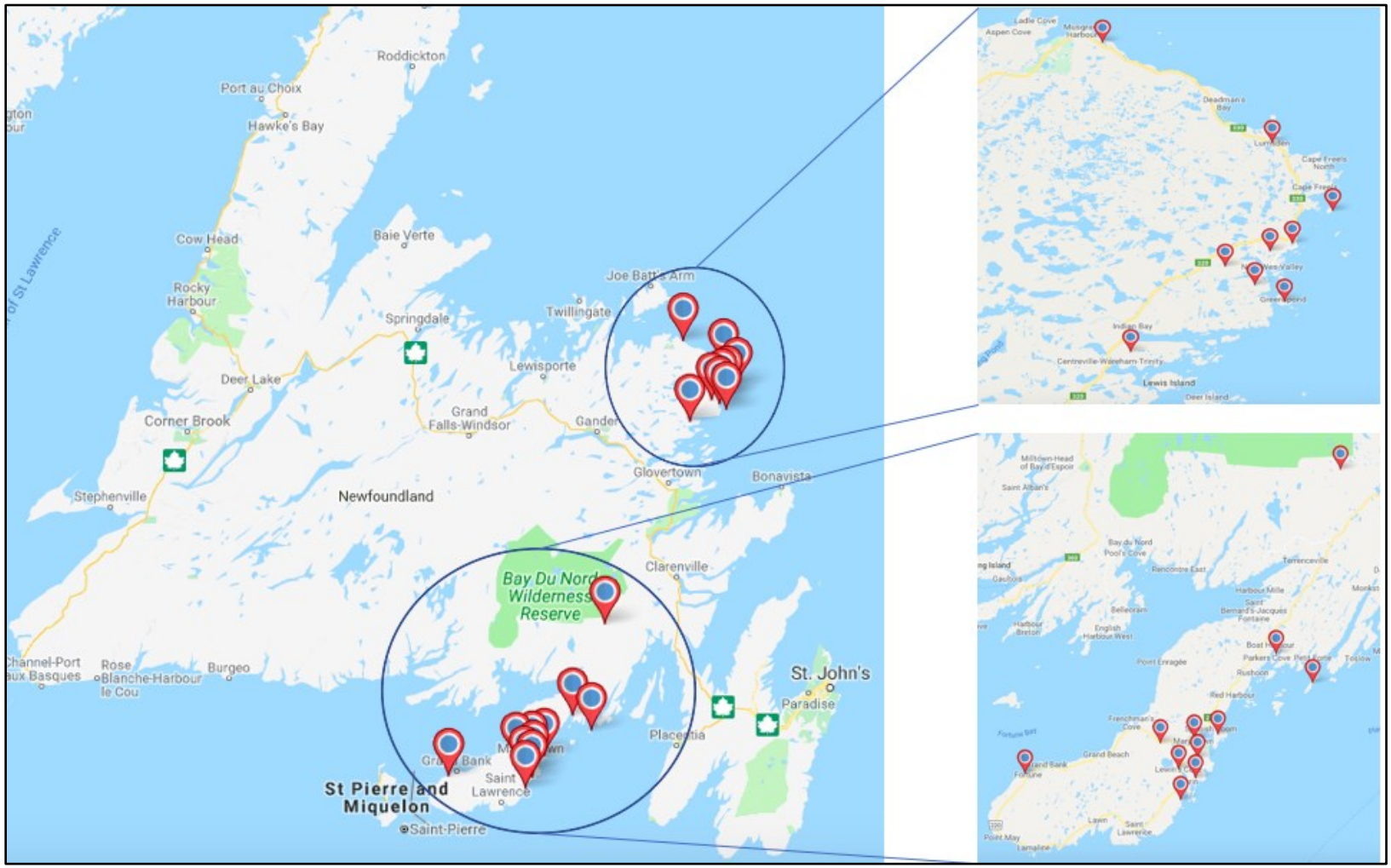



**Figure 4.3** Locations of participant residence as determined postal code (indicated by red marker). Maps data © 2020 Google.

The demographics of the study population are presented in Table 4.4. Briefly, the majority of participants were females (67.5%) compared to males (32.5%) and there were also more participants who were >50 years age at both locations (Table 4.5).

**Table 4.4** Demographic profile of the study population from NWV and Burin.

| **Demographic Characteristic** | **N (%)** | |
|---|---|---|
| **Sex** | | |
| Male | 26 (32.5) | |
| Female | 54 (67.5) | |
| | | |
| **Age** | **Male** | **Female** |
| 20-29 | 0 (0) | 6 (7.50) |
| 30-39 | 2 (2.50) | 5 (6.25) |
| 40-49 | 3(3.75) | 6 (7.50) |
| 50-59 | 8 (10.00) | 13 (16.25) |
| 60-74 | 8 (10.00) | 18 (22.50) |
| 75+ | 4 (5.00) | 5 (6.25) |
| No Answer | 1 (1.25) | 1 (1.25) |
| | | |
| **Residence duration** | **Male** | **Female** |
| Less than 1 year | 0 (0) | 0 (0) |
| 2-10 years | 2 (2.50) | 3 (3.75) |
| 10-20 years | 2 (2.50) | 4 (5.00) |
| 20+ years | 21 (26.25) | 46 (57.50) |
| No Answer | 1 (1.25) | 1 (1.25) |

**Table 4.5** Demographics of sex and age by location (Burin and NWV).

| Demographic variable | Burin | NWV |
|---|---|---|
| Age | 29.1% <50 years (n=14) | 25.0% <50 years (n=8) |
| | 66.7% >50 years (n=32) | 75.0% >50 years (n=24) |
| | 4.2% N/A (n=2) | 0% N/A (n=0) |
| Sex | 37.5% Male (n=18) | 25.0% Male (n=8) |
| | 62.5% Female (n=30) | 75.0% Female (24) |
| | 0% N/A (n=0) | 0% N/A (n=0) |

The greater recruitment and retention of female participants than male participants in this study is a trend supported by other studies (Smith, 2008; Ochoo et al., 2017). A further breakdown of the participant demographics showed that in the younger age group (<50 years), more females (n=15) than males (n=5) participated (25% males and 75% female), however for the older (>50 years) age group the ratios were slightly more equal (male n=20 [37%], female n=34 [63%]). Reviews of health research literature revealed low participation (20%) of males in health behaviour studies (Maher et al., 2014), however the likelihood of male participation in evidence-based programs has been shown to increase with age (Anderson, 2018), as seen in our study. The majority of participants in the study, namely 70%, were >50 years of age. This population distribution is similar to the population of Newfoundland as a whole, where the population is right-skewed (there is a greater number of older residents) and the largest proportion of the populations is in the >50 years age category (Statistics Canada, 2017). Smaller communities in rural Newfoundland are also right-skewed in their age distribution.

The average ages of residents in our study communities are similar; in Burin, it is 46 years, and in NWV it is 48 years (Simms and Ward, 2017). In our study population, the majority

(approximately 84%) of participants had lived in their respective communities for 20+ years, and the few that had lived there for shorter periods (6.25% in the 2 to10 year range) were generally younger adults who had moved for employment, mostly at the local healthcare centre where the blood collection and Seafood Consumption Questionnaire (SCQ) were administered.

### 4.2.2 Seafood consumption questionnaire (SCQ) results

All participants (n=80) completed the SCQ. While participant groups from both communities were given the same instructions prior to completing the SCQ, there were 11 participants (22%) all from the Burin site who missed answering at least one question on the seafood questionnaire. The majority of the missed responses were from the frequency of seafood consumption section of the questionnaire, and were likely the result of participants not eating a particular type of seafood but not coding it with the lowest possible consumption frequency response. Because we cannot be certain of their answer, unanswered questions were coded as “No Answer” (N/A) and not included in the final tallies of seafood consumption. The survey asked about consumption of local seafood only, and this was also explained to participants when they were handed the survey package (see Appendix A for SCQ used in this study). For the frequency of local species-specific seafood consumption by community, see Table 4.6. The most commonly consumed local seafood species were cod, along with salmon, shrimp, scallops and crabs.

**Table 4.6** Percentage (%) of participant responses to local seafood consumption frequency in the past month for Burin and NWV (B=Burin, N=New-Wes-Valley). Note: response frequencies are unconsolidated and directly from SCQ.

| Consumption Frequency (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Type of Seafood** | **Almost never** | | **Once/month** | | **2-3 times/month** | | **1-2 times/week** | | **3-5 times/week** | | **Almost every day** | | **No answer** | |
| | **B** | **N** | **B** | **N** | **B** | **N** | **B** | **N** | **B** | **N** | **B** | **N** | **B** | **N** |
| Capelin | 83.3 | 81.3 | 4.2 | 12.5 | 0 | 6.3 | 2.1 | 0 | 0 | 0 | 0 | 0 | 10.4 | 0 |
| Cod | 14.6 | 0 | 35.4 | 25.0 | 37.5 | 59.4 | 8.3 | 15.6 | 2.1 | 0 | 0 | 0 | 2.1 | 0 |
| Crab | 68.8 | 78.1 | 22.9 | 21.9 | 2.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.3 | 0 |
| Halibut | 68.8 | 90.6 | 20.8 | 6.3 | 4.2 | 3.1 | 0 | 0 | 0 | 0 | 0 | 0 | 6.3 | 0 |
| Herring | 91.7 | 90.6 | 0 | 9.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8.3 | 0 |
| Lobster | 79.2 | 87.5 | 12.5 | 9.4 | 0 | 0 | 0 | 3.1 | 0 | 0 | 0 | 0 | 8.3 | 0 |
| Mackerel | 89.6 | 87.5 | 2.1 | 6.3 | 0 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 8.3 | 0 |
| Salmon | 54.2 | 46.9 | 31.3 | 43.8 | 14.6 | 6.3 | 0 | 0 | 0 | 3.1 | 0 | 0 | 0 | 0 |
| Shrimp | 52.1 | 78.1 | 35.4 | 15.6 | 10.4 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 2.1 | 0 |
| Scallops | 41.7 | 87.5 | 50.0 | 9.4 | 4.2 | 3.1 | 2.1 | 0 | 0 | 0 | 0 | 0 | 2.1 | 0 |
| Smelt | 93.8 | 81.3 | 0 | 18.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.3 | 0 |
| Squid | 87.5 | 93.8 | 2.1 | 6.3 | 2.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8.3 | 0 |
| Trout | 79.2 | 84.4 | 10.4 | 15.6 | 4.2 | 0 | 0 | 0 | 2.1 | 0 | 0 | 0 | 4.2 | 0 |
| Turbot | 87.5 | 100 | 2.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10.4 | 0 |

### 4.2.3 SCQ results by demographic variables

There were numerous seafood species did not have many responses at the higher consumption frequencies (1-2 times/week, 3-5 times/week), which prompted us to consolidate consumption frequencies into two response categories: 1) no consumption and 2) consumed once or more in the past month (Table 4.7). We used these consolidated response categories to analyze differences in seafood consumption by hypothyroid status, sex, community and age using a chi square test. However, some species still violated the assumptions of the chi square test by having cells with expected counts less than five (>20% of responses). Therefore, a Fisher's exact test was used to look for a difference in seafood consumption by hypothyroid status, sex, community and age in these species.

#### 4.2.3.1 SCQ results by hypothyroid status

When comparing seafood consumption frequencies between hypothyroid and non-hypothyroid participants, all species violated the chi square test assumptions therefore all species were analyzed by Fisher's exact test. There was no difference in any species consumption frequencies between hypothyroid and non-hypothyroid participants, however, there were very few participants with hypothyroidism (n=6) compared with non-hypothyroid participants (n=74), therefore these results must be interpreted with caution.

**Table 4.7** Consolidated frequency and percentage (%) of participant responses to local seafood consumption frequency in the past month for Burin and NWV.

| Consumption frequencies N (%) | | | |
|---|---|---|---|
| **Species** | **No consumption** | **Once or more/month** | **No Answer** |
| Capelin | 66 (82.50) | 9 (11.25) | 5 (6.25) |
| Cod | 7 (8.75) | 72 (90.00) | 1 (1.25) |
| Crab | 58 (72.5) | 19 (23.75) | 3 (3.75) |
| Halibut | 62 (77.50) | 15 (18.75) | 3 (3.75) |
| Herring | 73 (91.25) | 3 (3.75) | 4 (5.00) |
| Lobster | 66 (82.50) | 10 (12.50) | 4 (5.00) |
| Mackerel | 71 (88.75) | 5 (6.25) | 4 (5.00) |
| Salmon | 41 (51.25) | 39 (48.75) | 0 (0.00) |
| Shrimp | 50 (62.50) | 29 (36.25) | 1 (1.25) |
| Scallops | 48 (60.00) | 31 (38.75) | 1 (1.25) |
| Smelt | 71 (88.75) | 6 (7.50) | 3 (3.75) |
| Squid | 72 (90.00) | 4 (5.00) | 4 (5.00) |
| Trout | 65 (81.25) | 13 (16.25) | 2 (2.50) |
| Turbot | 74 (92.50) | 1 (1.25) | 5 (6.25) |

#### 4.2.3.2 SCQ results by sex

We were able to perform a chi square test comparing seafood consumption frequencies between males and females for crab, salmon, shrimp and scallops; there was no association detected in this analysis. We then analyzed all remaining species by Fisher's exact test and found statistically significant higher reported consumption frequencies of herring ($p=0.029$) and trout ($p=0.026$) in males than females. No other species had different consumption frequencies by sex, however there was a general trend towards males reporting higher seafood consumption frequencies than females across most species (Table 4.8).

#### 4.2.3.3 SCQ results by community

Seafood consumption frequencies were analyzed by community (Burin vs. NWV; Table 4.9). Shrimp ($p=0.024$) and scallops ($p<0.001$) were analyzed by chi square test, and we found that participants from Bruin reported consuming these species more than participants from NWV. Capelin, crab, halibut, salmon, squid and trout were also analyzed by chi square test, and the results were non-significant. All remaining species were analyzed by Fisher's exact test. We found that participants from NWV consumed local cod ($p=0.038$) and smelt ($p=0.004$) more frequently in the past month than participants from Burin. There was no other difference in any other species consumption frequency by location.

**Table 4.8** Consolidated frequency and percentage (%) of participant responses to local seafood consumption frequency in the past month for males and females.

**Consumption frequencies N (%)**

| Species | No consumption | | Once or more/month | | No Answer | |
|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female |
| Capelin | 19 (73.1) | 47 (87.0) | 5 (19.2) | 4 (7.4) | 2 (7.7) | 3 (5.6) |
| Cod | 0 (0.0) | 7 (13.0) | 25 (96.2) | 47 (87.0) | 1 (3.8) | 0 (0.0) |
| Crab | 15 (57.7) | 43 (79.6) | 9 (34.6) | 10 (18.6) | 2 (7.7) | 1 (1.9) |
| Halibut | 17 (65.4) | 45 (83.3) | 8 (30.7) | 7 (13.0) | 1 (3.8) | 2 (3.7) |
| Herring* | 21 (80.8) | 52 (96.3) | 3 (11.5) | 0 (0.0) | 2 (7.7) | 2 (3.7) |
| Lobster | 21 (80.8) | 45 (83.3) | 4 (15.4) | 6 (11.2) | 1 (3.8) | 3 (5.6) |
| Mackerel | 22 (84.6) | 49 (90.7) | 3 (11.5) | 2 (3.7) | 1 (3.8) | 3 (5.6) |
| Salmon | 13 (50.0) | 28 (51.9) | 13 (50.0) | 26 (48.1) | 0 (0.0) | 0 (0.0) |
| Shrimp | 13 (50.0) | 37 (68.5) | 13 (50.0) | 16 (29.7) | 0 (0.0) | 1 (1.9) |
| Scallops | 13 (50.0) | 35 (64.8) | 13 (50.0) | 18 (33.3) | 0 (0.0) | 1 (1.9) |
| Smelt | 22 (84.6) | 49 (90.7) | 3 (11.5) | 3 (5.6) | 1 (3.8) | 2 (3.7) |
| Squid | 23 (88.5) | 49 (90.7) | 2 (7.7) | 2 (3.7) | 1 (3.8) | 3 (5.6) |
| Trout* | 18 (69.2) | 47 (87.0) | 8 (30.7) | 5 (9.3) | 0 (0.0) | 2 (3.7) |
| Turbot | 24 (92.3) | 50 (92.6) | 1 (3.8) | 0 (0.0) | 1 (3.8) | 4 (7.4) |

* Denotes proportions (%) that were statistically significantly different ($p<0.05$) between males and females by Fisher's exact test.

**Table 4.9** Consolidated frequency and percentage (%) of participant responses to local seafood consumption frequency in the past month for Burin and NWV.

| | **Consumption frequencies N (%)** | | | | | |
|---|---|---|---|---|---|---|
| **Species** | **No consumption** | | **Once or more/month** | | **No Answer** | |
| | Burin | NWV | Burin | NWV | Burin | NWV |
| Capelin | 40 (83.3) | 26 (81.2) | 3 (6.3) | 6 (18.8) | 5 (10.4) | 0 (0.0) |
| Cod* | 7 (14.6) | 0 (0.0) | 40 (83.3) | 32 (100.0) | 1 (2.1) | 0 (0.0) |
| Crab | 33 (68.8) | 25 (78.1) | 12 (25.0) | 7 (21.9) | 3 (6.3) | 0 (0.0) |
| Halibut | 33 (68.8) | 29 (90.6) | 12 (25.0) | 3 (9.4) | 3 (6.3) | 0 (0.0) |
| Herring | 44 (91.7) | 29 (90.6) | 0 (0.0) | 3 (9.4) | 4 (8.3) | 0 (0.0) |
| Lobster | 38 (79.2) | 28 (87.5) | 6 (12.5) | 4 (12.5) | 4 (8.3) | 0 (0.0) |
| Mackerel | 43 (89.6) | 28 (87.5) | 1 (2.1) | 4 (12.5) | 4 (8.3) | 0 (0.0) |
| Salmon | 26 (54.2) | 15 (46.9) | 22 (45.9) | 17 (53.1) | 0 (0.0) | 0 (0.0) |
| Shrimp** | 25 (52.1) | 25 (78.1) | 22 (45.8) | 7 (21.9) | 1 (2.1) | 0 (0.0) |
| Scallops** | 20 (41.7) | 28 (87.5) | 27 (56.3) | 4 (12.5) | 1 (2.1) | 0 (0.0) |
| Smelt* | 45 (93.8) | 26 (81.3) | 0 (0.0) | 6 (18.7) | 3 (6.3) | 0 (0.0) |
| Squid | 42 (87.5) | 30 (93.8) | 2 (4.2) | 2 (6.3) | 4 (8.3) | 0 (0.0) |
| Trout | 38 (79.2) | 27 (84.4) | 8 (16.7) | 5 (15.6) | 2 (4.2) | 0 (0.0) |
| Turbot | 42 (87.5) | 32 (100.0) | 1 (2.1) | 0 (0.0) | 5 (10.4) | 0 (0.0) |

* Denotes proportions (%) that were statistically significantly different ($p<0.05$) between Burin and NWV by Fisher's exact test.
** Denotes proportions (%) that were statistically significantly different ($p<0.05$) between Burin and NWV by chi square test.

#### 4.2.3.4 SCQ results by age

Seafood consumption frequencies were analyzed by age (<50 years and >50 years). Salmon, shrimp and scallops were analyzed by chi square test, and there was no association for these species. All remaining seafood species were analyzed by Fisher's exact test. Cod was the only species with any statistically significant association ($p=0.015$); we found greater reported consumption frequencies in older (>50 years, n=57) than younger (<50 years, n=21) participants (n=2 no age given). There were no associations for the remaining species.

### 4.2.4 SCQ results by season

Participants also answered questions about local seafood consumption frequency in four different seasons (fall, winter, spring, and summer). Summer was the season with the highest reported local seafood consumption frequency; 25.9% reported eating seafood 1-2 times per week, and 11.2% reported eating it 3-5 times per week. In fall and winter seasons, most participants reported consuming local seafood 1-2 times per week and very few reported higher frequencies of consumption, whereas in spring and summer a greater proportion of people reported consuming seafood 3-5 times per week (Table 4.10). Higher consumption in spring and summer correspond with both the opening of the seasonal commercial fisheries (spring and summer) and the provincial food fishery (summer), and is consistent with participants being able to obtain fresh seafood.

**Table 4.10** Seasonal local seafood consumption frequency and percent response (%) in males (M), females (F) and all participants (A) in Burin and NWV.

| Consumption frequency | N (%) Fall | | | Winter | | | Spring | | | Summer | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **M*** | **F** | **A** | **M** | **F** | **A** | **M*** | **F** | **A** | **M*** | **F** | **A** |
| Less than once/week | 11 (42.3) | 31 (57.4) | 42 (52.5) | 12 (46.2) | 34 (63.0) | 46 (57.5) | 11 (42.3) | 35 (64.8) | 46 (57.5) | 7 (26.9) | 30 (55.6) | 37 (46.3) |
| 1-2 times/week | 12 (46.2) | 18 (33.3) | 30 (37.5) | 10 (38.5) | 15 (27.8) | 25 (31.3) | 9 (34.6) | 13 (24.1) | 22 (27.5) | 9 (34.6) | 14 (25.9) | 23 (28.7) |
| 3-5 times/week | 1 (3.8) | 0 (0.0) | 1 (1.3) | 1 (3.8) | 2 (3.7) | 3 (3.8) | 3 (11.5) | 3 (5.6) | 6 (7.5) | 6 (23.1) | 6 (11.1) | 12 (15.0) |
| Every day | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| N/A | 2 (7.7) | 5 (9.3) | 7 (8.8) | 3 (11.5) | 3 (5.6) | 6 (7.5) | 3 (11.5) | 3 (5.6) | 6 (7.5) | 4 (15.4) | 4 (7.4) | 8 (10.0) |

* Denotes proportions (%) that were statistically significantly different ($p<0.05$) between males and females by chi square test using consolidated seafood consumption categories. Note: consolidated consumption categories (never, once/month or more/month) not presented in this table.

We used a chi square test to examine seasonal changes in local seafood consumption by age, sex and community. Seafood consumption frequency responses were again consolidated into three response categories (never, once/month or more/month), as there were very low reported consumption frequencies of more than once in the past month. We found that males from both communities reported eating seafood more frequently than females in the fall ($p=0.031$), spring ($p=0.026$) and summer ($p=0.049$), but not in winter. There was no effect of community or age on local seafood consumption frequencies by season.

### 4.3 Thyroid hormone and EDC results

Objective 3 of the study was to collect blood samples from participants who completed the SCQ, and test for thyroid hormone and EDC concentrations.

Histograms were used to assess the distributions of EDC (pmol/g) and thyroid hormone concentrations. All EDC and thyroid concentrations (with the exception of $FT_4$ and $FT_3$) were right-skewed and therefore not normally distributed (see Appendix D for serum thyroid hormone histograms and Appendix E for plasma EDC histograms). Because of this we used a Mann-Whitney U-test to examine the difference in EDCs and thyroid hormones by age, sex and community.

#### 4.3.1 Thyroid hormone concentrations

Thyroid stimulating hormone (TSH), free thyroxine ($FT_4$) and free triiodothyronine ($FT_3$) were measured in the serum of all 80 participants. Lab references ranges from the Clinical Biochemistry Laboratory at the Health Sciences Centre (HSC, St. John's) were used to determine if participants had abnormal thyroid hormone concentrations.

The HSC references ranges were:

- TSH: 0.35-4.94 mIU/L
- $FT_4$: 9.0-19.0 pmol/L
- $FT_3$: 2.6-5.7 pmol/L

There were 6 participants with diagnosed hypothyroidism (Burin=1 participant, NWV=5 participants), and all six confirmed that they were on thyroid hormone medication to treat their condition. From Burin, there were 2 participants (both female and >50 years) that had TSH values that were above lab reference range. One participant had a previous diagnosis of hypothyroidism (TSH=7.92 mlU/L), but the other did not report having been previously diagnosed with hypothyroidism (TSH=5.20 mlU/L). For both participants, their $FT_4$ and $FT_3$ values were within normal reference range. The 6 participants with diagnosed hypothyroidism were excluded from the thyroid hormone statistical analysis, as their hormone levels are artificially controlled through medication and may not be representative of the effects of EDCs on thyroid hormone concentrations in their communities. The medians and IQRs of TSH, $FT_4$ and $FT_3$ for all participants are presented in table 4.11.

**Table 4.11** Median and interquartile range (IQR) of thyroid hormone concentrations from participants of Burin and NWV (n=74). Hypothyroid participants not included.

| Thyroid Hormone | Range | Medin (IQR) |
|---|---|---|
| TSH (mIU/L) | 0.44-5.20 | 1.90 (1.32-2.31) |
| $FT_4$ (pmol/L) | 9.25-15.10 | 11.90 (11.25-13.18) |
| $FT_3$ (pmol/L) | 2.70-4.90 | 3.80 (3.40-4.10) |

Lab reference ranges: TSH 0.35-4.94 mIU/L, $FT_4$ 9.0-19.0 pmol/L, $FT_3$ 2.6-5.7 pmol/L.

The average thyroid hormone values for non-hypothyroid participants are all within the lab reference ranges. Only one participant (1.35% of total study population without hypothyroidism) had TSH levels above the normal reference range, which is close to the prevalence of hypothyroidism in the Canadian population (2-3%, Clemens et al., 2011).

4.3.2 Thyroid hormone concentrations by age, sex and community

One participant (Burin) had a TSH concentration (5.20 mlU/L) that was above the lab reference range, but their $FT_4$ and $FT_3$ concentrations were within normal range; because they did not have a known diagnosis of hypothyroidism they were included in the analysis. All participants with a known hypothyroidism diagnosis were removed from the remainder of the analyses as their thyroid levels are controlled through medication.

There was no effect of age on thyroid hormone concentrations in the study population. $FT_3$ was the only thyroid hormone where sex had an effect on concentration, with males (n=26) having statistically significantly higher median [IQR] $FT_3$ concentrations (4.00 pmol/L [3.65-4.23]) than females (n=54; 3.70 pmol/L [3.40-3.93]; U=438, p=0.046), however both male and female concentrations were within the lab reference range.

Some thyroid hormones varied between the two study communities (Burin n=48, NWV n=32); TSH concentrations (median [IQR]) were higher in the serum samples from NWV (2.02 mlU/L [1.53-2.72]) than those from Burin (1.74 mlU/L [1.17-2.14]; U=423.00, p=0.018), and $FT_4$ concentrations (median [IQR]) were also higher in participants from NWV (12.95 pmol/L [11.73-13.58]) than in those from Burin (11.73 pmol/L [11.01-12.72]; U=425.50, p=0.019). There was no difference in $FT_3$ concentrations between the two locations (Table 4.12).

**Table 4.12** Thyroid hormone concentrations by community.

| Thyroid hormone | Area | Range | Median (IQR) |
|---|---|---|---|
| TSH mIU/L | Burin | 0.65-5.20 | 1.74 (1.17-2.14) |
| | NWV* | 0.44-4.67 | 2.02 (1.53-2.72) |
| $FT_4$ pmol/L | Burin | 9.25-14.92 | 11.73 (11.01-12.72) |
| | NWV* | 9.8-15.10 | 12.95 (11.73-13.58) |
| $FT_3$ pmol/L | Burin | 2.90-4.60 | 3.85 (3.53-4.20) |
| | NWV | 2.70-4.90 | 3.50 (3.30-4.00) |

* denotes the statistically significantly higher concentration between communities (Burin [n=48] and NWV [n=32]) at the <0.05 significance level. Lab reference range: TSH 0.35-4.94 mIU/L, $FT_4$ 9.0-19.0 pmol/L, $FT_3$ 2.6-5.7 pmol/L.

When thyroid hormone concentrations were analyzed in the original survey age groupings (see Appendix A) by Kruskal-Wallis H-test, there was no difference in any of the hormone concentrations. We then grouped the study population into two age categories; <50 years of age and > 50 years of age. Differentiating age categories at 50 years reflects a change in thyroid hormone concentrations with age; TSH concentrations trend towards higher concentrations with increasing age, particularly after 50 years of age (Surks and Hollowell, 2007).

Furthermore, we explored the difference in thyroid hormone concentrations in the two age groups (<50 years and >50 years) by sex and community using a Mann-Whitney U-test. There was no difference in thyroid hormones by sex in the younger (<50 years) or older age (>50 years) age groupings. For the community comparison, some statistically significant trends did emerge:

- $FT_4$ was higher in Burin than in NWV in the younger (<50 years) age group (p=0.09, U=73.0).
- $FT_3$ was higher in Burin than in NWV in the older (>50 years) age group (p=0.026, U=206.5).
- TSH was higher in NWV than in Burin in the older (>50 years) age group (p=0.015, U=456.0).

4.3.3 Plasma EDC measurements

All 80 participants in the study had detectable levels of at least 11 different types of EDCs in their plasma. Each participant (including those with a known hypothyroidism diagnosis) was tested by INSPQ for the following:

- 1 polybrominated biphenyl congener (PBB-153)
- 9 polybrominated diphenyl ether congeners (PBDE-15, -17, -25, -28, -33, -47, -99, -100, -153)
- 13 polychlorinated biphenyl congeners (PCB-28, -52, -99, -101, -105, -118, -128, -138, -153, -156, -170, -180, -183)
- Dichlorodiphenyltrichloroethane (DDT)
- Dichlorodiphenyldichloroethylene (p,p'-DDE).

These congeners were chosen for our study as they were the EDCs that INSPQ had developed testing methods for in their facility. We selected all possible congers for testing so as to explore the greatest possible range of EDCs in Newfoundland residents.

Not all the EDCs tested in the participants were prevalent in the majority of the study population, therefore we restricted our remaining analyses to the most relevant EDCs for this

study, which included those associated with adverse health outcomes in the literature, those present in >70% of the plasma samples, based on methods used by Dallaire et al. (2009), and those EDCs with the majority of samples over the LOD. Several EDCs (PBDEs -15, -17, -25 and -33, PCBs -28, -52, -101, -156 and -183) were thus excluded from further analysis.

For EDC congeners where more than 5% of the total participants had undetectable plasma concentrations, a value (the limit of detection divided by 2 [LOD/2]) was assigned to those participants who had EDC concentrations below the LOD (based on similar methods by Dallaire et al., 2009). For the remainder of the analysis, we focused on 15 EDCs and two summed EDC groups (ΣPCBs and ΣPBDEs), as they had the highest levels of prevalence in the study population and have also been associated with changes in thyroid hormones concentrations in the literature.

Total plasma lipid concentrations were used to lipid-normalize EDC concentrations in the plasma of participants of this study. The mean total plasma lipid concentration (total triglycerides and total cholesterol) was 6.48 g/L, with a range from 3.92-10.23 g/L. An independent t-test was used to compare plasma lipid concentrations by location, sex and age (<50 years vs >50 years). It was important to test for a difference in plasma lipid concentrations by age, sex, and community as not all groups had equal/matched distributions of participants, but we did not find any difference.

Concentrations of lipophilic chemicals in tissues/serum/plasma are often adjusted based on difference in lipid content, however this correction can only be applied when concentrations of contaminants vary in direct proportion to tissue lipid content, such as with lipophilic EDCs (Hebert and Keenleyside, 1995). The most commonly used method to lipid-normalize contaminant concentrations is to divide each participant's individual EDC concentration by their

total lipid concentration, to determine the ng/g or pmol/g (lipid) concentration of the EDC in their plasma. This is frequently done for many EDCs including PBDEs, PCBs, PBBs and p,p'-DDEs to ensure EDC concentrations are reflective of serum lipid levels, which EDCs vary in direct proportion to in the plasma (Schisterman et al., 2005).

There is wide variability in the plasma levels of the tested EDCs, which made the analysis of relationships more difficult due to the large standard errors and deviations (Table 4.13). PCBs were found in much greater concentrations in the plasma samples than PBDEs, and the highest individual EDC concentrations were from one of the most chlorinated PCB congeners, PCB-180. For PBDEs, the congener with the greatest concentration in plasma was also the most brominated congener tested, PBDE-153.

PBDEs and PCBs all have varying degrees of bromination and chlorination, respectively, resulting in different molecular weights depending on the number of bromines or chlorines attached to aromatic moieties. Each EDC congener was originally measured in µg/L; this was divided by the molecular weight (g/mol) of the individual congener to determine the nmol/L (Appendix C - molecular weights congeners). This value was then lipid-normalized based on individual participant lipid concentrations (g/L) to determine the pmol/g concentration. All further statistical analysis of EDCs in this study were done using pmol/g, as this accounts for the effects of molar weight on concentration of the different congeners (see Table 4.14 for range, mean, median and 95% confidence interval in pmol/g).

**Table 4.13** Plasma concentrations (ng/g lipid) of PBDEs, PCBs, PBBs, and p,p'-DDEs tested in participants from Burin and New-Wes-Valley, Newfoundland.

| EDC congeners | Range | Median (IQR) |
|---|---|---|
| PBB-153 | <LOD-3.82 | 0.57 (0.00-1.14) |
| PBDE-28 | <LOD-1.86 | 0.00 (0.00-0.62) |
| PBDE-47 | 0.72-32.41 | 6.41 (4.04-11.29) |
| PBDE-99 | <LOD-7.45 | 1.86 (1.86-2.54) |
| PBDE-100 | <LOD-25.54 | 0.00 (0.00-2.16) |
| PBDE-153 | <LOD-95.38 | 9.03 (4.16-19.09) |
| ***∑PBDE*** | ***1.77-136.05*** | ***20.48 (12.52-40.37)*** |
| PCB-99 | <LOD-22.10 | 0.00 (0.00-1.81) |
| PCB-105 | <LOD-5.06 | 1.35 (0.80-2.58) |
| PCB-118 | 0.42-32.37 | 7.58 (4.10-13.96) |
| PCB-128 | <LOD-1.82 | 0.09 (0.00-0.22) |
| PCB-138 | 0.49-45.05 | 17.61 (8.50-40.81) |
| PCB-153 | 0.69-104.56 | 35.99 (17.74-87.66) |
| PCB-170 | 0.125-34.95 | 12.63 (4.63-25.31) |
| PCB-180 | 0.33-114.58 | 39.21 (13.27-87.18) |
| ***∑PCB*** | ***2.34-327.65*** | ***125.56 (55.65-272.01)*** |
| p'p-DDE | 10.48-498.99 | 189.49 (94.71-368.85) |

Only EDCs with >70% samples above LOD or known biological relevance were included in the table (n=80). Concentrations presented in ng/g lipid, IQR=interquartile range

**Table 4.14** Plasma concentrations (pmol/g lipid) of PBDEs, PCBs, PBBs, and p,p'-DDE tested in participants from Burin and New-Wes-Valley, Newfoundland based on molecular weight (see Appendix C for molecular weights of congeners).

| EDC congeners | Range | Median (IQR) |
|---|---|---|
| PBB-153 | <LOD-5.94 | 0.57 (0.00-1.14) |
| PBDE-28 | <LOD-5.94 | 0.00 (0.00-0.39) |
| PBDE-47 | 1.50-66.71 | 4.84 (3.05-8.52) |
| PBDE-99 | <LOD-13.19 | 1.63 (1.00-2.23) |
| PBDE-100 | <LOD-39.68 | 0.00 (0.00-2.16) |
| PBDE-153 | <LOD-148.20 | 9.03 (4.16-19.09) |
| ***∑PBDE*** | ***3.15-224.52*** | ***19.00 (11.28-36.12)*** |
| PCB-99 | <LOD-34.34 | 0.00 (0.00-1.81) |
| PCB-105 | <LOD-15.49 | 0.69 (0.40-1.31) |
| PCB-118 | 1.29-99.18 | 3.84 (2.08-7.08) |
| PCB-128 | <LOD-5.04 | 0.05 (0.00-0.12) |
| PCB-138 | 1.35-124.83 | 9.87 (4.76-22.88) |
| PCB-153 | 1.91-289.74 | 20.18 (9.95-49.15) |
| PCB-170 | 0.32-88.40 | 7.60 (2.84-15.54) |
| PCB-180 | 0.82-289.84 | 24.08 (8.15-53.55) |
| ***∑PCB*** | ***6.52-869.21*** | ***73.73 (32.36-159.99)*** |
| p'p-DDE | 32.94-1569.07 | 93.63 (46.80-182.26) |

Only EDCs with >70% samples above LOD or known biological relevance were included in the table (n=80). Concentrations presented in pmol/g lipid, IQR=interquartile range

### 4.3.4 Plasma EDC concentrations by demographic variables

#### 4.3.4.1 Plasma EDC concentrations by age

We analyzed the difference in EDC concentrations by age using a Mann-Whitney U-test (Table 4.15). There was some marked difference in mean concentrations of EDCs by age. There was a trend towards slightly higher PBDE-100, PBDE-153 and ∑PBDE concentrations the <50-year age group, however there were no PBDE congeners that were different by age. Concentrations of PBB-153 were statistically significantly higher in older (>50 years) participants than in younger participants ($U=1043.0$, $p<0.0001$). The PCBs that were statistically significantly higher in older participants were: PCB-105 ($U=970.0$, $p<0.0001$), PCB-118 ($U=999.0$, $p<0.0001$), PCB-138 ($U=1075.0$, $p<0.0001$), PCB-153 ($U=1115.0$, $p<0.0001$), PCB-170 ($U=1158.0$, $p<0.0001$), PCB-180 ($U=1156.0$, $p<0.0001$), and ∑PCBs ($U=1127.0$, $p<0.0001$). p,p'-DDE was also higher in older participants ($U=995.0$ and $p<0.0001$). We also compared ∑EDC concentrations by age (Mann-Whitey U-test), and found that older participants had statistically significantly higher ∑EDC concentrations than younger participants ($U=1057.0$, $p<0.0001$).

#### 4.3.4.2 Plasma EDC concentrations by sex

EDC concentrations were analyzed by sex (Mann-Whitney U-test), comparing plasma results between males and females (Table 4.16). There was a general trend towards higher plasma EDCs in males than in females with the exception of PCB-99 and -118, which were slightly higher in female than in male plasma samples.

**Table 4.15** Median EDC concentrations (pmol/g lipid) in participants by age.

| | Median pmol/g (IQR) | |
|---|---|---|
| **Compound** | **<50 years (n=21)** | **>50 years (n=57)** |
| **PBB-153** | 0.00 (0.00-0.00) | 0.90 (0.17-1.32)* |
| **PBDE-28** | 0.00 (0.00-0.30) | 0.00 (0.00-0.42) |
| **PBDE-47** | 5.16 (3.82-8.42) | 4.53 (2.68-8.49) |
| **PBDE-99** | 1.70 (1.33-2.23) | 1.53 (0.89-2.17) |
| **PBDE-100** | 0.98 (0.00-2.57) | 0.00 (0.00-2.01) |
| **PBDE-153** | 11.08 (4.54-25.78) | 9.00 (3.76-16.93) |
| **∑PBDEs** | 23.53 (18.19) | 18.93 (10.20-36.75) |
| **PCB-99** | 0.00 (0.00-0.00) | 0.00 (0.00-2.13) |
| **PCB-105** | 0.33 (0.25-0.58) | 0.86 (0.57-1.40)* |
| **PCB-118** | 1.61 (1.12-2.82) | 4.47 (3.16-8.30)* |
| **PCB-128** | 0.00 (0.00-0.06) | 0.07 (0.00-0.14) |
| **PCB-138** | 3.60 (1.71-5.90) | 15.01 (8.65-25.14)* |
| **PCB-153** | 6.81 (2.97-9.95) | 34.54 (18.16-61.71)* |
| **PCB-170** | 1.72 (0.64-2.42) | 13.33 (5.91-17.11)* |
| **PCB-180** | 4.93 (1.79-7.54) | 41.05 (18.41-57.82)* |
| **∑PCBs** | 23.87 (9.16-32.48) | 123.84 (60.26-192.44)* |
| **p,p'-DDE** | 41.86 (27.45-71.14) | 131.26 (70.41-222.72)* |
| **∑EDCs** | 107.40 (55.01-136.80) | 289.51 (169.63-471.49)* |

* denotes plasma congener concentrations that are statistically significantly higher ($p<0.05$) between age groupings across one type of EDC, IQR=interquartile range. N=2 participants did not provide age and were not included in this analysis.

**Table 4.16** Median EDC concentrations (pmol/g lipid) in participants by sex.

| | Median pmol/g (IQR) | |
|---|---|---|
| **Compound** | **Male (n=26)** | **Female (n=54)** |
| **PBB-153** | 1.03 (0.41-1.59)* | 0.00 (0.00-0.92) |
| **PBDE-28** | 0.00 (0.00-0.67) | 0.00 (0.00-0.34) |
| **PBDE-47** | 4.96 (3.67-13.43) | 4.74 (2.74-8.11) |
| **PBDE-99** | 1.84 (1.02-2.90) | 1.60 (0.98-2.11) |
| **PBDE-100** | 0.29 (0.00-3.59) | 0.00 (0.00-1.89) |
| **PBDE-153** | 16.93 (9.04-29.21)* | 6.70 (3.46-13.09) |
| **∑PBDEs** | 24.64 (14.20-45.45)* | 15.44 (8.58-27.69) |
| **PCB-99** | 0.00 (0.00-0.33) | 0.00 (0.00-2.03) |
| **PCB-105** | 0.87 (0.40-1.44) | 0.63 (0.41-1.21) |
| **PCB-118** | 4.34 (2.05-7.15) | 3.61 (2.07-7.01) |
| **PCB-128** | 0.08 (0.00-0.14) | 0.00 (0.00-0.09) |
| **PCB-138** | 14.44 (5.05-26.49) | 9.57 (4.55-21.79) |
| **PCB-153** | 36.55 (12.52-63-51)* | 18.78 (9.40-47.09) |
| **PCB-170** | 13.55 (3.51-18.89)* | 6.09 (2.48-14.40) |
| **PCB-180** | 42.90 (11.17-66.08)* | 19.04 (7.46-46.06) |
| **∑PCBs** | 117.92 (59.49-201.63)* | 59.94 (29.35-150.06) |
| **p,p'-DDE** | 114.92 (52.31-177.89) | 79.91 (41.56-185.98) |
| **∑EDCs** | 283.33 (171.20-425.23) | 179.53 (106.46-350.53) |

* denotes plasma congener concentrations that are statistically significantly higher ($p<0.05$) between males and females across one type of EDC, IQR=interquartile range.

However, a statistically significant difference was only found for PBB-153 (U=389.0, p=0.001), PBDE-153 (U=436.0, p=0.006), ∑PBDEs (U=443.0, p=0.008), PCB-153 (U=509.0, p=0.047), PCB-170 (U=496.0, p=0.034), PCB-180(U=490.0, U=0.029) and ∑PCBs (U=500.0, p=0.038). We also compared ∑EDC concentrations by sex (Mann-Whitey U-test), and found no difference.

4.3.4.3 Plasma EDC concentrations by community

We compared EDC concentrations (Mann-Whitney U-Test) by location (Burin versus NWV; Table 4.17). There were several EDCs that were higher in concentration in one community than in the other; PBB-153 (U=545.5, p=0.023), PBDE-28 (U=478.5, p=0.001), PBDE-99 (U=488.0, p=0.006) and PCB-128 (U=253.0, p<0.0001), were all higher in Burin participants than in NWV participants. Overall, there was a trend towards higher PBB and PBDE concentrations in Burin, and higher PCB (with the exception of PCB-128) and p,p'-DDE concentrations in NWV. ∑EDCs were also non-significantly higher in NWV than in Burin.

Both PCBs and DDT have been seen to decrease in St. Lawrence River sentinel species such as great blue heron eggs from 1991-2011 (Champoux and Boily, 2017a), however PBDE concentrations in belugas did not change over a similar time period (1997-2013; Simond et al., 2017), which aligns with our findings of lower PCB and p,p-DDE but higher PBDEs in the south coast residents. We also compared ∑EDC concentrations by community (Mann-Whitey U-test), and found no difference.

**Table 4.17** Median EDC concentrations (pmol/g lipid) in participants by community.

| | Median pmol/g (IQR) | |
|---|---|---|
| **Compound** | **Burin (n=48)** | **NWV (n=32)** |
| **PBB-153** | 0.81 (0.00-1.14)* | 0.00 (0.00-1.61) |
| **PBDE-28** | 0.24 (0.00-0.55)* | 0.00 (0.00-0.00) |
| **PBDE-47** | 5.11 (3.53-9.62) | 4.14 (2.46-6.93) |
| **PBDE-99** | 1.88 (1.20-2.46)* | 1.34 (0.00-1.79) |
| **PBDE-100** | 0.60 (0.00-2.63) | 0.00 (0.00-1.76) |
| **PBDE-153** | 9.03 (4.98-23.08) | 10.86 (2.93-16.36) |
| **∑PBDEs** | 21.35 (13.35-38.04) | 13.88 (7.75-24.87) |
| **PCB-99** | 0.00 (0.00-1.81) | 0.00 (0.00-2.62) |
| **PCB-105** | 0.62 (0.40-1.13) | 0.89 (0.42-1.55) |
| **PCB-118** | 3.40 (1.97-6.15) | 4.53 (2.28-8.49) |
| **PCB-128** | 0.09 (0.05-0.15)* | 0.00 (0.00-0.00) |
| **PCB-138** | 9.21 (4.61-16.70) | 18.76 (5.34-27.51) |
| **PCB-153** | 19.07 (10.07-39.06) | 39.21 (9.80-64.75) |
| **PCB-170** | 6.75 (2.63-14.25) | 11.57 (3.25-17.14) |
| **PCB-180** | 20.01 (8.15-46.67) | 34.75 (8.85-58.30) |
| **∑PCBs** | 61.68 (32.36-134.17) | 129.42 (32.06-199.80) |
| **p,p'-DDE** | 81.85 (45.11-135.48) | 121.94 (54.29-244.75) |
| **∑EDCs** | 191.79 (118.35-317.53) | 277.81 (124.11-469.63) |

* denotes plasma congener concentrations that are statistically significantly higher ($p<0.05$) between Burin and NWV across one type of EDC, IQR=interquartile range.

4.3.5 Plasma EDC concentrations by hypothyroid status

We tested for the difference between EDC concentrations of hypothyroid (n=6) and non-hypothyroid participants (n=74) using a Mann-Whitney U-test (Table 4.18), as the concentrations were not normally distributed (right-skewed). The results from this analysis must be interpreted with caution, due to the low number of participants with hypothyroidism. PBDE-99 concentrations were statistically significantly higher in non-hypothyroidism participants than hypothyroid participants (U=105.0, p=0.032). No other EDCs were different between hypothyroid and non-hypothyroid participants. We also compared ∑EDC concentrations by hypothyroid status (Mann-Whitey U-test), and found no difference.

**Table 4.18** Median concentrations (pmol/g lipid) of EDCs for hypothyroid and non-hypothyroid participants.

| | Median pmol/g (IQR) | |
|---|---|---|
| **EDC** | **No Hypothyroidism (n=74)** | **Hypothyroidism (n=6)** |
| **PBB-153** | 0.63 (0.00-1.16) | 0.00 (0.00-1.24) |
| **PBDE-28** | 0.00 (0.00-0.44) | 0.00 (0.00-0.10) |
| **PBDE-47** | 4.92 (3.07-8.70) | 3.32 (1.54-8.25) |
| **PBDE-99** | 1.68 (1.05-2.27)* | 0.36 (0.00-1.69) |
| **PBDE-100** | 0.00 (0.00-2.23) | 0.49 (0.00-3.29) |
| **PBDE-153** | 9.08 (4.60-20.03) | 2.68 (1.97-12.32) |
| **∑PBDE** | 19.32 (11.60-37.04) | 9.44 (5.87-19.88) |
| **PCB-99** | 0.00 (0.00-1.49) | 0.00 (0.00-2.94) |
| **PCB-105** | 0.69 (0.40-1.21) | 1.09 (0.41-2.00) |
| **PCB-118** | 3.84 (2.07-6.97) | 5.16 (1.97-10.91) |
| **PCB-128** | 0.05 (0.00-0.13) | 0.00 (0.00-0.02) |
| **PCB-138** | 9.87 (4.71-21.79) | 10.83 (7.88-29.66) |
| **PCB-153** | 21.40 (9.89-48.88) | 19.41 (14.40-57.38) |
| **PCB-170** | 7.99 (2.72-15.66) | 5.91 (3.07-15.57) |
| **PCB-180** | 25.35 (8.05-53.90) | 18.31 (9.13-48.04) |
| **∑PCB** | 76.74 (31.86-158.14) | 60.87 (46.60-182.86) |
| **p,p'-DDE** | 88.45 (46.30-177.89) | 118.88 (67.20-363.56) |
| **∑EDCs** | 254.41 (120.22-403.89) | 269.05 (121.68-519.48) |

* denotes plasma congener concentrations that are statistically significantly higher ($p<0.05$) between hypothyroid and non-hypothyroid participant across one type of EDC, IQR=interquartile range.

## 4.4 Analysis of relationships between EDCs, thyroid hormones, and SCQ results

Objective 4 of this study was to investigate relationships between the SCQ responses, the blood thyroid hormones and EDC concentrations, and to explore local seafood consumption as an exposure source in Newfoundland.

### 4.4.1 Data transformations (skewness and outliers)

Prior to testing for relationships between the different predictor and outcome variables, we checked for data normality using a Shapiro-Wilks test (see Wu et al., 2007 as an example) and by constructing histograms of the data to look for skew (see histograms in Appendix D and E). The Shapiro-Wilks test (which is appropriate for smaller sample sizes such as in this study) is used to determine if data were normally distributed; the null hypothesis of this test is that the values are normally distributed so if $p<0.05$ the null hypothesis is rejected and there is sufficient evidence to conclude that the population is not normally distributed.

In this study, the results of the Shapiro-Wilks test were $p<0.05$ for all EDC concentrations, indicating they were not normally distributed. TSH values were also not normally distributed ($p<0.05$), however $FT_3$ and $FT_4$ concentrations satisfied conditions of normality. As a result, all TSH and EDC concentrations were $\log_{10}$-transformed to improve normality in the data (as per methods from Chevrier et al., 2010). After log-transforming the data, we checked for outliers. There was one outlier in PBB-153 that was more than 3 standard deviations from the range of the mean, therefore this concentration was winsorized to the highest value contained within 3 standard deviations of the range of the mean based on methods from Verner et al. (2015). There were no other outliers in any other EDCs or thyroid hormones. Participants with hypothyroidism were also removed from further analysis, as their thyroid

hormone concentrations are altered by ingestion of thyroid medication and therefore will not have a relationship with EDC concentrations.

### 4.4.2 EDCs and seafood consumption

One of the main objectives of the study was to explore if local seafood consumption could be a potential exposure pathway for EDC contamination in the Newfoundland population. We consolidated consumption responses to never, once/month and more than once per month, as there were very few participants who reported consuming any species more than 2-3 times per month, and used only cod for this analysis as it was by far the most commonly consumed species. We used a Kruskal-Wallis H-test to explore whether EDC concentrations differed based on local seafood consumption (Table 4.19).

Some PCB and p,p'-DDE congener plasma concentrations were found to be associated with consumption of local cod. PBDE concentrations were not different by local cod consumption frequency. There was a general trend towards higher plasma EDC concentrations in the highest reported consumption frequency (2+/month) of local cod. Plasma concentrations of PCBs -118, -138, -153, -170, -180, ∑PCBs and ∑EDCs were all statistically significantly higher in participants who reported consuming local cod 2 or more times in the past month, compared with participants who reported no consumption or those that reported consuming cod once in the past month. Plasma PCB-105 and p,p'-DDE were statistically significantly higher in participants who reported consuming local cod 2+ times in the past month compared with participants who reported consuming local cod not at all in the past month.

**Table 4.19** Association between local cod consumption frequencies and EDC concentrations (pmol/g lipid).

| SCQ consumption frequency by EDC | Median (IQR) | Kruskal-Wallis H | P |
|---|---|---|---|
| **PCB-105** | | **16.53** | **0.0001** |
| 0/month* | 0.38 (0.23-0.47) | | |
| 1/month | 0.59 (0.35-1.03) | | |
| 2+/month** | 0.86 (0.60-1.58) | | |
| **PCB-118** | | **16.36** | **0.0001** |
| 0/month* | 2.06 (1.16-2.51) | | |
| 1/month* | 3.36 (1.72-4.62) | | |
| 2+/month** | 4.86 (3.05-9.50) | | |
| **PCB-138** | | **15.71** | **0.0001** |
| 0/month* | 4.57 (1.44-6.88) | | |
| 1/month* | 8.23 (3.45-16.30) | | |
| 2+/month** | 15.51 (8.03-25.99) | | |
| **PCB-153** | | **16.86** | **0.0001** |
| 0/month* | 9.41 (2.61-15.63) | | |
| 1/month* | 15.34 (5.79-33.83) | | |
| 2+/month** | 38.55 (18.08-61.40) | | |
| **PCB-170** | | **15.38** | **0.0001** |
| 0/month* | 2.77 (0.64-4.77) | | |
| 1/month* | 4.07 (1.48-12.03) | | |
| 2+/month** | 13.40 (5.89-17.04) | | |
| **PCB-180** | | **16.20** | **0.0001** |
| 0/month* | 8.36 (1.77-14.34) | | |
| 1/month* | 12.63 (4.43-36.75) | | |
| 2+/month** | 41.05 (17.77-56.86) | | |

Only statistically significantly positive associations were reported ($p<0.05$), indicated by * (lower) or ** (higher) within a congener, IQR=interquartile range.

**Table 4.19** Continued: Association between local cod consumption frequencies and EDC concentrations (pmol/g lipid).

| SCQ consumption frequency by EDC | Median (IQR) | Kruskal-Wallis H | P |
|---|---|---|---|
| **∑PCBs** | | **17.77** | **0.0001** |
| 0-1/month* | 30.06 (8.37-51.31) | | |
| 1-2/month* | 47.46 (21.72-105.74) | | |
| 2+/month** | 136.87 (58.79-193.54) | | |
| **p,p'-DDE** | | **10.86** | **0.004** |
| 0/month* | 46.58 (24.21-56.02) | | |
| 1/month | 73.91 (41.47-132.05) | | |
| 2+/month** | 124.50 (66.91-232.78) | | |
| **∑EDCs** | | **17.44** | **0.0001** |
| 0-1/month* | 107.82 (56.34-136.37) | | |
| 1-2/month* | 161.93 (107.66-311.88) | | |
| 2+/month** | 289.51 (175.68-474.69) | | |

Only statistically significantly positive associations were reported ($p<0.05$), indicated by * (lower) or ** (higher) within a congener, IQR=interquartile range.

4.4.3 EDCs and seasonal seafood consumption

We also tested for a difference in seasonal local seafood consumption and plasma EDC concentrations. For seasonal consumption frequency, there were very few responses of consumption frequencies more than once/month, therefore all answers were consolidated into three categories: never, once/month or more than once/month (2+/month) consumption frequencies. The Kruskal-Wallis H-test was used to analyze the difference in plasma EDC concentrations by seafood consumption in different seasons (fall, winter, spring and summer). Seasonal variation in local seafood consumption existed for both study locations (higher consumption of seafood species in the summer, see Table 4.10), however it was important to

explore the association with plasma EDC concentrations as seasonal variations in EDC concentrations in the environment exist (Makey et al., 2014).

We found that higher consumption of local seafood in fall was positively associated with plasma concentrations of PCB-118 (H=6.85, p=0.033), PCB-128 (H=4.94, p=0.034), PCB-170 (H=7.70, p=0.021), PCB-180 (H=9.11, p=0.011) and ∑PCBs (H=7.33, p=0.026). Higher consumption of local seafood in the winter was positively associated with plasma concentrations of PCB-105 (H=7.76, p=0.021), PCB-118 (H=8.32, p=0.016), PCB-128 (H=9.85, p=0.007), PCB-138 (H=7.25, p=0.027), PCB-153 (H=7.50, p=0.023), PCB-170 (H=6.95, p=0.031), PCB-180 (H=8.00, p=0.018), and ∑PCBs (H=8.11, p=0.017). In spring, only PBDE-100 (H=6.48, p=0.039) was associated with greater cod consumption. There was no difference in plasma EDC concentrations for the various seafood consumption frequencies in the summer. We also tested the association between EDCs and seasonal local seafood consumption frequency with only two consumption frequency categories (never and 1+/month), but because such a low number reported eating local seafood more than once/month this did not change the results.

Plasma EDC concentrations in humans have been shown to be consistent throughout the year, indicating the reliability of a single blood measurement for exposure studies; however non-significant changes in some PBDE congener concentrations between winter to summer have been observed in human serum samples of office workers from Boston (Makey et al., 2014). In the context of this study, it is possible that those that consume local seafood year-round may have higher average plasma EDC concentrations, however when year-round seafood consumption was compared with plasma EDC concentrations using a Kruskal-Wallis H-test, no EDC congener concentrations were associated with total seasonal seafood consumption.

4.4.4 Regression analysis of the relationship between EDCs and thyroid hormones

Simple (univariate) and multivariate linear regression analysis was used to explore relationships between predictors (age, sex, community, EDC concentration, seafood consumption) and outcomes (thyroid hormones, EDC concentrations). There was no relationship between EDCs and thyroid hormones in a simple linear regression analysis. A multivariate linear regression was also carried out to investigate the utility of EDCs (predictor) in predicting thyroid hormone concentrations (outcome) with community, sex and age as covariates. There was no relationship between any EDCs and thyroid hormones in the multivariate regression analysis when continuous variables were used for EDC and TH concentrations.

Multivariate regression was also performed with EDCs categorized into quartiles to account for non-monotonic dose-response curve thresholds in the relationships between EDCs and thyroid hormones (methods from Chevrier et al., 2010). Again, there was no relationship between EDCs and thyroid hormones. We also tested for the association between ∑EDCs (predictor) and thyroid hormones (outcome) using a multivariate regression analysis with age, sex and community as covariates, but there was no association. While the multivariate regression did not show an association between EDCs and thyroid hormones, there were some non-significant trends seen when these relationships are examined using EDC quartiles (Figures 4.4, 4.5 and 4.6).

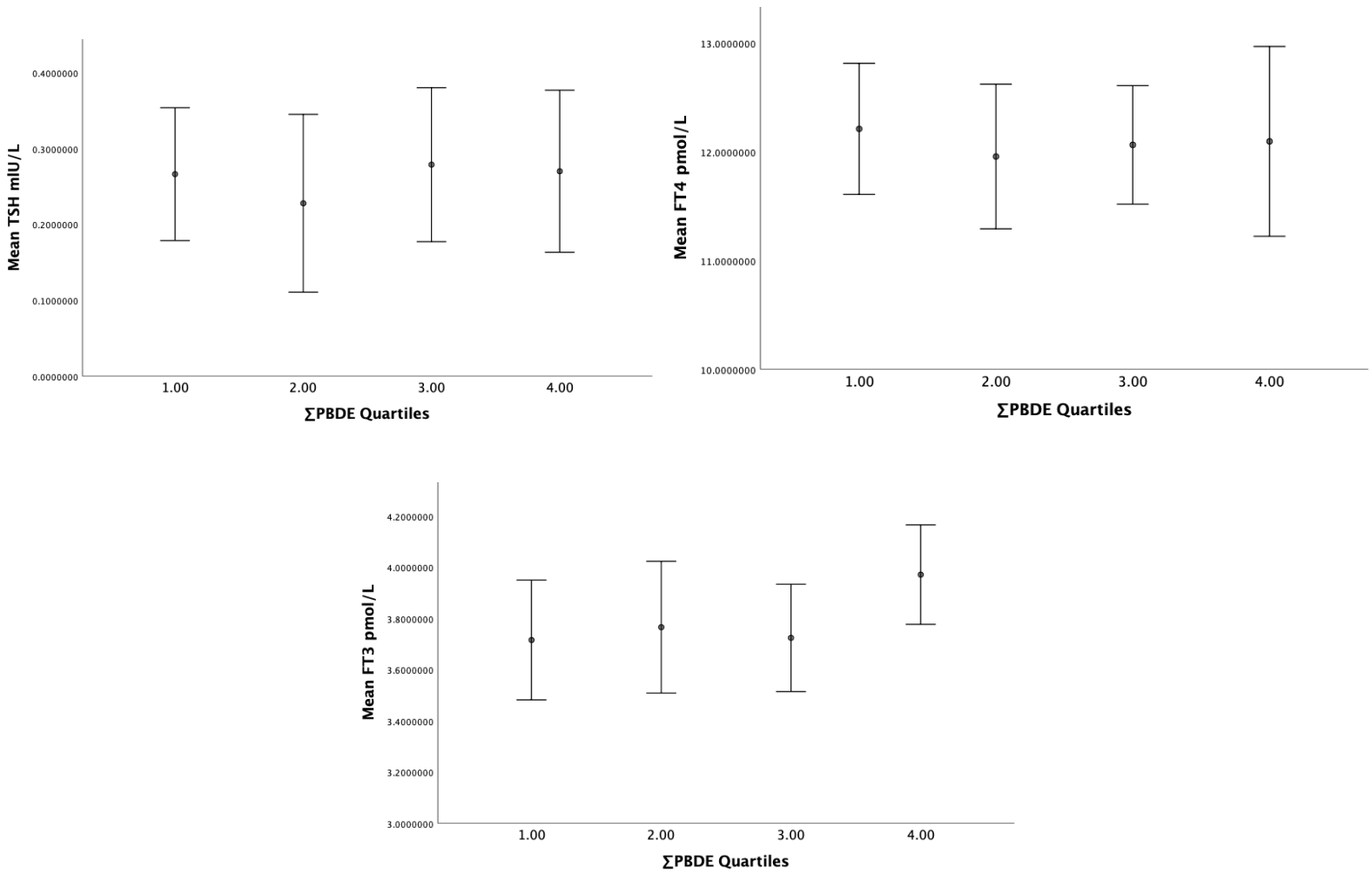



**Figure 4.4** Means and 95% Confidence Intervals of $Log_{10}$ TSH (mlU/L), $FT_4$ (pmol/L), $FT_3$ (pmol/L) by $Log_{10}$ ∑PBDE (pmol/g lipid) quartiles.

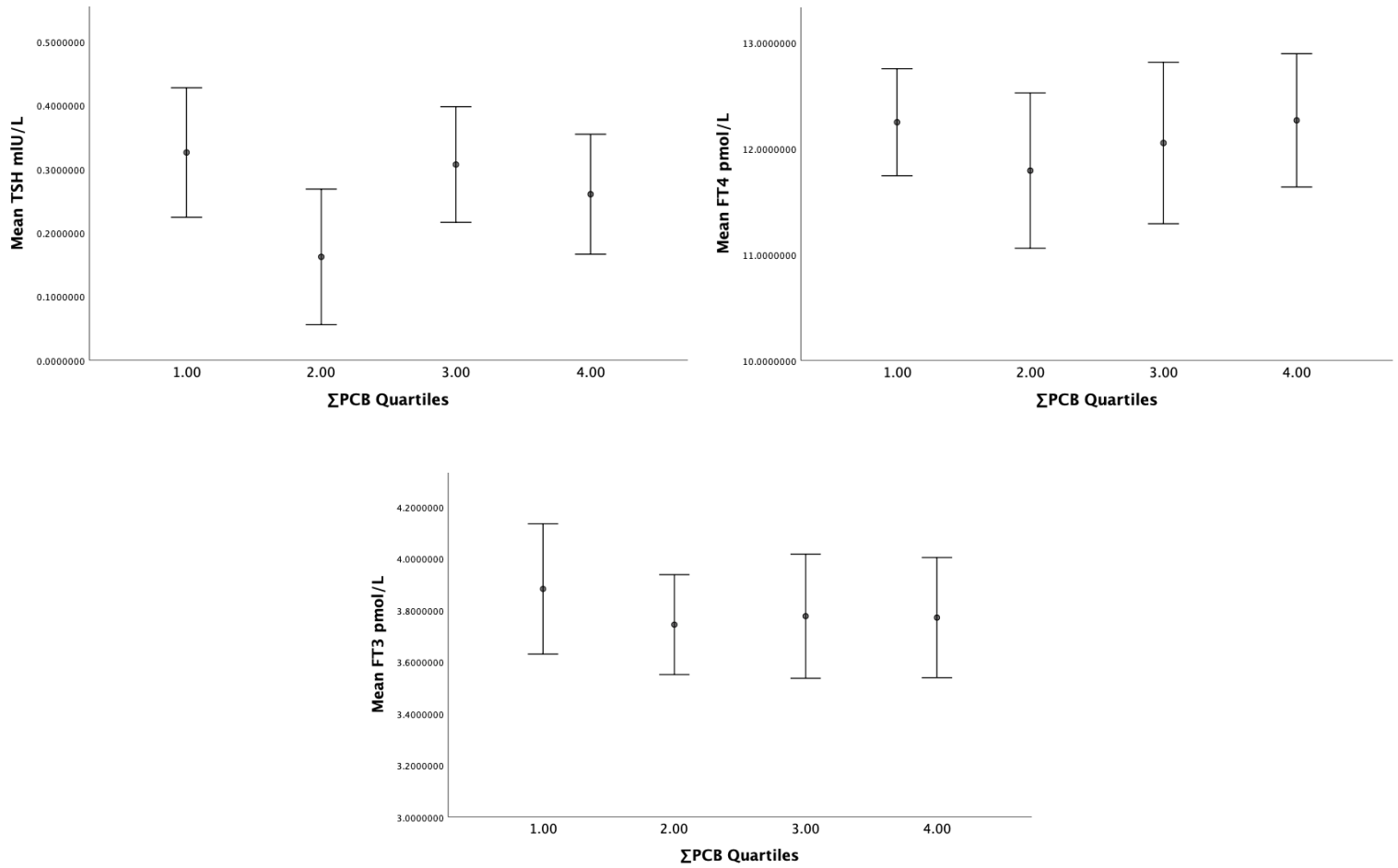



**Figure 4.5** Means and 95% Confidence Intervals of $Log_{10}$ TSH (mlU/L), $FT_4$ (pmol/L), $FT_3$ (pmol/L) by $Log_{10}$ ∑PCB (pmol/g lipid) quartiles.

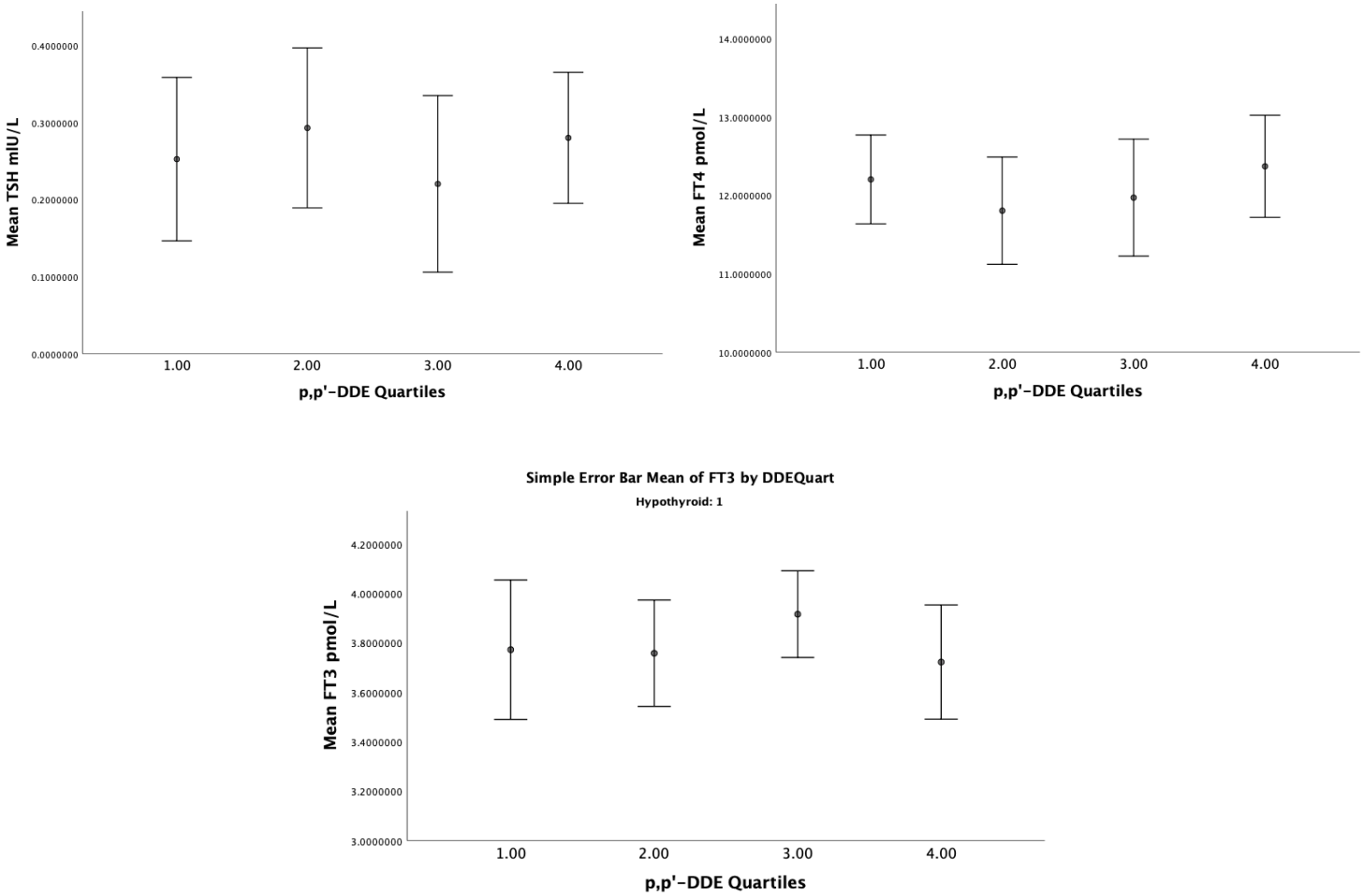



**Figure 4.6** Means and 95% Confidence Intervals of $Log_{10}$ TSH (mlU/L), $FT_4$ (pmol/L), $FT_3$ (pmol/L), by $Log_{10}$ p,p'-DDE (pmol/g lipid) quartiles.

Most of the thyroid hormones appeared to have slight non-monotonic curves associated with increasing quartiles of EDC concentrations. This means that the highest or lowest plasma EDC concentrations in our study population did not always correspond to the highest or lowest thyroid hormone concentrations. This is a common theme with EDCs, and makes it very complicated for researchers to infer endocrine disrupting effects from EDC concentrations as many of these relationships do not extrapolate back to a "no effect level" as they would in a linear relationship.

### 4.4.5 Regression analysis of the relationship between EDCs and seafood consumption

Relationships between EDC concentrations and local seafood consumption were also assessed, to explore local seafood as a potential source of exposure in the study population. Consumption of cod was analyzed separately from all remaining (non-cod) seafood species. This was done because cod was by far the most commonly consumed seafood species (most other local species had low reported consumption frequencies), and evidence of EDC contamination in this species has already been documented by our study. Additionally, when cod and all remaining seafood species were analyzed together, the results were the same as when cod was analyzed alone. This may be due to the high reported consumption frequencies of cod masking the association of other less commonly consumed species with the plasma EDC concentrations. Multivariate regression was run again, with cod consumption as the predictor and plasma EDC concentration as the outcome variable, with age, sex and community as covariates.

Frequency of local cod consumption was associated with several plasma PCB and p,p'-DDE concentrations in the study samples (Table 4.20).

**Table 4.20** Multivariate linear regression analysis of cod consumption (categorical predictor) and plasma EDC concentrations (continuous outcome). Community, age and sex are also included as covariates (only statistically significant covariates included in table).

| Predictors | Outcome | ß (95%CI) | P-value |
|---|---|---|---|
| Cod | PCB-105 | 0.385 (0.138-0.647) | 0.003 |
| Cod | PCB-118 | 0.443 (0.201-0.680) | 0.0001 |
| Cod | PCB-128 | 0.480 (0.142-0.763) | 0.006 |
| Cod | PCB-138 | 0.356 (0.122-0.593) | 0.003 |
| Age | | 0.263 (0.051-0.480) | 0.016 |
| Cod | PCB-153 | 0.393 (0.164-0.621) | 0.001 |
| Age | | 0.277 (0.070-0.486) | 0.010 |
| Cod | PCB-170 | 0.370 (0.144-0.593) | 0.002 |
| Age | | 0.338 (0.133-0.543) | 0.002 |
| Cod | PCB-180 | 0.381 (0.156-0.605) | 0.001 |
| Age | | 0.328 (0.124-0.533) | 0.002 |
| Cod | ∑PCBs | 0.397 (0.170-0.623) | 0.001 |
| Age | | 0.297 (0.091-0.505) | 0.005 |
| Cod | p,p'-DDE | 0.271 (0.020-0.530) | 0.035 |

PCBs in pmol/g, significance $p<0.05$, ß = Standardized coefficient, CI = confidence interval.

All statistically significant relationships between cod consumption and plasma PCBs and p,p'-DDE were positive, indicating that as local cod consumption increased, corresponding plasma EDC concentrations also increased. None of the cod consumption frequencies had relationships with plasma PBDE concentrations in these multivariate analyses.

Age was a statistically significant positive predictor of PCB-138, PCB-153, PCB-170 and PCB-180 concentrations in this multivariate analysis, and all congener concentrations were higher in older than in younger participants (see Table 4.15 for similar findings). No other covariates were associated with EDC concentrations. We analyzed the association between consumption of all other seafood species and plasma EDC concentrations. With the exception cod, all other seafood species had consumption frequencies summed, and therefore each species consumption frequency was one predictor variable. There was no association between frequencies of all seafood consumption (no cod) and participant plasma EDCs.

We also analyzed consumption of cod and all seafood (no cod) together in a multivariate regression analysis, with plasma EDC concentrations as outcome variables and age, sex, and community as covariates. When the cod and all seafood consumption frequencies of never, once/month, more than once/month were used in this analysis, the outcomes were the same as in Table 4.20; PCBs -105, -118 and -128 were all positively associated with cod consumption, but not with all seafood consumption or any other co-variates. PCBs -138, -153, -170, -180 and ∑PCBs were all positively associated with cod consumption and age, but not with all seafood consumption or any other covariates. No other EDC congeners had relationships with seafood consumption frequencies or covariates in this analysis. These results indicate that there are some statistically significant associations even when both categories of seafood species are analyzed together in a multivariate regression analysis.

### 4.5 Summary of results

In conclusion, there are some very robust trends that have emerged from the analysis of these data. There was strong evidence that PBDEs are present in the marine ecosystem around the island of Newfoundland, as most of the tested fish livers had PBDE congeners present in their tissues. There were statistically significantly higher concentrations of PBDEs seen in fish samples from the west coast, as compared with the northeast (offshore) coast of the island.

Seafood consumption varied amongst the two communities for some species, but cod emerged from both communities as the most commonly consumed species. In humans, there were a substantial number of EDCs detected in the plasma samples of residents from Burin and NWV. The plasma concentrations of PBDEs and PBB-153 were generally higher in residents of Burin, while the residents of NWV had higher concentrations of PCBs and p,p'-DDE. Males on the average had higher levels of EDCs in their serum. Younger participants (<50 years) generally had higher concentrations of PBDEs while older participants (>50 years) had higher concentrations of PBB-153, p,p'-DDE and PCBs. No association was detected between EDCs and thyroid hormones; however, some non-monotonic trends (slope of the outcome curve changes direction over increasing predictor concentrations) were seen.

There was no association between cod consumption and plasma PBDE concentrations, however cod consumption was associated with several PCB and p,p'-DDE concentrations. The all seafood (no cod) consumption, was not associated with any plasma EDC concentrations. Age was the only covariates that was also statistically significantly associated with EDC concentrations in these multivariate regressions.

Overall, the results indicate that PBDEs are present in seafood samples from the coastal waters of Newfoundland. We also observed that consumption of certain species of seafood was

associated with higher plasma PCB and p,p'-DDE concentrations, however no change in thyroid hormone concentrations was associated with EDCs levels. As well, there is evidence that local seafood is contaminated with PBDEs in Newfoundland and that the resident population is exposed to EDCs, although we were not able to demonstrate a direct correlation or impact of exposure on thyroid hormones. Additionally, there were some PBDE congeners that were found only in human plasma (PBDE-153) while others were found only in fish (PBDE-156, PBDE-209), indicating that there may be other potential sources of exposure. For fish, some PBDEs (i.e. PBDE 209) may not biomagnify well in the food chain (not easily passed to higher trophic-level organisms), and their presence in liver samples may be the result of acute exposure (i.e. a local pollution source). In humans, other potential sources of PBDE exposure includes meat consumption and house dust ingestion, and these should be explored in future studies of this population.

# Chapter 5 Discussion

This chapter discusses the significance of our findings from the four aspects of our study, namely the presence of PBDEs in fish livers (Objective 1), seafood consumption in two rural Newfoundland communities (Objective 2), concentrations of EDCs and thyroid hormones in human blood samples (Objective 3), and the association between seafood consumption, plasma EDCs and serum thyroid hormones (Objective 4).

This project was triggered by an earlier study by us (Sarkar et al., 2015), in which we analyzed extracted thyroid hormone data from hospitalized patients diagnosed with hypothyroidism in Newfoundland. We focused on hypothyroidism diagnosis rates by location (coastwise), looking for potential geographical differences in exposure. We found higher rates of hypothyroidism on the south and west coasts of Newfoundland, almost twice as high as rates on the northeast coast.

This led to our hypothesis that a possible cause for the varying thyroid diagnosis rates in these different locations might be due to EDC pollution reaching the west and south coasts of Newfoundland from the St. Lawrence River, and causing contamination in the local marine food chain. We theorized that this source of environmental contamination could be a potential contributor to this unequal distribution of hypothyroidism around the island, and found literature pointing to the St. Lawrence River as a source of EDCs into the Gulf of St Lawrence marine environment. We had initially planned to visit the west coast of Newfoundland to collect data and samples, however issues with securing a location for blood collection with the local health authorities led us to restrict the study to the south and northeast coasts only.

Newfoundland has a very long history of subsidence fishing, which continues to this day in the recreational food fishery as well as the presence of numerous fish harvesters and

processing plants around the island. Because of this we believed that residents of small coastal communities from different parts of the island would regularly consume local seafood, and that the geographical origin of seafood that was being consumed was contributing to the higher rates of hypothyroidism along the south and west coasts. We hypothesized that consumption of seafood contaminated with EDCs from St. Lawrence River pollution was contributing to higher hypothyroidism rates on the south and west coasts of Newfoundland, and that the northeast coast rates were lower due to less contamination of seafood from the clean Labrador current.

As our study progressed, from testing fish for EDCs to gathering information on seafood consumption and EDCs in participant's plasma, we realized that exposure to EDCs in Newfoundland was more complex than we initially hypothesized. This study has led to some interesting discoveries, which now lead to even more questions about EDC contamination in Newfoundland (discussed later in this chapter). Evidence from this study will hopefully help spur more research on EDC contamination and its environmental foot-print in Newfoundland and Atlantic Canada in the future

We began this study by evaluating whether the marine ecosystem around the Island of Newfoundland was contaminated with PBDEs. This was done by analyzing fish liver samples from four different locations around the island for the presence of PBDEs, and led to the finding that both cod and turbot livers contained PBDEs -28, -47, -99, -100, -156 and -209 as well as 6-MeO-BDE-47 and 2'-MeO-BDE-68. We also explored the association between consumption of local seafood species and potential EDC exposure by conducting a seafood consumption survey in two rural Newfoundland communities on the south and northeast coasts of the island. Blood samples collected from participants who completed the seafood consumption questionnaire were tested for EDC and thyroid hormone concentrations.

We were able to demonstrate for the first time, the presence of various EDCs including PBDEs, PCBs, PBBs, and p,p'-DDE in the plasma of residents living in two coastal communities of Newfoundland. Although these chemicals are all currently banned in Canada (and some have been restricted or banned for decades), they are still found in the environment and in the present Newfoundland population, as seen in our study. This contamination has also been documented in several different populations residing along the coast of the Gulf of St. Lawrence (GSL) including Inuit in the Quebec portion of the GSL (Dallaire et al., 2008) and Mohawk populations residing further upstream in the St. Lawrence River (Ryan et al., 1997; Schell et al., 2008). While no recent studies have tested for PBDEs, PCBs, PBBs or p,p'-DDE in populations residing along the St. Lawrence waterway, Caron-Beaudoin et al. (2019) found perfluoroalkyl substances (PFAS), a novel and persistent EDC, in Innu populations living along the north shore of the Gulf of St. Lawrence, indicative of ongoing environmental EDC contamination in many areas upstream from the Island of Newfoundland.

Upstream sources of water (and pollution) to the Island of Newfoundland include the St. Lawrence River, which empties into the Gulf of St. Lawrence and flows around the west and south coasts of Newfoundland, and the Labrador Current, which originates in the Eastern Canadian Arctic (Baffin Island and Norther Labrador) and flows down the coast of Labrador and over the northeastern coast of Newfoundland and the Grand Banks. Both of these currents may be sources of contamination to the Island of Newfoundland.

Current data on the association between EDCs and thyroid hormones are inconsistent (both a positive and negative association have been reported for the same EDCs and thyroid hormones), making the interpretation of data challenging. We failed to find any association between EDCs and thyroid hormones, similar to the studies by Byrne et al. (2018) who found no

PBDEs associated either with $T_4$ or $FT_4$ in their study population, and Dallaire et al. (2009) who reported no association between PBDEs and TSH. Additionally, Stapleton et al. (2011) found no association between PBDEs and $T_3$ or $FT_3$.

In contrast, other studies of the relationships between EDCs and thyroid hormones have revealed both a positive and negative (inverse) association. Lin et al. (2011) found an inverse relationship between human PBDE-153 and $FT_3$ concentrations, as did Gaum et al. (2016), who reported higher chlorinated PCBs were negatively associated with $FT_3$. However, positive relationships have been documented between PBDEs -47 and -28 and $FT_4$ (Vuong et al., 2015); PBDEs -28, -153, -183 and $FT_3$ (Li et al., 2011); and PCB-153, ∑PCBs and TSH (Donato et al., 2008). One possible explanation for these contradictory findings between EDCs and thyroid hormones is that exposure to a mixture of EDCs may result in synergistic and antagonistic effects all happening at the same time, and thus determining the outcomes of individual EDCs can be very difficult.

In comparison to European countries, Canadians and Americans are exposed to higher levels of PBDEs, and concomitantly, have higher blood levels of PBDEs (Trudel et al., 2011), making them an ideal population to study the effects of EDC exposure on thyroid hormones. As such, it is still difficult to predict the relationship between EDCs and thyroid hormones and more research is needed in this area.

### 5.1 Presence of PBDEs in Fish Liver

Cod and turbot from four different costal locations were tested for the presence of PBDEs and MeO-BDEs in liver, as a measure of PBDE contamination in the marine ecosystem. Two areas of sampling (Areas 3&4) were along the south and west coasts of Newfoundland, which

receive currents from the Gulf of St. Lawrence. The other sampling locations (Areas 1&2) were off the northeast coast of Newfoundland, on the North American continental shelf and the Grand Banks. Liver samples from cod and turbot were collected and tested for PBDEs from all four areas. Liver is an important tissue to test for environmental contaminant because it is very fatty, and contains a high concentration of lipid-soluble chemicals such as PBDEs and PCBs. Chemicals such as PBDEs and PCBs are detoxified in the liver through glycosidation and sulphation, rendering them water soluble so that they are readily excreted by the kidneys. Therefore, it is expected that the concentration of these chemicals is likely to be high in the liver and their presence will be easily detected.

### 5.1.1 Cod and turbot liver PBDE concentrations

In our fish liver samples, we detected PBDE-28, -47, -99, -156 and -209 as well as 6-MeO-BDE-47 and 2'-MeO-BDE-68. Liver generally has higher PBDE concentrations than fillet (see Vives et al., 2004 as an example), therefore it is a good tissue to initially test for the presence of PBDEs in marine species such as fish.

We were not able to analyze fish fillet (muscle) samples in this project as the Department of Fisheries and Oceans and the Marine Institute, who collected the fish, required the fillets for their own research purposes. However, we know from other studies (e.g. Boon et al., 2002; Weijs et al., 2009) that EDCs accumulate in both liver and muscle tissue of fish. Cod has relatively lean muscle (approximately 1% fat), and where many EDCs are lipophilic, they may accumulate less in muscle tissue and more in liver which has a higher fat content.

Julshamn et al. (2013) found PBDE and PCB concentrations were higher in liver than in muscle of Atlantic Cod from the Barents Sea. Boon et al. (2002) compared cod liver PBDE

concentrations to the sum PBDE concentrations in liver and muscle (liver/[liver+muscle]), and found a concentration ratio of 0.790 for PBDE-47, indicating that various tissues in the same organisms can have different PBDE (and other EDC) concentrations. If the Boon et al. (2002) PBDE-47 concentration ratio of 0.790 is applied to the cod samples in our study, the estimated muscle concentration of PBDE-47 would be 5.01 ng/g (based on a mean liver concentration of 18.86 ng/g). PBDE liver-to-muscle ratios (liver/[liver+muscle]) can also vary by congener; Weijs et al. (2009) found the cod liver-to-muscle ratio of PBDE-47 to be 0.78 while for PBDE-100 and -154 it was 0.67.

Greater accumulation of PBDEs in cod liver may be due to the high lipid content of this tissue, however this is known to vary from species to species. Herring (an oily fish) has a high lipid concentration in muscle stores but lower levels in the liver, and thus the PBDE-47 liver-to-muscle ratio is 0.445, which is the opposite of the lipid storage pattern in cod which has a fatty liver and lean muscle (Boon et al., 2002). Carp (*Cyprinus carpio)* and wels *(Silurus glanis)* have also been found to preferentially accumulate PBDEs in muscle (0.38 and 0.33 ratios respectively; Erdogrul et al., 2005), while nose-carp (*Chondrostoma regium*) has been found to preferentially accumulate PBDEs in the liver (0.58 liver-to-muscle concentration ratio). Again, this shows that accumulation of PBDEs in body tissues varies amongst species.

Canada's Minister of the Environment sets Federal Environmental Quality Guidelines (FEQGs) for PBDE concentrations in environmental samples (sediments, water, fish and wildlife tissues), to be used in risk assessment/management of substances identified under the Chemicals Management Plan (Environment and Climate Change Canada, 2013b). We compared the PBDE concentrations in cod and turbot livers from our study with the whole fish tissue Canadian FEQGs (Table 5.1).

**Table 5.1** PBDE concentrations from cod and turbot livers, and FEQG.

| PBDE | Cod | Turbot | FEQG (fish tissue) |
|---|---|---|---|
| Tri-BDE (PBDE-28) | 2.25 ng/g | 1.42 ng/g | 120 ng/g |
| Tetra-BDE (PBDE-47) | 16.98 ng/g | 3.51 ng/g | 88 ng/g |
| Penta-BDE (PBDE-99) | 10.14 ng/g | 1.98 ng/g | 1 ng/g |
| Hexa-BDE (PBDE-156) | 4.36 ng/g | 2.70 ng/g | 420 ng/g |

Both cod and turbot livers from our study had higher penta-BDE (PBDE-99) concentrations than those recommended in the FEQGs for whole fish tissue. All other PBDE congener concentrations were below FEQG recommended limits. If it were possible to compare the PBDE concentrations in fillets from our fish samples with the FEQG it is likely our PBDE fillet concentrations would be lower than the FEQG for whole fish, given the lower ratios of PBDEs found in fillet compared with liver.

We examined the literature to compare PBDE concentrations in our cod liver samples with other studies. For example, Bytingsvik et al. (2004) found lower PBDE-28 (0.75 ng/g) but greater PBDE-47 (22.7 ng/g) in cod livers sampled from the Norwegian North Atlantic Ocean to those seen in the cod livers in our study. ∑PBDE concentrations (7 congeners) from Northeastern Artic cod livers (1.8 µg/kg) sampled by Julshamn et al. (2013) were lower than ∑PBDE concentrations seen in our study (38.7 ng/g, 5 congeners), as were ∑PBDE concentrations (15 congeners) in cod livers from fish caught in the Barents Sea (5.6 µg/kg) sampled by Boitsov et al. (2019). We found PBDE-47 was the dominant congener in our cod liver samples and accounted for 49% of total PBDE content in the liver tissue; this is higher than

the profile seen in Baltic cod liver samples by Roszko et al. (2015), who found that PBDE-47 made up 38% of total PBDE content in their fish samples. Cod liver oil (often used as a health supplement) has also been shown to contain PBDEs (18.2 ng/g; Marti et al., 2010), although in lower concentrations than those seen in our study.

These foregoing studies provide strong evidence that cod from different geographical locations have varied PBDE concentrations, which may be the result of local sources of contamination or long-range transport of PBDEs that entered the environment elsewhere. Of note is that northern locations are more likely to receive PBDEs via atmospheric deposition than southern locations, due to global distillation of volatile PBDEs at lower and warmer latitudes to higher, colder latitudes.

We also found that some PBDE congeners varied by species; cod livers had statistically significantly higher concentrations of PBDEs-47, 99, 6-MeO-BDE-47, 2'-MeO-BDE-68, and ∑PBDEs than turbot livers. This is intriguing, as these fish species occupy similar positions (benthic) in the water column. Turbot bury themselves in ocean sediment and wait for their prey, while cod generally hunt near the bottom of the seabed, but have less contact with sediments. Contact with contaminated sediments has been shown to be a source of exposure for some species (Kilemade et al., 2009). Additionally, cod and turbot both occupy similar trophic positions in the food chain (Lee at al., 2010b). Age of the fish (not measured in this study) can also be a factor in EDC concentrations; PCBs, DDT and PBDE concentrations have all been found to increase with age in different species of fish (Pandelova et al., 2008; Youngs et al., 1972; Parmanne et al., 2006). While older fish are more desirable as a catch due to their size, they may also have higher body burdens of EDCs and be a greater source of exposure. We were

unable to adjust for the age of the fish in our study as this information was not available from our fish liver suppliers (Department of Fisheries and Oceans and the Marine Institute).

Several studies have also reported cod livers as having as much as 2 to 3 orders of magnitude greater PBDE levels than livers of other tested species (Bakke et al., 2008). The difference in liver concentrations of PBDEs between cod and other species could be due to different debromination and metabolism in other organisms. Studies of metabolite formation in rainbow trout, chinook salmon, and common carp have demonstrated that some species can have up to 100 times faster metabolism of major PBDE congeners than the other species (Roberts et al., 2011). Metabolism, lifecycle and trophic position are all potential contributors to the difference we saw between turbot and cod liver PBDE concentrations, and our study adds to the body of evidence that there was a statistically significant species-specific difference between liver PBDE concentrations, which is important to consider when examining human EDC exposure via seafood consumption.

PBDE-209 was detected rarely and only in very small concentrations in fish livers in this study. PBDE-209 has been noted not to biomagnify (accumulate in greater concentrations in higher trophic level organisms) in some fish species, including top predators such as pike, perch, and roach (Burreau et al., 2004). Therefore, it is not surprising that we did not see much PBDE-209 in our cod and turbot samples. Additionally, in North American Arctic waters Penta-BDE and Tetra-BDE are the most predominant congeners while in most other Arctic waters Deca-BDE (PBDE-209) is the dominant congener (Salvadó et al., 2016). Therefore, it seems likely that the PBDE-209 concentrations we detected in our fish liver samples were the result of direct exposure to this congener, and not as a result of bioaccumulation in the food chain.

MeO-BDE-47 and MeO-BDE-68 are naturally produced by cyanobacteria, red algae and marine sponges (Vetter et al., 2002; Malmvärn et al., 2008; Wiseman et al., 2011). The Grand Banks and Flemish Cap (located off the coast of northeastern Newfoundland) are very old deep-sea sponge grounds (Murillo et al., 2016), while the Straight of Belle Isle (Area 4, west coast of Newfoundland) has been documented to contain both red algae and sponges (Nalcor Energy, 2012). It is possible that these areas could naturally produce MeO-BDEs due to the presence of sponges, red algae and other marine life, but that has yet to be documented and verified.

There is also evidence that these chemicals may also be the result of metabolic breakdown products of anthropogenic PBDEs (Kelly et al., 2004). MeO-BDE liver concentrations in our study were higher than those found in Baltic cod (5.4 ng/g; Roszko et al., 2015). While less is known about the human health effects of MeO-BDEs, zebrafish have been shown to preferentially bioaccumulate 6-MeO-BDE-47 over PBDE-47, and to transform 6-MeO-BDE-47 into the metabolite 6-OH-BDE-47 (Liu et al., 2015). Additionally, studies in rats have shown that approximately 10% of MeO-BDEs are converted to 6-OH-BDE-47 (Wan et al., 2009). OH-BDEs are also known endocrine disruptors; they can compete with $T_3$ for binding to transthyretin (Kitamura et al., 2008) and can inhibit the gene transcription mediated by thyroid receptors (Kojima et al., 2009). It is unknown if the thyroid effects of MeO-BDEs seen in animal studies will translate to humans; MeO-BDEs have been detected in human serum samples, and seafood has been identified as a likely source of exposure (Wang et al., 2011). As such, it is important to consider these substances as potential environmental endocrine disruptors contributing to EDC body burden in humans and other organisms.

5.1.2 Cod PBDE concentrations by location

In this study, we measured liver PBDE concentrations in cod from different locations around the Island of Newfoundland. Although we had some turbot liver samples, there were too few to conduct a full analysis in this species. Geographically, the four areas where cod were sampled from (Figure 5.1) have very different currents and potential sources of environmental pollution.

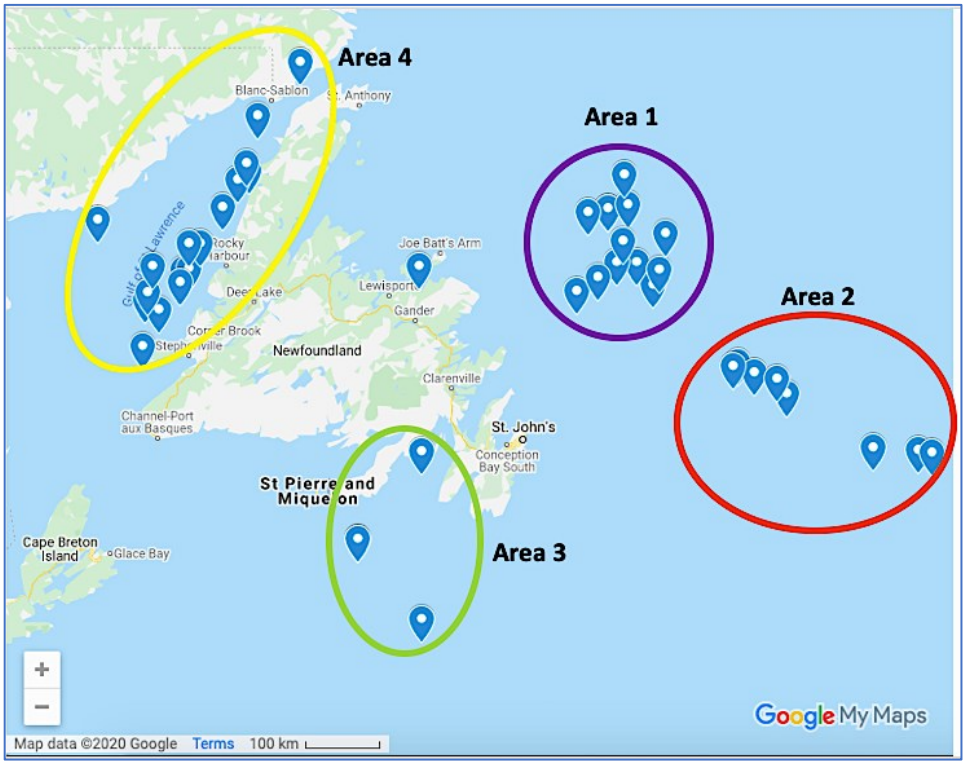



**Figure 5.1** Four main location of cod and turbot sampling for liver PBDE analysis. Map data ©2020 Google.

The cod liver samples with the highest PBDE concentrations were collected from Area 4, off the west coast of the island. Cod livers from this area had the highest average concentrations of PBDE-47, -99, and ∑PBDEs. Area 4 is a narrow channel between Labrador/Quebec and the west coast of the Island of Newfoundland which receives the St. Lawrence River and Labrador

Currents. Area 3, located off the south coast of Newfoundland in the Gulf of St. Lawrence, had the highest average concentrations of PBDE-156, as well as 2'-MeO-BDE-68, however none of the PBDE concentrations in this area were different from the other sampling locations.

Suspended particulate matter (both organic matter and sediment) in the St. Lawrence River was been tested for PBDEs by Environment and Climate Change Canada in 2005/2006, and was found to contain ΣPBDEs ranging from 1.35-25.37 ng/g, while surface sediments from the lakes located along the St. Lawrence River had ΣPBDEs ranging from 0.18-1.65 ng/g (Pelletier and Rondeau, 2013). While there have not been any recent studies of PBDE concentrations in the St. Lawrence River sediments, 2013-2014 sediment samples from the Huron-Erie Great Lakes corridor (which is part of the drainage basin which empties into the St. Lawrence River) had ∑PBDE concentrations ranging from 0.25-13.48 ng/g dry weight (Drouillard et al., 2019). This evidence points to legacy and ongoing PBDE contamination in this area, indicating that the St. Lawrence River is a potential source of PBDEs to the south and west coasts (sampling Areas 3&4) of Newfoundland. These areas also receive water currents from Labrador and the North Atlantic Ocean, meaning there are many potential sources of environmental pollution in these areas. It was not possible to determine which currents were responsible for the PBDE contamination seen in the cod and turbot liver samples in this study. However, there is evidence that PBDEs are present in the marine ecosystem and the lack of local pollution sources does imply upstream sources of PBDE contamination.

Liver samples from Area 1 (northeast coast of Newfoundland) did not have different PBDE concentrations when compared to livers tested from any other areas around Newfoundland. Area 1 is in the open North Atlantic Ocean and receives the Labrador Current. Area 2, also located in the open North Atlantic Ocean but further offshore and closer to an area known as the Grand

Banks, had some of the lowest concentrations of PBDEs and MeO-BDEs, indicating that fish in these areas may not be exposed to as much environmental pollution as some of the other areas in this study. This area was the furthest from any human settlements or urban/industrial activities, and although it receives waters of the Labrador Current it is further into the open North Atlantic Ocean than Area 1, which may dilute the Labrador Current pollutants and contribute to the low levels of PBDE contamination seen in fish livers collected from this area.

The Labrador Current brings down water from the Arctic, western Greenland, and Baffin Island into the North Atlantic Ocean by way of the coast of Labrador. The Arctic is a site of atmospheric deposition for volatile EDCs that travel through the atmosphere on wind currents and are deposited into remote locations. PBDEs have been detected in Arctic species for decades, and while concentrations of many EDCs including PBDEs are decreasing in Arctic biota (Rigét et al., 2019), EDCs trapped in snow and ice can still make their way back into ocean currents (such as the Labrador Current) and travel further south to more populated areas. For example, ringed seal blubber samples taken from Baffin Island and Nunatsiavut (Labrador), showed ∑PBDE concentrations ranging from 1.98-28.8 ng/g lipid weight (Houde et al., 2017). This indicates that the coast of Labrador may be receiving Arctic contamination; low urban and industrial densities along this coast make local sources of PBDEs contamination into the environment unlikely, and point to long-range transport of PBDEs from Artic currents as the most likely source of PBDEs in marine species.

Studies have also found EDCs in marine species in the open North Atlantic Ocean. Spinal fluid samples from dolphins from Massachusetts (US) contained PBDEs (Montie et al., 2009), and tissue samples from winter flounder, seals, and North Atlantic right whales (various high trophic level organisms in the marine food chain) also contained PBDEs (Montie et al., 2010).

Whole flounder PBDE-47 concentrations from East Cape Cod Bay (south of Newfoundland, Montie et al., 2010) were several orders of magnitude higher than fish from the present study (919.58 ng/g lipid versus 14.29 ng/g lipid). This may reflect the higher PBDE concentrations found in this species, the method of extraction (whole fish versus liver) and/or the geographical location.

While there is scant evidence of PBDEs or other environmental contamination in the marine environment around the Island of Newfoundland, our study confirms that PBDE contamination is present and has moved up the food chain into higher trophic level organisms such as turbot and cod. Cod movements around the island of Newfoundland have been monitored by Fisheries and Oceans Canada throughout the year to determine a seasonal difference in geographical location; many cod appear to overwinter offshore and return to inshore areas in the summer and fall. There does not appear to be much movement of cod to different parts of the island, and most return to the same bay from their offshore wintering areas (Lawson and Rose, 2000). As such, testing cod for PBDEs and other environmental contaminants should serve as an indication of the degree of pollution in the general vicinity, as there is not much movement of cod to different parts of the island.

Results from the fish liver samples demonstrate clear evidence of PBDE contamination in marine species around the Island of Newfoundland. The contamination likely originates from upstream sources, including the St. Lawrence River, the Labrador Current and the North Atlantic Ocean, all of which have evidence of PBDE contamination in higher trophic level species. PBDE contamination is higher in fish livers from the west coast compared with the northeast coast, indicating high pollution levels in the marine ecosystem in the Gulf of St. Lawrence. Further

investigation of PBDEs in different species is required to develop a more thorough understanding of EDC contamination around the island.

## 5.2 Seafood consumption in Newfoundland

Newfoundland has deep roots in the Atlantic fishery, and many of the first settlers came to Newfoundland seasonally to catch fish, eventually staying throughout the year and settling into communities. From its founding, the province's economy was built on the fisheries, and in addition to harvesting fish for export many people also consumed what they caught (Lowitt, 2013). Cod was the main fishery product and became the staple of many Newfoundland diets (Lowitt, 2013). This remains true today, as seen in our study of communities on the south and northeast coasts, and that by Lowitt (2013) on the west coast.

### 5.2.1. Seafood consumption by community

The main goal for this part of our study was to explore local seafood consumption in northern and southern Newfoundland. We did not attempt to quantify the amount of fish consumed, as this was not within the scope of the project, but we did document the reported local seafood consumption frequency over the past month, as well as over the course of the year, which has resulted in a greater understanding of seafood consumption patterns in the province. Cod was the most commonly consumed species, with a large proportion of participants from both communities reporting consuming local cod at least once in the past month (Burin=35%, NWV=59%) or 2 to 3 times in the past month (Burin=37.5%, NWV=15.6%). Other popular local seafood species included salmon, shrimp, scallops and crab.

NWV residents reported consuming local cod and smelt more frequently than those in Burin. It is possible that season may play a role in these results; the Burin SCQ survey was completed in February/March while those in NWV were completed in April. We know from our seasonal seafood consumption section of the SCQ that participants reported more local seafood consumption in spring than winter, which may influence the reported seafood consumption frequencies between the two communities.

Smelt fishing is open to the public between January-April therefore it is possible that these fish were being caught fresh in Burin while we visited this study location (February/March) however this would not apply to NWV (visited in April) as the smelt fishery would have been closed. The recreational fishery (which anyone can participate in, no license required) does not begin until June and the stewardship cod fishery (restricted to license holders in specific regions) does not open until August therefore neither location would have had access to self-harvested cod. However, Burin is close to a larger town (Marystown, population of 5,316), which has a Sobeys© grocery store and a Walmart©, while NWV is more isolated and has only small local grocery stores. NWV has an operational fish processing plant (Beothic Fish Processors©), which includes processing of Atlantic Cod and which may act as a source of local seafood for the residents of the surrounding communities. The closest seafood processing plant to Burin is in St. Lawrence (approximately 30 km from Burin).

Burin participants reported higher consumption of scallops and shrimp than NWV. This may be due to the scallop fishery in nearby Placentia and Fortune Bay (located on either side of the Burin Peninsula). Many fish harvesters turned to scallop dragging in Placentia Bay after the declaration of the cod moratorium in 1993 (Hickey, 2001), therefore, it is possible that residents from Burin had easier access to scallops than residents of NWV. Moreover, scallop harvesting

season is year-round, and it is possible that there was access to local scallops at the time of data collection. Additionally, Burin has reasonable access to a local seafood plant (Oceans Choice International in St. Lawrence) which processes shrimp and scallops, while in NWV the local plant (Beothic Fish Processors©) does not process shellfish (with the exception of crab). Processing plants and harvesters can sell directly to the community, which may explain the higher shrimp and scallop consumption in Burin.

### 5.2.2 Seafood consumption by age and sex

In our study, older participants (>50 years) reported eating more cod than younger participants (<50 years). We also found a general trend towards older participants reporting higher consumption frequencies of most seafood species than younger participants, which is similar to results found by Olsen (2003), in which frequency of seafood consumption and age were positively related based on assessments of studies done around the world. Studies of seafood consumption in Canada and the United States also found lower rates of seafood consumption in younger participants (Colletto et al., 2011; Jahns et al., 2014), indicating the trend of lower seafood consumption in younger generations extends beyond this study.

Gender was another variable affecting seafood consumption in this study. Males reported consuming local seafood more frequently than females in our study, with males reporting statistically significantly higher consumption frequency of herring and trout than females. This is similar to trends seen by Chen et al. (2015a), who found male Newfoundlanders were more likely to choose fish in their dietary patterns than their female counterparts. Studies in Canada and the US (Coletto et al., 2011; Jahns et al., 2014) have also found similar patterns in fish consumption by gender with men consuming more fish than women.

Previously, there was very little information about gender differences in seafood consumption in Newfoundland, however we found that for every species of fish we included in our survey, the average male consumption frequently was greater than females. It is possible that females are eating more imported fish; however, studies show that regardless of sex, Newfoundlanders prefer local over imported seafood (Lowitt, 2013). While the social factors behind this gendered pattern in fish consumption are unknown, the act of fishing itself is a male-dominated sport and profession. In Canada, recreational anglers are overwhelmingly men (73%; Fisheries and Oceans Canada, 2016), and males were also the traditional fish harvesters in Newfoundland. There are no other studies on gender-specific fish consumption in Atlantic Canada, however, Marushka (2017) found that fish consumption was higher in males from Ontario and Quebec First Nations groups, and that consumption was also correlated with EDC exposure.

### 5.2.3 Seafood consumption by season

In our two study communities, we found that there was a seasonal effect on seafood consumption, and that residents reported consuming local seafood (of any type) more frequently per week in the summer than any other time of year. Additionally, we found that male participants reported consuming more local seafood in the spring, summer and fall than female participants. This aligns with both the summer recreational fishery and commercial fishery timing, making fresh local seafood more available during the summer season, and with males being the predominant sex that was traditionally employed in the fishing industry.

The recreational fishery (also known as the food fishery) is open for approximately 39 days of the year (this varies annually) from June until September, during which residents and non-

residents of the province can catch up to 5 fish per day for their own consumption. This provides residents with potential to catch up to 195 individual fish per person during the season. As a result, many Newfoundlanders have a tradition of catching a supply of seafood during the food fishery to supplement store-bought food. We found some distinct trends in local seafood consumption in our study population, which may indicate greater risks of EDC exposure in these higher fish-consuming groups.

## 5.3 Serum thyroid hormone concentrations

All participants were tested for TSH, $FT_4$, and $FT_3$, the major thyroid hormones regulating endocrine control of the thyroid system. The majority (n=74) of our participants had no known thyroid condition, which was important to be able to investigate the endocrine effects of EDC concentrations in their blood samples. We also included a small number (n=6) of participants with diagnosed hypothyroidism, to explore EDC concentrations in those with hypothyroidism.

Thyroid hormones are essential for growth, development, and energy metabolism in humans. They are regulated between a very narrow set-point, therefore minor alterations can have large health implications. By measuring thyroid hormones in our study, we are able to explore changes in thyroid hormone concentrations that may be connected to environmental pollutant exposure through seafood consumption.

### 5.3.1 Thyroid hormones by community

TSH levels were statistically significantly higher in NWV (2.20 ± 0.19 mIU/L) than in Burin (1.99 ± 0.13 mIU/L), however both results were still below the upper limit for the lab's normal reference range (4.94 mIU/L). An upper limit of normal (euthyroid) concentration of 2.5 mIU/L

has been suggested as a guideline for diagnosing subclinical hypothyroidism, as 95% of humans with no known thyroid conditions will have TSH values below this level; individuals with values above this limit are more likely to have underlying causes artificially elevating their TSH levels (Wartofsky and Dickey, 2005). Using this amended upper TSH concentration limit has also been highly recommended by our consulting pediatric endocrinologist, Dr. Heather Power (Eastern Health, Health Sciences Centre, St. John's), therefore we used this concentration to measure the percentage of the participants in this study whose TSH values were above the adjusted normal upper limit.

In NWV, approximately 31% (n=8) of participants had TSH values that were above the subclinical hypothyroidism limit cutoff of 2.5 mIU/L, while the proportion in Burin was lower at approximately 15% (n=7), excluding participants with known hypothyroidism. For those above the subclinical cutoff, the average concentrations were 3.70 mIU/L (Burin) and 3.73 mIU/L (NWV), between which there is no difference. Therefore, it seems that being above the subclinical hypothyroidism cutoff point was more common in NWV than in Burin, even if the average TSH concentration above the subclinical thyroid threshold were not different. This may be explained by the higher proportion (78%) of participants >50 years of age in the NWV group compared with the proportion that were >50 years of age (69%) in the Burin group. It is likely that the skewed age distribution of our participants in this study may have impacted the thyroid hormones results, as age has been shown to be an important factor in TSH levels, and age is associated with an increased proportion of individuals with serum TSH concentrations above the 2.5 mIU/L euthyroid limit (Surks and Hollowell, 2007).

$FT_4$ and $FT_3$ were also different when concentrations were examined community-wise. $FT_4$ was statistically significantly higher in NWV, similar to TSH, and there was a (non-significant)

trend towards higher $FT_3$ concentrations in Burin. All average $FT_3$ and $FT_4$ concentrations from the study population were within the lab reference range for normal thyroid hormone concentrations ($FT_4$=9-19 pmol/L, and $FT_3$=2.6-5.7 pmol/L). In summary, there was a difference between thyroid hormone concentrations by community, but overall these concentrations were within lab references ranges.

5.3.2 Thyroid hormones by sex and age

We found that $FT_3$ was statistically significantly higher in males than in females within our study population, but there was no other difference in thyroid hormones by sex. These results align with the scientific literature, which points to males having naturally higher $FT_3$ concentrations than females (Strich et al., 2017). We did not find any difference in thyroid hormone concentrations with age in our study population; however, studies do point to a sex-specific difference in thyroid hormone with age, including a negative relationship between age and $FT_3$ in males (Suzuki et al., 2012). There is also a decline in $FT_3$ concentrations after 40 years of age, and the correlation between TSH and $FT_3$:$FT_4$ ratio also decreases with age (Strich et al., 2016). Considering the age difference in $FT_3$ levels found in the literature may also help to explain why Burin showed a trend towards higher serum $FT_3$ serum concentration than NWV. There was a slightly greater proportion of participants over age 50 in NWV (75%) than in Burin (70%), and the older (>50 years) participants from Burin had statistically significantly higher $FT_3$ concentrations than NWV participants, while TSH was higher in older NWV participants than in Burin participants. However, when thyroid hormone concentrations were analyzed by their age original groupings (see Appendix A Seafood Consumption Questionnaire), we did not see any

decline in thyroid hormones concentrations with age, although these thyroid hormone concentrations have been shown to decline with age (Strich et al., 2017).

Our participant group had a skewed age distribution, with a greater proportion of older participants, therefore relationships between $FT_3/FT_4$ and age may be difficult to discern due to the insufficient number of younger participants to compare hormone concentrations with. For future studies on the effects of EDCs on thyroid hormones in the Newfoundland population, it will be important to ensure that the study population is proportionally matched both by age and sex, to ensure that these biological factors (such as age and sex) are not confounders of endocrine disrupting effects in the population.

### 5.3.3 Thyroid disruption, iodine and genetic factors

Insufficient iodine consumption is another factor that must be considered when assessing thyroid hormone levels in the population. The National Institutes of Health recommend adult men and women consume around 150 mcg of iodine a day and recommend fish consumption as one of the highest dietary sources of iodine (National Institutes of Health, 2019). The seafood consumption frequencies in Newfoundland make hypothyroidism due to iodine deficiency highly unlikely in this province. Furthermore, research indicates that iodine status amongst most North Americans is sufficient (Pearce et al., 2013). This is mostly due to the introduction of iodized salt, which over 70% of households have access to, even in developing countries (Pearce et al., 2013). An older study of salt intake in Newfoundland indicates the population may be overconsuming salt, consuming 6.7-8.8g/day, well above the recommended intake of 5-6g/day (Fodor et al., 1973).

Another factor to be considered with populations that have pockets of elevated TSH levels, such as seen in our study in Newfoundland, is the role of inherited (genetic) thyroid disorders. There has been one haplotype (HLA-A9, Bw 16) identified in an extended family in the small community of Rencontre East on the south coast of Newfoundland which causes thyroid autoimmune disease leading to hyperthyroidism (Farid et al., 1976). This is unlikely to be a cause of thyroid disruption our study population, as the community in which the genetic condition was found is a small, isolated town (population 136) accessible only by boat and unlikely to make an impact on the genetic profile of the larger population of Burin (population 2,315) and surrounding area.

While we did not test for thyroid antibodies in our participants, it is possible that undiagnosed autoimmune thyroid disease could also be present in our study population. Studies estimate that as much as 5% of the adult population has undiagnosed hypothyroidism via thyroid dysfunction (Garmendia Madariaga et al., 2014). Interestingly, some EDC (such as PCBs and PBBs) are associated with autoimmune thyroid disorders (Benvenga et al., 2015), therefore it is possible that exposure to these contaminants could contribute to autoimmune thyroid dysfunction in the population. While this is beyond the scope of this project, it is a possibility that should be considered for future EDC exposure and thyroid research studies in Newfoundland.

## 5.4 Plasma EDC concentrations

We analyzed correlations between thyroid hormones and EDCs in pmol/g (SI units). While EDCs are measured in ng/g in a large proportion of the literature including studies done by the Canadian (Heath Canada, Environment and Climate Change Canada) and US (United States Environmental Protection Agency) governments, concentrations in pmol/g are more

accurate because they reflect the molecular weight of the individual congeners. Where congeners of PBDEs, PBBs and PCBs have different numbers of bromines (PBDEs) or chlorines (PCBs) attached to their main aromatic ring structure, the molecular weights of these chemicals vary. Accounting for molecular weight will give a more accurate assessment of the concentration of the congener in plasma.

We found a surprising number of EDCs in the plasma of participants of this study. A total of 19 different EDC congeners were detected in total, with 11 being found in all eighty participant's plasms samples. The highest concentrations were p,p'-DDEs, followed by PCBs, PBDEs and PBBs in that general order. These data provides strong evidence that EDC exposure has occurred in the Newfoundland population. While we did not test for other demographic characteristics aside from age and sex, variables such as health history, medication use, smoking status, and occupation can all have large impacts on EDC exposure, and should be considered for future studies of the Newfoundland population.

### 5.4.1 EDCs by age

In this study, PBDE concentrations were higher in younger participants (<50 years) while PBB-153, PCBs and p,p'-DDE were generally higher in older participants (>50 years), regardless of community. This may be a reflection of the sources of these chemicals, and the length of time they have been in production. PCBs have been in production and use since the 1930s, while DDT (the parent compound of p,p'-DDE) was widely used as an insecticide since 1939. PCB ad DDT were both restricted and/or banned by the Stockholm Convention in 2004 as part of the "Dirty Dozen" initial chemicals that were entered under the treaty. In Canada, DDT was phased out in the 1970s and completely banned by 1990; PCBs were banned from import,

manufacture and sale in 1977. However, due to their bioaccumulative nature and long half-lives, they have persisted in the environment for decades and thus tend to be found in higher concentrations in older individuals. It is possible that older participants have had a longer time to accumulate the legacy chemicals (PCBs and DDT), which peaked in use for much of their life span, but were phased out before some of the youngest participants were born. Age has also been shown to be a predictor for PCB and p,p'-DDE concentration in other studies (Laden et al., 1999).

PBBs (in production and use from the 1970's onwards) do not follow this trend, being "newer" flame retardants. However, other studies such as Lim et al. (2008) have observed a positive association between PBB-153 and age, corroborating our findings of higher PBB-153 in older participants. PBDEs were manufactured from the1960s onwards, and reached peak production and usage in the 1990's. They were banned under the Stockholm Convention in 2009, long after PCBs and DDT, but many PBDEs are still present in a variety of older consumer products (i.e. automobiles, furniture, carpet, electronics) to this day. PBDEs have been found to be inversely related to age, with younger populations having higher concentrations of these chemicals, likely due to high degree of EDC exposure that occurs in infants and younger children from breastmilk and hand to mouth activity (Garí and Grimalt, 2013). These results may possibly be indicative of an age-related cohort effect, particularly for PBDEs which were higher in younger participants. These people would have been infants or young children around the peak of PBDE production and use (1980-200s), and may have had high exposure to PBDEs through breastfeeding and hand-to-mouth activities. Since these chemicals have now been banned, this age group (20-50 years of age) would have had a unique exposure which would be different from

older (50+ years of age) or younger (<20 years) age groups. This possible age-cohort effect of plasma EDC concentrations requires a larger-scale study to explore this effect fully.

Another possible explanation for the difference in EDC concentrations by age is that the half-life of some EDCs can vary with age (Bates et al., 2004). Children have a plasma PCB concentration rate-of-decrease that is 2.6 times faster than that of their mother; the half-life of PCBs in the blood of children was 2.8 years while for the mothers it was 7.1 years (Yakushiji et al., 1984). Meta-analysis of global literature on half-life of EDCs shows a linear association between age and half-life duration, which is partially attributable to the dilution of body stores of EDCs. This can be the result of periods of rapid growth (which happen at younger ages), faster metabolism in younger individuals, and increased body fat with older age (Milbrath et al., 2009). While this may explain why PCBs, PBB-153 and p,p'-DDE concentrations were higher in older participants, we did not see the same pattern with PBDEs.

Additionally, half-life may decrease as EDCs concentrations in the body increase, thus lower concentrations of EDCs would have longer half-lives and take longer to eliminate (Bates et al, 2004). This is due to the disposition kinetics of some EDCs, which are non-linear, and as such elimination rates decrease as body burden decreases (Geyer et al, 2004). It is difficult to assess how this would impact our population, though it is possible that long-term, low level exposure would make it very difficult to eliminate EDCs from the body's system. There is still much uncertainty about the relationships between EDCs and age, but the more evidence there is (such as the results from our study), the greater our understanding will be.

5.4.2 EDCs by sex

Biological sex was another demographic variable that was related to both EDC and thyroid hormone concentrations in our study. We found that males generally had higher plasma concentrations of EDCs than females. Fraser et al. (2009) also found that males had higher individual PBDE congener and ∑PBDE concentrations than females, which they linked to higher consumption of red meat and poultry. Men have also been shown to have higher intake of PCBs/kg body weight than women from consumption of contaminated fish in Norway (Knutsen et al., 2008). A study of PBDE intake in the US population also found that males had higher dietary intake of PBDE (Schecter et al., 2006a). These studies give us insight into why we see greater EDC concentration in males in our study.

Greater consumption of seafood could be a contributor to the higher plasma EDC concentrations seen in the male participants in our study; research has found higher seafood consumption by males, and a higher estimated daily intake of DDT and PBDEs via seafood consumption (Guo et al, 2010). We also found that our male study participants from both locations reported statistically significantly higher consumption frequencies of local seafood over the past month than females. Additionally, males reported consuming local seafood in the summer more frequently than females from the same communities. This may put males at a greater risk of exposure to EDCs through consumption of contaminated seafood products.

5.4.3 EDCs by community

We found that PBBs and PBDEs were slightly higher in Burin participants, while PCBs and p,p'-DDE concentrations were (generally) slightly higher in NWV participants. The history of these different EDCs may play a role in the geographical differences in exposure. PBDEs and

PBBs are “newer” EDCs, having been banned from production and use much more recently than PCBs and DDT. As such, it is possible that many products containing PBBs and PBDEs are still found in regular consumer use or are transitioning into urban effluents and landfills. Abbasi et al. (2014) found that the largest flow of PBDEs from consumer products to their waste phase (deposition of EDC-containing products into landfills, recycling centers, waste water treatment plants) was between 2005 and 2008.

PBDEs and PBBs have been documented up and down the St Lawrence River in sediments (Pelletier and Rondeau, 2013), fish (Houde et al., 2014), birds (Gentes et al., 2012) and marine mammals (Simond et al., 2017). Therefore, it is plausible that higher concentrations of these EDCs in participants from the south coast of Newfoundland are a result of contamination from upstream in the St Lawrence River making its way into the marine food chain, where they then could be ingested by humans through local seafood consumption. This explains the higher concentrations of PBDEs and PBBs found in Burin participants, who would be exposed to pollutants from the St. Lawrence River.

PCBs and DDT have been banned from manufacture and use for a much longer time than the other EDCs in this study, and thus are not as actively transitioning from consumer products into the waste phase. They have a global distribution, with PCBs being documented in seafood species such as cod in different parts of the North Atlantic Ocean (Karl et al., 2016). PCBs and DDT/p,p’-DDE have also been found in Arctic ice (Gregor et al., 1995) and high trophic level Arctic organisms such as the polar bear (Letcher et al. 1995). This indicates the widespread presence of these chemicals both geographically and by trophic level.

Global distillation theory is an explanation for how these EDCs make their way to remote regions; EDCs produced at lower and/or warmer latitudes are volatized into the atmosphere and

travel to colder latitudes (and as far as the Arctic or Antarctic) where they are deposited via precipitation into the environment. EDCs with high mobility are relatively volatile (low octanol [O] to air [A] partitioning coefficient [log $K_{OA}$6.5-10]) and hydrophobic, which allows them to volatize into the atmosphere and travel long distances. Once they reach colder latitudes, these travelling EDCs are deposited via precipitation and can accumulate in ice/snow and in the food chain (Wania and Dugani, 2003). Furthermore, it now appears that climate change may be causing a re-volatilization of these (and other) EDCs back into the atmosphere (Ma et al., 2011).

This could be a potential source of Arctic PCBs and DDT/p,p'-DDE to the Island of Newfoundland. Once these EDCs are released from melting snow/ice, they could travel south on atmospheric or water currents (i.e. the Labrador Current) into marine ecosystems such as those around Newfoundland. If residents from NWV were to consume marine species from these contaminated areas, it could explain the high concentrations of these chemicals in this portion of the study population. This would also explain why we see lower concentrations of these chemicals in residents of Burin, which are mostly exposed to contamination from the St. Lawrence River and open North Atlantic Ocean.

In our earlier study (Sarkar et al., 2015), we found that the south coast of Newfoundland had hypothyroidism rates approximately twice as high as those on the northeast coast. It is possible that difference in EDC exposure in these coastal communities may be contributing to the geographical difference in hypothyroidism rates seen in our previous study. PBDE concentrations were much higher in residents of the south coast community (Burin) compared with residents of the Northeast coast community (NWV). PBDEs are known to be associated with increased incidence of hypothyroidism (Oulhote et al., 2016), however this does not fully explain the trends seen in our study, as other EDCs that are present in our study population

(PBBs, PCBs, p,p'-DDE) are also known to impact the thyroid system. Additionally, the ∑EDC concentrations were not different between the communities.

It is possible that there are also some local sources of EDCs in these areas, as this has been seen in two other parts of Newfoundland and Labrador; sculpins from northern Labrador were shown to have been contaminated with PCBs from a Canadian Air Force radar station (Kuzyk et al., 2005) and ground fish from Placentia Bay (south coast) have been documented to suffer adverse health effects from exposure to PCBs from a military/industrial waste site (Khan, 2003). While we can only speculate about the exact sources of marine food chain contamination around Newfoundland, it's possible that both global and local sources could be the causes of this pollution.

Other sources of human EDC exposure would have to be considered outside of seafood consumption to understand the patterns of exposure seen in the Newfoundland population. Dust has been shown to contain high levels of PBDEs, and to be a major contributor to exposure in humans (Wu et al., 2007). While not analyzed in this project, dust may provide insight into other sources which may be contributing to EDC exposure in Newfoundland.

### 5.4.4 EDCs in hypothyroid and non-hypothyroid participants

Some of our participants had hypothyroidism, and we included them in our study with the goal of assessing the difference in EDC concentrations between hypothyroid (n=6) and non-hypothyroid (n=74) groups. The general trend that emerged on comparing plasma EDC concentrations of these two groups were that some PCB congener concentrations (PCB-105, -118, and -138) as well as p,p'-DDE were higher in hypothyroid participants while PBB-153, all PBDE, and the remaining PCB concentrations were higher in non-hypothyroid participants.

Studies have found that serum PBDE concentrations are associated with increased prevalence of hypothyroidism (Oulhote et al, 2016). Other studies have examined the relationships between PCBs, PBB-153, or p,p'-DDE with thyroid hormones. Mixtures of some EDCs (including PBDEs, PBB-153, PCBs and p,p'-DDE) are associated with higher odds of hypothyroidism or autoimmune thyroid pathology (Dufour et al., 2019). In a cohort of people from Michigan exposed to PBBs in the early 1970s, increases in serum PBB-153 were associated with an increased odds ratio of hypothyroidism (Jacobson et al., 2017).

The trends in EDC concentrations between hypothyroid and non-hypothyroid participants were mixed, and our small sample size of hypothyroid participants may be affecting our ability to detect an association in our study population. The decision to include participants with hypothyroidism was made after Burin data collection was underway, and therefore we were unable to recruit greater numbers of individuals with hypothyroidism. The majority of participants with hypothyroidism we did manage to recruit were from NWV, which was visited at a later date than Burin. A larger number of participants from both communities is needed to conduct a more accurate assessment of EDC concentrations in hypothyroid and non-hypothyroid residents of Newfoundland.

### 5.4.5 EDCs in the Canadian population

Before this study was conducted, the prevalence of EDCs in the Newfoundland population was unknown, but some Canada-wide data have been collected. The Canadian Health Measures Survey (CHMS) collects nation-wide information on Canadian health and well-being, including the collection of physical samples such as blood for biomonitoring of contaminant exposure in the Canadian population. Data on EDCs in blood samples from CHMS collection cycles 1 (2007-

2009) were published by Haines et al. (2017), and the results from the CHMS were compared with those from our study, including 75$^{th}$ percentile and 95% confidence intervals [CI] (Table 5.2). We selected cycle 1 of CHMS sampling to compare with our population because it was the only cycle that analyzed PBBs, PBDEs, PCBs, and p,p'-DDE (Newfoundland was not included in cycle 1 CHMS sampling, but was in subsequent cycles).

Of note is that there is a 9-year gap between the most recent blood contaminant exposure data from the CHMS (2009) and the current study (2018), and as such is it not practical to make direct comparisons between the two sets of data without acknowledging that EDC concentrations in the Canadian population may have changed during this time. Additionally, the CHMS data are from a much wider age range of participants (3-79 years) than the current study (19-75+ years), which may affect the comparison between the two studies as higher PBDE and PCB serum concentrations have been reported in toddlers and children than in adults (Toms et al., 2009; Marek et al., 2013). Although the CHMS blood samples were collected in 2007 to 2009, the samples were analyzed more recently (2016 to 2017) and therefore reflect current instrumentation and analytic capabilities.

**Table 5.2** Comparison of EDC concentrations (75th percentile [95% CI]) between the present study and the Canadian Health Measures Survey (CHMS 2007-2009) from Haines et al. (2017).

| EDC congener | NL concentrations P75 (95% CI) | CHMS concentrations P75 (95% CI) |
|---|---|---|
| **PBB-153** | 0.73 (0.35-0.67) | <LOD |
| **PBDE-47** | 5.49 (3.81-6.30) | 120 (100-140) |
| **PBDE-99** | 1.43 (0.99-1.51) | 21 (<LOD-26) |
| **PBDE-100** | 1.39 (0.71-2.36) | 21 (<LOD-27) |
| **PBDE-153** | 12.28 (7.35-14.05) | 33 (27-39) |
| **PCB-99** | 1.43 (0.99-1.51) | <LOD |
| **PCB-105** | 0.84 (0.50-0.81) | <LOD |
| **PCB-118** | 4.56 (2.77-4.69) | 50 (41-58) |
| **PCB-128** | 0.08 (0.02-0.11) | <LOD |
| **PCB-138** | 14.73 (7.63-11.32) | 110 (97-130) |
| **PCB-153** | 31.63 (17.09-25.99) | 210 (180-240) |
| **PCB-156** | 4.40 (2.29-3.45) | 32 (29-36) |
| **PCB-170** | 10.00 (5.39-8.21) | 59 (52-65) |
| **PCB-180** | 34.46 (17.55-27.56) | 190 (170-220) |
| **PCB-183** | 2.36 (1.25-1.86) | 19 (15-22) |
| **p,p'-DDE** | 117.30 (69.55-106.65) | 1700 (1300-2100) |

LOD = limit of detection, concentrations in ng/g lipid

There is a clear trend in the present study of finding much lower plasma PCB, PBDE, PBB and p,p'-DDE concentrations than the Canada-wide CHMS from the previous decade. This may be reasonably explained by the decreasing concentrations seen across the globe in humans (i.e. decreasing PCB concentrations in breast milk; van den Berg et al., 2017) and environmental samples (i.e. PBDEs in the global environment; review from Law et al., 2014) since the wide-spread bans of these chemicals in the early 2000s. As such, this study demonstrates that a small

proportion of the Canadian population does still show evidence of EDC exposure in their serum, and while no time-trend comparison is possible in Newfoundland (the EDCs measured in our study were not tested during CHMS sampling in Newfoundland), when these data are compared to serum EDC concentrations from the past decade in the general Canadian population, the EDC concentrations do appear to be decreasing.

All participants in this study, who were randomly recruited from Burin or NWV, had detectable concentrations of at least 11 out of the 25 tested EDCs in their plasma. This shows the degree to which participants have been exposed to EDCs; every participant in the study had detectable concentrations in their plasma, indicating some contact with EDCs regardless of age, sex, community or consumption of local seafood. In the CHMS, p,p'-DDE and PBDE-47 were detected in >60% of samples, and PCBs -118, -138, -153 and -180 were detected in >90% of samples. In our study, p,p'-DDE, PBDE-47, PCB-118, -138, -153, -180 were detected in 100% of plasma samples, as were PCB-156, -170, and -183. This could indicate exposure to a wider range of EDCs in the Newfoundland population, however our sample size is small and the geographic range of our study is limited, which constrains our ability to make such an inference.

While our results do imply that Newfoundland residents are exposed to other sources of EDCs besides through consumption of local seafood, we did find a statistically significant association between consumption of certain seafood species and plasma EDC concentrations (discussed later in this chapter).

## 5.5 The association between EDCs and thyroid hormones

One of the main objectives of this study was to determine plasma EDC concentrations in residents of Burin and NWV, and to test for an association with thyroid hormone concentrations.

We did not find any relationships in the univariate and multivariate regression analysis of EDCs and thyroid hormones in our study population, however there were some non-significant trends that may indicate small effects of EDCs on thyroid hormones. Ours is one of many studies that have found no relationship between EDCs and thyroid hormones, however this does not mean that these EDCs do not affect the thyroid system, but that the relationship may be more complex and the effects more difficult to detect than we anticipated.

Epidemiological studies of the association between EDCs and thyroid hormones in human populations have long been used as a means to measure exposure and health outcomes, complimenting controlled exposure studies done in animal models. Generally, there are still mixed results about how EDCs are associated with the changes in the various thyroid hormones. Animal studies are important for investigating the association between EDC exposure and thyroid hormone concentrations, however extrapolating these results and applying them to humans is not straightforward and the correlations are not always reflected in population exposure studies.

It is difficult to discern why we did not see any association between EDCs and thyroid hormones in the rural Newfoundland population, as other research studies have found a statistically significant association. There are several possible explanations; 1) our sample size is quite small (74 participants were included in the EDC-thyroid hormone regression analysis), which could mask a statistically significant association due to low statistical power and insufficient population coverage , and 2) there were low contaminant levels in our plasma samples compared with past sampling of the general Canadian population (see Table 5.2 for comparisons with the CHMS), which would make it difficult to detect small changes in EDC concentrations and the corresponding association with thyroid hormones. It is also possible that

3) our study population did not have sufficient EDC exposure to cause changes in thyroid hormone concentrations, however this seems highly unlikely as all participants in this study had a minimum of 11 different EDCs present in the plasma sample they gave, which points to exposure to a "cocktail" of different chemicals in each individual person. This presents the issue of whether the mixture of endocrine disruptors is producing a combination effect, even if the individual EDCs are at low concentrations. This is of particular concern for environmental epidemiologists, because even if individual chemical concentrations in serum or plasma fall under their established no-observed adverse-effect level (NOAELs) concentration limits, they can still cause endocrine disruption especially when combined with other endocrine disruptors due to additive and/or synergistic effects (Kortenkamp, 2008).

Chemicals that act similarly (i.e. different EDCs that disrupt thyroid hormone concentrations) can substitute each other and cause disruptions to the endocrine system even if they act through different mechanisms (Kortenkamp, 2008). Because of this, the traditional methods of risk assessment for these chemicals are not accurate, and more and more researchers are starting to understand the need to examine mixture effects more than individual EDC effects. For example, Crofton et al. (2005) found that mixing 18 thyroid disrupting chemicals (all below their NOAELs) caused a stronger endocrine disrupting effect on $T_4$ concentrations in rats than estimated by more traditional Dose Additivity predictions. This may be the future of EDC and thyroid hormone relationship investigations, as it represents a more realistic exposure model.

Chevrier (2013) reviewed several challenges to exploring the relationship between PBDEs and thyroid hormones, which can also be attributed to a wider range of EDCs. Reverse causality, in which blood lipid levels play a deceptive role in determining PBDE concentrations, is the first potential challenge. Lipid metabolism is one of the many bodily processes regulated by thyroid

hormones, and as thyroid levels decrease serum lipid levels increase (Rizos et al., 2011). It has been suggested that this could potentially dilute lipid-normalized PBDE values, as PBDEs are very lipid-soluble. Therefore, as concentrations of PBDEs increased and thyroid hormone homeostasis was disrupted, serum lipid levels would also increase but would mask the corresponding PBDE levels when concentrations were lipid-adjusted. This may make the association between PBDEs and thyroid hormones difficult to detect. Many other EDCs, including those tested in this study, are also lipid-soluble and therefore may also be impacted by reverse causality.

Another challenge presented by Chevrier (2013) to assessing the relationships between PBDEs and thyroid hormones has to do with the measurement of thyroid hormones. Immunoassays may be biased by the amount of transport protein-bound $T_4$, causing $FT_4$ readings generated by immunoanalyzers to be higher than they actually are in the serum. $FT_3$ measurements have also been shown to vary amongst immunoassay methods (Giovannini et al., 2011). This could impact our ability to detect an association between EDCs and thyroid hormones.

As mentioned before, there have been many other peer-reviewed studies that have found no association between EDCs and thyroid hormones, similar to our findings. EDCs may exert endocrine disruption through a range of mechanisms, making it difficult to detect and determine exposure effects. Boas et al. (2012) makes a strong case that the consequences of EDC exposure in humans are not always easily predicted or measured, especially for the thyroid system as TSH feedback regulation also involves peripheral inputs and hormones, and that we have no "exposure free" human controls to measure effects of EDC exposure against. Below, we present

some other the "no effect" studies, explanations for why this may have arisen and connections to our study results.

A recent meta-analysis of correlations between PBDEs and thyroid hormones by Zhao et al. (2015) found that effects of PBDEs on thyroid hormones varied, and was dependent on exposure and serum concentrations. They observed that lower serum concentrations (<30 ng/g lipid) were negatively correlated with thyroid hormones (TSH and total $T_4$), while higher serum concentrations (>100 ng/g lipid) were positively correlated with serum thyroid hormones (TSH and total $T_4$). Additionally, mid-range (30-100 ng/g lipid) levels of PBDEs did not correlate with a change in thyroid hormones (TSH and total $T_4$). This sigmoid relationship between PBDEs and thyroid hormones is what makes determining health effects of EDCs so difficult; they often follow non-monotonic dose-response relationships with both high and low doses causing adverse health effects. In application to our study, we found that some of our PBDEs had ranges that would put them in this 30-100 ng/g lipid "no effect" range determined by Zhao et al., which may be contributing to the lack of significance between EDCs and thyroid hormones in our study population.

Zheng et al. (2017) found no association between PCBs and thyroid hormones in e-waste recycling workers, however they did find that PCBs were associated with an increase in thyroid receptor (TR) mRNA expression. TR may be responsible for some of the association (or lack thereof) seen between EDCs and thyroid hormones. OH-PCBs (common metabolites of PCBs) have been shown to compete with thyroid hormones for access to TR, and to suppress activation of TR by circulating thyroid hormones in cells (Iwasaki et al., 2002). Along these lines, lower-brominated OH-PBDEs bind weakly with TR whereas higher-brominated OH-PBDEs bind strongly to TR. However, Ren et al. (2013) have demonstrated that lower and higher brominated

PBDEs bind to different pockets of the TR, and this may be what causes their agonist (lower brominated PBDEs) or antagonistic (higher brominated PBDEs) effects on the TR. While we did not measure TR mRNA expression in our study, it is possible that this could be impacted by PCB (and OH-PCB) concentrations, and that this could also affect our ability to detect an association between EDCs and circuiting thyroid hormone concentrations via the thyroid system negative feedback loop, which is regulated by the TRs.

Organochlorines including DDT are restricted under the Stockholm Convention, with limited use allowed in some countries particularly to help control the spread of malaria. This chemical was used as a pesticide on agricultural crops, and became widespread in the environment, prompting concerns when it began to cause the thinning of eggshells in birds. While we did see some participants with DDT in our study, all participants had p,p'-DDE, the metabolite of DDT, in their plasma concentration. While there is no sale or use of DDT allowed in Canada today, the long-lasting legacy of these chemicals in the environment is telling of the ongoing exposure seen in our population, most likely through ingestion of contaminated foods.

There was no association between p,p'-DDE and thyroid hormones in our study population, and results from the literature are inconsistent in the relationships they report as well. Some have found no effect of p,p'-DDE on serum thyroid hormone concentrations (Abdelouahab et al., 2008), while other studies (i.e. Hernández-Mariano et al., 2017) have discovered a positive association between p,p'-DDE and total $T_3$, as well as between p,p'-DDE and $FT_3$ (Meeker et al., 2007). The lack of association between p,p'-DDE and thyroid hormones in our study could be in part due to the concentrations of p,p'-DDE seen in our study plasma samples; in Table 5.2, p,p'-DDE concentrations from the current study (117 ng/g) were compared with CHMS (1700 ng/g), which were more than 10-fold greater. There was no difference in p,p'-DDE concentrations by

age, sex or location in the study population, however participants diagnosed with hypothyroidism had higher plasma p,p'-DDE concentrations than those without hypothyroidism.

In conclusion to this section, while we did not find any association between EDCs and thyroid hormones it is possible that we may find different outcomes and trends with a larger sample size.

## 5.6 The association between EDCs and seafood consumption

Another objective of the study was to measure EDC concentrations and test for relationships with local seafood consumption, to explore local seafood consumption as an exposure source in the Newfoundland population. There were statistically significant correlations between cod consumption and several EDCs. We found several PCB congeners and p,p'-DDE were associated with local cod consumption, indicating that this species may be a source of EDC exposure in the rural Newfoundland population. Some of the higher chlorinated PCB congeners (PCB-138, -153, -170, -180) and ∑PCBs were also associated with age in the regression analysis, with corroborates with our earlier finding of PCB concentrations being higher in older (>50 years) participants.

Diet is one of the greatest sources of human exposure to environmental EDCs, and while we only examined the association between plasma EDCs and local seafood consumption, other dietary items such as meat, dairy, eggs, oils, and non-local seafood consumption likely also contribute to EDC exposure, along with ingestion or inhalation of indoor dust. The majority of local seafood had low reported consumption frequencies in our participants, therefore, their consumption data were aggregated together (with the exception of cod). Cod was the most

frequently consumed species, therefore we looked in depth at the correlation between consumption of this species and plasma EDC concentrations.

In the regression analysis between seafood consumption and EDCs, age (covariate) was also statistically significantly associated with plasma PCB concentrations. This is not surprising as we found that older (>50 years) participants had higher plasma PCB concentrations than younger (<50 years) participants. While we did not test for PCBs in our fish liver samples, other recent studies have found PCBs in fish from the North Atlantic (Karl et al., 2016) and other parts of the world (Greece – Renieri et al., 2019, Brazil – Santos et al., 2020), indicating the current and widespread distribution of these EDCs in seafood as an ongoing exposure issue. Additionally, similar studies have also found an association between PCBs (Sjödin et al., 2000), p,p'-DDE (Persky et al., 2001) and seafood consumption therefore our results do align with the literature. We did not find any association in the regression analysis between PBDEs and seafood consumption, although other studies have found this relationship to be statistically significant (example Knutsen et al., 2008). This could be due to other sources of PBDE exposure (such as indoor house dust ingestion) masking the effects of seafood consumption. Preliminary results from house dust analysis from participants in this study revealed high concentrations of organic bromines in the dust samples. This could be obscuring the relationships between frequency of local seafood consumption and plasma PBDE concentrations due to the ubiquity of the bromines in the house dust samples, negating the effect of geographical location and local seafood consumption on exposure and corresponding plasma PBDE concentrations, especially for less frequently consumed seafood.

House dust contains high concentrations of PBDEs (and other EDCs) which are shed into the household environment during the normal wear and tear of consumer products to which they are

applied. House dust concentrations in Canada have been shown to contain ∑PBDE concentrations as high as 61,000 ng/g (Shoeib et al., 2012). Exposure via house dust ingestion versus diet is a highly contested topic in the EDC research community; some studies have found diet as the main source of exposure (Fromme et al., 2009), while others have found house dust ingestion as the main pathway of PBDE exposure (Jones-Otazo et al., 2005). Most experts seem to agree that the route of exposure correlates with age; for example, toddlers are most likely to have the highest exposure to PBDEs through house dust ingestion, due to crawling on the floor and high rates of hand-to-mouth activity (Stapleton et al., 2012). In line with this, in the present study younger participants (<50 years) generally had higher plasma PBDE concentrations than older participants, which may correspond to higher exposure via household dust ingestion (containing PBDEs) when they were younger (this also aligns with peak production and use of PBDEs leading up to the 1990s). While local seafood consumption does contribute to EDC exposure in populations like those in our study, it is important to consider other sources of exposure which may also contribute to plasma EDC concentrations.

Fish consumption is categorized as an important part of a healthy diet, and seafood consumption is projected to rise until at least 2050 in industrial and developing countries (Kearney, 2010). In the Canadian population, fish consumption is estimated at 8.71 kg/person/year (Fisheries and Oceans Canada, 2016). A survey commissioned by the Canadian Aquaculture Industry Alliance (CAIA) found that Canadians ate finfish 3.7 times/month, and consumed shellfish 1.9 times/month (Colletto et al., 2011). While the estimated average Canadian fish consumption (24.4 kg/capita/year) sits slightly higher than the US (22.4 kg/capita/year), it is still much lower than European countries including Spain (42.2 kg/capita/year), Finland (43.9 kg/capita/year), Portugal (58.7 kg/capita/year), and Iceland (94

kg/capita/year), and also lower than Asian countries including Thailand (40 kg/capita/year), Taiwan (43 kg/capita/year) and Macao (49 kg/capita/year; Speedy, 2003).

Our hypothesis for this project was that consumption of seafood might put Newfoundlanders at a higher risk of exposure to environmental contaminants. Exploring human environmental pollutant exposure through seafood consumption has been documented in many different fish-eating populations around the globe, and there is evidence from many countries that seafood products are major contributors to human EDC exposure. In a market-basket study done in Sweden, fish contained the highest contaminant concentrations of all tested food products and contributed the greatest proportion to daily intakes of PBDEs, PCBs, and DDTs (Darnerud et al., 2006). In Spain, fish and shellfish were also found to contain the highest concentrations of contaminants (PBDEs) of all food products, and to contribute the greatest proportion of PBDE exposure through diet (Domingo et al., 2008). In Norway, oily fish contribute the most to dietary PBDE consumption (Knutsen et al., 2008). In south China, fish (and mollusks) also contributed the most to dietary intake of PBDEs and DDTs (Guo et al., 2010). South Korea has reported some of the highest rates of PBDE contamination via seafood consumption in the world, attributed in part to their high seafood consumption rate (Lee et al. 2013).

All these countries share attributes of being large fish consumers. Additionally, sport fishermen in the United States who fish in the Great Lakes and eat their catch were also found to have higher body burdens of PBDEs, matching the PBDE contaminant load found in local fish (Christensen et al., 2016). In our study, we were not able to test a wide variety of seafood products for the presence of EDCs, however, our regression analysis did show that consumption of local cod and all seafood (no cod) was positively correlated with serum PCB concentrations in our study population. As such, we must rely on results from previous studies that show exposure

through seafood consumption to strengthen our findings that local seafood consumption is a likely source of exposure to EDCs in our two study communities.

US exposure to PBDEs tends to be higher than Europe due to the higher flammability standards set for consumer materials (Schecter et al., 2006a). Fish in the US have the highest concentrations of PBDEs compared with other food products, however Americans consume a diet with a higher proportion of meat and dairy than Europeans, and thus meat contributes the most to daily PBDE intake from food, followed by dairy and fish (Schecter et al., 2006a). This is opposite the pattern seen in Europe and many Asian counties (as discussed above), where fish makes up a larger proportion of the diet. While there may be much similarity between Canadian and American diets, Newfoundland stands out as an exception to the rule, due to the traditional food fishery and the long ties to fish harvesting.

In Newfoundland, many small communities suffer from food insecurity due to long and expensive transport of food products to their location (Lowitt, 2014). As a result, local fisheries are very important to community food security, and locally sourced seafood is preferred and valued in the foodscape of small towns in Newfoundland (Lowitt 2014). The majority of Newfoundlanders prefer local over imported seafood, and the majority preserve their fish through freezing (Lowitt, 2013). New legislation from the provincial government of Newfoundland allows fish harvesters to sell directly to consumers, constituting another pathway for residents to buy local seafood.

Indigenous populations, many of whom consume a more traditional diet of harvested and hunted foods, are also at a risk of exposure to environmental contaminants from local sources and long-range transport (Kuhnlein and Chan, 2000). Canadian Inuit populations have been shown to be exposed to PCBs through diet (Dewailly et al., 1993). Similarly, Norwegians also

have a diet rich in seafood, and have been shown to consume the greatest amount of PBDEs through fish (Knutsen et al., 2008).

## 5.7 Limitations

There were 3 main limitations to this study which must be considered when interpreting our results. The first limitation was that it was only possible to test fish livers due to our collaboration with the Department of Fisheries and Oceans and Marine Institute who required fish fillet (and other tissues) for their sampling, thus we did not have access to any other fish tissues besides liver. While liver has the advantage of being the main site of metabolism for PBDEs and therefore a likely place for them to be detected, liver is not commonly consumed by humans as food. Fillet (muscle) is the predominant tissue that is consumed from fish, and therefore would be the main human ingestion pathway for PBDEs from this food source. The results from this study demonstrated the existence of PBDEs in fish species around the Island of Newfoundland; however, it was not possible to estimate human exposure from consumption because we do not know the concentrations of PBDEs in fillet. Lipid content of cod fillet is much lower than liver, therefore PBDE concentrations may vary between these tissues as PBDEs are lipophilic. Importantly, we were not able to test for PCBs, PBBs or p,p'-DDE in our fish liver samples, therefore, we do not have direct evidence of these contaminants in the marine ecosystem around Newfoundland and must rely on the literature for evidence of these EDCs around or upstream of Newfoundland.

Additionally, it would be beneficial to explore PBDE and other EDC contaminants in cod or fish liver oil; the presence of PBDEs in fish liver in our study lends evidence to the premise that without the specific removal of these contaminants, fish liver oil health products may also be

sources of human exposure to EDCs, as has been seen in a newly constructed global database of PBDEs in food and supplements (Boucher et al., 2018). Cod liver oil supplements have been found to have some of the highest concentrations of PBDEs (14.6-34.2 ng/g lipid) when compared with other fish oil supplements (Jacobs et al. 2004).

Our second limitation was our small sample size of rural Newfoundlanders. Our total number of participants was 80, however for some parts of the analyses (e.g. the association between EDCs and thyroid hormones) six participants with hypothyroidism were excluded bringing our sample number down to 74. While this is on par with other peer-reviewed studies (Byrne et al., 2018, n=85), it did make it difficult to account for more than 3-4 predictors/covariates in the regression analyses. Sample size calculation was possible in this study as the high costs of EDC sample analysis constrained the total number of participants. As such, the results from this study should be considered exploratory, providing evidence of the need for a larger-scale study of EDC exposure in the rural Newfoundland population. Additionally, there may be bias in the participants that did attend the study after recruitment, as they would (potentially) have a better memory (remembering appointment), have access to transportation and live within a reasonable distance to the healthcare center where the study occurred. These factors could have contributed to participants in better health or with a greater socio-economic status (SES) participating at a higher rate than those in poor health or low SES. While we did not account for this possible bias in our work, it is something that should be addressed in future studies of EDC exposure in rural Newfoundland populations.

Our third limitation was the narrow range of demographic and health data collected from participants. Only age, sex and location information could be collected from participants due to constraints from the Health Ethics Research Authority. However based on review of the

literature it is apparent that demographic data (income, education level, occupation) and personal health information (smoking, history of other diseases) are important in assessing the relationship between EDCs and thyroid hormones. In the absence of these data, this study focused more on describing geographical, age and sex-related differences in EDC concentrations in the study population.

## 5.8 Discussion conclusion

In Newfoundland, there is now confirmation of marine ecosystem contamination with PBDEs. Testing cod and turbot liver for PBDEs gave us conclusive evidence that there is environmental contamination all around the Island of Newfoundland marine habitats, and that it affects both benthic and (semi) pelagic fish species. The Canadian Federal Minister of the Environment has set Federal Quality Guidelines for PBDEs in the environment including in fish tissues; both cod and turbot liver samples from our study had Penta-BDE concentrations above quality guidelines (see Table 5.1). There were some areas that had fish that were more contaminated than others, which is likely the result of different currents (St. Lawrence River versus Labrador Current) bringing distinctive pollution to the different coasts of the Island of Newfoundland.

Newfoundlanders eat a wide variety of local seafood, with cod as the most frequently consumed species, and local seafood is consumed more in the summer than in any other time of the year. There were a wide variety of EDCs detected in study participant plasma samples (note: there are currently no recommended plasma concentration limits of PBDEs, PCBs, PBBs or p,p'-DDE), and there was a difference in congener concentrations by age, sex and community. PBB-153, PCBs, and p,p'-DDE were generally higher in concentration in older (>50 years)

participants, while PBDEs were higher in concentration in younger (<50 years) participants. Males had higher EDC concentrations of all congeners than female participants. Additionally, PBDEs and PBB-153 were higher in participants from Burin while PCBs and p,p'-DDE were higher in participants from NWV.

Thyroid hormone concentrations in the study population were within normal clinical range, and we were not able to detect any association between EDCs concentrations and thyroid hormone levels in our study participants, although some non-monotonic relations were observed when EDCs were grouped into quartiles and compared with thyroid hormone concentrations. The relationship between EDCs and local seafood consumption was also examined; some PCB congeners and p,p'-DDE were positively associated with cod consumption.

While there may be other sources of exposure contributing to the EDC body burden seen in our participants, this study demonstrates that local seafood consumption is a likely source of exposure to EDCs in Newfoundland. Although, it is not possible to provide exposure estimates from these data, there is enough evidence now to suggest that there is a need for more stringent monitoring of seafood species harvested around Newfoundland and to monitor exposure through consumption in the local population.

# Chapter 6 Summary and Conclusions

EDCs are long-lasting environmental pollutants with broad health implications for humans, particularly at low-dose exposures over long periods of time. Because of their ability to undergo long-range transport on atmospheric or ocean currents, they are ubiquitous worldwide and present an ongoing environmental health issue even in countries (such as Canada) where their manufacture and use may have been banned for decades. Exposure is now also occurring in utero for the newest generation of humans, and the associated health effects at the individual level are numerous.

In this study, we clearly demonstrated that exposure to EDCs continues to occur in two rural coastal communities of Newfoundland (Burin and NWV), even though all of the EDCs we tested for in study participants (PBBs, PBDEs, PCBs, and p,p'-DDE) are banned or restricted under Canadian federal law or the Stockholm Convention. While we were not able to find any association between EDCs and thyroid hormones in our study population, there were some non-monotonic dose response trends between EDC concentrations and thyroid hormones. There was also evidence of local seafood consumption as a source of exposure to some EDCs. Additionally, we were able to demonstrate the presence of one type of EDC (PBDEs) in two local seafood species. Our study provides evidence of EDC exposure in Newfoundland through the presence of EDCs in the plasma of all study participants, with seafood as a possible exposure source. However, it also raises more questions about alternate sources of exposure and origins of contamination into the environment, and demonstrates the need for a more comprehensive study of EDC exposure in Newfoundland and Atlantic Canada.

Although exposure to EDCs has been documented in many corners of the world, there have been limited assessments of EDC exposure in the Canadian population, including in the Atlantic

Canadian provinces of Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland and Labrador. These provinces sit along the St. Lawrence River and the Gulf of St. Lawrence, both of which have documented EDC contamination in higher trophic level organisms such as fish (Lebeuf et al., 2014) and marine mammals such as beluga whales (Houde et al., 2014).

The Labrador Current, which flows across Labrador and northeastern Newfoundland, originates in the Arctic and may be receiving EDCs distributed by the global distillation of volatile chemicals in this environment. Environmental contamination of EDCs has been documented in Arctic high trophic-level predators including PBDEs in the glaucous gull (Verreault et al., 2018), caribou, and wolves (Morris et al., 2018), and PCBs in polar bears (Letcher et al., 2018). As such, there is a wide area of Atlantic Canada that could be exposed to environmental contaminants from upstream sources. Newfoundland is exposed to both the Labrador Current and St. Lawrence River currents; therefore, it is unique from the other Atlantic Provinces and geographically situated for various types of environmental exposure from different sources. Our study is the first in Newfoundland and Atlantic Canada to test both environmental samples (fish liver) and human samples (plasma) for EDCs, as well as document seafood consumption as a possible exposure pathway.

We found wide-spread PBDE contamination in cod and turbot liver samples from different coasts of Newfoundland. PBDEs were higher in fish liver caught off the west coast of Newfoundland than those caught from the northeast offshore region. While these areas have very different pollution sources (St. Lawrence River on the west coast and Labrador Current/open North Atlantic Ocean on northeast coast), it is not surprising to find higher PBDEs in fish from the west coast due to the documented pollution from anthropogenic sources in the St. Lawrence River and the Great Lakes watershed which both drain into the Gulf of Lawrence. We also found

that cod had higher PBDE concentrations than turbot, indicating that there may be a species-specific difference in EDC levels.

These results signify the need for a more thorough investigation of PBDEs and other EDCs in seafood caught around the coasts of Newfoundland. The presence of PBDEs (long banned from production, sale and use in Canada) in fish alludes to their persistent and bioaccumulative nature, which is likely the case for other banned EDCs (such as PCBs, PBBs and p,p'-DDE). Because of the wide range and quantity of seafood products caught for local consumption and export, and the significant economic value of this industry to the province of Newfoundland (and the rest of Atlantic Canada), it is important that there is continuous monitoring for old and new EDCs which may pose health hazards for humans now and in the future.

We have shown that there is extensive human exposure to environmental EDCs in Newfoundland, as all eighty of the participants in our study had detectable concentrations of at least 11 different contaminants in their plasma. Male participants generally had higher plasma concentrations of all EDCs, which is mirrored in other studies and may be the result of lifestyle-based exposure. Older participants typically had higher plasma concentrations of PCBs, PBB-153 and p,p'-DDE, while younger participants had higher PBDE concentrations, which correlates with PCBs and p,p-DDE being in production and use much earlier than PBDEs. Burin participants had higher serum PBB-153 and PBDEs, while NWV participants had higher PCBs and p,p'-DDE, indicating a geographical difference in exposure which we attributed to the different currents (south coast – St. Lawrence River, north coast – Labrador Current) bringing different sources of pollution to the Island of Newfoundland.

Local seafood consumption was highest in older, male participants, and cod was the most commonly consumed species, with higher reported consumption frequencies in NWV than in

Burin. Several PCBs and p,p'-DDE had strong positive correlations with reported consumption frequency of cod consumption, therefore it is possible that consumption of this species plays a role in exposure to EDCs in Newfoundland.

Going forward, the risk/benefit analysis of seafood consumption will need to be strongly considered. Seafood has many nutritional qualities; it is high in protein, omega fatty acids, and essential nutrients such as iodine, however exposure to environmental contaminants including endocrine disruptors is a concern. Consistent monitoring of seafood species will be required to keep humans safe. There was a clear association between reported seafood consumption and plasma EDC concentration in this study, indicating that local seafood consumption may be one source of EDC exposure in rural Newfoundland. The next step in this research would be to conduct a wider-scale study including more locations (e.g. the west coast of Newfoundland and coastal Labrador) a greater number of participants, collect more demographic information (e.g. smoking status, income, history of medication use) and test for other possible sources of exposure including other dietary items known to contain EDCs (e.g. meat, eggs, dairy), and indoor dust.

In conclusion, there is clear evidence of EDC exposure in rural Newfoundland residents. Due to the small sample size of the study, it is difficult to extrapolate this to the wider provincial population, however this research serves as a pilot study of EDC contamination in Newfoundland, and strengthens the case for future monitoring of EDCs in seafood and of exposure in the human population in Newfoundland and other Atlantic Canadian provinces.

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**Appendix A Seafood Consumption Questionnaire**

# Seafood Consumption Questionnaire

**Introduction**

This survey is intended to collect information on local seafood consumption patterns of residents of Newfoundland. Where the term "local seafood" is used, it refers to seafood harvested close to the community, whereas as non-local seafood refers to seafood from other coasts (Newfoundland), countries or provinces, or seafood of which the participant is unsure of the source.

Please include all seafood eaten through different cooking methods (i.e. fried, baked, seafood stew, au gratin, etc.). Please reply to the best of your ability.

## Part One: Seafood survey

For each of the following types of **local seafood** you consume, please indicate how often you have consumed it in the past month (please check all categories of seafood):

| Type of Seafood | Consumption Frequency | | | | | |
|---|---|---|---|---|---|---|
| | Almost never | Once/ month | 2-3 times/ month | 1-2 times/ week | 3-5 times/ week | Almost every day |
| Capelin | | | | | | |
| Catfish | | | | | | |
| Cod | | | | | | |
| Crab | | | | | | |
| Halibut | | | | | | |
| Herring | | | | | | |
| Lobster | | | | | | |
| Mackerel | | | | | | |
| Salmon | | | | | | |
| Shrimp | | | | | | |
| Scallops | | | | | | |
| Smelt | | | | | | |
| Squid | | | | | | |
| Trout | | | | | | |
| Turbot | | | | | | |
| Other | | | | | | |

Other:_____

In the table below, please indicate how frequently your personally eat **local seafood** at different times of the year.

| | Fall | Winter | Spring | Summer |
|---|---|---|---|---|
| Less than once/week | | | | |
| 1-2 times/week | | | | |
| 3-5 times/week | | | | |
| Every day | | | | |
| Unsure | | | | |

Comments:

_____

## Part Two: Demographic Survey

Postal Code: _____

How long have you been a resident of this community?

| | |
|---|---|
| Less than 1 year | |
| 2-10 years | |
| 10-20 years | |
| 20+ years | |

Please indicate your age:

| | |
|---|---|
| 20-24 | |
| 25-29 | |
| 30-34 | |
| 35-39 | |
| 40-44 | |
| 45-49 | |
| 50-54 | |
| 55-59 | |
| 60-64 | |
| 65-74 | |
| 75 and over | |

Please indicate your sex:

| | |
|---|---|
| Male | |
| Female | |

## Appendix B Fish liver PBDE and MeO-BDE concentration distributions (histograms)

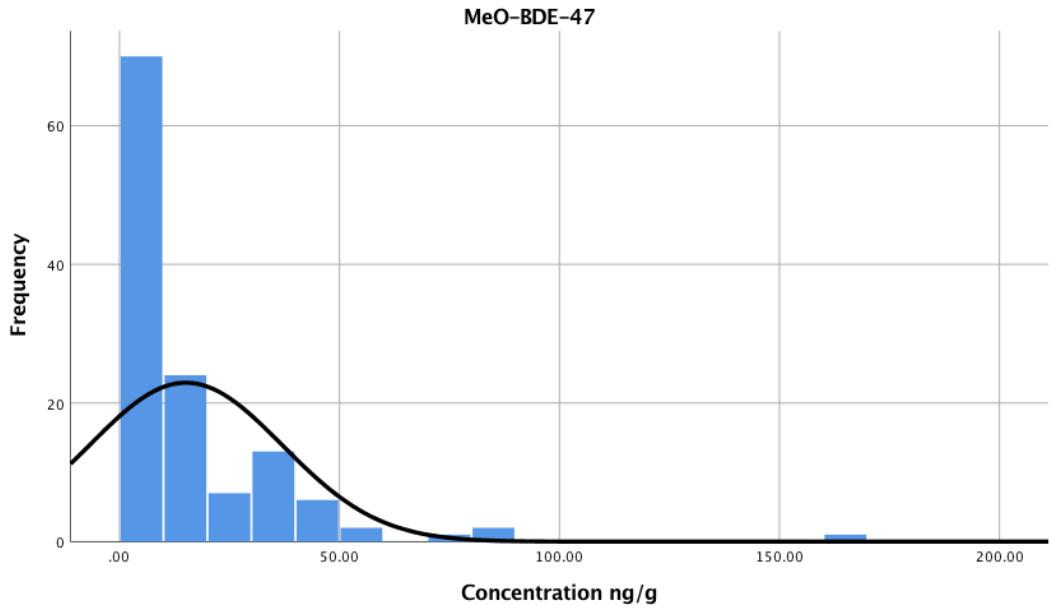


**Figure B.1** Histogram of MeO-BDE-47 concentration distributions.

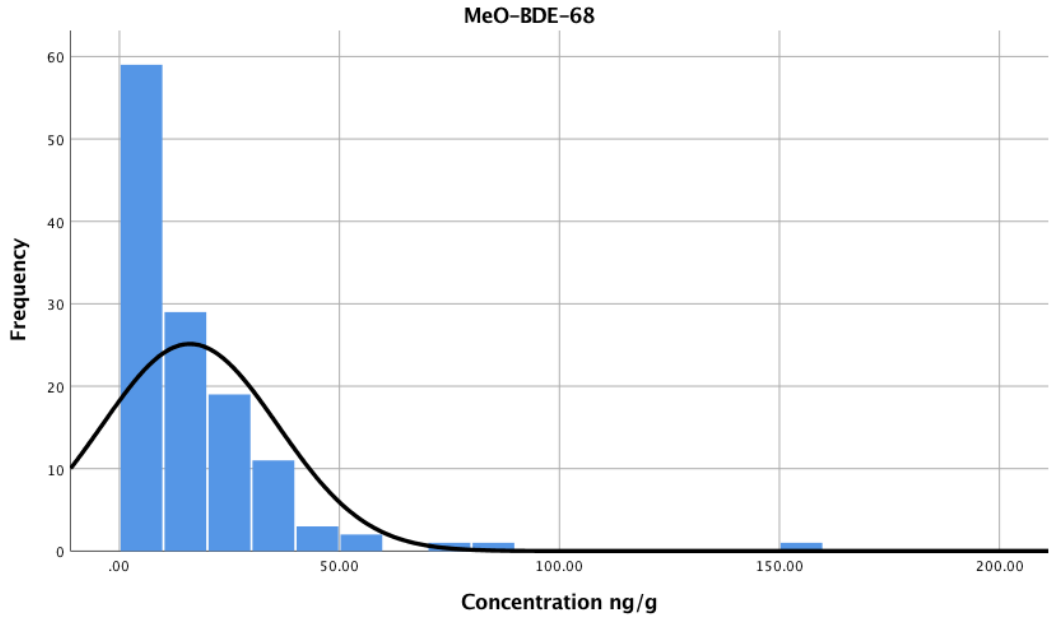

**Figure B.2** Histogram of MeO-BDE-68 concentration distributions.

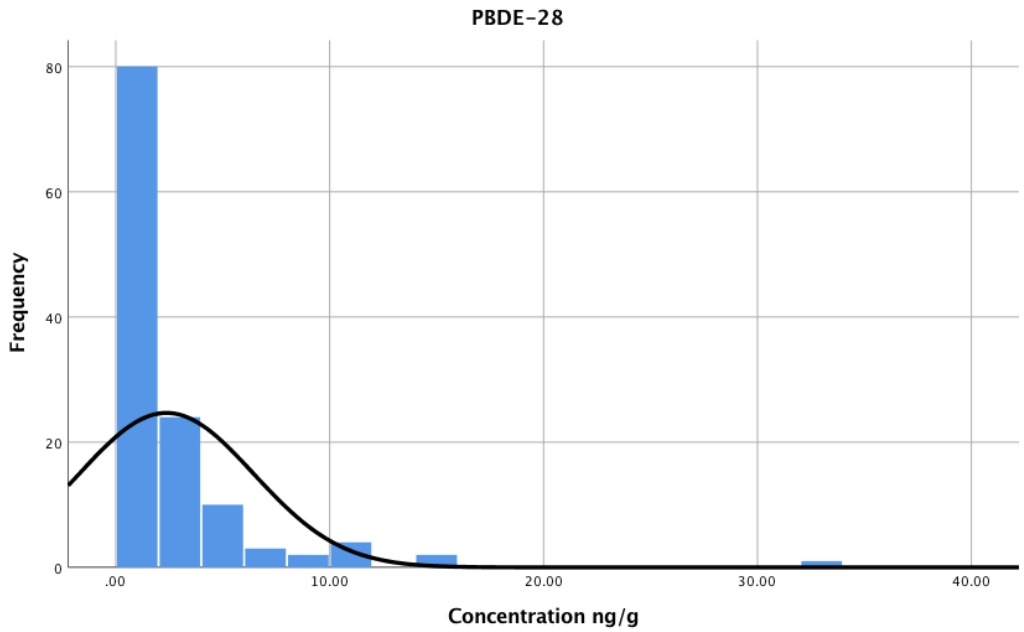


**Figure B.3** Histogram of PBDE-28 concentration distributions.

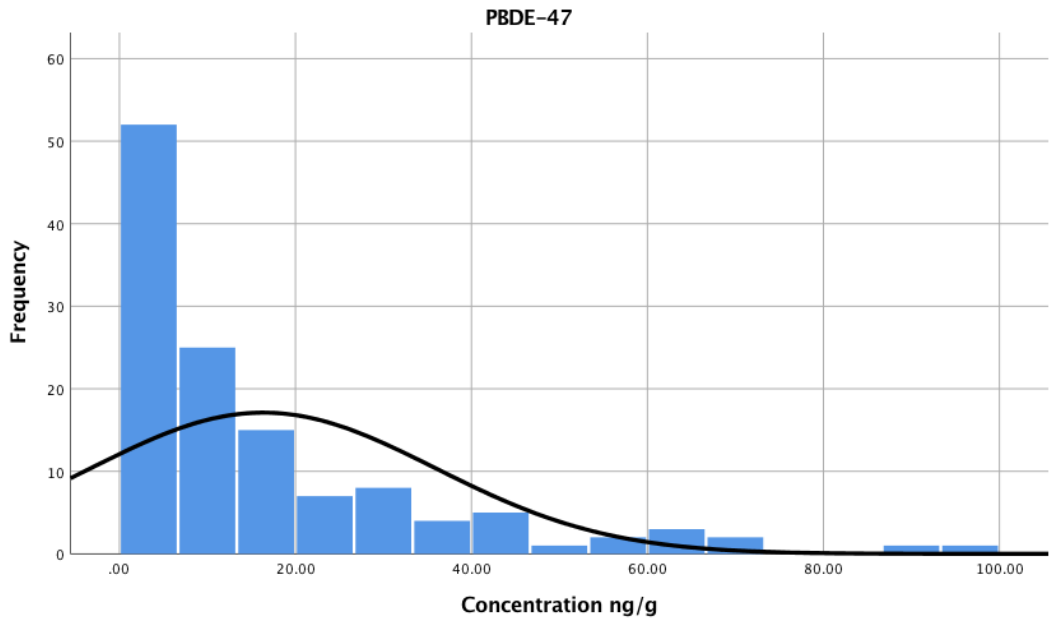


**Figure B.4** Histogram of PBDE-47 concentration distributions.

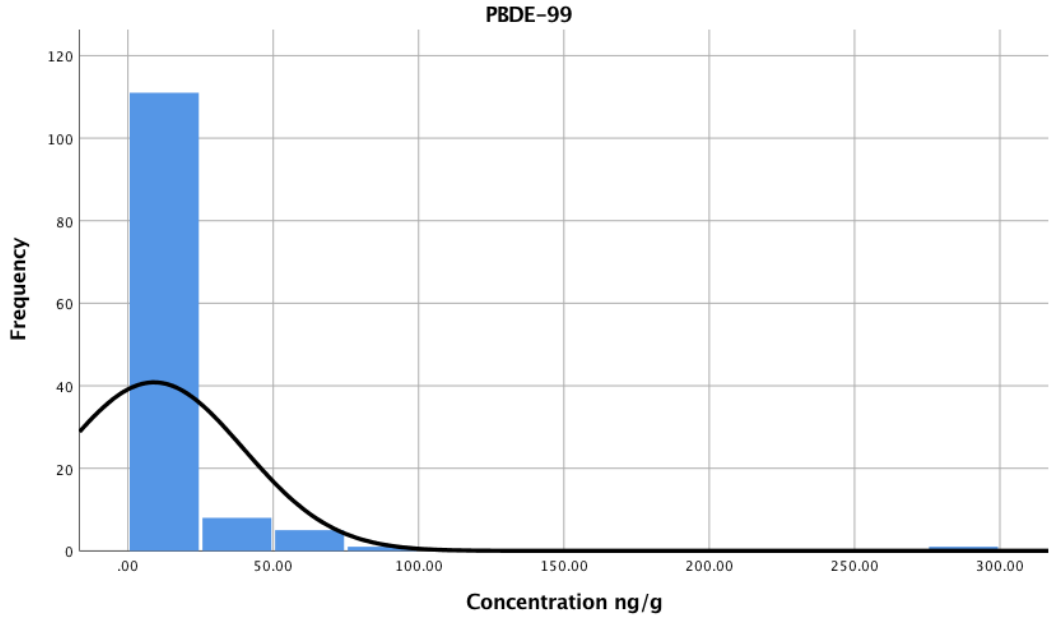


**Figure B.5** Histogram of PBDE-99 concentration distributions.

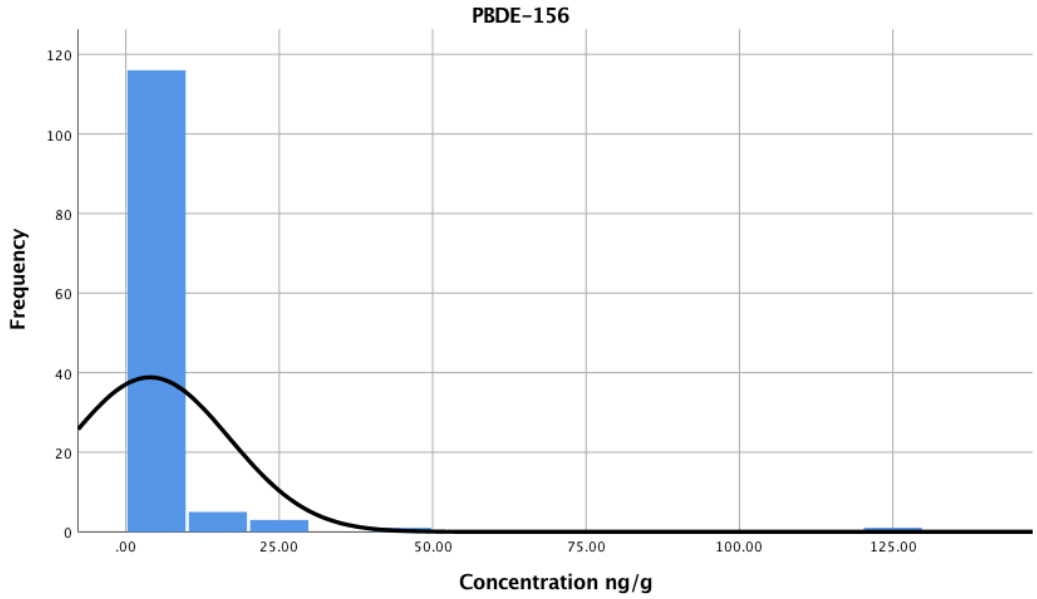


**Figure B.6** Histogram of PBDE-156 concentration distributions.

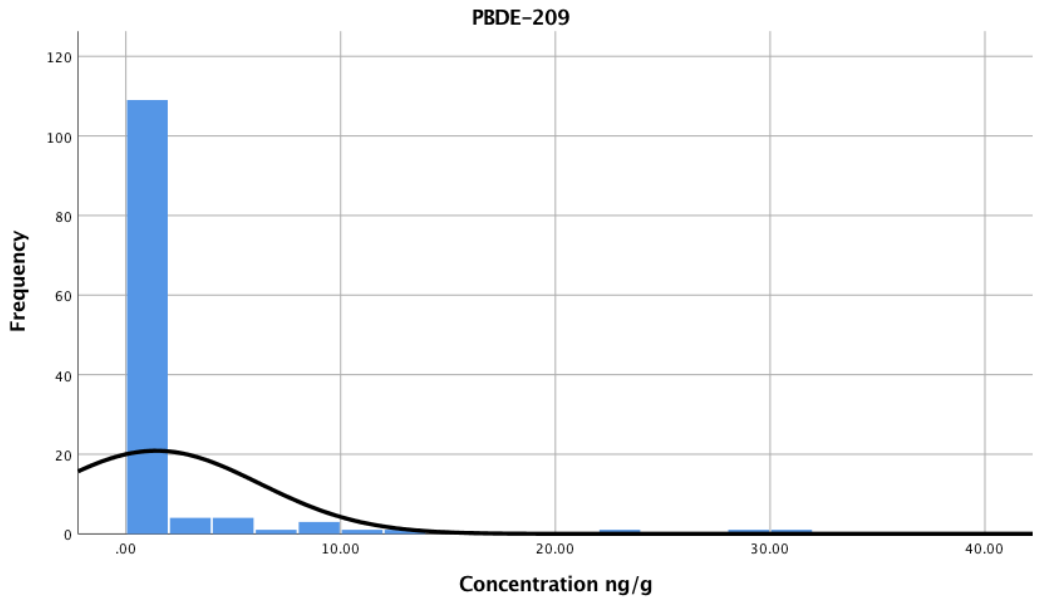


**Figure B.7** Histogram of PBDE-209 concentration distributions.

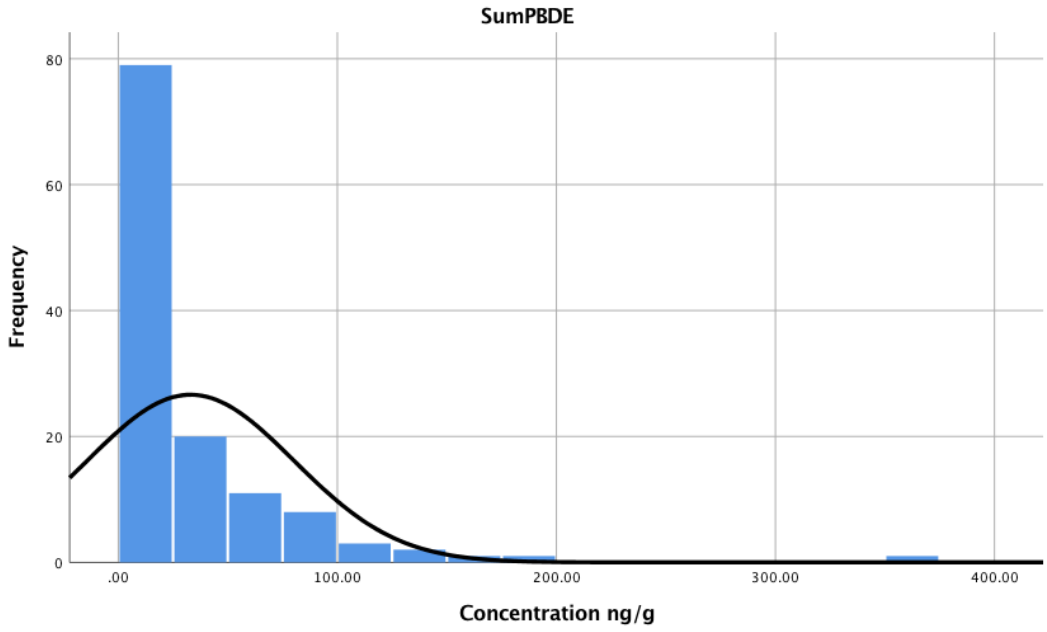


**Figure B.8** Histogram of ∑PBDE (28, 47, 99, 156, 209) concentration distributions.

## Appendix C Molecular Weights of EDCs (g/mol)

**Table C.1** Molecular weights of PBB, PBDE, PCB and p,p'-DDE congeners

| Congener | Molecular weight |
|---|---|
| PBB-153 | 627.588 g/mol |
| PBDE-28 | 406.899 g/mol |
| PBDE-47 | 485.795 g/mol |
| PBDE-99, PBDE-100 | 564.691 g/mol |
| PBDE-153 | 643.587 g/mol |
| PCB-99, PCB-105, PCB-118 | 326.422 g/mol |
| PCB-128, PCB-138, PCB-153 | 360.864 g/mol |
| PCB-170, PCB-180, | 395.306 g/mol |
| p,p'-DDE | 318.018 g/mol |

## Appendix D Serum thyroid hormone concentration distributions (histograms)

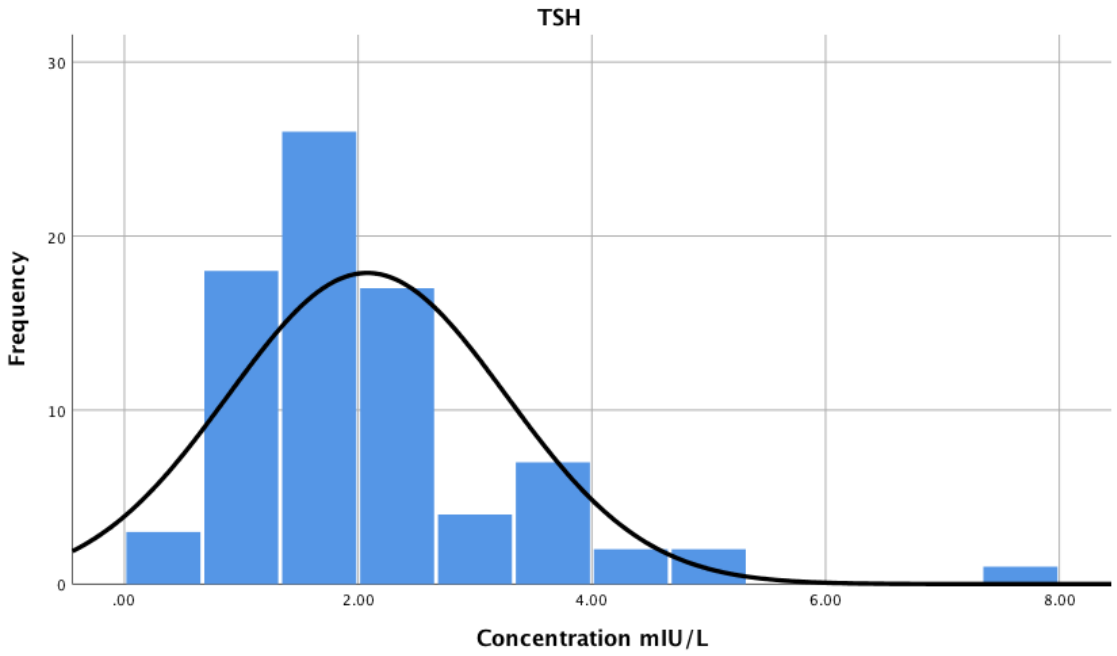


**Figure D.1** Histogram of serum TSH concentration distributions.

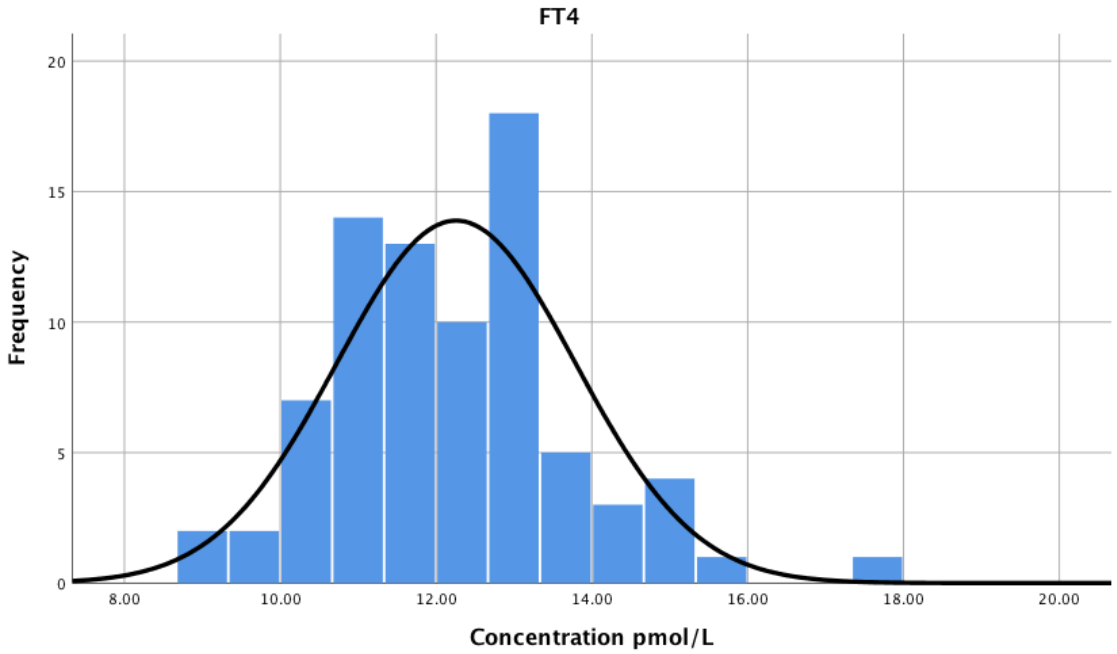


**Figure D.2** Histogram of serum $FT_4$ concentration distributions.

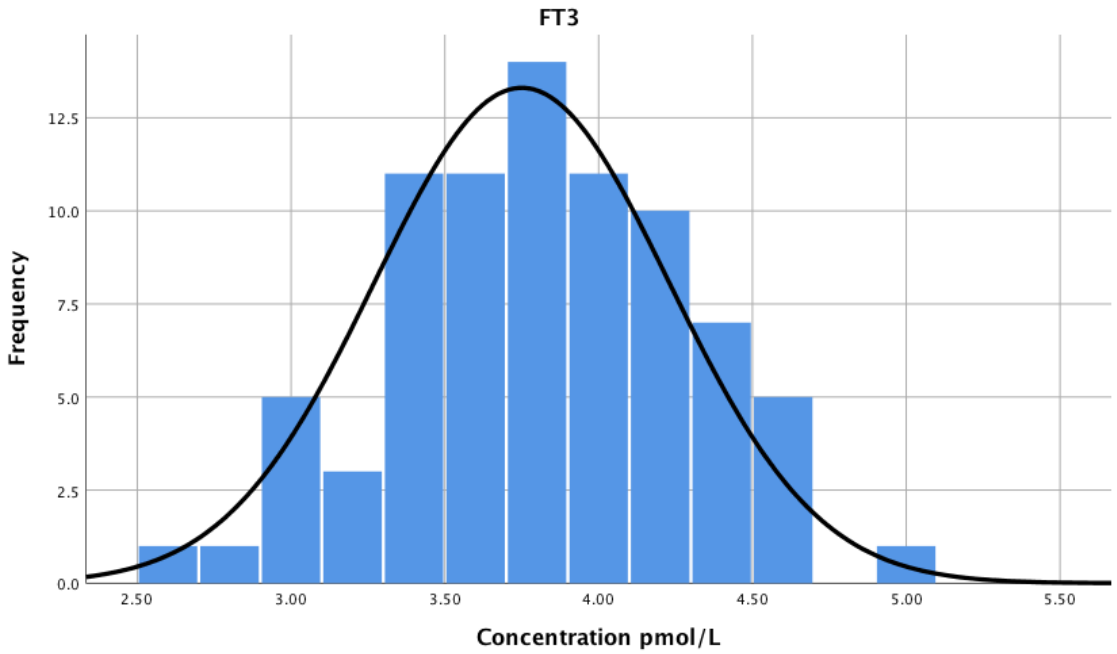


**Figure D.3** Histogram of serum $FT_3$ concentration distributions.

## Appendix E Plasma EDC concentration distributions (histograms)

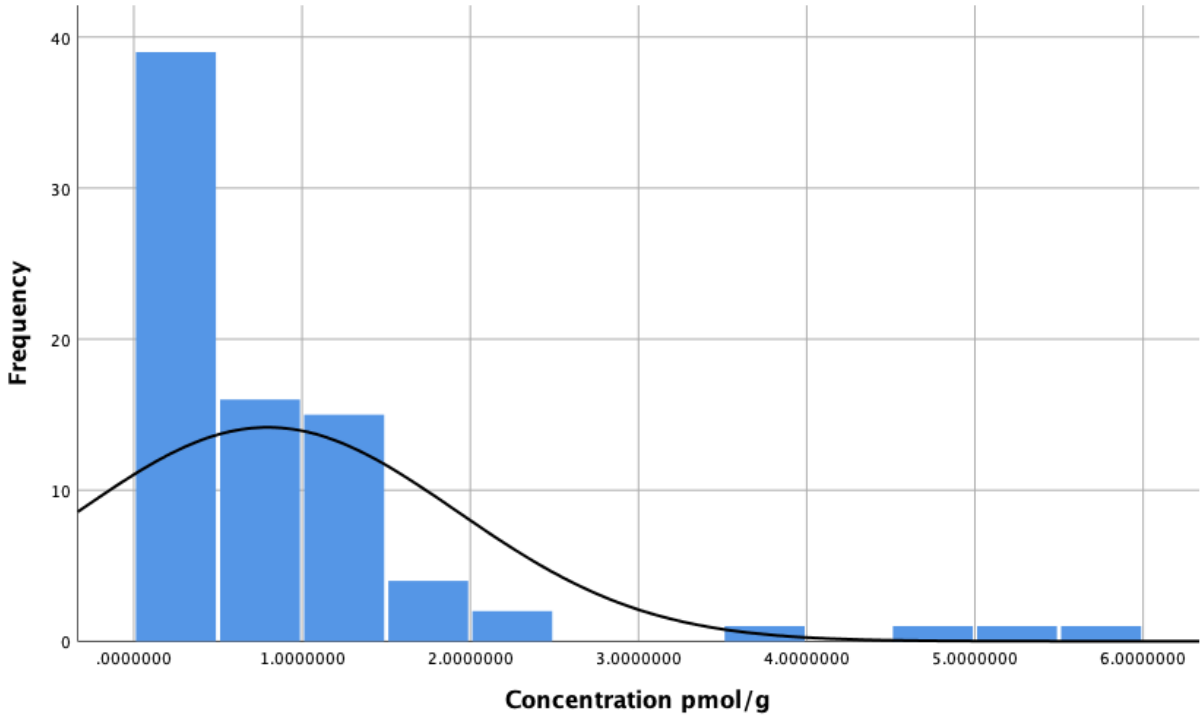


**Figure E.1** Histogram of plasma PBB-153 concentration distributions.

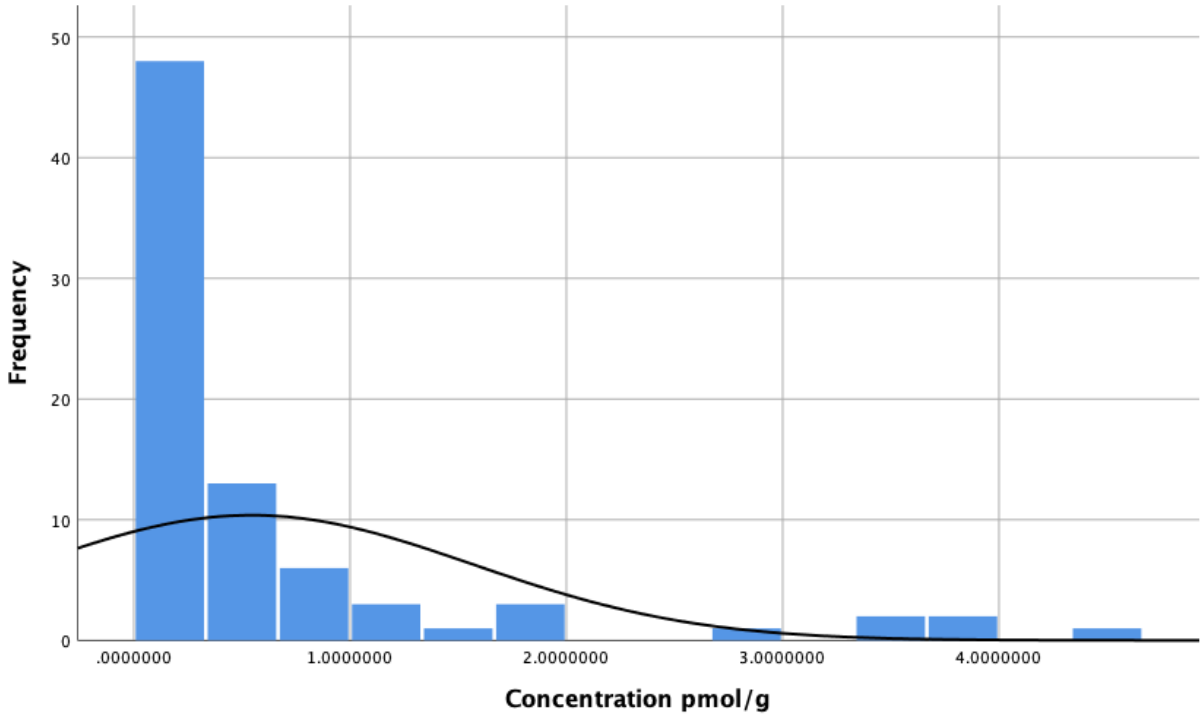


**Figure E.2** Histogram of plasma PBDE-28 concentration distributions.

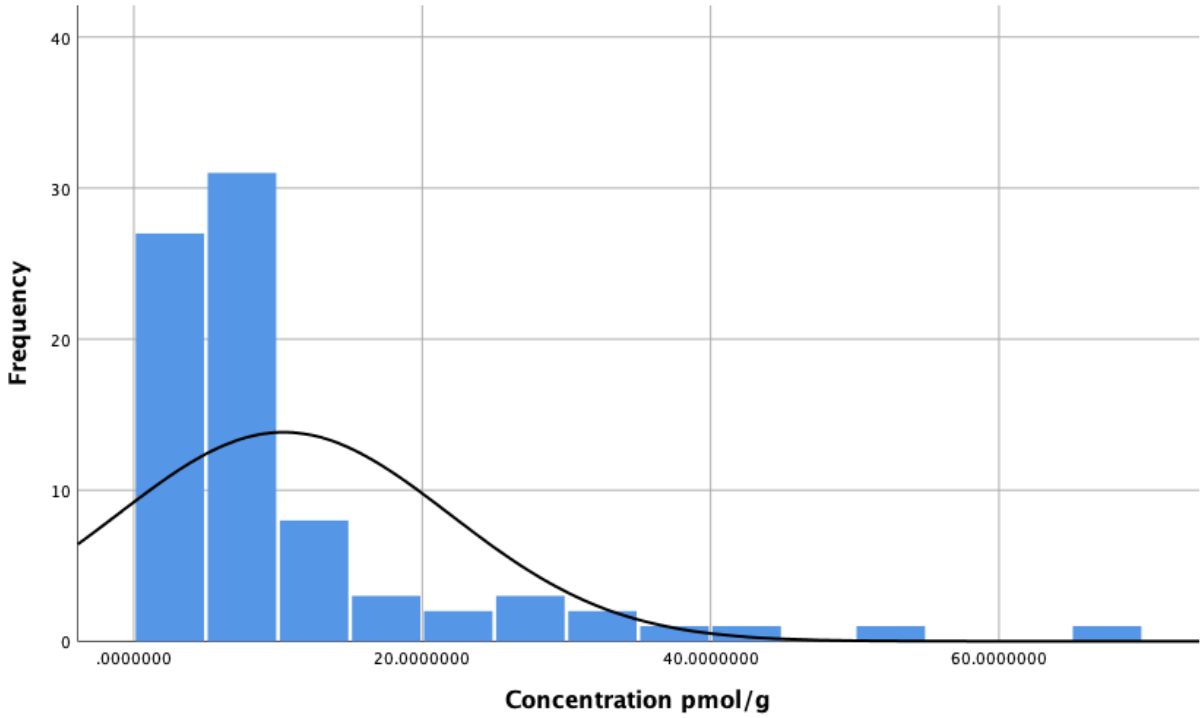


**Figure E.3** Histogram of plasma PBDE-47 concentration distributions.

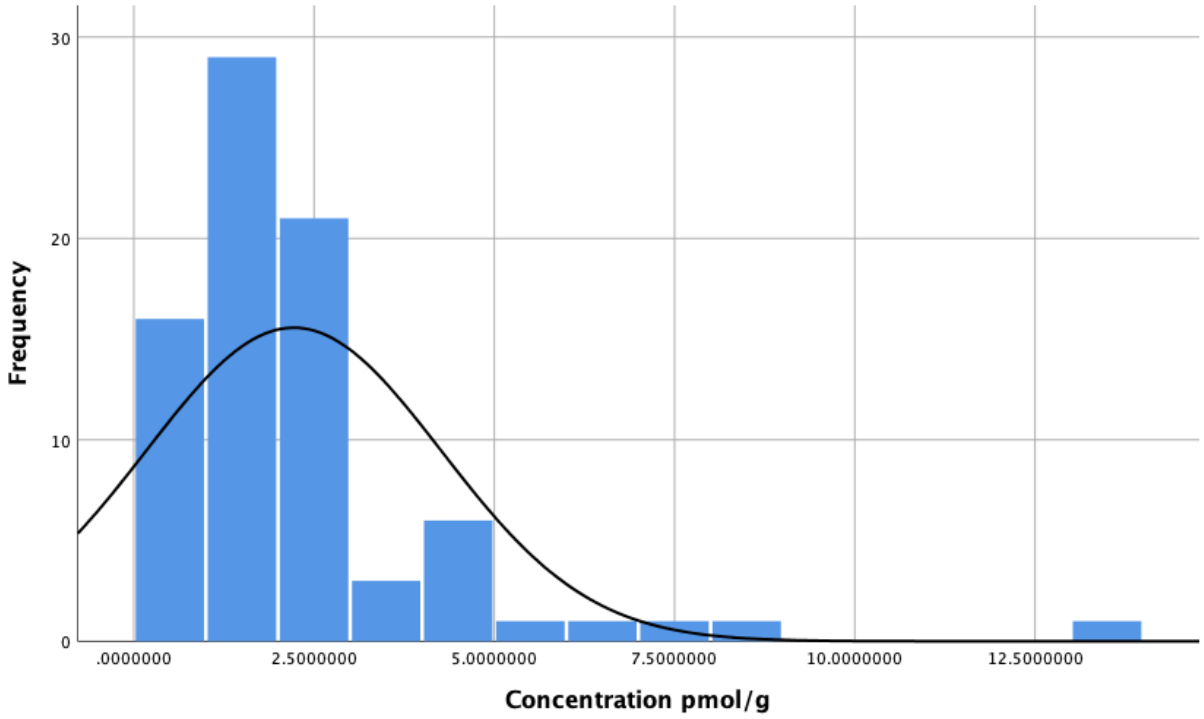


**Figure E.4** Histogram of plasma PBDE-99 concentration distributions.

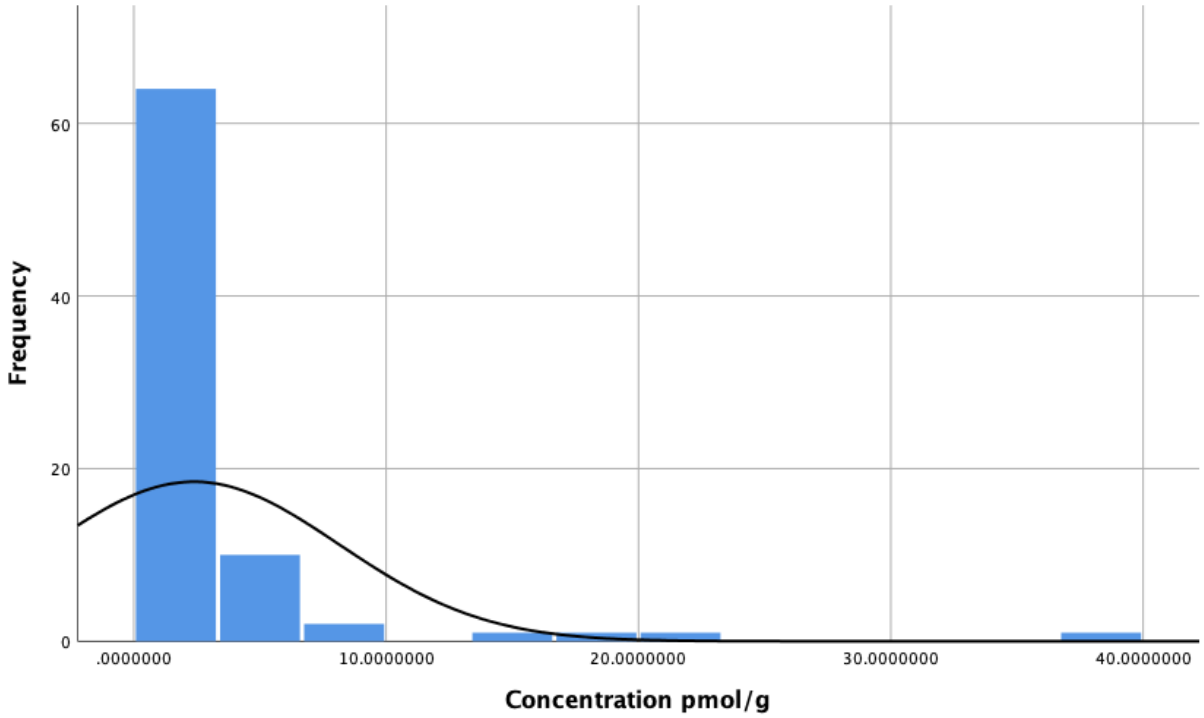


**Figure E.5** Histogram of plasma PBDE-100 concentration distributions.

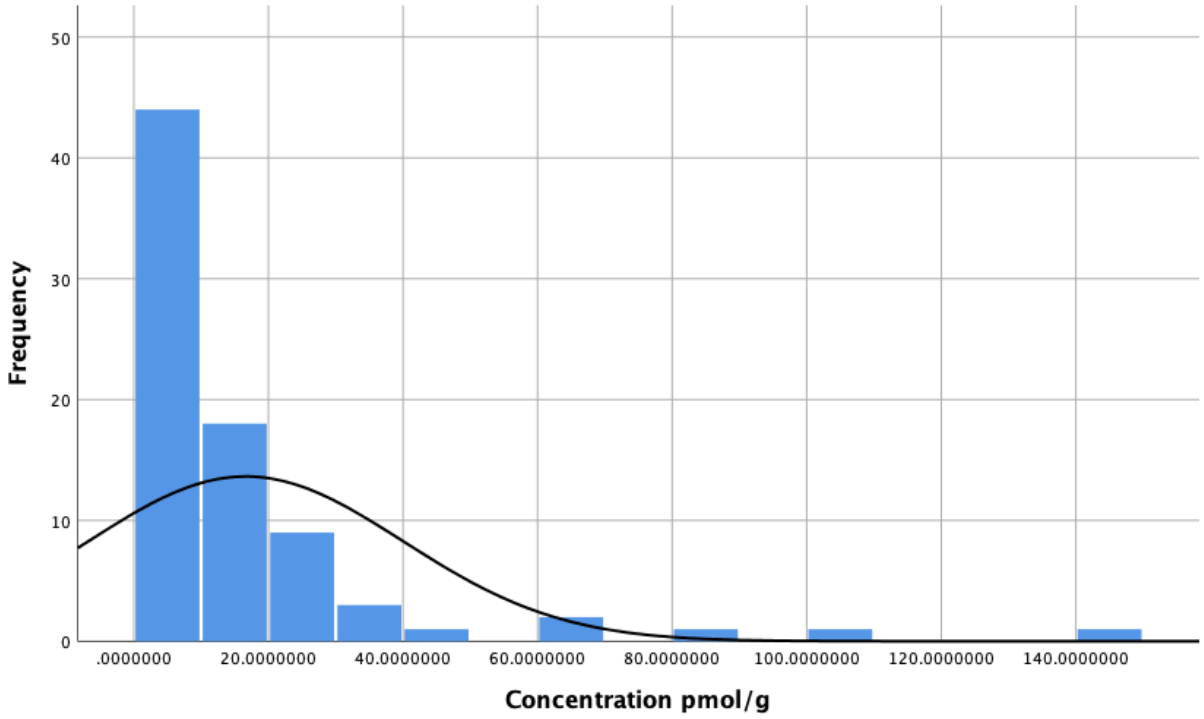


**Figure E.6** Histogram of plasma PBDE-153 concentration distributions.

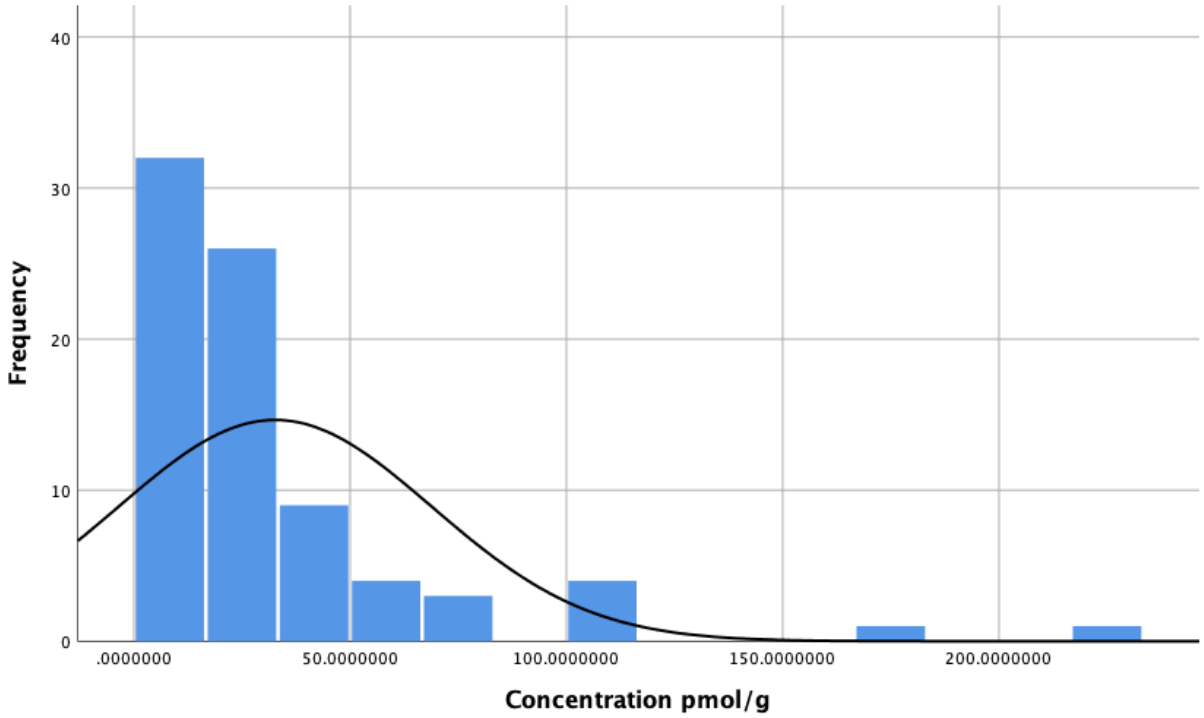


**Figure E.7** Histogram of plasma ∑PBDE concentration distributions.

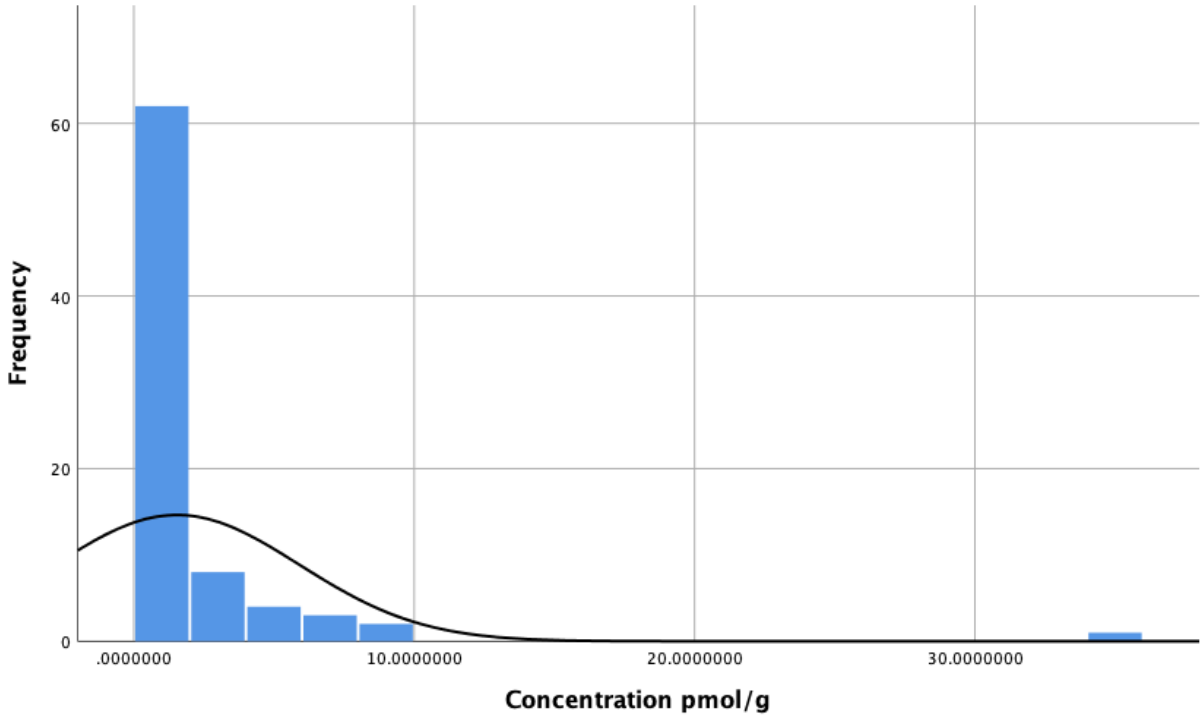


**Figure E.8** Histogram of plasma PCB-99 concentration distributions.

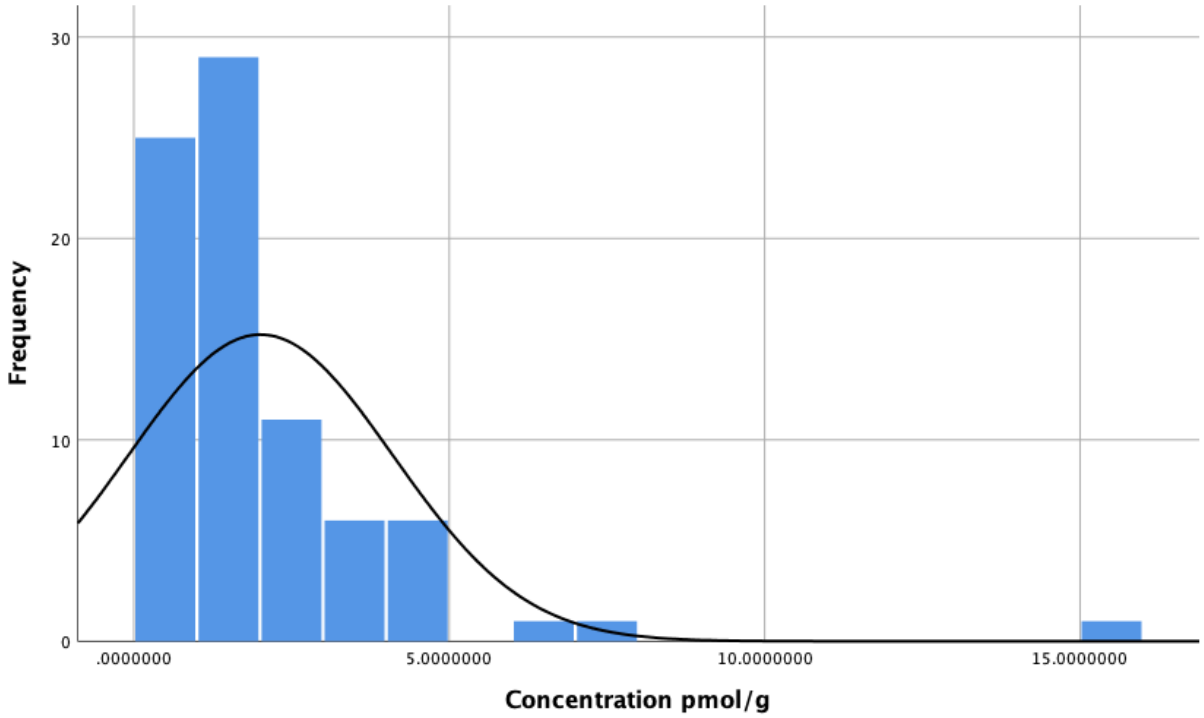


**Figure E.9** Histogram of plasma PCB-105 concentration distributions.

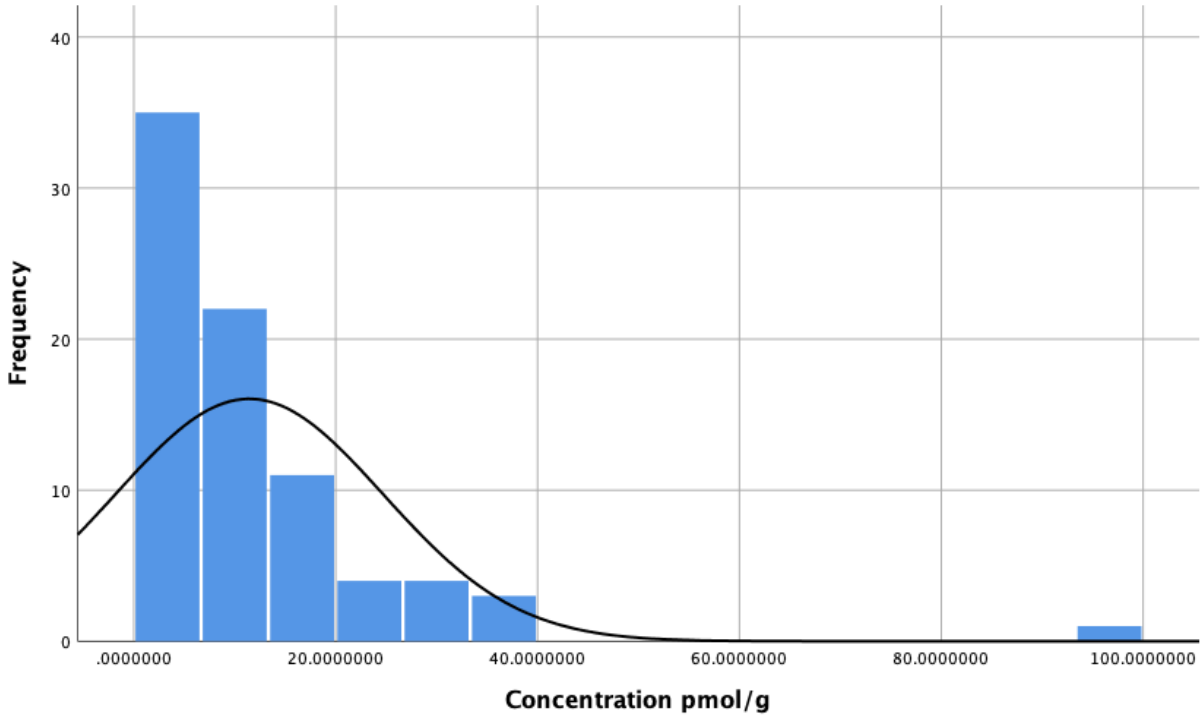


**Figure E.10** Histogram of plasma PCB-118 concentration distributions.

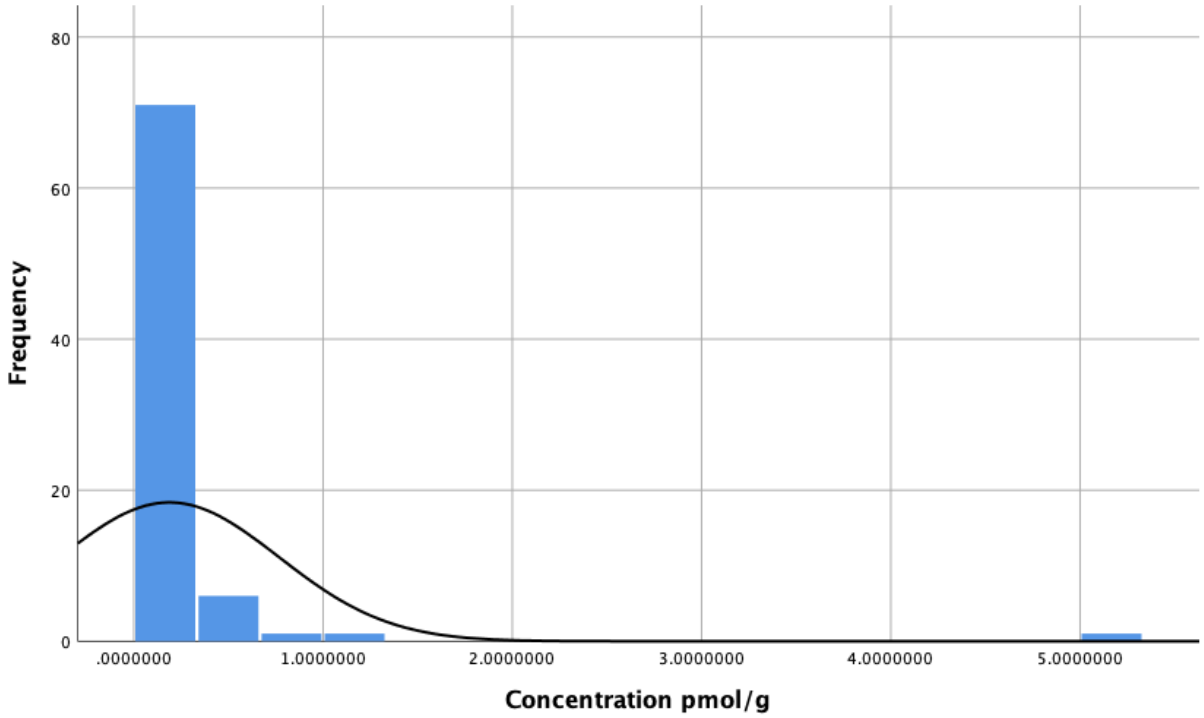


**Figure E.11** Histogram of plasma PCB-128 concentration distributions.

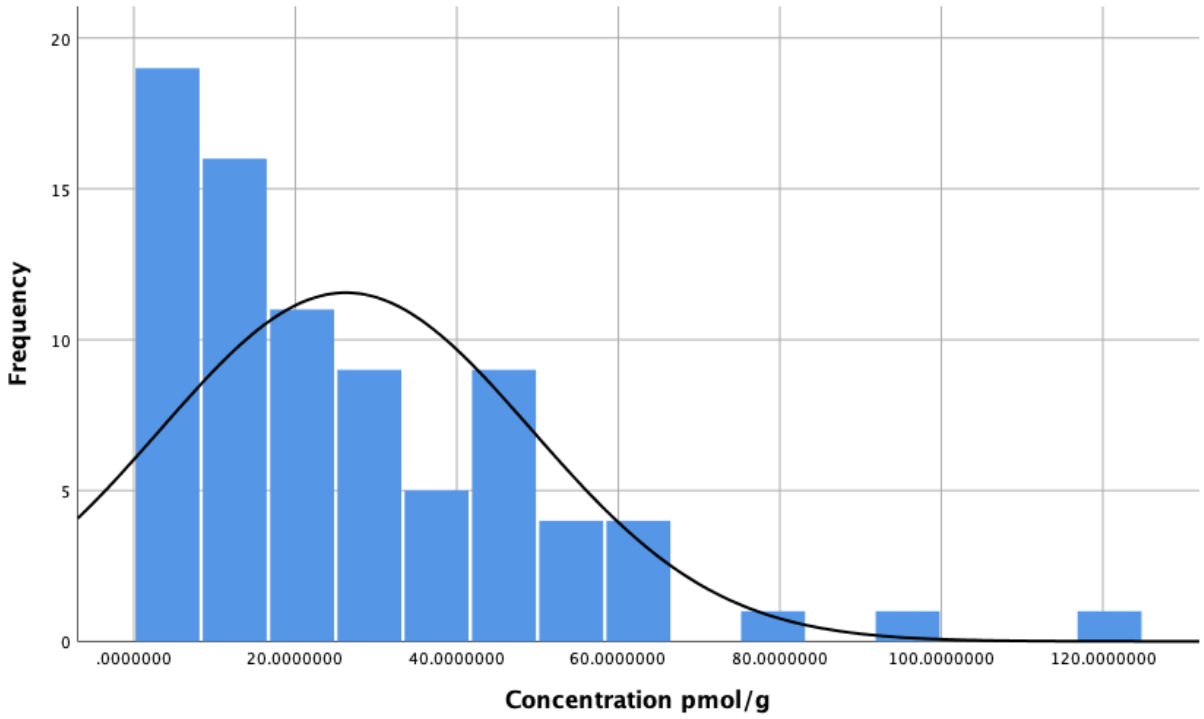


**Figure E.12** Histogram of plasma PCB-138 concentration distributions.

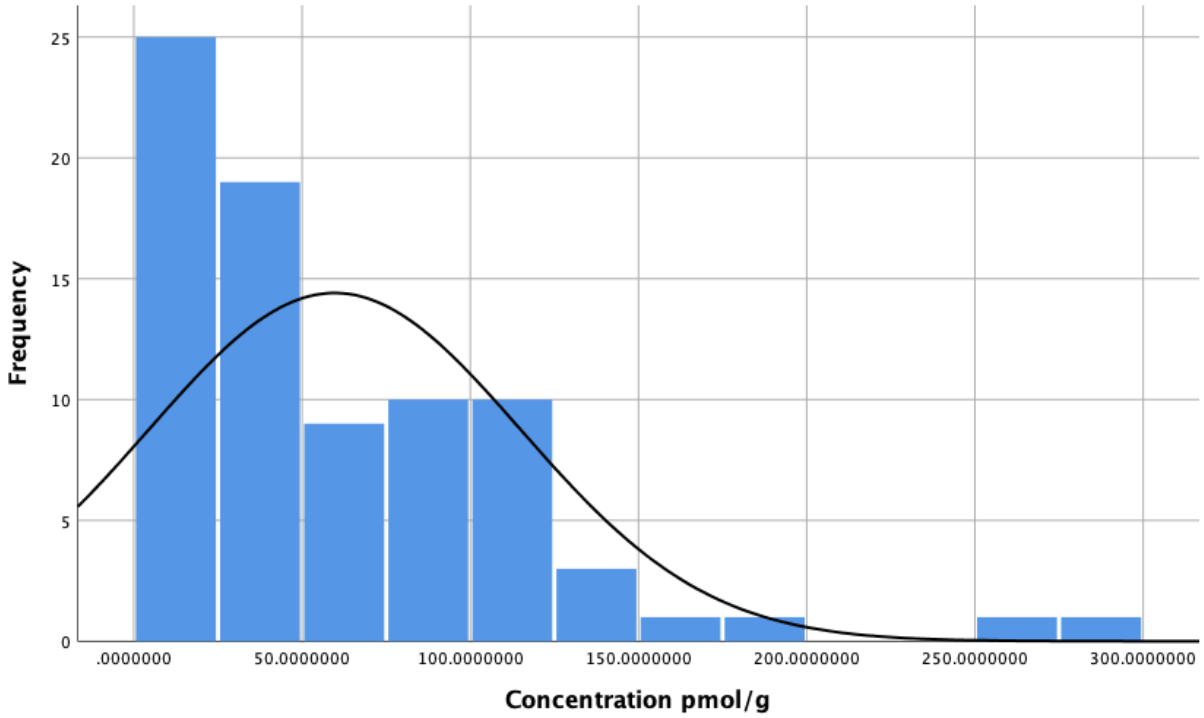


**Figure E.13** Histogram of plasma PCB-153 concentration distributions.

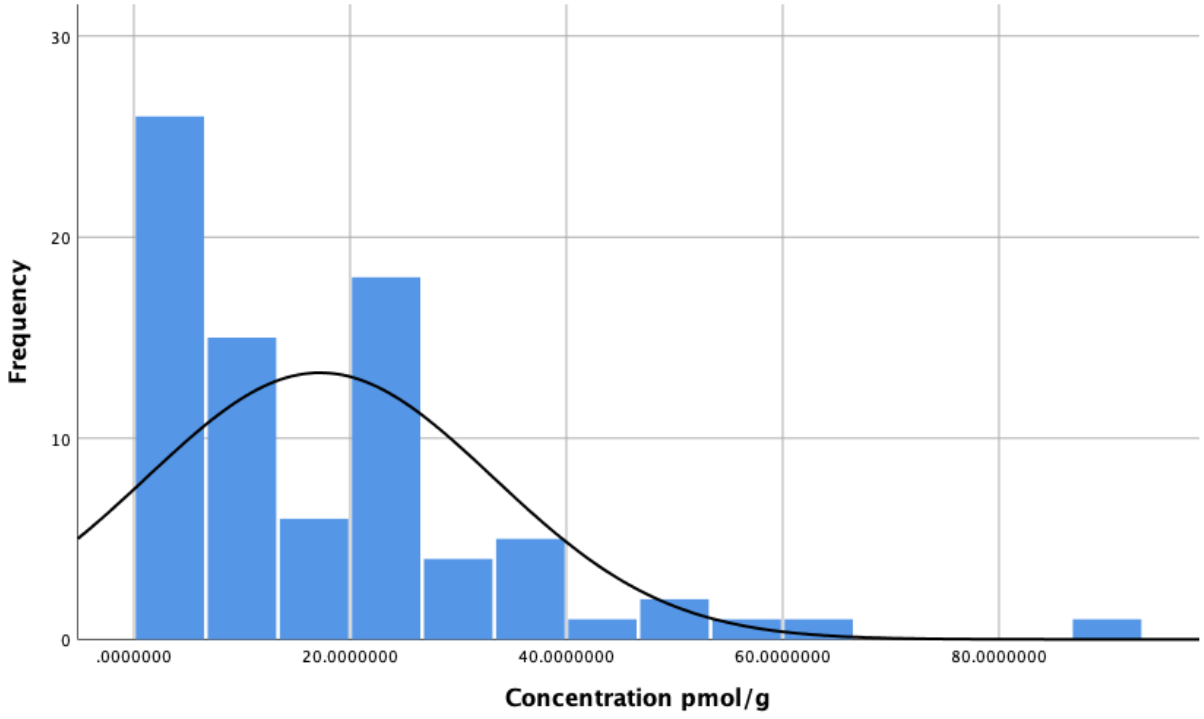


**Figure E.14** Histogram of plasma PCB-170 concentration distributions.

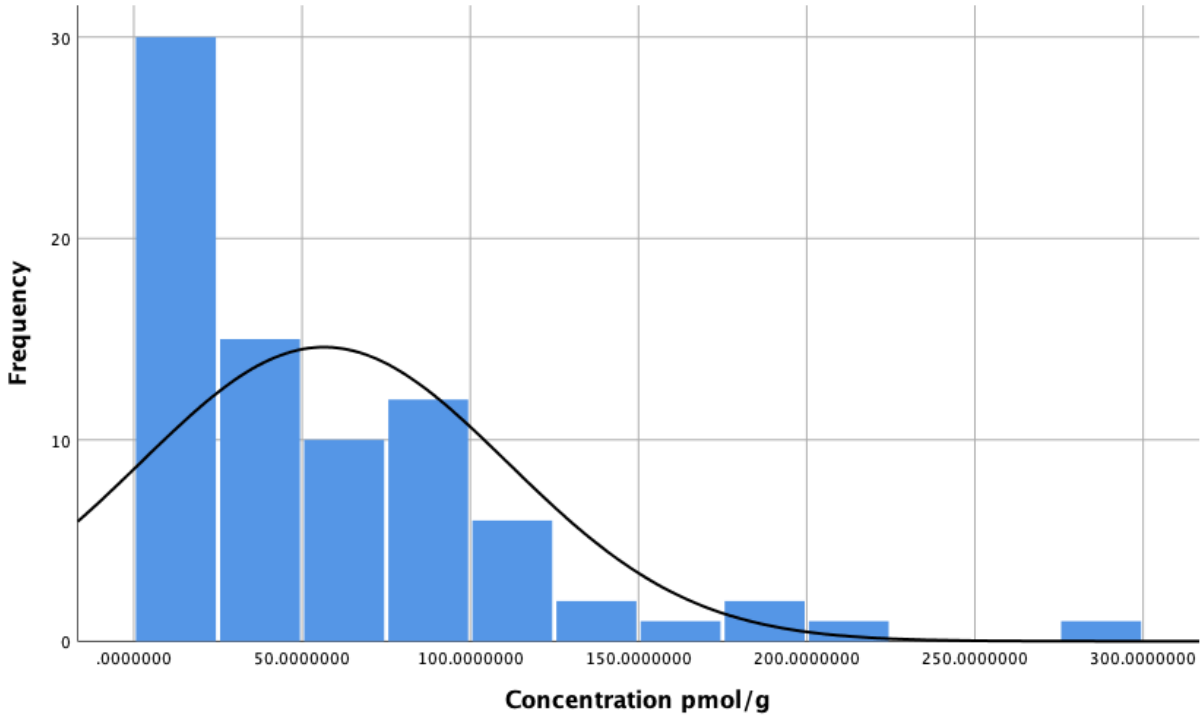


**Figure E.15** Histogram of plasma PCB-180 concentration distributions.

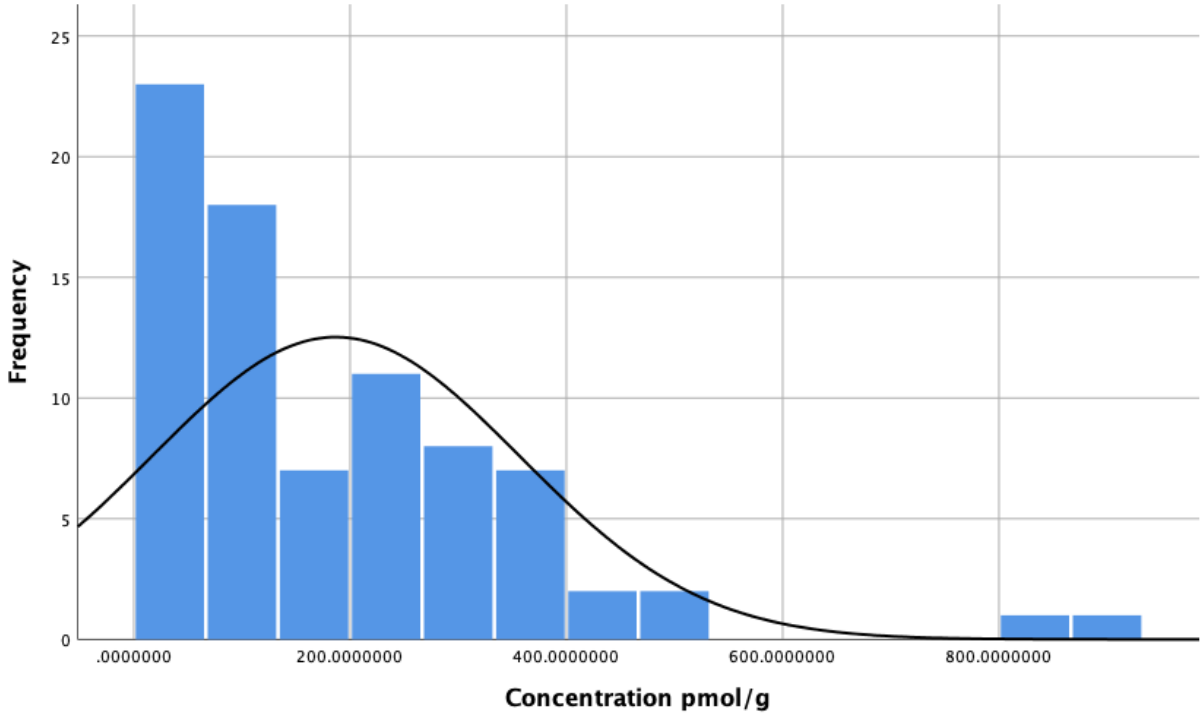


**Figure E.16** Histogram of plasma ∑PCB concentration distributions.

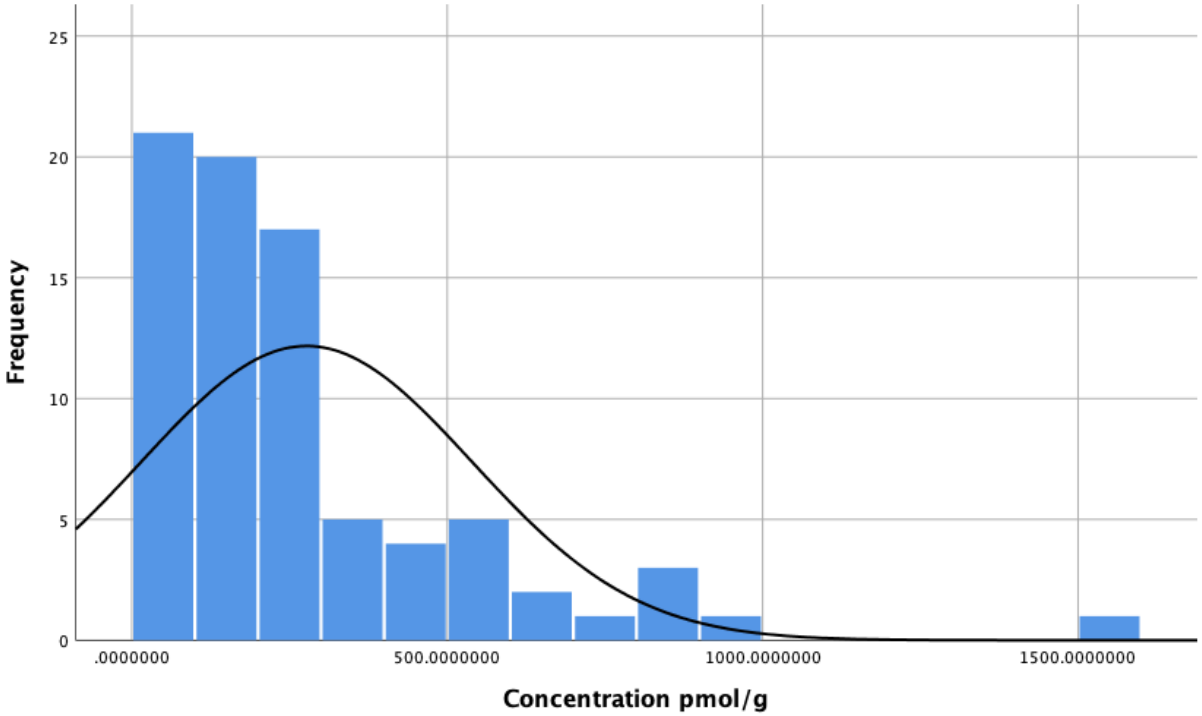


**Figure E.17** Histogram of plasma p,p'-DDE concentration distributions.