

**THE EFFECTS OF EARLY DEVELOPMENTAL EXPOSURE TO 3,3',4,4',5-  
PENTACHLOROBIPHENYL (PCB 126) ON DEVELOPING MINK (*NEOVISON*  
*VISON*) AND DOMESTIC CHICK (*GALLUS DOMESTICUS*) BRAINS**

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## **ABSTRACT**

Polychlorinated biphenyls (PCBs) are environmental toxicants that bioaccumulate in the body. PCB 126 is the most toxic PCB congener due to its ability to mimic the effects of dioxins. Here, I examined the effects of early developmental PCB 126 exposure in two animal models. In the first experiment, American mink kits were exposed prenatally and postnatally to PCB 126. The effects of early exposure were examined in the hippocampus, neocortex and corpus callosum. PCB 126 exposure had no effect on the hippocampus, but decreased volume of both the neocortex and corpus callosum. In the second experiment, chick eggs were injected with PCB 126 and the effects of embryonic exposure in the brain were examined in the telencephalon and hippocampus. Overall, I found no effects of PCB 126 on the chick brain. Taken together, my two experiments suggest PCB 126 has effects on the brain, but species sensitivity may differ.

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## LIST OF ABBREVIATIONS

µm –	micrometers
AHR –	aryl hydrocarbon receptor
ANOVA –	analysis of variance
CA –	cornu ammonis
CE –	coefficient of error
DF –	degrees of freedom
DG –	dentate gyrus
DNA –	deoxyribonucleic acid
EC –	entorhinal cortex
ED –	embryonic day
g –	grams
HAH –	halogenated aromatic hydrocarbon
HF –	hippocampal formation
Hp –	hippocampus
IQ –	intelligence quotient
LTP –	long term potentiation
MSU –	Michigan State University
PBS –	phosphate buffered saline
PCB –	polychlorinated biphenyl
PCB 126 –	3,3',4,4',5-pentachlorobiphenyl
PCDF –	polychlorinated dibenzofurans
PFA –	paraformaldehyde
REP –	relative effective potency
T4 –	thyroxine
TCDD –	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TEF –	toxic equivalency factor
TEQ –	toxic equivalency quotient
TSH –	thyroid stimulating hormone

## CHAPTER ONE: GENERAL INTRODUCTION

Polychlorinated biphenyls (PCBs) are environmental pollutants arising from decades of industrial use in coolants, paints, inks, caulking compounds and lubricants. Although PCBs were banned in Canada in the 1977, detectable levels of PCBs persist in the environment across North America (Kucewicz, 2004). PCBs are chemically inert, hydrophobic compounds with a half-life of up to 15 years so they are not easily biodegraded in the environment or metabolized in the body (Van den Berg et al., 2006). Due to the lipophilic and hydrophobic properties of PCBs, they accumulate in fatty tissue and biomagnify through the food chain as PCB-contaminated organisms are consumed. As a result, PCB concentrations are higher within the body than they are in the surrounding environment (Domingo & Bocio, 2007). The most common source of human exposure to PCBs is ingestion of fish and other PCB-contaminated foods, which causes a bioaccumulation of PCBs in fatty body tissue over time (Domingo & Bocio, 2007).

PCBs is a blanket term for 209 different congeners. All PCB congeners are composed of two benzene rings (Figure 1), but the congeners vary depending on the number of chlorines attached to the rings and the position of the chlorine atoms on the benzene rings (L. G. Hansen, 1998). The difference in chlorine number and positions makes some PCBs more toxic than others. The most toxicologically potent PCB congeners are the 12 dioxin-like PCB compounds. These compounds are coplanar halogenated aromatic hydrocarbons (HAHs). The HAH category of toxicants also includes 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is the most toxic dioxin and one of the most toxic exogenous chemicals in the world (Birnbaum, 1995). The potencies of dioxin-like PCBs are ranked using a Toxic Equivalency Factor (TEF) based on their

Relative Effective Potency (REP) as compared to TCDD (TEF = 1) (Consonni, Sindaco, & Bertazzi, 2012). The PCB congener with the highest REP (greater than 0.1) and highest TEF (0.1) is 3,3',4,4',5-pentachlorobiphenyl (PCB 126) (Van den Berg et al., 2006). Due to their high toxicity, both TCDD and PCB 126 are classified as group 1 human carcinogens (IARC, 2012).

PCB 126 is found in significant concentrations in fish collected from PCB-contaminated environments and consumption of contaminated fish leads to bioaccumulation (Bursian et al., 2006). In humans, detectable levels of PCBs are found from consumption of poultry, fish, beef, oils and fats, dairy products, eggs, bread and cereals, although the highest levels are in fish (Alcock, Behnisch, Jones, & Hagenmaier, 1998). Assessing the level of dioxin-like compounds is done by calculating a toxic equivalency quotient (TEQ); a TEF-weighted sum of lipid-adjusted concentrations of a compound (Consonni et al., 2012). In Canada and the US, PCB levels in seafood from retail markets have a TEQ ranging from 0.06 in shrimp to 0.92 in salmon (Domingo & Bocio, 2007). A meta-analysis of human TEQ levels from 1997 to 2010 found a positive correlation between age and human TEQ levels, which supports the bioaccumulation hypothesis of PCB-exposure (Consonni et al., 2012). That is, the more PCB-contaminated foods that are consumed over time, the higher the levels of PCBs in the body. While TCDD and other dioxin-like compound concentrations have declined in the general population over the last decade, PCB 126 concentrations (pg/g lipid) are the highest of any dioxin-like PCBs and levels have persisted since 1997 (Consonni et al., 2012).

In moderate to high concentrations, PCB exposure can result in reduced survival and fecundity in animal populations. For example, dietary administration of moderate to high concentrations of PCB 126 caused complete reproductive failure in American mink (*Neovison vison*) (Bursian et al., 2006). In other carnivorous mammals, such as red foxes (*Vulpes vulpes*) and wolves (*Canis lupus*), researchers found detectable levels of up to 15 different PCB congeners with concentrations varying depending on the sample area (Corsolini, Burrini, Focardi, & Lovari, 2000; Shore et al., 2001). In avian species, annual survival of adult tree swallows (*Tachycineta bicolor*) was reduced in areas of the Housatonic River (Massachusetts) with higher PCB contaminants, including PCB 126 (Custer, Custer, & Hines, 2012). Detectable levels of at least 11 non-dioxin PCBs were found in twelve species of wild bird eggs in levels indicating possible negative health effects for both insectivorous and scavenging birds (Quinn et al., 2013). Researchers expect that levels of dioxin-like PCBs will be found at appreciable levels in these species (Quinn et al., 2013).

Other physiological and anatomical effects of PCB 126 exposure have been characterized throughout the body. In rats (*Rattus norvegicus*), PCB 126 exposure increased heart weight, serum cholesterol and blood pressure, which put the rodents at higher risk for cardiovascular complications (Lind, Orberg, Edlund, Sjoblom, & Lind, 2004). Additionally, rates of cardiomyopathy and arteritis (inflammation of artery walls) in the heart increased with higher doses of PCB 126 (Jokinen et al., 2003). The same study also correlated chronic active arterial wall inflammation in the pancreas, rectum, liver, ovaries and uterus with higher doses of PCB 126. These studies support the results of human studies of cardiovascular disease and PCB exposure. Native American

populations with high lipid PCB levels had higher rates of cardiovascular disease (Goncharov et al., 2008). Similarly, high PCB and dioxin levels in human populations are correlated with increased mortality due to ischemic heart disease and cardiovascular disease (Humblet, Birnbaum, Rimm, Mittleman, & Hauser, 2008). The bones of rats exposed to PCBs tend to be lower in collagen concentration and have an altered bone composition (Lind et al., 2000). Additionally, PCBs impaired bone strength, increased bone fragility and decreased femur growth and cortical bone thickness (Cocchi et al., 2009). In the pancreas and liver, researchers have observed arteritis, cellular atrophy, carcinoma and increases in tumor promoting cells after PCB 126 exposure (Nyska et al., 2004; Vondracek et al., 2005). Finally, PCB exposure in human populations is associated with both effects related to immunosuppression, such as an increase in middle ear infections in children, and increased incidences of certain cancers, such as stomach and lung cancer (Crinnion, 2011; Pavuk et al., 2004).

The effects of dioxin-like PCB exposure are likely a result of the action of PCBs on the aryl hydrocarbon receptor (AHR). Second to TCDD, PCB 126 has the highest affinity for the AHR (Hahn, 2002). AHRs are widely distributed throughout the body and are part of a family of transcription factors referred to as ‘biological sensors’ (White & Birnbaum, 2009). Although its function is not entirely clear, the AHR plays some role in development, aging, hypoxia and circadian rhythms (White & Birnbaum, 2009). AHR signaling pathways can be activated without the presence of exogenous ligands by a number of relatively weak, low affinity, endogenous ligands (Denison & Nagy, 2003). Tryptophan and its metabolites are able to bind to and activate the AHR and may play a role in circadian rhythm activation by acting as a chemical messenger (Denison & Nagy,

2003). Other endogenous AHR activators include heme metabolites, arachidonic acid metabolites, some prostaglandins and several carotenoids, but none have a binding affinity comparable to the high affinity of TCDDs and dioxin-like PCBs (Denison & Nagy, 2003).

The AHR is thought to be responsible for almost all of the effects of dioxins and dioxin-like compounds, including PCB 126 (Hahn, 2002). Binding by exogenous chemicals to the AHR causes the receptor to undergo a conformational change and translocate into the nucleus. Through the release of ligands from the AHR complex, the AHR develops a high affinity for DNA binding. This new complex can bind to DNA recognition sites and stimulate gene transcription (Denison & Nagy, 2003). Most of the effects of dioxins and dioxin-like compounds are a result of alteration of gene expression and “continuous and inappropriate expression of specific genes” in susceptible cells (Denison and Nagy, 2003, pp. 311). These effects are strongest for exogenous chemicals that have a high molecular affinity for the AHR, such as TCDD and PCB 126 (Hankinson, 1995).

Binding of PCB 126 to the AHR causes changes in gene expression, which can result in changes in cell proliferation and cell signaling throughout the body and brain (Carlson & Perdew, 2002). Likely through changes in gene expression, one clear effect of PCB 126 exposure is that it increases cell growth and proliferation in peripheral areas of the body. Exposure to TCDD and PCB 126 causes tumour growth and enhanced cell proliferation in peripheral organs such as the liver, heart, and ovaries (Mandal, 2005). Wild mink exposed to PCB 126 and TCDD exhibited increased cell proliferation and epithelial lesions in the mandibles and maxillae of their jaw tissue (Beckett et al., 2005).

Additionally, PCB 126 exposure increased incidences of gingivitis and squamous cell carcinoma in rats (Yoshizawa et al., 2005). In humans, increased incidences of certain cancers following dioxin exposure may be a result of these changes to cell proliferation (Pavuk et al., 2004). Studies of populations exposed to dioxins reveal increased risk for carcinomas, altered growth factor signaling, and skin, tooth and nail abnormalities (White & Birnbaum, 2009).

Despite these widespread effects across a range of organ systems, the effects of PCB 126 and dioxins on the brain are not as clear despite evidence of AHRs in almost every region of the brain. AHRs are found in several regions including the olfactory bulb, basal ganglia, hypothalamus, hippocampus, neocortex, cerebellum and substantia nigra (Huang, Rannug, Ahlbom, Hakansson, & Ceccatelli, 2000). Presence of AHRs throughout the brain strongly suggests that PCBs will affect the brain in the same way they affect other organ systems. For example, dioxin exposure stimulates calcium ion uptake in hippocampal neurons and this was likely due to altered gene transcription (Hanneman et al., 1996). In fact, a variety of brain-related changes may be related to disturbances in transcription factors after AHR binding, which alter calcium signaling pathways, endocrine receptor binding and can mediate changes in the brain, such as alterations in neuronal branching pattern, spine density and receptor activity (Basha, Braddy, Zawia, & Kodavanti, 2006). In the hippocampus, PCB 126 exposure resulted in changes to cholinergic nicotinic receptors affinity in the hippocampus and manifested as behavioural deficits in spontaneous behaviour, and learning and memory functions (Eriksson & Fredriksson, 1998).

AHRs and AHR metabolites are found in every area of the brain, but the overall effects of the AHR activation on specific brain areas and the consequences for the anatomy and/or functions of these areas are not known (Huang et al., 2000). Evidence suggests that the AHR pathway interacts with estrogen receptors, androgen receptors and thyroid hormone receptors pathways (Carlson & Perdew, 2002). Therefore, alteration of transcription by AHR activation can cause endocrine disruption of sex steroids and thyroid hormones, which are important for proper brain development (Mandal, 2005). Dioxin exposure also induces AHR-mediated oxidative stress in the brain and liver (Hassoun, Li, Abushaban, & Stohs, 2000). Oxidative stress during development can cause a variety of issues in the brain including modification of gene expression and cell signaling, and disruption of normal neuronal development via alterations to the cell cycle (Dennerly, 2007). PCB-induced changes in the brain may be a result of altered gene transcription, endocrine disruption, oxidative stress, or a combination of these and other factors. Understanding how PCBs affect the brain is an important step in understanding the mechanism of action by which PCBs exert their toxic effects.

Assessing neurobehavioural deficits related to PCB 126 exposure is important and necessary because it allows researchers to correlate potential changes in the brain with altered reproductive behaviours, maternal behaviours or social behaviours, which may decrease survival and fecundity in wild populations. Particularly during development, the brain is extremely sensitive to toxic effects, which can permanently alter the brain and, consequently, behaviour (Bushnell et al., 2002). Furthermore, behavioural and neural measurements are far more sensitive to the toxic effects of contaminants than more commonly used methods in toxicology, such as mortality, fecundity, carcinogenicity and



endocrine assays (Ottinger et al., 2002). By assessing what changes occur in the brain following exposure, we can also extrapolate to how behaviour and long-term survival may be affected. With respect to PCB 126, exposure to it is associated with hyperactivity, decreased attention, impaired executive functions, lower IQ and deficits in learning and memory, but most of the neuroanatomical changes behind these life-altering deficits are unknown (Boucher, Muckle, & Bastien, 2009; Jacobson & Jacobson, 1996; Ten Tusscher et al., 2014; Vitalone et al., 2010; Winneke et al., 2014). In wild populations, these deficits in learning, memory and social behaviours may indirectly impair survival and reproduction. Similarly, in human populations, behavioural deficits can greatly reduce quality of life while increased risk for cancer and heart problems and decreased immune system can cause life-long health issues for individuals and place an increased burden on the health care system.

Although rodent models are often used to determine neurobehavioural effects of toxicants, such as PCB 126, rats and mice are not necessarily the best model for toxicological studies. An important component of assessing the effects of dioxins is extrapolating how exposure affects wild populations. Rodent models are lab-bred for generations and have no wild counterparts in North America. Additionally, many toxicological assessments, including PCB 126, focus on exposure through ingestion. The rat gastrointestinal system is physiologically and chemically different from the human gastrointestinal system, so comparing exposure effects through ingestion and metabolism is difficult (Kararli, 1995). Finally, due to the importance of biomagnification and bioaccumulation in assessing PCB-levels and exposure effects, carnivorous animal

models are often better toxicological indicators and can act as sentinel species for studying environmental toxicant exposure (Basu et al., 2007).

One alternative to typical rodent models is the American mink (*Neovison vison*). For many toxicological studies, mink offer several advantages over rodent models. For example, they have a digestive tract that is more similar in length and chemical physiology to the human digestive tract (Basu et al., 2007), so they metabolize and respond to dioxins in a similar way. Mink can be raised in a laboratory environment where oral doses of PCB 126 food contaminants can be controlled and delivered. Additionally, mink have a short gestation period and a short weaning period, so the effects of prenatal PCB exposure on development are relatively easy to study. Finally, from an environmental toxicology perspective, mink are one of the most widely distributed carnivores in North America and the collection of toxicological information allows them to be used as a biological indicator of toxicant levels in the environment (Basu et al., 2007).

Avian models also provide several advantages over rodent models as well as other mammalian models. The biggest advantage to avian models is that chemicals can be administered directly to the embryo without confounding maternal effects (Slotkin, Seidler, Ryde, & Yanai, 2008). A precise dose can be injected directly into each egg without worrying about litter effects or intrauterine position. The incubation period for chicks is comparable to gestational periods in rats, but the rate of development in chicks is faster so assessing neurodevelopment in chicks immediately after hatching is comparable to neurodevelopment in 2-week old rats (Slotkin et al., 2008). Additionally, the developmental trajectory of the avian brain, especially in species such as chickens

(*Gallus domesticus*), is well characterized, so any alterations in the brain are easy to compare with controls and quantify (Bellairs & Osmond, 2005). Avian models to study the developmental toxicity of PCB 126 have already been utilized in chickens, American kestrels (*Falco sparverius*), and common tern (*Sterna hirundo*). These species exhibited malformations, beak defects, changes in embryonic growth, and edema in hatchlings injected with PCB 126 during incubation (Hoffman, Melancon, Klein, Eisemann, & Spann, 1997), but neuroanatomical effects have yet to be assessed. By using an avian model to study changes in the brain related to PCB 126 exposure, I will be able to better assess the effects of developmental exposure to PCB 126 without the confounds of maternal metabolism and intrauterine position. Additionally, a second species model will allow me to determine whether neuroanatomical changes arising from PCB 126 exposure are similar across species.

Avian and mammalian brains are functionally and neuroanatomically similar in many ways, which allows for comparisons of the effects of PCB 126 on two different types of vertebrate brains. In both mammals and birds, the hippocampus has an important behavioural role in spatial memory (Broadbent, Squire, & Clark, 2004; Lee, Miyasato, & Clayton, 1998; Sherry, Jacobs, & Gaulin, 1992) and damage to the hippocampus produces similar spatial memory impairments in mammals and birds (Bingman & Mench, 1990; Broadbent et al., 2004; Colombo & Broadbent, 2000). Developmentally, the hippocampus arises from the same region of the telencephalon in the avian and mammalian brain (Colombo & Broadbent, 2000). Still, despite the similar behavioural functions and similar embryological origins, determining the subdivisions of the avian hippocampus that correspond to the mammalian is complicated, as the organization of the

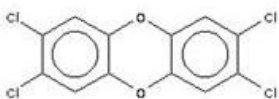
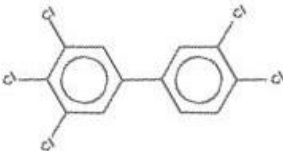
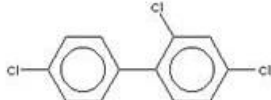
avian hippocampus is quite different. The avian hippocampus is divided into the ventromedial V-complex, the dorsomedial region and the dorso-lateral region, but which areas of these correspond to the major regions of the mammalian hippocampus including the dentate gyrus and the CA regions is still debated (Herold et al., 2014).

Neurotransmitter labelling, receptor autoradiography, Golgi staining of major hippocampal neurons, cell type analysis, studies of connectivity and zinc staining have revealed many similarities between the avian and mammalian hippocampus and allowed researchers to propose several models comparing regions of the avian and mammalian hippocampus (Erichsen, Bingman, & Krebs, 1991; Herold et al., 2014; Krebs, Erichsen, & Bingman, 1991; Tömböl, Davies, Németh, Sebestény, & Alpár, 2000). Most recent compilations of evidence proposes that the dentate gyrus and CA1 regions of the mammalian hippocampus corresponds to the ventromedial complex and the dorsal portion of the dorsomedial region. Meanwhile the mammalian CA2/CA3 regions correspond to the ventral dorsomedial region and the dorso-lateral region corresponds to the mammalian entorhinal cortex (Figure 1.2) (Herold et al., 2014). Overall, the evidence suggests that avian and mammalian hippocampi are functionally homologous, even if the subdivisions of the avian hippocampus are not completely delineated.

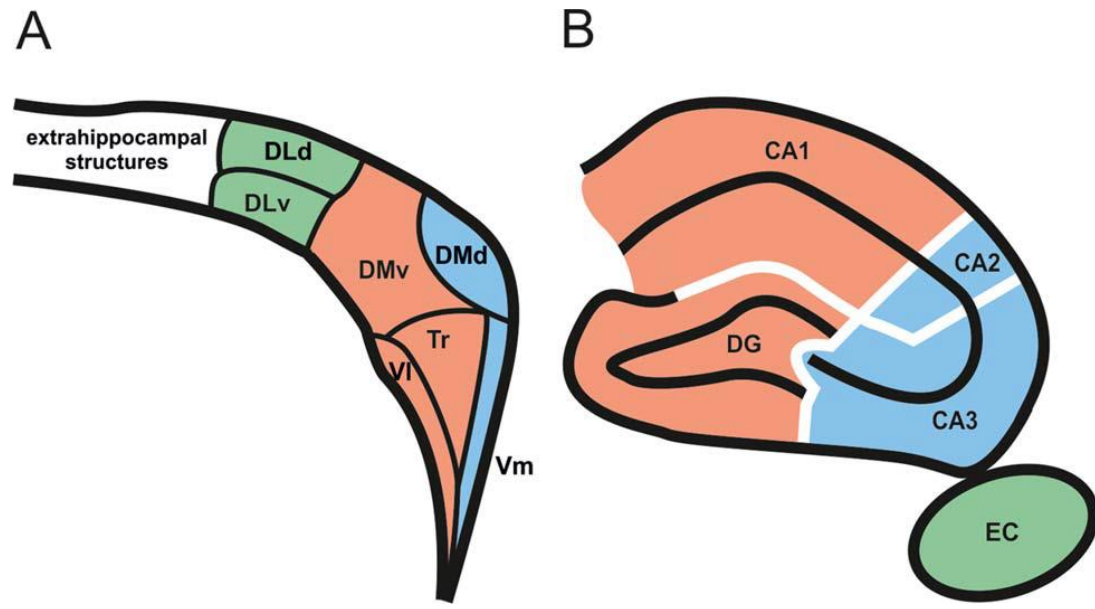
Much is known about how PCBs affect avian and mammalian bodies and behaviour, but little is known about how PCBs affect the brain. Despite their ban, PCBs still bioaccumulate at a high rate, especially in wildlife and human populations in highly contaminated areas. Understanding how PCBs affect the brain and whether these effects are different in mammals and birds helps to inform public policy on acceptable levels of PCBs in the body, in the environment and in foods. The following studies attempt to

understand some of the effects of PCBs by examining the effects of the most potent dioxin-like PCB, PCB 126 on the brain. The main objective of these studies was to begin to characterize the effects of dioxin-like PCBs on the brain. This is accomplished using two contrasting animal models, American mink as a mammalian animal model and domestic chickens as an avian mammal model.

## CHAPTER ONE FIGURES

2,3,7,8-Tetrachlorodibenzo- p-dioxin (TCDD)	3,3',4,4',5-Pentachlorobiphenyl (PCB 126) Non- <i>ortho</i> -substituted coplanar dioxin-like	2,4,4'-Trichlorobiphenyl (PCB 28) <i>Ortho</i> -substituted, non- coplanar, non-dioxin-like
		

**Figure 1.1** Chemical structures of TCDD, PCB 126 (a dioxin-like PCB) and PCB 28 (a non-dioxin-like PCB). Dioxin-like PCBs have chlorines in at least four lateral positions and none or one in the *ortho* positions and are therefore referred to as coplanar. This structure allows them to mimic the toxic responses of TCDD. Non-dioxin-like PCBs do not mimic TCDD, may have 2 to 10 chlorine atoms attached in several *ortho* positions (Henry & De Vito, 2003).



**Figure 1.2** Proposed subdivisions of the avian hippocampus from Herold et al., 2014 (A) and mammalian hippocampus (B) based on shared receptor densities. The same colours indicate overlap in receptor densities and give some clue of how the two may relate structurally and functionally. The avian hippocampus consists of the V-complex with ventrolateral (V1) and ventromedial (VM) cell bands, an inner triangular region (Tr), the dorsomedial region consisting of ventral (DMv) and dorsal (DMd) subdivisions, and the dorsolateral region consisting of ventral (DLv) and dorsal (DLd) subdivisions. In this figure, the mammalian hippocampus consists of the dentate gyrus (DG), pyramidal cell layers CA1, CA2 and CA3 and the entorhinal cortex (EC). From Herold, C., et al. (2014).

"Distribution of neurotransmitter receptors and zinc in the pigeon (*Columba livia*)

hippocampal formation: A basis for further comparison with the mammalian

hippocampus." *J Comp Neurol* **522**(11): 2553-2575.

## **CHAPTER TWO: THE EFFECTS OF DEVELOPMENTAL PCB 126 EXPOSURE ON THE AMERICAN MINK (*NEOVISON VISON*) BRAIN**

### **Introduction**

Polychlorinated biphenyls (PCBs) are environmental pollutants that were synthesized for a variety of uses including industrial coolants and lubricants from the early 1930s to the late 1970s. Despite a ban on PCBs in North America in the 1970s, chemical stability and a long half-life combined with the hydrophobic properties of PCBs have allowed them to persist in the environment (Grassman, Masten, Walker, & Lucier, 1998; Van den Berg et al., 2006). Furthermore, the hydrophobic and lipophilic properties of PCBs facilitate bioaccumulation in fatty tissue and biomagnification through the food chain resulting in a high burden of PCBs in the body, especially for species that consume large quantities of PCB contaminated foods, such as fish and other seafood (Domingo & Bocio, 2007). Given the classification of PCBs as a carcinogen and an endocrine disruptor, PCB exposure is an issue for both wild populations of birds and mammals in PCB contaminated areas as well as human populations in contaminated areas where consumption of fish and other PCB contaminated foods is common (Kimbrough & Krouskas, 2003).

All PCBs consist of a biphenyl molecule comprised of two benzene rings, but vary based on the number of chlorine atoms and the position of chlorine atoms attached to the rings (Crinnion, 2011). As a result, there are 209 different PCB congeners which fall into two main categories: highly toxic, coplanar, non-ortho substituted, dioxin-like PCBs or less toxic, non-coplanar, ortho substituted, non-dioxin-like PCBs (Carpenter, 2006). The mechanism of action for the toxic effects of dioxin-like versus non-dioxin like



PCBs is different; dioxin-like PCBs act via a specific pathway, whereas non-dioxin-PCBs act via multiple independent pathways (Henry & De Vito, 2003). The dioxin-like PCBs share a similar structure to the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) (Figure 1.1), and like TCDD, their effects are mediated through the activation of the aryl hydrocarbon receptor (AHR) (Mandal, 2005). The AHR is distributed throughout the body and brain where binding by exogenous toxicants alters transcriptional processes and stimulates a cellular response, such as cell growth (Hahn, 2002; Mandal, 2005). Of the 12 dioxin-like PCBs, PCB 126 has the highest AHR binding affinity, therefore making it the most potent PCB congener and a useful congener to study the health effects of dioxin-like PCB exposure (W. Zhang et al., 2012).

A variety of health effects related to PCB contamination have been documented in both animal and human populations, with many of the effects similar across species. In non-human mammals, PCB exposure is associated with decreased reproductive success, higher offspring mortality, decreased growth hormone expression, altered bone density, higher white blood cell and lymphocyte counts, cardiovascular malformations and higher incidences of cardiovascular disease, altered pancreatic and liver functions, increased cell proliferation leading to lesions and carcinomas in oral and endothelial tissues, increased body weight, altered social behaviour, impaired learning and memory and hyperactivity (Aulerich & Ringer, 1977; Beckett et al., 2005; Beckett, Yamini, & Bursian, 2008; Cocchi et al., 2009; Heaton et al., 1995; Jokinen et al., 2003; Jolous-Jamshidi, Cromwell, McFarland, & Meserve, 2010; Lind et al., 2004; Nyska et al., 2004; Vitalone et al., 2010; Vondracek et al., 2005; Yoshizawa et al., 2005). In humans, acute exposure leads to chloroacne (Yusho Disease), which results in endothelial lesions and epidermal cysts

(Aoki, 2001). Chronic exposure, however, is associated with lowered testosterone in males, altered thyroid hormone levels, increased incidence of cardiovascular disease, increased risk of stroke, elevated blood pressure, altered immune function and higher incidences of various cancers (Bergkvist et al., 2014; Goncharov et al., 2008; Goncharov, Pavuk, Foushee, Carpenter, & Anniston Environm Hlth Res, 2011; Goncharov et al., 2009; Pavuk et al., 2004; Schell et al., 2008). Much like the behavioural effects demonstrated in animal models, in humans, prenatal and perinatal exposure *in utero* and through breastmilk is associated with poor learning and memory, hyperactivity and impaired cognitive development in offspring (Boucher et al., 2009; Boucher et al., 2014; Schantz, Widholm, & Rice, 2003).

Despite extensive documentation of the health effects of PCB exposure in a variety of animal models and humans, the literature on neurological consequences of PCB exposure is much more limited. Documented effects of early PCB exposure on general cognitive abilities, verbal and spatial skills, memory, and attentional processes make it clear that the brain is affected, but little direct evidence of changes in the brain exists (Boucher et al., 2009; Bushnell et al., 2002; Curran et al., 2011; Eriksson & Fredriksson, 1998; Huisman et al., 1995; Ten Tusscher et al., 2014). Some evidence suggests that dioxin-like PCBs disrupt calcium homeostasis and signaling pathways in the brain (especially the hippocampus) and alter transcription via the AHR, somehow leading to long term neurological alterations (Basha et al., 2006; Koclavanti, 2004). In the hippocampus, these calcium alterations appear to contribute to disruptions in long-term potentiation (LTP) and reduced synaptic transmission in the CA1 and CA3 subfields (Niemi, Audi, Bush, & Carpenter, 1998; Ozcan, Yilmaz, King, & Carpenter, 2004). In

addition, PCB exposure can alter neurotransmitter receptor densities (Boix, Cauli, & Felipo, 2010). For instance, exposure to PCB 126 causes a decrease in the density of nicotinic acetylcholine receptors in the hippocampus and neocortex (Eriksson & Fredriksson, 1998). Disruption of thyroid and sex-steroid hormones by PCBs may alter cellular proliferation, synaptogenesis, apoptosis, and overall cell size and numbers in the cerebral cortex and the hippocampus (Parent, Naveau, Gerard, Bourguignon, & Westbrook, 2011; Tofighi et al., 2011).

Given its role in learning and memory and previous evidence that hippocampal LTP, receptors and signaling is affected, I investigated the effects of prenatal and perinatal PCB 126 exposure on hippocampal and neocortical volume, cell numbers and cell morphology (Basha et al., 2006; Curran et al., 2011; Hanneman et al., 1996; K. H. Kim & Pessah, 2011; Niemi et al., 1998; Ozcan et al., 2004; Tofighi et al., 2011). I chose PCB 126 because it is the most potent dioxin-like PCB and there has been no significant decrease in PCB 126 levels in humans over the last two decades, so it continues to be a potential public health concern (Consonni et al., 2012). Although rodents are often used to examine the toxicological effects of PCBs, this study focused on American mink (*Neovison vison*) as an animal model. American mink in the wild were one of the first documented examples of negative reproductive and survival consequences arising from PCB exposure and they have since become a sentinel species for environmental health (Aulerich & Ringer, 1977; Basu et al., 2007). Mink are carnivores that feed primarily on fish and, as a result, they are at a higher risk for the effects of PCB bioaccumulation than many other native species that can be examined in the lab (Basu et al., 2007; Giesy et al., 1994). Furthermore, mink have a digestive physiology that is more similar to humans

than that of rats, so ingested toxicants enter and accumulate in the body in a more similar manner to humans (Basu et al., 2007). Previous work shows that mink exposed to TCDD and PCB 126 show hematological and histological alterations in the liver, spleen and lungs. They also present with squamous epithelial jaw lesions, reduced growth, increased organ weights, anorexia, altered thyroid hormones and impaired reproduction (Basu et al., 2007; Beckett et al., 2008; Heaton et al., 1995).

Based on this evidence and the advantages of mink as an animal model for toxicant exposure, I tested the effects of prenatal and perinatal PCB 126 exposure on brain development, specifically focusing on the neocortex and the hippocampus. I chose to examine these two structures because learning and memory, attention and social activity are related to one or both of these structures (Gould, Reeves, Graziano, & Gross, 1999; Kaas, 1987). Furthermore, both of these structures are highly plastic during development and are prone to effects of the environment, including toxicants and early life stress (Andersen & Teicher, 2004; Birnbaum, 1995; Parent et al., 2011; Rice & Barone Jr, 2000). I hypothesized that if PCB 126 is acting like a dioxin by activating the AHR and disrupting various endocrine processes then I would see changes in the hippocampus and neocortex of mink kits exposed during development. Here, I specifically test these hypotheses in lab-reared American mink kits.

## **Materials and Methods**

### *Animals*

Female American mink were housed at the Michigan State University experimental fur farm. All procedures were approved by the Michigan State University

Institutional Animal Care and Use Committee and proper husbandry practices were followed to ensure animal welfare (Fur Commission USA, 2010). Females in their second or third year, which had successfully produced litters before, were housed in individual wire enclosures with front nesting boxes lined with aspen shavings. Females for this study were randomly assigned to either PCB 126 or control groups. Mink were fed once a day and received water ad libitum. PCB 126 contaminated food was administered throughout pregnancy and lactation to the treatment group at 0.9 ng PCB 126/g feed wet weight until kits were six weeks of age. The mink used in my study were used as a positive control for a larger mink study at MSU. This dose was chosen because it was previously identified as a low dose that causes detrimental changes in peripheral organs and tissue but is not lethal to the mink (Render, Aulerich, Bursian, & Nachreiner, 2000). These changes were also observed in mink captured from contaminated areas in the wild, so the dose is environmentally relevant for populations in contaminated areas (Bursian et al., 2006). In the Great Lakes, levels of PCBs detected in fish range from 21 – 50 ng/g wet weight, so the chosen dose 0.9 ng/g was much lower than levels in fish in the highly contaminated Great Lakes (Bhavsar et al., 2007). All food was prepared following guidelines for the nutritional requirements of mink (National Research Council, 1982). Diets consisted of a chicken-substituted food consisting of 38% water, 20% whole ground chicken, 16% GNF20 cereal mix (National Feeds), 6% spray-dried poultry liver (Van Elderen), 6% spray-dried egg (Van Elderen), 6% spray-dried fish meal (menhaden), 4% cheese (Michigan State University Dairy), 2% soybean oil (North American Nutrition), 2% spray dried blood protein (APC), and <1% vitamin and mineral mix (Akey), sodium bisulfate (Jones-Hamilton), biotin (Akey), and larvadex (Novartis). Fish

meal was kept to a minimum and substituted with chicken in these diets to limit the potential of outside contaminants in the food. PCB 126 (Accustandard, Lot # 082504MS-AC) was dissolved in soybean oil and added to the treatment group food.

At six weeks postnatal, after weaning, mink kits were anesthetized with an intramuscular injection of 1 mL/kg body weight ketamine (Veterinary Products) and euthanized by CO<sub>2</sub> asphyxiation. Brains were extracted and examined for gross abnormalities before immersion in 10% neutral buffered formalin in large centrifuge tubes (Corning Centristar) and shipped to the University of Lethbridge. Upon receipt, brains were weighed and switched to fresh PFA where they remained for 1-2 weeks until they were cryoprotected in 30% sucrose-PBS for 48 hours. Damage to some of the brains during the extraction and shipping processes necessitated the removal of the hindbrain including cerebella, and brainstem. As a result, the brain volumes used throughout this study consist of only forebrain (telencephalon and thalamus) and midbrain. The brains were coronally blocked rostral to the hippocampus. Each portion of the brain was embedded in gelatin and serially sectioned in the coronal plane at 40 µm on a freezing stage microtome. Free floating sections were collected in 0.1 M phosphate-buffered-saline with 0.01% sodium azide. Every 12<sup>th</sup> section (ie. every 480 µm) was mounted onto gelatinized slides, stained with thionin for Nissl substance and coverslipped with Permount. A separate series through the hippocampus was stained with green fluorescent Nissl (Neurotrace, Life Technologies, Cat# N21480) for granule cell counts.

#### *Volumetric Measurements*

The volume of the entire hippocampus (total Hp), dentate gyrus (DG) and cornu ammonis (CA) subfields were measured from every 12<sup>th</sup> section using the Cavalieri

method in StereoInvestigator (Microbrightfield, Williston, VT) on a Zeiss Axio Imager MT (Carl Zeiss, MicroImaging GmbH, Germany) with a 10x objective (Fig 2.1). Both left and right sides were measured simultaneously for all four measurements.

Total hippocampal volume included DG and all of the hippocampus proper and was measured on a 1000 x 1000  $\mu\text{m}$  grid. The coefficients of error (CE, Gundersen,  $m=1$ ) were all  $\leq 0.015$ . DG and CA volumes were measured with a 300 x 300  $\mu\text{m}$  grid. DG was measured from the entire granule cell layer and yielded a CE (Gundersen,  $m=1$ )  $\leq 0.021$ . The pyramidal layers of the CA subregions were easily distinguishable throughout the hippocampal sections. CA3 was measured separately from CA1 and CA2, but CA1 and CA2 were measured together as we were unable to reliably distinguish the borders between the two subfields. In contrast, CA3 is easily distinguishable by a marked change in pyramidal cell density (Figure 2.2),

The volumes of the brain (see above), neocortical and corpus callosum volumes were measured from digital photos of every 12<sup>th</sup> section using ImageJ (Rasband, Bethesda, MD: U.S. National Institutes of Health) (Figure 2.3). Total volumes for all measured brain areas were then calculated by multiplying the total area of the measured sections by the sampling interval (12) and the section thickness (0.04 mm).

#### *Cell Counts*

Cells were counted using the optical fractionator as implemented in StereoInvestigator (Microbrightfield, Williston, VT). Granule cells were quantified on every 12<sup>th</sup> section using fluorescent Nissl stained sections (Fig 2.4a) and a 400 x 400 micron counting grid. The dentate gyrus was outlined at 10x and cell counts were done at 40x with a counting frame of 15x15 microns placed on the grid (Fig 2.4b). Section

thickness was measured at every counting site. Granule cells were quantified based on their location within the dentate gyrus and visible nuclei (West & Gundersen, 1990).

Pyramidal cells were also quantified on every 12<sup>th</sup> section using brightfield Nissl stained sections and a 600 x 600 counting grid. The CA3 and CA1/2 regions were outlined at 5x and cell counts were done at 20x using a 31x31 micron counting frame. Section thickness was measured at every counting site. Pyramidal cells are easy to distinguish from other cells in the CA subregions based on their size, but were only counted if they exhibited the distinct fusiform shape and a visible nucleus (Fig 2.4c/d) (Slomianka, Amrein, Knuesel, Sorensen, & Wolfer, 2011). Coefficients of error (Gundersen, m=1) for all cell counts were  $\leq 0.09$ .

#### *Cell Sizes*

Average pyramidal and granule cell size was determined using the nucleator probe (Gundersen et al., 1988; Møller, Strange, & Gundersen, 1990) with five radial arms within the optical fractionator workflow as implemented in StereoInvestigator. Granule and pyramidal cell sizes were measured at the same time and subregions were traced with a 5x objective before switching to the 40x oil immersion lens for cell sizes. A 40x40 counting frame was used within a 600x600 grid on every 24<sup>th</sup> section. These sampling parameters allowed us to calculate an average cell size based on 100-150 cells sampled per cell type. Granule cells were only measured if they were intact, round and had a visible nucleolus. Cells with condensed chromatin and/or no visible nucleolus, which may have been pyknotic, were not included (Amrein, Slomianka, & Lipp, 2004). Pyramidal cells were only measured if they had the distinct triangular shape, a visible



nucleolus and at least one visible extension away from the cell body (Slomianka et al., 2011). Coefficients of error (Gundersen,  $m=1$ ) were all  $\leq 0.004$ .

### *Statistical analysis*

Statistical analysis was performed using a two-way analysis of variance (ANOVA) testing the effects of independent variables: treatment and sex and the interaction of the two on dependent variables: volumes of the total Hp, dentate gyrus, CA1/2 region, CA3 region, brain, neocortex and corpus callosum; total cell counts for DG granule cells and CA pyramidal cells; average cell areas for granule and pyramidal cells. I included sex as a variable to account for potential sex differences in brain volume, hippocampal volume and cell counts (Burger, Gulbrandsen, Saucier, & Iwaniuk, 2014; Burger, Saucier, Iwaniuk, & Saucier, 2013; Sawada, Horiuchi-Hirose, Saito, & Aoki, 2013; Spring, Lerch, & Henkelman, 2007).

Linear regression analysis was used to determine whether the size of each brain area was related to brain volume, minus the region of interest (Deacon, 1990). If a significant scaling relationship was detected, I then took the residuals of the regression line for the region of interest as a measurement of relative size. To ensure that allometric effects were removed, I then tested whether there was a significant correlation between the residuals and brain size. If no significant correlation was present (i.e., allometric effects were successfully removed), a two-way ANOVA was finally applied to the residuals to test for the effects of treatment, sex or the interaction on relative brain region volumes, when applicable.

## Results

### *Absolute Volumes*

Brain volume varies significantly by sex ( $F = 11.97$ ,  $df = 1, 12$ ,  $p = 0.005$ ), but not by treatment ( $F = 0.79$ ,  $df = 1, 12$ ,  $p = 0.78$ ) and no interaction was detected ( $F = 1.90$ ,  $df = 1, 12$ ,  $p = 0.19$ ) (Figure 2.5b). Specifically, brain volume was significantly larger in males than in females. Brain volume was not significantly related to body weight ( $F = 3.87$ ,  $df = 1, 14$ ,  $p = 0.07$ ,  $df = 1, 14$ ,  $r^2 = 0.22$ ).

Total hippocampal volume did not vary significantly between controls and PCB treatment groups ( $F = 0.11$ ,  $df = 1, 12$ ,  $p = 0.92$ ) or between males and females ( $F = 3.25$ ,  $df = 1, 12$ ,  $p = 0.097$ ) and there was no significant interaction effect ( $F = 0.08$ ,  $df = 1, 12$ ,  $p = 0.78$ ) (Fig 2.6a). Similarly, the DG did not vary by sex ( $F = 3.23$ ,  $df = 1, 12$ ,  $p = 0.10$ ) or treatment ( $F = 0.10$ ,  $df = 1, 12$ ,  $p = 0.75$ ) and no significant interaction was detected ( $F = 0.02$ ,  $df = 1, 12$ ,  $p = 0.89$ ) (Fig 2.6c). Hippocampal subfields CA1/2 also did not differ significantly by sex ( $F = 3.08$ ,  $df = 1, 12$ ,  $p = 0.11$ ) or treatment ( $F = 0.34$ ,  $df = 1, 12$ ,  $p = 0.57$ ) and showed no significant interaction ( $F = 0.46$ ,  $df = 1, 12$ ,  $p = 0.51$ ) (Fig 2.6e). Finally the CA3 subfield did not vary significantly by sex ( $F = 1.16$ ,  $df = 1, 12$ ,  $p = 0.30$ ) or treatment ( $F < 0.01$ ,  $df = 1, 12$ ,  $p = 0.98$ ) and no significant interaction was detected ( $F = 2.10$ ,  $df = 1, 12$ ,  $p = 0.17$ ) (Fig 2.6f).

Neocortex volume was significantly larger in males ( $F = 18.56$ ,  $df = 1, 12$ ,  $p = 0.001$ ) than in females and larger in the control group ( $F = 18.56$ ,  $df = 1, 12$ ,  $p = 0.008$ ) than in the PCB group (Fig 2.5c), however, there was no significant interaction effect ( $F = 1.54$ ,  $df = 1, 12$ ,  $p = 0.24$ ). Planned comparison t-tests within sex indicated that the neocortex of control males is significantly larger (13.69%) than PCB males ( $p = 0.011$ ),

but there is no significant difference in the neocortex of control females compared to PCB females ( $p = 0.18$ ). Within treatment groups, planned comparisons indicate that neocortical volume is significantly larger (8.77%) in control males than control females ( $p = 0.0008$ ), but PCB males do not differ significantly from PCB females ( $p = 0.07$ ).

Similarly, corpus callosum volume was also significantly larger in males ( $F = 12.55$ ,  $df = 1, 12$ ,  $p = 0.004$ ) than in females and in the control group ( $F = 13.71$ ,  $df = 1, 12$ ,  $p = 0.003$ ) than in the PCB group (Fig 2.5d), but there was no significant interaction effect ( $F = 0.16$ ,  $df = 1, 12$ ,  $p = 0.70$ ). Planned comparisons t-tests within each sex indicated that control males had significantly larger corpus callosum volumes than PCB males ( $p = 0.016$ ) and control females significantly larger corpus callosum volumes than PCB females ( $p = 0.03$ ) (Fig 2.5d). Corpus callosum volume was 13.07% smaller in the PCB treated, while female corpus callosum volume decreased by 11.87%. Planned comparisons within treatment indicated that control males differ significantly from control females ( $p = 0.008$ ), with a 12.53% larger corpus callosum volume, but PCB males do not differ significantly from PCB females (0.07).

### *Relative Volumes*

No significant relationship between neocortex and brain volume was detected ( $F = 3.19$ ,  $df = 1, 14$ ,  $p = 0.10$ ,  $r^2 = 0.19$ ). Similarly, no significant allometric relationship between corpus callosum volume and brain volume was detected ( $F = 0.89$ ,  $df = 1, 14$ ,  $p = 0.36$ ,  $r^2 = 0.06$ ). In contrast, there was a significant linear relationship between total Hp volume and brain volume ( $F = 11.64$ ,  $df = 1, 14$ ,  $p = 0.004$ ,  $r^2 = 0.45$ ) so hippocampus volume was regressed against brain volume minus hippocampus. The residuals of Hp volume (representing relative Hp volume) were tested using a two way ANOVA. There

was no significant effect of sex ( $F = 0.05$ ,  $df = 1, 12$ ,  $p = 0.82$ ) or treatment ( $F = 0.001$ ,  $df = 1, 12$ ,  $p = 0.97$ ) and no interaction ( $F = 1.42$ ,  $df = 1, 12$ ,  $p = 0.26$ ) on total relative hippocampal volume (Fig 2.6b). The same process was repeated with DG as it too had a significant relationship with brain volume ( $F = 8.04$ ,  $df = 1, 14$ ,  $p = 0.01$ ,  $r^2 = 0.36$ ). As with the absolute volume measurements, there was no significant effect of treatment ( $F = 0.05$ ,  $df = 1, 12$ ,  $p = 0.82$ ) or sex ( $F = 0.10$ ,  $df = 1, 12$ ,  $p = 0.75$ ) and no interaction ( $F = 0.71$ ,  $df = 1, 12$ ,  $p = 0.42$ ) on relative DG volume (Fig 2.6d).

### *Cell Counts*

Total estimated granule cell counts in the DG did not vary significantly by sex ( $F = 1.58$ ,  $df = 1, 12$ ,  $p = 0.24$ ) or treatment ( $F = 1.22$ ,  $df = 1, 12$ ,  $p = 0.29$ ) and there was no significant interaction ( $F = 0.01$ ,  $df = 1, 14$ ,  $p = 0.92$ ). There was a significant linear relationship between total brain volume (minus cerebellum) and granule cell count ( $F = 7.39$ ,  $df = 1, 13$ ,  $p = 0.02$ ,  $r^2 = 0.36$ ). To account for this relationship, the residuals of granule cells to represent relative cell counts were tested with a two-way ANOVA. The results remained the same, and there was no effect of treatment ( $F = 1.31$ ,  $df = 1, 12$ ,  $p = 0.28$ ) or sex ( $F = 0.0536$ ,  $df = 1, 12$ ,  $p = 0.82$ ) on granule cell count and no interaction effect ( $F = 0.31$ ,  $df = 1, 12$ ,  $p = 0.59$ ) (Fig 2.7a).

There was also no significant differences in the number of CA1/2 pyramidal cells between the sexes ( $F = 1.11$ ,  $df = 1, 12$ ,  $p = 0.32$ ) and treatments ( $F = 0.73$ ,  $df = 1, 12$ ,  $p = 0.41$ ) and no interaction ( $F = 0.38$ ,  $df = 1, 12$ ,  $p = 0.55$ ) nor was there a difference in number of CA3 pyramidal cells between sex ( $F = 0.83$ ,  $df = 1, 12$ ,  $p = 0.38$ ), treatment ( $F = 0.03$ ,  $df = 1, 12$ ,  $p = 0.86$ ) and no interaction ( $F = 0.86$ ,  $df = 1, 12$ ,  $p = 0.37$ ). No relationship between brain volume and CA1/2 counts ( $F = 0.20$ ,  $df = 1, 14$ ,  $p = 0.66$ ,  $r^2 =$

0.01) nor between brain volume and CA3 ( $F = 0.08$ ,  $df = 1, 14$ ,  $p = 0.08$ ,  $r^2 = 0.005$ ) was detected. No significant differences in total pyramidal cell counts were detected between sex ( $F = 1.84$ ,  $df = 1, 12$ ,  $p = 0.13$ ) nor between treatments ( $F = 0.42$ ,  $df = 1, 12$ ,  $p = 0.53$ ) and there was no significant interaction ( $F = 0.03$ ,  $df = 1, 12$ ,  $p = 0.87$ ) (Fig 2.7c).

Qualitatively, there was no distinct visual difference in the shape of any of the subfields, hippocampal lamination, distribution of cells or the morphology of the cells. The shape of the dentate gyrus, CA subfields and hippocampus were indistinguishable between control and treatment groups and between sexes. Similarly, there was no marked visual difference in cell distribution within either of the subfields or in overall cell morphology. Cell bodies, nuclei and cell extensions looked quantitatively the same.

#### *Cell sizes*

Average granule cell size did not vary significantly by sex ( $F = 0.09$ ,  $df = 1, 12$ ,  $p = 0.78$ ) nor by treatment ( $F = 3.76$ ,  $df = 1, 12$ ,  $p = 0.08$ ) and no interaction was detected ( $F = 3.85$ ,  $df = 1, 12$ ,  $p = 0.07$ ) (Fig 2.7b). Similarly, average pyramidal cell size did not differ significantly by sex ( $F = 0.002$ ,  $df = 1, 12$ ,  $p = 0.97$ ) or treatment ( $F = 1.11$ ,  $df = 1, 12$ ,  $p = 0.31$ ) and no interaction was detected ( $F = 0.36$ ,  $df = 1, 12$ ,  $p = 0.56$ ) (Fig 2.7d).

## **Discussion**

Although I predicted that developmental PCB 126 exposure would affect hippocampal volume, cell counts and cell sizes, no significant effects of developmental exposure to PCB 126 in American mink kits were detected. However, in the neocortex and corpus callosum, a sex specific effect of PCB 126 was detected in the neocortex males exposed to PCB 126. That is, male control mink have larger neocortical volumes

than their PCB-dosed counterparts. Furthermore, both male control mink and female control mink have a larger corpus callosum volume than their PCB-dosed counterparts.

### *Hippocampus*

Across all analyses, the size of the hippocampus, and its constituent regions, as well as cell numbers and sizes of pyramidal and granule cells did not differ significantly by treatment or sex, regardless of whether absolute or relative size measurements were analyzed. In some mammals, sex differences in hippocampal anatomy are well characterized, but depend largely on reproductive status, environmental demands and age (Burger et al., 2014; Burger et al., 2013; Galea, Perrot-Sinal, Kavaliers, & Ossenkopp, 1999; Juraska, Fitch, & Washburne, 1989; Lavenex, Steele, & Jacobs, 2000). The mink kits used in this study were sacrificed at six weeks of age immediately post-weaning and had likely not experienced any environmental pressures that would drive anatomical changes in the hippocampus. Additionally, sexual maturity in mink does not occur until 9-11 months of age (depending on time of year the kits were born), so it is perhaps unsurprising that I detected no morphological dimorphisms in the hippocampus as many of these dimorphisms seem to be driven by sex hormones and reproductive status and may not emerge until adulthood (Galea et al., 1999; B. K. Hansen, Jeppesen, & Berg, 2010; Larivière, 1999). Still, consideration of sex differences is important in neurotoxicology, because many sex differences in the brain are regulated by the neuroendocrine system (Juraska, 1991) and potential endocrine disruptors, such as dioxin-like PCBs, likely affect male and female brains differently (Weiss, 2011).

Along with no observed sex differences, no effects of PCB 126 were detected on hippocampal volume, cell numbers or cell morphology. This result was unexpected, as

the hippocampus tends to be sensitive to environmental toxicants, endocrine disruption and stress, especially during development (Andersen & Teicher, 2004; Rice & Barone Jr, 2000; Winneke, 2011). In peripheral organs, cell proliferation is altered as a result of AHR activation when animals are exposed to PCB 126, so I expected to see an effect in the hippocampus, where neurogenesis occurs through most of the lifespan of mammals (Beckett et al., 2005; Render et al., 2000; Vondracek et al., 2005; Yoshizawa et al., 2005). Hippocampal changes previously observed after dioxin or dioxin-like PCB exposure include alterations in transcription factors, increased sensitivity to excitotoxicity, reduced LTP, decreased dendritic length and mossy fiber growth, and increased apoptosis (Basha et al., 2006; K. H. Kim & Pessah, 2011; Niemi et al., 1998; Ozcan et al., 2004; Parent et al., 2011; Tofighi et al., 2011). Many of the observed behavioural deficits, such as deficits in visual recognition memory, poor performance in the Morris Water Maze, and impaired novel object recognition are all behaviours mediated at least partially by the hippocampus and behaviours which may also be affected by PCB exposure (Boucher et al., 2014; Curran et al., 2011; Ulbrich & Stahlmann, 2004). In sum, there were many reasons why hippocampal anatomy would be affected by PCB exposure. Molecular effects, such as changes in calcium signaling, alterations in LTP and alterations in transcription factors could occur without changes in hippocampal anatomy (Basha et al., 2006; Hanneman et al., 1996; Huang et al., 2000; Koclavanti, 2004; Ozcan et al., 2004). This is also not the first study to note no effects of PCB 126 on the brain. Rats that received up to 1 µg/kg/day of PCB 126 showed no spatial deficits in either the T-maze or radial arm maze and were overall behaviourally similar to control rats (Bushnell & Rice, 1999; Rice & Hayward, 1998; Schantz, Seo,

Moshtaghian, Peterson, & Moore, 1996). Furthermore, no levels of PCB 126 in these studies were detectable in the brain despite being detected in other fatty tissue (Rice, 1999). The lack of effects observed in the mink hippocampus may be because most other dioxin studies on PCB exposure utilize aroclors comprised of many dioxin-like and non-dioxin like PCBs or a combination of several dioxin-like PCBs (K. H. Kim & Pessah, 2011; Widholm et al., 2001). Perhaps effects of PCB 126 alone are not enough to affect hippocampal morphology and the interaction of different PCBs or an additive effect of several congeners is important.

It is also possible that the effects of early PCB 126 exposure on the hippocampus do not emerge until later in development or until adulthood. Studies of the effects of PCB and dioxin exposure often focus on adolescents whose brains are at later stages of development or on adults (Schantz et al., 2003; Ten Tusscher et al., 2014; Ulbrich & Stahlmann, 2004; Winneke et al., 2014). It is possible that at six weeks of age, hippocampal changes as a result of PCB 126 exposure are not detectable yet. The principle of delayed neurotoxicity suggests that some deficits may not appear immediately and may be unmasked as the brain changes with age long after the toxicant is removed (Rice & Barone Jr, 2000). Perhaps reaching sexual maturity and facing environmental demands on the hippocampus would unmask deleterious effects of PCB 126 on the hippocampus, when it is in higher cognitive demand. Finally, it is also possible that PCB 126 is simply not reaching the brain and activating AHRs during brain development, however, if this is the case I would expect there to be no changes in any area of the brain and I did find significant changes in the mink neocortex and corpus callosum.



### *Neocortex and corpus callosum*

Both neocortical and corpus callosum volumes were sexually dimorphic in size with males having larger volumes than females. Sexually dimorphic neocortical volumes are reported across many mammals (Juraska, 1991; Leonard et al., 2008; Lindenfors, Nunn, & Barton, 2007; Reid & Juraska, 1992), including other carnivores (Sawada et al., 2013), so this is unsurprising. Similarly, sex differences are frequently present in the corpus callosum (Fitch, Berrebi, Cowell, Schrott, & Denenberg, 1990; Franklin et al., 2000; Gur et al., 1999; Noonan, Smith, Kelleher, & Sanfilippo, 1998; Sawada et al., 2013) although this effect is not always retained when related to overall brain volume (Bishop & Wahlsten, 1997). These sexual dimorphisms are at least partially mediated by sex hormones, especially testosterone and estrogen (Juraska, 1991; MacLusky, Clark, Naftolin, & Goldman-Rakic, 1987; Roof & Havens, 1992; J. M. Zhang, Konkle, Zup, & McCarthy, 2008), which could suggest that PCB 126 is acting via disrupting sex hormones. This is further supported by the sex specific effect of PCB 126 on neocortical volumes in males, but not females. If PCB 126 is disrupting the endocrine pathways involved in male brain development, then males exposed to PCB 126 would have a brain more similar to females. This is what I observed; there were no significant differences between males exposed to PCBs and both groups of females in the neocortex and corpus callosum. PCB 126 may be disrupting sex hormones in the androgen pathway by decreasing the function of steroidogenic enzymes or altering aromatase activity through AHR activation (Cooke, Sato, Buchanan, Robertson, & Hansen, 2001; Moore, Potter, Theobald, Robinson, & Peterson, 1985). Indeed, PCB exposure is associated with lower concentrations of serum testosterone in males, decreased circulating androgens, and anti-

androgenic effects, such as smaller testes and decreased sperm production (Kuriyama & Chahoud, 2004; Moore et al., 1985; Roman & Peterson, 1998). Furthermore, corpus callosum may be size modulated by testosterone (Fitch et al., 1990). Female rats that received perinatal testosterone or estrogen blockers showed a significant increase in corpus callosum size while male rats who received testosterone blockers had a smaller corpus callosum than expected (Fitch et al., 1990). While this helps to explain the sex-specific effect of PCB 126 observed on the neocortex and may partially explain the effect on male corpus callosum, other mechanisms must be taking place in females.

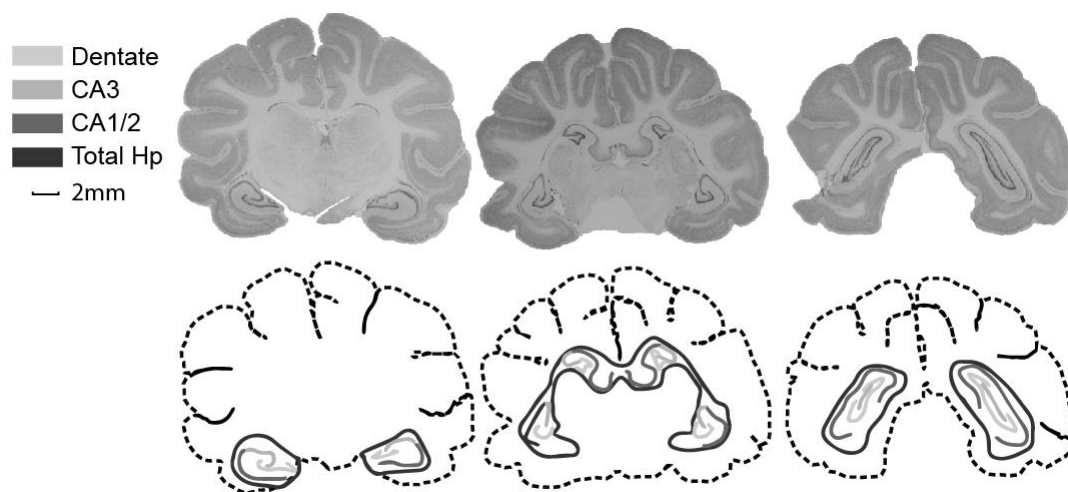
An alternative explanation for the effect of PCB 126 on the corpus callosum is thyroid hormone disruption. Enzymes activated in the AHR pathway by dioxin-like PCB exposure interfere with metabolism of both sex hormones and thyroid hormones and alter the availability of these hormones to the brain (Darras, 2008; Porterfield & Hendry, 1998; Sher et al., 1998). Dioxin-like PCBs can interfere with thyroid signaling during fetal development and may disrupt circulating thyroid hormones by altering the structure of the thyroid gland, altering thyroid hormone metabolism or bind to thyroid hormone binding proteins in the blood thus reducing the amount of circulating thyroxine (T4) and reducing the efficacy of thyroid stimulating hormone (TSH) (Gauger et al., 2004; Zoeller et al., 2002). PCB 126, specifically, is associated with depressed T4 levels in rats (Fisher et al., 2006; Seo et al., 1995; Van Birgelen et al., 1995). Decreased levels of circulating thyroid hormones affect axonal myelination and decrease the number of oligodendrocytes in developing white matter tracts, so if PCB 126 is altering thyroid levels, this may explain the differences in corpus callosum from controls who did not receive the dioxin-like PCB (Calza, Fernandez, Giuliani, Aloe, & Giardino, 2002; Schoonover et al., 2004).

Determining whether the volumetric differences in corpus callosum and neocortex are a result of thyroid hormones, sex hormones, some other mechanism, or a combination requires further study involving measurements of circulating hormones throughout development.

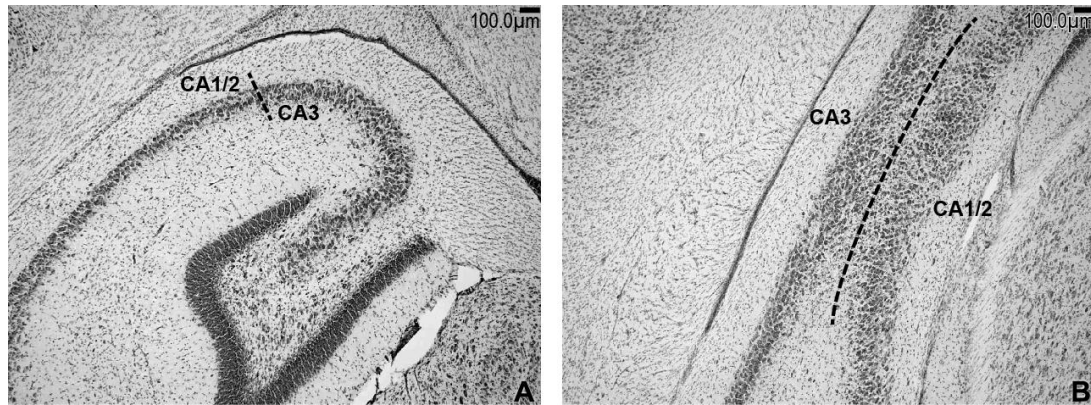
## **Conclusions**

Although no effects were observed on hippocampal anatomy, my analyses of neocortical and corpus callosum volumes suggest that early developmental exposure to PCB 126 affects the developing brain. Perhaps even more importantly, the mink exhibited these neuroanatomical effects after being exposed to a relatively low dose of PCB 126 (0.9 ng/g, PCB 126). A sample of fish and seafood from the Canadian retail market reported that total PCB contaminants in fish products are less than 2000 ng/g, which is the upper safe limit for food stuffs according to Health Canada (Rawn et al., 2006). Thus, the mink were given a dose of PCB that would be deemed acceptable for fish products in Canada. Although I examined only a single PCB congener, this nevertheless suggests that chronic exposure to levels of PCBs similar to that found in fish products can exert a significant effect on neural development. Furthermore, the sex differences observed herein suggest that these acceptable values may affect males and females differently. The effects observed in the neocortex and corpus callosum illustrate that despite their ban, PCBs are still a relevant environmental contaminant and food contaminant, especially for populations in highly contaminated areas.

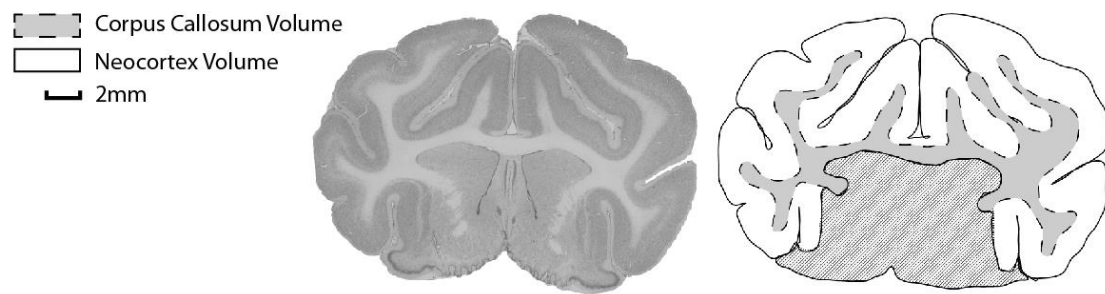
## CHAPTER TWO FIGURES



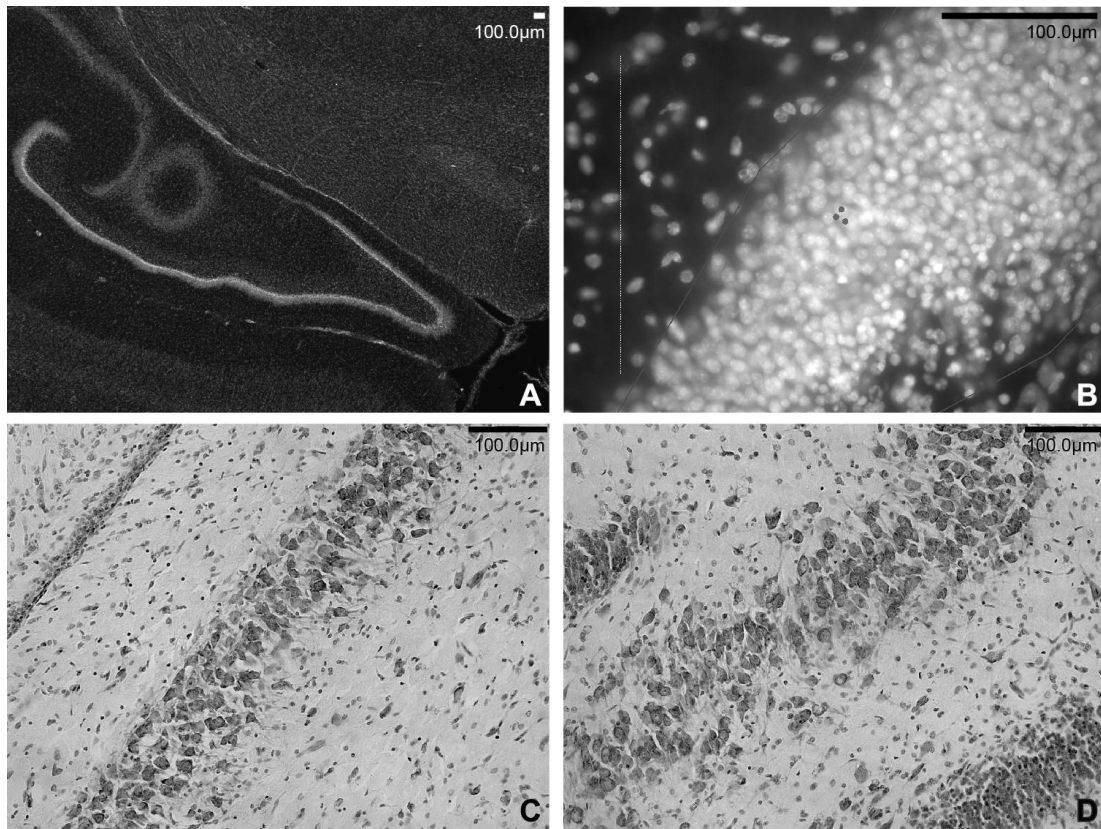
**Figure 2.1** – Coronal Nissl stained sections of the mink hippocampus from rostral (left) to caudal (right) accompanied by a schematic drawing representing the subregions of the hippocampus.



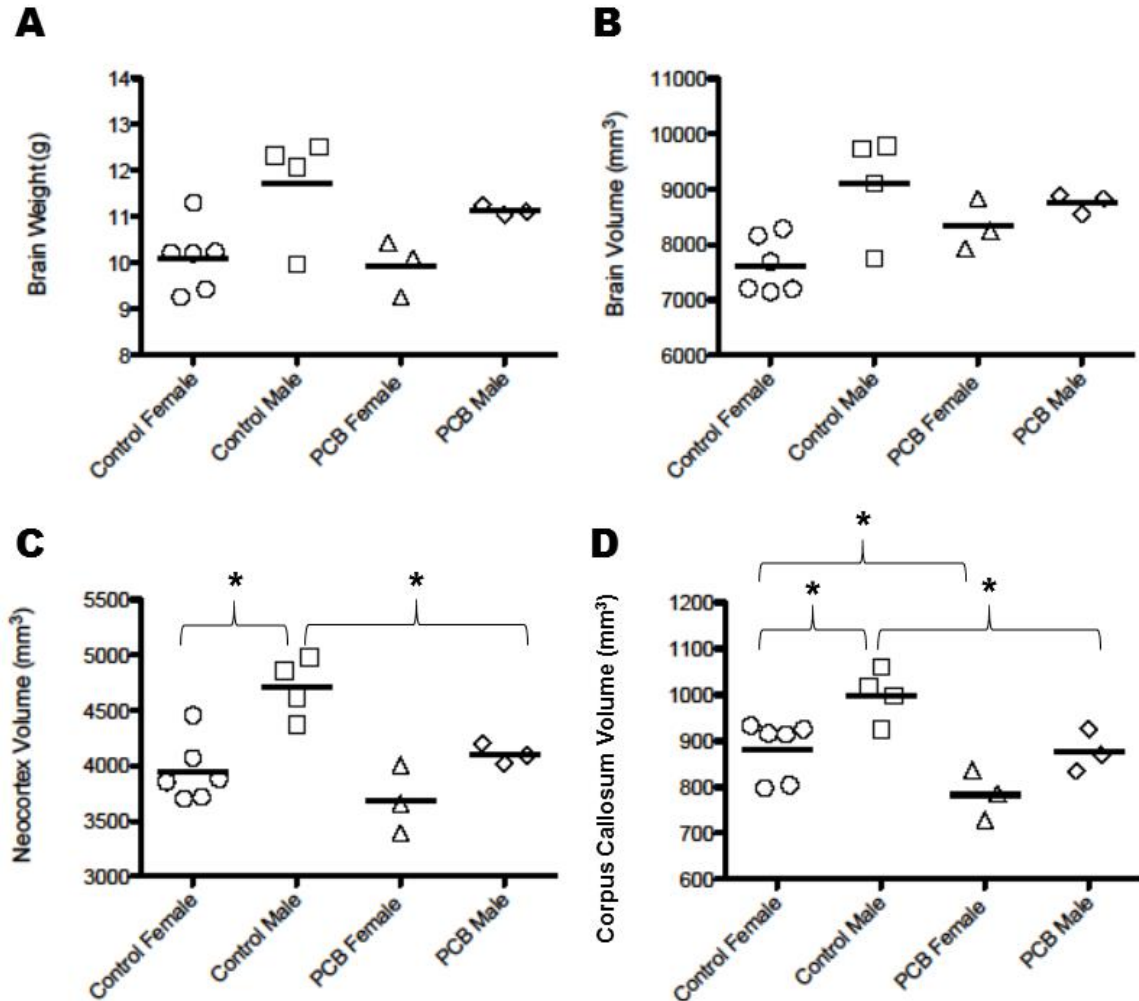
**Figure 2.2** – Nissl stained coronal section of the mink hippocampus under a 5x objective lens focusing on the pyramidal layer of the hippocampus. A) The border between CA3 and CA1/2 on a rostral section of dorsal hippocampus. B) The border between CA3 and CA1/2 on a caudal section of the medial hippocampus immediately before CA3 and CA1/2 split into two separate layers. In both cases, the border is distinguished by a change in pyramidal cell type from larger and more diffuse to smaller and more compact cells.



**Figure 2.3** - A coronal section of the Nissl stained mink neocortex accompanied by a schematic representation of the areas measured for total corpus callosum volume and total neocortical volume.

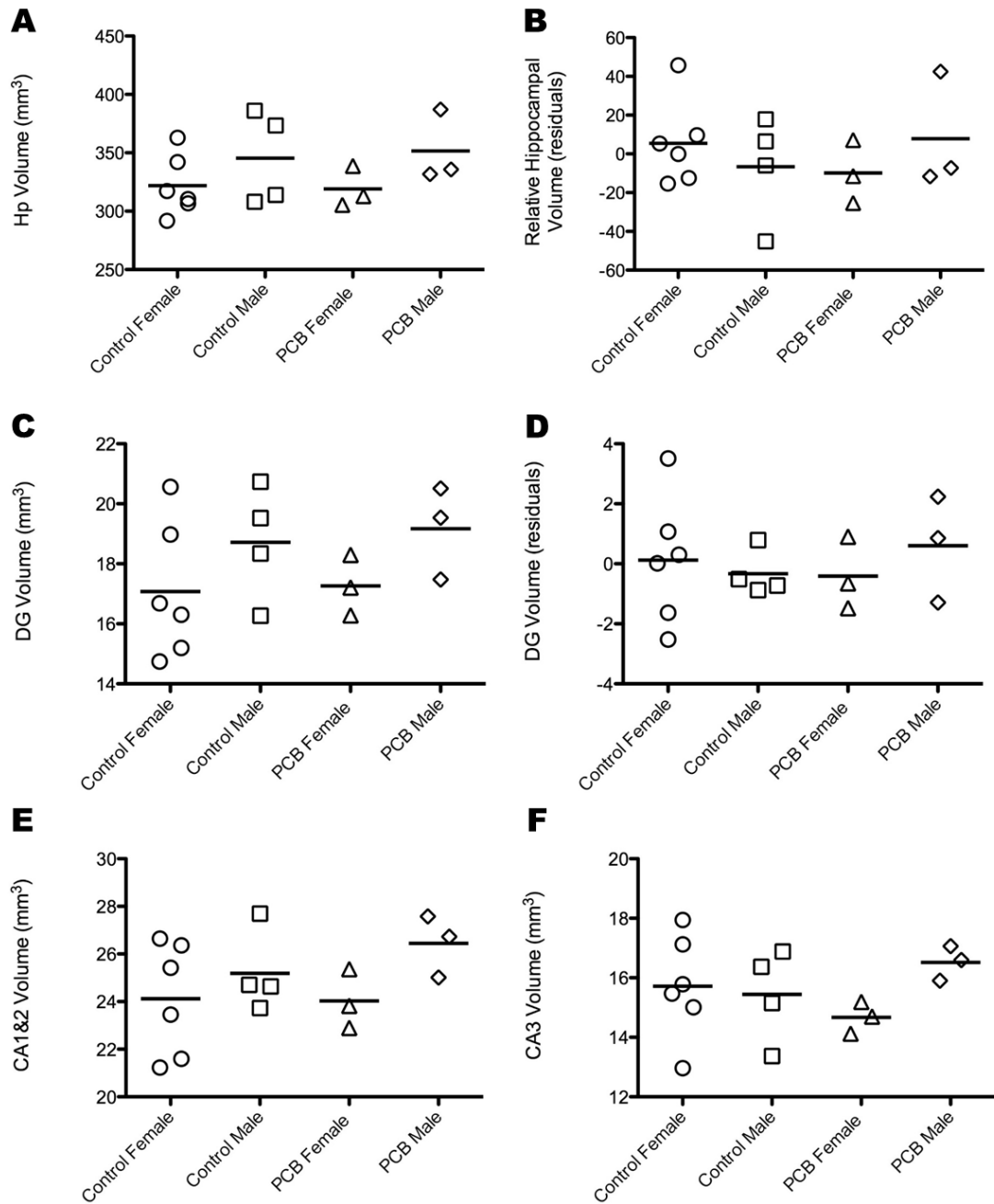


**Figure 2.4** – A) A coronal section of the ventral portion of the mink hippocampus depicting green fluorescent Nissl staining of the granule cells within the dentate gyrus under a 2.5x objective lens. B) Fluorescent Nissl stained granule cells in the dentate gyrus and the counting frame as implemented in StereoInvestigator’s optical fractionator workflow. C) Nissl stained pyramidal cells of the CA1/2 region under a 20x objective. D) Nissl stained pyramidal cells of the CA3 region under a 20x objective.



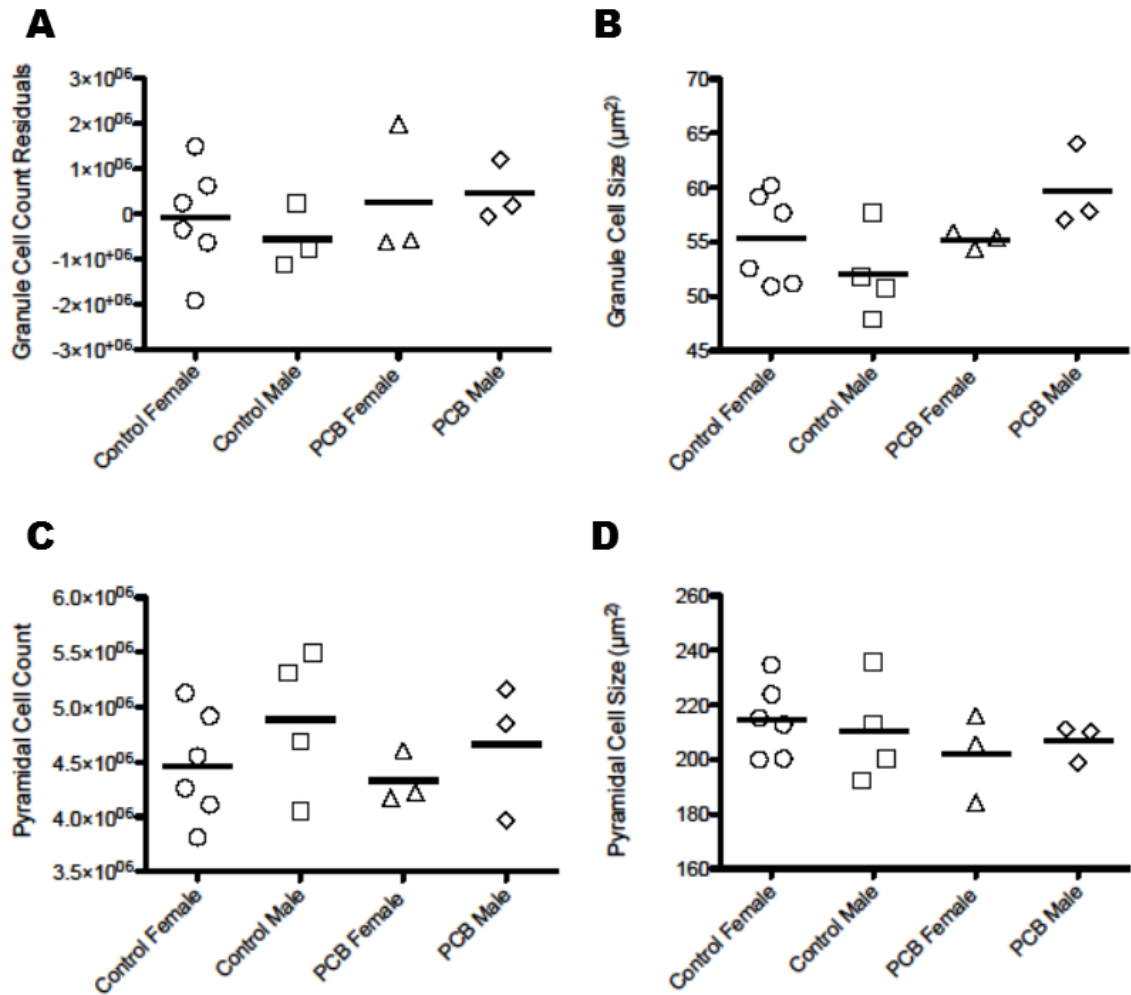
**Figure 2.5** - Plots of A) Brain weight, B) Brain volume, C) Neocortical volume, and D) Corpus callosum volume compared across groups divided by sex and treatment. Horizontal lines represent the mean volume for each group and each point represents an individual animal. \* denotes a significant difference between the indicated groups.





**Figure 2.6** - Plots of A) absolute hippocampal volume, B) relative hippocampal volume, C) absolute dentate gyrus volume, D) relative dentate gyrus volume, E) absolute CA1/2 volume and F) absolute CA3 volume compared across sex and treatment groups.

Horizontal lines represent the mean volume for each group and each point represents an individual animal.



**Figure 2.7** - Plots of A) relative granule cell count, B) relative granule cell size, C) absolute pyramidal cell count and D) absolute pyramidal cell size compared across sex and treatment groups. Horizontal lines represent the mean volume for each group and each point represents an individual animal.

### **CHAPTER 3: THE EFFECTS OF EMBRYONIC PCB 126 EXPOSURE ON THE BRAIN OF THE DOMESTIC CHICK (*GALLUS DOMESTICUS*)**

#### **Introduction**

Polychlorinated biphenyls are persistent environmental pollutants that contaminate both salt and fresh water sources as a result of their industrial use in coolants, oils, and pesticides until the 1970s and subsequent improper disposal (Crinnion, 2011). PCBs were banned in 1977 when researchers determined that a variety of negative health effects occurred from PCB exposure, including reproductive toxicity, immunotoxicity, tumour promotion, cardiovascular disease, endocrine disruption and neurobehavioural deficits relating to IQ, attention and memory (Goncharov et al., 2008; Goncharov et al., 2009; Humblet et al., 2008; Mandal, 2005; Schell et al., 2008; C. Zhang, Fang, Liu, Xia, & Qiao, 2002). The primary route of PCB exposure is through dietary intake (Rawn et al., 2006). PCBs are lipophilic so they tend to bioaccumulate in fatty body tissue and biomagnify through the food chain (Carpenter, 2006). Detectable levels of PCBs are still found in serum samples of humans, mammals, birds and fish and in breast milk samples of humans and mammals despite the ban on PCB production over three decades ago (Domingo & Bocio, 2007).

Although many health effects of PCBs are already well characterized, little is still known about the neurotoxic consequences of PCB exposure. PCBs can be divided into two broad classes based on their chemical structure and the number and position of chlorine atoms attached to the two benzene rings. Of the 209 possible congener combinations, 12 of these have a coplanar structure, which gives them the ability to mimic dioxins (such as TCDD, the most potent dioxin) and exert many of the same

dioxin-like effects by binding to the aryl hydrocarbon receptor (AHR) (Denison & Nagy, 2003). Dioxin-like PCBs likely exert their toxic effects by binding to the AHR, which activates a cascade to transcriptional processes resulting in a range of toxic effects (Hankinson, 1995). These effects can include stimulation of cell growth, altered tissue responses leading to increased cell growth and disruption of endocrine signaling processes involving thyroid and gonadal hormones (Grassman et al., 1998; Mandal, 2005). Both thyroid and gonadal hormones are important for proper brain development and early endocrine disruption could have long term neurological consequences on behaviour and survival (Cooke et al., 2001; MacLusky et al., 1987; J. M. Zhang et al., 2008; Zoeller et al., 2002). Behaviourally, early developmental exposure to dioxins and dioxin-like PCBs has been associated with decreased cognitive abilities, lower IQ, attentional deficits and impaired learning and memory, but the changes occurring in the brain that underlie these neurobehavioural changes are not well known (Boucher et al., 2009; Jacobson & Jacobson, 1996; Schantz et al., 2003; Ten Tusscher et al., 2014).

Although most research on the effects of PCBs on brain development focuses on mammals, avian studies offer many advantages over traditional rodent models of toxicant exposure. First, there are no effects of litter size or uterine position. Fetuses developing between two siblings of the opposite sex may experience different hormonal environments that may alter hormone levels, sex- specific behaviours and sensitivity to endocrine disruption (Ryan & Vandenberg, 2002). In birds, after maternal deposition of the egg, avian embryonic development is independent of the effects of litter size or maternal physiology so researchers do not have to try to account for maternal toxicant metabolism and the blood-placenta barrier when administering toxicants (De Groef,

Grommen, & Darras, 2008). Second, toxicants can be injected directly into the egg, allowing researchers to ensure that each bird receives the entire desired dose (Carro, Dean, & Ottinger, 2013; Henshel, Hehn, Wagey, Vo, & Steeves, 1997). In mammals, administering an entire dose to each fetus is impossible to do without invasive procedures that are further complicated by maternal stress and maternal toxicant responses. Third, the stages of embryonic development are well characterized, and development is easy to assess via egg candling, which also allows us to easily assess embryonic survival (Bellairs & Osmond, 2005). Finally, some endocrine changes in the chicken, such as thyroid hormone regulation, are actually more similar to the human endocrine changes during late development than the thyroid hormone changes in rodents (Darras, Hume, & Visser, 1999).

With respect to PCB exposure, the AHR in birds is also somewhat better characterized than the AHR in mammals (Farmahin et al., 2012). Although many avian and fish species have two types of AHR receptors (Hahn et al., 2006), chickens (*Gallus domesticus*) have only one AHR, which is orthologous to the mammalian AHR (Head, Hahn, & Kennedy, 2008). The exact function and evolutionary significance of the difference in AHRs across taxa is unclear, but two versions of the AHR, along with differences in amino acid sequences of the AHR, contribute to significant interspecific differences in dioxin-like PCB sensitivity (Head et al., 2008). For example, domestic chickens are much more sensitive to PCBs than most other avian species studied to date (Eng et al., 2014; Giesy & Kannan, 1998). This difference in sensitivity is useful for studies of neurotoxicology, because there is a better chance that dosages will produce neurological changes as compared to other species with higher sensitivity thresholds. Yet,

the results are still generalizable to other avian species, as we know the relative sensitivity of dioxins of many avian species compared to chickens and can assess these levels in the environment (Head et al., 2008).

Exposure to dioxins or dioxin-like PCBs in avian species results in reduced embryo survival, lowered hatching weights, delayed hatching, altered gonadal development, cardiac malformations, altered primary immune organ development and eye, head or beak malformations (Carro et al., 2013; Fox & Grasman, 1999; Hoffman et al., 1997; Jin, Kennedy, Di Muccio, & Moon, 2001; Lavoie & Grasman, 2007; W. Zhang et al., 2012). Additionally, exposure to dioxin-like PCBs can cause abnormalities in cardiac walls, higher rates of edema, liver necrosis, decreased immune organ masses and lymphocyte numbers, decreased bursa size, suppressed antibody responses, changes in thyroid levels, decreased size of reproductive organs and lower levels of serum testosterone (Blankenship et al., 2003; Carro et al., 2013; Fox & Grasman, 1999; Hilscherova et al., 2003; Lavoie & Grasman, 2007; Powell et al., 1996; Roelens et al., 2005; Yeager, Oleske, Millsap, & Henshel, 2006; C. Zhang et al., 2002). In the avian brain, researchers observed gross cerebral asymmetry related to the forebrain in wild great blue heron (*Ardea herodias*) and double-crested cormorant (*Phalacrocorax auritus*) hatchlings from environments contaminated with TCDD, PCBs and PCDFs (Henshel, 1998). However, of these three groups of contaminants, PCBs had the lowest correlation with degree of asymmetry and tectum size. In the same study, TCDD was administered to developing chickens and the asymmetry effect was reproduced in addition to significant differences in tectum angle and width at higher doses and suppressed myelination in the spinal cord (Henshel, 1998). Although PCB is structurally similar to TCDD, it is unclear

whether PCB might have similar effects on asymmetry in the developing brain. Indeed, (Lipsitz, Powell, Bursian, & Tanaka Jr, 1997) found no dose-related effects of PCBs exposure on brain asymmetry in domestic chicks.

Apart from these studies, there is very little information on whether developmental PCB exposure affects the avian brain. I specifically chose to test the effects of PCB 126 because it is the most toxic of the dioxin-like PCBs and levels of PCB 126 have shown no significant decline in human tissue samples for over a decade (Consonni et al., 2012). I administered PCB 126 in domestic chicks at four different doses to establish a dosage response and level of effect for our specific strain of chickens. A specific LD50 for chickens has not been established because there are many strain differences in toxicant response and the route of injection can change toxicant sensitivity (Henshel et al., 1997; McKernan, Rattner, Hale, & Ottinger, 2007). Although many brain regions could have been examined, I focused on the avian hippocampal formation because many of the neurobehavioural effects of dioxins and dioxin-like PCBs are related to learning and memory (Boucher et al., 2009; Rice, 1999; Vitalone et al., 2010). In mammals, dioxin and dioxin-like PCB exposure affects calcium signaling, long term potentiation, receptor density and synaptic transmission in the hippocampus (Basha et al., 2006; Boix et al., 2010; Eriksson & Fredriksson, 1998; Niemi et al., 1998; Ozcan et al., 2004). Despite the fact that avian and mammalian hippocampi are homologous, share similar functions in spatial learning and memory, and structurally share many of the same receptors, cell types, and subregions (Colombo & Broadbent, 2000; Herold et al., 2014), this is the first work examining the effects of dioxins on the avian hippocampus. In addition, I tested whether PCB 126 exposure affected telencephalon volume and

asymmetry because of the equivocal results of previous studies (Henshel et al., 1995; Lipsitz et al., 1997).

## **Materials and Methods**

### *Animals*

Fertile white leghorn chicken eggs were purchased from the University of Alberta Hatchery (Edmonton, AB), received on day of laying, and transported back to Lethbridge in a padded Styrofoam cooler kept at 4-8°C. At the University of Lethbridge, 3,3',4,4',5-pentachlorobiphenyl (PCB 126) (AccuStandard, Inc., New Haven, CT) was dissolved in charcoal stripped organic safflower oil (Spectrum Organic Oil, Boulder, CO) at four concentrations: minute dose (0.02 mg/μl), low dose (0.2 mg/ μl), medium dose (2.0 mg/μl), high dose (20.0 mg/μl). Eggs were randomly sorted into one of seven groups with a similar distribution of egg weights: high dose, medium dose, low dose, minute dose, vehicle dose, sham, or control. Eggs were weighed at embryonic day 0 (ED0) and were dosed according to weight. Eggs were then swabbed with an alcohol pad before a Dremel tool (Dremel Co., Racine WI) with a sterile diamond tip bit was used to drill a small hole into the air cell at the top of the egg, which was delineated with a pencil marking. All eggs, including controls, were swabbed with alcohol pads and marked with pencil. Solutions were injected into the air cell with an electronic micropipette (Hamilton SofTouch Electronic Precision Pipette, Hamilton Company). The drilled holes were sealed with melted paraffin wax and incubated at 38°C and 72% humidity. Sham eggs were drilled and sealed with paraffin, but not injected with any solution. Vehicle eggs were drilled and injected with the weight-appropriate amount of charcoal stripped



safflower oil containing no PCBs. Eggs were distributed randomly in the incubator and candled every 5 days, at which time egg trays were switched to new tray positions in the incubator.

After hatching, the chicks were weighed, euthanized via cranio-cervical dislocation and decapitated. The skull was partially opened and heads were immediately immersed in 4% buffered paraformaldehyde (PFA). The gonads were examined to determine sex and tarsus length and wing chord length measured to the nearest 0.01mm with digital calipers. Each animal was also examined for obvious deformities such as gross craniofacial malformations, crossed beaks, or deformities of the wings or legs; however, I found no such deformities (Blankenship et al., 2003; Lavoie & Grasman, 2007; Yeager et al., 2006).

### *Histology*

Brains were extracted from the skull 5-7 days after immersion in PFA. Extracted brains were returned back to fresh PFA and stored at 4°C until they were ready to be processed. Prior to slicing, brains were cryoprotected in 20% sucrose and embedded in gelatin. Gelatin embedded brains were frozen and sliced on a freezing stage microtome at a thickness of 40 microns. Sections were free-floated in individual 24-well trays in 0.1 M phosphate buffered saline and 0.01% sodium azide. Every fourth section was mounted on gelatinized slides, stained with thionin for Nissl substance and coverslipped with Permount.

### *Volumetric Measurements*

Every second section on the slides (i.e. every 8<sup>th</sup> section) was photographed using a digital camera. The images were exported to ImageJ (Schneider, Rasband, & Eliceiri,

2012) where they were measured for total brain volume and telencephalic volume. Left and right telencephalon were measured separately to determine the presence of asymmetry between the volumes of the two hemispheres (Lipsitz et al., 1997). Percent asymmetry was calculated by subtracting the volume of the smaller hemisphere from that of the larger hemisphere and dividing the difference by total telencephalic volume. Telencephalon borders were determined by consulting a stereotaxic chick brain atlas (Puelles, 2007).

Hippocampal volumes were calculated using the Cavalieri estimator (Gundersen & Jensen, 1987; West & Gundersen, 1990) as implemented in StereoInvestigator (MicroBrightfield, Williston, VT) and a Zeiss Axio Imager MT (Carl Zeiss, MicroImagine GmbH, Germany). Volumes were measured on a 500 x 500 micron grid. Coefficients of error were calculated for each brain (Gundersen,  $m = 1$ ) and were all low, ranging from 0.01 to 0.019. Every second section on the slides was measured by tracing hippocampal borders as defined by various chicken brain atlases (Kuenzel, Jarvis, & Karten, 2000; Puelles, 2007; Youngren & Phillips, 1978) (Fig 3.1). Parahippocampal areas were also included as part of the hippocampal formation, because the border between the hippocampal proper and parahippocampal areas are difficult to reliably determine with Nissl staining. Although the mammalian and avian hippocampus appear to be homologous (Colombo & Broadbent, 2000; Herold et al., 2014), the exact borders of the hippocampal subdivisions such as the CA subfields and dentate gyrus are still unclear, so I only measured the volume of the entire hippocampal formation (Colombo & Broadbent, 2000; Erichsen et al., 1991; Herold et al., 2014) (Fig 3.1). The same measurement is frequently used for other neuroanatomical studies of the avian

hippocampus (Clayton, Reboreda, & Kacelnik, 1997; Sherry & Hoshooley, 2010; Smulders, Sasson, & DeVoodg, 1995; Yaskin, 2011). Based on previous studies (Herold et al., 2014; Sherry, Vaccarino, Buckenham, & Herz, 1989), I defined the dorsal border of the hippocampal formation as the brain surface, the ventral border as the lateral ventricle and the medial border as the midline of the brain. The lateral border of the hippocampal formation extends ventrally past the lateral ventricle and is distinguished from the adjacent areas by a change in cell type between the two areas and a curved cell alignment throughout the hippocampal formation (Fig 3.2) (Herold et al., 2014; Sherry et al., 1989).

### *Statistical Analysis*

Mortality was assessed using  $\chi^2$  contingency tests and Fisher's exact tests comparing each treatment group to the other. One-way ANOVAs were used to determine the main effects of treatment and sex on: body weight, wing length, tarsus length, brain volume, telencephalon volume, telencephalon asymmetry and hippocampal volume. Interaction effects (i.e., the interaction between sex and treatment) could not be included because the sample sizes of each sex were low within each treatment group. In addition to analyzing absolute volumes, I also tested whether relative hippocampal and telencephalic volumes varied across treatments and between the sexes. To examine relative brain region size, I first tested for allometric scaling relationships between: telencephalon and brain; hippocampus and brain; and hippocampus and telencephalon. Linear regression analysis was used to determine whether the size of each brain area was related to brain volume, minus the region of interest (Deacon, 1990). If a significant scaling relationship was detected, I then took the residuals of the regression line for the region of interest as a

measurement of relative size. To ensure that allometric effects were removed, I then tested whether there was a significant correlation between the residuals and the independent variable used in the regression analyses (i.e., brain or telencephalon volume). If no significant correlation was present (i.e., allometric effects were successfully removed), a one-way ANOVA was finally applied to the residuals to test for the effects of treatment, sex or the interaction on relative brain region volumes, when applicable.

## **Results**

### *Mortality*

Controls experienced no mortality, while shams experienced 9% mortality and vehicles had 43% mortality. For the experimental groups, minute dose mortality was 32%, low dose mortality was 53%, medium dose mortality was 35% and high dose experienced 100% mortality. A chi-squared 2x7 contingency test, indicated that survival and treatment group were not independent ( $\chi^2 = 38.5$ ,  $p < 0.001$ ,  $df = 6$ ). Pairwise comparisons of each treatment group using Fisher's exact tests indicated that the vehicle treatment differed significantly from the control groups ( $p = 0.004$ , Fisher's exact test), but did not differ from the sham group ( $p = 0.09$ , Fisher's exact test). Furthermore, the control group and the sham group did not differ significantly ( $p = 0.39$ , Fisher's exact test). Given that the vehicle treatment resulted in a higher mortality than both other controls groups, mortality rates for the PCB groups were compared to vehicles only. However, there were no significant differences detected between the vehicle group and the minute dose ( $p = 0.72$ , Fisher's exact test), low dose ( $p = 0.7283$ , Fisher's exact test),

or medium dose ( $p = 0.73$ , Fisher's exact test) treatment groups. There was a difference between vehicle and the high dose group, which experienced 100% mortality ( $\chi^2=10.56$ ,  $p=0.0012$ ,  $df= 1$ ) and therefore could not be included in any further analysis.

### *Body size*

Body mass was unaffected by either sex ( $F = 0.22$ ,  $df= 1$ ,  $63$ ,  $p = 0.64$ ) or treatment ( $F = 0.91$ ,  $df = 5$ ,  $59$ ,  $p = 0.48$ ). No sex differences were detected in wing length ( $F = 2.95$ ,  $df = 1$ ,  $65$ ,  $p = 0.10$ ) and treatment did not affect wing length ( $F = 0.23$ ,  $df = 5$ ,  $63$ ,  $p = 0.95$ ). Tarsus length, however, was 3.2% shorter in female chicks ( $F = 23.55$ ,  $df = 1$ ,  $65$ ,  $p < 0.0001$ ). Tarsus length and body mass were significantly correlated ( $F = 9.63$ ,  $df = 1$ ,  $63$ ,  $p = 0.003$ ,  $r^2 = 0.13$ ), so I also calculated relative tarsus length based on the residuals of tarsus vs. body mass. The sex difference favouring males in tarsus length remained ( $F = 24.55$ ,  $df = 1$ ,  $63$ ,  $p < 0.0001$ ), but there was still no effect of treatment on tarsus length ( $F = 0.62$ ,  $df = 5$ ,  $59$ ,  $p = 0.62$ ).

Given the high rate of mortality in the vehicle group, I also analyzed the effects of treatment on these parameters excluding control and sham control groups. Despite this exclusion, I still detected no significant effects of treatment on body mass ( $F = 1.27$ ,  $df = 3$ ,  $40$ ,  $p = 0.30$ ), wing length ( $F = 0.44$ ,  $df = 3$ ,  $44$ ,  $p = 0.73$ ), or tarsus length ( $F = 0.71$ ,  $df = 3$ ,  $44$ ,  $p = 0.55$ ).

### *Asymmetry*

I observed no effects of treatment ( $F = 0.79$ ,  $df = 5$ ,  $53$ ,  $p = 0.56$ ) or sex ( $F = 0.14$ ,  $df = 1$ ,  $56$ ,  $p = 0.71$ ) on percentage of telencephalon asymmetry. Overall, the degree of asymmetry between the cerebral hemispheres was quite low. Controls varied in volume between the two sides by an average percent difference of 0.85%, shams by 1.23% and

vehicles by 1.31%. In the treatment groups, minute dose brains exhibited 1.23% asymmetry, low dose brains exhibited 1.3% asymmetry and medium dose brains exhibited 0.76% asymmetry.

### *Brain Volumes*

Total brain volume did not vary by sex ( $F=1.27$ ,  $df = 1$ ,  $62$ ,  $p = 0.26$ ) or by treatment ( $F = 1.82$ ,  $df = 5$ ,  $59$ ,  $p = 0.12$ ). Similarly, absolute telencephalon volume did not vary by sex ( $F = 1.90$ ,  $df = 1$ ,  $62$ ,  $p = 0.17$ ) or by treatment ( $F= 1.02$ ,  $df = 5$ ,  $59$ ,  $p = 0.41$ ). Total brain minus telencephalon and telencephalon volume were significantly related ( $F = 49.99$ ,  $df = 1$ ,  $63$ ,  $p < 0.0001$ ,  $r^2 = 0.44$ ). Still, relative telencephalon volume was unaffected by sex ( $F = 1.35$ ,  $df = 1$ ,  $62$ ,  $p = 0.25$ ) or treatment ( $F = 0.86$ ,  $df = 5$ ,  $58$ ,  $p = 0.51$ ).

Absolute hippocampus volume did not vary by sex ( $F = 0.20$ ,  $df = 1$ ,  $59$ ,  $p = 0.66$ ) or by treatment ( $F = 1.85$ ,  $df = 5$ ,  $56$ ,  $p = 0.12$ ). There was a significant scaling relationship between hippocampus (Hp) volume and telencephalon volume minus Hp ( $F = 8.81$ ,  $df = 1$ ,  $59$ ,  $p = 0.004$ ,  $r^2 = 0.13$ ). This relationship was also detected for Hp and total brain volume minus Hp ( $F = 8.12$ ,  $df = 1$ ,  $59$ ,  $p = 0.006$ ,  $r^2 = 0.12$ ). I therefore calculated two relative Hp volumes: one in relation to telencephalon minus Hp and one in relation to total brain minus Hp. There was no significant effect of treatment on relative Hp volume, regardless of whether Hp volume was corrected for brain ( $F = 2.31$ ,  $df = 5$ ,  $55$ ,  $p = 0.06$ ) or telencephalon size ( $F = 2.01$ ,  $df = 5$ ,  $55$ ,  $p = 0.09$ ). Similarly, there was no sex difference in Hp relative to total brain volume ( $F= 0.03$ ,  $df = 1$ ,  $58$ ,  $p = 0.85$ ) or relative to telencephalon volume ( $F = 0.003$ ,  $df = 1$ ,  $58$ ,  $p = 0.93$ ).

Again, given the high mortality of the vehicle control group, I also ran these analyses for effects of treatment while excluding sham and control groups. Still, no effect of treatment on brain volume ( $F = 1.68$ ,  $df = 3, 40$ ,  $p = 0.19$ ) or relative telencephalon volume ( $F = 0.43$ ,  $df = 3, 40$ ,  $p = 0.73$ ). Additionally, I still detected no significant effects on Hp volume relative to telencephalon ( $F = 0.91$ ,  $df = 3, 38$ ,  $p = 0.44$ ) or on Hp volume relative to total brain ( $F = 0.75$ ,  $df = 3, 38$ ,  $p = 0.53$ ).

## **Discussion**

Overall, I found no significant effects of three different doses of PCB 126 on chick body size, tarsus length, wing length, brain volume, telencephalon volume or hippocampal volume. Despite a previous report of PCB exposure increasing cerebral asymmetry (Henshel et al., 1995), I also found no differences in asymmetry between treatment and control groups

Mortality rates varied significantly among treatments, but the eggs that were injected with the vehicle experienced mortality that was as high as the PCB 126 treated groups. As a result of this high mortality in the vehicle group, it is unclear whether the mortality in the PCB treated eggs arose from PCB exposure or the safflower oil vehicle. Dissolving our contents in charcoal stripped safflower oil was necessary to ensure delivery of PCB 126 to the embryo, but vehicle injections carry a risk of higher mortality. Oil vehicles are generally associated with higher hatching success than other vehicles, such as acetone, ethylene glycol, ethanol and propylene glycol, all of which significantly reduce hatching success (Ameenuddin & Sunde, 1984). Further, some of the other chemicals used as vehicles for toxicology studies (e.g., dimethyl sulfoxide) are

neurotoxicants, which can confound neuroanatomical analyses (Cavaletti et al., 2000; Hanslick et al., 2009; Windebank, Blexrud, & De Groen, 1994). In some studies, oil vehicles significantly increase the rate of chick embryo mortality (Janczak, Braastad, & Bakken, 2006), while in others oil vehicles have little effect on mortality rates compared to other controls (Carro et al., 2013; Powell et al., 1996). The age of the embryos may also be a factor in the toxicity of a vehicle, as embryos injected with oil on embryonic day 1 experienced decreased survival when compared to embryos injected on embryonic day 3 or later (Heinz, Hoffman, Kondrad, & Erwin, 2006). The effects of vehicle on mortality could be due to batch or strain differences in sensitivity (Lindgren & Altimiras, 2011), egg incubation position after vehicle injection (McKernan et al., 2007), or the volume of vehicle delivered to the egg. In this study, eggs were all incubated in the same position, but the injection of oil into the air cell may have produced hypoxic conditions for the developing embryo by reducing the amount of oxygen available from the air cell (DeWitt, Meyer, & Henshel, 2005). The volume of oil injected was determined by the weight of our eggs and ranged from 5.1 to 7.3  $\mu\text{l}$  oil with a mean of 6.3  $\mu\text{l}$ , which may be a sufficient volume to cause hypoxia or anoxia in some of the developing embryos (DeWitt et al., 2005). Additionally, of the batch of chicken eggs I used may have just been more sensitive to vehicles than another batch due to factors such as genetics, transportation method, egg handling, or season (DeWitt et al., 2005).

Different sensitivities to toxicants, including dioxin-like PCBs, exist between different avian species (Giesy & Kannan, 1998; Head et al., 2008; Heinz et al., 2009). Species differences in the genotype of a specific amino acid sequence at the AHR binding site appears to partially account for differences in avian dioxin and dioxin-like PCB



sensitivities (Head et al., 2008). Chickens exhibit extensive genetic variation across strains (Ka-Shu Wong et al., 2004), which could result in different genotypes for the AHR binding site that would then confer different sensitivities to toxicants that act via the AHR in a similar fashion to interspecific differences. There is also variation in brain structure size and shape between different breeds, not only in overall brain volume, but also in relative volume of the hippocampus, olfactory bulbs, telencephalon and striatum (Rehkamper, Kart, Frahm, & Werner, 2003). Although not yet studied, these differences likely translate to differences in behaviour and possibly to the brain's responses to toxicants. Overall, this evidence suggests that although I found no significant effects of PCB 126 on the chick brain, reproducing this study in a different chicken breed could produce different results.

Reports on the effects of PCB 126 on the brain vary with regards to whether an effect is found. Perhaps a single dose in early development is not enough to allow PCBs to affect the brain even though similar doses seem to affect peripheral organs, such as the heart and liver, as well as immune function in avian studies (Carro et al., 2013; Fox & Grasman, 1999; Lavoie & Grasman, 2007). Egg injections of PCBs in a triolein vehicle before incubation in chicken eggs produced developmental abnormalities, lower body weights, lower heart weights, and increased liver weights, but did not affect the brain (Powell et al., 1996). In mammal species, offspring are exposed to PCBs throughout gestation and then through their mother's milk until weaning, unlike birds who only receive a single dose. Still, even in mammals PCB 126 is detected in other parts of the body, but is often not detectable in brain tissue (Holene, Nafstad, Skaare, & Sagvolden, 1998; Rice, 1999).

It should also be noted that PCB 126 alone may not exert the same effects as a combination of several PCBs. In the environment, PCBs never exist alone. Instead, they are found in complex combinations of PCB congeners called aroclors (L. G. Hansen, 1998). In wild bird eggs, more than 80% of eggs sampled contained at least 30 non-dioxin like congeners (Quinn et al., 2013) and dioxin-like congeners could be exerting toxic effects in concert with one another through a variety of different mechanisms to contribute to decreased hatchling success, increased reproductive failure, increased mortality and behavioural impairments (Ottinger et al., 2002; Ottinger et al., 2009; Quinn et al., 2013). Without studies of each individual toxicants and congener and then multiple permutations and combinations, it is impossible to know which is exerting which toxic effect and how these toxic effects are mediated or whether a combination of toxicants is to blame. So, although PCB 126 is the most toxic dioxin-like PCB, it may not exert toxicological effects in the brain as an individual contaminant.

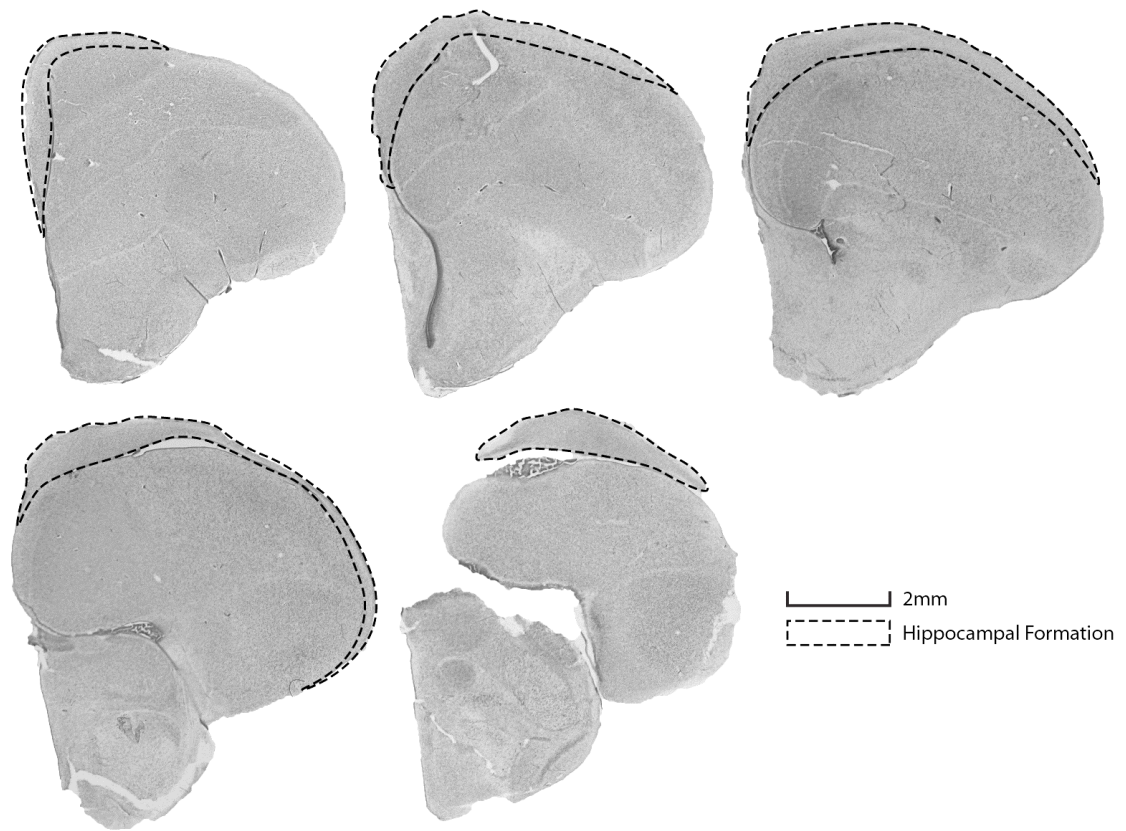
Finally, although PCB 126 is not affecting brain volume it could be affecting other aspects of the brain on a smaller scale such as receptor expression, cell signaling or cell morphology that would not manifest as an overall volumetric change. In mammal studies, molecular effects, such as changes in calcium signaling, transcription factors and physiology could occur without changes in anatomy (Basha et al., 2006; Hanneman et al., 1996; Huang et al., 2000; Koclavanti, 2004; Ozcan et al., 2004). Thus, PCB 126 could be affecting some other aspects of brain chemistry, physiology or ultrastructure without any significant changes in Hp size. For instance, memory impairments like those observed following PCB occur due to changes such as reduced synaptic plasticity (J. J. Kim & Diamond, 2002), altered receptor function (eg. NDMA) (Nakazawa et al., 2002; Tsien,

Huerta, & Tonegawa, 1996) or altered connectivity (Izquierdo & Medina, 1997), to name a few. Understanding whether these changes occur in avian species as well requires further study.

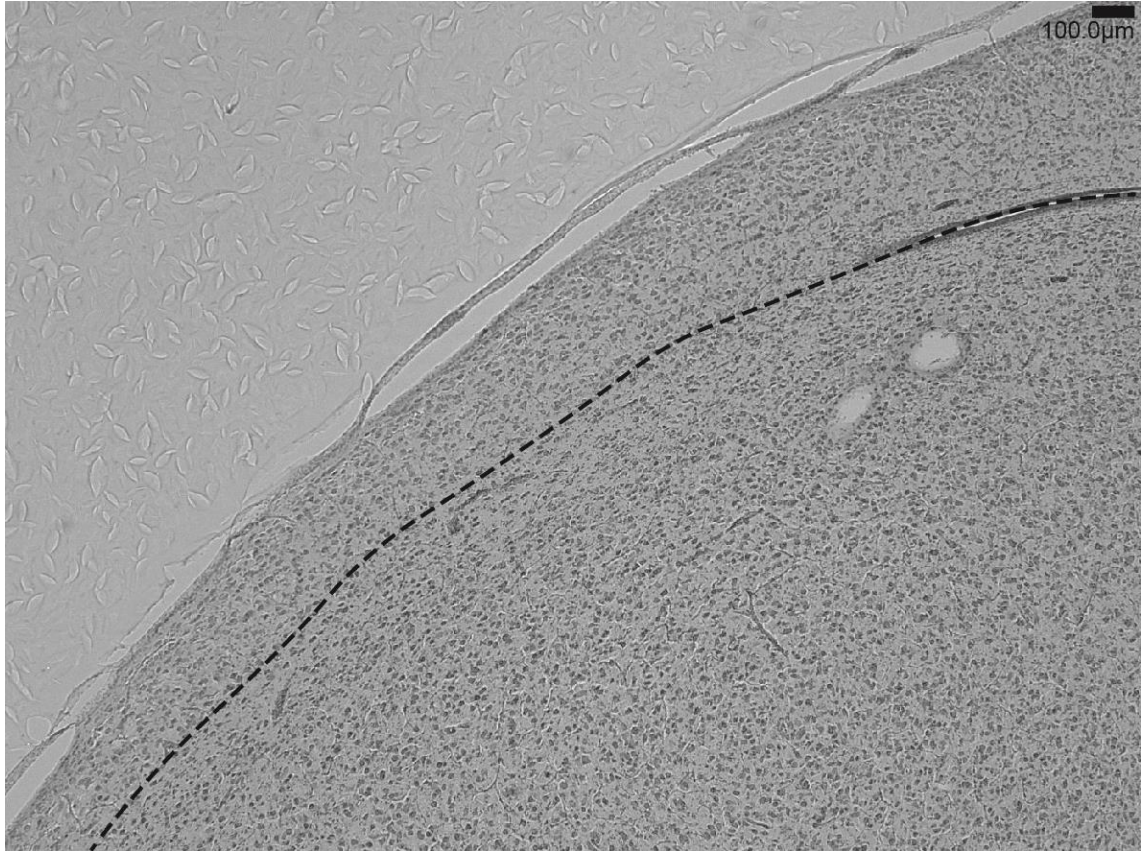
## **Conclusions**

Overall, we did not find evidence that embryonic PCB 126 exposure affects brain volume brain asymmetry, or hippocampal volume in the domestic chicken. Although this study found no effects, strain and species differences in response to dioxin-like PCBs may play a role in how the brain responds to exposure. Furthermore, future studies may wish to use different exposure routes such as yolk injections or maternal deposition and utilize several different vehicles to observe the effects on mortality. Investigating the effects of combined toxicants, including a number of dioxin-like and non-dioxin like PCBs, is a useful next step to determine the cumulative effects of toxicant exposure, which is more similar to what is experienced in the natural environment, rather than testing the effect of a single toxicant.

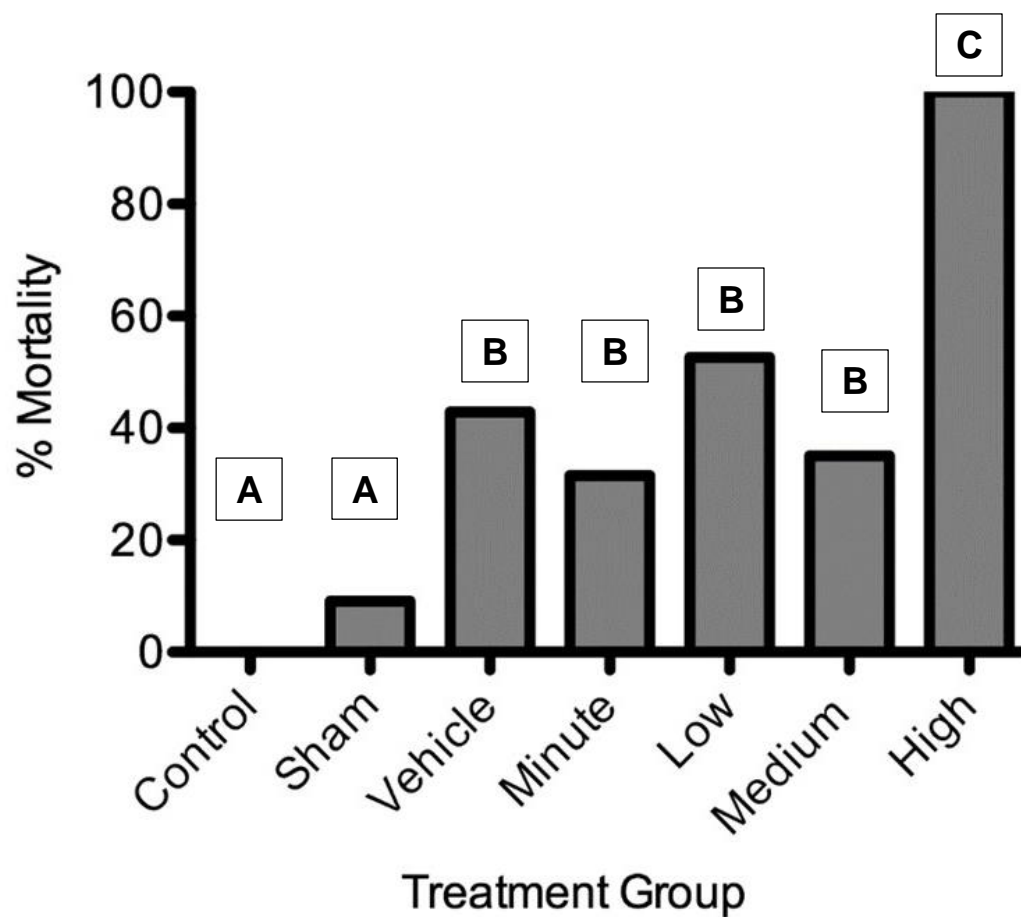
## CHAPTER 3 FIGURES



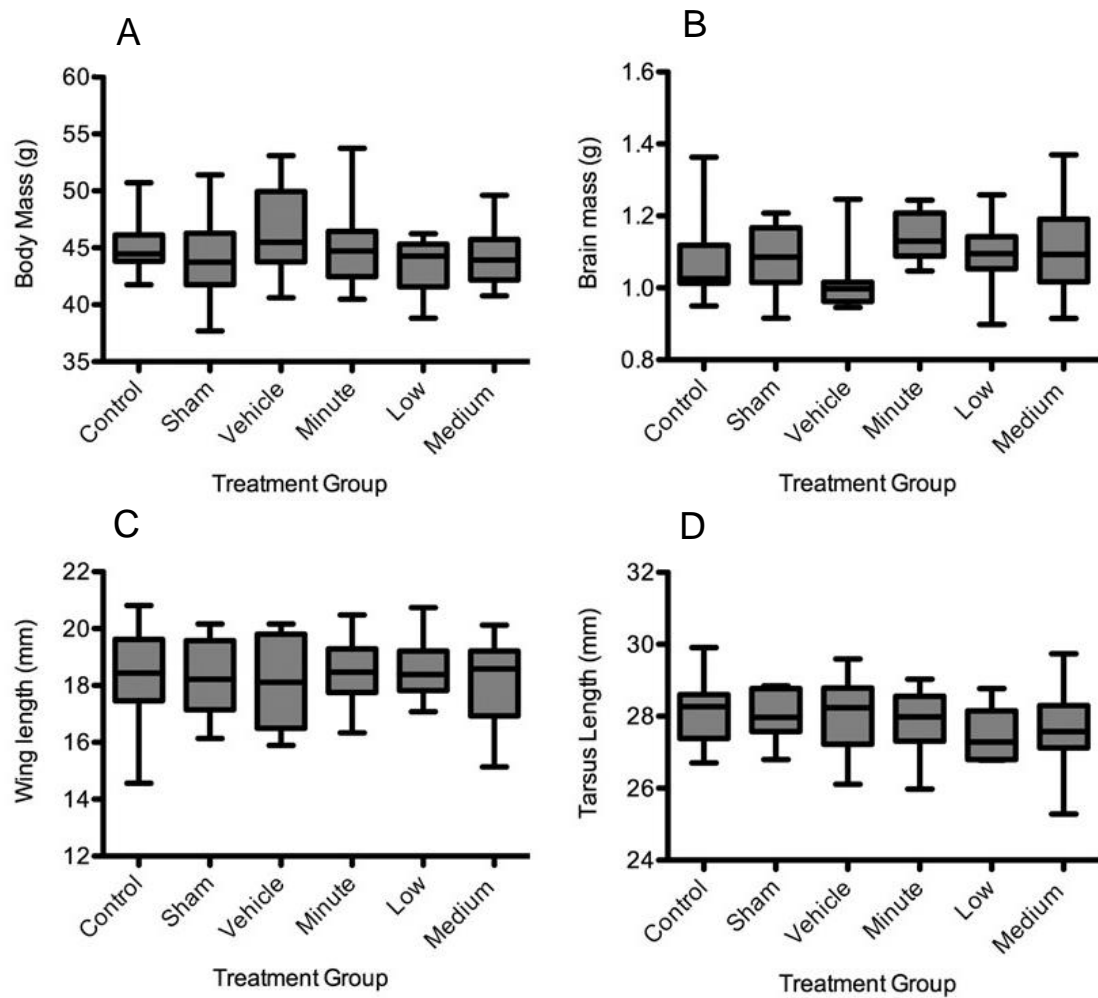
**Figure 3.1** - Coronal Nissl stained sections of the chick brain depicting the borders of the hippocampal formation rostrocaudally through the brain.



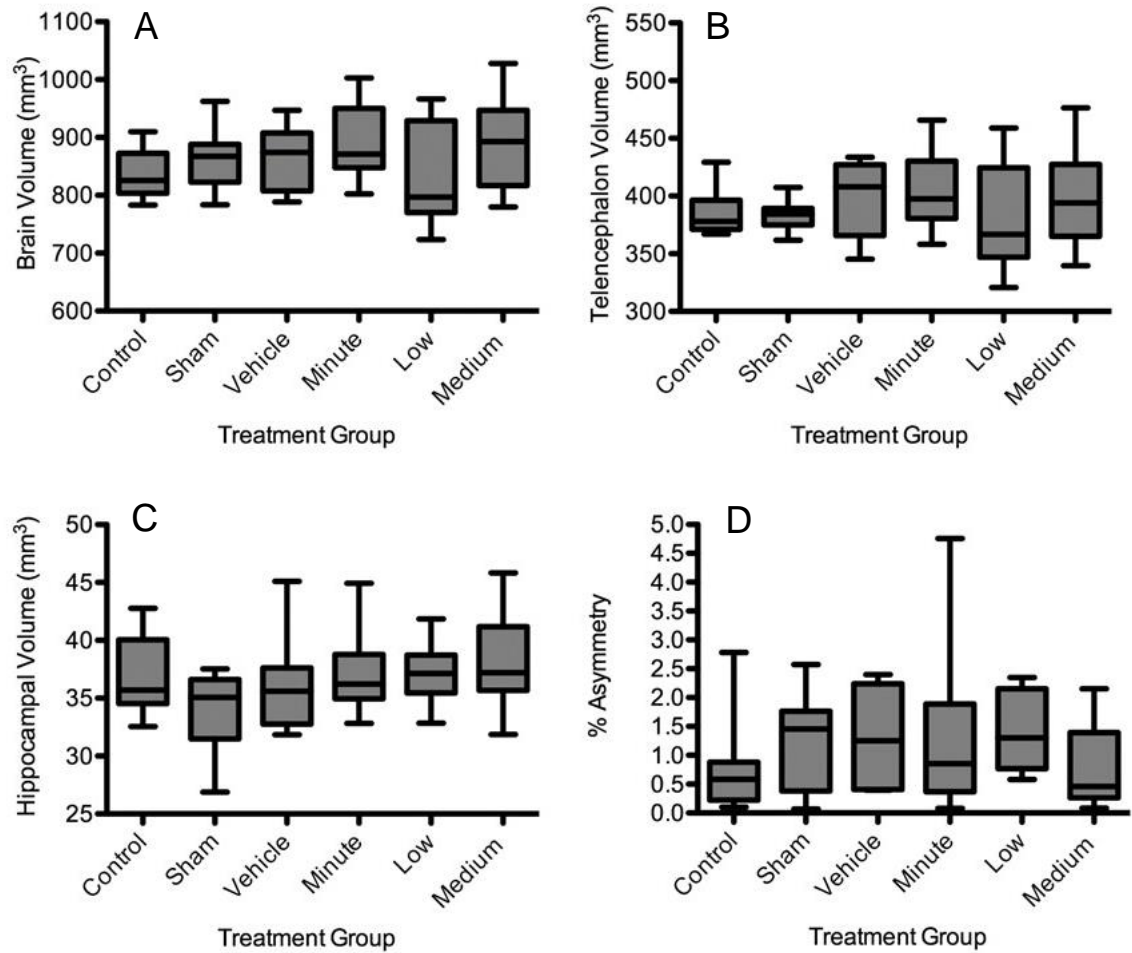
**Figure 3.2** - The chick hippocampal formation in coronal section, stained with Nissl, under a 5x objective, depicting the lateral border of the hippocampal formation which extends from the lateral ventricle and is distinguished by a more uniform cell distribution within the HF and a slightly curved alignment of cells.



**Figure 3.3** – Bar graph of the percentage of mortality for each treatment group. Control, n = 17. Sham, n = 11. Vehicle, n = 14. Minute, n = 19. Low, n = 19. Medium, n = 20. High, n = 13. Different letters denote groups with significantly different mortality, the same letter indicates that these groups did not differ significantly from each other

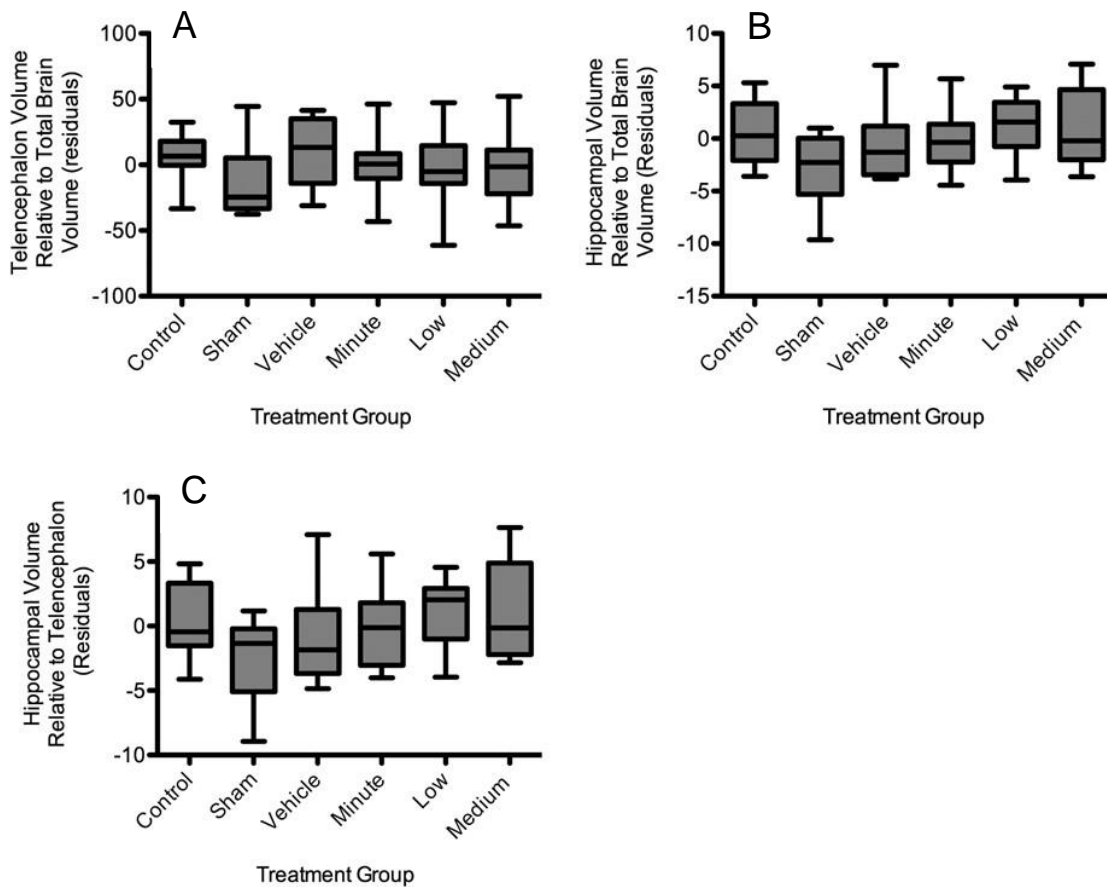


**Figure 3.4** - Box and whiskers plot of a) body mass, b) brain mass, c) wing length and d) tarsus length across each treatment group. Horizontal lines within the box represent the mean for each group and error bars represent the minimum and maximum values within each group.



**Figure 3.5** - Box and whiskers plot of A) absolute brain volume B) absolute telencephalon volume C) absolute hippocampal volume and D) percentage of asymmetry across each treatment group. Horizontal lines within the box represent the mean for each group and error bars represent the minimum and maximum values within each group.





**Figure 3.6** - Box and whiskers plot of A) relative telencephalon volume as compared to total brain volume B) relative hippocampal volume as compared to total brain volume and C) relative hippocampal volume as compared to telencephalon volume. Horizontal lines within the box represent the mean for each group and error bars represent the minimum and maximum values within each group.

## **CHAPTER FOUR: GENERAL DISCUSSION**

I examined the effects of early developmental exposure to PCB 126 in two animal models, the American mink and the domestic chicken. In mink, I measured volumes of the neocortex, corpus callosum, total hippocampus and hippocampal subfields to determine if developmental PCB 126 exposure affected brain volume. I also counted pyramidal and granule cells in the hippocampus and measured cell sized of both these types of cells. Overall, I found no effects of PCB 126 on the hippocampus. In the cortex, developmental exposure to PCB 126 decreased neocortical volume in males and decreased corpus callosum volume in both males and females. These effects may be a result of the endocrine disrupting properties of PCB 126 disrupting important hormones from the thyroid and gonads during neurodevelopment. In chickens, PCB 126 exposure did not affect brain volume, telencephalon volume, hippocampus volume or telencephalon asymmetry. In sum, my results indicate a much smaller effect of PCB 126 on brain development than expected and an obvious difference between the mammalian and avian species. Although these results could be interpreted as suggesting that PCB 126 is simply not very toxic, the myriad of deleterious effects documented in other organs and tissues such as the heart (Carro et al., 2013; Lind et al., 2004; Walker & Catron, 2000), pancreas (Nyska et al., 2004) and bones (Lind et al., 2000), as well as effects on the immune system (Fox & Grasman, 1999) and endocrine system (Crinnion, 2011; Schell et al., 2014 ; Schell et al., 2008) illustrates that it is indeed toxic. Although this research represents a critical first step in understanding the neuroanatomical effects of PCB exposure, there are a variety of factors to consider before drawing conclusions about the effects of PCB 126 and PCBs in general on brain development.

The hippocampus was chosen as the main brain area of assessment in both studies because of the reported effects of PCB exposure on learning and memory (Kakeyama, Endo, Zhang, Miyazaki, & Tohyama, 2014; Schantz et al., 2003; Ulbrich & Stahlmann, 2004) and because PCB 126 is thought to be an endocrine disruptor (Van den Berg et al., 1998). Through the activation of the aryl hydrocarbon receptor (AHR), PCB 126 is thought to affect thyroid and sex steroid hormones, both of which are important for the development of the hippocampus (Parent et al., 2011). Furthermore, PCB exposure increases cell death in the hippocampus (Tofighi et al., 2011), decreases nicotinic receptor density in the hippocampus (Eriksson & Fredriksson, 1998), reduces hippocampal long term potentiation (Niemi et al., 1998; Ozcan et al., 2004) and reduces hippocampal plasticity (Gilbert, 2003). Despite this evidence, I found no significant effects of developmental PCB 126 exposure on hippocampal volume in either species and no significant effects on cell density or sizes in the mink. Although this could imply that PCB 126 does not affect hippocampal anatomy, there are several other possible explanations for why no effects were detected.

First, despite its usefulness in assessing overall morphological differences in the brain, volumetric analysis is limited in what it can tell us and the biological relevance of variations in volume without additional measurements is somewhat unclear (Roth, Brodin, Smulders, LaDage, & Pravosudov, 2010). For instance, we can say that one area of the brain is larger or smaller, but we do not know whether this difference is a result of increased cell number, increased cell size, increases in glial cells, increased dendritic branching or other anatomical changes. That said, in the mink, there was also no evidence of a significant effect of PCB 126 exposure on cell sizes or numbers. A lack of

volumetric changes does not, however, mean that there may not be any effects at the cellular or molecular level. Changes in receptor densities, cell signaling, and cell distribution can occur without overall changes in brain morphology (Basu et al., 2010; DeVries & Panzica, 2006). Teasing out these changes requires a variety of techniques including receptor labelling, immunohistochemistry, and electrophysiology. Ideally, these questions about neurological effects would be addressed in concert with behaviour and endocrine studies to determine if changes in the brain are coincident with changes in hormone profiles and behaviour.

Second, the age of the animals in our study must be considered when drawing conclusions about the effects of PCB 126 on neurodevelopment. Mink kits were sacrificed at six weeks of age and chicks were sacrificed immediately after hatching. It is possible that effects may only be measurable in adult animals that experienced both developmental and dietary exposure throughout their lifetime (Rutkiewicz, Bradley, Mittal, & Basu, 2013). Furthermore, the principle of delayed neurotoxicity suggests that exposure to endocrine disruptors may cause damage that is invisible until the brain is challenged later in life by complex behavioural tasks or aging (Weiss & Reuhl, 1994). This effect is commonly observed following early methylmercury exposure (Stern, Cox, Cernichiari, Balys, & Weiss, 2001; Weiss, Stern, Cox, & Balys, 2005), but may also apply to dioxins and dioxin-like PCBs. Ideally, unmasking this effect in animals exposed to PCBs would require complex behavioural tasks that challenge the brain in concert with assessment for neuroanatomical changes throughout the lifespan of the animal. Allowing animals to age to adulthood and challenging them with behaviourally relevant tasks could unmask some of the effects of PCBs on the brain and behaviour.

Third, outside of the lab, animals and humans are never exposed to only a single PCB or endocrine disrupting chemical (Giesy & Kannan, 1998). As discussed in chapter 3, the toxicity of PCBs needs to be assessed with consideration of arcolors of dioxin-like and non-dioxin-like PCBs in concert with other contaminants commonly found in the body because the additive toxic effects of these contaminants and their various mechanisms of action may be much different than the individual effects of a single toxicant in a laboratory environment (Giesy & Kannan, 1998). However, we also need to understand the effects of individual toxicants and the mechanisms of action for each. Even within the same category, toxicants may have very different effects and mechanisms, as is the case with dioxin-like and non-dioxin-like PCBs (Giesy & Kannan, 1998; Henry & De Vito, 2003). The need for studies such as these are complicated by the large number of environmental toxicants, the individual effects and the interaction effects, and the variety of different chemical congeners with variable effects on the brain and body.

Fourth, PCBs may not be reaching high concentrations in the brain of either mink or chicks because they are being sequestered in areas of the body where they are doing less damage. Often, toxicants tend to accumulate in the liver, kidney and feathers (of birds) in much higher concentrations than in more sensitive organs such as the heart and brain (although the toxicants are still present in measurable concentrations) because sequestering them in these areas may do less damage (Chen, Hamm, Hass, & Birnbaum, 2001; Rutkiewicz & Basu, 2013). This may explain why PCBs and dioxins seem to have a much greater effect in peripheral areas of the body than in the central nervous system, due to higher concentrations of PCBs and more detrimental effects in areas like the liver

(Bursian et al., 2006; Cocchi et al., 2009; Heaton et al., 1995), bones (Cocchi et al., 2009), pancreas (Nyska et al., 2004), and heart (Carro et al., 2013; Jokinen et al., 2003). While this hypothesis does not account for the effects observed in the mink neocortex, perhaps the neocortex is more sensitive to PCB levels than the hippocampus and sequestration of PCBs in peripheral organs prevented PCBs from reaching a level of toxic effects in the hippocampus. Testing this would require a follow up experiment measuring PCB levels in both organs and the brain before measuring changes in the brain.

These considerations apply to both the mink study and the chick study, but unlike the mink study where I found an effect in the mink neocortex, in the chick study I found no significant effects of PCB 126 on any of the measurements. That is, early PCB 126 exposure did not affect brain volume, telencephalon volume, hippocampal volume or brain asymmetry. One difference between these two animal models was the length of PCB exposure. In mink, kits were exposed throughout gestation (through the pregnant mother's diet) and then until weaning at six weeks of age (through lactation) and had a period of exposure over two months long. In contrast, chicken eggs were injected on embryonic day 0 and were sacrificed immediately after hatching 19-21 days later, resulting in only approximately three weeks of exposure. Furthermore, it could be argued that the mink route of exposure was a much more natural route of exposure versus the egg injection, which could result in different types of toxic effects (Rutkiewicz & Basu, 2013). A more natural route of exposure for avian species may be feeding the mothers the toxicants or injecting them with the toxicants so that the toxicants are naturally deposited into the laid eggs (Heinz, Hoffman, Klimstra, & Stebbins, 2010). Although, in field situations the deposition of toxicants can sometimes be uneven depending on egg order

(Lukowski, 1978), this depends on the level of contamination in the mother and the number of eggs laid (Custer, Gray, & Custer, 2010; Van den Steen et al., 2006) and the levels of toxicants can be tested in each egg (Custer et al., 2010). Finally, chicks are much more precocial immediately after hatching while mink are altricial and the effects of toxicants may vary depending on the degree of altriciality.

The results of PCB 126 exposure on white matter volume and neocortex suggest that PCB 126 does have some effect on the brain, at least in mink. These changes observed in the mink likely reflect the effect of PCB 126 on thyroid and sex hormones, which are important for proper brain development. The effects on neocortical volume and corpus callosum volume in mink are still a concern for human populations. Many people in North America live in PCB contaminated areas and consume fish and food items contaminated with PCBs. High concentrations of PCBs in humans are associated with decreased IQ (Jacobson & Jacobson, 1996), delayed psychomotor and mental development (Park et al., 2010), impaired executive function (Boucher et al., 2009) and impaired visual recognition memory (Boucher et al., 2009). The volumetric changes in the mink brain might underlie these behavioural effects. For instance, corpus callosum damage is associated with cognitive dysfunction (Mesaros et al., 2009) and lowered verbal IQ and verbal fluency (Nosarti et al., 2004). Meanwhile neocortical damage can produce widespread deficits including motor impairments (Whishaw, Alaverdashvili, & Kolb, 2008) and impaired executive functions (Robbins, Weinberger, Taylor, & Morris, 1996), depending on which areas of the neocortex are affected. Future studies should assess for these deficits and perform behavioural tests on relevant animal models before

examining the brains for morphometric changes so that behavioural changes and changes in the brain following PCB exposure can be directly correlated.

Despite its limitations, this research is one of the first to characterize the neuroanatomical effects of early PCB exposure and represents a starting point for further research on the effects of dioxin-like PCBs, non-dioxin like PCBs and other endocrine disrupting chemicals with poorly understood neurological effects. PCBs may be banned, but as long as they exist in the environment and bioaccumulate in the bodies of humans and animals, they still warrant further study.



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