CHAPTER I

INTRODUCTION

1.1. Background

Bisphenol A or 2,2-bis(4-hydroxyphenyl)propane (BPA) is a human-made small chemical molecule consisting of two phenolic rings joined together with methyl bridges (Naderi & Kwong, 2020). It was first synthesized in 1891 and explored during the discovery of synthetic estrogen by Edward Charles Dodds, a researcher from the University of London. Instead of being used as a synthetic estrogen, BPA was commercialized as a raw material for manufacturing plastics and epoxy resins in the early 1950s(Vogel, 2009). It can be found in different daily products, including dental cement, food, beverage cans, protective coatings, thermal receipt papers, infant products, implanted medical devices, and other plastic products (Ben-Jonathan & Hugo, 2016; Vogel, 2009). The extensive use of BPA on these products has led to environmental contamination and further bioaccumulation in humans and other living organisms (Corrales et al., 2015). The concern about the health effect of BPA started to emerge in the late 1990s. Collective study results on BPA have shown that BPA exerts a potential endocrine disruption activity (Bhat et al.,2019). As a result, BPA use has been strictly regulated by health authorities and become one of the public health concerns in different countries (Mandel et al.,2019)

Following the public health concern and regulation on BPA usage, BPA analogs have gained attention by industries as replacement chemicals. Bisphenol S or 4-hydroxyphenyl sulfone (BPS), bisphenol F or 4,4'-methylenediphenol (BPF), and bisphenol AF or (4.4'-hexafluoroisopropylidene)diphenol (BPAF) are the most commonly used analogs for BPA substitutions(Zhang et al., 2017). A review by Chen et al. recorded that contamination of these BPA analogs was found in different media. The occurrence in environmental compartments, such as indoor dust, sediments, surface water, sewage effluent, and sludge, has been reported in various countries. Among BPA analogs, BPF and BPS were demonstrated to be the first and second most abundant BPA

analogues in the environment (Chen et al., 2016). Other groups of studies also detected the contamination of these analogues in food products with BPF being the first abundant analogue found. Whereas in other consumer products, the level of these analogues was found in variative levels according to the respective analogues (Dodson et al., 2020). Although these data suggest that BPF has the most extensive occurrence in different media, BPS was accumulated in the human body in significant amounts. A multinational study conducted in the US and seven Asian countries revealed that BPS was found in 81% of human urine samples. The median concentration of BPS in this study was slightly lower than BPA (Zifeng Zhang et al., 2011). In other studies, conducted in Saudi Arabia, BPS concentrations were above the BPA concentration in the urine samples (Asimakopoulos et al., 2016). Consistent with this result, the frequency of BPS was found in 78% of urine samples collected from American, which was higher than the BPF found in 55% of the samples. The concentration of BPS (0.13 ng/mL) was also found to be higher compared to the BPF (0.08 ng/mL) in this study (Zhou et al., 2014). Combined, these data indicate that BPS might significantly affect humans compared to other BPA analogues due to high bioaccumulation.

Unfortunately, the safety profile of BPS was not well studied prior to its application as a replacement for BPA. While as a chemical replacement, BPS is expected to be chemically inert and less toxic than BPA. BPS is considered a safer substitution due to high thermal and light stability, attributed to the strong O=S=O double bonds formation (Lee, Kim, Shin, Kho, & Choi, 2019). On the other hand, BPS still shares similar structural properties to BPA, which opens up the possibility for similar toxicological profiles. Several studies have demonstrated that BPS interferes with endocrine systems identical to BPA. As a member of the bisphenol family, BPS still shares a basic phenolic OH group that resembles the 3-hydroxyl group of estradiol (E2). It has been shown to interact with estrogen receptors (ER) and induce the alteration in estrogen-responsive genes, mostly through activation of membrane receptor-mediated pathways. In concordance with this, the alteration in other sex hormones, including testosterone and progesterone, were also observed. BPS has also been shown to interact with androgen receptors (AR) in weak binding affinity and exert a sex-specific androgenic effect (Naderi &

Kwong, 2020). Therefore, the BPS exposure is strongly linked to reproductive and developmental toxicity resulting in an alteration in reproductive system structure, reduced hatching success, increased mortality, and skewed sex ratio (Qiu et al., 2019).

A recent study by da Silva et al. reported that BPS exposure also interferes with thyroid hormone systems, in addition to the sex hormones. An increase in the T4 serum level and a decrease in serum T3 level were identified in the Wistar rats model exposed to low concentrations of BPS (da Silva et al., 2019). Previous *in vitro* studies also suggested that BPS can disrupt the thyroid hormone systems by interacting with thyroid hormone receptors, altering transcriptional regulation, and changing the thyroid hormone homeostasis. A crystallographic study conducted by Zhang et al. showed that BPS has a binding affinity to the transthyretin protein, which is responsible for T4 transportation. This binding is responsible for the increase in free T4 level in the serum (J. Zhang et al., 2016). Another study by Lu et al. demonstrated that BPS has the same binding site with T3 in the thyroid hormone receptor beta ligand-binding domain (LBD), thus suggesting competitive binding to thyroid hormone receptors (Lu et al., 2018). However, these mechanistic studies did not merely explain the causative relationship between BPS exposure and thyroid hormone disruption. Inconsistent effects of BPS on thyroid hormone level have been observed in several studies. In a transgenerational study, bisphenol S exposure has led to a significant decrease in serum T4 and an increase in serum T3 of F0 and F1 zebrafish generations, which is the opposite of a recent study by da Silva et al. (Wei et al., 2018). Surprisingly, another study conducted in the zebrafish observed that BPS exposure as high as 100 µg/l was able to significantly decrease the serum T4 and T3 level (Naderi et al., 2014). In addition, a recent study by Sangwoo et al. generated other conflicting results. In their study, the serum T4 and T3 levels were both increased in embryo-larval zebrafish at 120 hours post fertilization (Lee et al., 2019).

Despite the inconsistent BPS exposure and thyroid hormone level relationship, it has been clear that disruption in thyroid hormones by BPS is linked with neurodevelopmental impairment. During the neurodevelopmental period, the exposure of BPS has been investigated in previous studies in Chinese pregnant women, which showed that BPS was detected in 93.7% of maternal urine samples.

The concentration of BPS found was associated with the pregnancy duration, in which higher levels of BPS in maternal urine was found in pregnancy durations over 41 weeks (Wan et al., 2018). A recent longitudinal cohort study by Jiang et al. has revealed that this maternal exposure of BPS was associated with retardation of children's psychomotor at two years old, which suggested to be associated with impairment in estrogen or thyroid hormone signaling (Jiang et al., 2020). Meanwhile, in *in vitro* and *in vivo* setting, BPS prenatal exposure has been shown to interfere with serotonergic neurotransmission, axon guidance process, and transcription of the gene associated with neuronal regeneration, neuroplasticity, neuroprotection, and memory formation, as reviewed by Naderi & Kwong. These effects were shown to be mediated by direct modification of BPS in the hypothalamic-pituitary-thyroid axis (HPT) (Naderi & Kwong, 2020). This evidence suggests that prenatal BPS exposure can lead to serious adverse effects on neurodevelopment mediated by the disruption in thyroid hormone levels. However, there are still many uncertainties in the mechanistic aspect of how prenatal BPS exposure and thyroid hormone disruption can lead to neurodevelopmental defects.

A study by Nakamura et al. has demonstrated one of the mechanistic evidences of disruption of thyroid hormone of BPA that lead to neurodevelopmental disorder. They revealed that prenatal BPA exposure disrupts the murine cortical histogenesis by altering the gene expression through antagonistic activity towards THRα. It has been shown to alter the expression of *Ngn2*, *Mash1*, and *Math3* (Nakamura et al.,2006). The genes encode for basic-helix-loop-helix (bHLH) transcription factors which are responsible for neuronal proliferation and differentiation (Chen et al., 2012). Therefore, it can be concluded that BPA antagonistic activity towards THRα leads to the alteration in the neurogenesis process through transcriptional regulation. This argument is supported by a previous study that showed THRα activation contribute to neurodevelopment by altering the transcriptional activity (Kapoor, Fanibunda, Desouza, Guha, & Vaidya, 2015). Unfortunately, such analysis has not been conducted with BPS exposure. Therefore, the expression of *THRα* was analyzed in this study following maternal exposure to BPS. The expression study was conducted at two-time points of development during embryonic day 16 (E16) and postnatal day 1 (P1). Embryonic day 16 was

considered as the critical time point for thyroid hormone involvement in mice prenatal brain development. It is marked by establishment of hypothalamus-pituitary-thyroid axis and peak of *THRα* expression in the brain, which is observed to remain steady until the birth (Howdeshell, 2002). Therefore, further analysis at postnatal day 1 was also conducted to identify whether the prenatal effects persisted until postnatal behaviour. This analysis is necessary considering the possibility of reversible effect and time point-specific effect, which is possibly associated with the duration of the BPS exposure (Gilbert et al., 2012; Nesan et al., 2018; Rivollier et al., 2019). A further systematic review is also conducted to provide theoretical arguments on the neurodevelopmental effects of bisphenol S. This systematic review will analyze the significance of the neurodevelopmental effects of BPS compared to BPA in preclinical models. Combined, the data from molecular analysis and systematic review hopefully can improve the understanding of the mechanism of the neurodevelopmental effect of BPS.

1.2. Objectives

The general objective of this project is to identify neurodevelopmental changes in the brain following maternal exposure to bisphenol S, which will be achieved through:

1.2.1. Systematically reviewing the neurodevelopmental effects of bisphenol S and bisphenol A exposure in preclinical models.

1.2.2. RT-PCR analysis of *THRα* gene expression on E16 and P1 mice brains following maternal exposure to BPS.