CHAPTER 1

INTRODUCTION

1.1. Background

The neuroendocrine systems, particularly the hypothalamic-pituitary-thyroid (HPT) axis, are among the vital regulators of fetal brain development. The HPT axis orchestrates brain development through tight regulation of thyroid hormone during the development, resulting in an influential activity in the development of cognition, motoric function, and mood regulation (Moog *et al.*, 2017; Sahay & Nagesh, 2012). The proper development of these physiological features is possible through a finely tuned regulation of thyroid hormone, starting from the neurogenesis, migration, development of the cerebral cortex, to terminal differentiation of neuron and glia at the late stage of brain development, myelination, and synaptogenesis (Miranda & Sousa, 2018). The important role of thyroid hormone in these processes is related to its action on the nuclear receptors such as thyroid receptor α and thyroid receptor β (Gil-Ibáñez *et al.*, 2014). This action directly results in the upregulation of the Sonic Hedgehog (*Shh*) gene, which is responsible for developmental morphogenesis, the Hairless (*Hr*) gene, encoding for a transcription corepressor, and the transcription factor Krüpfel-like transcription factor 9 (*Klf9*). The varying level of *Shh* expression during cortical development has been demonstrated to guide the proliferation of neuronal progenitors to their differentiation to specific neuronal and glial lineage (Cohen *et al.*, 2013; Maatough *et al.*, 2018; Tichy *et al.*, 2015; Yabut & Pleasure, 2018). Hairless exerted its function by binding to free thyroid hormone receptors, allowing the autoregulation of the thyroid hormone functions. Despite the unknown significance of *Hr* in the brain, current studies indicated that the downregulation of *Hr* promotes the growth and survival of glioblastoma, suggesting the role of *Hr* in keeping neural growth and development in check (Brook *et al.*, 2019) The expression of *Klf9* plays a role in neuronal maturation, differentiation, neurogenesis, and synaptic plasticity (K. Yin *et al.*, 2015).

The disruption of thyroid hormone regulation within the HPT axis poses a threat of neurodevelopmental disorder, in which the clinical manifestations can be delayed several years after birth (Mughal *et al.*, 2018; Rivollier *et al.*, 2019). The disruption can shift the balance of thyroid hormone to either too little (hypothyroidism) or too much (hyperthyroidism), both of which are deleterious to neurodevelopment. For example, maternal hypothyroidism during pregnancy has been considered as a risk factor for reduced IQ score and neurodevelopmental diseases, leading to cognitive impairments and behavioral problems, in the conceived children (Andersen *et al.*, 2014; Endendijk *et al.*, 2017; Mughal *et al.*, 2018; Rayman & Bath, 2015). Furthermore, a recent meta-analysis even confirmed that mild hypothyroidism during pregnancy is positively associated with the intellectual disability of the offspring, suggesting the sensitivity of developing fetal brain to disrupted regulation of thyroid hormone (Thompson *et al.*, 2018). Thus, the disruption of the thyroid regulation by the HPT axis, including due to endocrine-disrupting chemicals with antagonistic activity to thyroid receptors, during pregnancy can be a driver of mental retardation, impaired cognitive performance, and neurobehavioral problems. (Gilbert & Lasley, 2013; Gilbert *et al.*, 2012; Kortenkamp *et al.*, 2020; Min *et al.*, 2016; Thompson *et al.*, 2018). In contrast, maternal hypothyroidism has been reported to contribute to the elevated risk of attention deficit hyperactivity disorders (ADHD), autism spectrum disorder, and intellectual impairment in the conceived children (Andersen *et al.*, 2013; Andersen *et al.*, 2014; Andersen *et al.*, 2018).

The threat of the disruption of the HPT axis is becoming even more apparent due to the ubiquitous presence of endocrine-disrupting chemicals, such as Bisphenol A (BPA), on daily basis. BPA is a chemical used as a precursor for epoxy resin and polycarbonate plastics (Almeida *et al.*, 2018). As BPA-based polycarbonate (PC) plastics offer desirable physical properties, such as exceptional rigidity, transparency, and stability of the material, BPA-based PCs are desirable for making food and beverage containers, including water and baby bottles. Furthermore, BPA-based epoxy resin is also used as an internal coating for canned food or beverages to prevent corrosion due to direct contact between the can with its content. Despite being extensively applied in various food-related applications, Bisphenol

A can still leach out from the container and contaminate its content. The leaching out of BPA is worsened by heating, contact with acids or alkaline, frequent use, and microwave heating (Bertoli *et al.*, 2015). Further findings of BPA and its metabolite in human urine further confirmed that BPA intake is possible through contaminated food and beverages (Thayer *et al.*, 2015).

The concern on the evidence of the ability of BPA to enter the body becomes even more worrisome as long-term BPA exposure, which is highly feasible in the real-life scenario, has been demonstrated to bring out deleterious health effects. The earliest health concern of BPA is related to its estrogenic properties. The estrogenic activity of BPA has been associated with increased risks of infertility, early puberty, breast cancer, prostate cancer, and polycystic ovary syndrome (Barrett & Sobolewski, 2014; Fenichel *et al.*, 2013; Konieczna *et al.*, 2015; Matuszczak *et al.*, 2019). However, the concern of the antagonistic effect of BPA on thyroid hormone receptors has at least an equal caliber as its deleterious estrogenic activity. The antagonistic effect of BPA on the thyroid hormone receptor is reflected in the ability of BPA to competitively bind to nuclear thyroid hormone receptor with the triiodothyronine (T3), the active form of thyroid hormone, and prevent gene expression by recruiting transcriptional repressors in vitro (Moriyama *et al.*, 2002). Several clinical studies had demonstrated that there is a correlation between maternal exposure to Bisphenol A, especially during pregnancy, with the occurrence of neurodevelopmental disorders, manifested as cognitive and behavioral problems in the conceived children at an early age of life (Barrett & Sobolewski, 2014; Braun *et al.*, 2011; Harley *et al.*, 2013; Perera *et al.*, 2012; Roen *et al.*, 2015). As a lot of evidence has suggested that maternal exposure to BPA may lead to neurodevelopmental disorders in infants and children, the use of BPA for producing baby bottles has been prohibited and strict limitation has been applied for food-grade applications (Adeyi & Babalola, 2019). However, the regulatory restrictions do not apply for the BPA analogs, such as Bisphenol F, Bisphenol S, and Bisphenol AF, allowing the manufacturers to use bisphenol analogs to produce polycarbonate-based plastic products and label their products as “BPA-free”.

The use of BPA analogs, such as Bisphenol S (BPS), has been hypothesized to be as risky as BPA itself. However, the bodies of evidence to support the validity of the risk are still lacking. Moreover, BPS becomes even more desirable to substitute BPA due to its lower estrogenic activity and superior resistance to heat and sunlight compared to BPA, suggesting the better suitability of BPS for food and beverage containers, including baby bottles, than BPA (Herrero *et al.*, 2018). This suggests a potential exodus from the widespread use of BPA to the extensive use of BPS in the plastic industry. However, BPS also shares a considerable structural similarity with BPA, suggesting similar hypothetical health consequences as BPA (Guo *et al.*, 2016). This suggests that BPS has a potential thyroid-disrupting capacity as observed in BPA, indicating the potential of maternal exposure to BPS can lead to neurodevelopmental disorders. Several recent in vitro and in vivo studies have demonstrated the potential of BPS as a thyroid disruptor and promote neurodevelopmental problems, albeit, the evidence is scarce (da Silva *et al.*, 2019; Jiyun Lee *et al.*, 2018; Zhang *et al.*, 2018). However, the potential threat of BPS is becoming an actual issue as BPS has been detected in human urine, blood, serum placental tissue, breast milk, maternal and cord blood plasma, and semen, suggesting evidence of widespread risk of BPS exposure in humans (Deceuninck *et al.*, 2015; Ghayda *et al.*, 2019; Li *et al.*, 2020; Liu *et al.*, 2017). Several clinical studies had demonstrated a positive association between maternal exposures to BPS with the potential occurrence of neurodevelopmental disorders in their offspring (Aker *et al.*, 2018; Jiang *et al.*, 2020). However, the number of studies is still very limited, showing the lack of understanding of the threat of BPS to child neurodevelopment.

A previous project by Moseselli (2019; unpublished) has attempted to investigate the potential threat of potential neurodevelopmental threat following maternal exposure to BPS in mice. The project evaluated the effect of prenatal expose to BPS on Day 14 (E14) of embryonic development and revealed several adverse effects, particularly the loss of intermediate zone (IZ) in the brains of the exposed embryos. However, two years since the completion of the project, it is still unknown whether the adverse developmental effects have deleterious consequences at the later stage of pregnancy or even after birth. Thus, further investigation is necessary to these potential maladies.

To address the health issues regarding the widespread use of BPS as a BPA substitute, this thesis project is aimed to investigate the health effect of maternal exposure to BPS through a systematic review of current studies on developmental effects of BPS and an *in vivo* study using mice model. Specifically, the systematic review is aimed to identify, evaluate, and summarize the current studies on the developmental effects of BPS, particularly neurodevelopment. Whereas, the *in vivo* study aims to investigate the effect of maternal exposure of BPS at two later points of development, day 16.5 of embryonic development (E16.5) and Day 1 of postnatal development (P1). The evaluation of the effect of the BPS exposure is assessed through structural evaluation and morphometric analysis of the cortical plate of the E16.5 embryos and P1 pups. This project is expected to contribute to providing another evidence regarding the potential detrimental health effect of maternal exposure to BPS at a later stage of fetal brain development and early life period.

1.2. Problem Formulation

Based on the background of this thesis project the problem formulations of this thesis is as follows:

- What are the current clinical studies that have been conducted to study the developmental effects of maternal BPS exposure, especially to neurodevelopment?
- How are the qualities of evidence provided by current clinical studies on the developmental effects of maternal BPS exposure, especially on fetal neurodevelopment?
- How is the current knowledge on the developmental effects of maternal exposure to BPS, particularly on neurodevelopment?
- How are the structural changes of the brain, particularly the cerebral cortex, following chronic maternal exposure to BPS in E16.5 mice embryos and P1 pups?
- How is the effect of chronic maternal exposure to BPS on the thickness of the cerebral cortex and its layers in E16.5 mice embryos and P1 pups?

1.3. Objectives

This thesis project was conducted to investigate the developmental effects following maternal exposure to Bisphenol S through a systematic review and experimental study. The systematic review covers the clinical studies on developmental effects, particularly neurodevelopment following maternal exposure to BPS. The systematic review was conducted to identify recent clinical literature on developmental and neurodevelopmental effects following maternal exposure to BPS, evaluate the quality of the literature, and conclude the current understanding of the developmental and neurodevelopmental toxicity following maternal exposure to BPS. The experimental study comprised of a histological investigation on the structural changes in the brain, particularly the cerebral cortex, of mice embryos at gestational day 16.5 (E16.5) and mice pups at postnatal day 1 (P1). The histological investigation was conducted to compare the morphological appearance and thickness of the cerebral cortex and its layers of the mouse litter exposed to BPS with those receiving corn oil as a control treatment at E16.5 and P1.

1.4. Scope of Work

The scope of this thesis project covers the development of a systematic review of the clinical studies on the developmental effects of BPS and an *in vivo* study. The development of systematic review covers the formulation of the research question, derivation of PICOS (population, intervention, comparison, outcome, and study design) aspects, development of search strategy, retrieval of search results, title and abstract screening, full-text screening, data extraction, risk of bias assessment, quality assessment, and qualitative synthesis, along with review writing and reporting. Meanwhile, the *in vivo* study covers the histological evaluation of the brain, particularly the cerebral cortex, of E16.5 mouse embryos and P1 pups, following daily administration of BPS. The evaluation includes histological analysis of the cerebral cortex and morphometric analysis for measuring the thickness of the layers of the cerebral cortex.

1.5. Research Urgency

The health concern regarding the use of BPA as the basic materials of polycarbonate plastics and epoxy resins for common plastic wares has led to the increasing awareness to avoid such products to even the banning of BPA for most food-grade plastic appliances. Despite the positivity of such intentions and policies, these intentions and policies have driven plastic manufacturers, especially those relying on polycarbonate- and epoxy resin-based products, to replace their basic materials with BPA analogs, such as BPS. The reasons for this replacement are mainly because of the lack of scientific studies on the health effect of BPA analogs and the incentive of being able to label their products as BPA-free without sacrificing product quality. However, the structural similarities of BPA and its analogs suggest that BPA analogs, including BPS, poses at least equivalent health risks to BPA. Thus, studies on the health effects of BPA analogs, such as BPS, are urgently needed for a timely health risk identification before the health effects of the unregulated use of these chemicals escalate to a larger scale.

1.6. Expected Outcomes

The expected outcomes of this thesis projects cover a systematic review of clinical studies on the developmental effects of maternal BPS exposure, histological analysis of the cortical plate of the E16.5 embryos and P1 pups following maternal exposure to BPS, morphometric analysis of the embryos and pups following maternal exposure to BPS, thesis presentation in the form of a PowerPoint Presentation, and thesis report.

1.7. Benefits of the study

The outcome of this thesis is expected to contribute to providing a brief evaluation and summary of the current clinical studies of the developmental effects of maternal exposure to BPS and preliminary evidence on the potential effect of maternal exposure to BPS, particularly on neurodevelopment. The evaluation and summary of the current clinical literature on the effect of BPS will be a useful reflection in the current clinical research progress on the developmental effect of BPS,

thus, providing some potential considerations for future studies on developmental effects, especially on neurodevelopment, of maternal exposure to BPS. If the project manages to indicate any potential harm in a developmental mice model. In contrast, if the project fails to identify any negative effect of BPS on development, the result is expected to at least contribute to proving some evidence that BPS is a potential BPA substitute from a safety perspective.