Chapter I

Introduction

1.1 Problem Background

BPS or Bisphenol S is a structural analog of BPA which has been proposed as a safer alternative compared to BPA. BPS is commonly found in a variety of paper products such as paper currency, flyers, tickets, mailing envelopes, and airplane boarding passes. Among paper products containing BPS, thermal receipt papers account for 88% of human exposure making it a notable source of BPS exposure (Liao, Liu & Kannan, 2012). It is estimated that the average individual is exposed to a BPS median value of 4.18ng/kg body weight on a daily basis (Liao, Liu & Kannan, 2012). Furthermore, as cited in Chen et al. (2016), BPS has been detected in various food products including vegetables, seafood, canned foods, soft drinks, and cereals as well as various personal care products such as toothpaste, makeup and hair care products. In addition, a study involving HPLC-MS/MS analysis of Urine samples collected from 7 different countries (USA, Japan, China, Korea, India, Kuwait, Malaysia, and Vietnam) revealed that traces of BPS is detected in 81% of the 315 Urine samples collected (Liao et al., 2012). The concentration of BPS in the Urine was found to be up to 21 ng/mL with a geometric mean of 0.168 ng/mL (Liao et al., 2012)

As aforementioned, BPS has been used as an alternative to BPA out of safety concerns. However, there is little data to prove that using BPS to substitute BPA is safe. On the other hand, data from various *In vitro* studies and *in vivo* studies show that BPS can exhibit endocrine disrupting activities at a degree which is similar to BPA (Rochester & Bolden, 2015; Chen et al., 2016; Rosenfeld, 2017). In a review involving 4 in vivo and 18 in vitro studies investigating BPS exposure, 17 studies which compared the Endocrine disrupting potency of BPA and BPS suggest that BPS has a similar potency to BPA in terms of adipocyte hormonal signaling inhibition, androgenic, antiandrogenic and antiestrogenic effects. BPS mimics the

action of the hormone Estradiol and affect cell proliferation, differentiation and apoptosis via membrane mediated pathways .17 *in vitro* studies also suggest that BPS may exhibit Estrogenic activity which could likely induce undesired Estrogenic physiological responses (Rochester & Bolden, 2015). In one *in vitro* study, fetal mouse testicular assays were performed to compare the antiandrogenic effects of BPS and BPA. 3 days of 100 nmol/L of BPS exposure caused a decrease in Testosterone production while the same effect was not observed with 3 days of 100 nmol/L of BPA exposure (Eladak et al., 2015). Moreover, daily administration 10 ug/kg of BPS is shown to pregnant Wistar rats since gestational day 12 up to postnatal day 12 is shown to influence female rat offspring reduced mRNA expression levels of 5α-reductase 3 (*Srd5a3*), increased the expression level of the corticosteroid synthesis regulating gene *Cyp2d4* in rat prefrontal cortex and affected Serotonin and Dopamine expression pathways (Catanese & Vandenberg, 2016).

In summary, BPS is expected to exert toxicity through multifactorial mechanisms involving Endocrine disruption, genotoxicity, and idiosyncratic mechanisms which remains to be elucidated. Thus, it is probably not a safe substitute to BPA.

1.2 Research Objectives

This *in vivo* study aims to study the potential Neurodevelopmental toxicity of Bisphenol S (BPS) by investigating whether prenatal exposure of BPS at dose under NOAEL (No observed adverse effect level) will produce significant change on the expression levels of *NGN2* and *THRα* in the embryonic mice brain.

1.3 Study Limitations

This study lacks a group of mice treated with BPA which will serve to compare the effect of prenatal BPA and BPS administration on embryonic brain gene expression.

Secondly, this study only evaluates brain gene expression profile of embryonic mice at a single time point during gestational development. The absence of embryonic brain gene expression profiling on multiple gestational timepoints is a confounder as the alteration in brain gene expression profile of NGN2 and *THRα* may not be the result of BPS exposure alone, but rather due to natural fluctuations caused by ongoing neurodevelopmental processes in the brain of the mice embryo itself.

Thirdly, the parameters of Neurodevelopmental toxicity are only evaluated by using two genes: namely NGN2 and *THRα.* As will be discussed later on in this study, both NGN2 and *THRα* play an important role in proper neurodevelopment of embryos in both mice and humans. However, aberrant expression of NGN2 and *THRα* are only correlated to the risk of developing certain Neurobehavioral disorders and are not predictive biomarkers of specific Neurological diseases or disorders. In other words, no causal link has been established between aberrant NGN2 and *THRα* expression and the occurrence or development of certain neurological disorders. As such, alteration of NGN2 and *THRα* expression alone may not be a sufficient parameter to measure or interpret whether any Neurodevelopmental toxicity that occurs is severe enough to produce detrimental effects which are of clinical significance.

Fourth, the whole brain of the Mice embryo is used to extract RNA in this study as opposed to dividing the embryonic mouse brain into several regions. Expression of neurodevelopmental genes are not only time point dependent; they are also often location dependent. This introduces another confounding factor in the interpretation of the data obtained.