Regulatory and academic studies to derive reference values for human health: The case of bisphenol S

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Claire Beausoleil, Brigitte Le Magueresse-Battistoni, Catherine Viguié, Sylvie Babajko, Marie-Chantal Canivenc-Lavier, et al.. Regulatory and academic studies to derive reference values for human health: The case of bisphenol S. Environmental Research, 2022, 204 (Part C), pp.112233. 10.1016/j.envres.2021.112233 . hal-03408038

HAL Id: hal-03408038
https://hal.science/hal-03408038

Submitted on 25 Nov 2021

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Title: Regulatory and academic studies to derive reference values for human health: the case of Bisphenol S

Authors

Claire Beausoleil [1#], Brigitte Le Magueresse-Battistoni [2*], Catherine Viguié [3*], Sylvie Babajko [4], Marie-Chantal Canivenc-Lavier [5], Nicolas Chevalier [6], Claude Emond [7], René Habert [8], Nicole Picard-Hagen [3], Sakina Mhaouty-Kodja [9]

Affiliations

- [1] ANSES, Risk Assessment Department, France
- [2] Univ-Lyon, CarMeN Laboratory, INSERM U1060, INRAE U1397, Université Claude Bernard Lyon 1, France
- [3] Toxalim, Institut National de la Recherche Agronomique et de l'Environnement (INRAE), Toulouse University, Ecole Nationale Vétérinaire de Toulouse (ENVT), Ecole d'Ingénieurs de Purpan (EIP), Université Paul Sabatier (UPS), Toulouse, France.
- [4] Centre de Recherche des Cordeliers, INSERM, Sorbonne Université, Université de Paris, Paris, France
- [5] Centre des Sciences du Goût et de l'Alimentation, INRAE, Université de Bourgogne-France-Comté, Dijon France
- [6] Université Côte d'Azur, Centre Hospitalier Universitaire (CHU) de Nice, INSERM U1065, C3M, Nice, France
- [7] University of Montreal, School of Public Health, DSEST, Montreal, Quebec, Canada
- [8] Unit of Genetic Stability, Stem Cells and Radiation, Laboratory of Development of the Gonads, University Paris Diderot, Institut national de la santé et de la recherche médicale (Inserm) U 967 – CEA, Fontenay-aux-Roses, France
- [9] Sorbonne Université, CNRS, INSERM, Neuroscience Paris Seine – Institut de Biologie Paris Seine, 75005 Paris, France.

*** Equal contribution**

 # Corresponding author: 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex, France. E-mail address: claire.beausoleil@anses.fr

Abbreviations:

ECHA: European Chemicals Agency; EFSA: European Food Safety Authority; IARC: International Agency for Research on Cancer; LOAEL: lowest-observed-adverse-effect level; NIOSH: National Institute for Occupational Safety and Health; NOAEL: no-observed-adverse-effect level; OECD: Organisation for Economic Co-operation and Development; SCCS: Scientific Committee on Consumer Safety; WHO: World Health Organization.

Abstract

The close structural analogy of bisphenol (BP) S with BPA, a recognized endocrine-disrupting chemical and a substance of very high concern in the European Union, highlights the need to assess the extent of similarities between the two compounds and carefully scrutinize BPS potential toxicity for human health. This analysis aimed to investigate human health toxicity data regarding BPS, to find a point of departure for the derivation of human guidance values.

A systematic and transparent methodology was applied to determine whether European or international reference values have been established for BPS. In the absence of such values, the scientific literature on human health effects was evaluated by focusing on human epidemiological and animal experimental studies. The results were analyzed by target organ/system: male and female reproduction, mammary gland, neurobehavior, and metabolism/obesity. Academic experimental studies were analyzed and compared to regulatory data including subchronic studies and an extended one-generation and reproduction study. In contrast to the regulatory studies, which were performed at dose levels in the mg/kg bw/day range, the academic dataset on specific target organs or systems showed adverse effects for BPS at much lower doses (0.5 to 10 µg/kg bw/day). A large disparity between the lowest-observed-adverse-effect levels (LOAELs) derived from regulatory and academic studies was observed for BPS, as for BPA. Toxicokinetic data on BPS from animal and human studies were also analyzed and showed a 100-fold higher oral bioavailability compared to BPA in a pig model. The similarities and differences between the two bisphenols, in particular the higher bioavailability of BPS in its active (non-conjugated) form and its potential impact on human health, are discussed. Based on the available experimental data, and for a better human protection, we propose to derive human reference values for exposure to BPS from the N(L)OAELs determined in academic studies.

Keywords

Bisphenol S, Endocrine-disrupting chemical, Health-based guidance value, Toxicity, Policy making.

1. Introduction

The identification of bisphenol A (BPA) as a substance of very high concern (SVHC) in the European Union (EU) (ECHA, 2017) has generated pressure for substitution of this substance. This substitution mostly involves the use of other bisphenols, which have a structure similar to that of BPA. One of the most widely used bisphenols is bisphenol S (BPS), in particular as a developer for thermal paper (Molina-Molina et al., 2019). Use of BPS has increased continually, even accelerating after the definitive ban on BPA in thermal paper, as shown by the 153% increase in a single year between 2018 and 2019 (ECHA, 2020b). In addition, BPS is currently used as a monomer in the manufacture of plastic food contact materials, including baby bottles, based on its supposed higher chemical stability and lower specific migration limit (SML) in comparison to BPA. Human biomonitoring data have reported median urinary values within the 0.1–10 μg/L range for the general population (Husoy et al., 2019; Sakhi et al., 2018).

The increasing use of BPS has drawn attention from both the scientific community and regulatory bodies, like the European Chemicals Agency (ECHA), as the compound is thought to affect the human reproductive and hormonal systems (Carvaillo et al., 2019; Rochester and Bolden, 2015). BPS has been included in the EU's Community rolling action plan (CoRAP) since 2014 as a suspected endocrine disruptor. It was also included among other bisphenols in the first list of prioritized substances for the European Joint Program on Human Biomonitoring (HBM4EU). This latter initiative aims to coordinate and advance human biomonitoring in Europe to generate knowledge on human exposure to specific chemicals and chemical groups and on their human health impacts (Ougier et al., 2021).

The concerns associated with BPS and/or other bisphenols and their high regulatory priority have generated an urgent need to improve our knowledge on BPS-induced adverse effects through regulatory toxicological evaluation, but also and most importantly by integrating the scientific data generated by academic research. In this context, the goal of this study was to determine the no-observed-adverse-effect level (NOAEL) and/or the lowest-observed-adverse-effect level (LOAEL) of BPS, and to select the points of departure (PODs) as key parameters to determine human reference values. For this purpose, all the available data from human epidemiological and animal experimental studies from both regulatory and published academic results were analyzed. The endpoints investigated for BPS exposure

included male and female reproduction, the mammary gland, neurobehavior, and metabolism/obesity. Moreover, available human and animal toxicokinetic studies were also reviewed as a critical element to help extrapolate external exposure to internal exposure, to determine human reference values that could be considered by risk assessors from different organizations when setting health-based external or internal toxicological values.

2. Methods

2.1 Methodology overview

Human biomonitoring guidance values (HBM-GVs) are body concentrations or internal exposure levels, related to external chemical exposure, below which no adverse effect on human health is expected to occur. According to the methodology developed by Apel et al., there are three possible options for deriving HBM-GVs. Selecting one of these options is conditioned by data availability, data quality, and their relevance for deriving HBM-GVs (Apel et al., 2020). As a first priority option, a POD determined from a relationship between internal concentration of the substance of concern or its metabolite(s) and a selected critical health effect observed in humans should be considered as a starting point for deriving values. When this first strategy is not achievable, the second option is to estimate the concentration of the substance's biomarkers, which corresponds to an external toxicity reference value (TRV) already set by a recognized body (preferably European), using a rigorous and transparent scientific methodology. This approach is similar to the biomonitoring equivalent approach developed in the United States by Hays et al. (Hays and Aylward, 2009; Hays and Aylward, 2012; Hays et al., 2007). Finally, if no TRV is available, the third option consists in extrapolating and adjusting a POD identified from an animal experimental study.

The first option is applicable when human data based on a relationship between internal concentrations and health effects are available. The most informative studies for deriving HBM-GVs are well-conducted human studies adequately reporting measured internal concentration levels of a substance, sampling times, analytical methods used, and the relationships between concentrations of a substance or its metabolites in human biological media and the occurrence of adverse effects. The second option refers to a TRV or occupational exposure limit (OEL) from a relevant EU or non-EU organization. However, no TRV for BPS has been published so far. Therefore, this option cannot be considered. The third option, based on a POD (e.g. N(L)OAEL or a benchmark dose (BMD)) identified from animal experimental

studies, can be considered to derive HBM-GVs for BPS for both the general and occupational populations, if the conditions of the first and second options are not met.

2.2 Scientific literature search

Peer-reviewed articles were identified through the PubMed database up to July 2020 using a single-concept literature search strategy as recommended (ECHA et al., 2018), with "Bisphenol S" or Bisphenol S synonyms as the main keywords. For detailed information, the reader is invited to refer to Figure 1 and to the supplementary data (see Table S1). Additionally, secondary literature from industry reports, European or International reports from ECHA, EFSA, OECD, IARC, and SCCS was also consulted and taken into account when available (ECHA, 2019; ECHA, 2020a; EFSA, 2020; OECD SIDS, 2013). Although a Klimisch or ToxRTool approach was not applied to evaluate the quality of the studies, the experimental design of the available studies was scrutinized considering the size of the experimental group, the experimental conditions of breeding and exposure, and the adequacy of the statistical analyses. The results of this expert-based analysis are given in the respective summary tables (see Tables S2-S8).

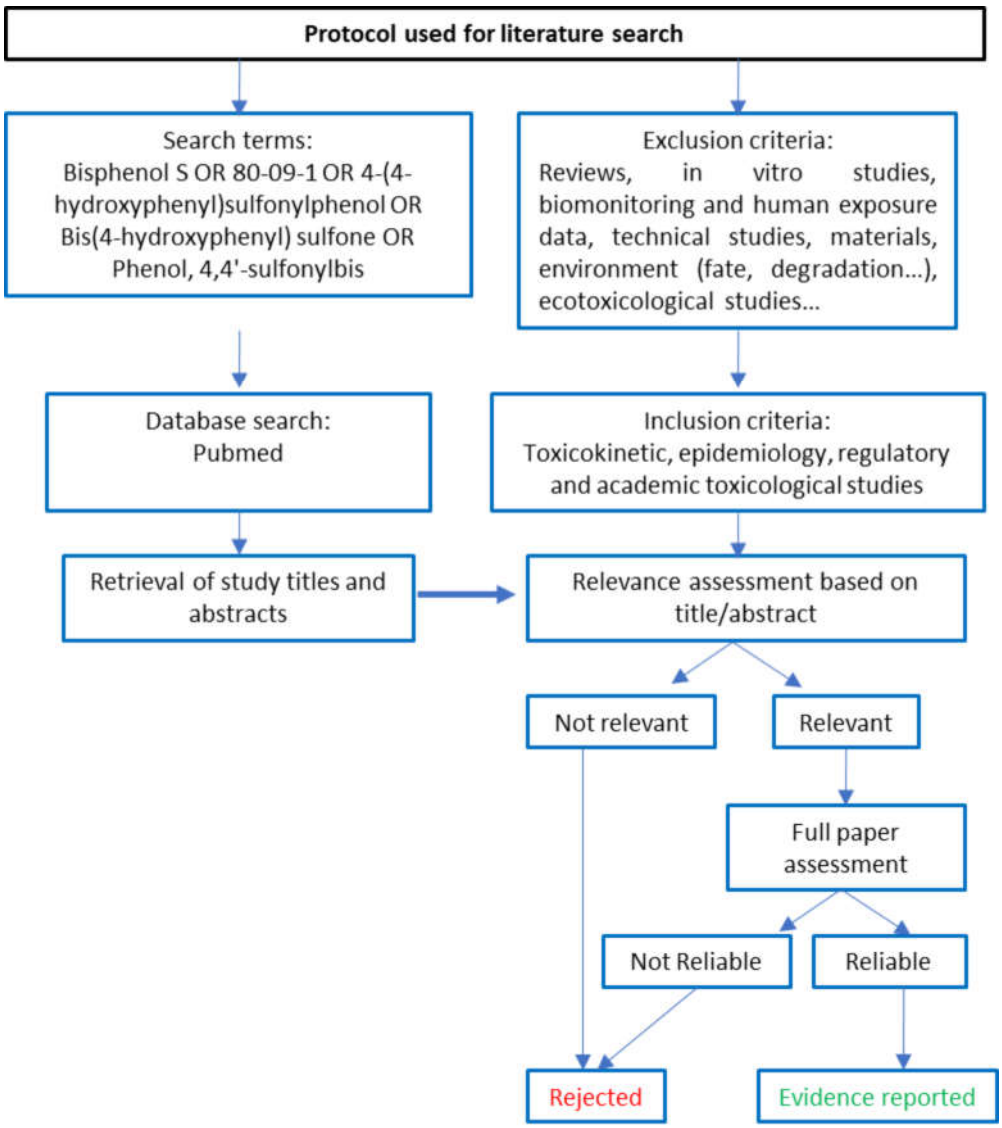

Figure 1: Protocol used for literature search. The exclusion and inclusion criteria are presented in details in Table S1.

3. Toxicokinetics of BPS and comparison with BPA

It is typically acknowledged that any adverse systemic effect of a compound is directly related to the plasma concentrations of its biologically active forms. However, there are critical gaps in our knowledge about the extent to which humans, especially human fetuses, are internally exposed to emerging bisphenols. Toxicokinetic studies are required to understand and predict internal exposure concentrations to the bioactive forms of BPS, and to derive acceptable exposure levels based on critical toxicological studies in animals. In *in vitro* studies, both BPA and BPS were largely detoxified by phase II enzymes, mainly into glucuronide conjugates (Gramec Skledar et al., 2015; Zalko et al., 2003). Table S2 summarizes the toxicokinetic data

obtained in different species, including mice, rats, piglets, sheeps, and humans, depending on the route of exposure in adults, and in the context of fetal exposure. In all species, BPS, like BPA, was rapidly metabolized, mainly into its glucuronide form.

3.1. Toxicokinetics of BPA and BPS by the intravenous route

Following a single intravenous administration, BPS was less efficiently eliminated than BPA. Importantly, the plasma clearance, which measures the overall ability to eliminate a drug, is 2 to 3.5 times lower than that of BPA (Gayrard et al., 2019; Grandin et al., 2017). In addition, BPS seemed to be more persistent in rats than in other species, as reflected by the mean residence time (MRT) range, which was 5 to 13 times higher due to a larger volume of steady-state distribution (Vss) (5 to 21-fold). From an allometric approach (Gayrard et al., 2020), human BPS clearance was estimated to be 0.79 L/kg/h, a value two-fold lower than that of BPA.

3.2. Bioavailability of BPA and BPS by the oral route

Bioavailability by the oral route quantifies the proportion of the parent substance actually reaching the systemic circulation. It is determined by both absorption and first-pass metabolism. Following oral administration, a large proportion of the dose was recovered in urine mainly as BPS glucuronide in human volunteers (Khmiri et al., 2020; Oh et al., 2018), in mice (Song et al., 2017), pigs (Gayrard et al., 2019), and in rats (EFSA, 2020; Waidyanatha et al., 2018), showing the considerable extent of BPS absorption by the oral route, and indicating that the total urinary BPS amount might be a good estimate of BPS internal exposure. The moderate efficiency of hepatic first-pass BPS glucuronidation yields bioavailability of active BPS that is about 100 times higher than that of BPA (57.4% and 0.50%, respectively) in pigs (Gayrard et al., 2019). Accordingly, *in vitro* data in humans indicated that the liver and intestinal intrinsic clearances of BPS were 11 and 6 times lower, respectively than those of BPA (Karrer et al., 2018). In accordance with data obtained in the pig model, human toxicokinetic studies using labelled BPS showed major differences between BPA and BPS kinetics, with much higher systemic levels of active BPS than BPA (Khmiri et al., 2020; Oh et al., 2018; Thayer et al., 2015). In addition, in rats and humans, enterohepatic recirculation of BPS was found (EFSA, 2020; Khmiri et al., 2020; Waidyanatha et al., 2018).

3.3. Bioavailability of BPA and BPS by the cutaneous route

Dermal BPS exposure through thermal paper is a question of great importance. Two *in vivo* toxicokinetic studies evaluated the disposition of BPS after cutaneous exposures (Khmiri et al., 2020; Liu and Martin, 2019). The results showed that dermal absorption of BPS was limited (< 0.2%) compared to oral absorption, but with a longer residence time after dermal absorption (Khmiri et al., 2020). Furthermore, *in vitro* experiments with fresh human skin samples showed that BPS absorption was much lower than that of BPA, with at least a 100-fold difference when the vehicle used was acetone or water or a 2-fold difference with the sebum. However, both BPS and BPA were mainly absorbed without biotransformation (Liu and Martin, 2019, Champmartin et al., 2020). This limited metabolic capability of the skin and the persistence of BPS after human dermal exposure should be considered in evaluating the relative risks for bisphenols.

3.4. Disposition of BPS in the materno-fetal unit

In view of potential BPS-related endocrine disruption, it is very important to evaluate fetal exposure. Fetal exposure to bisphenols results from a complex interplay between bidirectional placental clearances and maternal and fetal metabolism. A chronically catheterized fetal sheep model (Gauderat et al., 2016; Grandin et al., 2018) and an *ex vivo* human placenta model (Grandin et al., 2019) were used to evaluate the disposition of BPS and BPS-glucuronide (BPS-G) in the materno-feto-placental unit, and to compare them to those of BPA and BPA-glucuronide (BPA-G). In the maternal compartment, BPS was mainly metabolized into BPS-G and mostly eliminated in urine (Table S2 and Figure 2). It was striking that the fraction of the maternal BPS dose transferred from mother to fetus (0.40%) was about 10 times lower than that of BPA. This limited placental passage of BPS compared to BPA has also been observed using an ex-vivo model of perfused human placental cotyledon (Grandin et al., 2019). In sheep, once in the fetal compartment, only 26% of the BPS dose entering the fetal blood, against 74% for BPA, was rapidly cleared from the fetal compartment through its transfer into maternal blood. About half of the BPS remaining in the fetal compartment was metabolized by the fetus into BPS-G. The ultimate elimination of BPS-G from the fetal compartment required its hydrolysis and back-conversion into free BPS due to its limited placental transfer. Therefore, despite lower materno-fetal transfer of BPS compared to BPA, the higher persistence of BPS in the fetal compartment led to a fetal exposure to BPS in a range of concentrations similar to

those of BPA following repeated gestational exposure to the same bioavailable dose of BPA/BPS (Grandin et al., 2018).

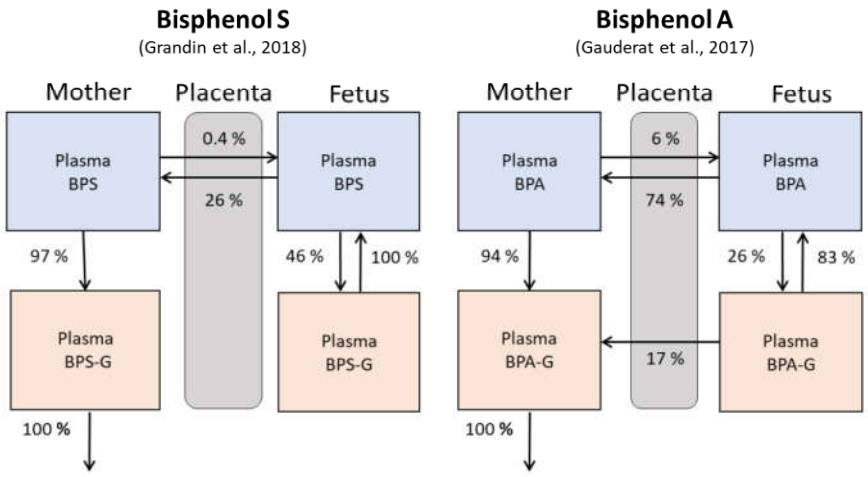

Figure 2: Transplacental exchange rates of BPA, BPS, and their main glucuronidated metabolites (BPS-G and BPA-G)

The percentage represents the fraction of a given dose that crosses the placental barrier, or the rate at which the parent compounds are metabolized to glucuronide conjugates

Overall, BPS toxicokinetic studies in several species, including humans, showed that BPS was well absorbed by the oral route, whereas dermal absorption was very limited. As previously shown for BPA, BPS clearance was mainly driven by glucuronidation of BPS into BPS-G, which was mainly excreted in urine. This means that urine constitutes the most optimal matrix for the characterization of human exposure to BPS (Gayrard et al., 2019; Khmiri et al., 2020; Oh et al., 2018). Notwithstanding, it should be noted that repeated urine samples (that can be pooled together before chemical assessment to maintain cost-effectiveness) are needed to provide an accurate human exposure assessment, especially to study exposure-health outcome associations (Vernet et al., 2019). In the animal model and in humans, after oral dosing, the major differences between BPA and BPS toxicokinetics led to a proportion of active parent (non-conjugated) BPS that was much higher than for BPA (Gayrard et al., 2019; Khmiri et al., 2020; Oh et al., 2018). Concerning the critical period of gestation, toxicokinetic studies showed that the blood-placental barrier was much more efficient in limiting fetal exposure to BPS than BPA. However, the higher persistence of BPS in the fetal compartment led to expected BPS concentrations in fetal plasma of the same order of magnitude as those of BPA for the same maternal exposure.

4. Data from human epidemiological studies

Forty eight human studies dealing with the health effects of BPS were identified. Four of them providing data on contamination levels, without any result on health outcomes, were thus excluded from our analysis (Asimakopoulos et al., 2016; Kolatorova et al., 2018; Liu et al., 2019b; Pell et al., 2017). Another one was excluded because urinary BPS was not detectable in any of the participants (Vitku et al., 2018). The main outcomes of the forty-three remaining publications were pregnancy/reproduction issues (n = 20), metabolic concerns (n = 17), neurocognitive development (n = 2) and other issues (n = 4).

4.1. Pregnancy and fetal growth

Seven studies evaluated the impact of exposure to BPS on fetal growth (Ferguson et al., 2018; Goodrich et al., 2019; Hu et al., 2019; Liang et al., 2020; Mustieles et al., 2018; Wan et al., 2018; Zhou et al., 2020). Among them, five studies did not report significant associations between maternal urinary or serum BPS concentrations and birth weight, birth length, or head circumference (Ferguson et al., 2018; Liang et al., 2020; Mustieles et al., 2018; Wan et al., 2018; Zhou et al., 2020). BPS concentrations in breast milk samples collected one week after delivery were not correlated with children growth parameters evaluated at a mean age of 5.3 months (Jin et al., 2020).

By contrast, in a cohort of 845 pregnant women, an interquartile range increase in urinary BPS concentrations in the first trimester was significantly associated with reduced birth weight (ß = -38 g [95% CI: -65, - 11 g]), as in the second trimester (ß= -43 g [95% CI: -71, - 15 g]) (Hu et al., 2019). Moreover, for newborns in the 10th percentile of birth weight and birth length, maternal exposure levels of BPS were relatively higher than for newborns in the 90th percentile, across the period of 10–36 weeks of gestation (WG) (Hu et al., 2019). In another cohort of 56 pregnant women from Michigan, maternal urinary BPS concentrations in the first trimester were significantly associated with reduced birth weight (ß = -150.42 g [95% CI: -294.85, - 6.00 g ; p = 0.04]) but only 37% (n = 21/56) of urine samples had detectable concentrations of BPS (LOD 0.2 ng/mL) (Goodrich et al., 2019).

In a sample of the Generation R cohort (n = 823 pregnant women included before 18 WG), urinary BPS concentrations decreased during pregnancy (68.1% of positive samples at 13.1 WG and 29.1% at 20.4 WG, with a median concentration of 0.35 ng/mL and 0.24 ng/mL respectively) and were not associated with maternal weight gain (Philips et al., 2020).

Also, the impact of exposure to BPS on other pregnancy-related health outcomes has been examined. Wan et al. reported that urinary BPS was correlated with increased gestational age and increased risk of late term birth for girls (Wan et al., 2018). However, these results must be interpreted with caution because urine samples were obtained just when the women attended hospital for delivery. In four other studies (Aker et al., 2020; Aung et al., 2019; Huang et al., 2019; Mustieles et al., 2020), urinary concentrations of BPS in mothers were correlated to the gestational length. Aung et al. reported a suggestive association between urinary BPS concentrations around 35 WG and preterm birth (Aung et al., 2019). In a cohort of 850 pregnant women, Huang et al. reported a positive correlation between urinary BPS concentrations during the third trimester of pregnancy and the gestational length, which didn't remain significant after multiparametric adjustment (Huang et al., 2019). In the American EARTH (Environment and Reproductive Health) cohort, Mustieles et al. analyzed 163 preconception mothers showing that an increase in preconception BPS was associated with a higher risk of preterm birth, even after adjustment for covariates (RR = 2.42 [95% CI: 1.01 – 5.77; p = 0.05]) (Mustieles et al., 2020). In a posterior study investigating prenatal exposure, higher first trimester urinary BPS concentrations tended to be associated with a higher risk of preterm birth, although not reaching statistical significance (RR = 1.25; 95% CI: 0.82, 1.89). This suggested that, in addition to prenatal development, preconception may constitute a particularly susceptible and understudied period (Zhang et al., 2021). In the Puerto Rican PROTECT cohort, Aker et al., 2020 reported that an interquartile range increase in maternal urinary BPS concentrations was significantly associated with a decrease in gestational length (Δ = -3.15 days [95% CI: -6.06, -0.24 days]) (Aker et al., 2020).

In 439 pregnant women in the United States, maternal urinary concentrations of BPS were associated with a suggestive increase in thyroid-stimulating hormone (TSH) levels, together with a decrease in those of free thyroxin (T4) before 15 weeks of pregnancy (Aker et al., 2018). However, in a population-based prospective study of 1,996 pregnant women, there were no associations between maternal urinary concentrations of BPS and absolute TSH, free T4, or free triiodothyronin (T3) concentrations (Derakhshan et al., 2019). In a sample of the PROTECT cohort (n = 609), an interquartile range increase in maternal urinary BPS concentrations between 16 and 30 WG was significantly associated with a 11% decrease in maternal corticotropin-releasing hormone (Δ = -11.35 pg/mL [95% CI: -18.71, - 3.33 pg/mL];

p = 0.008) (Aker et al., 2019). In a cohort of 764 pregnant women, Wang et al. reported a negative correlation between maternal urinary BPS concentrations at first trimester of pregnancy and newborn mitochondrial DNA copy number in cord blood, but it was significant only for boys (Wang et al., 2021).

4.2. Metabolism and obesity

Relationships between exposure to BPS and obesity (defined as a body mass index (BMI) ≥ 30 kg/m^2 or $\geq 85^{th}$ percentile of the age- and sex-adjusted *z*-scores in children and teens) were investigated in five studies in the United States. Three of them included only children or teens (Jacobson et al., 2019; Kataria et al., 2017; Liu et al., 2019a) and four of them analyzed a sample of the US National Health and Nutrition Examination Survey (NHANES) 2013–2014 (Jacobson et al., 2019; Liu et al., 2017; Liu et al., 2019a; Zhang et al., 2019b) as summarized in Table S3.

The results of these studies are conflicting. Overall, it was observed that individuals with obesity had higher median values of BPS in urine than those without obesity. In an analysis of NHANES 2013–2014, urinary BPS was positively associated with increased odds of general and abdominal obesity in adults (fourth vs. first quartile of urinary BPS: odds ratio 1.44 [95% CI: 1.01 – 2.07; $p = 0.045$] and 1.45 [95% CI: 1.01 – 2.16; $p = 0.045$], respectively) (Zhang et al., 2019b). However, these results were not significant after adjustments for lifestyle, demographic, and socioeconomic factors (Liu et al., 2017). Furthermore, in a cohort of 662 Dutch adults (Lifelines Cohort Study), urinary BPS was not associated with BMI, waist circumference or metabolic abnormalities but was detectable in only 9% of the sample (van der Meer et al., 2020).

In children and teens, urinary BPS concentrations were higher in teens with obesity from the NHANES 2015–2016 (Jacobson et al., 2019), but not in those from the NHANES 2013–2014 (Liu et al., 2019a), as in a very small US cross-sectional pilot study of 10–13-year-old teens (Kataria et al., 2017). In NHANES 2015–2016, BPS exposure was inversely correlated with a lower education level of the head of the household ($p = 0.05$), and with a lower poverty/income ratio ($p = 0.01$) (Jacobson et al., 2019). Log-transformed continuous BPS concentrations were associated with increased odds of general obesity (OR 1.16 [95% CI: 1.02 – 1.32]), severe obesity (OR 1.18 [95% CI: 1.03 – 1.35]), and abdominal obesity (OR 1.13 [95% CI: 1.02 –

1.27]). However, the BPS quartiles were not statistically significantly associated with any outcome (Jacobson et al., 2019).

Similarly, six studies investigated the association of exposure to BPS with incident diabetes, fasting blood glucose, or insulin resistance (Duan et al., 2018; Kataria et al., 2017; Lee et al., 2019; Ranciere et al., 2019; Song et al., 2019; Zhang et al., 2019a) (Table S3). Song et al., 2019 reported that serum BPS levels were not associated with hyperglycemia in a nested population of elderly patients living near an e-waste recycling facility in China. Kataria et al., 2017 did not find associations between urinary BPS concentrations and insulin resistance in their cross-sectional study of 10–13-year-old teens. In a cohort of pregnant women from Wuhan, China (Zhang et al., 2019a), log-transformed urinary BPS concentrations were not associated with incident gestational diabetes, but with increased fasting and post-load blood glucose (0.04 mmol/L for fasting blood glucose; 0.11 mmol/L for 1hr-post-load blood glucose, and 0.19 mmol/L for 2hr-post-load blood glucose; $p = 0.02$). In a case-control study, log-transformed urinary BPS concentrations were associated with a significantly increased risk of type 2 diabetes (OR 1.73 [95% CI: 1.37 – 2.18; $p < 0.001$] for the total sample, and OR 3.83 [95% CI: 2.37 – 6.20; $p < 0.001$] for people with BPS concentrations above the method detection limits [57.9%]) (Duan et al., 2018). In a *posthoc* analysis of the D.E.S.I.R. cohort in France, even though BPS-G was detectable only in 11% of the population, urinary BPS-G detection was significantly associated with a higher risk of incident type 2 diabetes at baseline (adjusted hazard ratio (aHR) 1.68 [95% CI: 1.09 – 2.58]) and at year 3 (aHR 2.81 [95% CI: 1.74 – 4.53]) (Ranciere et al., 2019). In a cohort of 459 women (20 – 48 years) from South Korea, in which BPS was detectable in 83.7% of the study population, urinary BPS concentrations were positively associated with an increase of insulin resistance evaluated by ln-transformed homeostatic model assessment for insulin resistance (ln-HOMA-IR) ($p = 0.017$) calculated as fasting insulin (μU/mL) x fasting glucose (mmol/L) x 22.5, but only in women without overweight or obesity (BMI < 25 kg/m^2) (Lee et al., 2019).

Five studies also investigated the association of BPS exposure with urinary markers of oxidative stress. Urinary BPS concentrations were positively correlated with urinary concentrations of 8-hydroxy-2'deoxyguanosine (8-OHdG), a marker of oxidative stress, in a nested case-control study conducted in a Chinese population residing near an e-waste recycling facility (Zhang et al., 2016), but not in a limited sample of men (Wang et al., 2019), nor in a small cohort of teens (Kataria et al., 2017) or in a cohort of women suffering from unclear

recurrent spontaneous abortion (Liang et al., 2020). In the American LIFECODES cohort, urinary BPS concentrations were positively correlated with 8-isoprostane but not with 8-OHdG (Δ = + 18.5% [95% CI: +7.68, + 30.5%]; $p < 0.001$) (Ferguson et al., 2019). However, BPS was detectable in this sample in only 21% of the participants (LOD 0.4 ng/mL).

In a cohort of 1437 patients from Wuhan (China), urinary BPS concentrations were significantly higher in participants with incident hypertension (n = 433) (0.334 ng/mL vs 0.243 ng/mL; $p < 0.001$). Urinary BPS concentrations were significantly associated with systolic blood pressure (third vs. first tertile of urinary BPS: odds ratio 3.89 [95% CI: 1.49 – 6.29; *P-trend* = 0.006]), diastolic blood pressure (third vs. first tertile of urinary BPS: odds ratio 1.70 [95% CI: 0.26 – 3.14; *P-trend* = 0.046]), mean arterial pressure (third vs. first tertile of urinary BPS: odds ratio 2.43 [95% CI: 0.79 – 4.07; *P-trend* = 0.013]) and mid blood pressure (third vs. first tertile of urinary BPS: odds ratio 2.80 [95% CI: 1.00 – 4.60; *P-trend* = 0.009]) (Jiang et al., 2020a). In a sample of Generation R Study including 1064 mother/child pairs, maternal urinary BPS measured at each pregnancy trimester (12.9 [12.1-14.5] ; 20.4 [19.9-20.9] and 30.2 [29.9-30.8] WG) was not associated with arterial blood pressure measured at the age of 10 years in children (Sol et al., 2020).

4.3. Neurocognitive development

The impact of exposure to BPS on neurocognitive development was evaluated in a sample (n = 312) of an ongoing prospective pregnancy cohort (Alberta Pregnancy Outcomes and Nutrition [APrON] Study). Urinary BPS concentrations at second trimester of pregnancy and at the third post-partum month (0.16 and 0.18 ng/mL respectively) were not correlated with neurocognitive development evaluated by Behavioral Rating Inventory of Executive Function (BRIEF-P) in children at the age of 2 and 4 years (England-Mason et al., 2021). By contrast, in a cohort of 456 mother/child pairs, psychomotor development index scores, evaluated by Bayley Scales of Infant Development in childrens of 2 years old, decreased across quartiles of urinary BPS concentrations (fourth vs. first tertile of urinary BPS: Δ PLDI = - 4.7 points; *P-trend* = 0.01]) (Jiang et al., 2020b).

4.4. Other health issues

Fecundability

Philips et al. evaluated the impact of BPA analogues on fecundability among 877 participants in the population-based Generation R pregnancy cohort (EU cohort). They reported no association between bisphenol analogues including BPS and fecundability; but total bisphenols (including 4,4-BPF, BPS, BPB, BPP, BPAF, BPAP, and BPZ) were associated with a longer time to pregnancy in women with inadequate folic acid supplement use (Philips et al., 2018a; Philips et al., 2018b).

In a case-control study including 124 women, urinary BPS concentrations were not associated with occurrence of endometriosis, but BPS was detected only in 14.8% of the samples (Peinado et al., 2020).

In the EARTH cohort, Ghayda et al. reported that urinary BPS concentrations in men (n = 158) were significantly associated with lower sperm volum (2.66 vs 2.91 mL; p = 0.03) and lower spermatozoa concentration (30.7 vs $38.3.10^6$/mL; p = 0.03), but not with motility and morphological modifications of spermatozoa. This association was described only in men with BMI $\geq$ 25 kg/m^2, who had also significant higher urinary BPS concentrations (compared to men with BMI < 25 kg/m^2) (Ghayda et al., 2019).

Hematological effects

In a cohort of 196 pregnant women, urinary BPS concentrations were not associated with significant modifications of haemato-biochemical alterations (including red blood cells, white blood cells, platelets, hemoglobin level and hematocrit) (Kang et al., 2020).

Respiratory effects

In a sample of the NHANES 2013-2016 including 3538 teens over 12 years and adults, Mendy et al. reported that a 10-fold increase in urinary BPS concentration was associated with increased odds of current asthma only in men (OR 1.64 [95% CI: 1.13 – 1.240]; p = 0.01) (Mendy et al., 2020). However, these results were not significant after adjustments for age, lifestyle, demographic, socioeconomic factors, exposure to cigarette smoke and history of asthma. In the prospective Mouse Allergen and Asthma Cohort Study (MAACS), urinary BPS concentrations were not associated with incident asthma in African American children of 5-17 years (Quirós-Alcalá et al., 2021).

Bone effects

Among 1,362 mother-child pairs participating in a population-based cohort study, van Zwol Janssens et al. reported that urinary BPS concentrations decreased during pregnancy (67.8% of positive samples at first trimester, 29.5% at second trimester and 19.3% at third trimester, with

a median concentration of 0.68 ng/mL, 0.13 ng/mL and 0.13 ng/mL respectively). In addition, urinary BPS concentrations at first trimester of pregnancy were associated with a significant decrease of bone mineral density (BMD) and area-adjusted bone mineral content (aBMC) evaluated in children at the age of 10 years, but not at the age of 6 years (ΔBMD = -6.08 mg/cm^2; $p < 0.01$ and ΔaBMC = -0.12 g; $p < 0.01$) (van Zwol-Janssens et al., 2020).

Overall, given the confounding factors and the discrepancies in the available epidemiological data, it remains impossible to derive human reference values from these results.

5. Effects of BPS in regulatory toxicological studies

A total of eight regulatory toxicology studies conducted in accordance with OECD Test Guidelines are available on BPS. Table 1 provides an overview of the main characteristic toxic effects of BPS and the corresponding N(L)OAELs. Five oral repeated dose toxicity studies (i.e. OECD TG 407; TG 408; 422 quoted as Anonymous 14, 15, 16, 17 and 18 in (ECHA, 2019)) used an exposure duration varying from 13 to 90 days, at doses ranging from 40 to 1,000 mg/kg bw/day. The main observed toxic effects were systemic toxicity with excessive salivation, decreased body weight, and kidney, adrenal and cecum effects, as described in Table 1. A decreased weight of prostate and seminal vesicles was also observed in some studies, together with diffuse atrophy of the male mammary gland. Four other regulatory studies (i.e. OECD TG 414, TG 421, 422 and 443 quoted as Anonymous 12, 13, 14 and 19 in ECHA, 2019 investigated developmental or reproductive toxicity of exposure to BPS in the rat at doses ranging from 10 to 300 mg/kg bw/day via gavage (ECHA, 2019). These results led to a classification of BPS in category 1B for reproductive toxicity (H360FD) (ECHA, 2020a). In addition, the recent evaluation of an extended one-generation reproductive toxicity study in rats led the EFSA Working Group to determine an NOAEL of 20 mg/kg bw/day for developmental toxicity, based on increased post-implantation losses in the F1 progeny, or for developmental immunotoxicity, based on decreased spleen weight in males at 60 mg/kg bw/day (EFSA, 2020).

6. Effects of BPS in academic studies

The analysis of academic studies is presented below by identified endpoint. Table 2 provides an overview of the main characteristic toxic effects of BPS and the corresponding N(L)OAELs.

6.1. *Effects of BPS on male reproductive function*

A total of twelve papers have described the effects of BPS on male testicular function. Among them, four papers were not taken into account in the present review because they were poorly informative (John et al., 2019; Ullah et al., 2019a; Ullah et al., 2019b; Ullah et al., 2018a). The eight remaining papers, originating from 4 distinct laboratories, reported a coherent picture of alterations in spermatogenesis in both Sprague-Dawley rats and CD-1, ICR and C57Bl/6 mice, exposed to BPS (see Table S4).

This review separates analyses depending on the period of exposure, i.e. before or after neonatal life. It is known that the germ stem cell population is established during fetal and neonatal life. More precisely, this period extends from gestational day (GD) 7 to postnatal day (PND) 8 in the mouse, and from GD 9 to PND 10 in the rat. After this period, setup of the stem spermatogonia population is complete and the meiosis process starts in the testis (de Rooij and Vergouwen, 1991; Olaso and Habert, 2000).

Four papers from 3 laboratories, using either rat or mouse models reported convergent results showing that fetal and/or neonatal exposure to BPS caused qualitative and quantitative alterations in spermatogenesis at adulthood (Horan et al., 2018; Shi et al., 2018; Ullah et al., 2019c), and that these alterations were transmitted to their descendants (Shi et al., 2019c). Specifically, the adverse effect of BPS on the quality and number of spermatozoa was clearly observed. The studies described decreased daily spermatozoa production (Ullah et al., 2019c), decreased number and motility of spermatozoa and alterations of testicular histology (Shi et al., 2018; Ullah et al., 2019c). These effects could result from meiosis disruption as shown by changes in the percentages of specific stages VII-VIII throughout the spermatogenic cycle of the seminiferous epithelium (Shi et al., 2017; Shi et al., 2018), and by the reduction in the homologous recombination rate (Horan et al., 2018). Concerning BPS effects on hormonal levels, an increase in plasma estradiol concentrations or in testicular *Cyp19* expression was observed (Shi et al., 2018; Shi et al., 2019c; Ullah et al., 2019c). Plasma testosterone levels were decreased in two of these studies (Shi et al., 2019c; Ullah et al., 2019c), in line with meiosis disruption at stages VII-VIII (Shi et al., 2017; Shi et al., 2018) as these stages are androgen-dependent (De Gendt et al., 2004). Other effects included an increase in oxidative stress in testis extracts (Shi et al., 2018; Ullah et al., 2019c). Importantly, epigenetic modifications were found by changes in the expression of testicular genes involved in DNA or

histone methylation (Shi et al., 2018), and confirmed by transgenerational inheritance of spermatogenic defects (Shi et al., 2019c).

In the key study of Shi et al. (2018), allowing the determination of an NOAEL/LOAEL for BPS, mice were orally exposed to 0.5, 20 or 50 µg/kg bw/day of this bisphenol from GD 11 to birth. This treatment altered the entire morphometric analysis of spermatogenesis studied on PND 60, except for the weight of the testes. In the 0.5 µg/kg bw/day BPS-treated group, spermatozoa numbers in the cauda epididymis were reduced by 34%, and the percentage of motile spermatozoa by 15%, as compared with controls. These disturbances were probably related to an alteration of germ cell line development. On PND 12, the number of apoptotic seminiferous cells was doubled and dysregulations in the expression of numerous genes involved in apoptosis, autophagy, oxidative stress, and histone methylation were observed with 0.5 µg/kg bw/day BPS and higher doses. As a result, although some dose-response curves were not monotonic in this study, a dose of 0.5 µg/kg bw/day BPS was sufficient to alter male reproductive function. This observation is strengthened by another study where Shi et al. (2019c) reported that oral exposure of F0 mice from GD 7 to birth to BPS at the lowest tested dose of 0.5 µg/kg bw/day reduced the spermatozoa number and the percentage of motile spermatozoa in the cauda epididymis, and altered the cycle of seminiferous epithelium on PND 60 in the F3 generation.

Only two papers using rats are available to estimate an NOAEL and LOAEL after neonatal exposure to BPS. The study of Ullah et al. (2016) investigated few parameters, which overlap with those explored in another more complete study (Ullah et al., 2018b) from the same laboratory. The last study included the height of the seminiferous epithelium, plasma testosterone levels, and oxidative and anti-oxidative activities, all evaluated in both studies on BPS. Therefore, only this second study was used for NOAEL and LOAEL estimations. BPS was added to the drinking water of Sprague-Dawley rats for a very long period (48 weeks) from PND 22 onwards. Adverse effects of BPS were clearly observed, such as a decrease in daily spermatozoa production and in the number and motility of spermatozoa in the epididymis. Furthermore, the testes showed reduced weight and histological alterations. BPS at 25 µg/L in the drinking water caused adverse effects, whereas 5 µg/L had no effect. Since the daily beverage intake of a male rat is around 300 mL/kg bw on PND 23 and 40 mL/kg bw on postnatal week 52 (Holdstock, 1973; McGivern R.F. et al., 1996), the NOAEL is estimated to

be between 0.2 and 1.5 µg/kg bw/day, and the LOAEL between 1 and 7.5 µg/kg bw/day. These values are slightly higher than those identified for fetal and/or neonatal exposure. Other effects after neonatal exposure to BPS included decreased plasma testosterone levels and increased oxidative stress (Ullah et al., 2018b; Ullah et al., 2016).

Recently a study was conducted to investigate whether testicular and sperm effects can be detected after exposure to very low BPS doses (Řimnáčová et al., 2020). Adult male mice were exposed to doses around 0.001, 1 or 100 µg/kg bw/day BPS via drinking water from 8 to 16 postnatal weeks (PNW). Some endpoints such as testicular histological parameters and testicular concentration in γH2AX, a marker of DNA damage and cellular stress, were affected only at high BPS doses. On the contrary, others such as sperm motility, sperm proteins methylation and acetylation were changed at 0.001 µg/kg bw/day of BPS and not at higher doses. This raises the question of the effects at very low doses which are generally not tested in most of the studies, and which may be more effective than higher doses in the context of nonmonotonic dose response relationship.

Importantly, in six of the eight studies, BPA was also tested and its effects were similar or slightly lower than those of BPS (Horan et al., 2018; Shi et al., 2017; Shi et al., 2018; Shi et al., 2019b; Ullah et al., 2019c; Ullah et al., 2018b), giving rise to similar estimated NOAEL/LOAELs to those calculated for BPS, for both exposure periods. The only striking difference was the minimum dose of bisphenol required to significantly reduce spermatozoa motility, since it was two-fold higher for BPA than for BPS, regardless of whether the exposure occurred during the neonatal period (Ullah et al., 2019c) or in adulthood (Ullah et al., 2018b) (see also Table S4). These *in vivo* data are in the same line with previous *ex vivo* studies showing that the responsiveness of human and mouse fetal testis to BPS is similar to that of BPA (Eladak et al., 2015).

6.2. Effects of BPS on female reproductive function

A total of fifteen studies investigated female reproductive function outcomes. Among them, five studies were not retained because they were poorly informative of reproductive function due to the specific nature of the model (Demacopulo and Kreimann, 2019), limited focus on reproductive function (da Silva et al., 2019), or major limitations related to statistical analysis (Hill et al., 2017) and/or experimental design (Nourian et al., 2017; Nourian et al., 2020). Table

S5 reports the 10 studies that were kept for the present analysis. Some of them may present certain limitations, but none of these were considered important enough to preclude consideration of the data. All the considered studies addressed the effects of exposure to BPS through developmental periods (fetal, postnatal, and peripubertal) and adulthood.

Certain studies investigated ultimate endpoints of reproductive function regarding puberty and/or estrous cyclicity as adverse effects. Results regarding the age at vaginal opening reported in six studies (Ahsan et al., 2018; Kolla and Vandenberg, 2019; Shi et al., 2017; Shi et al., 2019a; Shi et al., 2019b; Tucker et al., 2018) were not consistent. Discrepancies between studies may result from differences in the experimental design regarding the species, routes, doses, and periods of exposure. Two studies from two different laboratories in the CD-1 mouse with gestational exposure did, however, lead to diverging results (Kolla and Vandenberg, 2019; Shi et al., 2019a). Monitoring of the estrous cycle was performed in only three studies. Increased occurrence of irregular cycles after gestational exposure (Shi et al., 2019a) was reported in one study from 0.5 to 50 µg/kg bw/day, while no changes in the number and duration of estrous cycles were observed with a similar period of exposure but at much higher doses by Tucker et al. (2018). The last study reported unchanged estrous cyclicity in F3 animals born from exposed F0 males and females (Shi et al., 2019b). It is, therefore, not possible to draw any conclusions regarding the effect of BPS on these two parameters.

Among the ten evaluated studies, seven investigated the effect of BPS developmental exposure (gestational, postnatal, and peripubertal) on folliculogenesis and/or oocyte maturation and meiosis. There is a large body of evidence, i.e. six of the seven studies from four different laboratories using different species and strains, exposure paradigms, and periods of observation, that BPS can alter these processes (Ahsan et al., 2018; Horan et al., 2018; Ijaz et al., 2019; Nevoral et al., 2018; Prokesova et al., 2020; Shi et al., 2019a). The remaining study, looking at the effect of gestational F0 exposure to BPS (GD 7 to birth) on F3 reproductive function, failed to show an effect of BPS on the PND 4 follicular populations (Shi et al., 2019b).

When changes in folliculogenesis/meiosis processes were evaluated jointly with potential adverse effects on fertility issues, or *in vitro* and *ex vivo* fertilization rates (four studies), various effects could be found, including at low dosage below 100 µg/kg bw/day. Interestingly, the effect of gestational exposure on the reproductive capacities of aging animals persisted over the third generation after exposure limited to F0 animals (Shi et al., 2019a; Shi et al., 2019b).

Peripubertal exposure of mice at a low dose (10 µg/kg bw/day; Nevoral et al., 2018) was associated with decreased *in vitro* oocyte fertilization rates, with a possible non-monotonic dose-response relationship. Finally, the fourth study, performed in rats exposed to a high BPS dose, showed decreased litter size following postnatal exposure (Ahsan et al., 2018). Interestingly, *in vitro* data confirmed the ability of BPS to interfere directly with oocyte maturation at low doses in pigs (Zalmanova et al., 2017) and cows (Campen et al., 2018).

Among the potential mechanisms underlying these effects, epigenetic changes, ovarian oxidative stress, and changes in endocrine status were all put forward. Two studies from the same laboratory (Nevoral et al., 2018; Prokesova et al., 2020) reported changes in epigenetic profiles in association with decreased meiotic and fertilization abilities of *in vivo* and *in vitro* matured MII oocytes after peripubertal exposure. Changes in hormone concentrations were described in five studies (Ahsan et al., 2018; Ijaz et al., 2019; Shi et al., 2017; Shi et al., 2019b; Tucker et al., 2018), along with increased oxidative stress in the ovary after peripubertal exposure (Ijaz et al., 2019). However, these data are difficult to put in perspective. Samples were collected at different stages of life and/or, when specified, at different stages of the estrous cycle. Estradiol levels results were usually not reliable because of inadequate assay methods, with sensitivity not allowing precise determination of low levels commonly found in this type of experiment. The most reliable and reproducible result was increased testosterone concentrations following BPS exposure at high doses (within the 1–10 mg/kg bw/day range) during early postnatal life (Ahsan et al., 2018) or in the peripubertal period (Ijaz et al., 2019) in rats, or in mice with exposure encompassing the neonatal to adult stage (Shi et al., 2017), or even in 9 month old animals (Shi et al., 2019a)), but not younger ones (3 and 6 months old) exposed during gestation to a relatively low dose (50 µg/kg bw/day). Three studies from the same author (Shi et al., 2017; Shi et al., 2019a; Shi et al., 2019b) evaluated gene expression of key steroidogenic enzymes. Subtle changes in the expression patterns of these enzymes were found in two studies with gestational exposure, while the third study with prepubertal exposure did not show any effect. An isolated increase of *cyp 11a1* expression, encoding a critical enzyme for the initiation of steroidogenesis, could be observed at 3 months of age in both F1 (Shi et al., 2019a) and F3 generations (Shi et al., 2019b), but not in 6 or 9-month-old animals. Therefore, among the three proposed mechanisms of action of BPS on female reproductive function, none can be clearly confirmed from the available data.

When BPA and BPS were tested in the same experiment, the effects of both substances were quite similar. There are, however, some indications that their efficiency might differ, although there are insufficient data to establish a firm comparison.

Given the body of converging evidence, it can be considered that BPS alters oocyte maturation, meiosis capacity, and/or folliculogenesis following developmental exposure from gestation to peripubertal periods. As potential consequences on fertility were also reported, alteration of oocyte maturation and folliculogenesis can be considered adverse effects. Whether one and/or several of the investigated modes of action underlie such effects remains to be determined. A LOAEL of 0.5 µg/kg bw/day was determined based on the study by Shi et al. (2019a) for an effect on follicular populations at PND 4, along with alteration of the estrous cycle and decreased fertility in aging animals. Interestingly, the study by Nevoral et al. (2018) showed an effect on the follicular population with a LOAEL as low as 0.001 µg/kg bw/day. However, as this was observed only on a single parameter, and as there were uncertainties around the level of exposure as compared to probable environmental contamination, this LOAEL was not retained for reference values determination.

6.3. Effects of BPS on the mammary gland

A total of six studies reporting the effects of BPS exposure on the mammary gland in CD-1 mice were analyzed (Kolla et al., 2019; Kolla et al., 2018; Kolla and Vandenberg, 2019; LaPlante et al., 2017; Tucker et al., 2018; Wormsbaecher et al., 2020) (see Table S6). They used BPS doses ranging from 2 µg/kg bw/day to 5,000 µg/kg bw/day, and the oral route for exposure with BPS incorporated into wafers, or administered by pipettes or gavage. Four of the six studies were carried out in Vandenberg's laboratory on mammary gland involution after delivery (LaPlante et al., 2017), or mammary gland development in males and females (Kolla et al., 2019; Kolla et al., 2018; Kolla and Vandenberg, 2019). The two remaining studies mainly focused on mammary gland histology and occurrence of lesions. One compared long-term effects of prenatal exposure to BPA and BPS on mammary gland development and the subsequent occurrence of lesions (Tucker et al., 2018). The other examined BPA and BPS effects on mammary gland stiffness, which is known to be a risk factor for breast cancer (Wormsbaecher et al., 2020).

Kolla et al. (2018) studied the effects of gestational and lactational exposure (GD 9 to PND 20) of CD-1 mice to BPS at 2 and 200 µg/kg bw/day on the developing mammary gland of F1 females. Ethinyl estradiol (EE2) at 0.01 or 1 µg /kg bw/day was used as a positive control. They observed a significant increase in the total terminal end bud (TEB) area and size at PND 24 (pre-pubertal period) in females exposed to BPS doses from 2 µg/kg bw/day, but not to EE2. No effects were observed during puberty (PND 35). TEB-like structures were observed at 9 weeks of age (adulthood) in BPS-exposed animals, and to a lesser extent in EE2-exposed groups. Intra-ductal hyperplasia in adult mammary glands was also observed at both doses of BPS and EE2. Similar effects in adults were previously observed for BPA exposure (Soto et al., 2013).

Similar investigations were carried out in F1 males exposed to BPS (2 and 200 µg/kg bw/day) over the same exposure period (GD 9 – PND 20) (Kolla et al., 2019). At embryonic day (ED) 16, the number of androgen receptor (AR)-positive cells in the mesenchyme was reduced in a dose-dependent manner. Interestingly, at PND 24, the authors observed a dose-dependent decrease in ductal areas in the left mammary gland, whereas later in life at postnatal week 9, they reported a dose-dependent increase in ductal area in the right mammary gland, and for the highest BPS dose only for the left gland. These data showed that the lowest dose of BPS (2 µg/kg bw/day) disrupted male mammary gland development with different susceptibility between the right and left glands, leading the authors to propose male mice exposed to low-dose BPS as a model to study unilateral gynecomastia observed in boys during puberty. Lastly, Kolla & Vandenberg (2019) showed that perinatal treatment from GD 9 to PND 2, or a challenge with EE2 at a peripubertal period from PND 21 to PND 30, did not affect the number or total area of TEBs, whereas the ductal area was decreased. Similar exposures in males showed different susceptibility to BPS and EE2 in the right and left glands (Kolla et al., 2019).

LaPlante et al. (2017) studied the effects of gestational and lactational exposure (GD 9 to LD 20) to BPS on mammary gland involution in F0 female mice at different periods of lactation (LD2 and LD21). Exposure to 200 µg/kg bw/day of BPS led to a decrease in lobuloalveolar units, with a decrease in STAT-5 phosphorylation related to prolactin receptor activation at LD 21. This was consistent with the involution of mammary glands, described earlier for other xenoestrogens such as perfluorooctanoic acid (White et al., 2007). Interestingly, the increased time of nursing at LD 14 (see Section 6.4), together with the delayed development of pups,

suggested that BPS at the lowest dose (2 µg/kg bw/day) was associated with lower quality and/or quantity of lactation (i.e. milk production and/or composition).

These studies suggest that developmental exposure to BPS altered mammary gland development in females and males. In addition, in males, low doses of BPS decreased the effects of postnatal EE2 challenges, whereas higher doses enhanced these effects. Although these results might appear to be contradictory, they suggest that BPS may advance some aspects of gland development while delaying others.

Tucker et al. (2018) investigated the effects of prenatal exposure (GD 10 to GD 17) to 50, 500 and 5,000 µg/kg bw of BPS twice a day on mammary gland development. The developmental scoring of mammary gland development was increased from the lowest dose (50 µg/kg bw) at PND 35. At PND 56, which represents the theorical stage of mature mammary gland branching, advanced mammary development was maintained, with an enhanced branching density for the highest dose of 5,000 µg/kg bw. This accelerated pubertal mammary development and the extended presence of TEBs into adulthood occurred without changes in other pubertal indicators (age at vaginal opening and first estrus, or estrous cyclicity) or body weight. In addition, neither serum steroid concentrations, with the exception of a decrease in dehydroepiandrosterone in old mice, nor mammary steroid receptor expression (*Estrogen receptor 1: Esr1, Progesterone receptor: Pgr, Ar,* and *G protein-coupled estrogen receptor 1: Gper1*), could explain these results. Later in life, female mice at 14 months of age developed non-neoplasic lesions including inflammation, squamous metaplasia, and lobuloalveolar hyperplasia, mainly for the middle dose of 500 µg/kg bw. Moreover, neoplasic lesions such as fibroadenoma, carcinoma, and histiocytic sarcoma were diagnosed between 11 and 16 months of age at 500 and 5,000 µg/kg bw. BPS displayed more lesional effects on mammary glands than BPA at the same doses. The study by Tucker et al. (2018) showed that prenatal exposure to 50 µg/kg bw/day was sufficient to observe disrupted mammary gland development. BPS also induced long-lasting effects on the mammary gland that may increase the risk for mammary gland lesions. Additional studies are needed to evaluate the long-term consequences of BPS exposure, including its ability to induce or promote carcinogenesis.

Wormsbaecher et al. (2020) investigated BPA and BPS effects on mammary target genes involved in gland stiffness following *in utero* exposure (ED 9.5 to ED 18.5). Whereas 25

µg/kg bw/day of BPA (via IP or gavage) increased collagen production and mammary gland stiffness in 12-week-old mice, no similar effects (considered risk factors for breast cancer) were observed for BPS (via gavage) at the same dose level. This apparent discrepancy with the other two studies (Kolla et al., 2018; Tucker et al., 2018) may be due to the fact that a single BPS dose of 25 µg/kg bw/day was used here, and this dose may not be the most efficient. In addition, analyses were limited to young adult mice, whereas lesions associated with fetal exposure to BPS were recorded later in life.

These studies showed that female mice prenatally exposed to BPS developed proliferative epithelial lesions by midlife, concomitant with a significant inflammatory response that may predispose them to tumor formation later in life. In fact, animals in the high-dose BPS group developed adenocarcinomas prior to one year of life, and these diagnoses triggered necropsy at 14 months of age in the remaining animals (Kolla et al., 2018; Tucker et al., 2018). The extended presence of TEBs into adulthood and the altered immune responses (e.g., increased perivascular inflammation) may have been important modifiers of the persistent adverse effects observed later in life.

Overall, these studies show that exposure to BPS altered the mammary gland, confirming that it is a sensitive target organ for xenoestrogens like bisphenols (Fenton et al., 2012; Kass et al., 2012; Soto et al., 2013; Vandenberg et al., 2008; Vandenberg et al., 2007). A LOAEL dose of 2 µg/kg bw/day was determined for effects on mammary gland development in both females and males.

6.4. Behavioral effects of BPS exposure

Six experimental studies addressing behavioral effects of BPS in rodents were identified. Among these studies, one addressing feeding behavior (Rezg et al., 2018) was discussed in the section 6.5 of this article. Among the five remaining studies, two analyzed maternal behavior (Catanese and Vandenberg, 2017; LaPlante et al., 2017), two studied anxiety-related behavior (da Silva et al., 2019; Mornagui et al., 2019), and one assessed social interactions (Kim et al., 2015). Table S7 includes only 3 behavioral studies (Catanese and Vandenberg, 2017; da Silva et al., 2019; LaPlante et al., 2017); the works of Kim et al. (2015) and Mornagui et al. (2019) used only one BPS dose in addition to a small number of tested animals for Mornagui et al. (2019). The data reported in these two latter studies are, however, cited below.

The two studies conducted by the group of Vandenberg showed that adult or developmental exposure to BPS altered maternal behavior in mice. In particular, BPS-exposed F0 dams or their F1 descendants displayed several changes in spontaneous behavior (cleaning of pups, nest size, time spent in the nest, nursing, and high-crouch position) at the BPS doses indicated in Table S7. Effects on pup retrieval induced following mother/pup separation were observed only in F0 dams. Another observed effect was the delayed growth and development of pups born to F0 dams exposed to BPS at 2 µg/kg bw/day, suggesting deficient nursing and/or milk production, as shown by the altered lactating mammary gland analyzed in the same study ((LaPlante et al., 2017); see Section 6.3).

Maternal behavior is tightly regulated by a hormonal sequence involving, at the end of gestation, a decrease in progesterone levels and an increase in estradiol levels, which then promote the expression and release of other hormones and neuropeptides involved in the expression of this behavior, such as oxytocin. This hormonal sequence acts on the neural circuitry including hypothalamic structures (preoptic area, arcuate nucleus), connected to the mesolimbic reward system (ventral tegmental area). Interestingly, the maternal behavioral changes were associated with reduced estradiol levels and increased estrogen receptor α (ERα) immunoreactivity in the medial preoptic area, suggesting reduced estrogen sensitivity in BPS-exposed F0 dams. The analyses of other markers of key neuronal populations (ERα, Stat5, and tyrosine hydroxylase) in the arcuate nucleus or ventral tegmental area did not show differences between controls and BPS-exposed F0 or F1 dams.

In these studies, a LOAEL of 2 µg/kg bw/day was observed for both periods of exposure. In comparison, previous studies showed altered maternal behavior following developmental exposure to BPA at doses of 10 µg/kg bw/day in mice (Palanza et al., 2002), and 5 µg/kg bw/day and 40 µg/kg bw/day in rats (Boudalia et al., 2014; Della Seta et al., 2005). Exposure during adulthood to BPA also impacted maternal behavior, as shown in mice at a BPA dose of 200 µg/kg bw/day (Kundakovic et al., 2013), or in Cynomolgus monkeys at a dose of 10 µg/kg bw/day (Nakagami et al., 2009). In a more recent study, exposure of CD1 female mice (F0) to BPA or BPS at 0.5 µg/kg bw/day from GD 7 until delivery induced long-term effects in unexposed F3 females generated from unexposed F2 and prenatally exposed F1 dams (Shi et al., 2019b). In particular, pup loss after delivery was observed in F3 dams, suggesting potential insufficient maternal care.

Concerning anxiety-related behavior, the study conducted by da Silva et al. (2019) showed that developmental exposure of rats to BPS increased the anxiety state level in males, but not in females (Table S7). In agreement with these data, and despite a small number of animals used (5 per treatment group), Mornagui et al. (2019) also reported an increased anxiety state level measured in the elevated plus maze test for adult male mice exposed to BPS at 100 µg/kg bw/day, through drinking water, from postnatal week 3 to postnatal week 10. Another study assessing the effects of exposure to BPS at 200 µg/kg bw/day from GD 8 to PND 21 reported reduced social interactions in male adult mice, possibly due to an increased anxiety state level, but this behavior was not measured (Kim et al., 2015). The data obtained in female rats by da Silva et al. (2019) should be interpreted cautiously since the stage of the estrous cycle was not considered in these analyses. Clearly, anxiety-related behavior is modulated by estradiol levels in rodents; females are less anxious under estrogen dominance (proestrus and estrus phases) (Naulé et al., 2015).

BPS exposure also induced molecular changes, in particular in neurotransmitter and hormonal systems (serotonin, sex steroids, corticosteroids, or thyroid hormones), known to modulate anxiety-related behavior. In the study by da Silva et al. (2019), changes in thyroid hormone levels were detected in PND21 males. Another study assessing the effects of gestational and lactational exposure to BPA, BPS or BPF (10 µg/kg bw/day) showed a total of 25 genes involved in dopamine and serotonin pathways significantly regulated by BPA, and 24 genes by BPS in female rats (Castro et al., 2015). A common change induced by these susbtances was upregulation of *Cyp2d4*, which is involved in the hydroxylation of allopregnanolone and progesterone and thus the production of deoxycorticosterone. In addition, reduced cortical expression of 5-α reductase, involved in the metabolization of testosterone into 5-α dihydrotestosterone and biosynthesis of neurosteroids, was observed with changes in expression levels of 5-α reductase 3 for BPS, and 5-α reductase 2 and 3 for BPA.

These studies indicate that anxiety-related behavior can be affected by developmental exposure to BPS in males, with a LOAEL dose of 10 µg/kg bw/day. A very recent review of the literature suggests that perinatal exposure to relatively low doses of BPA may alter hormonal systems sensitive to stress and cause endocrine disruption, which translate to anxious and depressive states later in life (Wiersielis et al., 2020).

6.5. Effects of BPS on metabolism/obesity

Eighteen original studies investigating the *in vivo* metabolic effects of BPS were identified. One study was not evaluated (Xu et al., 2019) because it examined whether BPS modulated the development of type 1 diabetes using a non-obese diabetic mouse model, while the present investigation focuses on the possible obesogenic functions of BPS and metabolic disorders associated with obesity. The 17 remaining studies are characterized by a large disparity within the protocols in terms of exposure periods, BPS doses used, species analyzed, sex studied, and metabolic outcomes measured. For example, the exposure periods targeted pregnancy (Ahn et al., 2020; Ullah et al., 2019c), pregnancy and lactation (da Silva et al., 2019; Jing et al., 2019; Meng et al., 2019a; Meng et al., 2019b; Pu et al., 2017), the period ranging from pregnancy to adulthood (Brulport et al., 2020a; Brulport et al., 2020b; Ivry Del Moral et al., 2016), and weaning to adulthood (Azevedo et al., 2020; Azevedo et al., 2019; Gao et al., 2020; Rezg et al., 2018; Rezg et al., 2019; Zhang et al., 2018), or were limited to adulthood (Mandrah et al., 2020). Eleven studies evaluated only males, 4 studies evaluated both sexes and 2 studies evaluated only females (Table S8). Worthy of note, in several instances laboratories have published several studies using the same experimental model, e.g., Chagnon and colleagues (Brulport et al., 2020a; Brulport et al., 2020b; Ivry Del Moral et al., 2016); Veiga-Lopez and colleagues (Jing et al., 2019; Pu et al., 2017); Meng and colleagues (Meng et al., 2019a; Meng et al., 2019b); Azevedo and colleagues (Azevedo et al., 2020; Azevedo et al., 2019); and Rezg and colleagues (Rezg et al., 2018; Rezg et al., 2019).

The BPS doses used were based on BPA toxicological values to frame the tolerable daily intake and NOAEL doses, and ranged from 1 µg/kg bw/day to 50 mg/kg bw/day. Most studies used BPS at doses of 50 to 500 µg/kg bw/day. Routes of exposure included subcutaneous injections, oral gavage, drinking water or incorporation into diet. Species used included sheeps, rats (Wistar, Sprague-Dawley), and mice (Swiss Albino, ICR and C57Bl/6). The surveyed metabolic outcomes were highly variable as described in Table S8. In addition to measurement of body weight, fat adipose depots and liver, and measurement of biochemical parameters and plasma levels of hormones and adipokines, outcomes sometimes included metabolic tests to survey tolerance to glucose and sensitivity to insulin, and measurement of triglycerides, free fatty acids, and cholesterol to assess possible dyslipidemia, as well as lipid accumulation in the liver, which may result in steatosis. Several studies developed gene expression analysis to

assess lipid and carbohydrate metabolism in the liver and adipogenesis and inflammation in the adipose tissue. Food intake and preference was also evaluated in some studies.

Overall, in nine studies, BPS was found to significantly enhance body weight in males for doses ranging from 1.5 to 5,000 µg/kg bw/day (Ahn et al., 2020; Azevedo et al., 2020; Azevedo et al., 2019; Brulport et al., 2020a; Ivry Del Moral et al., 2016; Meng et al., 2019a; Rezg et al., 2018; Rezg et al., 2019; Ullah et al., 2019c). One study, which explored both sexes, reported enhanced body weight only in male mice chronically exposed from gestation until adulthood, and switched to a high-fat diet from weaning onwards (Ivry Del Moral et al., 2016). Similarly, two other studies (Ahn et al., 2020; Meng et al., 2019a) reported that BPS-induced effects were more pronounced in male mice fed a high-fat diet than in those fed a low-fat diet. Exposure to BPS during pregnancy (Ahn et al., 2020; Ullah et al., 2019c) or from pregnancy until adulthood (Ivry Del Moral et al., 2016) triggered body weight changes in males. One study reported that BPS was as efficient in enhancing body weight of the male offspring as its analogs that included BPA, BPB and BPF (Ullah et al., 2019c). In contrast, in two other studies performed in the same laboratory (Azevedo et al., 2020; Azevedo et al., 2019), a group of mice was exposed to BPA and in contrast to BPS, BPA did not elicit body weight enhancement. Within these studies, different outcomes were analyzed in addition to recording the body weights. This included analysis of lipid and glucose metabolism, food intake regulation, and mitochondrial dysfunction in the liver, as shown in Table S8. Conclusions favored the hypothesis of BPS acting as an obesogenic substance in males, especially when fed a high-fat diet with doses as low as 1.5 µg/kg bw/day.

A single study (Meng et al., 2019b) over the 6 studies performed on females reported enhanced body weight. In this study, female mice were exposed from GD 7 to PND 21 to BPS or to BPA, all at 100 µg/kg bw/day and fed a standard diet. Interestingly, only BPS was efficient at promoting gain weight. Unfortunately, the authors did not investigate as they did for males if BPS-induced effects were aggravated in females fed with a high-fat diet as they did for males.

Six studies reported unchanged body weight following BPS exposure (Ahn et al., 2020; da Silva et al., 2019; Gao and Kannan, 2020; Gao et al., 2020; Jing et al., 2019; Mandrah et al., 2020; Pu et al., 2017; Zhang et al., 2018) in addition to the female part of the study of Ivry Del Moral (2016). Zhang et al. (2018) did not observe body weight gain in male mice fed a standard diet in which BPS was incorporated resulting in BPS exposure ranging from 5 to 5,000 µg/kg bw/day. Unfortunately, the authors did not expose the mice to a high-fat diet in

addition to BPS, which would have made it possible to compare the results with those of Ivry Del Moral et al. (2016). Only hepatic oxidative stress was reported in mice exposed to the highest dose (Zhang et al., 2018). Gao et al. (2020) analyzed only female C57Bl/6 mice exposed through drinking water to BPS doses of 50 and 5000 µg/kg bw/day for 10 weeks from weaning. The effects of both BPA and BPS were evaluated as in the study of Meng et al. (2019b). In these two studies, mice were fed a standard diet. However, in the study of Gao et al. (2020), neither BPS nor BPA had any effect on body weight. Glucose tolerance was also unaffected. Interestingly, the authors reported enhanced expression of the genes encoding liver X receptor (LXR)α and fatty acid binding factor (FABP)4 in response to BPA and BPS exposure, with similar activity. LXRα is a transcription factor that regulates the expression of key genes involved in lipid and cholesterol metabolism. FABP4 plays an important role in fatty acid storage. These data suggested lipid accumulation in the liver, although steatosis was probably limited because glucose tolerance was not affected in exposed mice. In the study by da Silva et al. (2019), Wistar rats were exposed to BPS at doses of 10 and 50 µg/kg bw/day during gestation and lactation via intra-gastric gavage. Both sexes were studied. Rats exposed to BPS showed no change in body weight, in either dams or male and female offspring at weaning or adulthood (PND 180). Additionally, BPS did not interfere with glucose metabolism in males or females. However, BPS exposure lowered food intake in the adult offspring of both sexes. In females, BPS exposure induced thermogenesis, which was in line with the reduced lipid accumulation observed in the brown adipose tissue and the reduced size of lipid droplets in the white adipose tissue. Males exposed to BPS showed reduced visceral adiposity. It was suggested by da Silva et al. (2019) that these effects on visceral adiposity caused by BPS exposure could originate from dysregulation of thyroid hormone homeostasis and changes in feeding behavior. However, as BPS-exposed mice have increased preference for a fat-enriched diet, increased risk for obesity is likely in situations in which only high-calorie diets would be available. In the study of Mandrah et al. (2020), BPS did not trigger enhanced body weight but rather hyperglycemia, in line with the levels of various biomarkers measured in serum and urine samples of the BPS-exposed rats, and reflecting dysregulation of glucose homeostasis. The two studies on sheep were performed within the same laboratory (Jing et al., 2019; Pu et al., 2017). The authors characterized the metabolic phenotype of male and female sheep fetuses in response to exposure to both BPA and BPS, used at the dose of 500 µg/kg bw/day. Exposure occurred only during gestation and analyses were performed on fetuses harvested before term.

Neither BPS nor BPA had any effect on fetal body weight in Pu et al., 2017. However, primary cultures of dissected perirenal adipose tissue from fetuses revealed that gestational exposure to BPS led to dysfunctional adipocytes in males. Adipocytes showed reduced expression of adipogenic markers and enhanced expression of estrogen receptors (*Esr1, Esr2*) and the glucocorticoid receptor (*Nr3c1*). These effects were not described in the BPS-exposed females. In females exposed to BPA but not in those exposed to BPS, dysprogramming of adipocytes was demonstrated. The second study in sheep involved skeletal muscle development. The authors showed that programing of skeletal muscle development, assessed by measuring the size and type of myofibers in primary cultures of skeketal muscle cells, was disrupted in response to gestational exposure to BPA and BPS, and that the situation was more critical in females and under BPS exposure. Although muscle plays a major role in energy metabolism and insulin sensitivity, it was not demonstrated whether these changes persisted in adulthood. Taken as a whole, it can be concluded that male adipocytes are more sensitive to BPS than female adipocytes, but that female muscle cells are more sensitive than male muscle cells. As dysfunction of both adipocytes and muscle cells can lead to insulin resistance and thus metabolic disorders, these studies indicate that BPS exerts adverse metabolic effects in both sexes.

From the *in vivo* studies reviewed, it is not possible to establish an NOAEL or a LOAEL because the two studies that used various doses of BPS demonstrated that BPS did not trigger obesity in male mice fed a standard diet (Ivry Del Moral et al., 2016; Zhang et al., 2018). However, one of the 2 studies also tested the combined impact of a high fat diet and exposure to BPS and showed worsening of diet-induced obesity and metabolic phenotype in treated males (Ivry Del Moral et al., 2016). Collectively, it is concluded that BPS could be a metabolic disruptor targeting several metabolic organs at both the neural and peripheral levels (liver, adipose tissue, and muscle). Parameters affecting the significance of the outcomes include sex (males are more susceptible than females), the periods and duration of exposure, and the nutritional context (effects are more often observed in animals fed a high-fat diet). Metabolic disruption may concern enhanced body weight gain, but also disruption of glucose and lipid metabolism and altered food intake and/or behavior. Enhanced expression of adipogenic markers, including the expression of genes targeted by PPARg, the master gene of adipogenesis, was also demonstrated. Dysregulation of thyroid hormone synthesis, changes in plasma levels of estradiol and testosterone, altered expression levels of estrogen and

glucocorticoid receptors, and mitochondrial dysfunction in the liver have been reported. All these mechanisms could account for the adverse effects of BPS on metabolism and obesity.

7. Discussion

Due to growing concerns about the increasing use of BPS to replace BPA, the present study addressed establishment of NOAELs or LOAELs in order to possibly derive human reference values for BPS. The available epidemiological and animal toxicological studies were analyzed, and the NOAELs or LOAELs determined for the analyzed endpoints in academic and regulatory studies were compared between each other. Of note, the endpoints including reproduction, the mammary gland, neurobehavior, and metabolism/obesity were also analyzed for the classification of BPA as an SVHC, thus allowing a relevant comparison between the two bisphenols.

Given that the available human data analyzed were insufficient to derive human reference values, and that neither TRVs nor OELs have been proposed by relevant EU or non-EU organizations to date, we used toxicological studies described as an alternative third option according to the strategy of Apel et al. (2020). More robust toxicological data have been made available in recent years thanks to toxicokinetic studies.

In this context, the toxicokinetic studies performed in several species, including humans, showed that BPS was well absorbed by the oral route, with a clearance mainly driven by its glucuronidation to BPS-G, like in the case of BPA. The major difference with BPA in both animal models and humans was the moderate efficiency of the first-pass effect, leading to 100-fold higher oral bioavailability compared to BPA. In addition, despite the higher efficiency of the blood-placental barrier to limit fetal exposure to BPS compared to BPA, BPS residence for a longer time than BPA in the fetal compartment led to concentrations in the same range as those reported for BPA under similar experimental conditions.

Analysis of the academic experimental data addressing BPS exposure provided evidence of adverse effects on female and male reproduction, the mammary gland, neurobehavior, and metabolism/obesity. Developmental exposure to BPS induced alteration of meiosis, oocyte maturation, and folliculogenesis processes, suggesting a potential impact on female fertility. In males, all the analyzed studies showed reduced spermatogenesis in rats and mice, but this decrease may have no consequence in rodents as a reduction by 55% in spermatozoa production

does not impact fertility (Forand et al., 2009). In contrast, a decrease in spermatozoa concentrations in semen in the human population may lead to lower couple fecundability and an increased demand for assisted reproduction (Leridon and Slama, 2008; Slama et al., 2004). The mean concentrations of spermatozoa in human semen have declined over the last few decades, now reaching a level below which hypofertility begins (Bonde et al., 1998; Le Moal et al., 2014). BPS exposure also disrupted mouse mammary gland development in males and females, and increased lesions in prenatally-exposed females. Studies also suggested that BPS may induce metabolic disruption, including body weight gain, alteration of lipid metabolism, and food intake behavior. Further studies will certainly be needed to better determine the potential obesogenic and sex-dependent effects of BPS. A very recent study (Brulport et al., 2021) reported that perinatal exposure to BPS in C57Bl6/J mice fed a high-fat diet, while not affecting females of the F1 generation (Ivry Del Moral et al., 2016), triggered sex-dependent multigenerational obesogenic effects, especially in both sexes of the F2 generation and in females only of the F3 generation. At the neural level, developmental or adult BPS exposure also altered maternal behavior and increased anxiety-related behavior.

Interestingly, the majority of these effects were previously described for BPA and were included in the SVHC evaluation of its endocrine disruptive activities (Anses, 2017; Beausoleil et al., 2018; Le Magueresse-Battistoni et al., 2018; Mhaouty-Kodja et al., 2018; Perrot-Applanat et al., 2018; Pouzaud et al., 2018; Viguié et al., 2018). Exceptions are male reproduction and maternal and anxiety-related behaviors. The updated literature clearly shows adverse effects of BPA on male reproduction in animals (Cariati et al., 2019; Matuszczak et al., 2019; Siracusa et al., 2018), and on maternal behavior in rats, mice, and primates (Boudalia et al., 2014; Della Seta et al., 2005; Kundakovic et al., 2013; Nakagami et al., 2009; Palanza et al., 2002). Furthermore, since our previous evaluation, which reported inconclusive data on the effects of developmental exposure to BPA on anxiety state levels in rats and mice (ANSES, 2013), a very recent review of the literature suggests that perinatal BPA exposure may alter anxiety- and depressive-like behaviors (Wiersielis et al., 2020).

In comparison to the academic data, which detailed the analysis of effects on specific organs or systems, the regulatory studies addressed systemic hepatic, renal, splenic, and digestive tract toxicity, as well as reproduction and development. Another difference lies in the BPS doses used for exposure, which were higher in regulatory than in academic studies (200 to 1,500-

fold). These discrepancies could explain the differences in the determined PODs, with LOAELs between 60 and 300 mg/kg bw/day for developmental exposure in regulatory studies versus 0.5 to 10 µg/kg bw/day in academic studies (Tables 1 and 2). Inconsistency between the determined PODs was observed, regardless of the endpoint considered, with a ratio of regulatory/academic ranging from 30,000 to 120,000. Most of the reviewed academic studies converged on doses in the µg range that elicited adverse effects in rats or mice. It would be interesting to compare the human reference values calculated from the present determined N(L)OAELs with human biomonitoring data for BPS.

Altogether, these observations raise two main questions. The first is related to the current guidance, which was historically established by the OECD based on previous knowledge in toxicology. There is a need to adapt these regulatory studies to modern tools of investigation and to the more recent scientific findings of the last few decades, showing long-lasting adverse effects induced at low doses for some of the chemical substances, with possibile transmission across generations. The second question concerns the substitution of bisphenol A with other bisphenols of the same family. The present comparison between BPS and BPA shows that most of the effects on similar endpoints were induced by both substances, and in the same dose ranges. Moreover, a recent evaluation of another bisphenol, BPB, showed that this substance meets the WHO definition of an endocrine-disrupting chemical (Serra et al., 2019).

8. Conclusion

In conclusion, the toxicokinetic studies addressing BPS exposure in several species, including humans, indicated similarities and differences with BPA. In particular, higher oral bioavailability and persistence in the fetal compartment were observed for BPS, suggesting that replacement of BPA with BPS may lead to higher exposure to the active parent compound. Adverse effects of BPS were reported in several academic studies addressing male or female reproduction, the mammary gland, neurobehavior, or metabolism/obesity. In the absence of sufficient human studies, the analysis of animal toxicological studies to determine human reference values revealed striking differences between the values identified in regulatory versus academic studies. The LOAELs for developmental exposure to BPS were extremely low in academic studies compared to those reported in regulatory toxicity studies (0.5–10 µg/kg bw/day versus 60 mg/kg bw/day). For these reasons, we propose to derive human reference values for exposure to BPS from the N(L)OAELs determined in academic peer-

reviewed experimental studies. To better protect human health, the authors stress the importance of i) analyzing both regulatory and academic studies when setting PODs for reference values, and ii) adapting regulatory studies to be performed in the near future to recent scientific knowledge in toxicology.

Acknowledgements

We would like to thank the HBM4EU team including Petra Apel (UBA), Matthieu Meslin and Christophe Rousselle (Anses) for their scientific contribution, as well as the ANSES Working Group on endocrine disruption, and its scientific Anses coordinators François Pouzaud and Sandrine Charles.

Tables 1 and 2

Table 1: Overview of LOAELs or NOAELs identified in regulatory toxicological or reproductive toxicological studies conducted on the basis of OECD technical guidelines (orally applied dose) for BPS

Species, exposure period and duration	Endpoint/ target organ	LOAEL (mg/ kg bw /day)	NOAEL (mg/ kg bw/day)	OECD TG number and study reference
Adulthood, 13 days				
Rat	Kidney effects (histology and decreased weight) and other signs of toxicity (hematology, clinical chemistry, decreased liver organ weight and atrophy of the adipose tissue).		97 (in males)	ECHA CLP, 2019 quoted as Anonymous 18, 1973, and ECHA Dissemination, 2020 quoted as Eastman-Kodak, 1973
Adulthood, 28 days				
Rat (study including a reversibility period)	Suppressed bw gain, kidney (in both sexes) and caecum effects (intestinal bleedings in males, dilatation of the caecum in females, hyperplasia of the mucosa with single cell necrosis).	200	40	TG 407 (OECD SIDS 2013, ECHA CLP, 2019 quoted as Anonymous 16, 1999 and ECHA Dissemination, 2020.
Rat	Decreased final body weight, increased kidney, adrenal, liver weight and histopathological changes, decreased prostate and seminal vesicle weights, and diffuse atrophy of the mammary gland (in males).	100		TG 407 (or similar to) ECHA CLP, 2019 quoted as Anonymous 15, 2017.
Adulthood, 90 days				
Rat	Decreased body weight in males.	1000 (in males)		TG 408
	Mammary gland (change from the physiological lobulo-alveolar morphology to a tubulo-alveolar appearance with smaller more basophilic epithelial lining cells (atrophy) in males).	300 (in males)	100 (in males)	
	Uterus (focal squamous cell metaplasia of glandular epithelium)	1000 (in females)	300 (in females)	
	Adrenals (increase weights and cortical hypertrophy/hyperplasia).	1000 (in males)	300 (in males)	
	Liver (increase liver weight, hypertrophy and eosinophilic foci in high dose females and increase relative liver weight in high dose males with clinical pathology findings.	1000 (both sex)	300 (both sex)	
Reproductive and developmental toxicity				
Rat, gestational exposure, 14 days	Maternal toxicity such as reduced food consumption and corrected (net) body weight gain.		100	TG 414 (ECHA CLP report, 2019 (study quoted as Anonymous 19, 2014). ECHA Dissemination, 2020).
	Prenatal development: no toxicologically relevant adverse fetal findings evident.		300	
Rat, 45 days (males) and 53 days (females)	Parental systemic toxicity (suppressed body weight gain and decreased food consumption in both sexes). Liver (increase liver weight in high dose males and histological hypertrophy of hepatocytes in both sexes at the highest dose (300 mg/kg bw/day).		60	TG 421 (ECHA CLP, 2019 quoted as Anonymous 12, 2000, OECD SIDS 2013 and ECHA Dissemination, 2020).
	Parental effects on the caecum (distension of the caecum in both sexes and diffuse hyperplasia of the caecum mucosal epithelium).	60	10	
	Decreased seminal vesicle weights in high dose males.			
	Reproductive and developmental toxicity based on prolongation of estrous cycle and diestrus period, decreased fertility index and decreased implantation index.	300	60	
Rat, 12 weeks (males) or 14 weeks (females)	Systemic effects in F0 (more pronounced in males) on kidney, liver caecum (dilatation) effects identified by macroscopic and histopathological examinations.	100	30	TG 422 (ECHA CLP 2019 quoted as Anonymous 14, 2017, 2019).
	Developmental and reproductive toxicity, reduced number of implantations, increased post-implantation losses, decrease in total number of pups delivered, decrease in mean number of pups delivered.	300	100	
	Mammary gland histology (atrophy in males).	300	100	
Rat, EOGRT with F2, DNT (cohorts 2A and 2B) and DIT (cohort 3)[1]	General systemic toxicity based on increased kidney weight and histopathological changes in both F0 and F1 in both sexes.	180	60	TG 443 (ECHA 2019, CLP quoted as
	Mammary gland histology (atrophy in males in cohort 1A).	180		
	Rats, developmental toxicity based on post implantation loss in the F1 progeny.	60	20	
	Rats, fertility and reproductive performance for F0 and F1 parental rats.		180	
	Rats, developmental neurotoxicity (in both sexes).		180	
	Rats, developmental immunotoxicity for F1 progeny, based on decreased spleen weight at the mid-dose in males.		20	

Table 2: Overview of L(N)OAEL identified in BPS academic oral studies

Endpoint/ targeted organ or system, species, exposure period and duration	LOAEL (µg/kg bw/day)	NOAEL (µg/kg bw/day)	Reference
Male reproductive system:			
CD-1 mice exposed from GD11 to birth Increased germ cell apoptosis in neonates	**0.5**	**n.d.**	Shi et al., 2018
CD-1 mice exposed from GD7 to birth: Important reduction in sperm number and motility in cauda epididymis in adults; these alterations were trangenerationnally transmitted.	**0.5**	n.d.	Shi, Whorton, et al. 2019b
Female reproductive system:			
CD-1 mice exposed from GD11 to birth: Alteration of folliculogenesis in F1 females at PND4.	**0.5**	**n.d.**	Shi, Sekulovski, et al., 2019
Mammary gland:			
CD-1 Mice exposed from GD9 to PND20: Mammary gland physiology (morphology) effects showed a delayed growth.	**2**	**n.d.**	Laplante et al., 2017
CD-1 Mice exposed from GD9 to PND20: Mammary gland whole mount and histology analysis showed increased number of TEBs at PND20 and at 3 months).	**2**	**n.d.**	Kolla et al., 2018
CD-1 Mice exposed from GD9 to PND20: At the puberty (PND24), dose-dependent decrease in ductal areas in the left mammary gland only (no effect in the right gland), whereas later in life, a dose-dependent increase in ductal area was mainly observed in the right mammary gland (Week 9).	**2**	**n.d.**	Kolla et al., 2019
Nervous system			
CD-1 mice exposed GD8/9 to PND20/21 Infanticide and severe maternal neglect. Females spent less time in the nest on LD2 and LD7 at 2 or 200 µg/kg bw/day. On LD14, those exposed to 200 µg/kg bw/day spent more time in nest building.	**2**	**n.d.**	Catanese and Vandenberg, 2017
Wistar rat exposed from GD1 to PND 21: Anxiety-related behaviour observed in male rat (in the elevated plus maze, males spent less time in the open arms and entered it less frequently than controls).	**10**	**n.d.**	da Silva et al., 2019
Legend: * Estimated by Zhang, Lin et al. 2018 with the assumption of a food uptake of 100 g/kg bw/day. **Using standard factors according to REACH Guidance (ECHA 2012) for food consumption of mice (120 g food/kg bw/day), 1.2 fold higher values are obtained for the LOAEL and NOAEL. All the other N(L)OAEL were derived by the ANSES ED-EG.			

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Figure legends

Figure 1: Protocol used for literature search

Figure 2: Transplacental exchange rates of BPA, BPS, and their main glucuronidated metabolites (BPS-G and BPA-G)

Figure

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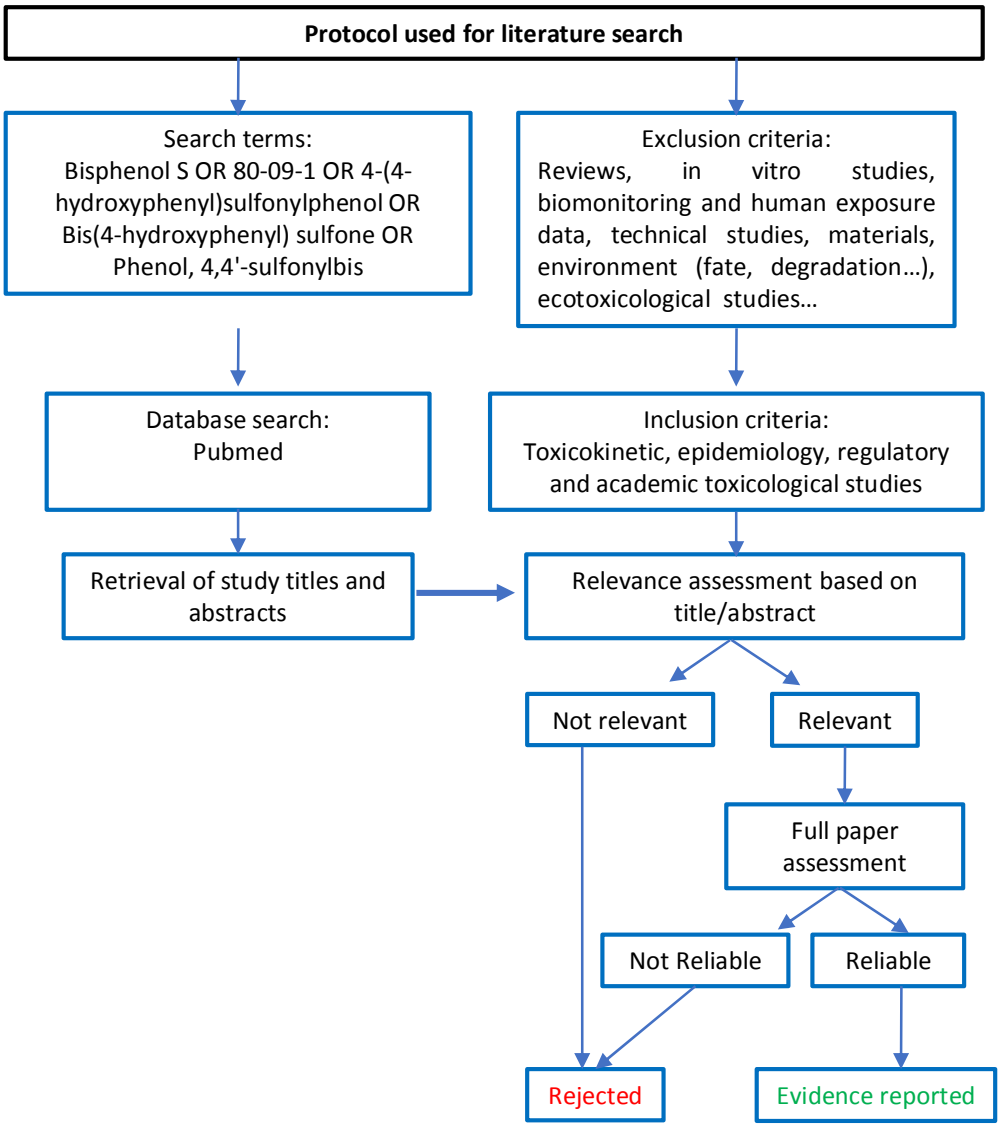

Figure 1

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Author contributions

CB performed the full literature search, which was reviewed by SMK. The assessment of relevance and reliability of studies was first shared between the authors according to their area of expertise: Epidemiology (NC), Toxicokinetics (NPH and CE), Regulatory studies (CB); Male reproduction (RH); Female reproduction (CV); Mammary glands (S.B and M-C C-L); Behavior (SMK); Metabolism/obesity (BLMB). Then, all the data were discussed between all the authors, and reviewed by C. Beausoleil and S. Mhaouty-Kodja. The article was written and corrected by all the authors.

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Conflict of interests

The authors declare that they have no competing interests to declare.

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☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Claire BEAUSOLEIL