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

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3 1 **Title: Regulatory and academic studies to derive reference values for human health: the case**
4 2 **of Bisphenol S**
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45
46 35 **Abbreviations:**

47 36
48 37 ECHA: European Chemicals Agency; EFSA: European Food Safety Authority; IARC:
49 38 International Agency for Research on Cancer; LOAEL: lowest-observed-adverse-effect level;
50 39 NIOSH: National Institute for Occupational Safety and Health; NOAEL: no-observed-adverse-
51 40 effect level; OECD: Organisation for Economic Co-operation and Development; SCCS:
52 41 Scientific Committee on Consumer Safety; WHO: World Health Organization.
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2
3 45 **Abstract**

4 46 The close structural analogy of bisphenol (BP) S with BPA, a recognized endocrine-disrupting
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6 47 chemical and a substance of very high concern in the European Union, highlights the need to
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8 48 assess the extent of similarities between the two compounds and carefully scrutinize BPS
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10 49 potential toxicity for human health. This analysis aimed to investigate human health toxicity
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12 50 data regarding BPS, to find a point of departure for the derivation of human guidance values.

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14 51 A systematic and transparent methodology was applied to determine whether European or
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16 52 international reference values have been established for BPS. In the absence of such values,
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18 53 the scientific literature on human health effects was evaluated by focusing on human
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20 54 epidemiological and animal experimental studies. The results were analyzed by target
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22 55 organ/system: male and female reproduction, mammary gland, neurobehavior, and
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24 56 metabolism/obesity. Academic experimental studies were analyzed and compared to regulatory
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26 57 data including subchronic studies and an extended one-generation and reproduction study. In
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28 58 contrast to the regulatory studies, which were performed at dose levels in the mg/kg bw/day
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30 59 range, the academic dataset on specific target organs or systems showed adverse effects for
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32 60 BPS at much lower doses (0.5 to 10 µg/kg bw/day). A large disparity between the lowest-
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34 61 observed-adverse-effect levels (LOAELs) derived from regulatory and academic studies was
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36 62 observed for BPS, as for BPA. Toxicokinetic data on BPS from animal and human studies were
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38 63 also analyzed and showed a 100-fold higher oral bioavailability compared to BPA in a pig
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40 64 model. The similarities and differences between the two bisphenols, in particular the higher
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42 65 bioavailability of BPS in its active (non-conjugated) form and its potential impact on human
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44 66 health, are discussed. Based on the available experimental data, and for a better human
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46 67 protection, we propose to derive human reference values for exposure to BPS from the
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48 68 N(L)OAELs determined in academic studies.

49
50 70 **Keywords**

51 71 *Bisphenol S, Endocrine-disrupting chemical, Health-based guidance value, Toxicity, Policy*
52 72 *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

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2 105 included male and female reproduction, the mammary gland, neurobehavior, and
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4 106 metabolism/obesity. Moreover, available human and animal toxicokinetic studies were also
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6 107 reviewed as a critical element to help extrapolate external exposure to internal exposure, to
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8 108 determine human reference values that could be considered by risk assessors from different
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10 109 organizations when setting health-based external or internal toxicological values.
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13 111 **2. Methods**

14 112 *2.1 Methodology overview*

15 113 Human biomonitoring guidance values (HBM-GVs) are body concentrations or internal
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17 114 exposure levels, related to external chemical exposure, below which no adverse effect on
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19 115 human health is expected to occur. According to the methodology developed by Apel et al.,
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21 116 there are three possible options for deriving HBM-GVs. Selecting one of these options is
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23 117 conditioned by data availability, data quality, and their relevance for deriving HBM-GVs (Apel
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25 118 et al., 2020). As a first priority option, a POD determined from a relationship between internal
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27 119 concentration of the substance of concern or its metabolite(s) and a selected critical health
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29 120 effect observed in humans should be considered as a starting point for deriving values. When
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31 121 this first strategy is not achievable, the second option is to estimate the concentration of the
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33 122 substance's biomarkers, which corresponds to an external toxicity reference value (TRV)
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35 123 already set by a recognized body (preferably European), using a rigorous and transparent
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37 124 scientific methodology. This approach is similar to the biomonitoring equivalent approach
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39 125 developed in the United States by Hays et al. (Hays and Aylward, 2009; Hays and Aylward,
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41 126 2012; Hays et al., 2007). Finally, if no TRV is available, the third option consists in
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43 127 extrapolating and adjusting a POD identified from an animal experimental study.

44 128 The first option is applicable when human data based on a relationship between internal
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46 129 concentrations and health effects are available. The most informative studies for deriving
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48 130 HBM-GVs are well-conducted human studies adequately reporting measured internal
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50 131 concentration levels of a substance, sampling times, analytical methods used, and the
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52 132 relationships between concentrations of a substance or its metabolites in human biological
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54 133 media and the occurrence of adverse effects. The second option refers to a TRV or occupational
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56 134 exposure limit (OEL) from a relevant EU or non-EU organization. However, no TRV for BPS
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58 135 has been published so far. Therefore, this option cannot be considered. The third option, based
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60 136 on a POD (e.g. N(L)OAEL or a benchmark dose (BMD)) identified from animal experimental
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137 studies, can be considered to derive HBM-GVs for BPS for both the general and occupational
138 populations, if the conditions of the first and second options are not met.

139
140 *2.2 Scientific literature search*

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

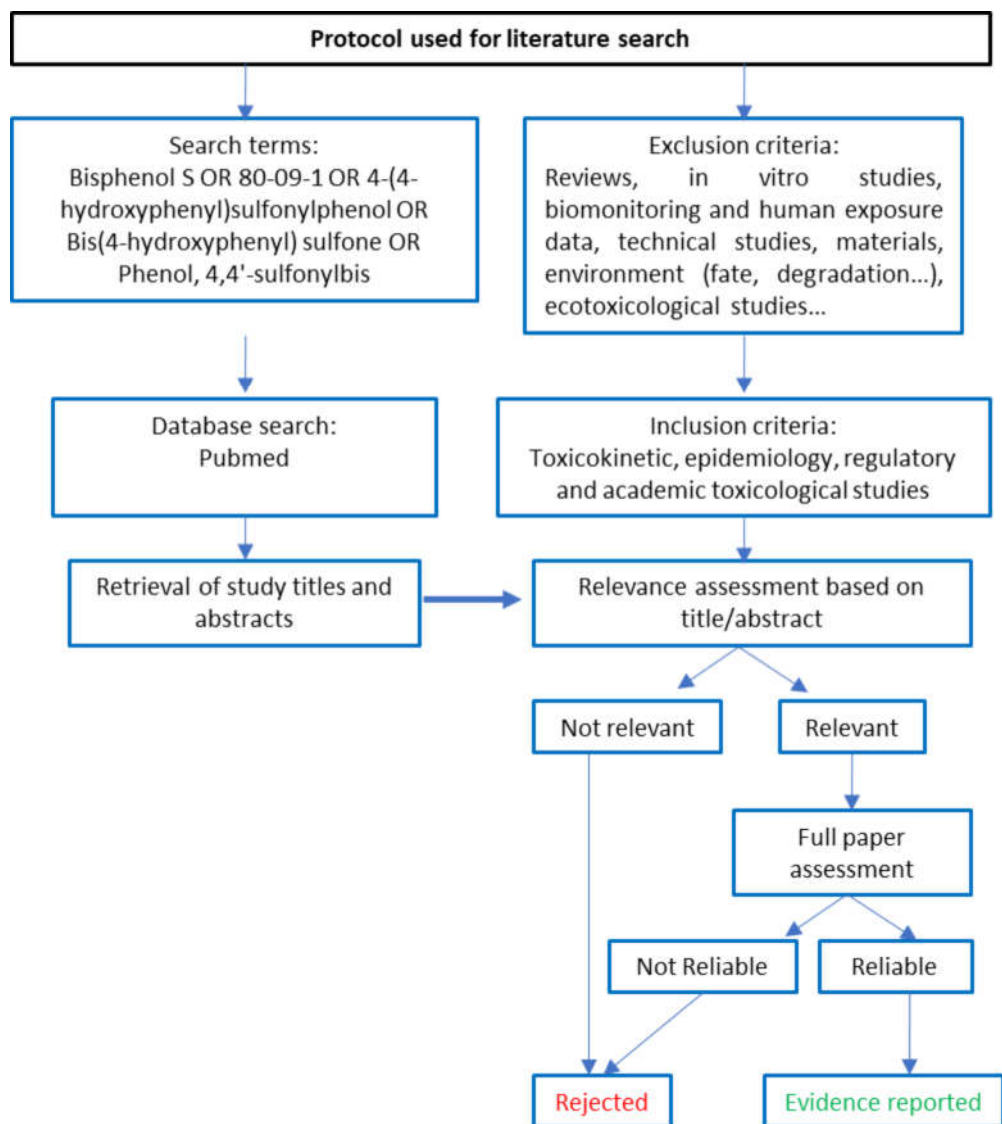


Figure 1: Protocol used for literature search. The exclusion and inclusion criteria are presented in details in Table S1.

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

1
2 165 obtained in different species, including mice, rats, piglets, sheeps, and humans, depending on
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4 166 the route of exposure in adults, and in the context of fetal exposure. In all species, BPS, like
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6 167 BPA, was rapidly metabolized, mainly into its glucuronide form.
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9 10 169 **3.1. Toxicokinetics of BPA and BPS by the intravenous route**

11 170 Following a single intravenous administration, BPS was less efficiently eliminated than BPA.
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13 171 Importantly, the plasma clearance, which measures the overall ability to eliminate a drug, is 2
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15 172 to 3.5 times lower than that of BPA (Gayrard et al., 2019; Grandin et al., 2017). In addition,
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17 173 BPS seemed to be more persistent in rats than in other species, as reflected by the mean
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19 174 residence time (MRT) range, which was 5 to 13 times higher due to a larger volume of steady-
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21 175 state distribution (V_{ss}) (5 to 21-fold). From an allometric approach (Gayrard et al., 2020),
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23 176 human BPS clearance was estimated to be 0.79 L/kg/h, a value two-fold lower than that of
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25 177 BPA.
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28 179 **3.2. Bioavailability of BPA and BPS by the oral route**

29 180 Bioavailability by the oral route quantifies the proportion of the parent substance actually
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31 181 reaching the systemic circulation. It is determined by both absorption and first-pass
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33 182 metabolism. Following oral administration, a large proportion of the dose was recovered in
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35 183 urine mainly as BPS glucuronide in human volunteers (Khmiri et al., 2020; Oh et al., 2018), in
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37 184 mice (Song et al., 2017), pigs (Gayrard et al., 2019), and in rats (EFSA, 2020; Waidyanatha et
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39 185 al., 2018), showing the considerable extent of BPS absorption by the oral route, and indicating
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41 186 that the total urinary BPS amount might be a good estimate of BPS internal exposure. The
42
43 187 moderate efficiency of hepatic first-pass BPS glucuronidation yields bioavailability of active
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45 188 BPS that is about 100 times higher than that of BPA (57.4% and 0.50%, respectively) in pigs
46
47 189 (Gayrard et al., 2019). Accordingly, *in vitro* data in humans indicated that the liver and
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49 190 intestinal intrinsic clearances of BPS were 11 and 6 times lower, respectively than those of
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51 191 BPA (Karrer et al., 2018). In accordance with data obtained in the pig model, human
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53 192 toxicokinetic studies using labelled BPS showed major differences between BPA and BPS
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55 193 kinetics, with much higher systemic levels of active BPS than BPA (Khmiri et al., 2020; Oh et
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57 194 al., 2018; Thayer et al., 2015). In addition, in rats and humans, enterohepatic recirculation of
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59 195 BPS was found (EFSA, 2020; Khmiri et al., 2020; Waidyanatha et al., 2018).
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1 2 197 **3.3. Bioavailability of BPA and BPS by the cutaneous route**

3
4 198 Dermal BPS exposure through thermal paper is a question of great importance. Two *in vivo*
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6 199 toxicokinetic studies evaluated the disposition of BPS after cutaneous exposures (Khmiri et al.,
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8 200 2020; Liu and Martin, 2019). The results showed that dermal absorption of BPS was limited
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10 201 (< 0.2%) compared to oral absorption, but with a longer residence time after dermal absorption
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12 202 (Khmiri et al., 2020). Furthermore, *in vitro* experiments with fresh human skin samples showed
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14 203 that BPS absorption was much lower than that of BPA, with at least a 100-fold difference when
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16 204 the vehicle used was acetone or water or a 2-fold difference with the sebum. However, both
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18 205 BPS and BPA were mainly absorbed without biotransformation (Liu and Martin, 2019,
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20 206 Champmartin et al., 2020). This limited metabolic capability of the skin and the persistence of
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22 207 BPS after human dermal exposure should be considered in evaluating the relative risks for
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24 208 bisphenols.

25 209 26 27 210 **3.4. Disposition of BPS in the materno-fetal unit**

28
29 211 In view of potential BPS-related endocrine disruption, it is very important to evaluate fetal
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31 212 exposure. Fetal exposure to bisphenols results from a complex interplay between bidirectional
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33 213 placental clearances and maternal and fetal metabolism. A chronically catheterized fetal sheep
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35 214 model (Gauderat et al., 2016; Grandin et al., 2018) and an *ex vivo* human placenta model
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37 215 (Grandin et al., 2019) were used to evaluate the disposition of BPS and BPS-glucuronide (BPS-
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39 216 G) in the materno-feto-placental unit, and to compare them to those of BPA and BPA-
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41 217 glucuronide (BPA-G). In the maternal compartment, BPS was mainly metabolized into BPS-
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43 218 G and mostly eliminated in urine (Table S2 and Figure 2). It was striking that the fraction of
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45 219 the maternal BPS dose transferred from mother to fetus (0.40%) was about 10 times lower than
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47 220 that of BPA. This limited placental passage of BPS compared to BPA has also been observed
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49 221 using an ex-vivo model of perfused human placental cotyledon (Grandin et al., 2019). In sheep,
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51 222 once in the fetal compartment, only 26% of the BPS dose entering the fetal blood, against 74%
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53 223 for BPA, was rapidly cleared from the fetal compartment through its transfer into maternal
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55 224 blood. About half of the BPS remaining in the fetal compartment was metabolized by the fetus
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57 225 into BPS-G. The ultimate elimination of BPS-G from the fetal compartment required its
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59 226 hydrolysis and back-conversion into free BPS due to its limited placental transfer. Therefore,
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61 227 despite lower materno-fetal transfer of BPS compared to BPA, the higher persistence of BPS
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63 228 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).

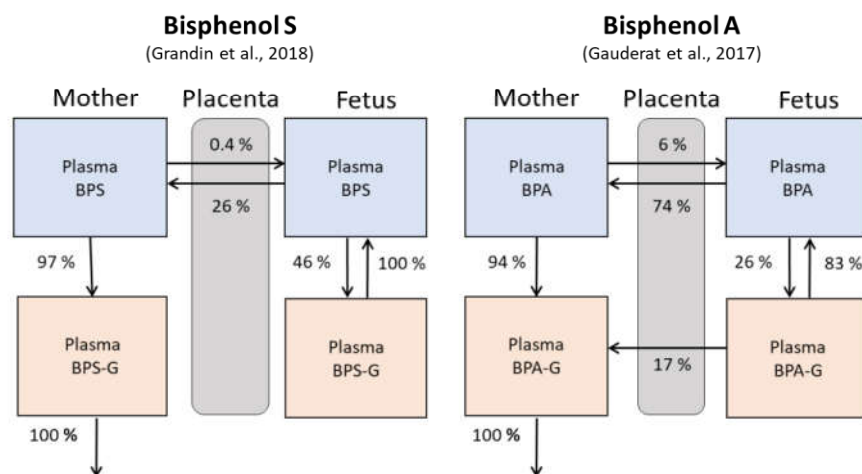


Figure 2: Transplacental exchange rates of BPA, BPS, and their main glucuronidated metabolites (BPS-G and BPA-G)

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 ($\beta = -38$ g [95% CI: -65, - 11 g]), as in the second trimester ($\beta = -43$ 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 ($\beta = -150.42$ 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).

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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 ($\Delta = -3.15$ 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 ($\Delta = -11.35$ pg/mL [95% CI: -18.71, - 3.33 pg/mL];

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2 316 p = 0.008) (Aker et al., 2019). In a cohort of 764 pregnant women, Wang et al. reported a
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4 317 negative correlation between maternal urinary BPS concentrations at first trimester of
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6 318 pregnancy and newborn mitochondrial DNA copy number in cord blood, but it was significant
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8 319 only for boys (Wang et al., 2021).
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10 320 11 321 **4.2. Metabolism and obesity**

12 322 Relationships between exposure to BPS and obesity (defined as a body mass index
13 323 (BMI) ≥ 30 kg/m² or $\geq 85^{\text{th}}$ percentile of the age- and sex-adjusted z-scores in children and
14 324 teens) were investigated in five studies in the United States. Three of them included only
15 325 children or teens (Jacobson et al., 2019; Kataria et al., 2017; Liu et al., 2019a) and four of them
16 326 analyzed a sample of the US National Health and Nutrition Examination Survey (NHANES)
17 327 2013–2014 (Jacobson et al., 2019; Liu et al., 2017; Liu et al., 2019a; Zhang et al., 2019b) as
18 328 summarized in Table S3.
19

20 329 The results of these studies are conflicting. Overall, it was observed that individuals with
21 330 obesity had higher median values of BPS in urine than those without obesity. In an analysis of
22 331 NHANES 2013–2014, urinary BPS was positively associated with increased odds of general
23 332 and abdominal obesity in adults (fourth vs. first quartile of urinary BPS: odds ratio 1.44 [95%
24 333 CI: 1.01 – 2.07; $p = 0.045$] and 1.45 [95% CI: 1.01 – 2.16; $p = 0.045$], respectively) (Zhang et
25 334 al., 2019b). However, these results were not significant after adjustments for lifestyle,
26 335 demographic, and socioeconomic factors (Liu et al., 2017). Furthermore, in a cohort of 662
27 336 Dutch adults (Lifelines Cohort Study), urinary BPS was not associated with BMI, waist
28 337 circumference or metabolic abnormalities but was detectable in only 9% of the sample (van
29 338 der Meer et al., 2020).
30

31 339 In children and teens, urinary BPS concentrations were higher in teens with obesity from the
32 340 NHANES 2015–2016 (Jacobson et al., 2019), but not in those from the NHANES 2013–2014
33 341 (Liu et al., 2019a), as in a very small US cross-sectional pilot study of 10–13-year-old teens
34 342 (Kataria et al., 2017). In NHANES 2015–2016, BPS exposure was inversely correlated with a
35 343 lower education level of the head of the household ($p = 0.05$), and with a lower poverty/income
36 344 ratio ($p = 0.01$) (Jacobson et al., 2019). Log-transformed continuous BPS concentrations were
37 345 associated with increased odds of general obesity (OR 1.16 [95% CI: 1.02 – 1.32]), severe
38 346 obesity (OR 1.18 [95% CI: 1.03 – 1.35]), and abdominal obesity (OR 1.13 [95% CI: 1.02 –
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1
2 347 1.27]). However, the BPS quartiles were not statistically significantly associated with any
3
4 348 outcome (Jacobson et al., 2019).
5
6 349 Similarly, six studies investigated the association of exposure to BPS with incident diabetes,
7
8 350 fasting blood glucose, or insulin resistance (Duan et al., 2018; Kataria et al., 2017; Lee et al.,
9
10 351 2019; Ranciere et al., 2019; Song et al., 2019; Zhang et al., 2019a) (Table S3). Song et al., 2019
11
12 352 reported that serum BPS levels were not associated with hyperglycemia in a nested population
13
14 353 of elderly patients living near an e-waste recycling facility in China. Kataria et al., 2017 did
15
16 354 not find associations between urinary BPS concentrations and insulin resistance in their cross-
17
18 355 sectional study of 10–13-year-old teens. In a cohort of pregnant women from Wuhan, China
19
20 356 (Zhang et al., 2019a), log-transformed urinary BPS concentrations were not associated with
21
22 357 incident gestational diabetes, but with increased fasting and post-load blood glucose (0.04
23
24 358 mmol/L for fasting blood glucose; 0.11 mmol/L for 1hr-post-load blood glucose, and 0.19
25
26 359 mmol/L for 2hr-post-load blood glucose; $p = 0.02$). In a case-control study, log-transformed
27
28 360 urinary BPS concentrations were associated with a significantly increased risk of type 2
29
30 361 diabetes (OR 1.73 [95% CI: 1.37 – 2.18; $p < 0.001$] for the total sample, and OR 3.83 [95%
31
32 362 CI: 2.37 – 6.20; $p < 0.001$] for people with BPS concentrations above the method detection
33
34 363 limits [57.9%]) (Duan et al., 2018). In a *posthoc* analysis of the D.E.S.I.R. cohort in France,
35
36 364 even though BPS-G was detectable only in 11% of the population, urinary BPS-G detection
37
38 365 was significantly associated with a higher risk of incident type 2 diabetes at baseline (adjusted
39
40 366 hazard ratio (aHR) 1.68 [95% CI: 1.09 – 2.58]) and at year 3 (aHR 2.81 [95% CI: 1.74 – 4.53])
41
42 367 (Ranciere et al., 2019). In a cohort of 459 women (20 – 48 years) from South Korea, in which
43
44 368 BPS was detectable in 83.7% of the study population, urinary BPS concentrations were
45
46 369 positively associated with an increase of insulin resistance evaluated by ln-transformed
47
48 370 homeostatic model assessment for insulin resistance (ln-HOMA-IR) ($p = 0.017$) calculated as
49
50 371 fasting insulin ($\mu\text{U/mL}$) x fasting glucose (mmol/L) x 22.5, but only in women without
51
52 372 overweight or obesity ($\text{BMI} < 25 \text{ kg/m}^2$) (Lee et al., 2019).
53
54 373 Five studies also investigated the association of BPS exposure with urinary markers of
55
56 374 oxidative stress. Urinary BPS concentrations were positively correlated with urinary
57
58 375 concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative stress, in a
59
60 376 nested case-control study conducted in a Chinese population residing near an e-waste recycling
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62 377 facility (Zhang et al., 2016), but not in a limited sample of men (Wang et al., 2019), nor in a
63
64 378 small cohort of teens (Kataria et al., 2017) or in a cohort of women suffering from unclear
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1
2 379 recurrent spontaneous abortion (Liang et al., 2020). In the American LIFECODES cohort,
3
4 380 urinary BPS concentrations were positively correlated with 8-isoprostane but not with 8-OHdG
5
6 381 ($\Delta = + 18.5\%$ [95% CI: +7.68, + 30.5%]; $p < 0.001$) (Ferguson et al., 2019). However, BPS
7
8 382 was detectable in this sample in only 21% of the participants (LOD 0.4 ng/mL).

9
10 383 In a cohort of 1437 patients from Wuhan (China), urinary BPS concentrations were
11
12 384 significantly higher in participants with incident hypertension ($n = 433$) (0.334 ng/mL vs 0.243
13
14 385 ng/mL; $p < 0.001$). Urinary BPS concentrations were significantly associated with systolic
15
16 386 blood pressure (third vs. first tertile of urinary BPS: odds ratio 3.89 [95% CI: 1.49 – 6.29; P -
17
18 387 *trend* = 0.006]), diastolic blood pressure (third vs. first tertile of urinary BPS: odds ratio 1.70
19
20 388 [95% CI: 0.26 – 3.14; P -*trend* = 0.046]), mean arterial pressure (third vs. first tertile of urinary
21
22 389 BPS: odds ratio 2.43 [95% CI: 0.79 – 4.07; P -*trend* = 0.013]) and mid blood pressure (third vs.
23
24 390 first tertile of urinary BPS: odds ratio 2.80 [95% CI: 1.00 – 4.60; P -*trend* = 0.009]) (Jiang et
25
26 391 al., 2020a). In a sample of Generation R Study including 1064 mother/child pairs, maternal
27
28 392 urinary BPS measured at each pregnancy trimester (12.9 [12.1-14.5] ; 20.4 [19.9-20.9] and
29
30 393 30.2 [29.9-30.8] WG) was not associated with arterial blood pressure measured at the age of
31
32 394 10 years in children (Sol et al., 2020).

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32 395

33 34 396 **4.3. Neurocognitive development**

35
36 397 The impact of exposure to BPS on neurocognitive development was evaluated in a sample (n
37
38 398 = 312) of an ongoing prospective pregnancy cohort (Alberta Pregnancy Outcomes and
39
40 399 Nutrition [APrON] Study). Urinary BPS concentrations at second trimester of pregnancy and
41
42 400 at the third post-partum month (0.16 and 0.18 ng/mL respectively) were not correlated with
43
44 401 neurocognitive development evaluated by Behavioral Rating Inventory of Executive Function
45
46 402 (BRIEF-P) in children at the age of 2 and 4 years (England-Mason et al., 2021). By contrast,
47
48 403 in a cohort of 456 mother/child pairs, psychomotor development index scores, evaluated by
49
50 404 Bayley Scales of Infant Development in childrens of 2 years old, decreased across quartiles of
51
52 405 urinary BPS concentrations (fourth vs. first tertile of urinary BPS: Δ PLDI = - 4.7 points; P -
53
54 406 *trend* = 0.01]) (Jiang et al., 2020b).

54 407

55 56 408 **4.4. Other health issues**

57 409 *Fecundability*

1
2 410 Philips et al. evaluated the impact of BPA analogues on fecundability among 877 participants
3
4 411 in the population-based Generation R pregnancy cohort (EU cohort). They reported no
5
6 412 association between bisphenol analogues including BPS and fecundability; but total bisphenols
7
8 413 (including 4,4-BPF, BPS, BPB, BPP, BPAF, BPAP, and BPZ) were associated with a longer
9
10 414 time to pregnancy in women with inadequate folic acid supplement use (Philips et al., 2018a;
11
12 415 Philips et al., 2018b).

13
14 416 In a case-control study including 124 women, urinary BPS concentrations were not associated
15
16 417 with occurrence of endometriosis, but BPS was detected only in 14.8% of the samples (Peinado
17
18 418 et al., 2020).

19 419 In the EARTH cohort, Ghayda et al. reported that urinary BPS concentrations in men (n = 158)
20
21 420 were significantly associated with lower sperm volum (2.66 vs 2.91 mL; p = 0.03) and lower
22
23 421 spermatozoa concentration (30.7 vs 38.3.10⁶/mL; p = 0.03), but not with motility and
24
25 422 morphological modifications of spermatozoa. This association was described only in men with
26
27 423 BMI \geq 25 kg/m², who had also significant higher urinary BPS concentrations (compared to
28
29 424 men with BMI < 25 kg/m²) (Ghayda et al., 2019).

30 425 *Hematological effects*

31
32 426 In a cohort of 196 pregnant women, urinary BPS concentrations were not associated with
33
34 427 significant modifications of haemato-biochemical alterations (including red blood cells, white
35
36 428 blood cells, platelets, hemoglobin level and hematocrit) (Kang et al., 2020).

37 429 *Respiratory effects*

38
39 430 In a sample of the NHANES 2013-2016 including 3538 teens over 12 years and adults, Mendy
40
41 431 et al. reported that a 10-fold increase in urinary BPS concentration was associated with
42
43 432 increased odds of current asthma only in men (OR 1.64 [95% CI: 1.13 – 1.240]; p = 0.01)
44
45 433 (Mendy et al., 2020). However, these results were not significant after adjustments for age,
46
47 434 lifestyle, demographic, socioeconomic factors, exposure to cigarette smoke and history of
48
49 435 asthma. In the prospective Mouse Allergen and Asthma Cohort Study (MAACS), urinary BPS
50
51 436 concentrations were not associated with incident asthma in African American children of 5-
52
53 437 17 years (Quirós-Alcalá et al., 2021).

54 438 *Bone effects*

55
56 439 Among 1,362 mother-child pairs participating in a population-based cohort study, van Zwol
57
58 440 Janssens et al. reported that urinary BPS concentrations decreased during pregnancy (67.8% of
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60 441 positive samples at first trimester, 29.5% at second trimester and 19.3% at third trimester, with
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1
2 442 a median concentration of 0.68 ng/mL, 0.13 ng/mL and 0.13 ng/mL respectively). In addition,
3
4 443 urinary BPS concentrations at first trimester of pregnancy were associated with a significant
5
6 444 decrease of bone mineral density (BMD) and area-adjusted bone mineral content (aBMC)
7
8 445 evaluated in children at the age of 10 years, but not at the age of 6 years (Δ BMD = -6.08
9
10 446 mg/cm²; $p < 0.01$ and Δ aBMC = -0.12 g; $p < 0.01$) (van Zwol-Janssens et al., 2020).

11 447
12
13 448 Overall, given the confounding factors and the discrepancies in the available epidemiological
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15 449 data, it remains impossible to derive human reference values from these results.
16

17 450 18 19 451 **5. Effects of BPS in regulatory toxicological studies**

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21 452 A total of eight regulatory toxicology studies conducted in accordance with OECD Test
22
23 453 Guidelines are available on BPS. Table 1 provides an overview of the main characteristic toxic
24
25 454 effects of BPS and the corresponding N(L)OAELs. Five oral repeated dose toxicity studies (i.e.
26
27 455 OECD TG 407; TG 408; 422 quoted as Anonymous 14, 15, 16, 17 and 18 in (ECHA, 2019))
28
29 456 used an exposure duration varying from 13 to 90 days, at doses ranging from 40 to
30
31 457 1,000 mg/kg bw/day. The main observed toxic effects were systemic toxicity with excessive
32
33 458 salivation, decreased body weight, and kidney, adrenal and cecum effects, as described in
34
35 459 Table 1. A decreased weight of prostate and seminal vesicles was also observed in some
36
37 460 studies, together with diffuse atrophy of the male mammary gland. Four other regulatory
38
39 461 studies (i.e. OECD TG 414, TG 421, 422 and 443 quoted as Anonymous 12, 13, 14 and 19 in
40
41 462 ECHA, 2019 investigated developmental or reproductive toxicity of exposure to BPS in the rat
42
43 463 at doses ranging from 10 to 300 mg/kg bw/day via gavage (ECHA, 2019). These results led to
44
45 464 a classification of BPS in category 1B for reproductive toxicity (H360FD) (ECHA, 2020a). In
46
47 465 addition, the recent evaluation of an extended one-generation reproductive toxicity study in
48
49 466 rats led the EFSA Working Group to determine an NOAEL of 20 mg/kg bw/day for
50
51 467 developmental toxicity, based on increased post-implantation losses in the F1 progeny, or for
52
53 468 developmental immunotoxicity, based on decreased spleen weight in males at 60 mg/kg bw/day
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55 469 (EFSA, 2020).

56 470 57 58 471 **6. Effects of BPS in academic studies**

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60 472 The analysis of academic studies is presented below by identified endpoint. Table 2 provides
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62 473 an overview of the main characteristic toxic effects of BPS and the corresponding N(L)OAELs.
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3 474 **6.1. Effects of BPS on male reproductive function**

4 475 A total of twelve papers have described the effects of BPS on male testicular function. Among
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6 476 them, four papers were not taken into account in the present review because they were poorly
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8 477 informative (John et al., 2019; Ullah et al., 2019a; Ullah et al., 2019b; Ullah et al., 2018a). The
9
10 478 eight remaining papers, originating from 4 distinct laboratories, reported a coherent picture of
11
12 479 alterations in spermatogenesis in both Sprague-Dawley rats and CD-1, ICR and C57Bl/6 mice,
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14 480 exposed to BPS (see Table S4).

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16 481 This review separates analyses depending on the period of exposure, i.e. before or after neonatal
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18 482 life. It is known that the germ stem cell population is established during fetal and neonatal life.
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20 483 More precisely, this period extends from gestational day (GD) 7 to postnatal day (PND) 8 in
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22 484 the mouse, and from GD 9 to PND 10 in the rat. After this period, setup of the stem
23
24 485 spermatogonia population is complete and the meiosis process starts in the testis (de Rooij and
25
26 486 Vergouwen, 1991; Olaso and Habert, 2000).

27
28 487 Four papers from 3 laboratories, using either rat or mouse models reported convergent results
29
30 488 showing that fetal and/or neonatal exposure to BPS caused qualitative and quantitative
31
32 489 alterations in spermatogenesis at adulthood (Horan et al., 2018; Shi et al., 2018; Ullah et al.,
33
34 490 2019c), and that these alterations were transmitted to their descendants (Shi et al., 2019c).
35
36 491 Specifically, the adverse effect of BPS on the quality and number of spermatozoa was clearly
37
38 492 observed. The studies described decreased daily spermatozoa production (Ullah et al., 2019c),
39
40 493 decreased number and motility of spermatozoa and alterations of testicular histology (Shi et
41
42 494 al., 2018; Ullah et al., 2019c). These effects could result from meiosis disruption as shown by
43
44 495 changes in the percentages of specific stages VII-VIII throughout the spermatogenic cycle of
45
46 496 the seminiferous epithelium (Shi et al., 2017; Shi et al., 2018), and by the reduction in the
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48 497 homologous recombination rate (Horan et al., 2018). Concerning BPS effects on hormonal
49
50 498 levels, an increase in plasma estradiol concentrations or in testicular *Cyp19* expression was
51
52 499 observed (Shi et al., 2018; Shi et al., 2019c; Ullah et al., 2019c). Plasma testosterone levels
53
54 500 were decreased in two of these studies (Shi et al., 2019c; Ullah et al., 2019c), in line with
55
56 501 meiosis disruption at stages VII-VIII (Shi et al., 2017; Shi et al., 2018) as these stages are
57
58 502 androgen-dependent (De Gendt et al., 2004). Other effects included an increase in oxidative
59
60 503 stress in testis extracts (Shi et al., 2018; Ullah et al., 2019c). Importantly, epigenetic
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62 504 modifications were found by changes in the expression of testicular genes involved in DNA or
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1
2 505 histone methylation (Shi et al., 2018), and confirmed by transgenerational inheritance of
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4 506 spermatogenic defects (Shi et al., 2019c).

5
6
7 507 In the key study of Shi et al. (2018), allowing the determination of an NOAEL/LOAEL for
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9 508 BPS, mice were orally exposed to 0.5, 20 or 50 µg/kg bw/day of this bisphenol from GD 11 to
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11 509 birth. This treatment altered the entire morphometric analysis of spermatogenesis studied on
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13 510 PND 60, except for the weight of the testes. In the 0.5 µg/kg bw/day BPS-treated group,
14
15 511 spermatozoa numbers in the cauda epididymis were reduced by 34%, and the percentage of
16
17 512 motile spermatozoa by 15%, as compared with controls. These disturbances were probably
18
19 513 related to an alteration of germ cell line development. On PND 12, the number of apoptotic
20
21 514 seminiferous cells was doubled and dysregulations in the expression of numerous genes
22
23 515 involved in apoptosis, autophagy, oxidative stress, and histone methylation were observed with
24
25 516 0.5 µg/kg bw/day BPS and higher doses. As a result, although some dose-response curves were
26
27 517 not monotonic in this study, a dose of 0.5 µg/kg bw/day BPS was sufficient to alter male
28
29 518 reproductive function. This observation is strengthened by another study where Shi et al.
30
31 519 (2019c) reported that oral exposure of F0 mice from GD 7 to birth to BPS at the lowest tested
32
33 520 dose of 0.5 µg/kg bw/day reduced the spermatozoa number and the percentage of motile
34
35 521 spermatozoa in the cauda epididymis, and altered the cycle of seminiferous epithelium on PND
36
37 522 60 in the F3 generation.

38
39 523 Only two papers using rats are available to estimate an NOAEL and LOAEL after neonatal
40
41 524 exposure to BPS. The study of Ullah et al. (2016) investigated few parameters, which overlap
42
43 525 with those explored in another more complete study (Ullah et al., 2018b) from the same
44
45 526 laboratory. The last study included the height of the seminiferous epithelium, plasma
46
47 527 testosterone levels, and oxidative and anti-oxidative activities, all evaluated in both studies on
48
49 528 BPS. Therefore, only this second study was used for NOAEL and LOAEL estimations. BPS
50
51 529 was added to the drinking water of Sprague-Dawley rats for a very long period (48 weeks)
52
53 530 from PND 22 onwards. Adverse effects of BPS were clearly observed, such as a decrease in
54
55 531 daily spermatozoa production and in the number and motility of spermatozoa in the epididymis.
56
57 532 Furthermore, the testes showed reduced weight and histological alterations. BPS at 25 µg/L in
58
59 533 the drinking water caused adverse effects, whereas 5 µg/L had no effect. Since the daily
60
61 534 beverage intake of a male rat is around 300 mL/kg bw on PND 23 and 40 mL/kg bw on
62
63 535 postnatal week 52 (Holdstock, 1973; McGivern R.F. et al., 1996), the NOAEL is estimated to

1
2 536 be between 0.2 and 1.5 µg/kg bw/day, and the LOAEL between 1 and 7.5 µg/kg bw/day. These
3
4 537 values are slightly higher than those identified for fetal and/or neonatal exposure. Other effects
5
6 538 after neonatal exposure to BPS included decreased plasma testosterone levels and increased
7
8 539 oxidative stress (Ullah et al., 2018b; Ullah et al., 2016).

9
10 540 Recently a study was conducted to investigate whether testicular and sperm effects can be
11
12 541 detected after exposure to very low BPS doses (Řimnáčová et al., 2020). Adult male mice were
13
14 542 exposed to doses around 0.001, 1 or 100 µg/kg bw/day BPS via drinking water
15
16 543 from 8 to 16 postnatal weeks (PNW). Some endpoints such as testicular histological
17
18 544 parameters and testicular concentration in γH2AX, a marker of DNA damage and cellular
19
20 545 stress, were affected only at high BPS doses. On the contrary, others such as sperm motility,
21
22 546 sperm proteins methylation and acetylation were changed at 0.001 µg/kg bw/day of BPS and
23
24 547 not at higher doses. This raises the question of the effects at very low doses which are generally
25
26 548 not tested in most of the studies, and which may be more effective than higher doses in the
27
28 549 context of nonmonotonic dose response relationship.

29
30 550 Importantly, in six of the eight studies, BPA was also tested and its effects were similar or
31
32 551 slightly lower than those of BPS (Horan et al., 2018; Shi et al., 2017; Shi et al., 2018; Shi et
33
34 552 al., 2019b; Ullah et al., 2019c; Ullah et al., 2018b), giving rise to similar estimated
35
36 553 NOAEL/LOAELs to those calculated for BPS, for both exposure periods. The only striking
37
38 554 difference was the minimum dose of bisphenol required to significantly reduce spermatozoa
39
40 555 motility, since it was two-fold higher for BPA than for BPS, regardless of whether the exposure
41
42 556 occurred during the neonatal period (Ullah et al., 2019c) or in adulthood (Ullah et al., 2018b)
43
44 557 (see also Table S4). These *in vivo* data are in the same line with previous *ex vivo* studies
45
46 558 showing that the responsiveness of human and mouse fetal testis to BPS is similar to that of
47
48 559 BPA (Eladak et al., 2015).

48
49 560

50 561 **6.2. Effects of BPS on female reproductive function**

51
52 562 A total of fifteen studies investigated female reproductive function outcomes. Among them,
53
54 563 five studies were not retained because they were poorly informative of reproductive function
55
56 564 due to the specific nature of the model (Demacopulo and Kreimann, 2019), limited focus on
57
58 565 reproductive function (da Silva et al., 2019), or major limitations related to statistical analysis
59
60 566 (Hill et al., 2017) and/or experimental design (Nourian et al., 2017; Nourian et al., 2020). Table

1
2 567 S5 reports the 10 studies that were kept for the present analysis. Some of them may present
3
4 568 certain limitations, but none of these were considered important enough to preclude
5
6 569 consideration of the data. All the considered studies addressed the effects of exposure to BPS
7
8 570 through developmental periods (fetal, postnatal, and peripubertal) and adulthood.

9
10 571 Certain studies investigated ultimate endpoints of reproductive function regarding puberty
11
12 572 and/or estrous cyclicity as adverse effects. Results regarding the age at vaginal opening
13
14 573 reported in six studies (Ahsan et al., 2018; Kolla and Vandenberg, 2019; Shi et al., 2017; Shi
15
16 574 et al., 2019a; Shi et al., 2019b; Tucker et al., 2018) were not consistent. Discrepancies between
17
18 575 studies may result from differences in the experimental design regarding the species, routes,
19
20 576 doses, and periods of exposure. Two studies from two different laboratories in the CD-1 mouse
21
22 577 with gestational exposure did, however, lead to diverging results (Kolla and Vandenberg, 2019;
23
24 578 Shi et al., 2019a). Monitoring of the estrous cycle was performed in only three studies.
25
26 579 Increased occurrence of irregular cycles after gestational exposure (Shi et al., 2019a) was
27
28 580 reported in one study from 0.5 to 50 µg/kg bw/day, while no changes in the number and
29
30 581 duration of estrous cycles were observed with a similar period of exposure but at much higher
31
32 582 doses by Tucker et al. (2018). The last study reported unchanged estrous cyclicity in F3 animals
33
34 583 born from exposed F0 males and females (Shi et al., 2019b). It is, therefore, not possible to
35
36 584 draw any conclusions regarding the effect of BPS on these two parameters.

37 585 Among the ten evaluated studies, seven investigated the effect of BPS developmental exposure
38
39 586 (gestational, postnatal, and peripubertal) on folliculogenesis and/or oocyte maturation and
40
41 587 meiosis. There is a large body of evidence, i.e. six of the seven studies from four different
42
43 588 laboratories using different species and strains, exposure paradigms, and periods of
44
45 589 observation, that BPS can alter these processes (Ahsan et al., 2018; Horan et al., 2018; Ijaz et
46
47 590 al., 2019; Nevoral et al., 2018; Prokesova et al., 2020; Shi et al., 2019a). The remaining study,
48
49 591 looking at the effect of gestational F0 exposure to BPS (GD 7 to birth) on F3 reproductive
50
51 592 function, failed to show an effect of BPS on the PND 4 follicular populations (Shi et al., 2019b).

52 593 When changes in folliculogenesis/meiosis processes were evaluated jointly with potential
53
54 594 adverse effects on fertility issues, or *in vitro* and *ex vivo* fertilization rates (four studies), various
55
56 595 effects could be found, including at low dosage below 100 µg/kg bw/day. Interestingly, the
57
58 596 effect of gestational exposure on the reproductive capacities of aging animals persisted over
59
60 597 the third generation after exposure limited to F0 animals (Shi et al., 2019a; Shi et al., 2019b).

1
2 598 Peripubertal exposure of mice at a low dose (10 µg/kg bw/day; Nevoral et al., 2018) was
3
4 599 associated with decreased *in vitro* oocyte fertilization rates, with a possible non-monotonic
5
6 600 dose-response relationship. Finally, the fourth study, performed in rats exposed to a high BPS
7
8 601 dose, showed decreased litter size following postnatal exposure (Ahsan et al., 2018).
9
10 602 Interestingly, *in vitro* data confirmed the ability of BPS to interfere directly with oocyte
11
12 603 maturation at low doses in pigs (Zalmanova et al., 2017) and cows (Campen et al., 2018).

13
14 604 Among the potential mechanisms underlying these effects, epigenetic changes, ovarian
15
16 605 oxidative stress, and changes in endocrine status were all put forward. Two studies from the
17
18 606 same laboratory (Nevoral et al., 2018; Prokesova et al., 2020) reported changes in epigenetic
19
20 607 profiles in association with decreased meiotic and fertilization abilities of *in vivo* and *in vitro*
21
22 608 matured MII oocytes after peripubertal exposure. Changes in hormone concentrations were
23
24 609 described in five studies (Ahsan et al., 2018; Ijaz et al., 2019; Shi et al., 2017; Shi et al., 2019b;
25
26 610 Tucker et al., 2018), along with increased oxidative stress in the ovary after peripubertal
27
28 611 exposure (Ijaz et al., 2019). However, these data are difficult to put in perspective. Samples
29
30 612 were collected at different stages of life and/or, when specified, at different stages of the estrous
31
32 613 cycle. Estradiol levels results were usually not reliable because of inadequate assay methods,
33
34 614 with sensitivity not allowing precise determination of low levels commonly found in this type
35
36 615 of experiment. The most reliable and reproducible result was increased testosterone
37
38 616 concentrations following BPS exposure at high doses (within the 1–10 mg/kg bw/day range)
39
40 617 during early postnatal life (Ahsan et al., 2018) or in the peripubertal period (Ijaz et al., 2019)
41
42 618 in rats, or in mice with exposure encompassing the neonatal to adult stage (Shi et al., 2017), or
43
44 619 even in 9 month old animals (Shi et al., 2019a)), but not younger ones (3 and 6 months old)
45
46 620 exposed during gestation to a relatively low dose (50 µg/kg bw/day). Three studies from the
47
48 621 same author (Shi et al., 2017; Shi et al., 2019a; Shi et al., 2019b) evaluated gene expression of
49
50 622 key steroidogenic enzymes. Subtle changes in the expression patterns of these enzymes were
51
52 623 found in two studies with gestational exposure, while the third study with prepubertal exposure
53
54 624 did not show any effect. An isolated increase of *cyp 11a1* expression, encoding a critical
55
56 625 enzyme for the initiation of steroidogenesis, could be observed at 3 months of age in both F1
57
58 626 (Shi et al., 2019a) and F3 generations (Shi et al., 2019b), but not in 6 or 9-month-old animals.
59
60 627 Therefore, among the three proposed mechanisms of action of BPS on female reproductive
61
62 628 function, none can be clearly confirmed from the available data.

1
2 629 When BPA and BPS were tested in the same experiment, the effects of both substances were
3
4 630 quite similar. There are, however, some indications that their efficiency might differ, although
5
6 631 there are insufficient data to establish a firm comparison.
7

8 632 Given the body of converging evidence, it can be considered that BPS alters oocyte maturation,
9
10 633 meiosis capacity, and/or folliculogenesis following developmental exposure from gestation to
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12 634 peripubertal periods. As potential consequences on fertility were also reported, alteration of
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14 635 oocyte maturation and folliculogenesis can be considered adverse effects. Whether one and/or
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16 636 several of the investigated modes of action underlie such effects remains to be determined. A
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18 637 LOAEL of 0.5 µg/kg bw/day was determined based on the study by Shi et al. (2019a) for an
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20 638 effect on follicular populations at PND 4, along with alteration of the estrous cycle and
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22 639 decreased fertility in aging animals. Interestingly, the study by Nevoral et al. (2018) showed
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24 640 an effect on the follicular population with a LOAEL as low as 0.001 µg/kg bw/day. However,
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26 641 as this was observed only on a single parameter, and as there were uncertainties around the
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28 642 level of exposure as compared to probable environmental contamination, this LOAEL was not
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30 643 retained for reference values determination.
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35 646 ***6.3. Effects of BPS on the mammary gland***

36 647 A total of six studies reporting the effects of BPS exposure on the mammary gland in CD-1
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38 648 mice were analyzed (Kolla et al., 2019; Kolla et al., 2018; Kolla and Vandenberg, 2019;
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40 649 LaPlante et al., 2017; Tucker et al., 2018; Wormsbaecher et al., 2020) (see Table S6). They
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42 650 used BPS doses ranging from 2 µg/kg bw/day to 5,000 µg/kg bw/day, and the oral route for
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44 651 exposure with BPS incorporated into wafers, or administered by pipettes or gavage. Four of
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46 652 the six studies were carried out in Vandenberg's laboratory on mammary gland involution after
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48 653 delivery (LaPlante et al., 2017), or mammary gland development in males and females (Kolla
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50 654 et al., 2019; Kolla et al., 2018; Kolla and Vandenberg, 2019). The two remaining studies mainly
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52 655 focused on mammary gland histology and occurrence of lesions. One compared long-term
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54 656 effects of prenatal exposure to BPA and BPS on mammary gland development and the
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56 657 subsequent occurrence of lesions (Tucker et al., 2018). The other examined BPA and BPS
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58 658 effects on mammary gland stiffness, which is known to be a risk factor for breast cancer
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60 659 (Wormsbaecher et al., 2020).
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2 660 Kolla et al. (2018) studied the effects of gestational and lactational exposure (GD 9 to PND 20)
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4 661 of CD-1 mice to BPS at 2 and 200 µg/kg bw/day on the developing mammary gland of F1
5
6 662 females. Ethinyl estradiol (EE2) at 0.01 or 1 µg /kg bw/day was used as a positive control.
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8 663 They observed a significant increase in the total terminal end bud (TEB) area and size at PND
9
10 664 24 (pre-pubertal period) in females exposed to BPS doses from 2 µg/kg bw/day, but not to EE2.
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12 665 No effects were observed during puberty (PND 35). TEB-like structures were observed at
13
14 666 9 weeks of age (adulthood) in BPS-exposed animals, and to a lesser extent in EE2-exposed
15
16 667 groups. Intra-ductal hyperplasia in adult mammary glands was also observed at both doses of
17
18 668 BPS and EE2. Similar effects in adults were previously observed for BPA exposure (Soto et
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20 669 al., 2013).

21 670 Similar investigations were carried out in F1 males exposed to BPS (2 and 200 µg/kg bw/day)
22
23 671 over the same exposure period (GD 9 – PND 20) (Kolla et al., 2019). At embryonic day (ED)
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25 672 16, the number of androgen receptor (AR)-positive cells in the mesenchyme was reduced in a
26
27 673 dose-dependent manner. Interestingly, at PND 24, the authors observed a dose-dependent
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29 674 decrease in ductal areas in the left mammary gland, whereas later in life at postnatal week 9,
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31 675 they reported a dose-dependent increase in ductal area in the right mammary gland, and for the
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33 676 highest BPS dose only for the left gland. These data showed that the lowest dose of BPS
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35 677 (2 µg/kg bw/day) disrupted male mammary gland development with different susceptibility
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37 678 between the right and left glands, leading the authors to propose male mice exposed to low-
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39 679 dose BPS as a model to study unilateral gynecomastia observed in boys during puberty. Lastly,
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41 680 Kolla & Vandenberg (2019) showed that perinatal treatment from GD 9 to PND 2, or a
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43 681 challenge with EE2 at a peripubertal period from PND 21 to PND 30, did not affect the number
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45 682 or total area of TEBs, whereas the ductal area was decreased. Similar exposures in males
46
47 683 showed different susceptibility to BPS and EE2 in the right and left glands (Kolla et al., 2019).

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49 684 LaPlante et al. (2017) studied the effects of gestational and lactational exposure (GD 9 to
50
51 685 LD 20) to BPS on mammary gland involution in F0 female mice at different periods of lactation
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53 686 (LD2 and LD21). Exposure to 200 µg/kg bw/day of BPS led to a decrease in lobuloalveolar
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55 687 units, with a decrease in STAT-5 phosphorylation related to prolactin receptor activation at
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57 688 LD 21. This was consistent with the involution of mammary glands, described earlier for other
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59 689 xenoestrogens such as perfluorooctanoic acid (White et al., 2007). Interestingly, the increased
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61 690 time of nursing at LD 14 (see Section 6.4), together with the delayed development of pups,
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2 691 suggested that BPS at the lowest dose (2 µg/kg bw/day) was associated with lower quality
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4 692 and/or quantity of lactation (i.e. milk production and/or composition).
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7 693 These studies suggest that developmental exposure to BPS altered mammary gland
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9 694 development in females and males. In addition, in males, low doses of BPS decreased the
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11 695 effects of postnatal EE2 challenges, whereas higher doses enhanced these effects. Although
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13 696 these results might appear to be contradictory, they suggest that BPS may advance some aspects
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15 697 of gland development while delaying others.
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19 699 Tucker et al. (2018) investigated the effects of prenatal exposure (GD 10 to GD 17) to 50, 500
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21 700 and 5,000 µg/kg bw of BPS twice a day on mammary gland development. The developmental
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23 701 scoring of mammary gland development was increased from the lowest dose (50 µg/kg bw) at
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25 702 PND 35. At PND 56, which represents the theoretical stage of mature mammary gland branching,
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27 703 advanced mammary development was maintained, with an enhanced branching density for the
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29 704 highest dose of 5,000 µg/kg bw. This accelerated pubertal mammary development and the
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31 705 extended presence of TEBs into adulthood occurred without changes in other pubertal
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33 706 indicators (age at vaginal opening and first estrus, or estrous cyclicity) or body weight. In
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35 707 addition, neither serum steroid concentrations, with the exception of a decrease in
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37 708 dehydroepiandrosterone in old mice, nor mammary steroid receptor expression (*Estrogen*
38
39 709 *receptor 1: Esr1*, *Progesterone receptor: Pgr*, *Ar*, and *G protein-coupled estrogen receptor 1:*
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41 710 *Gper1*), could explain these results. Later in life, female mice at 14 months of age developed
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43 711 non-neoplastic lesions including inflammation, squamous metaplasia, and lobuloalveolar
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45 712 hyperplasia, mainly for the middle dose of 500 µg/kg bw. Moreover, neoplastic lesions such as
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47 713 fibroadenoma, carcinoma, and histiocytic sarcoma were diagnosed between 11 and 16 months
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49 714 of age at 500 and 5,000 µg/kg bw. BPS displayed more lesional effects on mammary glands
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51 715 than BPA at the same doses. The study by Tucker et al. (2018) showed that prenatal exposure
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53 716 to 50 µg/kg bw/day was sufficient to observe disrupted mammary gland development. BPS
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55 717 also induced long-lasting effects on the mammary gland that may increase the risk for
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57 718 mammary gland lesions. Additional studies are needed to evaluate the long-term consequences
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59 719 of BPS exposure, including its ability to induce or promote carcinogenesis.
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65 720 Wormsbaecher et al. (2020) investigated BPA and BPS effects on mammary target genes
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67 721 involved in gland stiffness following *in utero* exposure (ED 9.5 to ED 18.5). Whereas 25

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2 722 $\mu\text{g}/\text{kg}$ bw/day of BPA (via IP or gavage) increased collagen production and mammary gland
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4 723 stiffness in 12-week-old mice, no similar effects (considered risk factors for breast cancer) were
5
6 724 observed for BPS (via gavage) at the same dose level. This apparent discrepancy with the other
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8 725 two studies (Kolla et al., 2018; Tucker et al., 2018) may be due to the fact that a single BPS
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10 726 dose of 25 $\mu\text{g}/\text{kg}$ bw/day was used here, and this dose may not be the most efficient. In addition,
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12 727 analyses were limited to young adult mice, whereas lesions associated with fetal exposure to
13
14 728 BPS were recorded later in life.

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16 729 These studies showed that female mice prenatally exposed to BPS developed proliferative
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18 730 epithelial lesions by midlife, concomitant with a significant inflammatory response that may
19
20 731 predispose them to tumor formation later in life. In fact, animals in the high-dose BPS group
21
22 732 developed adenocarcinomas prior to one year of life, and these diagnoses triggered necropsy
23
24 733 at 14 months of age in the remaining animals (Kolla et al., 2018; Tucker et al., 2018). The
25
26 734 extended presence of TEBs into adulthood and the altered immune responses (e.g., increased
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28 735 perivascular inflammation) may have been important modifiers of the persistent adverse effects
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30 736 observed later in life.

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32 737 Overall, these studies show that exposure to BPS altered the mammary gland, confirming that
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34 738 it is a sensitive target organ for xenoestrogens like bisphenols (Fenton et al., 2012; Kass et al.,
35
36 739 2012; Soto et al., 2013; Vandenberg et al., 2008; Vandenberg et al., 2007). A LOAEL dose of
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38 740 2 $\mu\text{g}/\text{kg}$ bw/day was determined for effects on mammary gland development in both females
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40 741 and males.

41 742

42 43 743 **6.4. Behavioral effects of BPS exposure**

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45 744 Six experimental studies addressing behavioral effects of BPS in rodents were identified.
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47 745 Among these studies, one addressing feeding behavior (Rezg et al., 2018) was discussed in the
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49 746 section 6.5 of this article. Among the five remaining studies, two analyzed maternal behavior
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51 747 (Catanese and Vandenberg, 2017; LaPlante et al., 2017), two studied anxiety-related behavior
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53 748 (da Silva et al., 2019; Mornagui et al., 2019), and one assessed social interactions (Kim et al.,
54
55 749 2015). Table S7 includes only 3 behavioral studies (Catanese and Vandenberg, 2017; da Silva
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57 750 et al., 2019; LaPlante et al., 2017); the works of Kim et al. (2015) and Mornagui et al. (2019)
58
59 751 used only one BPS dose in addition to a small number of tested animals for Mornagui et al.
60
61 752 (2019). The data reported in these two latter studies are, however, cited below.

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2 753 The two studies conducted by the group of Vandenberg showed that adult or developmental
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4 754 exposure to BPS altered maternal behavior in mice. In particular, BPS-exposed F0 dams or
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6 755 their F1 descendants displayed several changes in spontaneous behavior (cleaning of pups, nest
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8 756 size, time spent in the nest, nursing, and high-crouch position) at the BPS doses indicated in
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10 757 Table S7. Effects on pup retrieval induced following mother/pup separation were observed
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12 758 only in F0 dams. Another observed effect was the delayed growth and development of pups
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14 759 born to F0 dams exposed to BPS at 2 µg/kg bw/day, suggesting deficient nursing and/or milk
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16 760 production, as shown by the altered lactating mammary gland analyzed in the same study
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18 761 ((LaPlante et al., 2017); see Section 6.3).

19 762 Maternal behavior is tightly regulated by a hormonal sequence involving, at the end of
20
21 763 gestation, a decrease in progesterone levels and an increase in estradiol levels, which then
22
23 764 promote the expression and release of other hormones and neuropeptides involved in the
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25 765 expression of this behavior, such as oxytocin. This hormonal sequence acts on the neural
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27 766 circuitry including hypothalamic structures (preoptic area, arcuate nucleus), connected to the
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29 767 mesolimbic reward system (ventral tegmental area). Interestingly, the maternal behavioral
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31 768 changes were associated with reduced estradiol levels and increased estrogen receptor α (ER α)
32
33 769 immunoreactivity in the medial preoptic area, suggesting reduced estrogen sensitivity in BPS-
34
35 770 exposed F0 dams. The analyses of other markers of key neuronal populations (ER α , Stat5, and
36
37 771 tyrosine hydroxylase) in the arcuate nucleus or ventral tegmental area did not show differences
38
39 772 between controls and BPS-exposed F0 or F1 dams.

40
41 773 In these studies, a LOAEL of 2 µg/kg bw/day was observed for both periods of exposure. In
42
43 774 comparison, previous studies showed altered maternal behavior following developmental
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45 775 exposure to BPA at doses of 10 µg/kg bw/day in mice (Palanza et al., 2002), and 5 µg/kg
46
47 776 bw/day and 40 µg/kg bw/day in rats (Boudalia et al., 2014; Della Seta et al., 2005). Exposure
48
49 777 during adulthood to BPA also impacted maternal behavior, as shown in mice at a BPA dose of
50
51 778 200 µg/kg bw/day (Kundakovic et al., 2013), or in *Cynomolgus* monkeys at a dose of
52
53 779 10 µg/kg bw/day (Nakagami et al., 2009). In a more recent study, exposure of CD1 female
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55 780 mice (F0) to BPA or BPS at 0.5 µg/kg bw/day from GD 7 until delivery induced long-term
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57 781 effects in unexposed F3 females generated from unexposed F2 and prenatally exposed F1 dams
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59 782 (Shi et al., 2019b). In particular, pup loss after delivery was observed in F3 dams, suggesting
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61 783 potential insufficient maternal care.
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784 Concerning anxiety-related behavior, the study conducted by da Silva et al. (2019) showed that
785 developmental exposure of rats to BPS increased the anxiety state level in males, but not in
786 females (Table S7). In agreement with these data, and despite a small number of animals used
787 (5 per treatment group), Mornagui et al. (2019) also reported an increased anxiety state level
788 measured in the elevated plus maze test for adult male mice exposed to BPS at 100 µg/kg
789 bw/day, through drinking water, from postnatal week 3 to postnatal week 10. Another study
790 assessing the effects of exposure to BPS at 200 µg/kg bw/day from GD 8 to PND 21 reported
791 reduced social interactions in male adult mice, possibly due to an increased anxiety state level,
792 but this behavior was not measured (Kim et al., 2015). The data obtained in female rats by da
793 Silva et al. (2019) should be interpreted cautiously since the stage of the estrous cycle was not
794 considered in these analyses. Clearly, anxiety-related behavior is modulated by estradiol levels
795 in rodents; females are less anxious under estrogen dominance (proestrus and estrus phases)
796 (Naulé et al., 2015).
797 BPS exposure also induced molecular changes, in particular in neurotransmitter and hormonal
798 systems (serotonin, sex steroids, corticosteroids, or thyroid hormones), known to modulate
799 anxiety-related behavior. In the study by da Silva et al. (2019), changes in thyroid hormone
800 levels were detected in PND21 males. Another study assessing the effects of gestational and
801 lactational exposure to BPA, BPS or BPF (10 µg/kg bw/day) showed a total of 25 genes
802 involved in dopamine and serotonin pathways significantly regulated by BPA, and 24 genes
803 by BPS in female rats (Castro et al., 2015). A common change induced by these substances
804 was upregulation of *Cyp2d4*, which is involved in the hydroxylation of allopregnanolone and
805 progesterone and thus the production of deoxycorticosterone. In addition, reduced cortical
806 expression of 5-α reductase, involved in the metabolization of testosterone into 5-α
807 dihydrotestosterone and biosynthesis of neurosteroids, was observed with changes in
808 expression levels of 5-α reductase 3 for BPS, and 5-α reductase 2 and 3 for BPA.
809 These studies indicate that anxiety-related behavior can be affected by developmental exposure
810 to BPS in males, with a LOAEL dose of 10 µg/kg bw/day. A very recent review of the literature
811 suggests that perinatal exposure to relatively low doses of BPA may alter hormonal systems
812 sensitive to stress and cause endocrine disruption, which translate to anxious and depressive
813 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

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

849 Overall, in nine studies, BPS was found to significantly enhance body weight in males for doses
850 ranging from 1.5 to 5,000 µg/kg bw/day (Ahn et al., 2020; Azevedo et al., 2020; Azevedo et
851 al., 2019; Brulport et al., 2020a; Ivry Del Moral et al., 2016; Meng et al., 2019a; Rezg et al.,
852 2018; Rezg et al., 2019; Ullah et al., 2019c). One study, which explored both sexes, reported
853 enhanced body weight only in male mice chronically exposed from gestation until adulthood,
854 and switched to a high-fat diet from weaning onwards (Ivry Del Moral et al., 2016). Similarly,
855 two other studies (Ahn et al., 2020; Meng et al., 2019a) reported that BPS-induced effects were
856 more pronounced in male mice fed a high-fat diet than in those fed a low-fat diet. Exposure to
857 BPS during pregnancy (Ahn et al., 2020; Ullah et al., 2019c) or from pregnancy until adulthood
858 (Ivry Del Moral et al., 2016) triggered body weight changes in males. One study reported that
859 BPS was as efficient in enhancing body weight of the male offspring as its analogs that included
860 BPA, BPB and BPF (Ullah et al., 2019c). In contrast, in two other studies performed in the
861 same laboratory (Azevedo et al., 2020; Azevedo et al., 2019), a group of mice was exposed to
862 BPA and in contrast to BPS, BPA did not elicit body weight enhancement. Within these studies,
863 different outcomes were analyzed in addition to recording the body weights. This included
864 analysis of lipid and glucose metabolism, food intake regulation, and mitochondrial
865 dysfunction in the liver, as shown in Table S8. Conclusions favored the hypothesis of BPS
866 acting as an obesogenic substance in males, especially when fed a high-fat diet with doses as
867 low as 1.5 µg/kg bw/day.

868 A single study (Meng et al., 2019b) over the 6 studies performed on females reported enhanced
869 body weight. In this study, female mice were exposed from GD 7 to PND 21 to BPS or to BPA,
870 all at 100 µg/kg bw/day and fed a standard diet. Interestingly, only BPS was efficient at
871 promoting gain weight. Unfortunately, the authors did not investigate as they did for males if
872 BPS-induced effects were aggravated in females fed with a high-fat diet as they did for males.
873 Six studies reported unchanged body weight following BPS exposure (Ahn et al., 2020; da
874 Silva et al., 2019; Gao and Kannan, 2020; Gao et al., 2020; Jing et al., 2019; Mandrah et al.,
875 2020; Pu et al., 2017; Zhang et al., 2018) in addition to the female part of the study of Ivry Del
876 Moral (2016). Zhang et al. (2018) did not observe body weight gain in male mice fed a standard
877 diet in which BPS was incorporated resulting in BPS exposure ranging from 5 to
878 5,000 µg/kg bw/day. Unfortunately, the authors did not expose the mice to a high-fat diet in

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2 879 addition to BPS, which would have made it possible to compare the results with those of Ivry
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4 880 Del Moral et al. (2016). Only hepatic oxidative stress was reported in mice exposed to the
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6 881 highest dose (Zhang et al., 2018). Gao et al. (2020) analyzed only female C57Bl/6 mice
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8 882 exposed through drinking water to BPS doses of 50 and 5000 $\mu\text{g}/\text{kg}$ bw/day for 10 weeks from
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10 883 weaning. The effects of both BPA and BPS were evaluated as in the study of Meng et al.
11
12 884 (2019b). In these two studies, mice were fed a standard diet. However, in the study of Gao et
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14 885 al. (2020), neither BPS nor BPA had any effect on body weight. Glucose tolerance was also
15
16 886 unaffected. Interestingly, the authors reported enhanced expression of the genes encoding
17
18 887 liver X receptor (LXR) α and fatty acid binding factor (FABP)4 in response to BPA and BPS
19
20 888 exposure, with similar activity. LXR α is a transcription factor that regulates the expression of
21
22 889 key genes involved in lipid and cholesterol metabolism. FABP4 plays an important role in fatty
23
24 890 acid storage. These data suggested lipid accumulation in the liver, although steatosis was
25
26 891 probably limited because glucose tolerance was not affected in exposed mice. In the study by
27
28 892 da Silva et al. (2019), Wistar rats were exposed to BPS at doses of 10 and 50 $\mu\text{g}/\text{kg}$ bw/day
29
30 893 during gestation and lactation via intra-gastric gavage. Both sexes were studied. Rats exposed
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32 894 to BPS showed no change in body weight, in either dams or male and female offspring at
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34 895 weaning or adulthood (PND 180). Additionally, BPS did not interfere with glucose metabolism
35
36 896 in males or females. However, BPS exposure lowered food intake in the adult offspring of both
37
38 897 sexes. In females, BPS exposure induced thermogenesis, which was in line with the reduced
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40 898 lipid accumulation observed in the brown adipose tissue and the reduced size of lipid droplets
41
42 899 in the white adipose tissue. Males exposed to BPS showed reduced visceral adiposity. It was
43
44 900 suggested by da Silva et al. (2019) that these effects on visceral adiposity caused by BPS
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46 901 exposure could originate from dysregulation of thyroid hormone homeostasis and changes in
47
48 902 feeding behavior. However, as BPS-exposed mice have increased preference for a fat-enriched
49
50 903 diet, increased risk for obesity is likely in situations in which only high-calorie diets would be
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52 904 available. In the study of Mandrah et al. (2020), BPS did not trigger enhanced body weight but
53
54 905 rather hyperglycemia, in line with the levels of various biomarkers measured in serum and
55
56 906 urine samples of the BPS-exposed rats, and reflecting dysregulation of glucose homeostasis.
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58 907 The two studies on sheep were performed within the same laboratory (Jing et al., 2019; Pu et
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60 908 al., 2017). The authors characterized the metabolic phenotype of male and female sheep fetuses
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62 909 in response to exposure to both BPA and BPS, used at the dose of 500 $\mu\text{g}/\text{kg}$ bw/day. Exposure
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64 910 occurred only during gestation and analyses were performed on fetuses harvested before term.
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2 911 Neither BPS nor BPA had any effect on fetal body weight in Pu et al., 2017. However, primary
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4 912 cultures of dissected perirenal adipose tissue from fetuses revealed that gestational exposure to
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6 913 BPS led to dysfunctional adipocytes in males. Adipocytes showed reduced expression of
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8 914 adipogenic markers and enhanced expression of estrogen receptors (*Esr1*, *Esr2*) and the
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10 915 glucocorticoid receptor (*Nr3c1*). These effects were not described in the BPS-exposed females.
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12 916 In females exposed to BPA but not in those exposed to BPS, dysprogramming of adipocytes
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14 917 was demonstrated. The second study in sheep involved skeletal muscle development. The
15
16 918 authors showed that programming of skeletal muscle development, assessed by measuring the
17
18 919 size and type of myofibers in primary cultures of skeletal muscle cells, was disrupted in
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20 920 response to gestational exposure to BPA and BPS, and that the situation was more critical in
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22 921 females and under BPS exposure. Although muscle plays a major role in energy metabolism
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24 922 and insulin sensitivity, it was not demonstrated whether these changes persisted in adulthood.
25
26 923 Taken as a whole, it can be concluded that male adipocytes are more sensitive to BPS than
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28 924 female adipocytes, but that female muscle cells are more sensitive than male muscle cells. As
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30 925 dysfunction of both adipocytes and muscle cells can lead to insulin resistance and thus
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32 926 metabolic disorders, these studies indicate that BPS exerts adverse metabolic effects in both
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34 927 sexes.
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36 928 From the *in vivo* studies reviewed, it is not possible to establish an NOAEL or a LOAEL
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38 929 because the two studies that used various doses of BPS demonstrated that BPS did not trigger
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40 930 obesity in male mice fed a standard diet (Ivry Del Moral et al., 2016; Zhang et al., 2018).
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42 931 However, one of the 2 studies also tested the combined impact of a high fat diet and exposure
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44 932 to BPS and showed worsening of diet-induced obesity and metabolic phenotype in treated
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46 933 males (Ivry Del Moral et al., 2016). Collectively, it is concluded that BPS could be a metabolic
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48 934 disruptor targeting several metabolic organs at both the neural and peripheral levels (liver,
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50 935 adipose tissue, and muscle). Parameters affecting the significance of the outcomes include sex
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52 936 (males are more susceptible than females), the periods and duration of exposure, and the
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54 937 nutritional context (effects are more often observed in animals fed a high-fat diet). Metabolic
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56 938 disruption may concern enhanced body weight gain, but also disruption of glucose and lipid
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58 939 metabolism and altered food intake and/or behavior. Enhanced expression of adipogenic
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60 940 markers, including the expression of genes targeted by PPAR γ , the master gene of
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62 941 adipogenesis, was also demonstrated. Dysregulation of thyroid hormone synthesis, changes in
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64 942 plasma levels of estradiol and testosterone, altered expression levels of estrogen and
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2 943 glucocorticoid receptors, and mitochondrial dysfunction in the liver have been reported. All
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4 944 these mechanisms could account for the adverse effects of BPS on metabolism and obesity.
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7 946 **7. Discussion**

10 947 Due to growing concerns about the increasing use of BPS to replace BPA, the present study
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12 948 addressed establishment of NOAELs or LOAELs in order to possibly derive human reference
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14 949 values for BPS. The available epidemiological and animal toxicological studies were analyzed,
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16 950 and the NOAELs or LOAELs determined for the analyzed endpoints in academic and
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18 951 regulatory studies were compared between each other. Of note, the endpoints including
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20 952 reproduction, the mammary gland, neurobehavior, and metabolism/obesity were also analyzed
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22 953 for the classification of BPA as an SVHC, thus allowing a relevant comparison between the
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24 954 two bisphenols.

25 955 Given that the available human data analyzed were insufficient to derive human reference
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27 956 values, and that neither TRVs nor OELs have been proposed by relevant EU or non-EU
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29 957 organizations to date, we used toxicological studies described as an alternative third option
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31 958 according to the strategy of Apel et al. (2020). More robust toxicological data have been made
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33 959 available in recent years thanks to toxicokinetic studies.

34 960 In this context, the toxicokinetic studies performed in several species, including humans,
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36 961 showed that BPS was well absorbed by the oral route, with a clearance mainly driven by its
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38 962 glucuronidation to BPS-G, like in the case of BPA. The major difference with BPA in both
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40 963 animal models and humans was the moderate efficiency of the first-pass effect, leading to 100-
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42 964 fold higher oral bioavailability compared to BPA. In addition, despite the higher efficiency of
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44 965 the blood-placental barrier to limit fetal exposure to BPS compared to BPA, BPS residence for
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46 966 a longer time than BPA in the fetal compartment led to concentrations in the same range as
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48 967 those reported for BPA under similar experimental conditions.

49 968 Analysis of the academic experimental data addressing BPS exposure provided evidence of
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51 969 adverse effects on female and male reproduction, the mammary gland, neurobehavior, and
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53 970 metabolism/obesity. Developmental exposure to BPS induced alteration of meiosis, oocyte
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55 971 maturation, and folliculogenesis processes, suggesting a potential impact on female fertility. In
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57 972 males, all the analyzed studies showed reduced spermatogenesis in rats and mice, but this
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59 973 decrease may have no consequence in rodents as a reduction by 55% in spermatozoa production
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2 974 does not impact fertility (Forand et al., 2009). In contrast, a decrease in spermatozoa
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4 975 concentrations in semen in the human population may lead to lower couple fecundability and
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6 976 an increased demand for assisted reproduction (Leridon and Slama, 2008; Slama et al., 2004).
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8 977 The mean concentrations of spermatozoa in human semen have declined over the last few
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10 978 decades, now reaching a level below which hypofertility begins (Bonde et al., 1998; Le Moal
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12 979 et al., 2014). BPS exposure also disrupted mouse mammary gland development in males and
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14 980 females, and increased lesions in prenatally-exposed females. Studies also suggested that BPS
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16 981 may induce metabolic disruption, including body weight gain, alteration of lipid metabolism,
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18 982 and food intake behavior. Further studies will certainly be needed to better determine the
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20 983 potential obesogenic and sex-dependent effects of BPS. A very recent study (Brulport et al.,
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22 984 2021) reported that perinatal exposure to BPS in C57Bl6/J mice fed a high-fat diet, while not
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24 985 affecting females of the F1 generation (Ivry Del Moral et al., 2016), triggered sex-dependent
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26 986 multigenerational obesogenic effects, especially in both sexes of the F2 generation and in
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28 987 females only of the F3 generation. At the neural level, developmental or adult BPS exposure
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30 988 also altered maternal behavior and increased anxiety-related behavior.
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32 989 Interestingly, the majority of these effects were previously described for BPA and were
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34 990 included in the SVHC evaluation of its endocrine disruptive activities (Anses, 2017; Beausoleil
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36 991 et al., 2018; Le Magueresse-Battistoni et al., 2018; Mhaouty-Kodja et al., 2018; Perrot-
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38 992 Applanat et al., 2018; Pouzaud et al., 2018; Viguié et al., 2018). Exceptions are male
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40 993 reproduction and maternal and anxiety-related behaviors. The updated literature clearly shows
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42 994 adverse effects of BPA on male reproduction in animals (Cariati et al., 2019; Matuszczak et
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44 995 al., 2019; Siracusa et al., 2018), and on maternal behavior in rats, mice, and primates (Boudalia
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46 996 et al., 2014; Della Seta et al., 2005; Kundakovic et al., 2013; Nakagami et al., 2009; Palanza et
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48 997 al., 2002). Furthermore, since our previous evaluation, which reported inconclusive data on the
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50 1000 effects of developmental exposure to BPA on anxiety state levels in rats and mice (ANSES,
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52 1001 2013), a very recent review of the literature suggests that perinatal BPA exposure may alter
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54 1002 anxiety- and depressive-like behaviors (Wiersielis et al., 2020).

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56 1003 In comparison to the academic data, which detailed the analysis of effects on specific organs
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58 1004 or systems, the regulatory studies addressed systemic hepatic, renal, splenic, and digestive tract
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60 1005 toxicity, as well as reproduction and development. Another difference lies in the BPS doses
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2 1006 fold). These discrepancies could explain the differences in the determined PODs, with
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4 1007 LOAELs between 60 and 300 mg/kg bw/day for developmental exposure in regulatory studies
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6 1008 versus 0.5 to 10 µg/kg bw/day in academic studies (Tables 1 and 2). Inconsistency between the
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8 1009 determined PODs was observed, regardless of the endpoint considered, with a ratio of
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10 1010 regulatory/academic ranging from 30,000 to 120,000. Most of the reviewed academic studies
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12 1011 converged on doses in the µg range that elicited adverse effects in rats or mice. It would be
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14 1012 interesting to compare the human reference values calculated from the present determined
15 1013 N(L)OAELs with human biomonitoring data for BPS.

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17 1014 Altogether, these observations raise two main questions. The first is related to the current
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19 1015 guidance, which was historically established by the OECD based on previous knowledge in
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21 1016 toxicology. There is a need to adapt these regulatory studies to modern tools of investigation
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23 1017 and to the more recent scientific findings of the last few decades, showing long-lasting adverse
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25 1018 effects induced at low doses for some of the chemical substances, with possible transmission
26 1019 across generations. The second question concerns the substitution of bisphenol A with other
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28 1020 bisphenols of the same family. The present comparison between BPS and BPA shows that most
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30 1021 of the effects on similar endpoints were induced by both substances, and in the same dose
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32 1022 ranges. Moreover, a recent evaluation of another bisphenol, BPB, showed that this substance
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34 1023 meets the WHO definition of an endocrine-disrupting chemical (Serra et al., 2019).

35 36 1024 **8. Conclusion**

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38 1025 In conclusion, the toxicokinetic studies addressing BPS exposure in several species,
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40 1026 including humans, indicated similarities and differences with BPA. In particular, higher oral
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42 1027 bioavailability and persistence in the fetal compartment were observed for BPS, suggesting
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44 1028 that replacement of BPA with BPS may lead to higher exposure to the active parent compound.
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46 1029 Adverse effects of BPS were reported in several academic studies addressing male or female
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48 1030 reproduction, the mammary gland, neurobehavior, or metabolism/obesity. In the absence of
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50 1031 sufficient human studies, the analysis of animal toxicological studies to determine human
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52 1032 reference values revealed striking differences between the values identified in regulatory versus
53 1033 academic studies. The LOAELs for developmental exposure to BPS were extremely low in
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55 1034 academic studies compared to those reported in regulatory toxicity studies (0.5–
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57 1035 10 µg/kg bw/day versus 60 mg/kg bw/day). For these reasons, we propose to derive human
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59 1036 reference values for exposure to BPS from the N(L)OAELs determined in academic peer-

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2 1037 reviewed experimental studies. To better protect human health, the authors stress the
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4 1038 importance of i) analyzing both regulatory and academic studies when setting PODs for
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6 1039 reference values, and ii) adapting regulatory studies to be performed in the near future to recent
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8 1040 scientific knowledge in toxicology.

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1049 **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)OAEI 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 transgenerationally 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)OAEI were derived by the ANSES ED-EG.			

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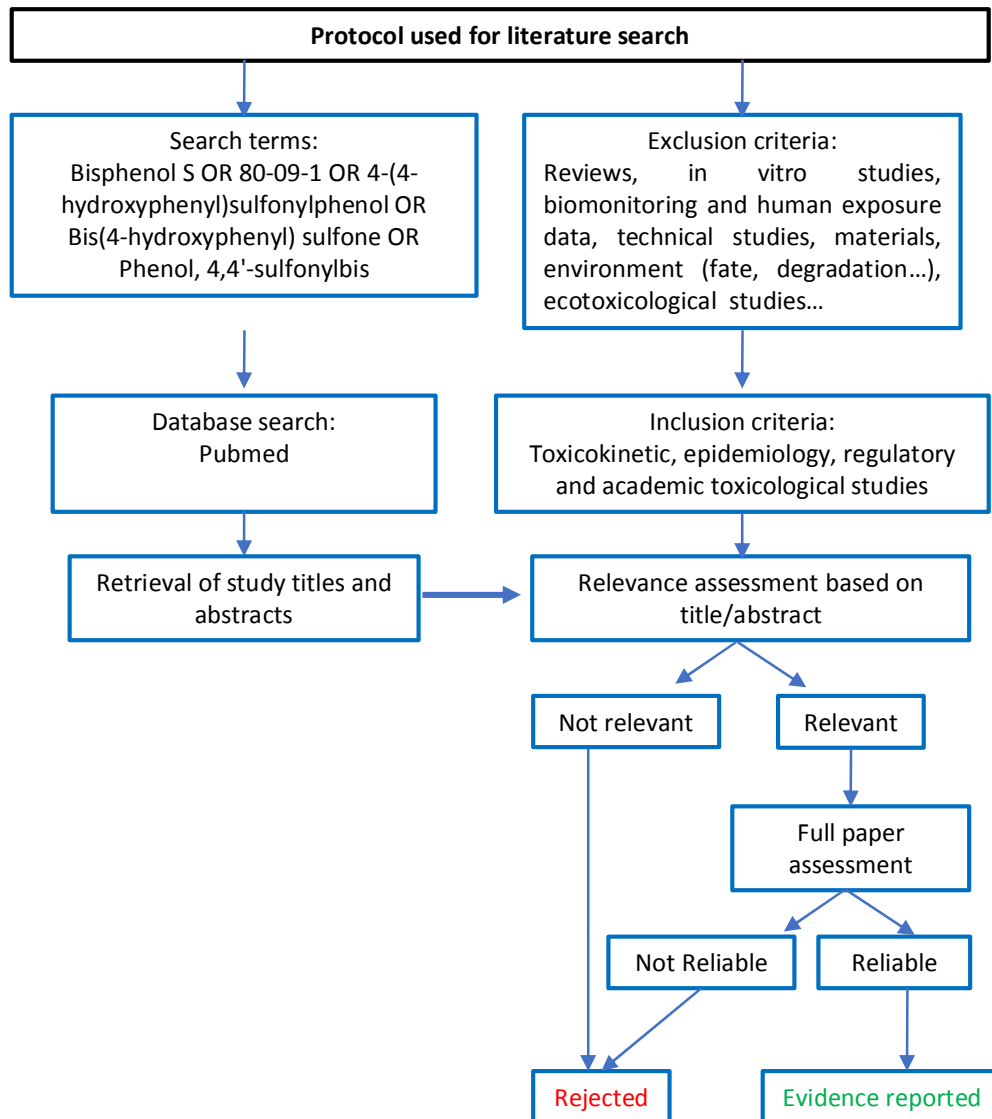
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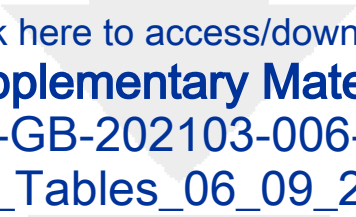
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Figure legends

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 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.

Declaration of interests

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:

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