SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Substance name
4,4’-sulphonyldiphenol (Bisphenol S; BPS)

EC No. 201-250-5

CAS RN 80-09-1

Evaluating Member State(s): Belgium

Dated: 4 April 2023

Evaluating Member State Competent Authority

Belgian Federal Public Service Health, Food Chain Safety and Environment, Risk Management Service
Address : Galileelaan 5/2
1210 Brussels
Belgium
Email: evaluation.reach@health.fgov.be

Year of evaluation in CoRAP: 2014

Before concluding the substance evaluation, a Decision to request further information was issued on: 13 June 2016.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

4,4'-sulphonyldiphenol, referred to hereinafter as BPS, was originally selected for substance evaluation to clarify concerns about:

- suspected CMR
- potential endocrine disruptor
- high aggregated tonnage

During the evaluation no other concerns were identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A CLH proposal submitted by BE CA for a more stringent reproductive toxicity classification (Repr. 1B H360FD) was adopted by the Risk Assessment Committee on 10 December 2020 and published on 16 February 2022[2].

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in table 1.

Table 1: Conclusion of Substance Evaluation

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	√
Harmonised Classification and Labelling	√
Identification as SVHC (authorisation)	√
Restrictions	√
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

[2] COMMISSION DELEGATED REGULATION (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (18th ATP)

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

BE CA submitted to ECHA a proposal for a more stringent reproductive toxicity classification (Repr. 1B H360FD instead of the self-classification of Repr. 2 H361f) on 10 October 2019. ECHA's Committee of Risk Assessment (RAC) agreed with the proposal of the BE CA and consequently the opinion of RAC has been adopted on 10 December 2020 (https://echa.europa.eu/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e182ed4414).

In the meanwhile, BPS is covered by index number 604-098-00-1 in the 18th ATP of Regulation (EC) No 1272/2008[3] in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) with reproductive toxicity classification: Repr.1B, H360FD.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

The eMSCA submitted on 4 August 2022 a proposal for the identification of BPS as SVHC (article 57 of REACH) by reason of:

- The substance is toxic for reproduction in accordance with article 57 c) of REACH based on the harmonised classification for reproduction: Repr. 1B, H360FD

and

- The substance has endocrine disrupting properties and can be identified as substance of equivalent level of concern to CMR/PBT substances in accordance with article 57 f) of REACH for
  - Human health and
  - Environment

Based on all available scientific evidence, it can be concluded that BPS fulfils the WHO/IPCS (2002) definition of an endocrine disruptor:

- It shows clear reproductive adverse effect in rodents and fish. The reproductive endocrine system is highly conserved not only between mammals, but also between mammals and other vertebrates like fish.

- It has endocrine modes of action: clear estrogenic mode of action and alteration of steroidogenesis.

- The adverse effects, including the recognised EAS-mediated effects (e.g., on oestrous cycle and sex ratio) and sensitive effects, but not diagnostic of EAS

[3] COMMISSION DELEGATED REGULATION (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (18th ATP)

(e.g., fecundity, fertility, implantation sites and number of pups), are a consequence of the endocrine modes of action.

4.1.3. Restriction

BPS is a potential candidate to be included in the intended restriction, which was submitted by the German Competent Authority (DE CA), on 7 October 2022:

Proposed restriction

Bisphenols, HO-(R1)-R2-(R3)-OH with R1 and R3 being phenylene groups bearing any substituents at any ring position and R2 being a methylene group being unsubstituted or bearing any substituents or another bridging unit bearing unspecified substituents, which are listed in Appendix X and their salts.

Further bisphenols may be added to Appendix X if they fulfil one or more of the following conditions:

- They have been identified as substances of very high concern due to their endocrine disrupting properties for the environment according to Article 57 and Article 59 of this Regulation.
- They are classified as endocrine disruptors for the environment category 1 in Part 3 of Annex VI to Regulation (EC) No 1272/2008.
- They have been identified as endocrine disruptors for the environment according to the Biocidal Products Regulation (EU) No 528/2012.
- They have been identified as endocrine disruptors for the environment according to the Plant Protection Products Regulation (EC) No 1107/2009.

Conditions of restriction

1. Shall not be placed on the market in mixtures and articles in a concentration equal to or greater than 10 ppm (0.001 % by weight). This limit value refers to the sum of all substances subject to this Annex XVII entry which are present in the respective mixtures and articles.

2. Paragraph 1 shall not apply to mixtures and articles where the bisphenols listed in Annex X are either covalently bound to any type of matrix (e.g. via functioning as a cross-linker) or are used as intermediates in the manufacture of polymers, and for which
   i. contact to aqueous media in any form can be excluded during their reasonable and foreseeable use throughout their service life or
   ii. the migration limit in the respective mixtures and articles does not exceed 0.04 mg/L over the entire service life. Conditions for migration testing are described in Annex Z below.

3. Paragraphs 1 and 2 shall apply from [EiF + 18 months] for entries 1 to 5 of Appendix X. Specific derogations are listed in Appendix Y.

4. Appendices X and Y shall be amended in accordance with the procedure referred to in Article 133(4). Articles 69 to 73 shall not apply.

BPS is falls under the scope of the restriction proposal for BPA (https://echa.europa.eu/documents/10162/6b2321cf-5334-9354-cbcd-57a9345ae0fb).

4.1.3. Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable, see section 4.

5.2. Other actions

Not applicable, see section 4.

6. FOLLOW-UP ACTIONS

In Table 2 the list of follow-up actions is reported.

Table 2: Follow-up actions

FOLLOW-UP		
Follow-up action	**Date**	**Actor**
RMOA	Concluded	BE CA
SVHC identification	Concluded	BE CA
Restriction	Submitted in October 2022	DE CA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

4,4'-sulphonyldiphenol (BPS) was originally selected for substance evaluation to clarify concerns about:

- suspected CMR
- Potential endocrine disruptor
- high aggregated tonnage

During the evaluation no other concerns were identified.

Table 3: Endpoints of concern for substance evaluation:

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Suspected Reproductive toxicity	*Concern confirmed: Harmonised classification published[4] : Repr. 1B, H360FD*
Suspected Mutagenicity	*Concern refuted. Based on the toxicokinetics study received as a result of the Substance Evaluation Decision, it can be concluded that the substance is not a mutagen.*
Potential Endocrine disruptor	*Concern confirmed : Based on all available data, incl. the EOGRTS and ZEOGRTS received as a result of the Substance Evaluation Decision, it can be concluded that the substance can be identified as ED HH and ED ENV*
Exposure data and exposure assessment	*Concern confirmed: uses of the substance lead to exposure of workers and consumers. eMSCA did only perform an exposure assessment for workers and it can be concluded that the risk management measures presented by the registrant are sufficient. Available human biomonitoring data show presence of BPS in urine, blood and human placenta and saliva of vulnerable population.*

Additionally, following the CLP-criteria for long-term toxicity, BPS should be classified for the aquatic environment as **Aquatic Chronic 2, H411**:

- chronic NOEC =0.25 mg/L and thus meeting the classification criterion ≤ 1 mg/L

[4] COMMISSION DELEGATED REGULATION (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (18th ATP)

- and following the degradation decision scheme: NOT RAPIDLY DEGRADABLE
  - ready biodegradability test :
    0% degradation in an OECD TG 301C
  - or ultimate degradation in surface water simulation test:
    NA
  - or primary biotic or abiotic degradation in the aquatic environment
    - Considered hydrolytically stable
    - No degradation in seawater (aerobic and anaerobic)

7.2. Procedure

Substance Evaluation and follow up:

Based on an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to Human health (Suspected CMR); Potential endocrine disruptor and Exposure/aggregated tonnage, BPS was included in the Community rolling action plan (CoRAP) for substance evaluation (article 44(2) of the REACH Regulation) to be evaluated in 2014.

Pursuant to Article 45(4) of the REACH Regulation, the registration dossier of BPS and all other relevant and available information (e.g. Scientific publications, QSAR predictions, …) were used as a basis for the evaluation. eMSCA considered that further information was required to clarify the concerns by means of an Extended One Generation Reproductive Toxicity Study, Toxicokinetics, in vivo alkaline Comet Assay (conditional to Toxicokinetics results), Medaka or Zebrafish Extended One Generation Reproduction test, Exposure data and exposure assessment. A decision was issued in 2016 with a deadline to provide the requested information in 2018.The registrant(s) submitted their updated registration dossier containing all requested information on 04/11/2021. In accordance with Article 46(3) of REACH, the evaluating Member State started the follow-up evaluation without undue delay. The eMSCA presented the postulated MoA (HH and ENV) for BPS during the open session of the 19th meeting of the Endocrine Disruptor (ED) Expert Group (13 April 2021) where a representative of the Registrants was also present. ED EG experts agreed that there was sufficient evidence to conclude that BPS acts as an ED for Human Health and that these data are also population relevant for mammalian wildlife and thus supporting identification as ED for environment.

Classification and labelling:

After receipt of the requested information concerning reprotoxicity and assessment of all available study results, BE CA decided to address this concern by submitting to ECHA a proposal for a more stringent reproductive toxicity classification (Repr. 1B H360FD instead of the self-classification of Repr. 2 H361f). The CLH proposal was submitted on 10 October 2019. ECHA's Committee of Risk Assessment (RAC) agreed with the proposal of the BE CA and consequently the opinion of RAC has been adopted on 10 December 2020 (https://echa.europa.eu/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e182ed4414).
This resulted in the inclusion of BPS in the 18th ATP of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) with reproductive toxicity classification Repr. 1B H360FD. The attributed index number is 604-098-00-1.

Identification as substance of very high concern:

BE CA concluded that based on all available scientific evidence, the substance should be identified as a substance of very high concern due to its endocrine properties for the human health and the environment. Therefore, the Annex XV dossier for identification of BPS

according to articles 57 (c) and (f) of REACH was prepared and submitted to ECHA on 4 August 2022.

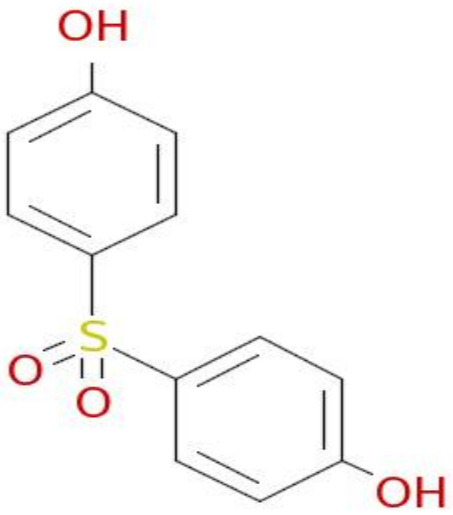
7.3. Identity of the substance

Table 4: Substance identity

SUBSTANCE IDENTITY	
Public name:	4,4'-sulphonyldiphenol (BPS)
EC number:	201-250-5
CAS number:	80-09-1
Index number in Annex VI of the CLP Regulation:	NA
Molecular formula:	$C_{12}H_{10}O_4S$
Molecular weight range:	250.27 g/mol
Synonyms:	Phenol, 4,4'-sulfonylbis- (9CI) Phenol, 4,4'-sulfonyldi- (6CI, 8CI) 1,1'-Sulfonylbis[4-hydroxybenzene] 4,4'-Bisphenol S 4,4'-Dihydroxydiphenyl sulfone 4,4'-Sulfonylbisphenol 4-Hydroxyphenyl sulfone Bis(4-hydroxyphenyl) sulfone Bis(p-hydroxyphenyl) sulfone Bisphenol S BPS BPS 1 Diphone C p,p'-Dihydroxydiphenyl sulfone Phenol, sulfonylbis- Bis(hydroxyphenyl)sulphone Dihydroxydiphenyl sulphone Phenol, sulphonyldi- Sulfonyl diphenol-

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



7.4. Physico-chemical properties

Table 5: Summary of physicochemical properties

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	A fine white odourless powder: solid at 20°C and 101.3 kPa
Vapour pressure	negligible The melting point of the substance is between 200 °C and 300°C. The calculated value of vapour pressure at 25°C is quite low as expected ($6.29x10^{-10}$ hPa at 25°C).
Water solubility	715 mg/L at 20 °C
Partition coefficient n-octanol/water (Log Kow)	1.2 at 23°C
Flammability	non flammable Based on chemical structure pyrophoric properties and flammability in contact with water are not to be expected. The substance or mixture does not need to be classified as self-reactive as the heat of decomposition is less than 300 J/g. The substance or mixture does not need to be classified as self-heating as the onset temperature is greater than 220 °C in the Grewer Oven test (screening test). The substance or mixture does not need to be classified as an organic peroxide as by definition based on their chemical structure the substance is no organic peroxide
Explosive properties	non explosive

	There are no chemical groups associated with explosive properties present in the molecule.
Oxidising properties	Non Oxidising The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested is lower than the maximum burning rate of the reference mixture.
Granulometry	particles <100µm approximate 55%, particles <10µm approximate 1.8%, particles <4µm approximate 0.4%
Stability in organic solvents and identity of relevant degradation products	The stability of the substance is not considered as critical.
Dissociation constant	pKa1 at 20°C: 8, pKa2 not reported

7.5. Manufacture and uses

7.5.1. Quantities

Table 6: Quantities

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	
☒ 10,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

> 10000 and < 100000 T on the ECHA dissemination website (24/06/2021)

7.5.2. Overview of uses

Table 7: Overview of uses

USES	
	Use(s)
Uses as intermediate	Yes (see uses at industrial sites)
Formulation	Manufacture of thermal paper - formulation into materials Formulation into solid matrix Formulation into mixture
Uses at industrial sites	Use as monomer for manufacture of PESU Use as monomer in the production of synthetic tanning agents (Syntans) Use as monomer in the production of synthetic tanning agents Recycling of thermal paper Industrial manufacture of paper Use of Syntants for leather tanning production Intermediate Industrial manufacture of thermal paper Uses at industrial sites

	Use as monomer for manufacture of polymers Industrial manufacture of thermal paper Use of Syntans for tanning in leather production Use of Syntants as fixation agents for dyed polyamide Industrial manufacture of polymer
Uses by professional workers	Professional use for leather products
Consumer Uses	/
Article service life	Service life of thermal paper (professional workers) Service life of thermal paper (consumers) Service life of thermal paper (professional workers – cashiers) Industrial manufacture of paper Use of leather articles Use of recycled paper

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

A proposal for harmonised classification and labelling of BPS (Annex VI of the CLP Regulation) was submitted by Belgium in October 2019. Based on the available information, the substance was proposed to be classified as Repr. 1B, H360FD.

In the RAC Opinion (of 10 December 2020), the proposed harmonised classification of BPS as Repr. 1B, H360FD was confirmed and adopted. The harmonised classification and labelling was published on 16 February 2022 (18th ATP of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) with index number 604-098-00-1.

Table 8: Classification according to Annex VI, Table 3 (list of harmonised classification and labelling of hazardous substances) of Regulation (EC) No 1272/2008

Index No	Chemical name	EC No	CAS No	Classification		Labelling			Spec. Conc. Limits, M-factors and ATEs[5]	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)	Pictogram, Signal Word Code(s)	Hazard statement code(s)	Suppl. Hazard statement code(s)		
604-098-00-1	Bisphenol S; 4,4'-sulphonyldiphenol	201-250-5	80-90-1	Repr. 1B	H360FD	GHS08 Dgr	H360FD			

[5] Acute Toxicity Estimate

7.6.2. Self-classification

- In the registration(s):

Due to the inclusion of the substance in the 18th ATP of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3, the classification in the REACH registration dossier was updated from Repr. 2, H361f to Repr. 1B, H360 FD.

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory[6] (25/7/2022) :

Repr. 2, H361
Eye Irrit. 2, H319
Skin Irrit. 2, H315
STOT SE 3, H335
Aquatic Chronic 3, H412
NC

7.7. Environmental fate properties

7.7.1. Degradation

Available studies and information on phototransformation (air and water), ready and inherent biodegradability, hydrolysis and simulation studies (seawater, soil) are summarised below.

Table 9: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Phototransformation			
Phototransformation in air QSAR estimation Rel. 2	DT50=26.5h.	EPIWIN SRC AOP v1.92 With assumption of degradation rate constant of 14.53 E-12 cm^3/molecule*sec, 24-h day and a mean OH radical concentration of 0.5E06 molecules per cm^3	REACH registration dossier: Epiwin calculation, 2007
Phototransformation in water : Equivalent or similar to OECD draft guideline (Phototransformation of Chemicals in Water - Direct and Indirect Photolysis) GLP : not specified	DT50=43.1 min	HPLC	REACH registration dossier: Cao *et al.*, 2012

[6] https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/51189

Method	Results	Remarks	Reference
Rel. 2			
Phototransformation in water : Equivalent or similar to OECD draft guideline (Phototransformation of Chemicals in Water - Direct and Indirect Photolysis) GLP : not specified Rel. 2	Phototransformation in water was readily Main product formed : p-hydroxybenzenesulfonic acid.	ESI-MS and GC-MS	Reach registration dossier: Wang *et al.*, 2014
Hydrolysis			
Hydrolysis Rel.2	Hydrolysis not expected as no hydrolysable functional groups are available	Theoretical evaluation (based on structural analogy where substances are assigned to one of three categories: 1. No hydrolysable functional groups 2. No labile functional groups 3. Hydrolysable groups	Kollig *et al.*,1993
Hydrolysis Rel.2	Hydrolysis not expected	Theoretical evaluation R-X + H2O -> R-OH + HX-method	Boethling and Mackay, 2000
Hydrolysis Rel.2	Phenols are generally regarded as stable towards hydrolysis	Theoretical evaluation: R-X + H2O -> R-OH + X- + H+	Harris, 1990
Biodegradation in water: screening tests			
Ready biodegradability OECD TG 301 C (Ready Biodegradability: Modified MITI Test (I)) GLP : not specified Rel. 2	0% degradation within 28d (TOC meas.)		Reach registration dossier: Unpublished study report, 1998
Ready biodegradability Enhanced OECD TG 301B (Ready Biodegradability: CO2 Evolution Test) GLP: yes Rel.1	32% degradation after 59d (mean value of 2 replicates)	Biodegradation was determined after 59 days instead of 28 days Duration of adaptation phase: 27d	REACH registration dossier: Unpublished study report, 2018a

Method	Results	Remarks	Reference
Equivalent or similar to OECD TG 302B (Inherent biodegradability: modified Zahn-Wellens/EMPA Test) GLP: no Rel. 2	47% degradation after 14d (initial concentration: 10 mg/L(nutrient-mineral rich, without any other source of organic carbon) 99% degradation after 14d (initial concentration: 10 mg/L (mineral-nutrient rich medium)) 100 after 14d (% degradation (initial concentration: 2 mg/L) 100 after 14d (% degradation (Initial concentration: 0.1 mg/L) 36% degradation within 7d (nutrient-mineral rich medium) and 35% within 7d in nutrient-mineral rich medium, without any other source of organic carbon)	Assessment of biodegradation kinetics during biological waste water treatment with activated sludge using GCMS/MS	REACH registration dossier Kovačič *et al.*, 2021
OECD TG 302B (Inherent biodegradability: Zahn-Wellens/EMPA Test) GLP: no Rel. 2	88% degradation within 77d (TOC removal) Role microbial adaptation: 67% elimination after 8d and up to 92% at day 105	Study performed with a mixture of 4,4'-dihydroxydiphenylsulfone and 2,4'-dihydroxydiphenylsulphone (1:1)	REACH registration dossier: Unpublished study report, 2006 Supportive data
Biodegradation in water and sediment: simulation tests			
Aerobic and anaerobic seawater simulation study TOC-Handai method Rel.2	Aerobic : 0% degradation after 22 days Anaerobic : 60% at ca. day 80		REACH registration dossier Ike *et al.*, 2006
Simulation study : degradation in seawater TOC Handai (TOC, potential test) and river (sea) die-away (SDA, simulation test) method	No degradation observed after 60d		REACH registration dossier Danzl *et al.*, 2009

Method	Results	Remarks	Reference
Rel. 2			
Biodegradation in soil			
Aerobic biodegradation in soil Similar to similar to those methods described by Mashtare *et al.*, 2013 GLP not specified Rel. 2	DT50 (at 22°C, PFO): Soil n°1: 0.935d Soil n°2: 0.649d 3 transformation products detected	2 surface clay loam soils from a forested area were used HPLC-MS/MS Analysis	REACH registration dossier: Choi and Lee, 2017a
Aerobic degradation in soil Rel. 2	T1/2=2.8d After 28d: biodegradation: 53.6% NER formation: 44.9%	^{14}C-BPS Soil from a Chinese paddy rice field: 46.7% clay, 37.9% silt and 15.4% sand	REACH registration dossier: Cao *et al.*, 2020

7.7.1.1. Abiotic degradation

7.7.1.1.1. Hydrolysis

Data waiving was applied in the registration dossier : Phenols are generally regarded as stable towards hydrolysis (Harris, 1990). Additionally based on the conclusions of Kollig *et al.* (1993) and Boethling and Mackay (2000), it can be assumed that BPS is hydrolytically stable since BPS does not contain functional groups that are susceptible to hydrolysis.

7.7.1.1.2. Phototransformation / photolysis

7.7.1.1.2.1. Phototransformation in air

Photodegradation was estimated by QSAR using EPIWIN SRC AOP v1.92.

A 24h day, a concentration of OH radicals of $0.5*10^6$ OH/cm^3 and a degradation rate constant of $14.5*10^{-12}$cm^3/molecule*sec were assumed for calculating the half-life.

The substance is relatively fast photochemically decomposed once released to air with a DT50 value of 26.5h.

Phototransformation is however no relevant degradation pathway as the substance shows low potential for volatilisation.

7.7.1.1.2.2. Phototransformation in water

Cao *et al.* (2012) demonstrated photolysis of BPS in water under UV light. The rate of photolysis increased with light source intensity. DT50 was 43.1 min.

In another study (Wang *et al.*, 2014) p-hydroxybenzenesulfonic acid was identified as major degradation product of BPS when phototransformed in water.

7.7.1.1.2.3. Phototransformation in soil

Not available.

7.7.1.2. Biotic degradation

7.7.1.2.1. Biodegradation in water

7.7.1.2.1.1. Estimated data

Not available.

7.7.1.2.1.2. Screening tests

A modified MITI test was conducted according to OECD TG 301C (REACH registration dossier: Unpublished study report, 1998) over a period of 28d with the use of 30 mg/l non-adapted activated sludge taken from 10 different sites (municipal STPs, industrial STPs, lakes and rivers) in Japan. 0% degradation was observed after 28d (TOC).

In another study performed according to OECD TG 301B (unpublished study report, 2018a; REACH registration dossier) 32 % degradation of BPS was observed after 59 days with a duration of the adaptation phase of +/- 27 days.

Therefore, the substance is not readily biodegradable.

In the inherent biodegradability study of Kovačič *et al*. (2021), similar to OECD 302B (modified Zahn-Wellens test), biodegradation kinetics of BPS was examined during aerobic degradation of activated sludge from a biological wastewater treatment plant within 14 days. The biodegradation pathway and products are shown in Figure 1. Kinetics were determined using MS/MS and biotransformation products by using LC-QTOF-MS. Degradation rate (kt) for BPS was determined to be 0.04-0.16 with a half-life of 4.3-17.3 days. 47% degradation was reached within 14 days. The absence of any additional organic carbon source significantly slowed down degradation of BPS (lag phase on day 18 instead of day 7).

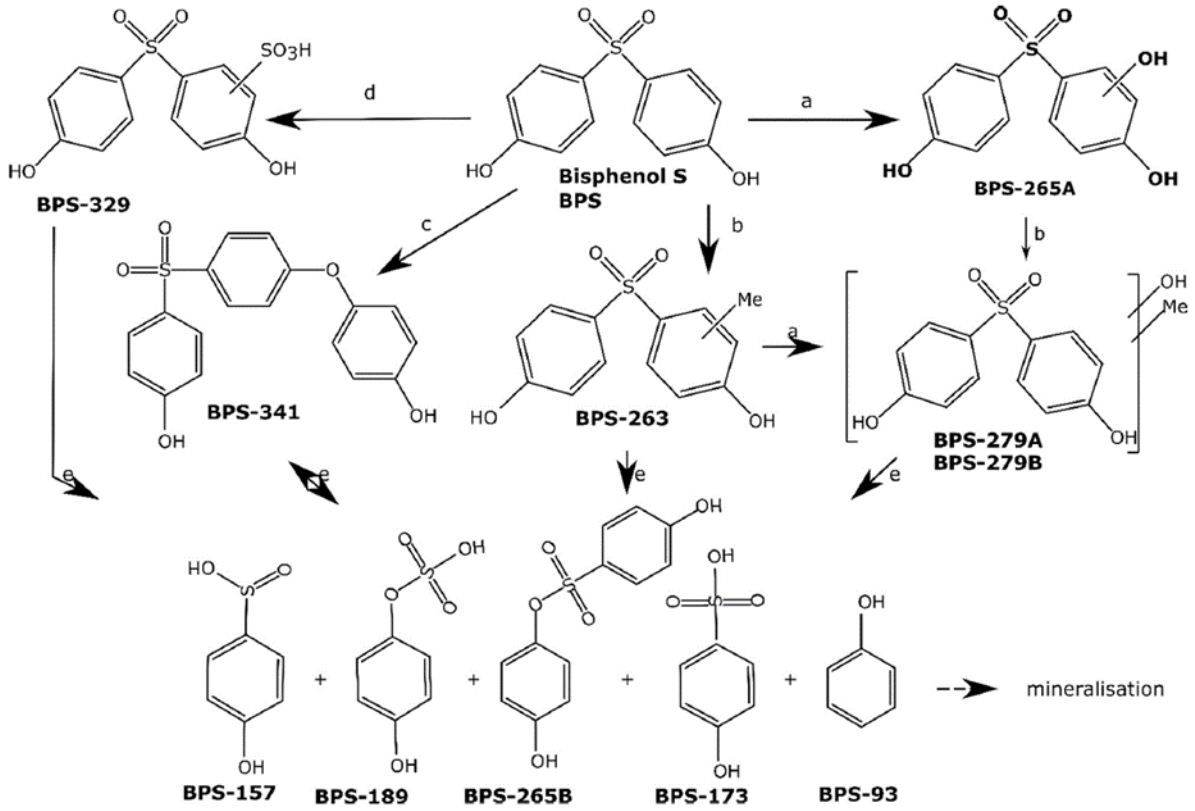


Figure 1: proposed biodegradation pathway of BPS in activated sludge
(a: hydroxylation, b: methylation, c: the coupling of smaller BPS moieties, d: sulphonation and e: cleavage of the S–C bond, dashed arrows; not identified) (from Kovačič *et al.*, 2021).

In the Zahn-Wellens test, a level of 70% mineralisation (DOC removal) must be reached within 7 days, the log phase should be no longer than 3 days, and the percentage removal in the test before degradation occurs should be below 15% (pre-adaptation of the inoculum is not allowed) (ECHA guidance IR/CSA, 2017a; chapter R.11). Therefore, BPS can be considered not inherently degradable.

7.7.1.2.1.3. Simulation tests (water and sediments)

Two literature studies, included in the REACH registration dossier, are available on the biodegradation of a variety of bisphenols, including BPS.

In the microcosm study of Ike *et al.* (2006), biodegradability of BPS is examined in an aerobic (according to the TOC-Handai method (Nasu *et al.*, 1993), a kind of river-die away method) and an anaerobic (according to a method similar to that of Kleerebenzem *et al.*, 1999) simulation test. Natural river water from three sites of four streams running (aerobic system) over Osaka and sediment from one pond (Inukai Pond- anaerobic system) in Japan was used. Test results supported the findings of the ready biodegradability study.

Under aerobic conditions the biodegradability of BPS is 0% after 22 days incubation (degradation in 0 out of the 24 microcosms tested).

Under anaerobic conditions biodegradation (only primary degradation) reached about 60% at ca. day 80 (study end) after a lag phase of ca. 60 days.

Another study examined the aerobic degradation of 3 bisphenols (BPA, BPF and BPS) in seawater using modified TOC Handai (TOC, potential test) and modified river (sea) die-away (SDA, simulation test) method (Danzl *et al.*, 2009). The TOC method uses seawater microorganisms collected through filtration from natural seawater. The retained organisms are then dispersed into artificial seawater at original levels of cell density. The SDA method uses samples of indigenous seawater microorganisms in their natural seawater.

Degradation of BPS was not observed by any of these methods in aerobic conditions after 60d, suggesting that BPS might accumulate and remain in the aquatic environment for a long time. A substance is considered persistent in marine waters when the degradation half-life in marine water is higher than 60 days (ECHA Guidance IR/CSA, 2017; Chapter R11).

BPS can be removed efficiently from municipal/industrial wastewater treatment with an average removal efficiency of 81.2% (Wang *et al.*, 2019a). In the study from Česen *et al.* (2018), BPS was found in the influent and sludge, but was below the detection limit in the effluent. Sun *et al.* (2017), concluded that based on the mass balance analysis, the mass loss can be attributed to biodegradation.

7.7.1.2.2. Biodegradation in soil

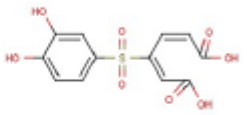
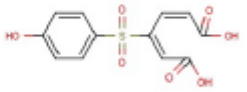
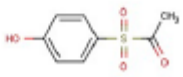
A literature study on biodegradation in soil is present in the REACH registration dossier:

Choi and Lee (2017a) investigated the aerobic soil biodegradation of BPA alternatives BPS and BPAF for up to 180d. Two surface clay loam soils (100 µg/kg) were used: one from a forested area close to the Purdue campus (FRST-50) and one sampled from the Purdue Student Organic Farm (PSF-51) (Indiana, USA). The study was conducted similar to those methods described by Mashtare *et al.*, 2013.

Based on compound mass recovered from soils compared to the mass applied, BPS had short half-lives of <1 day in both soils : 0.649d (FRST-50) and 0.935d (PSF-51). These DT50s are CAKE outputs for the times (d) required for the time 0 concentration to decline by 50%. The data were fitted using both Single First order (SFO) and in parallel for comparison Double First Order (DFO) kinetic model: DT50 were determined to be 1.10 and 0.0661 days respectively.

Metabolites were identified using uHPLC-TOF Analysis for Metabolites, an ultra-high performance liquid chromatography combined with a time-of-flight mass spectrometry, using non-target comparative screening. Three degradation products of BPS could be detected.

Table 10: Structure and MW of the metabolites of BPS (Choi and Lee, 2017a)

BPS Metabolites	
Formula Mass	**Structure**
$C_{12}H_{10}O_8S$ 314.0096 (U)	
$C_{12}H_{10}O_7S$ 298.0147 (J)	
$C_8H_8O_4S$ 200.0143 (K)	

Bold : metabolites qualified with both MS and MS/MS fragmentation data
Italic : metabolites tentatively identified with MS but insufficient MS/MS data to confirm

Degradation pathway:

Catechol is readily degraded by ortho- and metha-cleavage dioxygenases, common in soil bacteria.

For BPS, Ogata *et al.* (2013) observed hydroxylation and metacleavage of just one ring with no changes to the alkyl group connecting the two phenolic rings.

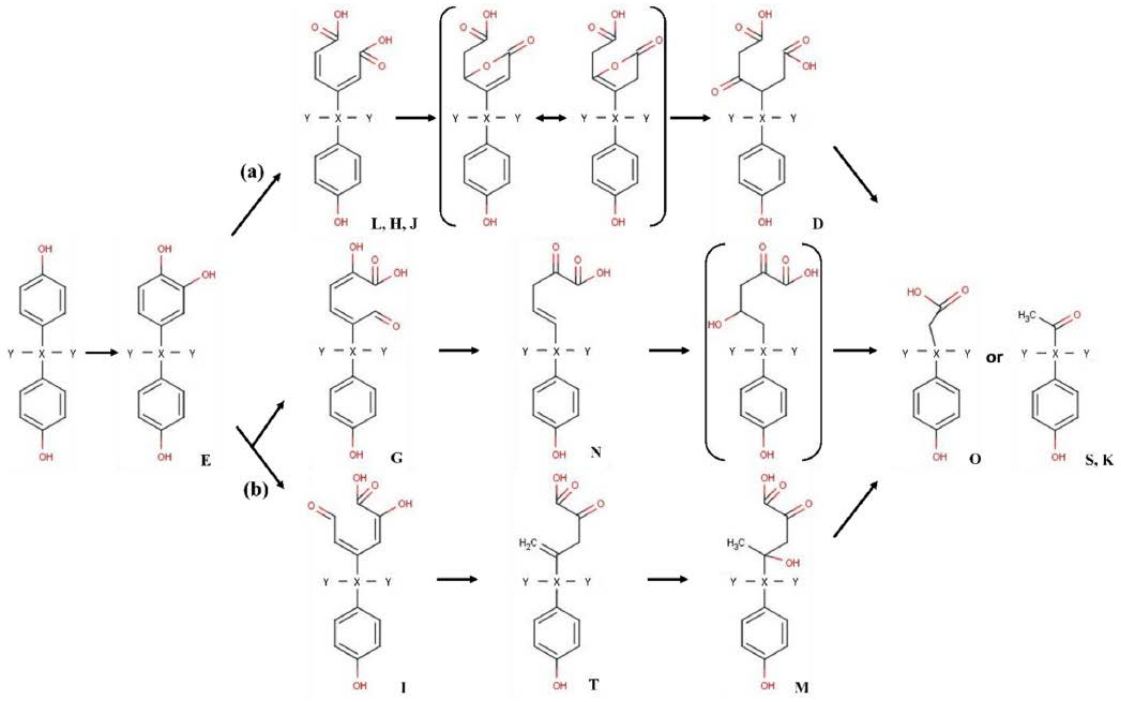


Figure 2: Proposed cleavage pathway postulated from oxidative degradation of catechol by (a) intradiol dioxygenase (ortho-cleavage) and (b) extradiol dioxygenage (meta-cleavage). X = carbon or sulphur linkages to 2 phenol groups, Y= CH3, CF3 or O groups to bridging atom. Letters under the structure represent the metabolites. (Choi and Lee, 2017a)

Cao *et al.* (2020) examined the degradation and NER formation of BPS in aerobic soil. The study was performed with a soil collected from a paddy rice field in Jiangsu (China). The soil consisted of 46.7% clay, 37.9% silt and 15.4% sand.
A half-life of 2.8d was determined. After 28d, the dissipation of BPS could be attributed to biodegradation (53.6 +/- 0.2%) and NER formation (44.9 +/- 2.9%). NER formation was reached before maximum mineralisation (after +/-6d vs +/-11d resp.). Formation of NERs occurred mostly via physicochemical entrapment (more than half) and ester-linkages (one third). Formation of ester-linkages NERs was especially attributed to microbial activities while physicochemical entrapment was attributed to the abiotic aging of BPS in soil. These last NER-type were unstable and became again bioavailable when mixed with fresh soil.
Degradation of BPS to metabolites (degradation constant k_{PM} = 0.186/d) was slower than mineralisation of the metabolites (K_{mv} = 0.773/d). Two major metabolites were formed. Both were detected at day 2 and increased until day 6. M1 remained stable thereafter, while M2 further decreased and was no longer detected at day 28.

7.7.1.2.3. Biodegradation in WWTP (Wastewater treatment plant)

The removal capacity/efficacy in WWTP was determined in several literature studies, although little information is available for Europe.

The mean concentration of eight bisphenols, including BPS (aqueous and suspended particulate matter combined) were measured in 5 Indian STPs by Karthikraj and Kannan (2017). Concentrations of BPS were found in the influent, effluent and sludge of the STPs (mean measured concentrations of 14.7, 2.4 and 185.7 ng/L respectively). The mean concentration of BPS in sludge was 10 times higher than that of BPA whereas, in the dissolved phase, the concentration of BPA was four-fold higher than that of BPS. These results suggest that BPS has a higher affinity for particulate matter/sludge than does BPA. Removal efficiency was calculated to be 77.7% (mean: range 69.6-96.7%).

Wang *et al.* (2019a) reviewed the occurrence and removal mechanisms of BPA and its analogues, including BPS, in municipal WWTPs from several countries/regions. BPS was measured in the influent of the municipal WWTPs with an average concentration of 54 ng/L and 5 ng/L in the effluent. The removal capacity of BPS in full-scale municipal WWTPs ranged between 3.6 to 100.0% (average 81.2%) which indicate a good removal performance of municipal WWTPs. Based on the relatively low Log Kow of BPS, it is suggested that biodegradation is likely to be an important route of removal. The concentration of BPS in the sewage sludge were in the range of not detected - 600 ng/g with an average concentration of 31.13 ng/g dw.

The study from Wang *et al*. (2019a) includes findings from Česen *et al.* (2018), for BPS in 5 municipal/industrial WWTPs in Slovenia. BPS was found above the LOD in 2 out of the 5 WWTP influent samples resulting in an average concentration of 21.3 ng/L, while in the effluent it was <LOD in all 5 samples, concluding a 100% removal capacity.

Also, findings from Sun *et al. (*2017), were included in this review. BPS was detected in the influent and the sludge of seven wastewater treatment plants in Xiamen (China) with a medium concentration of 48.0 ng/L and 1.01 µg/kg resp. The concentration of BPS was below the detection limit in the effluent. Total mass loads of BPS in the influent were 56.2 g with an adsorbed mass value of 1,19 g. After wastewater treatment, mass load in the effluent and sludge was 0.703 g and 0.259 g resp. The removal efficiency of BPS was 98.3% and based on the mass balance analysis it can be concluded that mass loss was through biodegradation.

BPS was one of the most abundant bisphenols found in the influent, primary effluent and final effluent of 2 WWTPs in Albany, NY (USA) (Xue and Kannan, 2019). The detection rate was 44% in the influent (raw wastewater) with concentrations ranging from MLOQ-707 ng/L. BPS was not detected in the suspended particulate matter phase of the wastewater influent in one WWTP but had a detection rate of 6.3% in the 2nd WWTP. The geom. mean concentration in the sludge was between 7.76 and 15.8 ng/g dw, with detection rate of 77% and 73% resp. Removal efficiency of BPS in WWTP after primary treatment was between 6.4 and 24% and -11 and 1.1% after secondary treatment. No BPS was detected in ash of incinerated sludge, demonstrating that this is a good removal method for BPS in WWTP sludge.

7.7.1.2.4. Additional biodegradation data

Sakai *et al.* (2007) investigated biodegradation of BPA and related compounds by a specific microorganism. In this study, *Sphingomonas sp.* strain BP-7 was not able to degrade BPS, while BPA was degraded within 20h.

7.7.1.2.5. Summary and discussion on degradation

Abiotic degradation

After evaporation or exposure to the air, BPS will be relatively fast degraded by photochemical processes with a DT50 of 26.5 hours. BPS shows low potential of volatilisation, thus photodegradation in air is not considered an important degradation pathway.

Under UV light, phototransformation in water is fast (DT50 = 43.1 min) and the rate increases with increasing light intensity. However, it cannot be excluded that BPS might accumulate in deeper and thus darker water layers.

Phenols are regarded as stable towards hydrolysis (Harris, 1990). Based on literature conclusions (Kollig *et al.*, 1993; Boethling and Mackay, 2000) it can be assumed that BPS is hydrolytically stable since it does not contain function groups susceptible to hydrolysis.

Biotic degradation

BPS is considered not readily degradable according to OECD TG 301 C and to an enhanced OECD TG 301 B (test duration prolonged to 59 days instead of 28 days). Furthermore BPS degraded only 47% after 14d (36% after 7d) in an inherent study equivalent or similar to OECD TG 302 B (modified Zahn-Wellens) and can thus be considered as non-inherently degradable (<70% biodegradation within 7 days; ECHA guidance IR/CSA, 2017; chapter R.11).

Simulation tests (water/sediment) performed according to the TOC Handai- and river (sea) die-away confirmed that BPS does not biodegrade rapidly under aerobic conditions:
Following the results of the biodegradation study on a variety of bisphenols (Ike *et al.*, 2006) according to the TOC-Handai method, BPS did not degrade (0% after 22d) under aerobic conditions using several river water samples. Under anaerobic conditions, primary biodegradation reached about 60% at ca. day 80.
In the simulation study using the TOC handai – and river (sea) die-away method (Danzl *et al.*, 2009) no degradation of BPS was observed.

Degradation in soil was examined by Choi and Lee (2017a) using a method similar to those described by Mashtare *et al.*, 2013 and resulted in a DT50 of 1.10 d, indicating that BPS is rapidly degraded. Three major degradation products could be identified.

This rapid degradation was confirmed by Cao *et al*. (2020), who determined a half-life of 2.8d for BPS in aerobic soil. After 28 days, dissipation of BPS was due to biodegradation (53.6%) and NER formation (44.9%). The reversibility of the NER formation for a large amount of NERs was suggested.

It can be concluded that BPS is not rapidly biodegraded under relevant environmental conditions.

Overall conclusion

BPS is hydrolytically stable, not readily and inherently degradable and shows low degradation in river water and seawater under aerobic conditions. The difference in half-life between aerobic soil and aerobic sea and river water might be due to the relatively low microbial activity in waters compared to soil (Cao *et al.*, 2020).

Primary degradation in soil was quick with reported DT50 between 0.0066 and 2.8 days.

Although photolysis in water is rapid (DT50: 43.1 min) it cannot be excluded that BPS might accumulate in deeper water layers and remain in the aquatic environment for a long time. p-hydroxybenzenesulfonic acid was identified as the major phototransformation product in water.

It can be concluded that BPS is not rapidly degraded under relevant environmental conditions.

Literature data confirm that BPS can be degraded by microbial organisms after acclimatisation. BPS was removed efficiently from municipal/industrial wastewater treatment plants mainly by biodegradation with an average removal of 81.2%. Also, the relatively low log Kow suggest that this is an important removal route.

However, it is most likely that at industrial sites microbial organisms in WWTP are adapted to BPS. Furthermore, BPS is a substitute for BPA, and it became already the main developer used in thermal paper since the restriction of BPA in thermal paper is in force. BPS is found

in several environmental compartments including water (Wu *et al.*, 2018). Therefore, it cannot be excluded that BPS ends up in municipal WWTP with unadapted microorganisms.

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

Three experimental studies examining the sorption of BPS are reported in the REACH registration dossier.

In a non-guideline study, Choi and Lee (2017b) examined the partitioning behaviour of Bisphenol alternatives BPS and BP AF compared to BPA in soil−water and octanol−water systems at different pH values (batch equilibrium method). 4 soils with varying chemical and physical properties were used.

Sorption was measured by independently quantifying aqueous and sorbed-solid phase concentrations.

Table 11: Summary of sorption coefficients of BPS (Choi and Lee, 2017b)

Soil system	Log Koc	Kd	pH	Temp (°C)	% OC	% clay
#1 (FRST-48)	2.81	15.34	5.5	23	2.38	9.0
#2 (PSF-49)	2.42	6.86	7.8	23	2.62	17.0
#3 (EPA-09)	2.91	0.89	8.6	23	0.11	17.4
#4 (EPA-14)	3.21	7.86	3.8	23	0.48	63.6

The highest sorption of BPS to soil was seen in the tested soils with the lowest % of Organic Carbon (OC) (<0.5%) and the highest % clay.

Geometric mean Log Koc = 2.82 (at 23°C), Koc at 20°C = 660.7

They also concluded that besides simple hydrophobic-type partitioning other additional sorption mechanisms impact BPS.

In another non-guideline literature study, the occurrence and partitioning of 9 bisphenol analogues in water and sediment from Taihu Lake and Liaohe River Basin including Liaohe River and Hunhe river (China) were examined (Jin and Zhu, 2016). This study measured the concentrations of BPS in the samples, estimated the partition coefficient between sediment and surface water using the concentrations in sediment and in overlaying water and determined the organic carbon fraction of sediment (foc, %) based on the wet chemistry technique (Potassium Dichromate Oxidation Ferrous Sulfate Titrimetry) as suggested by the U.S. EPA.

A log Koc of 3.5 was determined.

In a third study (REACH registration dossier : Unpublished study report, 2016), the adsorption on activated sewage sludge was examined according to the International Standard ISO 18749 Water Quality (Batch Test using specific analytical methods). <10% of DOC (mean value) was removed by adsorption onto the activated sludge after 72h of exposure.

7.7.2.2. Volatilisation

Henry´s law constant was estimated to be 2.73 E-010 Pa*m3/mole at 25°C by EPIWIN (SRC HENRYWIN v3.10).

BPS will very slowly evaporate from the water surface into the atmosphere.

7.7.2.3. Distribution modelling

Distribution was estimated according to Mackay, Level I (LEVEL 1, V3.00) by the registrant. Emission to water is expected because direct and indirect exposure of air, sediment and soil is unlikely.

Percent distribution in media:
Air (%): 0
Water (%): 99.3
Soil (%): 0.35
Sediment (%): 0.35
Susp. Sediment (%): 0
Biota (%): 0
Aerosol (%): 0

Over time, the substance will preferentially distribute into water.

The evaluating MSCA applied the Level III Fugacity Model (Episuite 4.1), also considering emissions to other compartments than water. According to the substance info card on the ECHA's dissemination website[7] (last consulted on 26/8/2022) release to the environment is likely to occur from article service life, industrial use and manufacturing. No public registered data are available on consumer and professional use. Furthermore literature data demonstrate the presence of BPS in the other environmental compartments than water (Wu *et al*., 2018). Application of the level III Fugacity Model resulted in a major distribution to soil instead of water when emissions to other compartments than only water are expected.

Log Kow of 1.2, WS of 715 mg/l

[7] ECHA's dissemination website: substance info card: https://echa.europa.eu/substance-information/-/substanceinfo/100.001.137

Level III Fugacity Model:

Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)	
Air	7.22e-007	26.5	1000
Water	16	360	1000
Soil	83	720	1000
Sediment	0.966	3.24e+003	0

Persistence Time: 788 hr

Level III Fugacity Model:

Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)	
Air	1.76e-006	26.5	1000
Water	3.7	360	0
Soil	96.1	720	0
Sediment	0.224	3.24e+003	0

Persistence Time: 968 hr

Level III Fugacity Model:

Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)	
Air	4.43e-017	26.5	0
Water	94.3	360	1000
Soil	2.42e-009	720	0
Sediment	5.69	3.24e+003	0

Persistence Time: 361 hr

Level III Fugacity Model:

Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)	
Air	1.06e-016	26.5	0
Water	0.195	360	0
Soil	99.8	720	1000
Sediment	0.0118	3.24e+003	0

Persistence Time: 1.03e+003 hr

Level III Fugacity Model:

Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)	
Air	1.28e-006	26.5	1000
Water	28.3	360	1000
Soil	70	720	0
Sediment	1.71	3.24e+003	0

Persistence Time: 664 hr

Level III Fugacity Model:

Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)	
Air	8.52e-007	26.5	1000
Water	1.89	360	0
Soil	98	720	1000
Sediment	0.114	3.24e+003	0

Persistence Time: 1e+003 hr

```
Level III Fugacity Model:
Mass Amount     Half-Life     Emissions
(percent)         (hr)         (kg/hr)
Air         9.04e-017         26.5          0
Water       24.5              360           1000
Soil        74                720           1000
Sediment    1.48              3.24e+003     0
Persistence Time: 698 hr
```

7.7.2.4. Summary and discussion on environmental distribution

BPS has a water solubility of 715 mg/L, a log Kow of 1.2 and a mean measured log K_{oc} of 2.82.

The substance shows very low volatilisation from water to air (H=2.73E-010 Pa-m3/mole).

According to the information provided in the REACH registration dossiers BPS is manufactured and used in closed systems and during industrial polymerisation process the substance is chemically bonded completely.

Only calculated data are available for the environmental distribution. If only emission to water is considered, BPS will remain in that compartment. Nevertheless, several literature studies demonstrate the presence of BPS in water, sediment, sludge and indoor dust/air (see 7.12 Exposure assessment).

7.7.3. Bioaccumulation

7.7.3.1. Aquatic bioaccumulation

Table 12: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Log Kow OECD TG 117 (Partition Coefficient (n-octanol / water), HPLC Method) GLP : no Rel. 2	Log Kow = 1.2 (at 23°C, pH 6.2)	/	REACH registration dossier: Unpublished study report, 2010
BCF OECD TG 305C (Bioaccumulation: Test for the Degree of Bioconcentration in Fish) Species : *Cyprinus carpio* GLP : not specified Rel. 2	BCF : <0.2 at test conc of 500 µg/L <2.2 at test conc of 50 µg/L	/	REACH registration dossier: Unpublished study report, 1998
Log BAF for aquatic organisms (plankton, invertebrates and	Log BAF between -1.70-0.650		*Wang et al.*, 2017b

Method	Results	Remarks	Reference
fish) from Lake Taihu, China			
BCF in different tissues of C*yprinus carpio*	Whole body BCF: Free form: 0.1 ± 0.0 Total: 0.3 ± 0.0	BPS free in the whole body was 20.9%, meaning that 79.1% was in the conjugated form (calculated based on the free and total forms)	Wang *et al.*, 2020a
BCF for algae *Navicula sp.* and *Chlorella vulgaris*	*Navicula sp.* at environmental relevant concentration of 0.5 mg/L: increased BCF from 157.83 ± 54.96 at 24 h to 2052.94 ± 732.13 at 72 h, but decreased to 72.22 ± 15.91 at 120 h At higher conc.: decreased BCF from 308.37 ± 2052.94 ± 732.13 at 0.5 mg/L to 110.01 at 72h to 194.60 ± 5.04 at 5 mg/L to 308.37 ± 110.01 at 10 mg/L. BCF was 322.50 ± 102.26 at 10 mg/L		Li *et al.*, 2021

The log K_{ow} is determined by HPLC method according to OECD TG 117: log Kow = 1.2, at 23°C and pH 6.2. This value is supported by a calculated Log Kow (KOWWIN v1.67) and reviewed database: 1.65 at 25°C.

The bioaccumulation of the test substance was determined in *Cyprinus carpio* under flow through conditions following a method similar or equal to OECD TG 305 C (Registration dossier: Unpublished study report, 1998). The study was run with at a concentration of 50 µg/l and 500 µg/l over a period of 6 weeks. The BCF for the test substance was measured to be very low : respectively < 2.2 and < 0.2 .

Wang *et al.* (2017b) examined the bioaccumulation and biomagnification of 9 bisphenols in aquatic organisms from Taihu Lake in China. BPS was found in all water samples and in 70.6% of the aquatic organisms (plankton, invertebrates and fish). Log BAF were between -1.70 and 0.650 in the aquatic organisms.

Wang *et al.* (2020a) determined BCFs in different tissues of C*yprinus carpio* after exposure to 8 bisphenols, including BPS. Both free and total forms of BPS were measured. The conjugated form was calculated based on the free and total forms. BPS free in the whole body was 20.9%, meaning that 79.1% was in the conjugated form.

Table 13: BCF in different tissues of Cyprinus carpio

BPS	Form	BCF (L/kg)
Blood	Free	0.2 ± 0.1
	Total	1.3 ± 0.3

Kidney	Free	0.2 ± 0.0
	Total	0.8 ± 0.1
Liver	Free	0.2 ± 0.0
	Total	0.3 ± 0.1
Muscle	Free	0.1 ± 0.0
	Total	0.1 ± 0.1
Whole body	Free	0.1 ± 0.0
	Total	0.3 ± 0.0

Extracted from Wang *et al*. (2020a)

Bioaccumulation of the test substance in aquatic organisms is not expected.

However in the literature study of Li *et al.*, (2021) BCF was determined for the typical freshwater algae *Navicula sp.* and *Chlorella vulgaris*. BCF was calculated by ratio of intracellular BPS concentration to extracellular BPS concentration.
Navicula sp. and *C. vulgaris* (initial density of $1.73x10^5$ and $4.29x10^5$ cells/mL resp.) were exposed to 0.5 and 10 mg/L. At environmental-related concentration (0.5 mg/L) BCF in *Navicula sp.* increased from 157.83 ± 54.96 at 24 h to 2052.94 ± 732.13 at 72 h, but decreased to 72.22 ± 15.91 at 120 h. This sharp increase in BCF at 72h may indicate that bioconcentration of BPS in this species can pose an ecological risk for aquatic predators of *Navicula sp.* in natural waters after short-term exposure.
Though, at higher BPS concentrations, BCF decreased from 308.37 ± 2052.94 ± 732.13 at 0.5 mg/L to 110.01 at 72h to 194.60 ± 5.04 at 5 mg/L to 308.37 ± 110.01 at 10 mg/L. BCF was 322.50 ± 102.26 at 10 mg/L showing lower ecological risk after exposure to higher concentrations of BPS.
Lower bioaccumulation was reported for *C.vulgaris*.

7.7.3.2. Terrestrial bioaccumulation

No experimental studies are available.

The Log K_{oa} was estimated to be 14.157, using the experimental log Kow of 1.2 and estimated Henry's law constant 2.70E-015 atm-m3/mole (2.73E-010 Pa-m3/mole) (Epiwin, AEROWIN v1.00) (eMSCA, 2018).

```
Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
 Vapor pressure (liquid/subcooled):  1.35E-005 Pa (1.01E-007 mm Hg)
 Log Koa (Koawin est  ): 14.157
  Kp (particle/gas partition coef. (m3/ug)):
      Mackay model           :  0.223
      Octanol/air (Koa) model:  35.2
  Fraction sorbed to airborne particulates (phi):
      Junge-Pankow model     :  0.889
      Mackay model           :  0.947
      Octanol/air (Koa) model:  1
```

Although a high log Koa (14.157) was estimated, in combination with a log Kow <2 this indicate that BPS has low potential to bio magnify in air-breathing (terrestrial) organisms.

7.7.3.3. Field data

BPS is found in remote areas like the Artic in detectable concentrations (<0.3-1.1 ng/g ww) in seabirds eggs of black-legged kittiwake and glaucous gull and in artic char muscle (n=10) at concentrations between <0.3-1.3 ng/g ww (Lucia *et al.*, 2016). Furthermore, BPS was detected in several organisms in urban fjord (Inner Oslofjord) in Norway by Ruus *et al.*, (2014). BPS was found in plankton (0.24-4.83 ng/g), bird eggs (ND-44.2 ng/g), polychaetes (0.06-2.35 ng/g), fish (<0.5-20.5 ng/g), prawns (1.34-2.87 ng/g) and mussels (<0.3-1.89 ng/g). However, in a follow-up study of 2016 and 2018, BPS was not detected in cod liver neither in blood and eggs from the herring gull resp. (Ruus *et al.*, 2017, and Ruus *et al.*, 2019, resp.). However, in 2019 BPS was detected in one liver and 3 biles of cod (Liver: <1 – 1.52 ng/g w/w; Mean: 0.8 ng/g w/w; Bile: <1 – 1.58 ng/g w/w; Mean: 0.3 ng) (Ruus *et al*., 2020).

The presence of 10 bisphenols, of which BPS, in the northern pike (*Esox lucius*) were analysed by Tian *et al*. (2019). Fish were collected in late May to early June 2014 and 2015 from the St. Lawrence River, Canada, 4 km upstream (n =12) and 4 km downstream (n = 14) of the point of discharge of a major primary WWTP. BPS was not detected in the muscle tissues.

Zhu *et al.* (2019), determined the concentrations of 45 substances in urine samples of various bovine breeds. 183 samples were collected in rural and agricultural areas (without point sources in the vicinity) in China, India and US between March and November 2018. Bovines from China were housed permanently in shelters and fed with commercial food while those from India and US were allowed to graze in open pastured/grassland and fed with a combination of grain and grass. The detection frequency of BPS for the urine was 77%, 82% and 100% resp. with a median concentrations resp. <LOQ (ND-3.7 ng/mL), <LOQ (ND-4.0 ng/mL) and 0.40 ng/mL (<LOQ-1.7 ng/mL). LOQs for the 8 measured bisphenols (BPA, BPAF, BPAP, BPS, BPF, BPP, BPZ and BPB) was between 0.12 and 1.2 ng/mL.

Liao and Kannan (2019), collected 11 molluscs species between 2006 and 2015 from coastal areas of five cities located along the Bohai Sea (China). They determined the concentrations of 8 bisphenols and 5 benzophenones in 186 samples. BPS was detected in <5% of the samples. Concentrations of BPS ranged between not detected and 4.68 ng/g dw, with a geom. mean and median value of 0.146 and 0.141 ng/g dw resp.

Wild-caught marine organisms were gathered from fisherman in the Pearl River Estuary in South China and comprised shellfish (n=11) and fish (n=10) (Zhao *et al.*, 2019). Concentration of BPS in the marine organisms ranged between not detected and 328 ng/g, with a median concentration of 1.28 ng/g.

Zhao *et al.* (2021) evaluated the occurrence of Bisphenols in marine organisms (13 species; n = 74), as well as seawater (n = 15), from East China Sea. In marine organisms (without hydrolysis), BPA and BPS were the predominant bisphenols with concentrations of BPA of 3.8 ng/g mean (range 1.2−7.7 ng/g) and BPS of 1.5 ng/g mean (range: 0.19−6.1 ng/g). After enzymatic hydrolysis treatment, mean concentrations of BPS increased 1.8 times in marine organisms.

7.7.3.4. Summary and discussion of bioaccumulation

The substance has a log K_{ow} of 1.2. The experimentally derived BCF in fish was determined to be <2.2. Therefore, no aquatic bioaccumulation is expected.

However a sharp increase in BCF in *Navicula* sp. (freshwater algae) at 72h and environmental-related concentration (0.5 mg/L) from 157.83 ± 54.96 at 24 h to 2052.94 may indicate a high risk for aquatic predators of this species (Li *et al.*, 2021). There is no indication of bioaccumulation in air-breathing organism, although the estimated Log K_{oa} is high (>5), the estimated log Kow <2.

There is no indication of bioaccumulation in air-breathing organism, although the estimated Log K_{oa} is high (>5), the estimated log Kow was <2.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

Table 14: Summary of relevant information on aquatic toxicity

Method	Species	Results	Remarks	Reference
AQUATIC TOXICITY				
Fish				
Acute Toxicity				
OECD TG 203 (Fish, Acute Toxicity Test) GLP Rel. 2	not specified	96h LC50 >100mg/L (nom)	Test type : not specified Analytical monitoring not specified	REACH registration dossier: Unpublished study report, 2010
Japanese Industrial Standard JIS K 0102-1986-71, "Testing methods for industrial waste water" GLP : not specified Rel. 2	*Oryzias latipes*	96h LC50 >500 mg/L (nom)	Test type: Semi-static Analytical monitoring not specified	REACH registration dossier: Unpublished study report, 1998
Long-term Toxicity				
OECD TG 210 (Fish, Early-Life Stage Toxicity Test) GLP : no Purity: 99.6% Rel. 2	*Danio rerio*	34d NOEC ≥ 10 mg/L (nom)- hatch rate, post hatch survival, growth	Deviations : Reduced number of replicates (2) per treatment group 8 test concentrations + control Growth assessment at day 33/34 instead of 30dph	REACH registration dossier: Unpublished study report, 2018
OECD TG 240 (Medaka Extended One Generation Reproduction Test, adapted for Danio rerio) ZEOGRT	*Danio rerio*	128d NOEC=250 µg/l (nom; male body length)	Test type: Flow-through	REACH registration dossier: Unpublished study report, 2020

Method	Species	Results	Remarks	Reference
GLP Purity: 99.87% Rel. 1				
Invertebrates				
Acute Toxicity				
OECD TG 202 (Daphnia sp. Acute Immobilisation Test) GLP : not specified Rel. 2	*Daphnia magna*	48h EC50=55 mg/L	Test type : static Analytical monitoring : not specified	REACH registration dossier: Unpublished study report, 2001
OECD TG 202 (Daphnia sp. Acute Immobilisation Test) GLP : yes Rel. 2	*Daphnia sp.*	48h EC50=100mg/L	Test type : not specified Analytical monitoring : not specified	REACH registration dossier: Unpublished study report, 2010
Not mentioned	*Daphnia magna*	48h LC50=179mg/L	Not described	Kienhuis and Geerdink, 2000
Long-term Toxicity				
OECD TG 211 (Daphnia magna Reproduction Test) GLP Rel. 2	*Daphnia sp.*	21d NOEC=2.7 mg/L	Test type : not specified	REACH registration dossier: Unpublished study report, 2010
Algae				
OECD TG 201 (Alga, Growth Inhibition Test) /EU C.3 (Algal Inhibition test) GLP Rel. 1	*Desmodesmus subspicatus* (previous name: *Scenedesmus subspicatus*)	72h ErC50=106 mg/L (nom) 72h NOErC=10.2 mg/L (nom)	Test type : static Analytical monitoring : yes	REACH registration dossier: Unpublished study report, 2010
OECD TG 201 (Alga, Growth Inhibition Test) GLP	Not specified	72h ErC50=65 mg/L 72h NOErC=4.6 mg/L	Test type : not specified Analytical monitoring : yes	REACH registration dossier: Unpublished study report, 2010

Method	Species	Results	Remarks	Reference
Rel. 2 (in the registration dossier) Rel. 3 for eMSCA (**as species, validity criteria, deviations, test condition unspecified**)				
Microorganisms				
OECD TG 209 (Activated Sludge, Respiration Inhibition Test) / EU C.11 (Biodegradation: Activated Sludge Respiration Inhibition Test) GLP Rel. 1	Domestic activated sludge	3h EC10 =200 mg/L (nom) 3h EC50=390 mg/L (nom)	Test type : static	REACH registration dossier: Unpublished study report, 2009
SEDIMENT TOXICITY				
/				

7.8.1.1. Fish

7.8.1.1.1. Short-term toxicity to fish

The acute toxicity in fish was tested according to OECD TG 203. The 96-hour LC50 value was determined to be above the limit dose of 100 mg/L (REACH registration dossier: Unpublished study report, 2010).

A supporting study “Testing methods for industrial waste-water” performed according to Japanese Industrial Standard JIS K 0102-1986-71 under semi-static conditions with *Oryzias latipes* resulted in a 96-hour LC50 value above the limit dose of 100 mg/L (> 500 mg/L) (REACH registration dossier: Unpublished study report, 1998).

7.8.1.1.2. Long-term toxicity to fish

A non-GLP chronic toxicity study with zebrafish was performed according to OECD TG 210 (Fish, Early-Life Stage Toxicity Test) in 2015 (REACH registration dossier: Unpublished study report 2018).
The study deviated from the test guideline:

- A reduced number of replicates per treatment group (2) was used
- 8 concentrations + control were used
- Growth assessment was measured at day 33/34 instead of 30dph

The 34d NOEC was determined to be 34d NOEC ≥ 10 mg/L (nom) for hatch rate, post hatch survival and growth.

Literature studies (Naderi *et al.*,2014 and Ji *et al.*, 2013) showed reduced egg production and sperm count (see details of these 2 studies in section 7.10.1). Effects on development

were also observed : among others, reduced body length and weight in males and increased malformation rates in exposed F1 embryos. Moreover, a skewing of phenotypic sex ratio was observed, which is a clear ED specific adverse effect.

In the frame of the Substance Evaluation a Zebrafish Extended One Generation Reproduction test (ZEOGRT) was performed to elucidate the environmental endocrine adverse effect as well as to determine a NOEC (REACH registration dossier: Unpublished study report, 2020).

Zebrafish were exposed to nominal concentrations of test substance of 2, 10, 50, 250 and 1250 µg/L. No effects were seen on survival, hatching, growth and reproduction in F0 and on survival in F1.

Although length (females and males) was significantly affected in F1 at ≥10 µg/L after day 35, the effect was transient and no longer observed at day 65. Length and body weight of females did not statistically significantly differ from control at day 65 and at the end of the experiment. The lowest NOEC of 250 µg/L was determined for male body length of F1 at the end of the study (day 125-128), which was significantly different from control at 1250 µg/L.

In this study, a non-significant decrease of the sex ratio was shown for all concentrations except for the treatment with 250 µg/L BPS. Nevertheless, the sex ratio at the 10 µg/L concentration fell below natural variation with only 29% of males. It should however be noted that, similar to the range-finding study, the percentage of males in the control group was very low (41%), impacting the sensitivity of the observations.

Histological analysis showed a dose-dependent decrease in the number of females with mature oocytes at the end of the experiment (total exposure duration of 170 days, ±150 d for F1) for all concentrations (calculated by DS using Cochrane-Armitage trend analysis) and was significantly affected at 1250 µg/L (recalculated by DS using Fisher exact test).

Furthermore, F1 showed a decrease in fecundity for all concentrations (reduced by 43, 21, 36, 21 and 7%, resp. at 2, 10, 50, 250 and 1250 µg/L) and the reduction was significant at 2 and 50 µg/L (Wilcoxon test-one sided). Additionally, the preliminary study (range-finding test) resulted in a (non-significant) decrease in fecundity for concentrations of 3.2, 10 and 32 µg/L with reductions of 36, 70 and 50% resp. However, statistical outcome is questionable as a Jonckheere-Terpstra-test was applied while the treatment mean had a non-ordering hypothesis (non-monotonic). Re-calculation by the dossier submitter using a Wilcoxon test showed, on the contrary, that fecundity was significantly reduced (by 70%) at 10 µg/L.

Furthermore, it should be noted that also fertility (non-significantly) decreased in the range-finding study but also here the Jonckheere-Terpstra-test was applied (the Jonkheere-Terpstra test is a trend test, i.e., it will test whether there is a monotonic dose-response relationship between increasing doses and the effect studied.) The reduction compared to the control was 91, 62, 64, 78 and 78%, response at 3.2, 10, 32, 100 and 320 µg/L). In the main study no effect on fertility in F1 was observed.

In the F2-generation, a statistically significant difference was determined on the hatching success (0-4 days) at 10 (94*%), 250 (95*%) and 1250 µg/L (94*%). This effect is to be considered minor.

For details see section 7.10.1.

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

The acute toxicity to *Daphnia magna* was tested according to OECD TG 202 under static conditions (2001). The 48 hour EC50 value was determined to be 55 mg/L (REACH registration dossier: Unpublished study report, 2001).

The 48 hour EC50 value was determined to be 100 mg/L in a second study (OECD TG 202, 2010) with a *daphnia sp.* (REACH registration dossier: Unpublished study report, 2010).

In a non-guideline study (US EPA report “Bisphenol A alternatives in thermal paper”, 2014) a 96h EC50=45 mg/l was determined, supporting the results found in the key study. Information regarding the measured test substance concentration was not reported.

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

The chronic toxicity to *Daphnia magna* was tested according to OECD TG 211 (21d Reproduction Test). The 21 days NOEC was determined to be 2.65 mg/L (meas. TWA). Measured values (time-weighted average values) were adopted for calculation of each effect concentration, because some measured concentrations of the test substance exceeded ±20% of nominal values (REACH registration dossier: Unpublished study report 2010).

7.8.1.3. Algae and aquatic plants

The study examined the effect of BPS on green alga (*Desmodesmus subspicatus*) for 72 hours in a growth inhibition test under static conditions. The study was conducted in accordance with the OECD TG 201 and all validity criteria were fulfilled. The cultures were exposed to nominal concentrations of 0, 1.02, 3.2, 10.2, 32, 102, 320 mg/L. Since the analytically determined concentrations of the test substance in the test solutions were within ±20% of the nominal concentrations, the effect concentration was expressed relative to the nominal concentration. The 72-hour NOErC value was determined to be 10.2 mg/L, the ErC50 to be 102 mg/L (REACH registration dossier: Unpublished study report 2010).

In a second study, also conducted in accordance with OECD TG 201, the toxicity of BPS was examined in green algae (species not specified) and resulted in a 72h ErC50 of 65 mg/L and a 72h NOErC of 4.6 mg/L (REACH registration dossier: Unpublished study report, 2010).

7.8.1.4. Sediment organisms

- Not evaluated.

7.8.1.5. Other aquatic organisms

Not available.

7.8.2. Terrestrial compartment

7.8.2.1. Toxicity to soil macro-organisms

Not evaluated.

7.8.2.2. Toxicity to terrestrial plants

Not evaluated.

7.8.2.3. Toxicity to other terrestrial organisms

Not evaluated.

7.8.3. Microbiological activity in sewage treatment systems

The toxicity of BPS to microorganisms was assessed in an activated sludge respiration inhibition test according to OECD TG 209 (REACH registration dossier : Unpublished study report, 2009). The EC10 and EC50 (3h) were 200 and 390 mg/L, respectively.

7.8.4. PNEC derivation and other hazard conclusions

Not evaluated.

7.8.5. Conclusions of the environmental hazard assessment and related classification and labelling

According to the CLP guidance (v.5.0, 2017), section I.2.1.2 chronic testing : "*Chronic or long-term tests with fish can be initiated with fertilized eggs, embryos, juveniles, or reproductively active adults. Tests consistent with OECD Test Guideline 210 (Fish Early Life Stage), the fish life-cycle test (US EPA 850.1500), or equivalent can be used in the classification scheme. Durations can vary widely depending on the test purpose (anywhere from 7 days to over 200 days). Observational endpoints can include hatching success, growth (length and weight changes), spawning success, and survival.*"

The lowest available chronic toxicity value was determined in the ZEOGRT (adapted OECD TG 240).
For classification purposes NOECs or other equivalent ECx (e.g. EC10) shall be used, however preference is given to ECx if available.
No EC10 was/could be determined in the ZEOGRT. The lowest NOEC (male body length F1) was determined to be 250 µg/L.
Following the CLP-criteria for long-term toxicity, BPS should be classified for the aquatic environment as **Aquatic Chronic 2, H411**:

- chronic NOEC =0.25 mg/L and thus meeting the classification criterion ≤ 1 mg/L
- and following the degradation decision scheme: NOT RAPIDLY DEGRADABLE
  - ready biodegradability test:
    0% degradation in an OECD TG 301C
  - or ultimate degradation in surface water simulation test:
    NA
  - or primary biotic or abiotic degradation in the aquatic environment
    - Considered hydrolytically stable
    - No degradation in seawater (aerobic and anaerobic)

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Table** 15**: Study on toxicokinetics

Method, guideline	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Observations	Reference
Toxicokinetics Gavage According to: OECD TG 417 Methods for the determination of toxicity and other health effects: Toxicokinetics; Official Journal of the European Union, No. L 142 U.S. EPA , Health effects Test Guidelines, OPPTS 870.7485, Metabolism and Pharmacokinetics, August 1998 Japan/MAFF: Guideline on the Compiling of the Test Results on Toxicity; Tests on *In vivo* Fate In Animals, 2001 GLP	Rat, Crl:WI (Han), males and females, Blood/Plasma kinetics: 4/dose/sex Balance/Excretion: 4/dose/sex Excretion via Bile: 6/dose/sex Tissue distribution: 12/dose/sex	^{14}C-4,4'-sulphonyldiphenol Radio label: phenyl-U-C14 Vehicle: 0.5% sodium carboxymethyl cellulose (CMC) in tap water	Conc.: 30 and 300 mg/kg bw Blood/Plasmakinetics: once high and low dose Balance/Excretion: Once high and low dose; non-labelled once per day for 14 days; radio-labelled once on day 15 (low dose) Excretion via bile: once high and low dose Tissue distribution: once high and low dose	Oral adsorption: at 300 mg/kg bw: 93 % and 96 % for male and female rats At 30 mg/kg bw: 95% and 87% for males and females. Distribution: residues of substance in organs and tissues showed sublinear correlation between radioactive residues and administered oral doses. Supralinear correlation between radioactive residues in carcass and the external dose was observed. Metabolism: The unchanged parent compound was the main component in faeces, and was detected in lower amounts in urine and absent in bile. Three phase II metabolites of substance were found after oral administration Excretion: Main excretion of substance occurs within 48h post dosing. Total excreted radioactivity reflects a total excretion of substance.	REACH registration dossier: Unpublished study report, 2019

The toxicokinetics study was conducted in male and female Wistar rats (Crl:WI (Han)). Dosing was performed once orally with 30 and 300 mg/kg bw. For balance/excretion experiments unlabelled BPS was administered for 14 days at 30 mg/kg bw/day followed by the administration of single dose of labelled ^{14}C-BPS at day 15. The blood- and plasma kinetics, balance/excretion, bile excretion, tissue distribution and metabolism were investigated within the toxicokinetics study.

Blood- and plasma kinetics:

4 animals per dose and sex were used. Blood samplings were performed on 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168 hours after oral administration for all dose groups. The maximum plasma concentrations were reached at 1 and 4h post dosing of 300 mg/kg bw of BPS in males and females and declined under the limit of quantification (loq) after 96h post dosing. Terminal half-lives were 10.3 and 14.7h, for males and females, respectively. After dosing of 30 mg/kg bw of BPS maximum plasma concentration occurred after 1 and 4h post administration for males and females. The values were under loq at 72h post dosing for males, whereas for 3 out of 4 females plasma levels were under loq after 96h. Terminal half-lives were 9.2 and 8.9h for males and females, respectively. Fast absorption of BPS after oral administration lead to a dose dependent increase in maximum plasma concentration with first T_{max} of generally 1h post dosing. The second C_{max}-value at later T_{max} for both dose levels and genders indicate a potential enterohepatic recirculation of the test substance and/or its metabolites. At high dose, mean maximum plasma concentration in female animals 1h after administration was almost twice as high as the mean plasma concentration of male animals at the corresponding time point. At low dose, mean plasma concentrations were comparable for male and female animals. Internal doses are slightly higher for females than for males. The internal dose increases slightly over proportional to the actual nominal administered dose. These data indicate a potential saturation of kinetics at higher doses, which may be caused by a potential active transport of the test substance and/or its metabolites.

Balance/Excretion:

4 animals per dose and sex were used. Animals were dosed and then placed in metabolism cages in order to collect urine after 6, 12 and 24h and subsequently in time intervals of 24h up to 168h and faeces was collected in intervals of 24h up to 168h post dosing. During the low dose experiment, two male animals were placed in closed metabolism cages in order to additionally collect exhaled air for 48h. The detection of less than 2% of the total radioactive dose in exhaled air justified to perform all balance/excretion experiments in open systems. Within 48h after administration of 300 mg/kg bw, 41.30 and 34.00% of administered radioactivity were found in urine of male and female rats, respectively. Total excretion of radioactivity via urine after 168h was 48.05 and 39.02% of dose for males and females, respectively. During the first 48h after administration, 38.74 and 50.13% of the administered radioactivity were excreted via faeces by males and females, respectively. After 168h total amount of excreted radioactivity via faeces was 44.20 and 55.71% of dose for males and females, respectively. Highest total radioactive residues 168h post dosing (except gastrointestinal tract (GI)) were found in thyroid and carcass for male animals and in carcass for female animals and lowest level in plasma for both sexes. At dose level of 30 mg/kg bw of BPS were 56.94 and 47.09% of the administered radioactivity found in urine of males and females, respectively. Total excretion via urine after 168h was 60.13 and 51.45% of dose for males and females, respectively. During the first 48h after administration 41.75 and 39.40% of the administered radioactivity were excreted via faeces by males and females, respectively. 168h after administration total amount of radioactivity excreted via faeces was found to be 43.00 and 40.85% of dose for males and females, respectively. Highest total radioactivity residues 168h post dosing (except GI tract) were found in carcass for both sexes. In the multiple doses experiment (14 unlabelled, 1 labelled) at 30 mg/kg bw/day, within 48h after dosing of males and females, 46.56 and 46.88% of the administered radioactivity were found in urine of males and females, respectively. Total excretion of radioactivity via urine after 168h was 51.09% for males and 51.82% of dose for females. Via faeces 40.16 and 42.59% of radioactive dose

were excreted via faeces by males and females within 48h post dosing. 168h after administration of radioactive dose, the total amount of 42.33 and 43.99% of administered dose were excreted via faeces for males and females, respectively. Highest total radioactive residues 168h post dosing (except GI tract) were found in carcass for both sexes.

Bile excretion: 6 animals per sex and dose were used. Bile ducts of rats were cannulated in a surgery for bile collection. Animals were placed in metabolism cages in order to collect bile at 3h intervals as wells as urine and faeces at 24h intervals up to 72h. At high dose, excretion of BPS via bile was found to be 43.81 and 45.65% for males and females, respectively within 72h post administration. Total excretion via urine until 72h post dosing was 47.52 and 48.91% and excretion of faeces was 1.02 and 1.03% of dose for males and females, respectively. At low dose, excretion via bile was found to be 56.39 and 38.21% of administered dose within 72h for males and females, respectively. Excretion via urine was 37.72 and 46.37% of administered dose and via faeces 1.28 and 3.03% of administered dose until 72h post dosing for males and females, respectively.

Tissue distribution: 12 animals per sex and dose were used. 3 animals were sacrificed at 4 defined time points, based on the findings of the blood and plasma kinetic experiments: maximum plasma concentration (MPC), second MPC, ½ MPC, and ¼ MPC. At high dose, male animals were sacrificed at 1, 4, 36, and 46h and female animals were sacrificed at 1, 4, 37, and 50h after administration of BPS, plasma and tissue levels were measured at these time points. At all observation time points after oral administration of 300 mg/kg bw BPS to male and female rats, highest tissue concentrations (mean) were found in the GI tract/ GI tract contents. With exception of the GI tract (including its content), highest and lowest residues (mean) were found in different tissues for males and females respectively 1h after oral administration of BPS.

Table 16: Highest residues of radioactivity (µg Eq/g) in tissues (except GI tract and its content) 1h post dosing of 300 mg/kg bw of BPS

Males	Females
Kidney 100.61	Brain 78.51
Liver 67.88	Plasma 65.22
Plasma 57.39	Liver 64.40
Carcass 49.42	Thyroid 44.63
Lung 33.22	Pancreas 43.33
Skin 30.64	Lung 40.89
	Skin 40.83
	Carcass 39.87

Table 17: Lowest residues of radioactivity (µg Eq/g) in tissues (except GI tract and its content) 1h post dosing of 300 mg/kg bw BPS

Males	Females
Adipose tissue 3.49	Adipose tissue 5.46
Brain 4.30	Bone 5.74
Bone 4.81	Kidney 6.61

In both sexes, radioactive residue concentrations generally declined in organs and tissues parallel to the radioactive residues in plasma with the exception of mean residues in carcass samples in which the concentrations declined until the 4h sampling time point and increased thereafter to the 37 and 38h time point and dropped at the last sampling time point to a value which was still above the 4h sampling time point. Males and females dosed with 30 mg/kg bw of BPS were sacrificed at 1, 4, 18, and 25h and at 1, 4, 17, and 22h time points, respectively. At 1h post dosing highest tissue concentrations (means) were found in the GI tract/GI tract contents in both sexes. With exception of the GI tract (including its content) highest residues (means) of radioactivity was found in liver and kidney of male animals and in liver, kidney and thyroid in female animals. Lowest tissue concentrations (means) of radioactivity 1h post dosing were found in brain, adipose tissue and bone between 0.42 and 0.79 µg Eq/g in male animals and in brain, bone adipose tissue and muscle between 0.18 and 0.57 µg Eq/g in female animals.

Table 18: Highest residues of radioactivity (µg Eq/g) in tissues (except GI tract and its content) 1h post dosing of 30 mg/kg bw of BPS

Males	**Females**
Liver 19.25	Liver 8.76
Kidney 16.17	Kidney 7.12
	Thyroid 5.39

In male and female animals, radioactive residues concentrations generally declined in organs and tissues parallel to the radioactive residues in plasma. There were slight increases in the concentrations in carcass samples of male animals and in liver samples of female animals at the 4h time point. Concentrations in both tissues decreased to the last observation time points.

Metabolism:

Urine, methanol extracts of faeces and bile with a sufficient level of radioactivity were analysed and residues were quantified. Biotransformation steps in the metabolic pathway are, glucuronidation and sulphation of BPS. Glucuronidation of BPS leads to metabolite SES24606, sulphation of BPS leads to metabolite SES24604, and glucuronidation of SES24604 or sulphation of SES24606 leads to metabolite SES24628.BPS was almost completely unmetabolized in faeces, mostly metabolized in urine and almost completely metabolized in bile. Main metabolite in urine was glucuronidated BPS for all dose groups, except for high dosed male animals where BPS was the highest detected compound (24.14%), followed by sulphated BPS (10.38%) and glucuronidated BPS (6.42%) was the lowest detected metabolite. The unchanged parent compound was in all dose groups of both sexes the only or the most abundant component excreted via faeces. In high dosed female animals BPS (44.10%) was the highest compound followed by glucuronidated BPS (1.26%). Repeated dosed animals showed differences in amount of detected BPS in faeces of males (37.99%) and females (40.42%). Main metabolite in bile was glucuronidated BPS of all dose groups. In high dosed animals amount of glucurondiated BPS was higher in females (40.07%) than in males (27.30%). Sulphated BPS was also detected in male animals of high (8.79%) and low dose (16.04%) groups. Further investigation of liver, kidney, and plasma has been performed to assess the radioactive residues in these organs. According to low portion of applied dose detected in these tissues (<5%) no metabolites were identified. Gender specific difference in liver of high dose group was detected, higher portion of radioactivity was detected in males (1.14%) than females (0.57%). In kidney and plasma no dose and gender specific differences have been shown. In total, up to 0.37% and 0.06-0.10% of the dose were characterized in kidney and plasma, respectively.

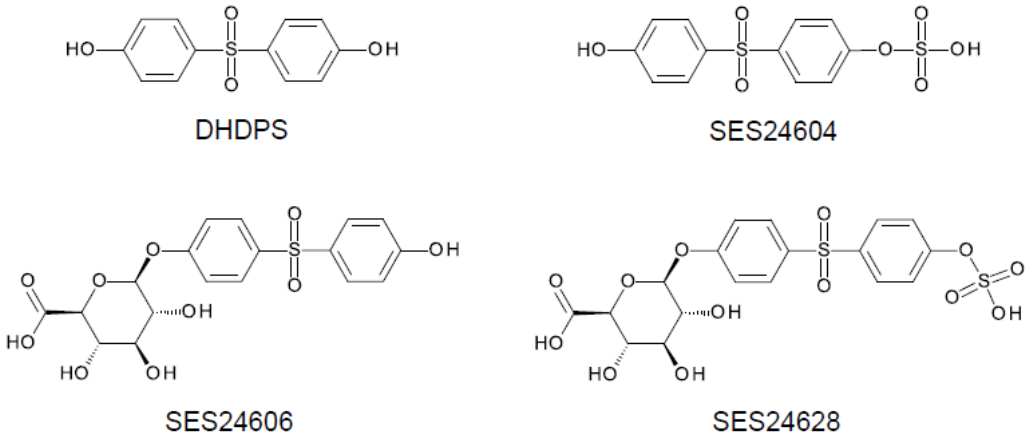


Figure 3: Molecular structures of BPS and its metabolites after glucuronidation and sulphation

Mortality:

One female of the low dose group of bile experiment was found dead in the metabolism cage 48h post dosing. The death of this animal was assessed not to be test substance related.

Necropsy:

During necropsy of two female animals of low dose group and one female of high dose group of bile experiment, suppurations were observed in the abdomen of the bile experiment that were attributed to the surgery procedure and assessed not to be test substance related.

Conclusion on Toxicokinetics:

There was a fast absorption of BPS found with maximum plasma concentrations at 1 and 4 hours. The plasma peak at 4h might indicate a enterohepatic circulation of BPS in rats. The absorbed dose is between 87-100% of the oral dose with terminal half lives in the range of 9.2-14.7h. A sex related difference was found at high test concentration indicated by two times higher plasma concentration in females one hour post dosing. Indications from kinetics saturation point to a potential involvement of active transport mechanism.

The highest concentration of BPS was found in the GI tract, whereas the concentration in the other organs were found to be gender specific. In males and females highest residues of BPS were detected in kidney and brain respectively and no residues were detected in thyroid and pancreas of male animals. Tissue distribution data demonstrated for both sexes a general decline in organs and tissues from 1h post application and was in parallel to the radioactive residues in plasma. Only the radioactive residues in carcass showed delayed clearance, especially in the high dose groups tested, residues in carcass were over proportional to administered dose.

Two main metabolites of BPS were found, namely a glucuronidated BPS and a sulphated BPS. A glucuronidated-sulphated metabolite was only found in bile. In urine extracts of the high dose group, metabolism data showed gender and dose specific differences in the composition. While the amounts of glucuronidated BPS were higher in females, the concentration of parent compound and sulphated BPS were higher in males. Only parent compound was found in faeces of male animals of both dose groups and females of low dose. Glucuronidated BPS was detected in faeces of female rats of the high dose group at a low percentage range. Gender specific difference of the metabolites profile were detected in bile extracts. Glucuronidated-sulphated BPS was only detected in male biles and the

glucuronidated BPS was higher in female animals than in male animals. In plasma, liver and kidney only a low portion of residues of BPS (<5% of dose) were found and therefore no metabolites could be identified in this compartments.

Overall, the balance/excretion data from urine and faeces indicate a major excretion of BPS within 48 hours. The urinary elimination is the predominate route of excretion except for females of the high dose group. While the urine contained unmetabolized parent compound as well as fractions of glucuronidated BPS and sulphated BPS, the faeces mainly consisted of parent compound. Only the excretion of BPS in the low dose group is independent from frequency of treatment and gender.

7.9.2. Acute toxicity and Corrosion / Irritation

7.9.2.1. Acute toxicity

Table 19: Summary of animal studies on acute toxicity

Method, guideline	Species, strain, sex, no / group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute toxicity study Gavage Similar to OECD TG 401 No GLP Rel. 2	Rat / Wistar / male 10 males/group	4,4'-sulphonyldiphenol Vehicle: lutrol	Conc.: 1000, 1500, 2000, 3100, 3500, 4000, 5000, 5500 mg/kg bw Obs. period: 14d	LD50: 2830 mg/kg bw	REACH registration dossier: Unpublished study report, 1978
Acute toxicity study Gavage Similar to OECD TG 401 No GLP Rel. 2	Rat / Tif: RAIf (SPF) 5 males and 5 females	4,4'-sulphonyldiphenol Vehicle: 0.5% CMC and 0.1% polysorbate 80	Conc.: 5000 mg/kg bw Obs. period: 14d	LD50: >5000 mg/kg bw	REACH registration dossier: Unpublished study report, 1984
Acute toxicity study Gavage Similar to OECD TG 401 GLP Rel. 2	Rat / Crj: CD (SD) / both sexes 5/sex/dose	4,4'-sulphonyldiphenol Purity: 99.80% Vehicle: 0.5% methylcellulose	Conc.: 0 and 2000 mg/kg bw Obs. period: 14d	LD50: >2000 mg/kg bw	OECSEH, 1999
Acute toxicity study Oral No guideline followed GLP compliance : unknown	Rat / strain unknown / both sexes	4,4'-sulphonyldiphenol Vehicle: unknown	Conc.: not specified	LD50 (male): >3200 mg/kg bw LD50 (female): 2540 mg/kg bw	REACH registration dossier: Unpublished study report, 1983

Method, guideline	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Rel. 4					
Acute toxicity study Oral No guideline followed GLP compliance : not specified Rel. 4	Mouse / strain unknown / sex unknown	4,4'-sulphonyldiphenol	Conc.: not specified	LD50 (males): 1600 mg/kg bw	REACH registration dossier: Unpublished study report, 1973

In an acute toxicity study (REACH registration dossier: Unpublished study report, 1978), groups of 10 male rats were given a single dose of BPS (conc. : 1000, 1500, 2000, 3100, 3500, 4000, 5000 or 5500 mg/kg bw). Animals were observed during 14 days.

During the observation period, mortality was observed in all dose levels (except in the low dose (1000 mg/kg bw)). Clinical signs were noted in all dose level such as diuresis, salivation, sedation, dyspnoea, lateral and prone position, decreased general condition. Based on the results, the LD50 was of 2830 mg/kg bw.

Table 20: Data on mortality

Dose level (in mg/kg bw)	1000	1500	2000	3100	3500	4000	5000	5500
Mortality	0/10	1/10	2/10	4/10	7/10	9/10	9/10	10/10
Time of death	/	2d	2d	3h – 7d	2d	2d	3h – 2d	3h – 3d

In acute toxicity study (REACH registration dossier: Unpublished study report, 1984), 5 male and 5 female rats were exposed to BPS at a concentration of 5000 mg/kg bw. Animals were observed during 14 days. During the observation period, no animals died. All animals exhibited dyspnea, exophthalmos, ruffled fur and curved body position. Based on the results, the LD50 was higher than 5000 mg/kg bw.

In acute toxicity study (OECSEH, 1999), groups of 5 male and 5 female rats were given by gavage BPS at a concentration of 0 or 2000 mg/kg bw. Animals were observed during 14 days. During the observation period, no dead animals were found. Moreover, animals did not exhibited clinical signs. Based on the results, the LD50 was higher than 2000 mg/kg bw, the highest dose tested.

Other studies with minimal description of methods and results are also available. In acute toxicity study (REACH registration dossier: Unpublished study report, 1983), groups of male and female rats were exposed to BPS (no more information available). Animals exhibited clinical signs (blood in urine, anorexia, ataxia, tremors and prostration). The LD50 was of 2540 mg/kg bw in females and higher than 3200 mg/kg bw in males. In the other acute toxicity study of reliability 4 (REACH registration dossier: Unpublished study report, 1973), groups of mice were exposed to BPS (no more information available). Animals exhibited clinical signs such as blood in urine, ataxia, anorexia, tremors and prostration. The LD50 was of 1600 mg/kg bw in males.

Conclusion on Acute toxicity:

The registrant concluded that the substance is not acutely toxic, and based on the available information, the eMSCA can support this conclusion and considers that there is no concern for acute toxicity.

7.9.2.2. Corrosion/irritation

7.9.2.2.1. Skin

Table 21: Summary of *in vitro* study on corrosion / irritation

Type of study / data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro/ex vivo skin corrosion/irritation study EpiDerm skin corrosivity/irritation test OECD TG 431 GLP	4,4'-sulphonyldiphenol Vehicle: unchanged	Conc.: 0 and 15 mg Duration of exposure: 3min or 1hour	Corrosivity test: Mean viability: 112% after 3min of exposure and 105% after 1 hour Irritation test: Mean viability: 106% after 1 hour of exposure period and 42h of post incubation period	REACH registration dossier: Unpublished study report, 2010

Table 22: Summary of animal studies on corrosion / irritation

Method, guideline	Species, strain, sex, no / group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores / animal -Reversibility	Reference
Acute dermal irritation study Type of coverage: occlusive OECD TG 404 No GLP Rel. 1	Rabbit / NZW / male 3 males/group	4,4'-sulphonyldiphenol Vehicle: /	Conc.: 0.5g Exposure period: 4h	erythema and oedema scores of 0 in all animals at all obs. time	REACH registration dossier: Unpublished study report, 1984
Skin irritation test Type of coverage: occlusive Similar to OECD TG 404 No GLP Rel. 2	Rabbit / strain unknown / sexes unknown 3 animals	4,4'-sulphonyldiphenol	Conc.: unknown Duration of exposure: 24h	erythema and oedema scores of 0 in all animals at all obs. time	REACH registration dossier: Unpublished study report, 1961
Skin irritation test Type of coverage: occlusive GLP compliance:	Guinea pig / strain unknown 5 animals	4,4'-sulphonyldiphenol Vehicle: water	Conc.: unknown Duration of exposure: 24h	Slight skin irritation signs observed	REACH registration dossier: Unpublished study report,

Method, guideline	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
unknown Rel. 4					1983
Skin irritation study Type of coverage: semi-occlusive No GLP Rel. 4	Rabbit / NZW / both sexes 2 animals	4,4'-sulphonyldiphenol Vehicle: /	Conc.: 500 mg Duration of exposure: 24h	No irritation signs observed	REACH registration dossier: Unpublished study report, 1979

An *in vitro/ex vivo* skin irritation study was performed following OECD TG 431 (REACH registration dossier: Unpublished study report, 2010). Tissues were exposed to BPS at a concentration of 0 or 15 mg. 2 parts were performed (irritation and corrosion). To analyse the corrosive properties, tissues were exposed to the test substance during 3 min or 1 hour. Whereas to analyse the irritation properties, tissues were exposed to the test-substance during 1 hour and were evaluated after un post-incubation period of 42 hours.

Concerning the corrosion test, the mean viability of the treated tissues determined after an exposure period of 3 minutes was of 112%. After an exposure period of 1 hour, the mean viability was of 105%. Regarding the irritation test, the mean viability of the test-substance treated tissues was of 106%.

In an acute dermal irritation study (REACH registration dossier: Unpublished study report, 1984), 3 male rabbits were exposed to BPS at a concentration of 0.5g during 4 hours. During the observation period, no signs of irritation was observed. The erythema and edema score were of 0 at all observation times (1, 24, 48 and 72h).

In a skin irritation test (REACH registration dossier: Unpublished study report, 1961), 3 rabbits were exposed to BPS during 24 hours (no more information available). During the observation period, no signs of irritation was observed. The erythema and edema scores were of 0 at all observation times (24, 48 and 72h).

Other studies with minimal description of methods and results are available. In a skin irritation study (REACH registration dossier: Unpublished study report, 1983), 5 guinea pigs were exposed to BPS during 24 hours (no more information available). All animals exhibited slight skin irritation signs. In another skin irritation study (REACH registration dossier: Unpublished study report, 1979), 2 rabbits were exposed to 500 mg of BPS during 24 hours (no more information). No signs of irritation were noted.

Conclusion on Skin corrosion/irritation:

The registrant concluded that the substance is not irritating to skin, and based on the available information, the eMSCA can support this conclusion and considers that there is no concern for skin irritation.

7.9.2.2.2. Eye

Table 23: Summary of animal studies on eye irritation

Method, guideline	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference

Eye irritation study Similar to OECD TG 405 No GLP Rel. 2	Rabbit / NZW / female 3 females	4,4'-sulphonyldiphenol	Conc.: 0.1g Single application	Mean score: 0.33 for corneal opacity, 0 for iritis, 1.55 for redness and 1.33 for chemosis	REACH registration dossier: Unpublished study report, 1984
Eye irritation study Similar to OECD TG 405 No GLP Rel. 2	Rabbit / strain unknown / sex unknown 2 animals	4,4'-sulphonyldiphenol	Conc.: 50 mg Single application	Slight irritation signs mean score of: 0/4 for corneal opacity, 0/2 for iris score, 0.17/3 for conjunctivae and 0/4 for chemosis	REACH registration dossier: Unpublished study report, 1979
Eye irritation study No guideline followed No GLP Rel. 3	Rabbit / strain unknown / sex unknown 3 animals	4,4'-sulphonyldiphenol Vehicle: /	Conc.: unknown Single application	Mild discomfort after application, slight lachrymation, mild to moderate redness of the conjunctivae, very mild oedema, trace of dullness developed in 1h Recovery within 72h	REACH registration dossier: Unpublished study report, 1961
Eye irritation study No guideline followed No GLP Rel. 4	Rabbit / strain unknown / sex unknown 6 animals	4,4'-sulphonyldiphenol Vehicle: /	Conc.: unknown Duration of exposure : single application Eyes of 3 rabbits were immediately washed and eyes of 3 animals were kept unwashed	Slight irritation Recovery within 48h	REACH registration dossier: Unpublished study report, 1973

In an eye irritation study (REACH registration dossier: Unpublished study report, 1984), performed following OECD TG 404, 3 female rabbits were exposed to 0.1g of BPS. Slight to moderate reactions were observed (see table 25). All effects were fully reversible within 72 hours.

Table 24: Mean eye irritation scores (obs. time: 24, 48 and 72h)

	Corneal opacity	Iris	Conjunctivae	Chemosis
Rabbit 1	0/4	0/2	1.33/3	1.33/4
Rabbit 2	0/4	0/2	1.33/3	1.33/4
Rabbit 3	1/4	0/2	2/3	1.33/4
Mean	0.33/4	0/2	1.55/3	1.33/4

In another eye irritation study (REACH registration dossier: Unpublished study report, 1979), 2 rabbits were given a single application of BPS at a concentration of 50 mg. The observation revealed only slight signs of irritation. The mean score (observation time : 24,

48 and 72h) was of 0/4 for corneal opacity, 0/2 for iris score, 0.17/3 for conjunctivae and 0/4 for chemosis.

Other studies with minimal description of methods and results are available. In an eye irritation study (REACH registration dossier: Unpublished study report, 1961), 3 rabbits were given BPS. After application, mild discomfort was observed such as slight lachrymation, mild to moderate redness of the conjunctivae, very mild edema and trace of dullness developed in 1h. Animals recovered within 72h (mean total irritation score 12.3, 12.3, 5.3, 3.3, 2.0 and 0.0/110 respectively after 1, 4, 24, 48, 72 and 120 hours). In another eye irritation study (REACH registration dossier: Unpublished study report, 1973), 6 rabbits were exposed to BPS. Eyes of 3 animals were immediately washed and eyes of the 3 remaining animals were kept unwashed. Rabbits exhibited signs of slight irritation and recovered within 48h (no more information available).

Conclusion on Eye irritation:

The registrant concluded that the substance is not irritating to eye, and based on the available information, the eMSCA can support this conclusion and considers that there is no concern for eye irritation.

7.9.3. Sensitisation

Table 25: Summary of animal studies on skin sensitisation

Method, guideline	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
LLNA OECD TG 429 GLP Rel. 1	Mouse / CBA / female 5 female/group	4,4'-sulphonyldiphenol Vehicle: methyl ethyl ketone	Conc.: 5, 10 and 25 %	Mean DPM: 661.1 ± 240.0, 904.1 ± 205.0 and 799.3 ± 560.4 respectively at 5, 10 and 25% (Positive control : 5918.7 ± 2521.5) SI: 1.39, 1.90 and 1.68 respectively at 5, 10 and 25% (positive control : 6.49)	REACH registration dossier: Unpublished study report, 2010
GPMT OECD TG 406 No GLP Rel. 2	Guinea pig / Pirbright White / both sexes 10/sex/group	4,4'-sulphonyldiphenol Vehicle: Vaseline	Induction: ID and epicutaneous, 0.4g of 30% test substance Challenge: epicutaneous, occlusive, 0.2g of 10% test substance	Nb of positive reactions after challenge: 2/20 and 3/20 respectively after 24h and 48h Nb of positive reactions after rechallenge : 4/20 and 3/20 respectively after 24 and 48h	REACH registration dossier: Unpublished study report, 1988
GPMT No guideline followed No GLP Rel. 4	Guinea pig / strain unknown / sex unknown 10 animals/group	4,4'-sulphonyldiphenol	Induction: no data available Challenge: no data available	No positive reactions	REACH registration dossier: Unpublished study report, 1973

In a Local Lymph Node Assay (LLNA, REACH registration dossier: Unpublished study report, 2010), performed following OECD TG 429, groups of 5 female mice were exposed by topical application to BPS at a concentration of 5, 10 or 25%. Animals did not exhibited any signs of systemic toxicity or any signs of skin irritation during the study. The mean DPM were of

661.1, 904.1 and 799.3 respectively at 5, 10 and 25 % (vs 5918.7 in the positive control group). The SI were of 1.39, 1.90 and 1.68 respectively at 5, 10 and 25 %.

In a Guinea Pig Maximisation Test (GPMT, REACH registration dossier: Unpublished study report, 1988), performed following OECD TG 406, groups of 10 male and 10 female guinea pigs were exposed to BPS. The induction was performed in 2 steps (intradermal and epicutaneous, 0.4g of 30% test substance). Two weeks after the induction, animals were tested with 10 % of the test item (epicutaneous, occlusive). Five and four animals of the tested group reacted positively respectively 24 and 48h after removing of the challenge application. Due to the slight reactions, a second challenge application was performed after 10 days. Four and three animals reacted positively respectively after 24 and 48h.

In another GPMT with minimal description of methods and results (REACH registration dossier: Unpublished study report, 1973), groups of 10 guinea pigs did not exhibited positive reactions.

Conclusion on skin sensitisation:

The registrant concluded that the substance is not sensitising, and based on the available information, the eMSCA can support this conclusion and considers that there is no concern for skin sensitisation.

7.9.4. Repeated dose toxicity

Table 26: Summary of animal studies on repeated dose toxicity

Method, guideline, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Subacute repeated dose toxicity study including 2-weeks observation of reversibility Rat / Crj:CD (SD) 6/sex/group (for main group) + 6/sex/group (for recovery group) Similar to OECD TG 407 GLP Rel. 2	4,4'-sulphonyldiphenol (purity: 99.80 %) Vehicle: 0.5 % aqueous solution of methylcellulose Gavage Conc.: 0, 40, 200 and 1000 mg/kg bw/d for main groups 0, 200 and 1000 mg/kg bw/d for recovery groups Duration of exposure: 28d, daily Observation period: 2w for recovery groups	Mortality: 2 males exposed to the highest dose (dilatation of cecum and signs of intestine haemorrhage at necropsy) Clinical signs: 1000 mg/kg bw/d : abdominal distension in 1 female after 15 days and in 5 females after 28 days (this effect disappeared during the recovery period) BW: lower value at 1000 mg/kg bw/d during the dosing period (sign in males). Recovered at the end of the recovery period BWG: sign. lower in both sexes for the exposure period and sign. reduce in males for the recovery period Animals necropsied at the end of dosing period: Gross pathology findings: 1000 mg/kg bw/d: dilatation of	REACH registration dossier: Unpublished study report, 1999

		cecum in all animals Organ weight: few modification (see table 31) Histopathological findings observed in cecum (hyperplasia of mucosa, single cell necrosis of mucosal epith.), liver (hypertrophy centrilobular), adrenals (hypertrophy of zona fasciculate), femur (increase spongy bone) and thymus (atrophy) <u>Animals necropsied at the end of recovery period:</u> Gross pathology findings : dark red spots in the glandular stomach in 2 females of the mid dose and in 2 females of the high dose. Organ weight: few modifications Histopathological findings observed in cecum (hyperplasia of mucosa and single cell necrosis of mucosal epith.), liver (microgranuloma) and femur (increase spongy bone)	
Sub chronic repeated dose toxicity study Rat / Crl: WI(HAN) / males + females 10/sex/dose Following OECD TG 408 GLP Rel. 1	4,4'-sulphonyldiphenol (purity: 99.4 %) Vehicle: 1 % CMC Gavage Conc.: 0, 100, 300 and 1000 mg/kg bw/d (in males the highest dose was changed to 600 mg/kg bw/d after 70D) Duration of exposure: 90 days	Mortality: no animals died Clinical signs: soft and discoloured faeces and salivation in all animals exposed to the mid and high doses. Bw: significant increase in males at the highest dose. The bwg (D0 – 91) was significantly lower in males in the mid and high dose level. Gross necropsy findings: dilatation of cecum in all males of the high dose Enlargement of liver in 8 females at the high dose level Organ weight: sign. changes observed in both sexes (see table 36) Sign. lower male reproductive organ	REACH registration dossier: Unpublished study report, 2014

		weight (testes and epididymides) Histopathology: few changes (see table 37) Dilatation of cecum in all males and females at the highest dose + increase incidence of apoptosis Mammary glands: in males: increase incidence of multifocal atrophy at the mid and high dose Uterus: increase incidence of squamous metaplasia	
13-day repeated dose toxicity study Rat / strain not specified / male 5 males/group No guideline followed No GLP Rel. 4	4,4'-sulphonyldiphenol (purity not specified) Vehicle: 1 % corn oil Diet Doses: 0, 0.1 and 1 % (± 0, 97 and 810 mg/kg bw/d) Duration of exposure: 13D	Bw: decreased at the highest dose (no more information available) Organ weight: lower kidneys and liver weight at 1 % Histopathology: adipose tissue atrophy and cytoplasmic basophilia of epith. of the renal distal convoluted tubule at 1%	REACH registration dossier: Unpublished study report, 1973

In a 28-day repeated dose toxicity including 2-week observation of reversibility (REACH registration dossier: Unpublished study report, 1999), performed similarly to OECD TG 407, main groups of 6 male and 6 female rats were given diets containing BPS at a concentration of 0, 40, 200 and 1000 mg/kg bw/d for 28 days. Additionally of these main groups, recovery groups of 6 male and 6 female rats were given diets containing BPS at a concentration of 0, 200 and 1000 mg/kg bw/d for 28 days and thereafter were observed during 2w. During the dosing period, 2 males exposed to the highest dose died (1 after 13D and 1 after 21D). The clinical sign examination revealed an abdominal distension at the highest dose group in 1 female after 15 days of exposure and in 5 females after 28 days of exposure. During the recovery period, this effect disappeared. Significant lower body weight value was recorded at the highest dose level in males during the dosing period. This significant reduce disappeared during the recovery period. In females, only a slight decrease was observed. However, the body weight gain (D1 – D28) was significantly reduced at the highest dose in both sexes. (See table 28)

Table 27: Body weight data during dosing and recovery period (in g)

	Males				Females			
Dose level (mg/kg bw/d)	0	40	200	1000	0	40	200	1000
Dosing period								
D1	217	214	215	215	165	165	166	168
D14	330	324	325	281**	216	216	210	206
D28	409	402	401	337**	258	256	244	240
BWG (1 – 28)	192	187	186	122**	93	91	78*	72**
Recovery period								
D1	398	/	411	330**	258	/	250	242
D14	457	/	465	416	286	/	278	269

BWG (1 – 14)	59	/	54	86**	28	/	58	27

*: p < 0.05 ; ** : p < 0.01

Examination of the haematological and clinical chemistry parameters revealed significant changes (see table 29 and table 30). Significant decrease of Red Blood Cell (RBC), haemoglobin and haematocrit were observed in both sexes at the highest dose level and a significant reduced prothrombine time was noted in females. Furthermore, in males, a significant higher Alkaline Phosphatase (ALP) activity and a significant lower Lactate Dehydrogenase (LDH) activity were noted at the highest dose level. Whereas, in females, a significant increase of total protein, albumin and calcium and a significant decrease of total cholesterol were observed.

Table 28: Haematological findings during dosing and recovery period

		Males				Females			
Dose level (mg/kg bw/d)		0	40	200	1000	0	40	200	1000
Dosing period	RBC (10^4/mm³)	770	764	763	687*	773	766	776	705**
	Hb (g/dL)	15.9	15.9	15.8	14.3**	16.2	15.9	15.9	13.9**
	Ht (%)	47	47	46	42**	47	47	47	42**
	MCHC (%)	34.1	33.9	34.1	33.7	34.5	34.0*	33.9*	33.6*
	PT (sec)	12.9	13.4	13.6	12.9	12.0	12.2	12.0	11.4*
Recovery period	RBC (10^4/mm³)	804	/	793	735**	809	/	801	762
	Hb (g/dL)	16.0		15.9	15.3	16.2		15.8	15.2**
	Ht (%)	47		47	45	47		47	45
	MCHC (%)	33.8		33.9	33.8	34.1		33.3*	33.4
	PT (sec)	13.5		13.2	11.8**	11.9		11.6	11.7

* : p<0.05 ; ** : p<0.01

Table 29: Blood chemical findings during dosing and recovery period

		Males				Females			
Dose level (mg/kg bw/d)		0	40	200	1000	0	40	200	1000
Dosing period	GOT (IU/L)	44	47	55	56	64	59	57	52
	LDH (IU/L)	43	40	39	25**	27	25	27	28
	ALP (IU/L)	307	298	289	424*	205	209	197	222
	Tot. prot. (g/dL)	6.3	6.1	6.1	6.4	6.2	6.2	6.3	7.2**
	Albumin (g/dL)	3.7	3.7	3.6	3.8	3.7	3.8	3.8	4.2**
	Tot. chol (mg/dL)	65	61	64	25**	85	64	71	42**
	Ca (mg/dL)	9.4	9.3	9.4	9.7	9.5	9.4	9.5	10.1**
Recovery period	GOT (IU/L)	43	/	43	24*	60	/	57	55
	LDH (IU/L)	53		40	55	23		26	23
	ALP (IU/L)	249		260	271	152		144	142
	Tot. prot. (g/dL)	6.4		6.4	6.1	6.8		6.5	6.7
	Albumin (g/dL)	3.7		3.8	3.7	4.0		3.9	4.0
	Tot. chol. (mg/dL)	72		73	69	86		75	100
	Ca (mg/dL)	9.1		9.1	9.1	9.4		9.2	9.3

* : p<0.05 ; ** : p<0.01

Concerning the gross pathology observation, in cases necropsied at the end of the dosing period, a dilatation of cecum was observed in all animals of the highest dose level (5 out of 5 males and 6 out of 6 females at 1000 mg/kg bw/d. This change is not observed in the other dose level groups and in the control group. Whereas, in cases necropsied at the end of the recovery period, dark red spots in the glandular stomach were observed in 2 females of the mid dose level and 2 females of the high dose level. No abnormalities were seen in males necropsied at the end of the recovery period.

Regarding the organ weight examination, animals necropsied at the end of dosing period showed, at 1000 mg/kg bw/d, significant changes of absolute thymus and lung weights in both sexes and significant changes of absolute heart and adrenal glands weights in males (see table 31). While, animals necropsied at the end of recovery period exhibited, at 1000 mg/kg bw/d, significant higher absolute adrenal glands weight in males (59, 60 and 80** mg, resp. at 0, 200 and 1000 mg/kg bw/d), significant higher relative kidneys weight in males and females (0.70/0.65, 0.73/0.75** and 0.81**/0.72* mg%, resp. in females/males at 0, 200 and 1000 mg/kg bw/d), significant higher relative liver weight in females (2.79, 2.82 and 3.21* g%, resp. at 0, 200 and 1000 mg/kg bw/d).

Table 30: Absolute and relative organ weights data of animals necropsied at the end of dosing period

		Males				Females			
Dose level (mg/kg bw/d)		0	40	200	1000	0	40	200	1000
Nb of animals examined		6	6	6	6	6	6	6	6
FBW (g)		389	369	364	311**	234	235	223	218
Adrenals	Abs.(mg)	70	71	66	101**	72	74	65	74
	Rela. (mg%)	18	19	18	33**	31	32	29	34
Brain	Abs. (g)	2.07	2.06	2.07	1.99	1.90	1.89	1.86	1.82
	Rela. (g%)	0.53	0.56	0.57	0.64**	0.81	0.80	0.84	0.84
Heart	Abs. (g)	1.30	1.18	1.21	0.99**	0.84	0.87	0.79	0.81
	Rela. (g%)	0.34	0.32	0.33	0.32	0.36	0.37	0.36	0.37
Kidneys	Abs. (g)	2.79	2.68	3.09	2.76	1.84	1.76	1.73	1.83
	Rela. (g%)	0.72	0.73	0.85**	0.89**	0.79	0.75	0.77	0.84
Liver	Abs. (g)	11.98	11.35	11.14	10.94	7.23	6.83	6.99	8.46
	Rela. (g%)	3.07	3.07	3.06	3.54**	3.09	2.90	3.14	3.89**
Lung	Abs. (g)	1.33	1.28	1.33	1.13*	1.09	1.06	1.04	0.92**
	Rela. (g%)	0.34	0.35	0.37	0.36	0.47	0.45	0.47	0.42**
Thymus	Abs. (mg)	438	428	493	252**	475	521	441	259**
	Rela. (mg%)	113	117	135	82	203	221	199	119**
Testes	Abs. (g)	3.07	2.94	3.06	2.96	/	/	/	/
	Rela. (g%)	0.79	0.81	0.84	0.96**	/	/	/	/
Ovaries	Abs. (mg)	/	/	/	/	86.1	92.0	85.1	76.5
	Rela. (mg%)	/	/	/	/	36.7	38.9	38.1	34.9

* : $p < 0.05$; ** : $p < 0.01$

Weight (absolute and/or relative) of adrenals, liver and thymus were modified. These organs were also affected at the histological level. Furthermore, cecum examination revealed a hyperplasia of the mucosa and single cell necrosis of the mucosal epithelium at 200 and 1000 mg/kg bw/d. Whereas, the histopathological examination of animals necropsied at the end of the recovery period revealed significant changes in femur (increase spongy bone in 4* females at 1000 mg/kg bw/d), in spleen (significant increase of extramedullary haematopoiesis). Moreover, hyperplasia of cecum mucosa was observed in 3 males and in 1 female at the highest dose level (vs in 0 males and in 0 females in control group). (see table 32)

Table 31: Histopathological findings in animals necropsied at the end of the dosing period

			Males				Females			
Dose level			0	40	200	1000	0	40	200	1000
Main groups										
Cecum	Hyperplasia mucosa	P	0/6	0/6	6*/6	5*/5	0/6	0/6	5**/6	6**/6
		Slight			2	2			4	5
		Mild			4	3			1	1
		P	0/6	0/6	4*/6	5*/5	0/6	0/6	4*/6	4*/6
		Slight			3	2			4	1

	Single cell necrosis, mucosal epith.	Mild			1	3				2
		Moderate								1
liver	Hypertrophy centrilobular	P	0/6	0/6	0/6	3*/5	0/6	0/6	0/6	3/6
		Slight				3				3
adrenals	Hypertrophy, zona fasciculata	P	0/6	0/6	0/6	4*/5	0/6	0/6	0/6	0/6
		Slight				4				
thymus	Atrophy	P	0/6	0/6	0/6	4**/5	0/6	0/6	0/6	4*/6
		Slight				4				4
Femur	Increase spongy bone	P	0/6	0/6	0/6	5**/5	0/6	0/6	0/6	4*/6
		Slight				5				4
Spleen	Haematopoiesis, extramedullary	P	6/6	6/6	6/6	5/5	0/6	0/6	6/6	6/6
		Slight	6	5	5	2			5	4
		Mild		1	1	2			1	2
		Moderate				1				
Recovery groups										
cecum	Hyperplasia, mucosa	P	0/6	/	1/6	3/5	0/6	/	1/6	1/6
		Slight				1				1
		Mild			1	2			1	
Femur	Increase spongy bone	P	0/6	/	0/6	1/5	0/6	/	0/6	4*/6
		Slight				1				4
Spleen	Haematopoiesis extramedullary	P	6/6	/	6/6	5**/5	6/6	/	6/6	6*/6
		Slight	3		2		5		5	1
		Mild	3		4		1		1	3
		Moderate				5				2

* : p < 0.05 ; ** : p < 0.01 ; P : present

In a 90-day repeated dose toxicity study similar to OECD TG 408 (REACH registration dossier: Unpublished study report, 2014), groups of 10 male and 10 female rats were exposed via gavage to BPS at a concentration of 0, 100, 300 or 1000 mg/kg bw/d. The group of male rats receiving 1000 mg/kg bw/d was changed to 600 mg/kg bw/d onwards 70 days. The animals of the 2 highest dose presented soft and discoloured faeces and excessive salivation. The body weight decreased in males at the mid dose level and was significantly lower in males at the highest dose. Furthermore, the body weight gain reduced significantly in males at the 2 highest dose levels. (see table 33)

Table 32: Body weight and body weight gain examination (in g)

	Males				Females			
Dose level (mg/kg bw/d)	0	100	300	1000/600	0	100	300	1000
D0	158.4	157.1	158.1	158.2	126.1	127.0	126.0	126.7
D42	351.4	343.8	326.0	293.3**	208.4	204.7	202.8	205.2
D91	417.1	400.7	377.3	334.7**	237.3	231.7	225.0	222.5
BWG (D 0 - 91)	258.7	243.6	219.2*	176.4**	111.2	104.6	99.0	95.9

* : p < 0.05 ; ** : p < 0.01

Blood examination revealed significant haematological changes and significant modification of enzymes. RBC and haemoglobin were significantly lower at the highest dose in both sexes. Other changes were observed however not in both sexes. (see table 34 and table 35)

Table 33: Haematological findings (examined at the end of the administration period)

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000/600	0	100	300	1000
RBC (tera/L)	8.71	8.83	8.46	8.08**	7.89	7.82	7.76	7.45**
Hb (mmol/L)	9.0	9.0	8.8	8.6**	8.8	8.6	8.5	8.0**
Ht (L/L)	0.427	0.426	0.420	0.412	0.408	0.406	0.402	0.380**
MCV (fL)	49.1	48.2	49.6	51.0**	51.8	52.0	51.8	51.0
MCHC (mmol/L)	21.05	21.17	20.97	20.95	21.62	21.28	21.24*	21.07**
RET (%)	1.5	1.2	1.6	1.9*	1.8	2.0	2.2	2.3
WBC (giga/L)	5.51	5.11	4.59*	4.28**	4.09	4.35	3.85	3.56

* : p < 0.05 ; ** : p < 0.01

Table 34: Enzyme data (examined at the end of the administration period)

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000/600	0	100	300	1000
ALT (µkat/l)	0.68	0.80	0.91**	0.92	0.58	0.63	0.58	0.79
AST (µkat/l)	1.63	1.42	1.77	1.81	1.38	1.54	1.36	1.19
ALP (µkat/l)	1.25	1.43	1.40	1.41	0.66	0.55	0.69	1.01*
GGT_C (nkat/l)	0	0	0	0	0	0	0	0
Chol (mmol/L)	1.85	1.65	1.23**	1.03**	1.62	1.56	1.30	1.33
Trig (mmol/L)	0.97	1.53**	1.48**	2.32**	0.72	0.81	0.79	0.99

* : p < 0.05 ; ** : p < 0.01

At necropsy, a dilatation of cecum was observed in all males exposed to 1000 mg/kg bw/d of BPS. While the liver was enlarged in 8 females out of 10 at this dose level. An uterus dilatation was also observed in 3 females at the mid dose level. After this external examination, the organ weight was recorded and revealed a significant reduce of the male reproductive organs. Moreover, significant lower brain and thymus weights were observed in both sexes at the highest dose whereas a higher adrenal glands weight was noted. (see table 36)

Table 35: Organ weight (relative weight in %)

		Males				Females			
Dose level (mg/kg bw/d)		0	100	300	1000/600	0	100	300	1000
FBW (g)		394.02	376.95	356.6**	311.89**	221.72	214.72	207.95	205.6
Adrenal glands (g)	Abs	64.5	59.1	63.7	90.1**	65.6	64.5	74.6	80.4**
	Rela	0.016	0.016	0.018	0.029**	0.03	0.03	0.036*	0.039**
Brain (g)	Abs	2.212	2.098**	2.074**	2.084**	2.007	1.992	1.99	1.913*
	Rela	0.565	0.564	0.583	0.675**	0.91	0.931	0.962	0.932
Heart (g)	Abs	1.115	1.039	1.026*	0.958**	0.752	0.739	0.755	0.763
	Rela	0.284	0.277	0.288	0.309*	0.341	0.344	0.364	0.371*
Kidneys (g)	Abs	2.507	2.646	2.762	2.485	1.5	1.489	1.584	1.644*
	Rela	0.636	0.702*	0.775**	0.795**	0.679	0.695	0.765*	0.799**
Liver (g)	Abs	8.936	8.402	8.415	8.347	5.106	5.39	5.688	7.043**
	Rela	2.269	2.226	2.359	2.676**	2.297	2.502	2.75**	3.433**
Spleen (g)	Abs	0.628	0.585	0.535**	0.595	0.44	0.447	0.465	0.454
	Rela	0.16	0.156	0.15	0.19**	0.198	0.209	0.224**	0.22*
Thymus (mg)	Abs	327.5	269.4	271.3	226.1**	303.2	292.4	245.3	222.7**
	Rela	0.084	0.071	0.076	0.073	0.136	0.136	0.118	0.108*
Epididymides (g)	Abs	1.209	1.16	1.126	1.072**	/	/	/	/
	Rela	0.308	0.31	0.316	0.346**	/	/	/	/
Testes (g)	Abs	3.914	3.862	3.636*	3.592*	/	/	/	/
	Rela	0.999	1.035	1.021	1.162**	/	/	/	/

Ovaries (mg)	Abs	/	/	/	/	104.7	104.0	106.9	126.9
	Rela	/	/	/	/	0.047	0.048	0.052	0.061*
Uterus (g)	Abs	/	/	/	/	0.724	0.864	1.284	0.648
	Rela	/	/	/	/	0.332	0.41	0.615	0.315

* : p < 0.05 ; ** : p < 0.01

Concerning the histopathological examination, changes were observed in few organs such as cecum, kidneys, liver, mammary gland, spleen and uterus. In spleen, an extramedullary haematopoiesis was observed in both sexes at 1000 mg/kg bw/d. Furthermore, changes in cecum were observed particularly in the highest dose level in both sexes. Squamous metaplasia in uterus was observed in all tested dose level. Moreover in females, modification in liver (centrilobular hypertrophy and cellular alteration) were observed in all tested dose levels. While in males, an atrophy multifocal observed in mammary was noted at the 2 highest dose levels and a hypertrophy/hyperplasia of the adrenal cortex was noted at 1000 mg/kg bw/d.

Table 36: Incidence of histopathological findings

		Grade	Males				Females			
Dose level (mg/kg bw/d)			0	100	300	1000/600	0	100	300	1000
Adrenal cortex	Hypertrophy/ hyperplasia	P	0/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10
Cecum	Dilatation	P	0/10	0/10	0/10	10/10	0/10	0/10	1/10	10/10
	Parasite(s) in lumen	P	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	Increased apoptosis (all of grade 1)	P	0/10	3/10	4/10	7/10	0/10	1/10	4/10	7/10
Kidneys	Mineralisation, medulla	P	0/10	7/10	9/10	6/10	5/10	NE	NE	3/10
		1		4	6	6				
		2		3	2					
		3			1					
	Tubules, basophilic	P	8/10	8/10	9/10	8/10	2/10	NE	NE	3/10
Liver	Centrilobular hypertrophy	P	0/10	0/10	0/10	0/10	0/10	2/10	5/10	10/10
		1						1	1	0
		2						1	3	0
		3							1	10
	Hyperplasia, bile duct	P	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
	Cellular alteration	P	0/10	1/10	0/10	2/10	1/10	1/10	1/10	6/10
Mammary gland	Atrophy multifocal	P	0/10	0/10	7/10	10/10	0/10	NE	NE	0/10
		1			7	0				
		2				4				
		3				2				
		4				3				
		5				1				
Spleen	Haematopoiesis extramedullary	P	0/10	0/10	0/10	8/10	2/10	1/10	4/10	10/10
		1				5	2	1	4	3
		2				3				7
Uterus	Squamous metaplasia	P					0/10	2/10	2/10	5/10
		1						2	2	4
		2								1
	Dilatation of horn(s)	P					0/10	0/10	3/10	0/10

P : present ; grade 1 : minimal ; grade 2 : slight ; grade 3 : moderate ; grade 4 : marked (severe) ; grade 5 : massive (extreme)

In a 13-day repeated dose toxicity study which not followed an OECD guideline (REACH registration dossier: Unpublished study report, 1973), groups of 5 male rats were given diets containing BPS at a concentration of 0, 0.1 or 1% (corresponding to 0, 97 and 810 mg/kg bw/d). A lower body weight was noted at the highest dose level (no more information available). Blood examination revealed a slight increase in RBC count, haemoglobin concentration and haematocrit and a lower aspartate aminotransferase value at the highest dose. Moreover, slight increase in haemoglobin concentration was already observed at the low dose level. (no more information available). At necropsy, an adipose

tissue atrophy was observed in 1 male at 0.1% and in all males at 1%. The organ weight recording revealed a lower absolute liver and kidneys weights at the highest dose level. The adipose tissue atrophy was confirmed at the histopathological examination. Moreover, a cytoplasmatic basophilia of epithelium of the renal distal convoluted tubule was noted at 1%. (no more information available).

Conclusion on Repeated dose toxicity:

The registrant concluded that the substance is not toxic after repeated dose exposure as effects occurred well above the guidance values for specific target organ toxicity-repeated dose. Based on the available information, the eMSCA can support this conclusion and considers that there is no concern for repeated dose.

7.9.5. Mutagenicity

Table 37: Summary of *in vitro* studies on mutagenicity

Method, guideline	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro gene mutation assay in bacteria With and without met. act. OECD TG 471 GLP Rel. 2	4,4'-sulphonyldiphenol Vehicle: DMSO	*S. Typh*. TA98, TA100, TA1535, TA1537 and TA1538 Conc.: 0.32, 1.6, 8, 40, 200 and 1000 µg/plate for experiment I and 1.6, 8, 40, 200, 1000 and 5000 µg/plate for experiment II	Genotoxicity: negative Cytotoxicity: yes (at 1000 and 5000 µg/plate)	REACH registration dossier: Unpublished study report, 1989
In vitro gene mutation assay in bacteria With and without met. act. OECD TG 471 No GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	*S. typh.* TA98, TA100, TA1535, TA1537 and TA1538 Conc. : 20, 80, 320, 1280 and 5120 µg/ml	Genotoxicity: negative Cytotoxicity: yes	REACH registration dossier: Unpublished study report, 1987
In vitro gene mutation assay in bacteria With and without met. act. OECD TG 471 GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	*S. typh.* TA98, TA100, TA1535 and TA1537 *E. Coli* WP2uvrA Conc.: 20, 39, 78, 156, 313, 625, 1250, 2500 and 5000µg/plate	Genotoxicity: negative Cytotoxicity: yes (at 2500 and 5000 µg/plate)	REACH registration dossier: Unpublished study report, 1996

Method, guideline	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro gene mutation assay in bacteria With and without met. act. OECD TG 471 GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	*S. typh.* TA98, TA100, TA1535 and TA1537 *E. Coli* WP2uvrA Conc.: 78.1, 156, 313, 625, 1250, 2500 and 5000 µg/plate for S. typh. and 156, 313, 625, 1250, 2500 and 500 for E. coli	Genotoxicity: negative Cytotoxicity: yes (for *S. typh.* at 5000 µg/plate)	OECSEH, 1999
In vitro gene mutation assay in bacteria With and without met. act. OECD TG 471 GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	Histidine operon *S. typh.* TA98, TA100, TA1535 and TA1537 Conc.: 8, 40, 200, 1000 and 5000 µg/plate for Experiment I and 30, 60, 120, 240, 480 and 960 µg/plate for experiment II	Genotoxicity: negative Cytotoxicity: yes (≥120µg/plate)	REACH registration dossier: Unpublished study report, 1991
In vitro gene mutation assay in bacteria With and without met. act. No information on guideline and GLP compliance Rel. 3	4,4'-sulfonyldiphenol Vehicle: unknown	Histidine operon *S. typh*. TA98, TA100, TA1535, TA1537 and TA1538 Conc.: 0.1 to 3333µg/plate	Genotoxicity: negative Cytotoxicity: not specified	Seifried *et al.*, 2006
In vitro chromosome aberration test in mammalian cells OECD TG 473 GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	Chinese hamster ovary Conc.: 125, 250, 500, 750 and 1000 µg/ml for main assay and 300, 400, 500, 600 and 700 µg/ml for confirmation assay	With met. Act.: Genotoxicity: negative Cytotoxicity: yes (at 750 and 1000 µg/ml) Without met. Act.: Genotoxicity: positive Cytotoxicity: yes (at 750 and 1000 µg/ml)	REACH registration dossier: Unpublished study report, 1991

Method, guideline	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro chromosome aberration test in mammalian cells With and without met. act. OECD TG 473 GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	Chinese hamster lung cells Conc.: 0.05 to 0.4 mg/ml	Genotoxicity: ambiguous Cytotoxicity: yes (at 0.4 mg/ml)	OECSEH, 1999
In vitro gene mutation test in mammalian cells With and without met. act. OECD TG 476 GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	HGPRT Chinese hamster ovary Conc.: 62.5, 125, 250, 500, 750 and 1000 µg/ml (with and without S9 mix) for main assay and 31.25, 62.5, 125, 250, 500 and 600 µg/ml (without S9 mix) and 31.25, 62.5, 125, 250, 350 and 500 µg/ml (with S9 mix) for confirmatory assay)	Genotoxicity: negative Cytotoxicity: yes (at ≥600 µg/ml for without met. act and ≥750µg/ml for with met. act.)	REACH registration dossier: Unpublished study report, 1990
Microtubules polymerization assay in free-cell system Without met. Act. No OECD guideline No GLP Rel. 2	4,4'-sulfonyldiphenol Vehicle: DMSO	MT protein in cell free system Conc.: 50 to 200 µM	Genotoxicity: negative	Pfeiffer *et al.*, 1996

Table 38: Summary table of *in vivo* study on mutagenicity

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vivo mammalian erythrocyte micronucleus assay Gavage OECD TG 474 GLP	4,4'-sulfonyldiphenol Vehicle: DMSO	Mouse / NMRI/ 5 males/dose Conc.: 0, 500, 1000 and 2000 mg/l bw Frequency of treatment: twice (interval of 24h)	Genotoxicity: negative Toxicity: no effects (Evidence of bone marrow exposure not been shown by the lab)	REACH registration dossier: Unpublished study report, 2010

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Rel. 1				

In few *in vitro* gene mutation assays in bacteria, BPS did not induce any significant reproducible increases in the observed numbers of revertant colonies.

An *in vitro* chromosome aberration test in mammalian cells (REACH registration dossier: Unpublished study report, 1991) was performed following OECD TG 473.

In the main assay, significant higher % of cells with aberrations was observed at the highest dose tested without metabolic activation. Due to the cytotoxicity noted at this dose level, a confirmation test was performed and exhibited also significant higher % of cells aberrations at the 2 highest dose.

Table 39: Cells with aberrations (main experiment)

Without S9 mix							
Dose level (µg/ml)	Control -	Solvent	125	250	500	750	Control +
Mitotic index	5.7	5.8	5.2	4.8	3.8	0.0	3.2
Cells scored	100	100	100	100	100	0	100
Mean aberration per cell	0.000	0.000	0.000	0.020	0.160	/	0.390
% cells with aberrations	0	0	0	2	13*	/	27*
With S9 mix							
Dose level (µg/ml)	Control -	Solvent	125	250	500	750	Control +
Mitotic index	6.5	6.8	6.6	6.7	6.8	0.0	2.0
Cells scored	100	100	100	100	100	0	100
Mean aberration per cell	0.000	0.000	0.000	0.010	0.020	/	0.400
% cells with aberrations	1	0	0	1	2	/	25*

* : p<0.01

Table 40: Percentage cells with aberrations (confirmation assay)

Without S9 mix								
Dose level (µg/ml)	Control -	Solvent	30	400	500	600	700	Control +
Mitotic index	4.2	3.6	2.9	3.1	2.7	3.1	0.0	2.6
Cells scored	100	100	100	100	100	100	0	100
Mean aberration per cell	0.020	0.010	0.010	0.030	0.160	0.250	/	0.330
% cells with aberrations	2	1	1	3	13*	14*	/	19*

* : p<0.01

In an *in vitro* chromosome aberration test in mammalian cells (OECSEH, 1999), performed following OECD TG 473, cells with structural chromosomal aberrations including gaps were weakly higher at the highest dose level after 24h of treatment (frequency 5.5%).

Moreover, after a short-term treatment, polyploidy was weakly higher (frequency of 1.63 and 1.75 % respectively at 0.18 and 0.35 mg/ml). Cytotoxicity was only noted at 0.4 mg/ml.

In an *in vitro* gene mutation test in mammalian cells (REACH registration dossier: Unpublished study report, 1990), performed following OECD TG 476, no significant increases in the mutant frequencies in any of the test groups was noted.

In an *in vitro* genetic toxicity study (Pfeiffer *et al.*, 1996) measuring the potential to interfere with the formation of microtubule (MT) in a cell-free assay, BPS did not inhibit MT assembly.

In an *in vivo* mammalian somatic cell study (micronucleus assay) (REACH registration dossier: Unpublished study report, 2010), performed following OECD TG 474, groups of 5 males were given by gavage BPS at a concentration of 0, 500, 1000 or 2000 mg/l bw.

No dose-dependent inhibition of erythropoiesis was observed. Moreover, the ratio of polychromatic to normochromatic erythrocytes was in the same range than the control group.

Literature (not available in the registration dossier):

Literature studies revealed contradictory results regarding the mutagenic potential of BPS. An Ames Test performed in *Salmonella Typhimurium* with and without S9 metabolic activation did not reveal any mutagenic activity of BPS (Fic *et al.*, 2013). Similarly, a H2AX phosphorylation assay in HepG2 cells (to detect early DNA damages) did not reveal any genotoxic activity of BPS (Hercog *et al.*, 2019).

However, a chromosomal aberration assay performed by Lee *et al.* (2013) showed that BPS induces DNA double-strand breaks in mutant cells lacking DNA repair mechanism (no effect in wild-type cells). Moreover, Fic *et al.* (2013) showed that BPS induces significant DNA damages after 24h exposure in an in vitro Comet assay in HepG2 cells (no linear dose-response). Another Comet assay performed in RWPE-1 cells resulted also in a significant increase of DNA double-strand breaks. In the same study, co-exposure of BPS with Fpg protein, which is used to assess oxidative DNA base damages, caused a significantly higher tail intensity, suggesting that oxidative stress might be one of the underlying reasons responsible for the DNA damages (Kose *et al.*, 2019).

Indeed several studies showed that BPS increases oxidative stress. Ullah *et al*. investigated the effects of BPS on the male reproductive system of rat in different studies. Rat testes cells exposed in vitro showed a strong increase of reactive oxygen species (ROS) (Ullah *et al.*, 2016 and 2018a). This was confirmed in vivo in male rats under different exposure conditions (doses, duration, window of exposure). Antioxidant enzymes are also affected as all in vivo studies revealed a significant decrease of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities, and a significant increase of lipid peroxidation (LPO) (Ullah *et al.*, 2016, 2018a, 2018b and 2019).

The higher oxidative stress linked to increase of ROS has also been observed in fish. In vitro, primary macrophages isolated from kidney of Red common carps (Cyprinus carpio) showed a significant increase of ROS level after exposure to BPS (Qiu *et al.*, 2018a and 2019). This increase of ROS has also been confirmed in vivo in Red common carps (Qiu *et al.*, 2019) and in Zebrafish (Qiu *et al.*, 2018b). Finally, antioxidant enzymes activity was also altered in Zebrafish after BPS exposure, but not similarly to rats, with a significant increase of CAT activity, whereas SOD activity was enhanced at mid dose, but significantly reduced at higher dose (Gu *et al.*, 2019).

Finally, a biomonitoring study performed by Asimakopoulos *et al.* (2016) demonstrated a correlation between BPS urinary concentration and 8OHDG, a known oxidative stress biomarker in human ($r = 0.30$, $p = 0.0005$).

Conclusion on mutagenicity:

Due to the negative results of the *in vivo* mammalian somatic cell study (micronucleus assay, OECD TG 474) (REACH registration dossier: Unpublished study report, 2010) the registrant concluded that the substance is not a mutagen.

Whereas some literature studies confirmed the negative results, some other showed a potential mutagenic activity of BPS, particularly due to the increase of oxidative stress and higher level of ROS, resulting in DNA damages.

Based on the available information (positive results in two in vitro mammalian chromosome aberration tests and literature), the eMSCA concludes that the existing data are not sufficient to exclude a genotoxic potential of BPS. Therefore, a tiered approach was proposed in the substance evaluation, to confirm the negative results of the in vivo study in mammalian somatic cells (micronucleus assay, OECD TG 474) and the conclusion reached by the registrant. If BPS cannot be detected in plasma through the toxicokinetic study (OECD TG 417), an in vivo alkaline comet assay (OECD TG 489) was requested as part of the substance evaluation. The presence of BPS in plasma should be investigated in advance by the toxicokinetic study (OECD TG 417) (section 7.9.1).

Based on the toxicokinetics data (see section 7.9.1), the substance has been shown to reach the plasma. Given the negative results of the in vivo study in mammalian somatic cells (micronucleus assay, OECD TG 474), the in vivo alkaline comet assay (OECD TG 489) was therefore not necessary. The substance is not considered as a mutagen.

7.9.6. Carcinogenicity

No information available

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Table 41: Summary of animal studies on reproductive toxicity

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reproductive toxicity test Rats / Crj: CD (SD) 12/sex/group Gavage Following OECD TG 421 GLP Rel. 2	4,4'-sulphonyldiphenol (purity: 99.87 %) Vehicle: 0.5% aqueous sodium CMC solution with 0.1% Tween 80 Doses: 0, 10, 60 and 300 mg/kg bw/d Exposure: a total of 45 days for males (including 14D of premating period, through mating to the day before necropsy) and a total of 40 to 46D for females (from premating mating through gestation and until lactation D3) (females	Parental generation: Clinical signs: excessive salivation at 300 mg/kg bw/d Bw: lower at the highest dose in both sexes Gross necropsy findings: distension of cecum was observed in 1 male and 1 female in the mid dose level and in all males and 4 females at the highest dose level Organ weight: in males : sign. increase of rela. pituitary and rela. liver weight and significant decrease of seminal vesicle weight In females: no sign. changes at the highest dose Histopathology: cecum : sign. increase incidence of hyperplasia of the mucosal epithelium (in 11 males) and sign. higher incidence of single cell necrosis (in 5 males) in males at the highest dose	REACH registration dossier: Unpublished study report, 2000

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	without delivery were exposed until D25 after confirmation of copulation)	Liver: centrilobular hypertrophy of hepatocytes observed in 5 males at 300 mg/kg bw/d Reproductive data: copulation index, parturition index, delivery index, number of corpora lutea, gestation period : no effects Mean duration of oestrus cycle was sign. increased at the highest dose (5.57**d vs 4.08d in control group). + Increase number of animals showing irregular oestrus cycle (5 females at 300 mg/kg bw/d vs 0 females in control group) Decrease number of implantation sites and significant lower implantation index at 300 mg/kg bw/d (64.89** % vs 95.80 % in control group) Severe decrease of fertility index: 58.3 % at the highest dose vs 91.7 % in control group <u>Offspring:</u> Decrease mean nb of offspring at birth at 300 mg/kg bw/d (9.1 at 300 mg/kg bw/d vs 14.3 in control group) No sign. difference in viability index and in anogenital distance No abnormalities in external appearance and clinical signs nor bw, bwg, anogenital distance, …	
Extended-one-generation reproductive toxicity study (EOGRTS) with F2, DNT (cohorts 2A and 2B) and DIT (Cohort 3) Rats/ SD F0 generation: 24/sex/dose F1 generation: 20/sex/dose for cohort 1A, 24/sex/dose for cohort 1B, 10/sex/dose for cohorts 2A, 2B and 3. Gavage Following OECD TG 443 GLP Rel. 1	4,4'-sulphonyldiphenol Vehicle: 0.5 % CMC Doses: 0, 20, 60 and 180 mg/kg bw/d Duration of exposure: Min. 10 w after the beginning of exposure, males and females from the same dose group were mated. Shortly before weaning of the F1 pups, the F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups. Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after	<u>Parental generation:</u> Bw: sign. higher only in females during the in-life period (D 7 and 14 in the mid dose) Male reproductive data: sign. reduction in % of motile sperm in all tested dose group (88, 84*, 85* and 86* %, respectively at 0, 20, 60 and 180 mg/kg bw/d) Female reproductive data: mean duration of oestrus cycle: sign. increase at 180 mg/kg bw/d (4.1*d vs 3.9d in control) Mean nb of implantation site: reduced at the highest dose (14.3 vs 15.3 in control) Mean nb of post-implantation loss sign. affected (1.5** vs 0.5 in control) Necropsy: enlarged cecum and changes in kidneys observed in males at 180 mg/kg bw/d <u>F1 pups:</u> Sign. lower tot. nb. of liveborn pups (285* at 180 mg/kg bw/d vs 340 in control) and sign. higher nb of stillborn pups (8* at 180 mg/kg bw/d vs 2 in control)	REACH registration dossier: Unpublished study report, 2019

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	weaning, placed into cohorts.	Cohort 1A: FBW: slightly reduced at 180 mg/kg bw/d in males BW: in females, body weight was sign. higher at D14 and D28 in the mid and high dose groups Necropsy: adrenal glands, kidneys, liver, spleen, thymus and prostate showed a significant deviation in abs. or rela. values An atrophy of the mammary gland was noted in males of the highest dose Cohort 1B: higher mean duration of oestrus cycle at 180 mg/kg bw/d (4.5 d vs 3.9 d in control) Lower mean nb of implantation sites and sign. higher incidence of post-implantation loss at 180 mg/kg bw/d Necropsy: FBW slightly reduced in males at the highest dose and a few organ weights modified Cohort 2A: Auditory startle response, home cage observations, open field observations, sensorimotor tests/reflexes, motor activity measurements and learning and memory tests : unaffected Cohort 2B: Necropsy examination no effects observed Cohort 3: Clinical and bw examination: unaffected Necropsy examination: sign lower rela. thymus weight at 180 mg/kg bw/d T-cell dependent antibody response: slight change in the low and mid dose groups in females F2 pups: Decrease tot. nb of pups delivered at the highest dose	
Range finding study of the EOGRTS Similar to combined repeated dose toxicity study with the reproduction/developmental toxicity screening test Rats / SD 10/sex/dose	4,4'-sulphonyldiphenol (purity: 99.87 %) Vehicle: CMC Doses: 0, 30, 100 and 300 mg/kg bw/d Duration of	F0 generation: Mortality: no Clinical signs: excessive salivation observed at the highest dose Bw: lower bw at the highest dose (-7% in males and -6% in females compared to the control group)	REACH registration dossier: Unpublished study report, 2017

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
gavage Similar to OECD TG 422 GLP Rel. 2	exposure: 10w for males and continued through premating, gestation, lactation and post-lactation periods for females	Haematology and clinical biochemistry: no effects (no more information available) Gross pathological findings: increase incidence of dilatation of cecum, enlarged and discoloration of kidneys and enlarged liver in males exposed to 300 mg/kg bw/d Organ weight: sign. higher rela. kidneys weight was observed in males (+11.5 and +35% respectively at 100 and 300 mg/kg bw/d) and sign higher rela. liver weight in males at the highest dose (+11%). In females, uterus weight was modified at 300 mg/kg bw/d. Histopathology: changes were observed in kidneys in both sexes and in mammary glands and in cecum of males Reproductive data: Oestrus cycle: prolonged at the highest dose (5.16** vs 4.02 in control group) The mean nb. of implantation sites was sign. lower at 300 mg/kg bw/d (10.4** vs 15.8 in control group) % of post-implantation loss : sign. higher at 300 mg/kg bw/d (34.6* vs 3.6% in control group) <u>F1:</u> Mean of pups delivered was sign decreased at the highest dose (10.8** vs 15.2) Bw was sign higher in male of the low dose at PND 21 (+6.6% compared to the control group) (no more information available) Gross pathological findings: no effects observed (no more information available)	
Range finding study for EOGRTS, similar to 28D repeated dose toxicity study Rat / SD 5 rats/sex/dose Gavage No guideline followed GLP: no Rel. 2	4,4'-sulphonyldiphenol (purity: 99.87 %) Vehicle: CMC Dose: 0, 100, 300 and 600 mg/kg bw/d Duration of exposure: 28D	Clinical signs: excessive salivation BWG: significantly lower at the highest dose in males (no more information available) FBW: lower at the mid and high dose levels (-9 and -12% respectively at 300 and 600 mg/kg bw/d) Gross necropsy findings: enlarged kidneys was observed in 4 males exposed to 600 mg/kg bw/d and in 3 males exposed to 300 mg/bw/d. Moreover, cecum dilatation was noted in 2 males of the highest dose. Organ weight: few changes observed in kidneys, adrenals, liver, prostate and sem. ves. Histopathology: few changes observed in kidneys, adrenal glands, liver, cecum and mammary gland.	REACH registration dossier: Unpublished study report, 2017

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study rat / Crl:WI(HAN) 25 pregnant females/group Gavage Following OECD TG 414 GLP Rel. 1	4,4'-sulphonyldiphenol (purity: 99.3 - 99.5 %) Vehicle: 1% CMC Conc.: 0, 30, 100 and 300 mg/kg bw/d Exposure: GD 6 to 19 Sacrificed at GD20	<u>Dams:</u> Clinical signs: excessive salivation was noted in 7 out of 25 females exposed to 300 mg/kg bw/d Bw: no sign change (however bwg GD 6–19 and GD 8-10 were sign lower) Uterus weight: no effects Necropsy observation: no treatment-related effects Reproduction data (nb. of dams with viable foetuses, corpora lutea, implantation sites, pre and post implantation loss, resorption): no effects <u>Offspring:</u> Sex ratio: unaffected Mean nb. of live foetuses: no effects Bw: unaffected Few skeletal variations observed	REACH registration dossier: Unpublished study report, 2014

* : p<0.05; ** : p<0.01

In a reproductive toxicity study (REACH registration dossier: Unpublished study report, 2000), following OECD TG 421, groups of 12 male and 12 female rats were given BPS via gavage at concentrations of 0, 10, 60 or 300 mg/kg bw/d. Males were exposed for a total of 45 days including 14 days of pre-mating period, through mating period to the day before necropsy. While, females were exposed for a total of 40 to 46 days (from mating period through gestation to lactation day 3). Excessive salivation was observed just before or immediately after administration of the test substance in 7 males and 1 female exposed to the highest dose, however all of them recovered within 30 minutes after administration. The food consumption was examined during the premating period (day 3, 7, 14), during the gestation and during the lactation. A statistically significant decrease of food consumption was only observed at day 3 of the premating period in males and females at the high dose group (24.3 mg/kg bw/d vs 30.7 mg/kg bw/d in control group and 14.8 mg/kg bw/d vs 20.2 mg/kg bw/d in control group, respectively in males and females). The body weight was analysed in males and females at the same time as food consumption examination. A statistically significant decreased body weight was noted in females at the highest dose at the end of the gestation period (see table 43).

Table 42: Body weight data (in g)

Dose level (mg/kg bw/d)			0	10	60	300
Males		D0	351.9	354.3	352.5	354.2
		D14	435.9	435.9	437.9	404.5**
		D42	511.8	514.5	523.5	486.0
		BWG D0-42	159.9	160.2	171.0	131.8
Females	Premating period	D0	229.1	228.4	228.2	230.3
		D14	264.2	263.8	262.3	251.7
		BWG D0-14	35.1	35.4	34.1	21.4**
	Gestation period	D0	272.8	277.1	266.8	264.4
		D20	436.5	433.1	418.6	390.4**

		BWG D0-20	163.6	156.0	151.8	126.0**
	Lactation period	D0	325.5	327.5	314.3	316.0
		D4	360.1	354.1	333.0*	338.6
		BWG D0-4	34.5	26.5	18.7	22.6

* : p<0.05; ** : p<0.01

No abnormalities were observed in parental animals with regard to copulation index, parturition index, delivery index, number of corpora lutea and gestation period. However, an increased number of animals showed irregular oestrus cycle (5 females exposed to 300 mg/kg bw/d exhibited a longer dioestrus period of 6 to 10 days). The mean duration of oestrus cycle was significantly higher at the highest dose (see table 44). Most of the females, which had a continued dioestrus, were not fertilized and the fertility index decreased severely (58.3 % at the highest dose vs 91.7 % in control group). Furthermore, a declining tendency in the number of implantation sites and a significant decrease of implantation index were observed at the highest dose level.

Table 43: Reproductive performance

Dose level (mg/kg bw/d)	0	10	60	300
Nb of pairs	12	12	12	12
Mean oestrus cycle (in D)	4.08	4.01	4.14	5.57**
Inc of females with irregular oestrus cycle	0/12	0/12	1/12	5/12*
Fertility index (in %)	91.7	91.7	100.0	58.3

* : p<0.05 ; ** : p<0.01

Table 44: Female reproduction delivery data

Dose level (mg/kg bw/d)	0	10	60	300
Nb of animals examined	11	11	12	7
Gestation length (d)	22.9	23.0	22.8	22.9
Mean nb of corpora lutea	16.6	15.9	17.3	15.7
Mean nb of implantation sites	15.9	13.3	14.8	10.7
Total nb of offspring	14.3	12.5	13.5	9.1
Implantation index (%)	95.80	80.84	86.15	64.89**
Delivery index (%)	90.03	94.60	91.22	89.57
Gestation index (%)	100.00	100.00	100.00	100.00

** : p<0.01

At necropsy, distension of the cecum was observed in 1 male and 1 female of the mid dose level and in all males (12) and 4 females of the highest dose group. The cecum examination revealed a significant increased incidence of diffuse hyperplasia of the mucosal epithelium and of single cell necrosis in males at 300 mg/kg bw/d. At the highest dose, the relative liver weight increased and a centrilobular hypertrophy of hepatocytes was observed in 5 males of the highest dose. In males and females, a tendency to decreased thymus weight was detected. Furthermore, in males, an increased relative pituitary weight and a decreased absolute seminal vesicle weight were observed.

Table 45: Organ weights

	Males				Females			
Dose level (mg/kg bw/d)	0	10	60	300	0	10	60	300
FBW (g)	513.4	517.3	526.7	488.1	360.1	354.1	333.0*	338.6
Pituitary (mg)	14.92	13.60	15.12	16.68	21.18	21.64	21.45	21.07
Pituitary rela. (mg%)	2.89	2.63	2.88	4.43**	5.90	6.14	6.45	6.21
Thymus (mg)	289.6	336.3	332.1	254.5	263.1	312.7	253.5	221.7

Liver (g)	16.373	16.246	16.803	17.439	15.289	14.114	14.490	14.393
Liver rela. (g%)	3.185	3.135	3.185	3.562**	4.247	3.989	4.359	4.246
Testes (g)	3.559	3.480	3.554	3.503				
Prostate (g)	0.723	0.746	0.777	0.708				
Sem. Ves. (g)	2.825	2.718	2.860	2.428**				
Epididymis (g)	1.355	1.292	1.328	1.292				
Ovaries (mg)					110.35	116.02	114.86	105.63
Uterus (g)					0.691	0.683	0.713	0.700

* : p<0.05; ** : p<0.01

Table 46: Incidence of histopathological findings

		Males				Females			
Dose level (mg/kg bw/d)		0	10	60	300	0	10	60	300
Cecum	Diffuse hyperplasia, mucosal epith.	0/12	/	0/1	11/12**	0/1	/	1/1	4/4
	Single cell necrosis, absorptive epith.	0/12	/	0/1	5/12*	0/1	/	0/1	1/4
Liver	Extramedullary haematopoiesis	2/12	2/12	3/12	2/12	6/12	6/12	7/12	5/12
	Centrilobular hypertrophy, hepatocytes	0/12	0/12	0/12	5/12*	0/12	0/12	0/12	3/12

* : p<0.05; ** : p<0.01

Regarding offspring examination, a tendency to decrease in the total number of offspring at birth, number of live offspring at birth and number of live offspring on day 4 of lactation were observed in the highest dose group (see table 48). However, no significant difference in live birth index and viability index were noted (at PND 0 : 99.35, 100.00, 99.48 and 100.00 % respectively at 0, 10, 60 and 300 mg/kg bw/d and at PND 4 : 99.30, 95.45, 99.48 and 100.0 % respectively at 0, 10, 60 and 300 mg/kg bw/d). External examination did not reveal any clinical signs, body weight change (see table 49), nor anogenital distance modification. Furthermore, no abnormalities were observed in dead offspring on lactation day 0 to 4 and live offspring on lactation day 4 in each group.

Table 47: Mean number of pups

Dose level (mg/kg bw/d)	0	10	60	300
Tot nb of offspring at birth	14.3	12.5	13.5	9.1
Tot nb of live offspring at birth	14.2	12.5	13.4	9.1
Nb of live offspring at D4	14.1	12.4	13.3	9.1

Table 48 : Pups body weight data (in g)

Dose level (mg/kg bw/d)		0	10	60	300
Males	D0	7.4	7.5	7.3	7.8
	D4	12.0	12.4	12.1	14.1
Females	D0	6.9	7.0	6.9	7.3
	D4	11.7	11.7	11.5	13.3

In an extended-one generation reproductive toxicity study (REACH registration dossier: Unpublished study report, 2019), performed following OECD TG 443, groups of male and female rats were given BPS at a concentration of 0, 20, 60 and 180 mg/kg bw/d.

For the F0 parental generation, minimum 10 weeks after the beginning of exposure, 24 males and 24 females from the same dose group were mated. Shortly before weaning of

the F1 pups, the F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups. Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after weaning, placed into cohorts.

- Cohort 1A was composed of 20 males and 20 females per dose group and animals were sacrificed approximately when 13 weeks old.
- Cohort 1B was composed of 24 males and 24 females per dose group and was selected to produce F2 pups. As for the F0 parental generation, minimum 10 weeks after assignment of the F1 parental animals, males and females were mated. F1 males were sacrificed shortly before weaning and F1 females shortly after the weaning.
- Cohort 2A (neurotoxicity) was composed of 10 males and 10 females per dose group and animals were sacrificed approximately when 11 weeks old.
- Cohort 2B (neurotoxicity) was composed of 10 males and 10 females per dose group and animals were sacrificed approximately when 3 weeks old.
- Cohort 3 (immunotoxicity) was composed of 10 males and 10 females per dose group and animals were sacrificed approximately when 8-9 weeks old.
- Pups, which were not chosen for cohorts or for blood sampling on PND 4 and 22, were sacrificed after weaning.

F0 parental and F1 pups (before weaning):

Regarding the F0 parental generation, 1 female of the low dose group was sacrificed on study day 63 due to poor general condition. Thirteen males and 6 females exposed to 180 mg/kg bw/d exhibited transient salivation during the first weeks of exposure. However, the maternal care was not affected during gestation and lactation periods. Furthermore, a higher significant body weight value was only observed in females of the mid dose group at D 7 and 14 of the premating period (premating D 7 : 141.3, 144.8, 148.3* and 145.2g respectively at 0, 20, 60 and 180 mg/kg bw/d ; premating D 14 : 162.8, 169.9, 172.4* and 169.6g respectively at 0, 20, 60 and 180 mg/kg bw/d).

Male reproduction parameters were examined and revealed a significant lower percentage of sperm motility (88, 84*, 85* and 86* % respectively at 0, 20, 60 and 180 mg/kg bw/d). Other parameters were not affected (total spermatids/gram testis, total sperms/gram cauda epididymis, % of abnormal sperms, male mating index and male fertility index).

Regarding female reproduction parameters, the mean oestrus cycle duration was significantly increased at the highest dose (see table 50). At 20 mg/kg bw/d, 1 female exhibited a mean oestrus length of 5.3 days and one other female had a mean cycle length of 4.0 days; however, this last female showed an oestrus cycle with a dioestrus period of 9 days. Two females exposed to 180 mg/kg bw/d exhibited a mean cycle length of 4.7 and 5.0 days. This last one had one cycle with a dioestrus period of 5 days. Fertility index (96, 91, 100 and 96 % respectively at 0, 20, 60 and 180 mg/kg bw/d) and duration of gestation (22.0 days in all groups) were not affected. However, a decreasing trend in the mean number of implantation sites was noted (15.3, 14.8, 14.9 and 14.3 respectively at 0, 20, 60 and 180 mg/kg bw/d).

Table 49: Oestrus cycle data

Dose level (in mg/kg bw/d)	0	20	60	180
Mean day from oestrus to oestrus (in day)	3.9	3.9	3.9	4.1*
Mean nb of days in stage: prooestrus[A]	4.7	3.5	3.8	2.2

Mean nb of days in stage: oestrus[A]	5.1	5.1	5.0	5.2
Mean nb of days in stage: metoestrus[A]	5.8	6.0	5.8	5.9
Mean nb of days in stage: dioestrus[A]	6.3	7.4	7.7	9.0

* : p<0.05
[A] : Oestrus cycle data was generated during the last 3 weeks prior to mating. These means were calculated by the eMSCA

At necropsy, enlarged cecum and enlarged kidneys were observed in males of the highest dose (resp. in 3 males and in 6 males). Absolute and relative adrenal glands, kidneys and thymus weights were significantly modified in males and relative liver weight was significantly higher in females (see table 51).

Table 50: Modified organ weights and microscopic findings

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (in g)		521.575	514.408	521.35	507.229	272.125	278.826	275.429	273.408
Adrenal glands	Abs (mg)	54.0	55.958	58.75*	60.625*	80.208	70.391	77.208	71.625
	Rela	0.01	0.011	0.011*	0.012**	0.029	0.025	0.028	0.026
Kidneys	Abs (g)	3.543	3.391	3.673	4.124**	2.083	2.135	2.137	2.148
	Rela	0.68	0.663	0.705	0.817**	0.767	0.768	0.776	0.787
	Inc. of medullar mineralisation	0	0	1	21	14	2	1	15
	Inc. of nuclear crowding	0	0	0	22	0	0	0	0
	Inc. of tubular dilatation	0	0	0	13	0	0	0	0
Liver	Abs (g)	12.572	13.298	13.003	12.46	8.08	8.259	8.348	8.695
	Rela	2.413	2.575	2.491	2.455	2.968	2.964	3.029	3.181**
Thymus	Abs (mg)	250.167	283.375	283.292*	233.708	239.167	224.391	233.625	218.875
	Rela	0.048	0.056	0.054*	0.046	0.088	0.081	0.085	0.08

* : p<0.05 ; ** : p<0.01

Regarding offspring examination, due to the higher resorptions, the mean number of F1 pups per dam was lower in all tested groups and was dose related (14.9, 14.0, 13.5, 12.7 respectively at 0, 20, 60 and 180 mg/kg bw/d). Furthermore, the number of liveborn pups was significantly reduced at the highest dose (340, 289, 322 and 285* pups respectively at 0, 20, 60 and 180 mg/kg bw/d) and the number of stillborn pups was also significantly increased at the highest dose (2, 5, 3 and 8* pups respectively at 0, 20, 60 and 180 mg/kg bw/d). Moreover, the mean pup body weight was significantly higher at the 2 highest dose levels (see table 52). Sex ratio, clinical observations, viability index, anogenital distance, vaginal opening, preputial separation and presence of areolas/nipples were not modified by exposure to the test substance.

Table 51: Pup body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
PND 1	Males	7.1	7.4	7.7*	7.7[A]
	Females	6.7	7.0	7.2*	7.3*
	M+F	6.9	7.2	7.5*	7.5*
PND 4 (post-culling)	Males	10.5	10.9	11.5*	11.4*
	Females	9.9	10.3	10.9*	10.9*
	M+F	10.2	10.6	11.2*	11.2*
PND 21	Males	54.0	56.8	57.4*	55.7
	Females	52.0	54.3	54.8*	53.7
	M+F	53.0	55.5	56.0*	54.7

* : p<0.05
[A] : S.d : 0.52, 0.76, 0.74 and 0.76

Cohort 1A:

1 female of the highest dose was found dead on study day 0 (necropsy revealed a slight fibrinous inflammation in the lung, focal hyperplasia in the mammary gland and an atrophic uterus). 12 males and 14 females of the highest dose exhibited transient salivation immediately after dosing. Body weight was significantly higher in females exposed to 60 and 180 mg/kg bw/d at D 14 and 28 (see table 53).

Table 52: Body weight data (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
D 0	86.8	86.8	85.5	85.9	77.5	78.4	79.9	78.2
D 14	208.0	203.0	204.9	203.7	149.0	153.4	159.6*	159.6*
D 21	266.4	262.7	263.9	254.0	173.9	176.1	184.2	183.2
D 28	326.7	322.0	323.5	315.5	193.5	196.0	207.3*	207.1*
D 42	408.4	404.1	408.4	394.1	226.9	228.4	237.6	241.0
D 63	488.3	490.6	472.9	459.2	264.1	256.3	265.1	277.0

* : p<0.05

Regarding reproduction parameters, sperm was examined and did not show any modification. In females, during the 2 weeks of observation, oestrus cycle was of 4.1 days in all groups, but showed prolonged dioestrus stage, as in the other cohorts observed (see table 54).

Table 53: Oestrus cycle data

Dose level (in mg/kg bw/d)	0	20	60	180
Mean day from oestrus to oestrus (in day)	4.1	4.1	4.1	4.1
Mean nb of days in stage :	2.2	2.0	2.2	1.3

prooestrus[A]				
Mean nb of days in stage : oestrus[A]	3.5	3.6	3.5	3.2
Mean nb of days in stage : metoestrus[A]	3.8	3.9	3.6	4.1
Mean nb of days in stage : dioestrus[A]	4.5	4.6	4.8	5.4

* : p<0.05
[A] : Oestrus cycle data was generated during the last 3 weeks prior to mating. These mean were calculated by the eMSCA

At the end of the exposure period (approx. 90 days), animals were sacrificed and necropsied. No macroscopic dose related findings were observed. Some organs exhibited weight differences (see table 55). As in the F0 parental generation histopathological changes were observed in kidneys (medullar mineralisation, nuclear crowding and tubular dilatation). Moreover, an increased incidence in atrophy of mammary gland was observed at the highest dose (in 1, 0, 2 and 7 males respectively at 0, 20, 60 and 180 mg/kg bw/d).

Table 54: Organ weight changes

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (g)		455.095	449.15	452.61	433.11	242.17	240.59	248.375	251.237
Adrenal glands	Abs (mg)	65.0	63.2	63.6	70.5	69.05	69.15	71.5	76.737
	Rela	0.014	0.014	0.014	0.016**	0.029	0.029	0.029	0.031
Kidneys	Abs (g)	3.224	3.137	3.335	3.599**	1.797	1.791	1.86	1.91
	Rela	0.712	0.701	0.737	0.832**	0.745	0.745	0.747	0.759
Liver	Abs (g)	13.032	13.349	12.923	11.265**	6.828	6.725	6.906	7.238
	Rela	2.863	2.973	2.858	2.601**	2.814	2.794	2.78	2.88
Spleen	Abs (g)	0.876	0.817	0.801*	0.726**	0.524	0.494	0.529	0.502
	Rela	0.194	0.182	0.177*	0.168**	0.216	0.206	0.213	0.2
Thymus	Abs (mg)	435.7	418.45	435.35	350.85*	354.05	356.75	381.8	355.158
	Rela	0.095	0.094	0.096	0.08	0.146	0.148	0.154	0.142
Prostate	Abs (g)	1.163	1.118	1.053*	1.046**	-	-	-	-
	Rela	0.257	0.252	0.233	0.242	-	-	-	-

* : p<0.05 ; ** : p < 0.01

Cohort 1B :

During the study period, 1 female of the mid dose group was found dead on premating D 3 (histopathological examination not performed). Clinical observation revealed excessive salivation immediately after exposure to the test substance in 11 males and 9 females during the in-life period and in 10 females during gestation period. Significant body weight changes was observed in both sexes during the in-life period (see table 56 and 57).

Table 55: Male body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
In-life period	D 0	79.8	80.1	82.3	78.9
	D 14	190.0	179.2	177.6*	173.7**
	D 21	253.9	250.3	260.4	248.4

	D 49	422.2	416.4	437.7	405.5
	D 70	489.2	481.8	502.7	466.8
Parental period	W 0	503.0	498.2	517.7	479.7
	W 5	564.3	559.2	579.7	541.6

* : p<0.05 ; ** : p<0.01

Table 56: Female body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
In-life period	D 0	73.4	71.5	75.4	73.6
	D 21	170.9	170.7	185.8**	184.7**
	D 49	237.6	233.0	252.7*	258.4**
	D 70	265.6	260.9	280.9	284.4*
Gestation period	GD 0	276.8	270.5	292.2	291.4
	GD 14	345.9	335.1	356.3	355.7
	GD 20	426.0	412.3	436.5	415.7
Lactation period	LD 0	330.6	323.8	343.8	341.3
	LD 10	359.3	350.6	371.4	367.0
	LD 21	342.3	332.9	356.6	353.0

* : p<0.05 ; ** : p<0.01

Regarding reproduction data, fertility index was not affected (100, 100, 96, 96 % respectively at 0, 20, 60 and 180 mg/kg bw/d). However, a slightly reduced number of females with liveborn pups was observed at 60 and 180 mg/kg bw/d. The oestrus cycle was also modified at the highest dose level. Furthermore, the mean number of implantation sites tended to decrease at 180 mg/kg bw/d (see table 58).

Table 57: Fertility data

Dose level (in mg/kg bw/d)	0	20	60	180
Female mating index (in %)	100	100	100	100
Mean mating day until DPC 0	3.0	2.4	2.5	3.0
Female fertility index (in %)	100	100	96	96
Nb of females with liveborn pups	24	24	21	21
Nb of females with stillborn pups	6	2	2	6
Mean day from oestrus to oestrus	3.9	4.0	4.0	4.5
Mean nb of days in stage : prooestrus[A]	4.7	2.8	2.2	1.3
Mean nb of days in stage : oestrus[A]	5.4	5.2	5.4	4.6
Mean nb of days in stage : metoestrus[A]	6.0	6.0	6.3	5.9
Mean nb of days in stage : dioestrus[A]	6.8	8.4	9.2	11.2
Mean nb of implantation sites	15.2	14.6	15.4	13.7
Duration of gestation (in day)	22.0	21.9	22.0	22.0

Statistical examination was not performed regarding the mean day of oestrus's stage ; ** : p<0.01
[A] : Oestrus cycle data was generated during the last 3 weeks prior to mating. These mean were calculated by the eMSCA

Shortly before weaning, parental animals were sacrificed. Necropsy revealed enlarged kidneys in 1 male of the mid dose and in 10 males of the highest dose. 3 organs showed weight modifications (see table 59). All other weight parameters did not show significant differences. Regarding the histopathological examination, an atrophy of the mammary gland was only noted in 1 male of each group.

Table 58: Organ weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (g)		536.054	530.863	548.279	510.363	291.842	284.588	304.817*	308.2[A]
Adrenal glands	Abs (mg)	59.792	62.625	67.708**	64.708	76.708	72.292	77.435	80.083
	Rela	0.011	0.012	0.012*	0.013**	0.026	0.026	0.025	0.026
Kidneys	Abs (g)	3.375	3.43	3.807**	4.252**	2.158	2.115	2.212	2.31*
	Rela	0.632	0.649	0.696**	0.832**	0.741	0.746	0.726	0.752
Liver	Abs (g)	14.813	15.395	14.677	13.272*	9.455	9.326	9.5	9.716
	Rela	2.758	2.902	2.669	2.6*	3.237	3.28	3.119	3.175

* : p<0.05 ; ** : p<0.01
[A] : S.d : 24.228, 20.968, 15.011 and 30.56, respectively at 0, 20, 60 and 180 mg/kg bw/d

Regarding offspring examination, due to the higher incidence of post-implantation loss, the number of liveborn pups was considerably reduced at the highest dose (336, 330, 311 and 234 pups, resp. at 0, 20, 60 and 180 mg/kg bw/d). Sex ratio, viability index, pup body weight and anogenital distance were not affected. Necropsy of pups was performed and did not reveal significant changes.

Cohort 2A:

No mortality occurred during the study period. As in the other cohorts, excessive salivation was observed immediately after exposure to the test substance in 3 males and 1 female of the highest dose. No body weight change was observed. In this cohort, neurotoxicity was examined. Auditory startle response, home cage observations, open field observations, sensorimotor tests/reflexes, motor activity measurement and learning and memory test did not show test-related effects.

Cohort 2B:

Necropsy examination did not reveal abnormalities.

Cohort 3:

One female of the lowest dose was found dead on the study day 18. During the exposure period, clinical observation and body weight examination were not affected. At necropsy, a sign. lower relative thymus weight was observed at the highest dose in males (0.152 vs 0.187 in control) (microscopic examination not performed). In this cohort, T-cell dependent antibody response (SRBC) was examined and revealed slight changes in the low and mid dose groups in females (3737, 3727, 4414 and 3599 U/ml in males, respectively at 0, 20, 60 and 180 mg/kg bw/d ; 13647, 8329, 9598 and 14555 U/ML in females, respectively at 0, 20, 60 and 180 mg/kg bw/d).

In a reproduction/developmental toxicity screening study *(*REACH registration dossier: Unpublished study report, 2017*),* performed as a range finding for the EOGRTS, groups of 10 male and 10 female rats were given BPS at a concentration of 0, 30, 100 and 300 mg/kg bw/d. Animals were exposed for 10w (for males : 6w of premating period, 2w of mating period, and 4w of post mating period ; and for females : 6w of premating period, 2w of mating period, and continued through gestation and lactation periods). No premature death occurred during the exposure period. At the highest dose, animals exhibited excessive salivation and lower bw value (-7% and -6%, resp. in males and females, compared to the control group). Reproductive data was examined and revealed effects at the highest dose. Females exhibited prolonged oestrous and pregnant females had a significantly lower mean number of implantation sites. Moreover, post-implantation loss

was significantly higher and 2 out of 8 pregnant females had complete intrauterine litter losses. These effects resulted in a significantly lower litter size.

Table 59: Reproductive data

Dose level (in mg/kg bw/d)	0	30	100	300
Mean duration of estrous cycle (d)	4.02	3.97	4.01	5.16**
Fertility index (%)	100	90	100	80[8]
Mean nb of implantation sites	15.8	15.0	15.5	10.4**
Females without implantation sites	0	0	0	2
% of post-implantation loss	3.6	5.2	6.5	34.6*
Mean duration of gestation (d)	22	22.1	22	22
Tot. nb of pups delivered	152	127	145	65
Nb of stillborn	2	1	3	3
Mean nb of pups delivered	15.2	14.1	14.5	10.8**
Mean perinatal loss (%)	1.3	0.6	2	5.3

* p<0.05 ; ** : p<0.01

Necropsy revealed treatment related effects. Macroscopic examination revealed changes in males. Cecum was dilated in 3 males exposed to 300 mg/kg bw/d. Moreover, kidneys was discolored in 8 males and enlarged in 9 males of the highest dose, and liver was enlarged in 1, 2 and 3 males respectively at 30, 100 and 300 mg/kg bw/d). Significant higher relative kidneys weight was observed in males (+11.5 and +35% respectively at 100 and 300 mg/kg bw/d) and significant higher relative liver weight was noted in males at the highest dose (+11%) (see table 61). Microscopic examination revealed also changes in these organs as well as in mammary gland in males (see table 62).

Table 60: Organ weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	30	100	300	0	30	100	300
FBW		548.6	530.2	546.3	497.5*	304.5	301.8	292.6	286.6
Adrenals	Abs	0.014	0.014	0.014	0.015	0.027	0.029	0.03	0.03
Kidneys	Rela	0.662	0.692	0.748**	1.013**	0.722	0.731	0.762	0.752
Liver	Rela	2.375	2.378	2.519	2.668**	2.846	2.938	3.297[A]	2.927
Prostate	Rela	0.302	0.303	0.278	0.297	/	/	/	/
Sem. ves.	Rela	0.357	0.366	0.336	0.348	/	/	/	/
Testes	Rela	0.685	0.663	0.663	0.734	/	/	/	/
Ovaries	Rela	/	/	/	/	0.035	0.035	0.037	0.034
Uterus	Rela	/	/	/	/	0.197	0.224	0.224	0.307[B]

* p<0.05 ; ** : p<0.01
[A] : S.d : 0.154, 0.395, 0.677 and 0.189, respectively at 0, 30, 100 and 300 mg/kg bw/d
[B] : S.d : 0.026, 0.088, 0.099 and 0.152, respectively at 0, 30, 100 and 300 mg/kg bw/d

Table 61: Microscopic data

	Males				Females			
Dose level (in mg/kg bw/d)	0	30	100	300	0	30	100	300

[8] Note that this specific effect was initially mentioned to be 60% at the highest dose tested in the CLH dossier and on IUCLID, but during the consultation it was noted that this effect size was incorrect and should be changed into 80% fertility at the highest dose tested. The information in IUCLID was updated to this regard as well (ECHA dissemination website). The eMSCA was unable to verify the exact number as it had no access to the underlying study report. However, the eMSCA wants to highlight that, either way, a dose-dependent decrease in fertility index is observed in this study

Cecum	Dilatation	0	0	0	3	0	0	0	0
	Thickening of wall	0	0	5	9	0	0	0	0
	Increased apoptosis	0	0	3	9	0	0	0	0
Kidneys	Degeneration/regeneration	0	0	6	10	0	0	0	0
	Mineralisation	0	0	2	2	1	0	0	4
	Tubular distension	0	0	5	10	0	0	0	0
Liver	Infiltration lymphoid	10	1	2	10	10	0	0	10
	Multifocal necrosis	1	0	1	1	0	0	0	0
Mammary gland	Diffuse atrophy	0	0	0	10	0	0	0	0

Pups were recorded and examined. At the highest dose, the total number of pups delivered was reduced (152, 127, 145 and 65 pups, resp. at 0, 30, 100 and 300 mg/kg bw/d) and the mean number of pups delivered was significantly lower (15.2, 14.1, 14.5 and 10.8**, resp. at 0, 30, 100 and 300 mg/kg bw/d). At PND21, a higher body weight value was noted in male pups of the low dose group (+6.6% compared to the control group). Necropsy did not revealed gross pathological findings (no more information available).

In a 28-day repeated dose toxicity study (REACH registration dossier: Unpublished study report, 2017), performed as a range finding of the EOGRTS, 5 male and 5 female rats were given 0, 100, 300 or 600 mg/kg bw/d of BPS. Animals were exposed daily during 28 days. A significantly lower bwg value was noted in males of the highest dose level (no more information available). At the end of the exposure period, animals were sacrificed and examined. Males exhibited a significantly decrease of Final Body Weight (FBW) (-9 and -12% respectively at 300 and 600 mg/kg bw/d, compared to control). Enlarged kidneys were noted in 3 males exposed to 300 mg/kg bw/d and in 4 males exposed to 600 mg/kg bw/d. The relative kidneys weight was higher at the mid and high dose in both sexes (+33% and +12% respectively in males and females, compared to the control group). Microscopic examination revealed also changes in kidneys. In 2, 5 and 5 males exposed respectively to 100, 300 and 600 mg/kg bw/d, minimal to moderate tubular degeneration/regeneration in kidneys was noted. Moreover, tubular hypertrophy was observed in 5 males of the highest dose (moderate hypertrophy), in 5 males of the mid dose (minimal hypertrophy) and in 1 male of the low dose (minimal). The relative adrenals weight was higher in males (+18 and +39% respectively at 300 and 600 mg/kg bw/d, compared to the control group) and minimal hypertrophy/hyperplasia in the adrenal cortex was observed in 3 males exposed to 600 mg/kg bw/d. Liver exhibited also changes. The relative liver weight was increased in females (+9 and +12% respectively at 300 and 600 mg/kg bw/d, compared to the control group). Centrilobular hypertrophy of the liver was noted in 1 males of the low dose (minimal hypertrophy), in 4 males of the mid dose (slight hypertrophy) and in all animals of the high dose (moderate hypertrophy in males and slight in females). Examination of the reproductive organs revealed lower relative prostate and seminal vesicles weights at the highest dose (-15% for prostate and -16% for seminal vesicles). 3 males exposed to 300 mg/kg bw/d and 4 males exposed to 600 mg/kg bw/d exhibited diffuse atrophy of the mammary gland.

In a prenatal developmental toxicity study following OECD TG 414 (REACH registration dossier: Unpublished study report, 2014), groups of 25 pregnant rats (Crl:WI(HAN)) were given BPS via gavage at a concentration of 0, 30, 100 or 300 mg/kg bw/d. The animals were exposed to the test substance from gestational day 6 to 19 and were sacrificed at gestational day 20. No mortality was noted during the exposure period. Furthermore, the clinical examination revealed only an increased incidence of females with excessive salivation. However, the maternal care was not affected. No significant body weight change was observed during the dosing period. However, the body weight gain value for GD 6 – 19 and GD 8 - 10 were significantly decreased at the highest dose (see table 63).

Table 62: Body weight data (in g)

Dose level (mg/kg bw/d)	0	30	100	300
GD 0	164.9	167.5	168.7	165.6

GD 6	195.9	199.1	199.2	198.3
GD 15	239.3	243.5	240.6	236.1
GD 20	295.9	302.4	297.8	291.0
GD 8 – 10	9.6	9.3	9.4	6.8*
GD 6 – 19	85.2	89.8	84.3	78.6*
Corrected bwg (terminal bw on GD 20 minus uterus weight minus bw on GD6)	40.9	43.7	40.0	36.9

* : $p<0.05$

For dams, no test substance related changes were observed for the gravid uterus weight (59.1, 59.6, 58.7 and 55.8 respectively at 0, 30, 100 and 300 mg/kg bw/d), nor for the necropsy observation and nor for the reproduction data parameters (see table 64).

Table 63: Reproductive data

Dose level (mg/kg bw/d)	0	30	100	300
Nb of females mated	25	25	25	25
Conception rate (%)	100	100	100	96 (24/25)
Nb of females aborted	0	0	0	0
Nb of dams with viable foetuses	25	25	25	24
Mean nb. of corpora lutea	11.5	11.8	11.7	11.4
Mean nb. of implantation sites	11.1	11.0	11.1	10.8
Mean pre-implantation loss (%)	3.6	6.1	5.4	5.3
Mean post-implantation loss (%)	4.7	3.9	3.9	6.3
Mean early resorption	0.5	0.4	0.4	0.6
Mean late resorption	0.0	0.0	0.1	0.0
Mean total resorption	0.5	0.4	0.5	0.7
Nb of dead foetuses	0	0	0	0
Mean live foetuses (females/males)	10.6 (5.2/5.4)	10.6 (5.0/5.6)	10.6 (6.0/4.6)	10.1 (4.8/5.3)

The foetus examination revealed no differences in sex distribution and body weight compared to control group (see table 65). Skeletal variations were observed in all dose. At 300 mg/kg bw/d, these variations were significant however were comprise within the range of the historical control data (see table 66). No treatment-related skeletal external malformation and variation nor soft tissue malformation and variation were observed.

Table 64: Sex ratio and mean foetal weight (in g)

Dose level (mg/kg bw/d)	0	30	100	300
Sex ratio (in % females/males)	48.9/51.1	47.2/52.8	56.6/43.4	47.3/52.7
Mean weight of all viable foetuses	3.6	3.6	3.4	3.4
Mean weight of male foetuses	3.6	3.7	3.5	3.5
Mean weight of female foetuses	3.5	3.5	3.4	3.3

Table 65: Skeletal variations data

Dose level (mg/kg bw/d)	0	30	100	300	HCD mean % (range)
Nb of litter	25	25	25	24	
Nb of foetuses	137	137	140	127	
Total skeletal variations					
Foetal incidence : nb (%)	136 (99)	135 (99)	139 (99)	127 (100)	
Litter incidence : nb (%)	25 (100)	25 (100)	25 (100)	24 (100)	
Mean affected foetuses/litter	99.2	98.3	98.7	100.0	

Incidence of significant increased foetal skeletal variations					
Incomplete ossification of supraoccipital (unchanged cartilage)	34.1	35.2	37.6	45.2*	43.5 (10.3 – 64.3)
Dumbbell ossification of thoracic centrum (unchanged cartilage)	0.7	3.0	0.0	5.6**	6.9 (0.0 – 14.5)
Unossified sternebrae (unchanged cartilage)	1.5	5.0	4.6	11.0**	8.2 (2.6 – 20.7)
Incomplete ossification of pubis (cartilage present)	0.0	0.8	2.0*	1.7	0.3 (0.0 – 2.4)
Incomplete ossification of ischium (cartilage present)	0.0	0.0	2.0*	1.7	0.2 (0.0 – 0.8)

* : p<0.05 ; ** : p<0.01

Conclusion on Toxicity to reproduction:

Based on the available data, BE CA has submitted a CLH proposal to classify the substance as Repr. 1B H360FD. This proposal has been discussed and adopted by RAC on 10 December 2020. In the meanwhile, BPS is covered by index number 604-098-00-1 in the 18th ATP of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances).

7.9.8. Hazard assessment of physico-chemical properties

BPS is not explosive, non-flammable upon ignition and has no oxidising properties.

7.9.9. Selection of the critical DNEL(s) / DMEL(s) and / or qualitative / semi-quantitative descriptors for critical health effects

Not evaluated in this dossier.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

Based on the available data, BE CA has submitted a CLH proposal to classify the substance as Repr. 1B H360FD. This proposal has been discussed and adopted by RAC on 10 December 2020. BPS is listed in the 18th ATP of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) and covered by index number 604-098-00-1 (See section 7.6.1)

7.10. Assessment of endocrine disrupting (ED) properties

Most of the available literature studies (until 30/8/2021) are described in this section. For the environmental part and human biomonitoring, a few additional articles relevant for the SVHC identification have been included after this date.

OECD CF level 1 : Existing data and non-test information

It was decided not to perform a thorough scientific literature search for *in silico* data because sufficient amount of *in vitro* data on oestrogen modality and steroidogenesis are available. However some of the data are listed underneath.

➔ **General information**

Based on structure activity relationships (SARs) of diverse Oestrogens, BPS is likely to be an ER ligand:

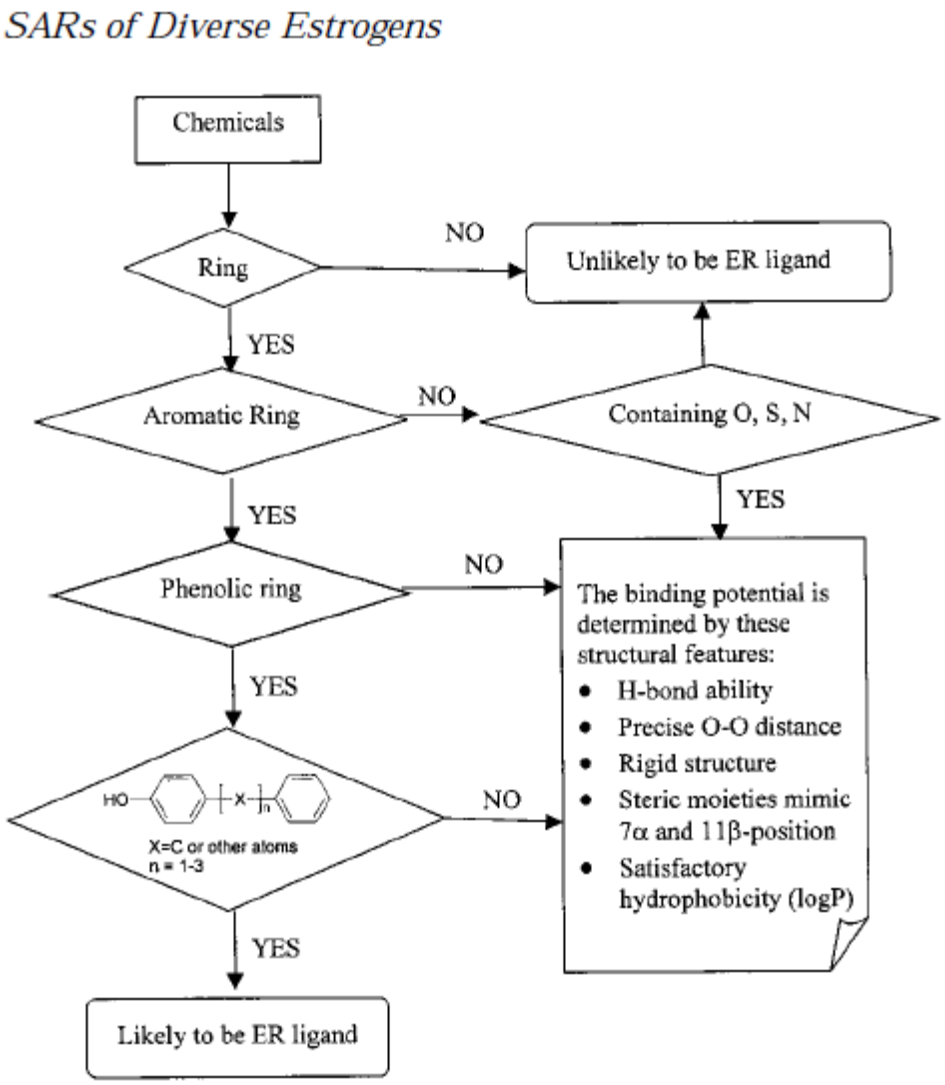


***Figure* 4: Flowchart for identification of ER ligands (Fang *et al*., 2001)**

Structural requirements for endocrine-disrupting activity of BPA and related compounds were investigated:

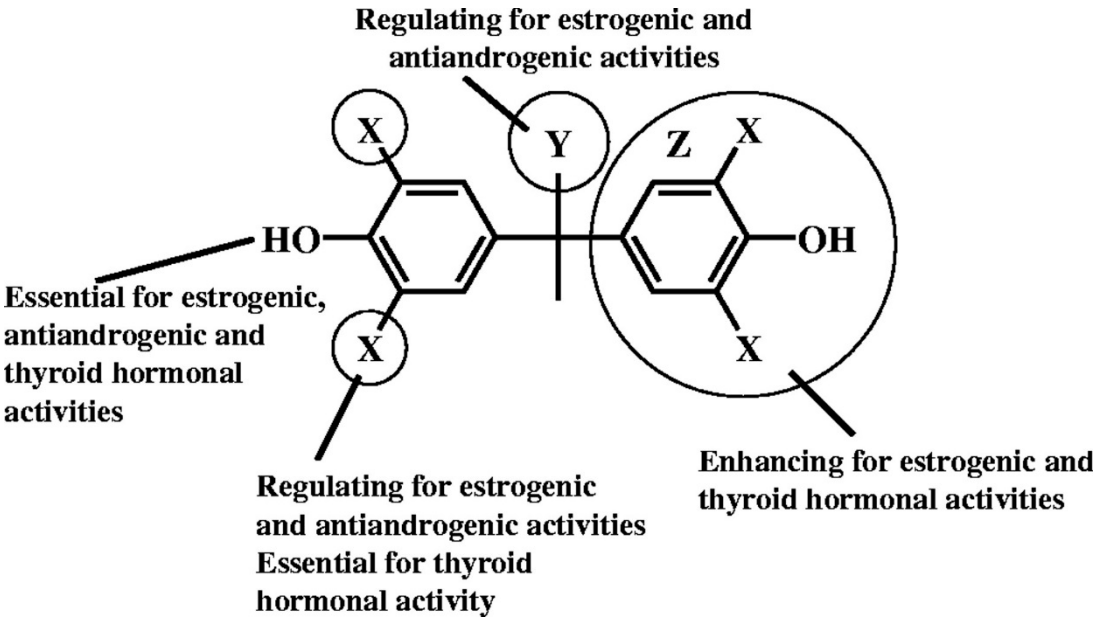


Figure 5: Endocrine-disrupting activity of BPA and related compounds based on their structure (Kitamura *et al.*, 2005)

Following tendencies were deduced:

To exert estrogenic activity an unhindered hydroxyl group on an aryl ring and a hydrophobic group on the para to hydroxyl group is required (Blair *et al.*, 2000; Elsby *et al.*, 2000; Fang *et al.*, 2000; Hong *et al.*, 2002; Nishihara *et al.*, 2000), for bisphenol derivates this means a phenolic hydroxyl group.

A hydroxyl group on the A-phenyl ring is essential for Anti-androgenic activity. The activity of phenyl phenols was in the order of 3-hydroxyl>4-hydroxyl>2-hydroxyl (Kitamura *et al.*, 2005; Paris *et al.*, 2002)

Experimental data suggested that not only the distance between para hydroxyl groups but also the nature of the bridging carbon substituent determined their oestrogenicity. Furthermore it is concluded that higher polarity reduces the oestrogenicity (Molina-Molina *et al.*, 2013)

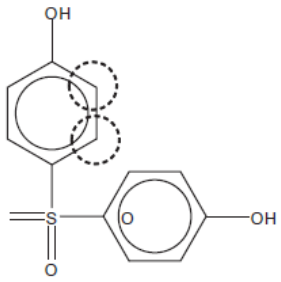
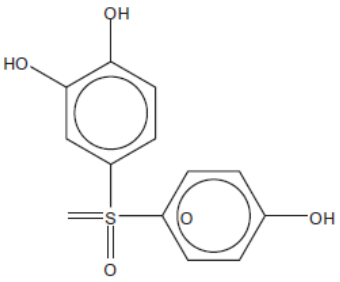
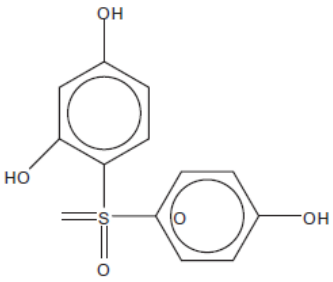
Thyroid receptor shows a rigid substrate specificity compared to the estrogenic and androgenic receptor, because of the relatively smaller size of the active site (Wagner *et al.*, 1995 and 2001). Kitamura *et al.* (2005), demonstrated in their study that a 4-hydroxyl group and double substitution by a halogen or methyl group at 3,5-positions of the A-phenyl-group were essential for thyroid hormonal activity.

➔ ***In silico*** **:**

Grignard *et al.* (2012)

The potential site(s) of liver CYP enzyme mediated metabolism of BPS was predicted using the MetaSite software. The main site of phase-1-metabolism is the ortho position of the phenol rings.

Table 66: Overview of metabolites (Grignard *et al.*, 2012)

Substrate	Sites of metabolism	Rank-1 metabolite	Rank-2 metabolite
Bisphenol S		 3-Hydroxy-BPS	 2-Hydroxy-BPA

In silico (MetaSite predictions of liver CYP mediated metabolism using consensus models of CYP29, CYP2D6 and CYP3A4. The predicted sites are indicated by dotted circles on the substrate structure, and the highest ranking metabolites formed according to the predicted sited of metabolism are depicted.

Based on the knowledge on BPA and his liver phase-I biotransformation products on the one hand and the structural similarity between BPA and BPS it can be hypothesize that BPS metabolites could also show lower oestrogenicity than their parent molecule.

Table 67: Overview of oestrogen activity (some literature data)

Non-test data			
Oestrogen activity			
Method	**Result**	**Description of Result**	**References**
Absolute hardness and absolute electronegativity	+	weak ER binding potential	Kobayashi *et al.*, 2001
MultiCASE	+	Weak ER binding activity	Klopman and Chakravarti, 2003
ACD/ToxSuite Software v. 2.95	+	Weak binder to the ER-α	Grignard *et al.*, 2012
QSAR toolbox 3.2.0	+	Very strong ER binder	
MultiCASE v. 2.4.1.4 and Leadscope v.3.04-10	+ -	Positive for ER binding, Estrogenicity reporter gene Antiandrogen	Rosenmai *et al.*, 2014
VirtualToxLab	+	Moderate binding affinity for 16 proteins, strongest binding affinity for oestrogen receptor β	Goldinger *et al.*, 2015
docking programs (GOLD and AutoDock), scoring functions (Gold Score, ChemScore, HintScore; Autodock software)	+	ERα, ERβ, ERRγ,AR (wild type) and AR^{W741L}: low interaction compared to BPA AR^{T877A}: medium interaction compared to BPA	Cavaliere *et al.*, 2020

Oestrogen activity:

Kobayashi *et al.* (2001)

In this study, an attempt was made to classify the relationship between electronic structures and biological activities of endocrine disruptors using the parameters of absolute hardness (η) (or global softness (S)) and absolute electronegativity (χ).
By means of the η-value, the toxicity and ER binding as well as the receptor binding affinity of the sex hormones can be predicted. It is suggested that the smaller the η-value, the softer the compound is and the activity of environmental hormones increases.

Figure 6: Conclusion from the η– χ activity diagram (Kobayashi *et al.*, 2001):

(i) The environmental hormones belonging to electronic coordinates in group I have estrogen activity. A necessary condition is that a phenol-like aromatic system be contained in the molecule.

(ii) The condition for the electronic structure between ligand (χ_1^0, η_1^0) and LBD (χ_{LBD}^0, η_{LBD}^0):

$$\Delta\eta=\eta_1^0-\eta_{LBD}^0\leqq 0.0,\quad \Delta\chi=|\chi_1^0-\chi_{LBD}^0|\leqq 0.0$$

(iii) The condition for the electronic structure among ligand (χ_1^0, η_1^0), agonist (χ_1^a, η_1^a) and antagonist (χ_1^t, η_1^t):

$$\Delta\eta=\eta_1^0-\eta_1^a=\sim\pm 0.0,\quad \Delta\chi=|\chi_1^0-\chi_1^a|=\sim\pm 0.0$$

$$\Delta\eta=\eta_1^0-\eta_1^t=>0.0,\quad \Delta\chi=|\chi_1^0-\chi_1^t|=\sim\pm 0.0$$

(iv) Coordinates of the electronic structure for environmental hormones are located in a position complementarily to that of amino acids (estrogen receptor, *etc.*) in the η–χ activity diagram.

Based on the results of this study, the electronic structures of environmental hormones were classified into four main groups:

- 17β-oestradiol type (I)
- testosterone type (II)
- thyroxine type (III) :
- hexachlorocyclohexane type (IV)

BPS was shown to belong to group III and was described as a soft acid with an absolute hardness η=4.544 eV, absolute electronegativity χ =5.186 eV and a global softness S = 0.2200 eV . Since the strength of affinity for the oestrogen receptor is in the order group III < group II < group I, BPS is predicted to have a weak potential for binding to the oestrogen receptor.

Klopman and Chakravarti (2003)

Klopman and Chakravarti (2003), screened 2526 high production volume chemicals for their oestrogen receptor binding activity using a quantitative structure-activity knowledge database generated by the MultiCASE expert system. The program aims to discover substructures or biophores that appear mostly in active molecules and may therefore be responsible for the observed activity. MultiCASE also identifies substructures and physicochemical parameters as modulators of activity and uses them in the construction of local QSARs. The knowledge that the program gained during the training process can be used to predict the biological activity of new chemicals that were not included in the training set. The authors used a training set of 313 chemicals from different chemical groups.

The oestrogen receptor binding data was placed on a common 'relative binding affinity' (RBA) scale, where the RBA is defined as 100 times the ratio of the molar concentrations of [3H]oestradiol and the competing chemical required to decrease the receptor bound radioactivity by 50%.

The RBA of the training set ranges from ~398 to no binding at all. The breakpoint between what is considered an oestrogen active and oestrogen inactive was set as follows: chemicals were considered:

- active if their RBA value is > 0.0004,
- marginal if their RBA value is <= 0.0004 and > 0.0001, and
- inactive if their RBA is <=0.0001.
-

The model performed well during validation (r2 = 0.881 and an average 84.04% correct classification in 10 leave-10%-out cross-validations).

For BPS, the predicted RBA was 0.0006, with a probability of 87%, indicating weak oestrogen receptor binding activity.

Grignard *et al.* (2012)

Besides examining *in vitro* the estrogen activity of BPA and BPS by using two highly standardized transactivation assays, the authors reported also in silico predictions in their study.

The estrogen receptor-α binding was predicted using the ACD/ToxSuite Software v. 2.95. Compounds having a log RBA > 0 may be classified as strong ERα binders, compounds having a log RBA < -3 may be classified as non-binders, whereas a log RBA value between 0 and -3 indicates weak binding activity.

The log Relative Binding Affinity used in this training set for BPS was -2.26, indicating a weak binding affinity. The calculated probability for overall binding (logRBA>-3) was 61%, the probability for strong binding (logRBA>0) was calculated to be 1%.

Table 68: Predicted probabilities of ERα binding

In silico predictions of estrogen receptor-α binding.

Predicted probabilities of ERα binding	BPA (%)	BPS (%)
Probability of overall binding (LogRBA > −3)	95	61
Probability of strong binding (LogRBA > 0)	37	1

Estrogen receptor binding profiler (OECD (Q)SAR toolbox 3.2)

The OECD (Q)SAR toolbox for Grouping Chemicals into Categories (hereafter called the OECD toolbox or Toolbox) contains a profiler for endocrine disruption, based on data from receptor binding.

Oestrogen receptor (ER) binding is a molecular initiating event much like protein binding. It is an endpoint where several comprehensive databases exist, which has led to the development of several approaches for using (Q)SARs to predict ER binding and possible subsequent endocrine disruption (OECD toolbox, Getting Started document).

The incorporated ER binding profiling scheme is based on structural and parametric rules extracted from literature sources and supported by experimental data. The Oestrogen Receptor Binding grouping method contains simple categories for ER binding. This method is relevant for reproductive toxicity endpoints in fish and mammals.

The ER-binding profiler classifies chemicals as non-binders or binders depending on molecular weight (MW) and structural characteristics of the chemicals i.e. a cyclic molecular structure with a single non-impaired hydroxyl- or amino group in the para- or meta-position on the ring. Substances with these features are ER binders. Binding potency is related to the size and shape of the molecule, which can be grossly measured by molecular weight. The ER profiler defines four categories of ER-binders:

1. Very strong binders: Chemicals with MW between 200 and 500 Da and two rings with a hydroxyl group connected to each of them.
2. Strong binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 200 and 500 Da;
3. Moderate binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 170 and 200 Da;
4. Weak binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW less than 170 Da;

If a chemical does not meet the structural and parametric requirements of category 1-4, it is classified as non-binder.

BPS has a MW of 250.27 and two cyclic molecular structures each with a single non-impaired hydroxyl group. BPS is thus estimated, by this model, to be a very strong ER binder.

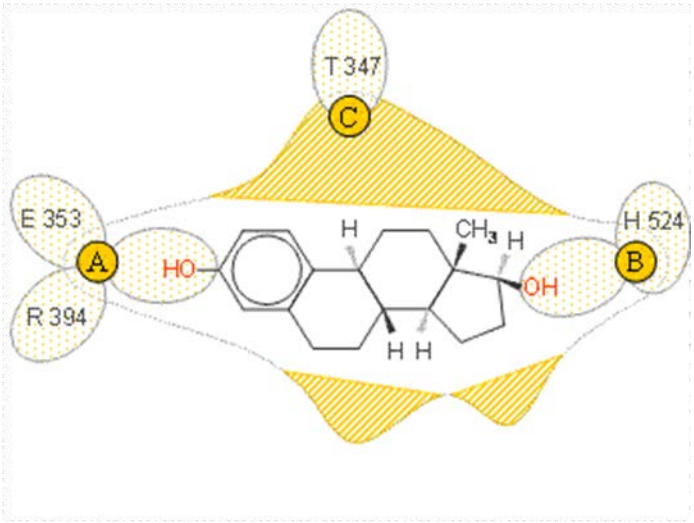


Figure 7: Scheme that reflects the ER binding of BPS (Grignard *et al.*, 2012)

<u>Rosenmai *et al.* (2014)</u>

Authors applied two QSAR models (MultiCASE and Leadscope) to predict the endocrine disruption of Bisphenol A an it's structural analogues, like BPS. BPS was found positive for ER binding and estrogenicity reporter gene.

Table 69: QSAR model prediction for BPA and its analogues (Rosenmai *et al.*, 2014)

QSAR Predictions for Test Co

			BPA	BPB	BPE	BPF	BPS	HPP
Metabolism[a]	CYP2D6	Substrates	0.69	0.69	0.69	0.69	0.34	0.74
		Inhibitors	0.64	0.73	0.64	0.62	0.16	0.67
	CYP3A4	Substrates	0.82	0.83	0.80	0.80	0.36	0.84
		Inhibitors	0.59	0.62	0.58	0.57	0.37	0.55
	CYP2C9	Substrates	0.64	0.65	0.64	0.62	0.73	0.70
		Inhibitors	0.27	0.33	0.26	0.25	0.29	0.26
	PXR binding[b]		0.64	0.75	0.60	0.53		0.74
Endocrine disruption	ER binding[b]		*		*	*	*	*
	Estrogenicity reporter gene[b]		*		*	*	*	*
	Antiandrogen[b]		*			*		
Reprotoxicity	Teratogenicity FDA TERIS							
Genotoxicity	Ashby structural alerts for DNA reactivity		*					
	Reverse, mutation test, Ames[b]		*				*	
	Chromosomal aberrations in CHO[b]		*					
	Chromosomal aberration in CHL[b]							
	Mouse lymphoma[b]		*					
	HGPRT/CHO[b]							
	UDS Rat hepatocytes[b]							
	SHE cell transformation[b]		*					
	Rodent dominant lethal[c]							
	Drosophila melanogaster SLRL[c]		*					
	SCE Mouse[c]							
	Mouse micronucleus[c]		*					
	COMET assay[c]							
Cancer	Carcinogenicity	Male rats (AF1)	*					
		Female rats (AF2)	*					
		Male mice (AF3)	*					
		Female mice (AF4)	*					
	RCA overall QSAR call (AF1-4)							
Sensitization	Skin		*					
	Respiratory							
Irritation	Skin		*				*	

Notes. Color code: red, positive; green, negative; white, out of domain. (*) Included in the training set of the model and the experimental result is indicated.
[a]Leadscope model, $p \geq 0.7$ and $p \leq 0.3$ is a positive and negative prediction, respectively.
[b]*In vitro*.
[c]*In vivo*.

Goldinger *et al.* (2015)

In this study the endocrine and metabolic disruption of BPS was predicted using VirtualToxLab™. This tool estimates the toxic potential and binding affinity to 16 proteins. BPS showed, with a Toxic potential of 0.380, a moderate risk of binding the proteins. The strongest binding affinity of BPS was for the oestrogen receptor β (0.742 µM).

Cavaliere *et al.* (2020)

26 bisphenols were computationally analysed for their capability to bind to 4 different sex steroid nuclear receptors (Erα, Erβ, ERRγ and AR) and 2 adenocarcinoma-relevant AR mutated forms (AR^{T877A} and AR^{W741L}) which are commonly developed by patients with AR-dependent adenocarcinoma prostate cancer treated with AR antagonist.

The analysis was performed with 2 different docking programs (GOLD and AutoDock) and 4 scoring functions (Gold software to generate the poses of each chemical which were scored with GoldScore, ChemScore and HintScore; Autodock software to generate and score the poses with the internal scoring function). BPA was used as reference compound.

- Computational Erα binding affinity:

BPM > MBP > BPP > BPTMC > TBBPA > BPAP > BPG > E2 > TCBPA > TMBPA > BPC > BPB > BPA > BPPH > BPBP > BPZ > BPE > BPA quinone > BPAF > BPC2 > BPA catechol > BPF > BPA_ol > BPA carboxylic > BPA sulfate > **BPS** > BPA glucuronide.

- Computational ERβ binding affinity:

BPM > BPP > MBP > E2 > BPG > BPBP > BPTMC > BPAP > TBBPA > BPAF > BPE > TCBPA > BPZ > BPC > BPA > BPB > BPA catechol > BPA quinone > BPPH > TMBPA > BPC2 > BPF > BPA_ol > BPA sulfate > BPA carboxylic > **BPS** > BPA glucuronide

- Oestrogen Related Receptor γ (ERRγ):

TCBPA > TBBPA > BPTMC > BPA catechol > BPAF > BPE > MBP > BPF > BPA glucuronide > **BPA** > BPB > BPC2 > BPBP > BPC > BPAP > BPZ > TMBPA > BPM > BPA quinone > BPA sulfate > BPA carboxylic > BPPH > BPP > **BPS** > BPG > E2 > BPA_ol.

- AR (Wild Type) binding affinity prediction:

BPPH > BPM > BPA sulfate > BPA > BPAF > TBBPA > BPB > MBP > BPG > BPC > BPA catechol> BPE>BPZ>BPAP>TCBPA>TMBPA>BPF>BPTMC> BPP > BPA quinone > BPC2>DHT > BPA ol > T > 2OHFTA > **BPS** > BPBP > BPA carboxylic > BPA glucuronide.

- computational AR^{T877A} binding affinity

BPB > BPA catechol > MBP > BPM > BPG > BPZ > BPE > BPAP > BPF > BPP > TBBPA > BPPH > BPA > BPC2 > BPAglucuronide > 2OH-FTA > BPC > BPTMC > TCBPA >DHT > BPA sulfate > T > TMBPA > **BPS** > BPAF > BPA quinone > BPA carboxylic > BPA ol > BPBP

- computational AR^{W741L} binding affinity:

BPPH > BPP > BPA catechol > TBBPA > MBP > TCBPA > BPM > BPAF > BPE > BPA glucuronide > BPC > BPF > BPAsulfate>2OHFTA > TMBPA > BPB > BPAP > BPG > BPA > BPZ > T > BPC2 > BPTMC > BPBP > BPA ol > DHT > BPA quinone > BPA carboxylic > **BPS**.

Substances were predicted to be H (higher interactors= better ligands) , M (medium) and L (lower interactors=worse ligands) compared to BPA.

Table 70: Overview of higher and lower interactors

BPS	ERα	ERβ	ERRγ	AR	AR^{T877A}	AR^{W741L}
	L	L	L	L	M	L

The computational findings for Erα and Erβ are in line with the *in vitro* findings that BPS has a weaker binding affinity than BPA. BPS was also found to show less binding affinity than BPA for ERRγ, AR and AR^{W741L} but was predicted as a medium interactor for AR^{T877A}. It should be highlighted that although BPS was predicted a lower interactor for AR, it was found to have similar binding capacity as the pharmacological anti-androgen 2OH-FTA and therefore authors conclude that BPS does not appear a completely safer alternative to BPA.

From molecular dynamic simulation performed with BPA, BPS and BPF, it can be concluded that BPS and BPF form more stable hydrogen bonds than BPA. The latter binds the protein establishing mainly hydrophobic interactions. In the presence of BPS protein and binding pocket flexibility were reduced. For the estrogen activity it seems that hydrophobic interactions are preferred to hydrogen bonds (BPA > BPF > BPS) which might be related to greater protein flexibility in presence of hydrophobic interactions which could be

necessary to allow interaction with the different proteins, like co-activators, needed to manifest real receptor activation.

Androgen activity :

See estrogen activity : Rosenmai *et al*., 2014 and Cavaliere *et al*., 2020

Conclusion : OECD CF level 1 data

* Estrogen activity :
  BPS is predicted to have weak to very strong ER binding potential

* Androgen activity:
  Very low binding affinity, no anti-androgen activity

OECD CF Level 2 : *In vitro* assays providing data about selected endocrine mechanisms/pathways

The available *in vitro* assays for BPS are summarized hereafter. Further description of the different assays are provided after the summary.

1. **Estrogenic MoA**

Table 71: Overview of estrogenic MoA (literature data)

Short Method description	Result (positive/negative)	Description of results (positive/negative)	References
Tox Cast Pathway model (ER) prediction	Estrogenic agonistic activity No estrogenic antagonistic activity	Agonist (AUC) :0.263 agonist Antagonist (AUC): 0 Cytotoxicity limit 13.64 µM	ToxCast, EPA
ER competitive binding assay (Rat uterine cytosol) US EPA OPPTS 890.1250	ER binder	Very weak affinity for ER IC50 = 1.05×10^{-4} M RBA (compared to E2) = 0.0009%	Blair *et al.*, 2000
Yeast two-hybrid system (ERα + coactivator TF2)	No agonist estrogenic activity	No estrogenic activity up to 1×10^{-3} M (i.e. 10% of the agonistic activity of 10^{-7} M E2 was not reached)	Nishihara *et al.*, 2000
Yeast two-hybrid system (ERα)	No agonist estrogenic activity	No estrogenic activity between 10-7 and 10^{-3} M (i.e. the relative β-galactosidase activity (rate of β-galactosidase activity divided by that of 10^{-7} M E2) was below 0.1)	Hashimoto and Nakamura, 2000
Fluorescence polarization (hERα)	ER binder	~70% displacement of the fluorescence ligand ES1 from the hERα-ES1 complex at 10^{-3} M	

E-screen (MCF-7)	Agonist estrogenic activity	MCF-7 cells proliferation at 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M	
Yeast two-hybrid system	Agonist estrogenic activity No agonist estrogenic activity	Weak estrogenic activity at 10^{-3} M with S9 mix, with a relative β-galactosidase activity of 0.3 No estrogenic activity at concentration between 10^{-7} and 10^{-3} M without S9 mix	Hashimoto *et al.*, 2001
Fluorescence polarization (hERα)	ER binder	~70% displacement of the fluorescence ligand ES1 from the hERα-ES1 complex at 10^{-3} M	
E-screen (MCF-7)	Agonist estrogenic activity	MCF-7 cells proliferation at 10^{-6} and 10^{-5} M (1.5 fold)	
Yeast two-hybrid system (Erα)	Agonist estrogenic activity	Very weak estrogenic activity at 200 mg/L with a relative β-galactosidase activity of 0.0105 (positive if > 0.01)	Chen *et al.*, 2002
Estrogen receptor competitive binding assays Human ERα reporter gene transcription	ER binder Agonist estrogenic activity	Weak estrogen receptor binding affinity RBA=0.0055% Weak relative activity (RA) of BPS regarding E2. The RA is the ratio between the concentration giving 50% of the maximum transcriptional activity of E2 1nM (PC50) of BPS divided by the PC50 of E2. RA = 0.000254	METI, 2002 (ER binding results also reported in Yamasaki *et al.*, 2004)
Estrogen receptor competitive binding assays (human recombinant Erα)	ER binder	Weak estrogen receptor binding affinity RBA=0.00549%	Yamasaki *et al.*, 2004
Fluorescent protein (GFP) expression system (MCF-7)	Agonist estrogenic activity No antagonistic activity	Weak estrogen potency, with an EC50 value of $1.75x10^{-6}$ (concentration showing 50% of the maximum fluorescence obtained with 50 pM E2) and an EEF of $5.54x10^{-6}$ (estradiol equivalency factor, i.e. EC50 of E2 divided by EC50 of BPS) No antagonistic estrogenic activity	Kuruto-Niwa *et al.*, 2005
Estrogen responsive element-luciferase reporter assay (MCF-7)	Agonist estrogenic activity No antagonist estrogenic activity	Significant estrogen agonist activity (EC50=1.1 µM) No significant antagonist activity	Kitamura *et al.*, 2005

Estrogen receptor competitive binding assay (rat uterine cytosol)	ER binder	Weak estrogen receptor binding activity IC50=64.0 µM Ki=82.4 µM 50-74% displacement of 17β-estradiol	Laws *et al.*, 2006
Estrogen receptor binding assay using recombinant human estrogen receptor α	ER binder	Weak estrogen hERα receptor binding activity Log RBA = -2.26 (RBA=0.00549%)	Akahori *et al.*, 2008
Transactivation assays using MELN cells Transactivation assays using BG1Luc4E2 cells (comparable to OECD TG 455)	Agonist estrogenic activity Agonist estrogenic activity	weak estrogenic activity MELN: EC50=4.24x10^{-6} M weak estrogenic activity BG1Luc4E2: relative response >50% EC50=4.93x10^{-6} M	Grignard *et al.*, 2012
Estrogen-induced (via membrane ERα) non-genomic signalling in a rat GH3/B6/F10 pituitary cell line	Agonist estrogenic activity Antagonist estrogenic activity	BPS can induce nongenomic signalling in pituitary cells at low concentration, mainly via activation of membrane ERα, leading to ERK, but not JNK kinase activation, in range similar to E2. BPS can also alter the timing of response to E2 (PRL release) at very low concentrations.	Viñas and Watson, 2013
Transient transfection luciferase assays using Monkey kidney CV1 cells	Agonist estrogenic activity No antagonist estrogenic activity	weak effect on ERα transcriptional activity EC50 =2.2 x 10^{-6} M No inhibition of ERα function (from 3 x 10^{-8} to 10^{-13} M) No effect	Teng *et al.*, 2013
Transactivation assays (luciferase) using MELN cells Transactivation and competitive binding assays using HELN-hERα and –hERβ E-screen (MCF-7)	Agonist estrogenic activity Agonist estrogenic Agonist estrogenic activity No antagonist estrogenic activity	EC50=1.21 x 10^{-5} M in HELN-hERα: EC50=3.96 x 10^{-6} M , IC50=6,56 x 10^{-6} M, RBA=0.001% in HELN-hERβ: EC50=1.72 x 10^{-6} M, IC50= 3.452 x 10^{-6} M, RBA=0.006% Increased proliferation (~3.7-fold) at 10^{-5} M compared to control	Molina-Molina *et al.*, 2013

		No antagonistic activity is reported (when co-incubated with 10^{-10} M E2)	
Luciferase reporter gene assay using MVLN cells (OECD TG 455)	Agonist estrogenic activity	BPS showed dose-dependent estrogenic activity : EC50=6.97 x 10^{-5} M REP50=4.09 x 10^{-9} at 0.5 µM of BPS (REP = relative potency to E2) The oestrogenic activity of the metabolites formed by S9 fraction showed increasing estrogenicity	Kang *et al.*, 2014
Estrogen receptor (ER) reporter gene assay using BG1Luc cells (OECD 457)	Agonist estrogenic activity	ER activity increased Emax: 222% EC50: 1.17 µM	Rosenmai *et al.*, 2014
Yeast reporter assay based on BPA-targeted receptor (BPA-R) Yeast reporter assay based on hERα	ER binder ER binder	Weak affinity for ER (2.5 orders of magnitude less potent than BPA) Weak affinity for ER (2 orders of magnitude less potent than BPA)	Rajasärkkä *et al.*, 2014
Fragment complementation assay (PCA) PRL assay Cell proliferation assay (MCF-7)	Erα and Erβ binding activity (ERα-LBD and Erβ-LBD stably expressed by fusion protein constructs in HEK93T cells) Estrogenic activity	Low binding activity of Erα and Erβ (log EC50=-4) Erα: no activity ERβ: EC50: 10^{-5}M Increased proliferation RPE= 97.54% RPP= 0.000105%	Stossi *et al.*, 2014
Chicken in vitro screening assay together with the Avian ToxChip PCR array (chicken embryonic hepatocyte)	Increased gene expression of estrogenic biomarker	At 300µM, BPS significantly up-regulated ApoII (by 30.7 fold), Vtg (by 10.3 fold) and other genes for which the relevance for ED MoA is not clear. No cell cytotoxicity up to 300 µM	Ma *et al.*, 2015

Bioluminescent Yeast reporter assay (BLYES) based on estrogen-response elements	Estrogenic activity (ER binder)	Weak estrogenic activity (2 orders of magnitude less potent than BPA) EC_{50} = 4.13 x 10^{-5} µM EEF = 1.05 x 10^{-6}	Ruan *et al.*, 2015
Estrogen-response element (ERE-luciferase) transcription assay (COS-7)	Estrogenic activity	Increased activation of ERE-luciferase reporter up to 3-fold	Boucher *et al.*, 2016
Yeast reporter assay (Xenometrix) based on hERα	Estrogenic activity (agonist)	EC_{50} = 8.4 x 10^{-5} M	Skledar *et al.*, 2016
ER transactivation assay (ERα) (T47D-KBluc cells (ERα and Erβ))	Estrogenic activity	EC_{50} = 6.43 x 10^{-7} M RPF (relative potency factors , to reference compound E2) = 1.55 x 10^{-6}	Conley *et al.*, 2016
Estrogen receptor transactivation assay (OECD TG 455/457) (VM7Luc4E2 (formerly BG1Luc4E2) Yeast based reporter gene assay (YES) (hERα)	Estrogenic activity (agonist) No antagonistic estrogenic activity Estrogenic activity (agonist)	Estrogenic activity observed No effect Estrogenic activity observed	Dvořáková *et al.*, 2016
luciferase gene expression with BG1Luc4E2 and MVV-luc (MCF-7)	Estrogenic activity (agonist) No antagonistic estrogenic activity	Agonistic activity: complete inhibition of the cell response: relative response >50% Antagonistic activity: no effect	Simon *et al.*, 2016
Competitive ER binding assay (Human U251 glia cells transfected with Zebrafish ERα, ERβ1 and Erβ2) Fluorescence in situ hybridisation (Human U251 glia cells transfected with zebrafish ERα, ERβ1 and ERβ2)	Estrogen binder No effect	BPS was almost not able to move [3H]-E2 from the zfERα, zfERβ 1 and zfERβ 2-bindingsite (probably due to the absence of binding to Erα): very weak estrogen receptor binder No stimulation of transcriptional activity of zebrafish nuclear receptors (ERa, ERb1,and ERb2) transfected in U251MG cells	Cano-Nicolau *et al.*, 2016
ER transactivation assay using MCF-7 and SK-BR-3	Not significant antagonistic estrogenic activity	Reduced transcriptional activity, but not statistically significant	Okazaki *et al.*, 2017
Pig oocytes exposed to BPS *in vitro*	Estrogenic activity	Reduced ERα and aromatase mRNA	Zalmanova *et al.*, 2017
MCF-7 proliferation assay	Estrogenic activity	Increased proliferation of MCF-7 cells (10^{-6} to 10^{-5}M)	Kim *et al.*, 2017
E-screen (MCF-7, T47-D, MDA-MB-231)	Estrogenic (agonistic) activity Estrogenic (agonistic) activity	MCF-7 AC_{50} = 1.33 µM T47-D Proliferation increased	Mesnage *et al.*, 2017

ERE-mediated luciferase assay (T47D-KB-luc cells)	 No estrogenic effect Estrogenic (agonistic) activity No antagonistic estrogenic activity	Same trend as seen in MCF-7 but at a lesser extend MDA-MB-231 No cell proliferation AC_{50} = 1.5 µM Addition of ICI 182,780 (100 nM) antagonised the effect of BPS No antagonistic effect	
Zebrafish ER transactivation assay (Zebrafish hepatic reporter cells: zfERα, zfERβ1 and zfERβ2)	Estrogenic (agonist)	EC50 of 4.058 µM in zfERα, 1.016 µM in zfERβ1 and 2.468 µM in zfERβ2 (REPE2) to each receptor was $3.7x10^{-5}$, $3.0x10^{-5}$ and $2.4x10^{-5}$ resp. => slightly more potent towards zfERβs than zfERα	LeFol *et al.*, 2017
Fish primary macrophages (Fish primary macrophages of *Cyprinus carpio*)	Estrogenic	Significant increase of ERα and ERβ expression at conc ≥ 1 µg/L	Qiu *et al.*, 2018a
Yeast estrogen screen (YES) assay (hERα)	Estrogenic	EC_{50} = 5.88 x 10^{-4} M EE (estradiol equivalent) = 1.07 x 10^{-6}	Conroy-Ben *et al.*, 2018
Luciferase reporter assay (MCF-7, hepG2, BG-1FR) ERE-mediated luciferase reporter assay (human enodmerialadenocarcinoma, Ishikawa/Erα)	Estrogenic (agonist) activity (Erα) No estrogenic activity (Erβ)	MCF-7 ERα specific binding: ERE-mediated activation at $1x10^{-6}$M (3-fold) HepG2 BPS only weakly activated the ERα ERE at 100 nM (8-fold), at 1000 nM (30-fold) No ERβ ERE mediated activation BG-1FR ERE-mediated activation at 1000 nM (± 5-fold) Ishikawa/ERα ERE-mediated activation at 1000 nM (3-fold) No Erβ-mediated activation	Li *et al.*, 2018
Luciferase reporter assay based on ERα or Erβ (CHO-K1)	Estrogenic (agonist) No agonistic activity	ERα: REC_{50} = 5.4 x 10^{-7} M ERβ: REC_{50} = 5.4 x 10^{-7} M No effect	Kojima *et al.*, 2018
Fluorescence polarization assay using hERα-LBD	Estrogenic binding activity	IC50=5.78 µM	Zhang *et al.*, 2018a
Estradiol and progesterone production in bovine granulosa and theca cells	Effect on steroidogenesis	Increased production of estradiol at high dose in granulosa cells.	Campen *et al.*, 2018

	(estrogenic activity) No antagonistic activity	 ERα Rela EC50= - ERβ Rela EC50= -	
Proliferation and migration assays MCF-7 (ER-Positive) and MDA-M-231 (ER-negative) breast cancer calls	Estrogen activity	Increased proliferation and migration in MCF-7, but not in MDA-MB-231	Awada *et al.*, 2019
Estrogen Receptor Transactivation assay (HEKT 293 (ERα and ERβ)	Estrogenic binding activity	ERα : IC50 = 0.97 µM ERβ: IC50 = 8 µM	Eilebrecht *et al.*, 2019
E-screen using MCF-7 cells	Estrogenic	No positive correlation between BPS concentrations in thermal paper and estrogenic activity	Molina-Molina *et al.*, 2019
Luciferase reporter assay based on ERα or Erβ (HepG2)	Estrogenic (agonist) No antagonist estrogenic activity	ERα: REC_{50} = 1.3 x 10^{-6} M ERβ: REC_{50} = 2.1 x 10^{-6} M No antagonistic effect	Pelch *et al.*, 2019
Cell proliferation MCF-12A, MCF-7 and MCF-10A Luciferase reporter assay based on ERα and ER β	Estrogenic (agonist) activity No estrogenic activity	<u>MCF-12A</u> Increased activation of Erα at 1 µM (2.5-fold) and at 10 µM (5-fold), Erβ followed a similar trend <u>MCF-7</u> Proliferation of MCF-7 (expressing ERα and ERβ) at 1 µM <u>MCF-10A</u> no proliferation of MCF-10A (expressing only ERβ) at 1 µM	Atlas and Dimitrova, 2019
Reporter gene assay (COS-7 cells (from monkey kidney) transfected with VP16-ERα or VP-16-Erβ)	Estrogenic activity	ERα: Sign. increased at 1.0 (2.5-fold) and 10 µM (5-fold) ERβ: sign. increased 10 µM: similar trend as ERα	
Luciferase assay based on ERα, ERβ and ERRγ	Estrogenic (agonist)	ERα: EC_{50} = 4.47 x 10^{-6} M ERβ: EC_{50} = 1.72 x 10^{-6} M ERRγ: no activity	Grimaldi *et al.*, 2019
Competitive binding assay (ERα and Erβ)	ER binding	ERα: IC50>10 µM Erβ: IC50>10 µM	Liu *et al.*, 2019b
Cells proliferation assay Aromatase activity (MCF-7, ZR-75-1 and HMF3A)	Erα-dependent proliferation No Erβ-dependent proliferation	Increased ERα-dependent cell proliferation: stat. sign. at 10^{-8}M in MCF-7, ZR-75-1 cell lines, but not in Erβ-dependent cell line HMF3A	Williams and Darbre, 2019

Protein isolation and western blotting (MCF-7) Luciferase assay (STTA, OECD TG 455) (HeLa9903 (hERα)	Estrogenic (agonist)	Significant decrease of ERα by 0.53-fold after exposure to 10 µM EC_{50} = 5.98 x 10^{-6} M PC50= 0.43 µM (response that is 50% of the maximal positive control response)	Park *et al.*, 2020
Fluorescence polarisation (Polar screen E-TM wcompetitor assay)ERα and ERβ	No effect	No inhibition of binding to the fluorescent compound(ERα nor ERβ)	Keminer *et al.*, 2020

Tox Cast Pathway model (ER) prediction

Agonist (AUC): 0.263 agonist and 0 antagonist.
Cytotoxicity limit 13.64 µM

Blair *et al.*, 2000

A validated (standardized) estrogen receptor (ER) competitive binding assay using Rat Uterine Cytosol (OPPTS 890.1250) was used to determine the ER affinity for a large, structurally diverse group of chemicals. In this study ovariectomised retired breeders were used which have comparable ER levels as both immature intact animals and adult ovariectomised rats. Cells were exposed for 20h to a BPS concentration range of 10^{-11} -10^{-3} M. Relative binding affinity (RBA) values were determined as well as the IC50 values, i.e., the concentrations at which the maximal binding of the reference chemical (3H)-estradiol was reduced by 50%. Overall, 188 chemicals were assayed. In general, the results indicate that chemicals with 2 ring structures separated by 2 carbon atoms have higher RBAs compared to chemicals with a single ring structure or 2 rings separated by 1 carbon atom.

Substances with log RBA>0 were considered as “strong binders”

Substances showing log RBA between 0 and -2 were considered as “moderate binders”

And substances with log RBA<-2 were considered as “weak binders”

The IC50 value for BPS was determined to be 1.05 x 10^{-4}M (compared to 8.99 x 10^{-10} M for (3H)-estradiol). The RBA was 0.0009% and the log RBA was reported to be -3.07, indicating that the substance has a very weak affinity for the estrogen receptor.

Nishihara *et al.* (2000)

Nishihara *et al.* (2000) developed a method to screen the estrogenic activity of 517 chemicals : the yeast two-hybrid method with ERα oestrogen receptor and the coactivator TIF2. Introduction of the coactivator TIF2 ensures a closer resemblance to the mammalian hormone system (in comparison to the YES assay, another reporter gene assay using yeast cells).

Genetically modified yeast cells (typically *Saccharomyces cerevisiae*) containing the gene for the human estrogen receptor, coupled to two expression plasmids (pGBT9-ERLBD and pGAAD424-TIF2) which carry a β-galactosidase reporter gene. If a substance binds to the estrogen receptor the coupled reporter gene is also read. Typical reporter genes encode for an enzyme (β-galactosidase), which converts a dye and thus induces a colour reaction. By measuring β-galactosidase activity an indication can be obtained of the estrogenic activity of the test substance.

In this study yeast cells were exposed for 4h. A chemical substance was considered positive when its activity was more than 10% of the activity van 10^{-7} M E2 (REC10).

BPS did not reach REC10 up to the highest concentration tested (1 x 10^{-3} M). This means that BPS didn't show estrogenic activity under the conditions of the test.

Hashimoto and Nakamura (2000)

In this study, 28 chemicals were tested for oestrogenicity at concentrations ranging from 10^{-7} to 10^{-3} M. 17β-estradiol at 10^{-7} M was used as a positive control. Three different assays were performed.

- The yeast two-hybrid system according to a technique originally described by Nishikawa *et al.* (1999). The yeast two-hybrid system appears to be less sensitive that the fluorescence polarization method or the E-screen test. Test duration: 4h.

- The fluorescence polarization method (a competitive ER binding assay) developed by Bolger *et al.* (1998). This method measures the capacity of a competitor chemical to displace a high affinity fluorescent ligand from purified, recombinant human estrogen receptor-[alpha]. Test duration: 1h.

- The E-screen test of estrogenicity according to a technique originally described by Villalobos *et al.* (1995). The E-screen test is based on the ability of MCF-7 human breast cancer cells to proliferate in the presence of estrogens. When a chemical was cytotoxic to MCF-7 cells, the E-screen could not be used. Test duration: 144h.

In the yeast two-hybrid system, BPS did not induce any significant β-galactosidase activity, indicating no estrogen activity with this test design.

In the fluorescence polarization study, BPS could significantly displace the ligand from the hERα-ES1 complex at a concentration of 10^{-3} M, indicating its potential to bind to the estrogen receptor. Finally, BPS significantly increased MCF-7 cell growth at concentrations of 10^{-7} up to 10^{-4} M (the latter also resulting in cytotoxicity).

Discrepancies between the studies are reported. This is possible due to the fact that in the Yeast two-hybrid assay, yeast cells are more robust than mammalian cells and have a rigid cell wall. Some chemicals may not be able to permeate trough such a cell wall; Furthermore co-activator proteins involved in the estrogenic activity might be different between the assays.

BPS did not show estrogenic activity at concentrations of 10^{-7}, 10^{-6} and 10^{-5} M in the yeast two-hybrid system and the fluorescence polarization method, while such activity was seen at those concentrations in the E-screen.

Hashimoto *et al.* (2001)

In this study the estrogenic activities of 13 bisphenol A-related chemicals were examined using three *in vitro* bioassays (similar to those used by Hashimoto and Nakamura, 2000). Concentrations of 10^{-7} to 10^{-3} M were used in the yeast two-hybrid assay (test duration: 4h) and in the fluorescence polarization system (test duration: 1h). Dilutions of 10^{-9} to 10^{-4} M were assayed in the E-screen test (test duration: 144h). The yeast two-hybrid assay was performed in the absence and presence of metabolic activation (S9 mix).

BPS did induce significant β-galactosidase activity at concentrations of 10^{-3} M (i.e., the highest concentration tested) in the yeast two-hybrid assay in the presence of metabolic activation, whereas it did not in the absence of the S9 mix. A β-galactosidase activity above

0.1 was adopted as criterion for estrogenic activity. Estrogenic activity of the BPS metabolites increased after 60 min incubation with the S9 fraction.

Overall, the activity of other chemicals was (also) higher in the presence of S9 mix.

In the fluorescence polarization assay similar observations were made as those reported by Hashimoto and Nakamura (2000). BPS was able to significantly displace the fluorescence ligand FES from the ER–FES complex at the highest concentration tested (i.e., 10^{-3} M).

In the E-screen test, a 1.5-fold increase in cell growth over the control (DMSO) was adopted as a criterion for estrogenic activity. BPS did yield a 1.5-fold increase in cell growth or more at 10^{-6} and 10^{-5} M (difficulty to assess the 1.5-fold increase with the concentration 10^{-7} and 10^{-4} M due to the fact that the results are only provided in a figure). No raw data are available.

Chen *et al.* (2002)

Chen *et al.* (2002), investigated the estrogenic activity of bisphenol A and related compounds using the yeast two-hybrid (*Saccharomyces cerevisiae*) assay using ERα and the coactivator TIF2. Estrogenicity is detected by the expression of the β-galactosidase reporter gene. In this study, a chemical is regarded as positive (or showing estrogenic activity) when it shows β-galactosidase activity higher than the 10% level of the activity given by 17β-estradiol at the optimum concentration (i.e., 1230 units at a concentration of 10 µg/L). The β-galactosidase activity of the negative control was 37.7 units. Yeast cells were exposed for 4h to a concentration range of 0.001 to 200 mg/L BPS.

BPS showed only 130 units at the highest concentration tested (200 mg/L) and was identified as a chemical with positive, but very weak, estrogenic activity (seven orders of magnitude lower than 17β-estradiol and only slightly higher that the negative control).

From all bisphenol compounds tested, BPS had the lowest estrogenic activity in this test.

METI (2002)

The Japanese Ministry of Economy, Trade and Industry reported results of estrogen receptor competitive binding assays and reporter gene experiments for a large number of chemicals, including BPA and BPS. Based on the results of the estrogen receptor competitive binding assay, the IC50 values were determined (50% inhibition of maximal binding of reference compound). The relative binding affinity (RBA) is then calculated by comparison with the results for the reference compound 17β-estradiol.

For BPS the RBA was determined to be 0.0055%, showing very weak binding affinity. Based on the results from the reporter gene experiment, the EC50 values were determined (concentration at which 50% of the transcriptional activity of the positive control is attained) and the relative activity (RA) was then calculated by comparison with the results of the reference compound. For BPS, the RA was reported to be 0.000254%, which is in agreement with the results of the other assay.

Yamasaki *et al.* (2004)

Yamasaki *et al.* (2004), performed both receptor binding assays and immature rat uterotrophic assays with 14 chemicals including BPS. For the description of the immature rat uterotrophic assay see “OECD CF Level3”

In the human estrogen receptor binding assay, chemicals were tested for 1h over the 1 x 10^{-11} to 1 x 10^{-4} M concentration range and added to the test solution together with 17β-estradiol. The percent ratio (B/B0 (%)) of standard ligand (17β-estradiol) bound to the receptor was calculated from the radioactivities of the solutions with and without the test

substance, subtracting the radioactivity due to non-specifically bound standard ligand to the receptor. The B/B0 values as a function of the concentration were fit to the logistic equation and the fifty percent inhibitory value (IC50) of each chemical was calculated by the least-squares method using computer software. The binding abilities of test chemicals to the receptor were evaluated by relative binding affinity (RBA), ratio of IC50 values to 17β-estradiol.

The RBA of BPS was 0.0055% (relative to 17β-estradiol), which confirms that BPS has a very weak (but positive) estrogen activity.

The outcomes of this assays were in agreement with that of the immature uterotrophic assay and indicated that BPS has a (weak but positive) estrogen activity.

Kuruto-Niwa *et al.* (2005)

Kuruto-Niwa *et al.* (2005), investigated estrogenic activity of bisphenols in a green fluorescent protein (GFP) expression system. In this assay, a GFP reporter vector regulated by an estrogen response element (ERE) was constructed and transfected into human breast carcinoma MCF7 cells which are estrogen responsive.

Cells were exposed for 3 days to a concentration range of 10^{-9}-10^{-4} M. The EC50 values for each chemical were determined as the concentration yielding 50% of the maximum fluorescence intensity. Estrogenic potency was then expressed as relative estradiol equivalency factor (EEF) by dividing the EC50 for 17β-estradiol by the EC50 of the chemical under consideration.

For BPS the EC50 was 1.75 x 10^{-6} M, yielding an EEF of 5.54 x 10^{-6}. This demonstrates the weak (but positive) estrogenic potency of BPS.

BPS could be chlorinated during wastewater treatment (disinfecting drinking water).

Chlorination of BPS can decrease its estrogenic activity.

Kitamura *et al.* (2005)

In this study, the estrogen, androgen and thyroid activity of 10 related compounds was examined using three different *in vitro* assays (see also OECD CF Level 2: 2. Androgen activity and 3. Thyroid hormonal activity).

The estrogenic activity was studied using an ERE (Estrogen Response Element)-luciferase reporter assay with human breast cancer cell line MCF-7 cells. As false positives and false negatives can occur in a single cell line due to certain cross talk pathways in the cell. Other cell lines were also used, including rat pituitary cell line expressing a high level of ERα, MtT/E2 and a mouse fibroblast call line, NIH3T3, transiently transfected with ERα or β. Generally estrogenic activity with one of these cell lines was confirmed after screening in MCF-7 cells.

In this study, estrogenic activity was related to the luciferase activity measured in the test system. The test substances were added at concentrations of 10^{-4} to 10^{-9} M for a test duration of 24h.

For the anti-estrogens assay, the inhibitory effect of the test substances on the estrogenic activity of 17β-estradiol (at the concentration of 10^{-10} M, test duration: 24h) was examined.

The EC50 of BPS (i.e., the effective dose that half maximally activates the estrogen receptor), was 1.1 x 10^{-6} M, compared to an EC50 of 8.6 x 10^{-6} M for 17β-estradiol. No significant antagonist activity was observed either.

Laws *et al.* (2006)

In this study, the estrogenic activity of 50 structurally diverse chemicals was evaluated using the rat uterine cytosolic (RUC) ER-competitive binding assay.

Chemicals were tested over a concentration series of 10^{-7} to 10^{-4} M for 18h. Radiolabelled 17β-estradiol was used for competition and bound radioligand was quantified during the test by scintillation counting. The IC50 was calculated for each chemical and test chemicals that demonstrated the ability to reduce ER binding by 40% or more were further evaluated in a Ki experiment to confirm whether or not they are true competitive inhibitors (Ki = the concentration of the test chemical at which half of the ER will be occupied in absence of another competitor). Chemicals that were not able to reduce ER binding by more than 20% were considered non-binders.

BPS was tested at a concentration series of 10^{-5} to 10^{-5} M and was shown to belong to a group of chemicals showing partial competitive binding curves with 50-74% displacement. The IC50 value was 6.4×10^{-5} M (compared to 5.2×10^{-10} M for 17β-estradiol) and the Ki was reported to be $8.2.4 \times 10^{-5}$ M (compared to 7.7×10^{-10} M for 17β-estradiol). This demonstrates that BPS has a rather weak affinity for the estrogen receptor.

Akahori *et al.* (2008)

In a comparative study with 65 chemicals the relationship between the results of *in vitro* binding assay to human estrogen receptor α and *in vivo* immature rat uterotrophic assay was examined.

For the receptor binding assay, the recombinant human hERα ligand binding domain fused with glutathione-S-transferase was expressed in *E. Coli*. Chemicals were tested between concentrations of 10^{-11} and 10 M for 1h. The relative binding affinity was calculated as

$$RBA = \frac{(IC50 \text{ for } E2) \times 100}{(IC50 \text{ BPS})}$$

LogRBA of BPS was -2.26 (RBA=0.00549%), compared to 2.00 for 17β estradiol, indicating weak estrogen activity.

Estrogenic and anti-estrogenic response was demonstrated for BPS. LogLED for estrogen activity was 1.90 µmol/kg/day (<-2.43 µmol/kg/day for 17βestradiol, Padilla-Banks *et al.*, 2001) and 3.30 µmol/kg/day for anti-estrogen activity.

Furthermore, it was demonstrated that logRBAs were correlated with both logLEDs in estrogenic and anti-estrogenic assay systems at $r^2=0.67$ (n=28, P<0.0001) and 0.79 (n=23, P<0.0001) resp. The results strongly suggesting that both assays detect the same ER mediated biological response, but extrapolation of the in vitro data should be done with caution because interaction of the ER with other endocrine related systems and metabolism *in vivo* cannot be neglected.

Grignard *et al.* (2012)

Grignard *et al.* (2012), used two highly standardized transactivation assays comparable with OECD TG 455 for comparing the estrogenic activity of BPA and BPS. Cells were exposed for 24h exposure to concentrations ranging from 10^{-15} to 10^{-4} M.

- The first assay used MELN cells derived from human breast cancer MCF-7 cells after stable transfection with the estrogen responsive gene ERE_Glob_Luc-SVNeo.

- The second assay used BG1Luc4E2 cells. The human ovarian cancer cell line BG-1, which expresses both human hER-alpha and hER-beta were stably transfected

with a plasmid containing a firefly luciferase reporter gene under the control of four estrogen response elements placed upstream of the mouse mammary tumour viral (MMTV) promotor.

In order to decrease their endogenous estrogenic signal, cells were adapted to hormone-free medium before starting the experiments. The experiments were performed as described in Witters *et al.* (2010) and http://iccvam.niehs.nih.gov/methods/endocrine/IVSdocs/LUMIAg12Mar09.pdf.

In both tests estrogenic activity was measured by monitoring luciferase activity (microplate reader). The test chemicals were tested at non-cytotoxic concentrations as defined in parallel in a cytotoxicity assay.

The dose-response curve obtained with the MELN test for BPS showed a significantly lower estrogenic activity than the reference compound 17β-estradiol. The EC50 value for BPS was reported to be 4.24 x 10^{-6} M (compared to 4.1 x 10^{-11} M for the reference compound). Similar results were obtained with the test based on BG1 Luc4E2 cells. There, the EC50 value for BPS was reported to be 4.93 x 10^{-6} M (compared to 1.24 x 10^{-12} M for the reference compound).

Viñas and Watson (2013)

In this study the effect of BPS on the non-genomic signalling in a rat $GH_3/B_6/F_{10}$ pituitary cell line was investigated.

BPS was tested for 24h, in the presence and absence of E2, in a concentration range likely to be found in the environment: 10^{-15} to 10^{-7} M

Extracellular signal-regulated kinase (ERK)– and c-Jun-N-terminal kinase (JNK)–specific phosphorylations were examined for their correlation to three functional responses: proliferation, caspase activation, and prolactin (PRL) release.

In order to examine the ERK phosphoactivation, cells were pre-treated with receptor selective inhibitors: MPP (10^{-8} M) for ERα, PHTTP (10^{-7} M) for ERβ and G15(10^{-7} M) for GPR30.

BPS caused ERK-activation similar to that caused by E2 (concentration and time). The lowest tested BPS concentration showed a higher pERK response than did 10^{-9} M E2. A non-monotonic dose was observed: rising concentrations of BPS resulted in a decreasing response. In the presence of E2, increasing BPS concentrations evoked a lower pERK activity than in the absence of E2.

No significant JNK activation was seen. The highest BPS concentration (10^{-7} M) caused deactivation significantly below vehicle levels. When tested in the presence of E2, JNK activation was higher than with E2 alone. Also, here a non-monotonic dose response curve was observed.

At optimal response concentration of 10^{-14} M, BPS phosphoactivated ERK after 2,5 min but showed no oscillation as E2 (first peak at 5 min, second peak at 30min.), while the combination of BPS and E2 showed a slight oscillation but no significant differences between stimulated points. It can be concluded that BPS can alter the timing of response to a E2 at very low concentrations.

Teng *et al.* (2013)

Teng *et al.* (2013), performed transient transfection luciferase assays for examining the potential androgenic or estrogenic agonism or antagonism of bisphenol A and several other chemicals.

Monkey kidney CV1 cells or HepG2 cells (hepatocellular carcinoma, human) were transfected with pRST7-ERα and 3X-ERE-TATA-luc and exposed for 24h to 10^{-8}-10^{-4} M BPS. The EC50 value for agonistic estrogenic activity was reported to be 2.2 x 10^{-6} M, whereas that for the reference compound 17β-estradiol was 1.26 x 10^{-10} M, indicating that BPS has only a weak estrogen agonist activity.

No ERα antagonist activity was measured for BPS, when cells were incubated together with 2 x 10^{-10} M of E2 and different concentrations of BPS (3 x 10^{-8} to 10^{-13} M).

Molina-Molina *et al.* (2013)

Agonistic and antagonistic nuclear receptor activities of BPS and other BPA-congeners and derivatives were examined in vitro using competitive binding nuclear receptor binding assays, reporter gene expression systems and cell proliferation assays.

The oestrogenic activity of BSF was tested using two different cell lines:

- The first assay (test duration: 16h) used the transfected luciferase reporter MELN cell line: ERα-positive breast cancer MCF-7 cell which are transfected with the estrogen responsive gene ERE-βGlob-Luc-SV-Neo.

- The second assay used the HELN-hERα and –hERβ reporter cell line: firstly HeLa cells were stably transfected with estrogen responsive reporter gene (AF-1 deleted hER), subsequently these cells were transfected with –hERα or –hERβ plasmid constructs. This study (test duration: 3h) was aimed to find out if BPS could act as a specific ER-modulator by determination of the Receptor Binding Affinity, the ratio of 17β-estradiol to the competitor concentration required to reduce specific radiolabelled binding by 50%. RBA for 17β-estradiol is set at 100.

Agonistic activities were tested for concentrations of BPS between 10^{-2} and 10^{-5} M. Results were expressed as a percentage of maximal luciferase activity (100% at 10 $^{-8}$ M for E2).

Estrogen activity was seen in both assays with an EC50 for BPS in the MELN cell line = 1.21 x 10^{-5} M, and resp. 3.96x 10^{-6} M and 1.72 x 10^{-6} M in the HELN-ERα and HELN-ERβ cell line, indicating that BPS binds to hERα and hERβ.

Deletion of the A/B domain in hERβ altered the transactivation potency pointing out that BPS is dependent on AF-1 (Delfosse *et al.*, 2012).

The IC50 value for BPS, i.e. the concentration necessary to reduce maximal 17β-estradiol-binding by 50%, was resp 6.560 x 10^{-6} M and 3.452 x 10^{-6} M for hERα and hERβ confirming the weak estrogenic activity of BPS. IC50 of 17β-estradiol is resp. 1.2 x 10^{-10} M and 2.1 x 10^{-10} M for hERα and hERβ

The binding affinity towards hERβ (RBA = 0.006%) was almost 2-fold higher than for hERα (RBA=0.001%). This may be explained by the polarity of BPS. It is suggested that ligand polarity modifies the affinity to ER isoforms. Bulky substitutions below the D-ring in the E2 molecule led to ERα agonists and substitution above the B and C-ring preferentially produce ERβ agonists (Hillisch *et al.*, 2004)

E-screen bioassays were also performed with MCF-7 cells, with BPS concentrations of 10^{-2} to 10^{-5} M. Agonist and antagonist (co-incubation with 10^{-4} M E2) activities were assessed. At 10^{-5} M, BPS significantly induced the proliferation of MCF-7 cells (~3.7-fold compared to the control). No antagonist ER activity is reported for BPS in this assay.

Kang *et al.* (2014)

The estrogenic potency of BPA, BPS and PESU (polyethersulfone) and their metabolites generated by the rat liver S9 fractions on a MVLN cell using a luciferase reporter gene (test duration: 72h) were estimated in this study.

The MVLN cell line was a stable transfected MCF-7 cell line (a human breast carcinoma cell line) with a luciferase reporter gene plasmid consisting of a *Xenopus laevis* vitellogenin promotor region containing four estrogen responsive elements.

Concentrations of 5, 0.5, 0.05, 0.01, 0.001 and 0.0001µM of BPS were used.

BPS was incubated with a rat liver S9 fraction to evaluate the estrogenic activity of the metabolites.

The result at 5µM BPS exposure was excluded to calculate REP (relative potency) due to the observation of death cells. BPS showed a dose-dependent estrogenic activity. No estrogenic effects were seen at 0.001 and 0.0001µM. BPS EC50 has been determined at 6.97×10^{5} µM, and its relative potency (REP50) to E2 was 4.09×10^{-9}.

ER gene expression level was not affected by BPS.

The estrogenic activity of the metabolites increased after 20 min incubation with rat liver S9 and was again similar to parent BPS at 40 min.

Rosenmai *et al.* (2014)

ER agonist and antagonist of Bisphenol A and its analogues was examined according to OECD TG 457, using a stable transfected human ovarian adenocarcinoma cell line (BG1Luc4E2).

Increased ER activity was seen after exposure for 22h to BPS (EC50= 1.17 µM), which supported the positive prediction of the QSAR models (see OECD level 1, table 71 above).

Table 72: Reporter gene assays performed with BPA and its analogues (Rosenmai *et al.*, 2014)

Tentative Maxi Efficacy (E_{max}) and Values for Concentrations Causing 50% of the Maximum Response (EC_{50}) for Test Compounds in Reporter Gene Assays

		BPA	BPB	BPE	BPF	BPS	HPP
ER	E_{max} (%)	**286**	**357**	**386**	**377**	**222**	**156**
	SD (%)	42	70	133	173	151	128
	EC_{50} (μM)	**0.08**	**0.12**	**0.47**	**0.82**	**1.17**	**0.10**
	SD (μM)	0.02	0.03	0.08	0.36	0.04	0.01
AR	E_{max} (%)	**91**	**92**	**72**	**89**		**92**
	SD (%)	5	3	11	6		2
	EC_{50} (μM)	**3.8**	**3.4**	**1.9**	**3.0**		**5.1**
	SD (μM)	1.3	1.8	0.9	1.5		0.7
AhR	E_{max} (%)	**47**[a]		**83**[a]	**86**		
	SD (%)				34		
	CI (%)	—		—			
	EC_{50} (μM)	**54.8**[a]		**53.0**[a]	**48.8**		
	SD (μM)				10.2		
	CI (μM)	VW		VW			
Nrf2	E_{max} (%)				**108**		**62**[a]
	SD (%)				23		
	CI (%)						—
	EC_{50} (μM)				**42.2**		**25.5**[a]
	SD (μM)				4.9		
	CI (μM)						VW
P53	E_{max} (%)	**82**	**61**				
	SD (%)	26	30				
	EC_{50} (μM)	**50.9**	**33.6**				
	SD (μM)	14.2	29.1				

Notes. E_{max} and EC_{50} values (bold) based on means of predicted values from dose-response curve fits for independent experiments. Standard deviations (SD) are shown if more than one experiment was modeled and 95% confidence intervals (CI) if one experiment was modeled. Color code: green, activation; red, deactivation; white, no significant change. VW: very wide; —: the constraints of the model were reached and thus no confidence interval could be predicted.
[a]Value based on one experiment, as other significant experiment could not be fitted to the nonlinear regression model applied.

Rajasärkkä *et al.*, 2014

The aim of this study was to assess the use of a yeast-based reporter assay to monitor the quantity and bioavailability of BPA and other estrogenic compounds in thermal papers. The authors used two different yeast strains, one expressing the BPA-targeted receptor (BPA-R), a mutated human estrogen receptor α with enhanced affinity towards BPA and low affinity towards 17β-estradiol and other estrogens, and one expressing the wild-type human hERα. Potencies of BPA, BPS and BPF haven been tested with both strains in concentration range 10^{-7} to 10^{-2} mol/L.

BPS showed the lowest potency in both BPA-R and hERα strains, showing affinity for the receptors 2.5 and 2 orders of magnitude lower than BPA, respectively.

Stossi *et al.* (2014)

Endocrine activity of several bisphenols (e.g., BPB, DM BPA, TM BPA, BPF, BPS) was examined in cell-based assays using high throughput microscopy and multi-parametric automated image analysis, which is highly complementary to those assays used in ToxCast.

Estrogen activity was examined in HeLa cells: PRL-HeLa cells (Erα) and PRL array cell line (ERβ) under agonist (17β-estradiol) and antagonist (4-hydroxy-tamoxifen, 4OHT) treatments. Erα and ERβ selectivity was further examined using fragment complementation (PCA) and PRL array platforms.

BPS was unable to induce Erα binding to PRL arrays, showed very little Erα dimerization and showed low activity for Erβ. After treatment with 10 μM for 30 minutes in the PRL array BPS caused an intermediate response (~ 50% of E2) in Erβ containing cells.

The effect of BPS (10 µM) was further examined in a MCF-7 cell proliferation assay and resulted after 24h exposure in a Relative Proliferative Effect RPE of 97.54% indicating that BPS is able to induce similar cell proliferation to E2. However, the Relative Proliferative Potency (min [17β-Estradiol] needed for max cell yield/min [compound] needed to achieve similar effect) RPP of 0.000105% was significantly lower than that of E2.

Ma *et al.* (2015)

Molecular and toxicological effects of tetrabromobisphenol-A, BPA, TBBPA-bi(2,3-dibromopropylether) and BPS were studied using a chicken *in vitro* screening assay together with the Avian ToxChip polymerase chain reaction (PCR) array. The mRNA expression level for the ApolipoproteinII (ApoII) and Vitellogenin (Vtg) genes, both encoding egg yolk precursor proteins in birds and associated with the sex steroid pathway and avian reproduction, were examined by probe-based RT-RCR assay. They are used as biomarkers to detect estrogenic MoA. The acryl hydrocarbon receptor (Ahr)-mediated transcriptional activity was studied by luciferase reporter gene assay.

The genome of chicken embryonic hepatocytes is fully sequenced and annotated, which makes it a very useful model to study molecular toxicological effects. Chicken embryonic hepatocytes were exposed for 35h to BPS concentrations in the range of 0.01 µM to 300 µM. BPS did not affect cell viability up to 300 µM, but actual cellular uptake concentrations were not analysed.

A concentration-dependent increase in apoII and Vtg mRNA levels, which were similar to the response provoked by 17β-estradiol, were seen after exposure to BPS. At 300µM, BPS significantly up-regulated ApoII (by 30.7-fold) and Vtg (by 10.3 fold) in comparison to DMSO vehicle control.

Ruan *et al.* (2015)

The authors assessed the estrogenicity of 8 bisphenols (including BPS) identified in sewage sludge collected from wastewater treatment plants in China, using a bioluminescent yeast estrogen screen assay (BLYES), based on a yeast cell line expressing an estrogen response element. This method measures the EC50 and the estradiol equivalency factor (EEF), calculated as following: EEFBPS = (EC50E2)/(EC50BPS). Cells were exposed for 12h to 10^{-8} to 10^{-4} M.

BPS showed significant bioluminescence induction when the concentrations were between 5×10^{4} and 1×10^{6} nM. BPS EC50 has been determined at 4.13×10^{5} µM, and its EEF was 4.09×10^{-9}. There was a marked reduction in E2- or BPS-induced bioluminescence when ICI182780, a strong estrogen receptor antagonist, was added.

Boucher *et al.* (2016)

The aim of this study was to see if BPS can induce adipogenesis as BPA. The authors exposed human preadipocytes to different concentration of BPS (0.1 nM to 25 µM) for 14 days. Lipid accumulation significantly increased after exposure to 10 and 25 µM BPS (up to 4.2-fold). This was confirmed by an increased expression of several adipogenic markers (mRNA and protein).

As estrogenic, glucocorticoid and PPARɣ receptors are known to potentially induce adipocyte differentiation, the authors performed different transcriptional assays in COS-7 cells. Results regarding glucocorticoid and PPARɣ are described below. BPS induced the transcriptional activity of ER reporter (up to 3-fold) after 24h. The exposition simultaneous of the preadipocytes to 25 µM BPS and 1 µM of ICI (ER antagonist) significantly inhibited BPS-induced lipid accumulation by 37.5%, confirming that ER is involved in this BPS-induced lipid accumulation.

Skledar *et al.* (2016)

This study focusses on BPS metabolites. As for BPA, BPS-glucoronide is the main BPS metabolite. Hydroxylation can also occur to a lesser extent, leading to the formation of BPSM1. The authors assessed then the estrogenic, androgenic and thyroid disrupting activities of BPS and its main metabolites. Results regarding androgen and thyroid are described below.

Using a yeast cell assay expressing hERα, the authors examined the estrogenic or anti-estrogenic activity of BPS and its metabolites for 48h from 30 nM to 300 µM. Both BPS and BPSM1 (hydroxylated BPS) exhibited agonistic activities in the ER assay, with values of 8.4 x 10^{-5} and 6.7 x 10^{-4} M, respectively. BPS-glucoronide did not show any activity.

Conley *et al.* (2016)

The aim of this study was to check the predictive capacity of *in vitro* data, comparing the estrogenic activity determined using *in vitro* ERα-mediated transcriptional activation reporter assay and *in vivo* rat uterotrophic assay. Results of the uterotrophic assay are described below (OECD level 3 assays).

In vitro estrogenic activity was assessed using the T47D-KBluc estrogen receptor transcriptional activation (ERTA). Cells were exposed for 24h to 10^{-10} to 10^{-5} M BPS, showing agonist activity with an EC50 at 6.43 x 10^{-7} M, i.e., about 6 orders of magnitude lower than EE2 (relative potency = 1.55 x 10^{-6}).

Dvořáková *et al.* (2016)

The aim of this study was to compare the results of in silico approach (based on the OECD QSAR toolbox) for the prediction of potential ligands of hERα and the results obtained in two *in vitro* methods, i.e., the ER transactivation assay (OECD TG 455/457) and a commercially available YES/YAS assay. Test substances have been tested from 10^{-7} to 10^{-2} mg/mL and 10^{-8} to 10^{-4} mg/mL in ER transactivation and YES/YAS assays, respectively.

BPS has been identified as agonist for hERα with both in silico and in vitro methods. This correlated the ERα very strong binding affinity predicted with OECD QSAR Toolbox. No anti-estrogenic nor androgenic activity has been shown for BPS.

Simon *et al.* (2016)

65 compounds, migrating from polycarbonate replacement materials for plastic baby bottles, were examined for their endocrine activity. Authors investigated human oestrogen receptor (ER), human androgen receptor (AR), human progesterone receptor (PR), human glucocorticoid receptor (GR), human peroxisome proliferator-activated receptor gamma (PPARγ), human thyroid receptor beta (TRβ) and the mouse aryl hydrocarbon receptor (AhR) in *in vitro reporter gene* assays. Effects on ER were examined in two different cell lines: BG1Luc4E2 and MVV-Luc (MCF-7). For agonistic activity cell lines were exposed for 24 or 48h to 1 µM, 10 µM, 100µM and 1 mM BPS dissolved in 1% DMSO. For antagonistic activity cells were exposed for 24 or 48h to 10 mM, 1mM and 100 µM BPS dissolved in DMSO and in presence of an agonistic reference ligand at a concentration inducing 50% of the maximal response.

BPS showed agonistic estrogenic activity (relative response >50%) and no antagonistic estrogen activity in both cell lines.

Cano-Nicolau *et al.* (2016)

In a competitive binding assay the estrogen binding potential of BPS with zfERα, zfERβ1 and zfERβ2 was tested at concentrations of 10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M (test duration: 24h). Results were compared to the solvent control (0.1% DMSO). BPS was

almost not able to move [3H]-E2 from the zfER binding site and it is suggested that this is probably due to the absence of binding to ERα.

Furthermore a cyp19a 1b-luceferase reporter gene assay was run to investigate the transcriptional activity of zebrafish nuclear receptors (ERa, ERb1, andERb2) transfected in U251MG cells which are an ER-negative human glial cell line. BPS did not stimulate reporter gene. The lack of estrogenic activity for BPS could be linked to the absence of key transcriptional co-factors required for efficient estrogen receptor-dependent trans-activation.

Okazaki *et al.* (2017)

The authors investigated the effects of BPA alternatives (including BPS) on estrogen signalling in human breast cancer cells. Exposure of MCF-7 cells to 25 µM BPS reduced the transcriptional activity mediated by ER/ERE, but these effects were not statistically significant.

Zalmanova *et al.* (2017)

The aim of this study was to explore the effects of BPS on the *in vitro* maturation of porcine oocytes. Pig oocytes were exposed to 3 nM, 300 nM or 30 µM BPS and this leaded to disrupted and blocked maturation of oocytes at all concentrations after 72h of culture. BPS also dramatically affected the formation and structure of the meiotic spindle.

The meiotic maturation of the oocytes depends on maternal reserves of gene transcripts. After BPS exposure, ERα and aromatase transcripts were considerably decreased. Exposure to BPS also disturbed the expression and distribution of ERα and ERβ.

Kim *et al.* (2017)

Using an MCF-7 CV breast cancer cells expressing both ERα and ERβ, the authors evaluated the estrogenic activity of BPA, BPS and BPF after 48h. Exposure to BPS (10^{-7}-10^{-5} M) significantly increased the proliferation of MCF-7 CV cells in a dose-dependent manner (from 10^{-6} to 10^{-5} mol/L). This proliferation was inhibited when the cells were co-exposed to ICI 182,780, an ER antagonist.

Further analyses on protein expression involved in cell cycle progression (Cyclin D1 and E1), cell morphology, cell epithelial marker, and cell migration confirmed the estrogenic effect of BPS. In all these assays, co-exposure to ICI 182,780 blocked the effect of BPS.

Mesnage *et al.* (2017)

The authors investigated the estrogenicity of BPA and 6 bisphenol analogues, including BPS, in 3 human breast cancer cell lines. 2 of these cell lines were hormone dependent (MCF-7 and T47D), whereas the third one was hormone independent (MDA-MB-231).

For the E-screen assay, the cells were exposed to 10^{-11} to 10^{-4} M BPS for 6 days. BPS induced the proliferation of MCF-7 (AC50 = 1.33 µM) and T47-D, but to a lesser extent which can be explained by the lower levels of ER expression in this cell lines. As expected, no cell proliferation was observed with the hormone independent MDA-MB-231 cell line.

The estrogenic activity of BPS was confirmed using a luciferase reporter gene assay allowing measurement of ERE-mediated transcription. Exposure for 24h to 10^{-11} to 10^{-4} M resulted in an AC50 of 1.5 µM. Addition of ICI 182,780, an ER antagonist, antagonized the BPS estrogenic activity observed in both E-screen and luciferase reporter gene assay.

Full transcriptome profiling was performed on MCF-7 cells exposed for 48h to BPA and its analogues. Interestingly, BPS showed the most different profile, quite different from the other bisphenols.

Le Fol *et al.* (2017)

The authors assessed the estrogenic activity of BPA, BPF and BPS in zebrafish, by testing the ERs transactivation potential *in vitro*, but also *in vivo* by quantifying the aromatase activity in the developing brain of embryos. The *in vivo* results are described below.

To determine the transactivation potential of BPS, the authors used ZELH-zFERs cell lines, i.e., zebrafish hepatic cell lines ZFL expressing the luciferase gene under the control of ERE and each of the three zebrafish estrogen receptors (zfERα, zfERβ1 and zfERβ2). BPS was weakly active in all cell lines, showing an EC50 of 4.058 µM in zfERα, 1.016 µM in zfERβ1 and 2.468 µM in zfERβ2. The relative estrogenic potency in comparison to E2 (REP_{E2}) to each receptor was 3.7 x 10^{-5}, 3.0 x 10^{-5} and 2.4 x 10^{-5}, respectively.

Qiu *et al.* (2018a)

In this study, primary macrophages isolated from the head kidney of red common carps (*Cyprinus carpio*) were exposed to 0, 0.1, 1, 10, 100 and 1000 µg/L BPS for 6 hours.

After exposure, the cells showed an upregulated immune response, showing an increased oxidative stress and cytokine genes in an approximately concentration-dependent manner. Cells exposed to 100 and 1000 µg/L showed significantly reduced phagocytic activity and increased apoptosis rate.

Expression of ERα and ERβ2 genes were significantly induced by exposure to BPS at concentrations ≥ 1 µg/L. Interestingly cotreatment with BPS and the ER antagonist ICI 182,780 significantly inhibited erα, but not ERβ2 upregulation. It confirmed that ER receptors are involved in the mode of action of BPS.

Conroy-Ben *et al. (*2018)

The authors of this study compared the results of *in vitro* assays and in silico predictions regarding the estrogenic and androgenic activities of several bisphenols. They used the Biograf3 VirtualToxLab multi-dimensional QSAR for the in silico modelling and compared the results with Yeast estrogen and androgen screen assays (YES/YAS assays).

In the YES assay, yeast cells (hER) were exposed for 4h to 10^{-10}-10^{-3} M BPS. The substance showed weak estrogen agonist activity, showing an EC50 of 5.88 x 10^{-4} M. Compared to 17β-estradiol, BPS showed a potency of 1.07 x 10^{-6}. The YAS results are described hereafter.

The weak estrogenic and anti-androgenic activity of BPS were confirmed in silico.

Li *et al.* (2018)

The authors analysed the binding of BPA, BPAF and BPS to estrogenic receptors. Diverse cell lines expressing ERα, ERβ (MCF-7, hepG2, BG-1FR) were tested in a luciferase reporter assay with chemicals concentrations of 1, 10, 100 and 1000 nM. Further analyses have been performed to affine information on binding.

In a ERE-mediated luciferase reporter assay using human enodmerialadenocarcinoma, Ishikawa/Erα a 3-fold increase of ERE-mediated activity was observed after exposure or 18h to a range of 1-1000 nM.

BPS only weakly activated the ERα at 100 nM, and a strong luciferase activity was observed at 100 nM. Interestingly BPS did not show ERβ activation.

Kojima *et al.* (2018)

The authors assessed the transcriptional activity of BPA and 8 analogues using luciferase receptor assays based on human ERα, ERβ, AR, GR, PXR and CAR. Transiently transfected cells (CHO-K1) with specific expression plasmids were exposed to 10^{-9} to 10^{-5} M of chemical. Results were expressed as REC_{50}, i.e., 50% of relative effective concentration in case of agonistic activity, and RIC_{50}, i.e. 50% relative inhibitory concentration.

BPS had no agonist nor antagonist effect on AR, GR, PXR and CAR. REC_{50} = 5.4 x 10^{-7} M for both ERα and ERβ. No antagonist activity was observed on ERs.

***Zhang et al.* (2018a)**

To examen the competitive binding of BPS to the hERα receptor a fluorescence polarization assay was conducted.

After a 2h exposure to 1-10 µM of BPS an IC50 was determined of 5.78 µM indicating estrogen binding activity.

Campen *et al.* (2018)

This study investigated the effects of BPS on bovine granulosa and theca cells. The cells were isolated from bovine ovaries and exposed for 6 days to vehicle control or BPS at concentration from 1 µM increasing in 10-fold increments up to 100 µM (12 concentrations total). Granulosa cells were cultured in the presence or absence of 0.33 ng/mL FSH, whereas the theca cells were cultured in presence or absence of 100 pg/mL LH. After 6 days exposure, the authors measured the concentrations of estradiol in granulosa cells, of androstenedione in theca cells and of progesterone in both types of cells. Increased production of estradiol at high dose in granulosa cells.

No effect on progesterone.

Awada *et al.* (2019)

In this study proliferation (using MTT assay) and cell migration (using cell scratch assay) was investigated in MCF-7 (ER-negative) and MDA-MB-231 (ER-Positive) breast cancer cells after exposure to BPS. Cells were exposed to 10^{-5} and 10^{-9} M with and without ICI 182,780 (estrogen receptor inhibitor-100 nM) in the proliferation assay and to 10^{-5}M and 10^{-9}Mwith and without ERI in the cell migration assay.

BPS increased cell metabolic activity in MCF-7 cells in a time- and dose-dependent manner after 24, 48 and 72h. After 48-72h, this increase was statistically significant at 10^{-5}M BPS ($p<0.001$). This effect was completely annulled in the presence of ICI 182,780 at 48 and 72h. Also, cell migration increased in a time- and dose-dependent matter and was statistically significant at 48 and 72h at both concentrations tested. The increased proliferation and migration were not associated with morphological changes in the MCF-7 cells.

No effect was seen on proliferation and migration in the MDA-MB-231 cells which confirms that exposure to BPS induce an ER-mediated effect.

Eilebrecht *et al.* (2019)

The endocrine potential of BPA and selected substitution candidates were tested through validated and commercially available test systems (competitive receptor-ligand binding assays, coactivator recruiting assays, and cell-based reporter gene assays).

In a fluorescence polarisation test receptor-ligand binding assays BPS was found to bind to ERβ with an IC50 of 7.8 µM (RBA: 0.012%). ERα was not tested.

In the estrogen receptor co-activator assay no agonist nor antagonist activity was observed for ERα, while a positive response was observed in the Estrogen Receptor Transactivation assay (GeneBLAzer Estrogen Receptor A Test: EC50 Erα = 0.97 µM).

Both in the Estrogen receptor co-activator assay and Estrogen Receptor Transactivation assay, ERβ activity was recorded:

ER co-activator: agonistic mode (IC50 ERβ ~3400 µM) and antagonist mode (IC50 ERβ ~380 µM).
Estrogen Receptor Transactivation assay (GeneBLAzer Estrogen Receptor A Test: EC50 ERβ = 8 µM.

Molina-Molina *et al.* (2019)

In this study 112 thermal paper receipts from Brazil, Spain and France were examined for the presence of BPA, BPS and BPF as well as for their estrogenic and anti-androgenic activity. BPS was only found in 2 samples from Brazil, 2 from Spain and 10 from France, in concentrations between 6.46 and 13.29 mg/g.

Estrogen activity of the thermal paper samples was examined in an E-screen bioassay using MCF-7 cells. No positive correlation was found between BPS concentrations in the samples and estrogenic activity.

Pelch *et al.* (2019)

The transcriptional activity of 22 bisphenols was assessed using luciferase receptor assays based on human ERα, ERβ and AR. Transiently transfected HepG2 (ER) and MDA-kb2 (AR) cells with specific expression plasmids were exposed for 18h to 3 x 10^{-9} to 1 x 10^{-5} M of chemical. Results were expressed as REC_{50}, i.e., 50% of relative effective concentration in case of agonistic activity, and RIC_{50}, i.e. 50% relative inhibitory concentration.

BPS showed an agonist activity on both ERα and ER β. REC_{50} = 1.3 x 10^{-6} M for ERα and REC_{50} = 2.1 x 10^{-6} M for ERβ. No antagonist activity was observed. BPS did not show any AR agonist nor antagonist activity.

Atlas and Dimitrova (2019)

The authors assessed the effects of BPA and BPS on MCF-12A human mammary epithelial cells, particularly on the cell's organization. MCF-12A cells were exposed to 0.1, 1 or 10 µM BPS or BPA, or to 1 nM E2 as positive control. When grown in 3D, BPS exposure resulted in a disruption in acini formation at all concentration tested. The cells showed a statistically different increase of cell volume and a reduce number of acini with lumen. Interestingly, when grown as monolayers, BPS (as BPA or E2) has no effect on proliferation rates of the MCF-12A cells. The authors also checked the effects of BPS and BPA on 2 other cell lines: MCF-7 (which is E2 dependent) and MCF-10A (expressing ERβ but not ERα). Whereas BPS promote MCF-7 cell proliferation (in a lesser extent than BPA, confirming that BPS is less oestrogenic), no effect was observed on MCF-10A, suggesting that ERα is needed for the effects.

The ability of BPS and BPA to activate the ERα and ERβ was also assessed in a luciferase assay using expression vectors for both receptors. BPS was able to transactivate the receptor at 1 µM (2.5-fold) and at 10 µM (5-fold). Similar activity was observed with ERβ.

Grimaldi *et al.* (2019)

The transcriptional activity of 24 bisphenols was assessed using luciferase receptor assays based on human ERα, ERβ, ERRγ (oestrogen related receptor), AR, PXR (pregnane X receptor), PR (progesterone receptor), GR (glucocorticoid receptor) and MR (mineralocorticoid receptor). Stably transfected HELN or HG5LN cells expressing specific expression plasmids were exposed for 16h to 0.001 to 10 µM bisphenols. Results were expressed as EC_{50}, i.e., 50% of relative effective concentration in case of agonistic activity, and IC_{50}, i.e. 50% relative inhibitory concentration.

BPS showed an agonist activity on both ERα and ER β. EC_{50} = 4.47 x 10^{-6} M for ERα and EC_{50} = 1.72 x 10^{-6} M for ERβ. No agonist nor antagonist activity was observed for ERRγ, PXR, PR, GR nor MR.

Liu *et al*. (2019b)

The receptor binding affinities of 11 bisphenols were evaluated in competitive binding assays (test duration: 1h; test concentration: 10 µM).

BPS was found to be completely inactive to RARα, RARβ, RARγ, PPARγ, RORα, RORβ, RORγ, LXRα, LXRβ, VDR, CAR, RXRα, RXRβ, RXRγ and GR.

IC50 for PR and AR were not determined. IC50 for ERRγ was 4090 nM +/- 238

IC50 for Erα, Erβ were >10µM (indicating that the inhibitory constant IC50 cannot be calculated up to the 10 µM concentration because high concentrations could not be prepared) and thus extremely weakly active.

Williams and Darbre (2019)

The authors evaluated the effect of several endocrine disruptors including BPS on aromatase in three different human breast cell lines: MCF-7, ZR-75-1 (ERα-dependent) and HMF3A (ERα-independent). Cells were exposed for 7 days to chemicals at environmentally relevant concentrations: 10^{-11}-10^{-4} M.

A significant proliferative growth response was seen in ERα-dependent MCF-7 and ZR-75-1 cell lines after exposure to 10^{-8} M BPS. The ERα-negative HMF3A cell line did not show any cell proliferation.

Park *et al*. (2020)

In this study the oestrogenic, androgenic, and AhR potencies of BPA, BPF and BPS were evaluated using stably transfected transcriptional activation assay (STTA). The luciferase activities were determined in accordance with the OECD TG 455 in HeLa9903 cells (ERα), with the OECD TG 458 in AR-EcoScreen cells (AR), and similarly in DR-EcoScreen cells (AhR). Moreover, the effect of the bisphenols on expression of ERα receptors in MCF-7 cells was determined by Western-blot (test duration: 24h, test concentration: 0.01-10 µM).

BPS showed an agonistic activity on ER, showing an EC50 of 5.98 x 10-6 M. The expression of ERα was also significantly reduced to 0.53-fold in comparison with the control after exposure to 10 µM. Results regarding AR and AhR are described below.

Keminer *et al*. (2020)

The authors evaluated the potential endocrine activity of several BPA substitutes, including BPS. They first screened the ability of the 33 alternatives identified, using commercial PolarScreenTM kits, for competitive ligand binding assay (fluorescence polarization) for

ERα, ERβ and AR. At 10 µM, BPS was not able to inhibit significantly the binding of fluorescent compound neither to ERs nor AR.

2. Androgenic Activity

Table 73: Overview of androgenic activity (literature data)

Short Method description	Result (positive/negative)	Description of results (positive/negative)	References
Tox Cast Pathway model (AR) prediction	No agonistic and antagonistic activity	Agonist (AUC) :0, Antagonist (AUC): 0. Cytotoxicity limit 13.64 µM	ToxCast, EPA
AR competitive binding assay using PanVera AR BLD (identical to hAR LBD): recombinant rat protein expressed in E. coli	Weak AR binding	IC50=3.75 x 10-4 µM RBA=0.0008 (IC50) 3.07 x 10-9 M for R1881= RBA of 100) Log RBA=-3.09 (log RBA of R1881=2)	Fang *et al.*, 2003
Androgen Response Element luciferase reporter assay	No agonist androgen activity Anti-androgenic activity	No androgenic activity. Anti-androgenic activity at concentrations between 10^{-6} and 10^{-4} M, IC50 = 17 µM	Kitamura *et al.*, 2005
Transient transfection luciferase assays using Monkey kidney CV1 cells	No hAR agonistic nor antagonistic activity	BPS does not compete with R1881 to bind hAR (10^{-8} to 10^{-4} M) BPS does not inhibit hAR activity, even at the highest dose tested ($3x10^{-8}$ to 10^{-13} M)	Teng *et al.*, 2013
Luciferase assay using PALM cells (transfected with Androgen receptor) Proliferation assay using MCF-7 AR1 cells	Agonist androgenic activity No antagonist androgenic activity Agonist androgenic activity	Weak agonistic androgen activity at 10^{-5} M (showing 15% of maximal activity) EC50 = 7.054 x 10^{-5} M No antagonistic activity in presence of R1881 Weak inhibition of proliferation at 10^{-5} M, revealing agonistic activity	Molina-Molina *et al.*, 2013
Androgen receptor (AR) reporter gene assay	Antagonist androgenic activity	Slight decrease of AR activity (not significant)	Rosenmai *et al.*, 2014
Yeast Androgen Receptor bioassay	No effect on AR (agonist or antagonist)	BPS did not affect AR activity up to 10-3M (agonist/antagonist)	Roelofs *et al.*, 2015
Luciferase reporter gene assay using MDA-kb2 cell line	Antagonistic activity	Inhibition of the transcriptional activity induced by DHT Cell viability over 90% at >50 µM	Kolšek et al., 2015

Yeast reporter assay based on hAR	No activity (agonist nor antagonist)	No effect observed	Skledar *et al.*, 2016
Reporter gene assay	No activity (agonist nor antagonist)	No effect observed	Simon *et al.*, 2016
Investigation of AR Binding mechanism (interaction with coregulators)	AR binder	AR binding at alternative sited	Perera *et al.*, 2017
AR Transactivation assay Proliferation activity in human prostate cells	No agonist nor antagonist activity	Synergetic effect observed with DHT in transactivation assay but not confirmed in cells	Zenata *et al.*, 2017
Yeast androgen screen (YAS) assay	Anti-androgenic	IC_{50} = 600 µM Hydroxyflutamide equivalent = 0.02	Conroy-Ben *et al.*, 2018
Luciferase reporter assay based on AR	No agonist nor antagonist AR activity	No effect	Kojima *et al.*, 2018
Fluorescence polarisation test receptor-ligand binding assays AR- Co activator assay AR transactivation assay	No AR binding No AR activity No AR activity	No effect No activity No activity	Eilebrecht *et al.*, 2019
PALM cell luciferase assay	No androgen activity (anti)	No positive correlation was found between BPS concentrations in the thermal paper samples and anti-androgenic activity	Molina-Molina, 2019
Luciferase reporter assay based on AR	No agonist nor antagonist AR activity	No effect	Pelch *et al.*, 2019
Luciferase assay based on AR	No activity	No activity	Grimaldi *et al.*, 2019
Competitive binding assay (PolarScreen Kit) Reporter gene assay using MDA-kb2 cells	No binding No agonistic nor antagonistic activity	No binding (AR) No AR activity	Chen *et al.*, 2019
Luciferase assay (STTA, TG458)	Anti-androgenic activity	IC_{50} = 4.33 x 10^{-5} M	Park *et al.*, 2020
Competitive ligand binding AR	No binding	No binding (AR)	Keminer *et al.*, 2020

Tox Cast Pathway model (AR) prediction

Agonist (AUC) :0,
Antagonist (AUC): 0.
Cytotoxicity limit 13.64 µM

Fang *et al.* (2003)

AR binding was examined in a AR competitive binding assay using PanVera AR BLD (identical to hAR LBD): recombinant rat protein expressed in E. coli.

RBA was calculated by dividing the IC50 of R1881 by the IC50 of the competitor and was expressed as a percent. For R1881, the mean IC50 was 3.07×10^{-9}, the RBA was set to 100 or its logRBA = 2.

A substance is considered a strong binder if RBA > 1, moderate binder if1 > RBA > 0.01 or a weak binder if 0.01 > RBA > 0.0001.

In this study an IC50 of 3.75×10^{-4} µM, an RBA of 0.0008 and a log RBA of -3.09 were reported for BPS. It can be concluded that BPS shows weak AR binding.

Kitamura *et al.*, 2005

In this study, the estrogen, androgen and thyroid activity of bisphenol A and 19 related compounds was examined using three different *in vitro* assays (see also OECD CF Level 2: 1. Estrogen activity and 3. Thyroid hormonal activity).

The androgenic activity of the test compounds was tested by means of an ARE (Androgen Response Element)-luciferase reporter assay using NIH3T3 cells (mouse embryonic fibroblast cell line). The test compounds were added at concentrations of 10^{-4} to 10^{-8} M.

For the assay of anti-androgenic activity, the inhibitory effect of the test chemicals on the androgenic activity of 10^{-10} or 10^{-11} M dihydrotestosterone (DHT) was examined.

BPS did not show androgenic activity in this test. However, the substance was shown to have anti-androgenic activity at concentrations between 10^{-6} and 10^{-4} M with an IC50 of 17 µM (at 10^{-10} M DHT). BPS was found to be a potent anti-androgen in this test.

Teng *et al.* (2013)

Teng *et al.* (2013) performed transient transfection luciferase assays for examining the potential androgenic or estrogenic agonism or antagonism of BPS and several other chemicals.

For the androgenic activity assay, monkey kidney CV1 cells were transfected with pSG5-AR and MMTV Luciferase. In the agonist mode assay, cells were incubated with different concentrations of BPS (10^{-8} to 10^{-4} M). For the antagonist mode assay, cells were incubated with 5×10^{-10} M of the synthetic agonist R1881 in addition to different concentrations of BPS (3×10^{-8} to 1×10^{-13} M). The results of the assay showed no agonistic or antagonistic androgenic activity for BPS under the tested conditions.

Molina-Molina *et al.* (2013)

Agonistic and antagonistic nuclear receptor activities of BPS and other BPA-congeners and derivatives were examined in vitro using competitive binding nuclear receptor binding assays, reporter gene expression systems and cell proliferation assays.

- PALM cells were used to examine the androgenic activities of BPS. These are PC3 cells co-transfected with the androgen responsive gene MMTV-Luc-SV-Neo and an androgen receptor expressing plasmid (pSG_5AR-puro).

  Agonistic activities were tested for concentrations of BPS between 10^{-8} and 10^{-5} M. Results were expressed as a percentage of maximal luciferase activity (100% at 10 nM for R1881).

  The potency corresponding to the concentration yielding half-maximal luciferase activity (EC50 value) was calculated: EC50 for BPS = 70.54 ± 2.21 µM.

  BPS showed weak agonistic androgen activity at 10 µM (15% of maximal activity), but no antagonistic activity was seen. IC50 = no effect.

- Androgenic activity was further characterised by using MCF-7 AR1 cells, which are MCF-7 cells stably transfected with hAR. Agonistic androgen activity is assigned when a substance inhibits the cell proliferation in the MCF-7 cells. Cells were treated with 100pM E2 in the presence of increasing R1881 and BPS for 5 days. Results were expressed as proliferative effect, which is the ratio between the highest cell yield obtained with the chemical and the proliferation of hormone-free control cells.

  10µM BPS induced significant inhibitory effects in the MCF-7 cells and was therefore considered as a weak androgen agonist.

BPS showed weak agonistic androgen activity, but no antagonistic androgen activity under the conditions of the test.

Rosenmai *et al.* (2014)

AR activation of BPA and its analogues was tested in an AR reporter gene assay (described by *Vinggaard et al.* (2002)), using Chinese hamster ovary cells. BPS showed a not significant decreasing trend in AR activity (not modelled for the assay), while it was predicted negative in the QSAR-models (see table 71 OECD level 1 and table 74 in 1. Estrogenic activity)

Roelofs *et al.* (2015)

Yeast Androgen Receptor bioassays were performed with different concentration of BPS: 10^{-11} to 10^{-3} M (for agonistic activity), 10^{-9} to 10^{-3} M (for Antagonistic activity). Testosterone was used as positive control for AR activation.

BPS did neither show agonistic nor antagonistic AR activity under the conditions of the test.

Kolšek *et al.* (2015)

To examine the androgen and glucocorticoid receptor activity of 15 industrial chemicals reporter gene assay were performed utilising MDA-kb2 cell line which expresses both receptors.

BPS showed no cytotoxicity at 50 µM (conc with over 90% cell viability).

MDA-kb2 Cells were incubated for 24h with 50 µM of BPS. BPS inhibited the transcriptional activity induced by DHT and thus showed androgen antagonistic activity.

Skledar *et al.* (2016)

This study focusses on BPS metabolites. As for BPA, BPS-glucoronide is the main BPS metabolite. Hydroxylation can also occur to a lesser extent, leading to the formation of BPSM1. The authors assessed then the estrogenic, androgenic and thyroid disrupting

activities of BPS and its main metabolites. Results regarding estrogen and thyroid are described above and below, respectively.

Using a yeast cell assay expressing hAR, the authors examined the androgenic or anti-androgenic activity of BPS and its metabolites from 30 nM to 300 µM. None of the compounds tested showed an activity.

Simon *et al.* (2016)

BPS was one of the 65 compounds, migrating from polycarbonate replacement materials for plastic baby bottles, which were examined for their endocrine activity.

Besides, human oestrogen receptor (ER), human progesterone receptor (PR), human glucocorticoid receptor (GR), human peroxisome proliferator-activated receptor gamma (PPARγ), human thyroid receptor beta (TRβ) and the mouse aryl hydrocarbon receptor (AhR), human androgen receptor (AR) was investigated in an *in vitro reporter gene* assays. T47-D cell line (TARM-Luc, TM-Luc and TGRM-Luc) were used.

For agonistic activity cell lines were exposed to 1 µM, 10 µM, 100µM and 1 mM BPS dissolved in 1% DMSO. For antagonistic activity cells were exposed to 10 mM, 1mM and 100 µM BPS dissolved in DMSO and in presence of an agonistic reference ligand at a concentration inducing 50% of the maximal response.

BPS showed no androgenic agonistic nor antagonistic AR activity.

Perera *et al.* (2017)

The aim of this study was to determine the binding mechanism of BPA and its analogues BPAF and BPS to the androgen receptor (AR), and particularly the interactions of coregulator peptides with the substances. The authors used the Micro Array for Real-time Coregulator Nuclear Receptor Interaction (MARCoNI) assay to assess the binding of the bisphenols to the AR, in presence or absence of the AR agonist R1881 or the AR antagonist CPA.

Results showed that BPS is able to bind the AR but interact with it at alternative surface sites. This explains why BPS did not compete with R1881 binding the ligand binding domain (LBD) as shown in previous study (Teng *et al.*, 2013).

Zenata *et al.* (2017)

The aim of this study was to see if BPS can activate several nuclear receptors, i.e., androgen receptor (AR), thyroid receptor (TR), aryl hydrocarbon receptor (AhR), glucocorticoid receptor (GR), pregnane X receptor (PXR) and vitamin D receptor (VDR). The authors used several transfected cell lines expressing the targeted receptor in a transactivation assay to determine the agonist and antagonist activity of BPS. Then in case of positive results, different human cell lines specific to the receptor assessed were used to confirm or not the effect.

Results regarding TR, AhR, GR, PXR and VDR are described hereafter. BPS did not show any agonist nor antagonist transcriptional activity of AR reporter. But co-exposure of BPS and DHT (a known AR binder) for the antagonist assay resulted in a synergetic increase of DHT-inducible AR-dependent luciferase activity by 10-30 % at all BPS concentrations (from 0.001 to 100 µM). However, this effect was not confirmed in prostate cancer cells which were treated for 24h with 0.001-10 µM of BPS and 100 nM DHT. The authors considered thus the results as negative.

Conroy-Ben *et al.* (2018)

The authors of this study compared the results of *in vitro* assays and in silico predictions regarding the estrogenic and androgenic activities of several bisphenols. They used the Biograf3 VirtualToxLab multi-dimensional QSAR for the in silico modelling and compared the results with Yeast estrogen and androgen screen assays (YES/YAS assays).

The YES results are described above. Regarding androgenic activity, BPS did not show any agonist activity but a weak anti-agonist activity, with an IC50 of 600 µM. Compared with hydroxyflutamide, which was used as positive control in this study, BPS showed a potency of 0.02.

The weak estrogenic and anti-androgenic activity of BPS were confirmed in silico.

Kojima *et al.* (2018)

The authors assessed the transcriptional activity of BPA and 8 analogues using luciferase receptor assays based on human ERα, ERβ, AR, GR, PXR and CAR. Transiently transfected cells with specific expression plasmids were exposed to 10^{-9} to 10^{-5} M of chemical. Results were expressed as REC50, i.e., 50% of relative effective concentration in case of agonistic activity, and RIC50, i.e. 50% relative inhibitory concentration.

BPS had no agonist nor antagonist effect on AR, GR, PXR and CAR. REC50 = 5.4×10^{-7} M for both ERα and ERβ. No antagonist activity was observed on ERs.

Eilebrecht *et al.* (2019)

The endocrine potential of BPA and selected substitution candidates were tested through validated and commercially available test systems (competitive receptor-ligand binding assays, coactivator recruiting assays, and cell-based reporter gene assays).

In a fluorescence polarisation test receptor-ligand binding assays AR binding of BPS could not be confirmed.

In the androgen receptor co-activator assay and Androgen Receptor Transactivation assay, no AR activity was observed.

Molina-Molina *et al.* (2019)

In this study 112 thermal paper receipts from Brazil, Spain and France were examined for the presence of BPA, BPS and BPF as well as for their estrogenic and ani-androgenic activity. BPS was only found in 2 samples from Brazil, 2 from Spain and 10 from France, in concentrations between 6.46 and 13.29 mg/g.

Anti-androgenic activity of the thermal paper samples was examined in a PALM cell luciferase assay. No positive correlation was found between BPS concentrations in the samples and anti-androgenic activity.

Pelch *et al.* (2019)

The transcriptional activity of 22 bisphenols was assessed using luciferase receptor assays based on human ERα, ERβ and AR. Transiently transfected HepG2 (ER) and MDA-kb2 (AR) cells with specific expression plasmids were exposed to 3×10^{-9} to 1×10^{-5} M of chemical. Results were expressed as REC_{50}, i.e., 50% of relative effective concentration in case of agonistic activity, and RIC_{50}, i.e. 50% relative inhibitory concentration.

BPS did not show any AR agonist nor antagonist activity. BPS showed an agonist activity on both ERα and ER β. $REC_{50} = 1.3 \times 10^{-6}$ M for ERα and $REC_{50} = 2.1 \times 10^{-6}$ M for ERβ.

Grimaldi *et al.* (2019)

The transcriptional activity of 24 bisphenols was assessed using luciferase receptor assays based on human ERα, ERβ, ERRγ (oestrogen related receptor), AR, PXR (pregnane X receptor), PR (progesterone receptor), GR (glucocorticoid receptor) and MR (mineralocorticoid receptor).

Authors used HELN cells expressing a chimeric AR, where the DNA binding domain was replaced by that of Erα. The EC50 value of R1881 for AR is 0.57 nM in such cells. The same cells were used to examen whether BPS was able to inhibit the R1881-induced luciferase expression (anti-androgen activity).

BPS showed no agonist nor antagonistic androgen activity.

Chen *et al.* (2019)

15 bisphenols were tested for their potential interference with the AR receptor.

AR binding was investigated in a competitive binding assay (PolarScreen Kit). BPS did not bind to the androgen receptor.

Furthermore, in a reporter gene assay using MDA-kb2 cell line BPS did not show AR activity (no agonistic not antagonistic activity).

Park *et al.* (2020)

The authors of the study evaluated the oestrogenic, androgenic, and AhR potencies of BPA, BPF and BPS using stably transfected transcriptional activation assay (STTA). The luciferase activities were determined in accordance with the OECD TG 455 in HeLa9903 cells (ERα), with the OECD TG 458 in AR-EcoScreen cells (AR), and similarly in DR-EcoScreen cells (AhR). Moreover, the effect of the bisphenols on expression of ERα receptors in MCF-7 cells was determined by Western-blot.

BPS showed no agonistic activity on AR, but an antagonistic activity with an IC_{50} of 4.33 x 10^{-5} M. Results regarding ER and AhR are described in corresponding sections.

Keminer *et al.* (2020)

The authors evaluated the potential endocrine activity of several BPA substitutes, including BPS. They first screened the ability of the 33 alternatives identified using commercial PolarScreenTM kits for competitive ligand binding assay (fluorescence polarization) for ERα, ERβ and AR. At 10 µM, BPS was not able to inhibit significantly the binding of fluorescent compound neither to ERs nor AR.

3. Steroidogenesis

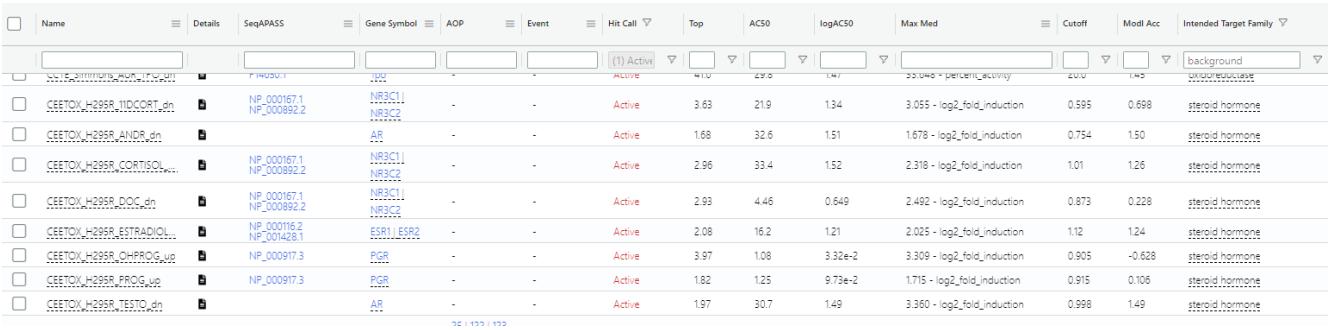
Table 74: Overview of steroidogenesis (literature data)

Short Method description	Result (*positive*/*negative*)	Description of results (*positive*/*negative*)	References
ToxCast (CEETOX H295R)	Effect on steroidogenesis	Active but above cytotoxicity limit of 13.64 µM • 11-deoxycoticosterone: AC50= 21.9 µM • Androstenedione: AC50= 32.6 µM • Cortisol: AC50= 33.4 µM • Estradiol: AC50= 16.2 µM	ToxCast, EPA

		• Testosterone: AC50 =30.7 µM Active below cytotoxicity limit: • 11-Deoxycortisol: AC50= 4.46 µM • Progesterone: AC50= 1.25 µM • 17-alpha-hydroxypregnelone: AC50= 1.08 µM	
H295R steroidogenesis assay	Effect on steroidogenesis (estrogenic and anti-androgenic)	Significant increase in production of: • Progesterone (Emax = 744%, EC50 = 1.47 µM) • 17-α-OH progesterone (Emax = 1676%, EC50 = 8.0 µM) Significant decrease in production of: • Dehydroandrosterone (Emax = 70%, EC50 = 12.5 µM) • Androstenedione (Emax = 57%, EC50 = 14.9 µM) • Testosterone (Emax = 62%, EC50 = 14.5 µM) • Cortisol (Emax = 74%, EC50 = 4.8 µM) • Corticosterone (Emax = 70%, EC50 = 4.7 µM) No effect on Estrone and 17β-estradiol production	Rosenmai *et al.*, 2014
Testosterone secretion assay using MA-10 Leydig cells Gene expression study, using MA-10 Leydig cells Steroid hormone profile using MA-10 Leydig cells	No effect on testosterone production Increased gene expression Effect on steroidogenesis	BPS did not affect testosterone secretion in MA-10 cells at concentrations from 10-2 to 3x10-5 M BPS upregulated 5aRed1 gene expression by 8.4-fold at 10-5 M BPS (10-5M) induced a significant increase in pregnenolone and progesterone	Roelofs *et al.*, 2015 (eMSCA comment: cell line not suitable to assess testosterone production)
H295R Steroidogenesis assay (OECD TG 456)	Effect on steroidogenesis (anti-androgenic)	No increase of estradiol level. Significant decrease of testosterone (Emax: 0.33, LOEC: 30 µM)	Goldinger *et al.*, 2015

Fetal testis assay (basal testosterone concentration)	Effect on steroidogenesis (anti-androgenic)	Inhibition of testosterone secretion (more potent than BPA)	Eladak *et al.*, 2015
H295R cells assay	Steroidogenesis inhibitor	Increase of progesterone production Decrease of aldosterone, cortisol and testosterone production	Feng *et al.*, 2016
Rat testis exposed to BPS	Not significant effect on steroidogenesis (anti-androgenic)	Light decrease of testosterone level (not significant)	Ullah *et al.*, 2016
Human testis explants exposed to BPS *ex vivo*	No clear activity	Production of testosterone not clearly affected (NMDR)	Desdoits-Lethimonier *et al.*, 2017
Rats' testis explant exposed to BPS *ex vivo*	Not significant effect on steroidogenesis (anti-androgenic)	Decreased testosterone level, but not significant	Ullah *et al.*, 2018a
Exposure of mouse spermatocyte GC-2 cells	Increased steroidogenesis gene expression	Increased expression of StAR, Cyp11a1, Hsd17b3, Cyp17a1 and Cyp19a1	Sidorkiewicz *et al.*, 2018
Cells proliferation assay Aromatase activity	Increased aromatase activity	Up-regulation of aromatase, increased estradiol biosynthesis, increased ERα-dependent cell proliferation	Williams *and Darbre* 2019
Testosterone release in TM3 Leydig cell line	No significant effect on steroidogenesis	Decrease in production of testosterone at 10 µg/ml (91.2%), 25 µg/ml (93.1%) and 50 µg/ml (80.6% but not sign. different from control	Jambor *et al.*, 2019
Hormones production in granulosa cells	Effect on steroidogenesis (Estradiol and progesterone production affected)	Decreased progesterone production (by 22% after exposure to 20 µM) Increased estradiol production (by 2-fold after exposure to 10 µM). Changed expression of PR and ER	Téteau *et al.*, 2020
In vitro maturation and fertilization of ewe oocytes	Effect on steroidogenesis (progesterone production affected)	Reduced progesterone production after exposure to 1 µM BPS. Affected oocyte maturation after in vitro fertilization	Desmarchais et al., 2020

ToxCast, EPA (2021)



Name	Details	SeqAPASS	Gene Symbol	AOP	Event	Hit Call	Top	AC50	logAC50	Max Med	Cutoff	Modl Acc	Intended Target Family
						(1) Active							background
CEETOX_H295R_11DCORT_dn		NP_000167.1 NP_000892.2	NR3C1 | NR3C2	-	-	Active	3.63	21.9	1.34	3.055 - log2_fold_induction	0.595	0.698	steroid hormone
CEETOX_H295R_ANDR_dn			AR	-	-	Active	1.68	32.6	1.51	1.678 - log2_fold_induction	0.754	1.50	steroid hormone
CEETOX_H295R_CORTISOL_...		NP_000167.1 NP_000892.2	NR3C1 | NR3C2	-	-	Active	2.96	33.4	1.52	2.318 - log2_fold_induction	1.01	1.26	steroid hormone
CEETOX_H295R_DOC_dn		NP_000167.1 NP_000892.2	NR3C1 | NR3C2	-	-	Active	2.93	4.46	0.649	2.492 - log2_fold_induction	0.873	0.228	steroid hormone
CEETOX_H295R_ESTRADIOL...		NP_000116.2 NP_001428.1	ESR1 | ESR2	-	-	Active	2.08	16.2	1.21	2.025 - log2_fold_induction	1.12	1.24	steroid hormone
CEETOX_H295R_OHPROG_up		NP_000917.3	PGR	-	-	Active	3.97	1.08	3.32e-2	3.309 - log2_fold_induction	0.905	-0.628	steroid hormone
CEETOX_H295R_PROG_up		NP_000917.3	PGR	-	-	Active	1.82	1.25	9.73e-2	1.715 - log2_fold_induction	0.915	0.106	steroid hormone
CEETOX_H295R_TESTO_dn			AR	-	-	Active	1.97	30.7	1.49	3.360 - log2_fold_induction	0.998	1.49	steroid hormone

Active but above cytotoxicity limit of 13.64 µM

- 11-deoxycoticosterone: AC50= 21.9 µM
- Androstenedione: AC50= 32.6 µM
- Cortisol: AC50= 33.4 µM
- Estradiol: AC50= 16.2 µM
- Testosterone: AC50 =30.7 µM

Active below cytotoxicity limit:

- 11-Deoxycortisol: AC50= 4.46 µM
- Progesterone: AC50= 1.25 µM
- 17-alpha-hydroxypregnelone: AC50= 1.08 µM

Rosenmai *et al.* (*2014*)

In this study, Rosenmai *et al.* investigated the activities of bisphenols analogues on diverse receptors using receptor-specific reporter gene assays, as well as their effects on steroidogenesis.

BPS showed an increase of ER activity (up to 222%) with an EC50 of 8 x 10-8 M. A light dose-dependent decrease of AR activity has been observed, but this was not significant. No effect has been observed on Aryl hydrocarbon receptor (AhR) nor retinoic acid receptor (RAR).

In a H295R steroidogenesis assay, the level of 17β-estradiol or estrone did not change after 48h of BPS exposure (0.8-50 µM). However, the level of male hormones (dehydroandrosterone, androstenedione and testosterone) was significantly lower (70%, 57% and 62%, respectively). Similarly, the levels of cortisol and corticosterone were also decreased (74% and 70%, respectively). The main effect, however, was an important increase of progesterone and 17-α-OH progesterone levels up to 744% and 1676%, respectively.

Goldinger *et al.* (2015)

This study examined endocrine activity of alternatives of BPA found in thermal paper In Switzerland. A steroidogenesis assay was performed according to OECD TG 456 (GLP). using a H295R human adrenocortical carcinoma cell line. BPS was tested with concentrations of 0.1, 0.3, 1, 3, 10, 30 and 100 µM.

After 48h exposure, BPS inhibited free testosterone level at 30 µM (maximal level in comparison with original concentration : 0.33) but had not significant effects on 17β-estradiol level. The authors indicate that endocrine activity might have been missed due to the possible low metabolic activity of H295R cells because BPS showed negative estrogenic activity in the E-screen without metabolic activation and positive after metabolic

activation in the Hashimoto *et al.*, 2001's study. Therefore, it might possible that BPS first need to be metabolized to show estrogenic activity.

Roelofs *et al.* (2015)

Testosterone secretion assay and gene expression study were performed with Mouse Leydig tumorigenic cells (MA-10) and BPS concentrations between 0.01 and 30 µM. MA-10 Leydig cells appear to be very useful to examine potential ED effects on foetal testicular steroidogenesis.

BPS did not affect T secretion by MA-10 Leydig cells at the highest tested concentration.

After 48h exposure to BPS (10 µM), levels of pregnenolone (P5) and progesterone (P4) increased (0.77 +/-0.17 and 7.14+/-1.36 in BPS treated cells vs 0.37+/-0.18 and 1.77+/-0.70 in DMSO control cells, respectively. BPS predominantly changed the levels of progestogens that are formed in the beginning of the steroidogenic pathway.

BPS did not upregulate the StAR gene expression suggesting that the effect on testicular steroidogenesis is not mediated by a cAMP-dependent pathway. 5αRed1 gene expression was upregulated by BPS (by 8.4 fold).

Upregulation of 5αRed1 gene expression suggests a redirection of steroidogenesis, which might lead to important effect in foetal testis development and function.

eMSCA comment on Roelofs *et al*. (2015):

As MA-10 cells do not express CYP17A1, this cell line is not suitable to assess the production of testosterone (Engeli *et al.*, 2018). Moreover, another *in vitro* study showed a decreased secretion of basal T in human and mouse even if it was not seen in rat foetal testis explant cultures (Eladak *et al.*, 2015).

Eladak *et al.* (2015)

The authors developed an *in vitro* assay to assess the effect of a substance on the production of testosterone using rat, mouse or human foetal testis explants (r/m/h FeTA). This method allows maintaining *in vitro* the development of Leydig cells, gonocytes and Sertoli cells. Changes in the expression of diverse foetal-Leydig cells specific genes can also be monitored. The determination of basal testosterone secretion reflects the testis activity and testosterone secretion.

Cells were exposed for 3 days to a concentration range of 10-10 000 nM BPS.

BPS significantly reduced testosterone secretion in mouse foetal testis explants exposed to 100 nM. BPS is here more potent than BPA (decrease observed at 1000 nM). Whereas in mouse the effect is dose-dependent (10 to 10000 nM), a non-monotonic dose-response was observed in hFeTA. Moreover, BPS (as BPA) tended to reduce the expression of foetal Leydig cell-specific genes involved in testosterone biosynthesis.

Feng *et al.* (2016)

The authors used a steroidogenesis assay to evaluate the effects of BPS and 3 other bisphenols (BPA, BPF and BPAF) on the production of five steroid hormones (progesterone, aldosterone, cortisol, testosterone and 17β-estradiol), and on transcription of genes encoding the steroidogenic enzymes. Cells were exposed to 0, 0.1, 1, 10, 30, 50 and 70 µM BPS for 48h. Hormones were quantified using commercially available radioimmunoassay kits, and gene transcription level measured by RT-PCR.

48h BPS exposure (0, 0.1, 1, 10, 30, 50, and 70 µM) resulted in non-monotonic dose-response, showing a significant elevation of progesterone at 1 and 10 µM (50.3% and

91.0%) but a decrease at 50 and 70 µM (53.8% and 70.3%). A dose-dependent decrease in aldosterone (from 20.5% to 75.2% between 0.1 and 70 µM), cortisol (from 55.9% to 79.0% between 30 and 70 µM) and testosterone production (from 34.0% to 86.8% between 10 and 70 µM).

BPS did not disrupt genes of hormones involved in progesterone production (StAR, FDX-1, CYP11A1 and HSD3B2). However, BPS downregulated single genes involved in aldosterone production (CYP11B1) and testosterone/17β-estradiol production (CYP17A1).

Ullah *et al.* (2016)

The aim of the study was to assess the effect of BPS on the rat male reproductive system *in vitro* and *in vivo* (see *in vivo* description hereafter). After removal, rat testes have been exposed to 0, 0.5, 1, 10 and 100 ng/mL BPS for 2h. The level of antioxidant activity (catalase CAT, peroxidase POD, superoxide dismutase SOD), reactive oxygen species (ROS) and testosterone have been measured.

Results indicate a light increase of the oxidative stress, even if the parameters measured were not always significantly higher than the controls.

The concentration of testosterone measured per gram of testis was reduced in the treated groups compared to the control group, but the reduction was not statistically significant (56.06, 53.17, 53.68, 54.56, 52.93 ng/g tissue after exposure to 0, 0.5, 1, 10 and 100 ng/mL, respectively).

The *in vivo* analysis (see hereafter) confirmed the increased oxidative stress and the reduced testosterone concentration.

Desdoits-Lethimonier *et al.* (2017)

The authors used adult human testes explants to assess the impact of BPA and some BPA-analogues (including BPS). Tissues were exposed to 10^{-9} to 10^{-5} M substances for 24h or 48h.

Contrary to BPA and other analogues, BPS exposure exhibited a non-monotonic dose response regarding testosterone production. At 24h, the production was significantly increased at 10^{-6} M but slightly decreased at 10^{-5} M. After 48h, the production was significantly reduced at 10^{-8} M, but slightly increased at higher doses.

The results were clearer for the production of insulin-like factor 3, which was increased at all concentrations and time, but only significantly at 10^{-9} and 10^{-8} M after 48h.

Ullah *et al.* (2018a)

The aim of the study was to compare the effect of several bisphenols, i.e., BPA, BPS, BPB and BPF on the rat male reproductive system *in vitro* and *in vivo* (see *in vivo* description hereafter). The experiment is similar to the study of the same group published in 2016 (Ullah *et al.*, 2016). After removal, rat testes have been exposed to 0, 1, 10 and 100 ng/mL BPS for 2h. The level of antioxidant activity (catalase CAT, peroxidase POD, superoxide dismutase SOD), reactive oxygen species (ROS) and testosterone have been measured.

Results indicate an increase of the oxidative stress, particularly at 100 ng/mL BPS. At this dose, the level of LPO and total ROS were significantly higher than in the control.

The concentration of testosterone measured per gram of testis was reduced in the treated groups compared to the control group, but the reduction was not statistically significant (54.27, 53.15, 51.65 and 52.00 ng/g tissue after exposure to 0, 1, 10 and 100 ng/mL, respectively).

The *in vivo* analysis (described hereafter) confirmed the increased oxidative stress and the reduced testosterone concentration.

Sidorkiewicz *et al.* (2018)

The aim of this study was to assess the impact of BPS, BPA and BPF, individually and in mixture, on the mouse spermatocyte GC-2 cells. Exposure to 10^{-10} to 10^{-6} M BPS did not affect the cell viability but revealed a significant increase of cells with permeabilised mitochondria, which is a marker of early apoptosis.

Mouse spermatocytes exposed to 10^{-10} or 10^{-8} M BPS showed increased expression of genes coding for ERα and ERβ, as well as genes involved in steroidogenesis, i.e., StAr, Cyp11a1, Hsd17b3, Cyp17a1 and Cyp19a1.

Williams and Darbre (2019)

The authors evaluated the effect of several endocrine disruptors including BPS on aromatase in three different human breast cell lines: MCF-7, ZR-75-1 (ERα-dependent) and HMF3A (ERα-independent). Cells were exposed to chemicals at environmentally relevant concentrations for 8h.

All 3 cell lines showed a significant increase of CYP19A1 mRNA synthesis and a significant increase of aromatase activity after exposure to 10^{-8} M BPS. Accordingly, a significant increase of 17β-estradiol synthesis has been also observed in all cell lines at 10^{-6} M. Finally, a significant proliferative growth response was seen in ERα-dependent MCF-7 and ZR-75-1 cell lines after exposure to 10^{-8} M BPS. The ERα-negative HMF3A cell line did not show any cell proliferation.

Jambor *et al.* (2019)

The impact of testosterone production in TM3 Leydig cell line was determined for BPA, BPB, BPS and BPF after cells were exposed for 24h to 0.04-50 µg/mL bisphenols.

The production of testosterone decreased, although not significantly different from the control, at 10 µg/mL BPS (91.2%), 25 µg/mL (93.1%) and 50 µg/mL (80.6%). At those concentrations BPS did affect cell viability significantly resp. with 84.81%, 79.47% and 61.30%.

Téteau *et al.* (2020)

In this study, the authors compared the effects of BPA and BPS on ovine granulosa cells. They collected approximately 1000 ovaries of adult ewes, extracted their antral follicles and isolated the granulosa cells. BPS had no effect on cell viability (up to 200 µM). Cell proliferation was also not affected below 200 µM BPS exposure (8% decrease).

The authors first evaluated the effects of bisphenols exposure on hormone production. Progesterone secretion was significantly reduced after exposure to BPS whereas estradiol expression was significantly increased. Progesterone plays an important role in the maturation and development of oocytes. Low ovulation levels in women were associated with low serum progesterone levels. Therefore it might be suggested that BPS reduction of progesterone has an harmful effect on oocyte quality and consequently on fertility.

The authors then observed no effect on steroidogenic enzyme protein nor gene expression. Only the expression of genes coding for estradiol receptors (ESR1 and ESR2 were significantly increased. Interestingly, PR gene expression was also enhanced at 10 and 50 µM without reaching statistical significance.

Table 75: Different doses of BPS and corresponding protein and gene expression levels (Téteau *et al.*, 2020)

Dose (µM)	ng Progesterone / mg protein	ng Estradiol / mg protein	Ratio ESR1 gene expression	Ratio ESR2 gene expression	Ratio PR gene expression
0	36.23 ± 3.88	28.94 ± 6.08	1.000 ± 0.120	1.000 ± 0.151	1.000 ± 0.157
10	28.91 ± 3.78*	61.97 ± 11.83*	1.236 ± 0.123*	1.058 ± 0.114	1.497 ± 0.267
50	22.31 ± 2.50**	n.t.	1.557 ± 0.278*	1.817 ± 0.278*	1.445 ± 0.248
100	17.13 ± 1.92**	78.65 ± 11.28**	2.062 ± 0.357*	2.574 ± 0.578*	0.982 ± 0.219

* = $p < 0.05$, ** = $p < 0.001$, n.t. = non tested

Finally, the authors assessed the effects of BPS on signalling pathways in granulosa cells. BPS transiently and significantly affected the MAPK3/1 involved in cell viability and proliferation, and the AMPKα phosphorylation involved in lipid metabolism.

Demarchais *et al.*, 2020

The authors assessed the effects of low doses of BPS on ewe oocyte quality and developmental competence, and the impact on the production of progesterone. Ewe cumulus-oocyte complexes were maturated in vitro for 24h, in absence or in presence of BPS (1 nM, 10 nM, 100 nM, 1 µM or 10 µM). Oocytes were then subjected to in vitro fertilisation and development.

Two days after in vitro fertilisation, the oocytes exposed to 1 nM showed a significant increase of cleavage rate (70.1% vs 54.6% in the control, $p<0.001$), whereas a significant decrease was observed in oocytes exposed to 1 µM BPS (47.6 %, $p<0.01$). After 7 days, the blastocyst rate was reduced at all doses but not in a dose-dependent manner (21.8, 18.7, 14.3*, 19.8. 12.5*, 14.8 % after exposure to 0, 1, 10, 100, 1'000 or 10'000 nM).

Progesterone was measured in 24h in vitro maturation spet culture media. A significant reduction of 41% was observed after exposure to 1 µM BPS (0.02 ng/mL) compared to the control conditions (0.034 ng/mL, $p<0.05$). The progesterone secretion was also reduced after exposure to 10 µM, but the difference was not statistically significant.

4. Thyroid MoA

Table 76: Overview of thyroid MoA (literature data)

Short Method description	Result (*positive*/*negative*)	Description of results (*positive*/*negative*)	References
Induction of growth hormone production in GH3 cells (rat pituitary tumor cells)	No thyroid hormonal activity	No significant increase of T3 thyroid hormone production.	Kitamura *et al.*, 2005
GH3.TRE-Luc reporter gene assay (TRα and TRβ)	No activity (agonist nor antagonist)	No effect observed	Skledar *et al.*, 2016
Reporter gene assays using stably transfected U-2 OS cells (human osteoblast)	No agonistic nor antagonistic activity	No agonistic nor antagonistic activity for TRβ	Simon *et al.*, 2016
Binding affinity to TTR using isothermal titration calorimetry	Binding to transthyretin (TTR-thyroid hormone transporter)	Binding affinity: Kd=52 µM Cocrystalization with wild type TTR: Compared to T4 BPS binds deeper in	Zhang *et al.*, 2016

Cocrystalization with wild type TTR		the two identical thyroxine binding sites due to their smaller molecular size.	
TR Transactivation assay	No agonist nor antagonist activity	No activity observed	Zenata *et al.*, 2017
Binding assay Coactivator recruitment assay TR-mediated reporter gene assay T-screen (GH3 proliferation) assay	Thyroid agonist and antagonist activity	Binding to TRα (IC_{50} = 2650 µM, RP_{T3} = 6.0 x 10^{-6}) Binding to TRβ (IC_{50} = 2294 µM, RP_{T3} = 8.0 x 10^{-6}) Ability to recruit coactivator to TRβ Agonist and antagonist activity in luciferase and T-screen assays	Zhang *et al.*, 2018b
Binding to TRβ Thyroid yeast two-hybrid assay	No thyroid agonist activity Thyroid antagonist activity	No agonistic activity Antagonistic activity: IC_{10} = 312 nM RP_{T3} = 2.8 (rel. potency)	Lu *et al.*, 2018a
GH3 cells proliferation assay	Thyroid agonist and antagonist activity	Increased cell proliferation up to 159% at 10^{-6} M	Lee *et al.*, 2018

Kitamura *et al.* (2005)

In this study, estrogen, androgen and thyroid activity of bisphenol A and 19 related compounds were examined using three different *in vitro* assays (see also OECD CF Level 2: 1. Estrogen activity and 2. Androgen activity).

The thyroid hormonal activity was investigated by measuring the induction of growth hormone production in GH3 cells (rat pituitary tumour cells) with triiodothyronine (T3) in concentrations between 10^{-12} and 10^{-9} M.

For the assay of anti-thyroid hormonal activity, the inhibitory effect of the test compounds on the activity of 10^{-7} or 10^{-8} M triiodothyronine (T3) was examined.

BPS did not show a significant thyroid hormonal activity under the conditions of this *in vitro* test.

Skledar *et al.* (2016)

This study focusses on BPS metabolites. As for BPA, BPS-glucoronide is the main BPS metabolite. Hydroxylation can also occur to a lesser extent, leading to the formation of BPSM1. The authors assessed then the estrogenic, androgenic and thyroid disrupting activities of BPS and its main metabolites. Results regarding estrogenic and androgenic activities are described above.

The authors used a thyroid hormone receptor reporter gene assay based on GH3.TRE-Luc cells, expressing the thyroid hormone receptors TRα and TRβ and a luciferase reporter gene. BPS and its metabolites were tested at concentrations from 30 nM to 300 µM. None of the compounds tested showed agonistic activity. Only BPSM1 (hydroxylated BPS) showed a weak antagonistic activity.

Simon *et al.* (2016)

65 compounds, migrating from polycarbonate replacement materials for plastic baby bottles, were examined for their endocrine activity. Authors investigated human oestrogen

receptor (ER), human androgen receptor (AR), human progesterone receptor (PR), human glucocorticoid receptor (GR), human peroxisome proliferator-activated receptor gamma (PPARγ), human thyroid receptor beta (TRβ) and the mouse aryl hydrocarbon receptor (AhR) in *in vitro reporter gene* assays.

Effects on TRβ were examined in U-2 OS cells (human osteoblast) stably transfected with human TRβ and a luciferase reporter construct.

For agonistic activity cell lines were exposed to 1 µM, 10 µM, 100 µM and 1 mM BPS dissolved in 1% DMSO. For antagonistic activity cells were exposed to 10 mM, 1mM and 100 µM BPS dissolved in DMSO and in presence of an agonistic reference ligand at a concentration inducing 50% of the maximal response.

BPS showed no agonistic nor antagonistic thyroid activity.

Zhang et al. (2016)

Transthyetrin (TTR) binding affinity of 12 chemicals was studied using isothermal titration calorimetry. TTR is a thyroid hormone transporter in vertebrates. One TTR tetramer can bind two ligands but ITC thermograms were fitted using one-site binding model as binding of the first ligand dominates the total binding energy and its affinity is approximately 100 times stronger than the second ligand.

BPS showed high binding affinity to TTR, with kd=52 µM. Following analysis of the thermodynamics data, the binding of BPS was largely driven by the enthalpic component.

Furthermore, BPS was co-crystalized with the human wildtype TTR. Compared to T4 BPS binds deeper in the two identical thyroxine binding sites due to their smaller molecular size. Hydroxyl groups of BPS interact with Lys15 and form water-bridged H-bonds with Thr119 and Ser117.

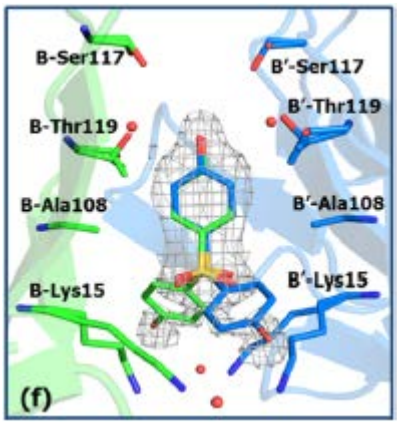


***Figure* 8** from Zhang *et al*., 2016: Crystallographic binding conformations of BPS and T4 in the thyroid-binding site of TTR: The refined (2|Fo|−|Fc|) electron density is shown in gray mesh at the 1σ level. The water molecules present around the ligands are shown as red spheres. The electron density of the second ring of BPS was poorly defined probably due to high mobility.

Zenata *et al.* (2017)

The aim of this study was to see if BPS can activate several nuclear receptors, i.e., androgen receptor (AR), thyroid receptor (TR), aryl hydrocarbon receptor (AhR), glucocorticoid receptor (GR), pregnane X receptor (PXR) and vitamin D receptor (VDR). The authors used several transfected cell lines expressing the targeted receptor in a transactivation assay to determine the agonist and antagonist activity of BPS. Then in case of positive results, different human cell lines specific to the receptor assessed were used to confirm or not the effect.

Results regarding AR, AhR, GR, PXR and VDR are described elsewhere. BPS did not show any agonist nor antagonist transcriptional activity of TR reporter.

Zhang *et al.* (2018b)

The authors of this study assessed the thyroid disrupting potential of BPA, BPF and BPS in several *in vitro* and *in vivo* assays. Results of *in vivo* assay are shown 7.10.1 (ED Environment, OECD level 3).

First, the authors evaluated the binding affinity of BPS to TRα and TRβ ligand binding domains (LBD) using a fluorescence competitive binding assay, showing the ability of BPS to inhibit the binding of fluorescein-T3 conjugate to the specific TR-LBD. BPS was able to compete with T3 for both receptors with a slightly higher affinity for TRβ (IC50 for TRα = 2650 µM, IC50 for TRβ = 2294 µM). The binding potency is weak in regard to T3 (RPT3 for TRα = 6.0 x 10-6, RPT3 for TRβ = 8.0 x 10-6).

The ability of BPS to recruit coactivator to TR-LBDs was also evaluated, measuring the amount of fluorescent probe binding to the LBD. The assay showed that 4000 µM BPS induced the recruitment of coactivator to TRβ-LBD. However, no coactivator recruitment was observed to TRα-LBD.

Transactivation ability was also tested in a TR-mediated luciferase reporter gene assay. BPS was able to induce the luciferase concentration from 1 µM to 10 µM, no effect being observed at 50 µM. And in presence of T3, BPS was able to inhibit the T3-mediated luciferase induction, showing both agonist and antagonist activity.

Finally in a T-screen assay, the higher concentration of BPS induced GH3 cell proliferation up to 1.9-fold. This proliferation was inhibited in presence of AM, a known TR antagonist. In presence of T3, the highest concentration slightly inhibited the T3-induced GH3 cell proliferation, confirming both agonist and antagonist activity.

Lu *et al.* (2018a)

The aim of this study was to assess the thyroid receptor β disrupting potential of BPA, BPS and some brominated analogues (TBBPA, TBBPS, BPS-DAE and OBBPS).

First, the authors tested *in vitro* if the substances were able to bind ligand binding domain (LBD) of TRβ. The binding was observed by fluorescence spectroscopy using a plasmid expressing the TRβ LBD. Using in silico modulation techniques, the authors confirmed the binding and showed that binding BPS to TRβ changes the configuration of the LBD, which could consequently affect the transcriptional activity of TRβ.

Using a recombinant human TRβ two-hybrid yeast assay, which is highly specific to TRβ ligand, the authors tested the potential TR disruption of BPS. The cells were exposed to 5 x 10^{-7} to 50 µM BPS but did not show any agonistic activity of BPS. However, when the yeasts were co-exposed to BPS and T3, the level of expressed β-galactosidase was significantly decreased, in a dose-dependent manner, suggesting the antagonistic effect of BPS toward TRβ. BPS exposure showed an IC10 of 312 nM and a relative potency in comparison to T3 of 2.8.

The authors also tested the impact of BPS on the expression of TRβ mRNA *in vivo* in zebrafish embryos. The TRβ mRNA were significantly upregulated after exposure to 1 µM.

Lee *et al.* (2018b)

The authors investigated the potential of several bisphenols to affect the thyroid endocrine system. The GH3 cell proliferation assay was used for the detection of TH-agonistic or TH-antagonistic properties of the chemicals in the absence or in the presence of T3, respectively. Cells were exposed to 10^{-9} to 10^{-6} M bisphenols for 48 h or 96 h.

In absence of T3, BPS stimulated cell proliferation in a dose-dependent manner, showing a significant increase of 159% at 10^{-6} M after 96h compared to the negative control. This shows that BPS is an agonist of thyroid hormone.

When co-exposed with T3 (at EC50, i.e., 6.4 x 10^{-10} M), BPS did not expand or even slightly reduced the T3-induced cell proliferation after 48 h, suggesting an antagonizing effect. However, after 96 h, high doses (10^{-7} and 10^{-6} M) BPS increased the T3-induced cell proliferation, suggesting again an agonistic effect. This indicates that TH-antagonistic effects of BPS depend on the tested dose and the exposure time.

5. Other MoA

Table 77: Overview of other MoA (literature data)

ED MoA	Short Method description	Result	Description of results	References
AhR	Aryl hydrocarbon receptor (AhR) reporter gene assay	No effect on AhR activity	No activation of AhR observed	Rosenmai *et al.*, 2014
AhR	Reporter gene assay using H1L7.5c1 cells (mouse hepatoma hepa1c1c7)	No effect	No antagonistic nor antagonistic activity	Simon *et al.*, 2016
AhR	AhR transactivation assay Effect on human hepatocytes or HeLa cells	No agonist nor antagonist activity	Weak agonist activity in transactivation Not confirmed in human cells, thus considered negative	Zenata *et al.*, 2017
AhR	Luciferase assay (STTA, DR-EcoScreen)	AhR activity	Low but significant 1.4-fold increase at 100 µM	Park *et al.*, 2020
CAR	Luciferase reporter assay based on CAR	No agonist nor antagonist activity	No effect	Kojima *et al.*, 2018
CAR	Competitive receptor-binding assay	No CAR activity	Completely inactive	Liu *et al.*, 2019b
ERRγ	Competitive receptor-binding assay	Very weak activity	IC50=4090 nM +/-238	Liu *et al.*, 2019b
GR	Yeast Glucocorticoid Receptor bioassay	No effect on GR (agonist or antagonist)	BPS did not affect GR activity up to 10^{-3} M (agonist/antagonist)	Roelofs *et al.*, 2015
GR	Luciferase reporter gene assay using MDA-kb2 cells	No GR activity	No GR agonistic nor antagonistic activity at 50 µM	Kolšek *et al.*, 2015
GR	GRE-luciferase transcription assay	No GR activity	No effect on GRE-luciferase reporter	Boucher *et al.*, 2016
GR	Reporter gene assay using T47-D (TARM-Luc, TM-Luc and TGRM-luc) cell line	No GR activity	No agonistic nor antagonistic activity	Simon *et al.*, 2016
GR	GR transactivation assay Effect on human hepatocytes or HeLa cells	No agonist nor antagonist activity	Antagonist activity in transactivation assay Not confirmed in human cells, thus considered negative	Zenata *et al.*, 2017

GR	Luciferase reporter assay based on GR	No agonist nor antagonist activity	No effect	Kojima *et al.*, 2018
GR	Luciferase assay based on GR	No activity	No activity	Grimaldi *et al.*, 2019
GR	Competitive receptor-binding assay	No GR activity	Completely inactive	Liu *et al.*, 2019b
LXR	Competitive receptor-binding assay	No LXRα and LXRβ activity	Completely inactive	Liu *et al.*, 2019b
MR	Luciferase assay based on MR	No activity	No activity	Grimaldi *et al.*, 2019
PPAR	PPRE-luciferase transcription assay	PPARG activity	Activation of PPRE-luciferase reporter up to 1.6-fold	Boucher *et al.*, 2016
PPAR	Murine adipocyte differentiation PPARγ reporter gene assay	Adipogen PPARγ activation (agonist)	Enhance lipid accumulation Increased expression of key adipogenic marker Activation of PPARγ	Ahmed and Atlas, 2016
PPAR	Reporter gene assay using stably transfected U-2 OS cells (human osteoblast)	Agonistic and antagonistic PPARγ activity	Agonistic and antagonistic activity observed (increase of the relative response of more than 10 % or decrease of the relative response for two consecutive points of more than 10 %)	Simon *et al.*, 2016
PPAR	3D-adipose tissue model	PPARγ activation	Increased expression of PPARγ gene expression	Wang *et al.*, 2017c
PPAR	Fish primary macrophages	PPRAγ mediated activity	PPARγ-mediated disruption of immune response	Qiu *et al.*, 2019
PPAR	Competitive receptor-binding assay	No PPARγ activity	Completely inactive	Liu *et al.*, 2019b
PPAR	Luciferase assay PPARγ and target genes expression	PPARγ activity	Dose-dependent increase of luciferase expression (significant at 100 µM) Dose-dependent increase of PPARγ mRNA (1.72-fold, sign. at 100 µM) Sign. increase of PPARγ-target genes at 100 µM	Gao *et al.*, 2020
PPAR	Lipid storage in 3T3 cells and expression of adipogenic markers	Obesogenic activity PPAR expression	Increased lipid storage Increased expression of adipogenic markers	Martinez *et al.*, 2020
PR	Reporter gene assay using T47-D (TARM-Luc, TM-Luc and TGRM-luc) cell line	No activity	No agonistic nor antagonistic PR activity	Simon *et al.*, 2016

PR	Luciferase assay based on PR	No activity	No activity	Grimaldi *et al.*, 2019
PXR	Competitive receptor binding assay using HG5LN-hPXR cells	No activity	No induction or inhibition of hPXR activation at concentrations up to 10µM	Molina-Molina *et al.*, 2013
PXR	PXR Transactivation assay	No activity	No transactivation of hPXR	Peyre *et al.*, 2014
PXR	PXR transactivation assay Effect on human hepatocytes or HeLa cells	No agonist nor antagonist activity	Significant agonist and antagonist activity in transactivation. Not confirmed in human cells, thus considered negative	Zenata *et al.*, 2017
PXR	Luciferase reporter assay based on PXR	No agonist nor antagonist activity	No effect	Kojima *et al.*, 2018
PXR	Luciferase assay based on PXR	No activity	No activity	Grimaldi *et al.*, 2019
PXR	Competitive receptor-binding assay	Extremely weak activity	IC50>10µM	Liu *et al.*, 2019b
RAR	Nrf2, retinoic acid receptor (RAR), and p53 reporter gene assays	No effect on RAR activity	No change in Nrf2 nor p53 response after exposure to BPS	Rosenmai *et al.*, 2014
RAR	Competitive receptor-binding assay	No activity of RARα, RARβ and RARγ	Completely inactive	Liu *et al.*, 2019b
ROR	Competitive receptor-binding assay	No RORα, RORβ and RORγ activity	Completely inactive	Liu *et al.*, 2019b
RXR	Competitive receptor-binding assay	No RXRα, RXRβ and RXRγ activity	Completely inactive	Liu *et al.*, 2019b
VDR	Competitive receptor-binding assay	No VDR activity	Completely inactive	Liu *et al.*, 2019b
Vit. D	Vit D receptor transactivation assay Effect on human hepatocytes or HeLa cells	No agonist nor antagonist activity	No activity in transactivation nor human cells	Zenata *et al.*, 2017

Molina-Molina *et al.* (2013)

Agonistic and antagonistic nuclear receptor activities of BPS and other BPA-congeners and derivatives were examined *in vitro* using competitive binding nuclear receptor binding assays, reporter gene expression systems and cell proliferation assays.

PXR is one of the nuclear receptors coordinating the detoxification of xenobiotic substances, which is strongly expressed in the liver, intestine and primarily exposed organs (Lamba *et al.*, 2004).

- Agonistic and antagonistic thyroid activity was examined by using HG_5LN-hPXR cell line. Firstly HeLa cells were stably transfected with a GAI4RE5-βGlob-Luc-SVNeo plasmid, subsequently stably transfected with the pSG5-GAL4(DBD)-hPXR (LBD)-puro. Results were expressed as a percentage of maximal luciferase activity (100% at 3 µM SR12813).
- HG_5LN cell line was used as a control for non-PXR specific activity.

BPS did not induce hPXR activation at concentrations up to 10 µM, neither did it show antagonistic activity.

Sui *et al.* (2012) detected that the presence of at least one para phenolic group, the number and position of methyl groups in the bridge between the two phenolic rings played an important role in hPXR activity. Agonistic activity was abolished by the loss of both methyl groups (BPF) or by the replacement of SO_2 (BPS).

Rosenmai *et al.* (2014)

In this study, Rosenmai *et al.* investigated the activities of bisphenols analogues on diverse receptors using receptor-specific reporter gene assays, as well as their effects on steroidogenesis.

No effect has been observed on Aryl hydrocarbon receptor (AhR) nor retinoic acid receptor (RAR).

Roelofs *et al.* (2015)

Yeast Glucocorticoid Receptor bioassays were performed with different concentration of BPS: 10^{-11} to 10^{-3} M (for agonistic activity), 10^{-9} to 10^{-3} M (for Antagonistic activity). Dexamethasone was used as positive control for GR activation

BPS did neither show agonistic nor antagonistic GR activity under the conditions of the test.

Kolšek et al., 2015

To examine the androgen and glucocorticoid receptor activity of 15 industrial chemicals reporter gene assay were performed utilising MDA-kb2 cell line which expresses both receptors.

BPS showed no cytotoxicity at 50 µM (conc with over 90% cell viability).

MDA-kb2 Cells were incubated for 24h with 50 µM of BPS. To assess GR antagonistic activity also 500 nM hydrocortisone was added, while 5µM flutamide was added to assess GR agonist activity. BPS showed no agonistic nor antagonistic GR activity.

Ma *et al.* (2015)

The PCR array contains genes involved in i.e., thyroid hormone pathway, xenobiotic metabolism, oxidative stress, and lipid homeostasis.

At 300µM, BPS revealed transcriptional changes in following genes:

- BPS downregulated Spot 14α, involved in the regulation of adipogenic enzymes and thought to play a role in thyroid stimulation of lipogenesis, by 2.11-fold.
- BPS also affected Insulin-like growth factor 1, which was significantly down regulated. It plays a role in normal growth and also a vital role via crosstalk with the thyroid hormone pathway and is associated with thyroid hormone status and lipid homeostasis.

- Upregulation of Cyp1a4 (40.6-fold). Cyp 1a4 mRNA levels were unchanged at concentrations <300 µM. At 300 µM cell viability decreased in the COS-7 cell assay, resulting in a depletion of luciferase activity.
- Mt4 expression level was increased by 10-fold. Its induction is a protective mechanism in the liver against oxidant damage and free radicals.
- Cyp3a37 and Atlas1, both regulated by the pregnane X receptor (PXR) in mammals, were upregulated 4.29-fold and 4.16-fold resp. after exposure to 300 µM BPS.
- Slco1a2, a solute transporter which mediates the cellular uptake of organic ions in the liver, including bile acids and some steroidal compounds was upregulated by 2.33-fold.
- Acs15, involved in the conversion of fatty acids to acyl-Co-A thioester and an intermediate for oxidation, elongation and desaturation of fatty acids was upregulated by 2.31-fold.
- mRNA level of Lbfabp (liver basic fatty acid binding protein), involved in the metabolism and intracellular transport of lipids, was increased by 4-fold.
- Il16, an immunomodulatory gene was down-regulated by 5.38-fold by BPS.

Peyre *et al.* (2014)

The authors evaluated the potential hepatotoxicity of BPS in comparison with BPA. Hepatocytes (HepG2) were exposed to 1 pM to 1 mM of BPS or BPA. BPS generally showed less hepatotoxicity than BPA.

Using a HepG2/PXR cell line expressing the human pregnane X receptor (hPXR), the authors performed a transactivation assay of the hPXR. BPS was not able to transactivate the PXR receptor after exposure to BPS (1 pM to 500 µM), in contrary to BPA.

Boucher *et al.* (2016)

The aim of this study was to see if BPS can induce adipogenesis as BPA. The authors exposed human preadipocytes to different concentration of BPS (0.1 nM to 25 µM). Lipid accumulation significantly increased after exposure to 10 and 25 µM BPS (up to 4.2-fold). This was confirmed by an increased expression of several adipogenic markers (mRNA and protein).

As estrogenic, glucocorticoid and PPARG receptors are known to potentially induce adipocyte differentiation, the authors performed different transcriptional assays in COS-7 cells (test duration: 24h). Results regarding estrogenic activity are described above. Whereas no effect has been reported in GR-transcription assay, BPS induced the transcriptional activity of PPARG reporters (up to 1.6-fold). The exposition simultaneous of the preadipocytes to 25 µM BPS and 1 µM of RU486 (GR antagonist) caused only a small but not statistically significant reduction, confirming that GR is not involved in this BPS-induced lipid accumulation.

Ahmed and Atlas (2016)

The authors evaluated the adipogenic potential of BPS and BPA. They first induced the adipocyte differentiation of 3T3-L1 mouse embryonic fibroblast in presence of BPS (0.01 – 50 µM) or BPA (0.01 – 25 µM). This resulted in an increased expression of adipogenic markers in the mature adipocyte from 10 µM, and a higher lipid accumulation. Interestingly, BPS was even more efficient than BPA at 25 µM (earlier adipogenesis and significantly higher lipid accumulation). In presence of the PPARγ antagonist GW9662 showed a significant decrease of adipogenic markers, suggesting that the adipogenic activity of BPS and BPA is PPARγ-mediated.

To confirm this, the authors investigated whether the substances could activate PPARγ in a PPRE-dependent luciferase assay. COS-7 cells were transfected with mPPARγ, mRXR, and a PPRE-luciferase reporter plasmid. BPS exposure caused a modest but significant increase of luciferase activity at 25 and 50 µM. Experiment performed in presence of the

PPARγ agonist ROSI showed that BPS was able to bind to PPARγ and displace ROSI, and thus functions as a partial agonist.

Simon *et al.* (2016)

Authors used *in vitro reporter gene* assays to investigate human oestrogen receptor (ER), human androgen receptor (AR), human progesterone receptor (PR), human glucocorticoid receptor (GR), human peroxisome proliferator-activated receptor gamma (PPARγ), human thyroid receptor beta (TRβ) and the mouse aryl hydrocarbon receptor (AhR) for 65 compounds which migrate from polycarbonate replacement materials for plastic baby bottles.

Effects on PR and GR were examined in T47-D (TARM-Luc, TM-Luc and TGRM-luc) cell line, AhR in H1L7.5c1 (mouse hepatoma heap 1c1c7) cells and PPARγ in stably transfected U-2 OS cells (human osteoblast).

For agonistic activity cell lines were exposed to 1 µM, 10 µM, 100 µM and 1 mM BPS dissolved in 1% DMSO. For antagonistic activity cells were exposed to 10 mM, 1mM and 100 µM BPS dissolved in DMSO and in presence of an agonistic reference ligand at a concentration inducing 50% of the maximal response.

BPS showed no agonistic nor antagonistic activity for PR, GR and AhR. However agonistic and antagonistic PPARγ activity was found after exposure to BPS (increase of the relative response of more than 10 % or decrease of the relative response for two consecutive points of more than 10 %).

Zenata *et al.* (2017)

The aim of this study was to see if BPS can activate several nuclear receptors, i.e., androgen receptor (AR), thyroid receptor (TR), aryl hydrocarbon receptor (AhR), glucocorticoid receptor (GR), pregnane X receptor (PXR) and vitamin D receptor (VDR). The authors used several transfected cell lines expressing the targeted receptor in a transactivation assay to determine the agonist and antagonist activity of BPS. Then in case of positive results, different human cell lines specific to the receptor assessed were used to confirm or not the effect.

Results regarding AR and TR are described above. BPS did not show any agonist nor antagonist transcriptional activity of VDR (vitamin D receptor) reporter.

In transcriptional assay, the luciferase activity dependent on AhR was weakly but insignificantly induced at 10 and 50 µM concentrations BPS (agonist), whereas no antagonist activity was observed. However, BPS had no effect on the level of RNA of CYP1A1, a gene controlled by AhR, in human hepatocytes.

Regarding the PXR, transcriptional assays showed that a significant reduction of luciferase activity in both agonist (35 and 45 % decrease at 50 and 100 µM BPS, respectively) and antagonist assays (34 and 43 % decrease at 50 and 100 µM BPS, respectively). However, the effects were again not confirmed on CYP3A4 mRNA in human hepatocytes, whereas this gene is controlled by PXR.

GR-transactivation assay did not show any agonist activity, but the GR-dependent luciferase activity was significantly decreased by 39 and 54% with 50 and 100 µM concentrations of BPS, respectively, showing an IC50 of 52 µM. One GR-target gene showed a reduced mRNA level when co-exposed with DEX, a GR binder, and 10 µM BPS, whereas the mRNA level was higher at 100 µM BPS. The two other GR-regulated genes tested did not show any mRNA change.

As all the effects observed in the transactivation assays were not confirmed in human cells, the authors considered the results as negative.

Wang *et al.* (2017c)

Tissue models in 2D or 3D can be useful for screening of potential toxic effect or determining mechanisms of action. Regarding obesogenicity, neither the exposure of embryonic stem cells in a 2D-model, nor the exposure of adult stem cells in 3D-model to BPS and other known obesogens (TBT, BPA) did not show any effect. The authors then developed a 3D-adipose tissue assays using human embryonic-derived stem cells.

Exposure to 10 µM BPS in this 3D-model showed significant accumulation of triglycerides. Expression of genes known as adipose markers was significantly increased, including the expression of PPARγ.

Kojima *et al.* (2018)

The authors assessed the transcriptional activity of BPA and 8 analogues using luciferase receptor assays based on human ERα, ERβ, AR, GR, PXR and CAR. Transiently transfected cells with specific expression plasmids were exposed to 10^{-9} to 10^{-5} M of chemical. Results were expressed as REC50, i.e., 50% of relative effective concentration in case of agonistic activity, and RIC50, i.e. 50% relative inhibitory concentration.

BPS had no agonist nor antagonist effect on AR, GR, PXR and CAR. REC50 = 5.4 x 10^{-7} M for both ERα and ERβ. No antagonist activity was observed on ERs.

Qiu *et al.* (2019)

The main part of this study concerns the exposure of red common carps (*Cyprinus carpio*) to 0, 0.1, 1, 10, 100 and 1000 µg/L BPS *in vivo*. Results of the *in vivo* experiments are described below. The study included an *in vitro* experiment, for which primary macrophages isolated from the fish head kidney were exposed to 0, 0.1, 1, 10, 100 and 1000 µg/L BPS for 6 hours.

After exposure, the cells showed an upregulated immune response, showing an increased oxidative stress (increased ROS level) and as well increased gene expression of interleukin-1β, interleukin-6 and tnfα1. This upregulation was inhibited (totally or partially) in presence of rosiglitazone, a known PPARγ antagonist.

Both *in vivo* and *in vitro* experiments showed a decreased gene expression of *pparγ* and *rxrα*, whereas *nfκb* was significantly increased.

Grimaldi *et al.* (2019)

The transcriptional activity of 24 bisphenols was assessed using luciferase receptor assays based on human ERα, ERβ, ERRγ (oestrogen related receptor), AR, PXR (pregnan X receptor), PR (progesterone receptor), GR (glucocorticoid receptor) and MR (mineralocorticoid receptor). Stably transfected HELN or HG5LN cells expressing specific expression plasmids were exposed to 0.001 to 10 µM bisphenols. Results were expressed as EC_{50}, i.e., 50% of relative effective concentration in case of agonistic activity, and IC_{50}, i.e. 50% relative inhibitory concentration.

BPS showed an agonist activity on both ERα and ER β (EC_{50} are indicated above). No agonist nor antagonist activity was observed for ERRγ, PXR, PR, GR nor MR.

Liu *et al.* (2019b)

The receptor binding affinities of 11 bisphenols were evaluated in competitive binding assays.

BPS was found to be completely inactive to RARα, RARβ, RARγ, PPARγ, RORα, RORβ, RORγ, LXRα, LXRβ, VDR, CAR, RXRα, RXRβ, RXRγ and GR

IC50 for ERRγ was 4090 nM +/- 238 and considered very weakly active

Park *et al.* (2020)

The authors of the study evaluated the oestrogenic, androgenic, and AhR potencies of BPA, BPF and BPS using stably transfected transcriptional activation assay (STTA). The luciferase activities were determined in accordance with the OECD TG 455 in HeLa9903 cells (ERα), with the OECD TG 458 in AR-EcoScreen cells (AR), and similarly in DR-EcoScreen cells (AhR). Moreover, the effect of the bisphenols on expression of ERα receptors in MCF-7 cells was determined by Western-blot.

BPS showed a weak agonistic activity on AhR, with a significant 1.4-fold increase of AhR-mediated activity after exposure to 100 µM. Results regarding ER and AR are described above.

Gao *et al.* (2020)

The aim of this study was to investigate the activity of BPA and BPS on PPARγ pathway in human macrophages, as these cells interact closely with adipocytes and hepatocytes to control lipid metabolism.

First, they evaluated if BPS could activate PPARγ as BPA, performing a luciferase assay in transiently transfected HEK293T cells. After exposure to 1, 10 or 100 µM BPS, the cells showed a slight dose-dependent increase of luciferase activity. The increase was significant at 100 µM only. Similar results were obtained using THP-1 cells, which were previously differentiate into macrophages after treatment with PMA.

Then, the authors assessed the transcriptional expression of PPARγ in THP-1 macrophages. Here also, they observed a dose-dependent increase of PPARγ mRNA level. At 100 µM, this increase reached 1.72-fold and was statistically significant. Afterwards, the expression of PPARγ target genes was also measured in THP-1 macrophages. The authors showed a statistically significant increase of CD36, FABP4 and NR1H3 mRNA after exposure to 100 µM BPS. The implication of PPARγ was confirmed as this upregulation was almost completely inhibited in THP-1 PPARγ knock-out cells.

Finally, the authors confirmed the upregulation of some PPARγ-target genes and the disturbance of metabolism profiles in mice *in vivo* (results presented below).

Martinez *et al.* (2020)

The authors assessed the obesogenic potential of BPA, BPF and BPS in 3T3-L1 cell line, by evaluating the lipid accumulation in cells determined using red oil O staining to determine the level of adipocyte differentiation. After 8 days of maturation, exposure to 32 µM BPS induced a significant increase in the number of differentiated 3T3-L1 adipocytes (199 ± 4% compared to the control, $p < 0.001$). This value was strongly higher than BPA (127 ± 3%).

The effect of BPS on the expression of adipogenic markers at the end of the differentiation process was also evaluated. After exposure to 32 µM BPS, the expression of PPARγ, C/EBPα and FABP4 was significantly increased (about 2-fold compared to control). No change was observed on the active form of phosphorylated AKT protein. This study suggests that BPS has a higher obesogenic potential than BPA.

Conclusion: OECD CF level 2 data

* Estrogen activity: clear agonistic estrogenic activity via ER-binding and ER activation

*Androgen activity: weak anti-androgenic activity (but contradictory results)

* Steroidogenesis: BPS affects steroidogenesis, by decreasing testosterone level and increasing progesterone level. No effect on estradiol level is observed.

*Thyroid activity: some antagonist activity on thyroid receptors has been observed (but contradictory results).

*Other: Several studies showed that Bisphenol S activates PPARγ (agonist activity). No activity was observed on AhR, GR and PXR in multiple studies. The few available studies examining the interaction with receptors for Constitutive Androstone (CAR), Retinoid acid (RAR), Vitamin D (VDR), Mineralocorticoid (MR), RAR related orphan receptor (ROR), liver X receptor (LXR), were inactive.

7.10.1. Endocrine disruption – Environment

OECD Level 3: *in vivo* assay providing data about selected endocrine mechanism(s)/pathway(s)

Table 78: Overview of endocrine disruption – environment (OECD Level 3, literature data)

Method (guideline)	Short description of Method	Endpoint	Result (positive/negative/trend)	Description of Result (positive/negative/trend)	References
OECD TG 229 with minor modifications	21d reproduction assay	Steroidogenesis/ Estrogen	+	Disturbed balance of sex steroid hormone and impaired reproduction of adult zebrafish and malformations in offspring	Ji *et al.*, 2013
-	embryonic zebrafish exposure (0-5 dpf)	steroidogenesis	+	Increase in neuronal birth in the hypothalamus at 24 hpf, through an AR -mediated mechanism (up-regulation of aromatase), and subsequent hyperactive behaviour on dpf 5 (following exposure to 0.0068 µM BPS)	Kinch *et al.*, 2015
-	Juvenile brown trout (2 and 8 weeks of exposure)	Thyroid Estrogen	-	Exposure via implantation of cholesterol pellets containing 2 and 20 mg/kg BPS resp. No effect on T3 and T4 levels Slight increase of VTG at highest dose but not significant	Ribera, 2015
-	Embryonic zebrafish (7dpf and 4dpf exposure)	Steroidogenesis	+	41-fold sign. induction of Cyp19a 1b expression after exposure to 1µM BPS compared to the solvent DMSO. BPS is able to affect aromatase expression. A 6-fold induction of the GFP fluorescence in the brain of the transgenic zebrafish line after exposure to 1µM BPS, although not statistically significant.	Cano-Nicolau *et al.*, 2016

				a correlation between the cyp19a 1b gene expression and the induction of the cyp 19a 1b promotor activity was observed. cyp19a 1b transcripts distribution patterns in the whole brain were over-expressed (identical to EE2 and BPA)	
-	Embryonic zebrafish exposure (2 to 25 or 120 hpf)	EATS	+	Increase number of hypothalamic GnRH3 neurons and increased expression of reproduction-related genes (GnRH and ER) after exposure to 100 µg/L BPS. Indication of estrogenic, thyroid and aromatase mediated activity	Qiu *et al.*, 2016
-	Embryonic chicken embryos exposure (up to 207 µg/g)	Thyroid	+	Decreased viability, reduced growth. Increased expression of some mRNA involved in Thyroid hormone pathway. No effect on circulating free T4	Crump *et al.*, 2016
-	Embryonic zebrafish exposure (2 to 168 hpf)	Thyroid	+	Delayed hatching. Decreased T4 and T3, increased TSH levels Change in mRNA involved in Thyroid hormone pathway	Zhang *et al.*, 2017
-	Guppy fish exposure for 21 days	Estrogen	+	Increased vitellogenin in caudal fin, liver and whole body	Wang *et al.*, 2017a
-	Transgenic ER-GFP zebrafish exposure for 120 hpf	Estrogen	+	ER-dependent induction of GFP	Moreman *et al.*, 2017
-	Transgenic aromatase-GFP zebrafish exposure for 96 hpf	Estrogen	+	Increased ER-regulated aromatase activity resulting in increased GFP	Le Fol *et al.*, 2017
-	P. nigromaculatus (amphibian) TH-response gene transcription assay	Thyroid	+	Up-/down-regulation of TH-regulated genes in absence/presence of T3	Zhang *et al.*, 2018b
-	Embryonic zebrafish exposure (2 to 120 hpf)	Estrogen	+	Early hatching Increased oxidative stress ER-dependent change in immunity-related gene expression	Qiu *et al.*, 2018b
-	Embryonic zebrafish exposure (2 to 96 hpf)	Estrogen	-	No teratogenic effect No induction of ERα protein nor ER-coding mRNA	Mu *et al.*, 2018
-	24h exposure of Chironomus riparius larvae (midge), followed by	EAS	+	Effect on transcriptional activity of several genes: n general, upregulation after exposure to BPS, down-	Herrero *et al.*, 2018

	recovery period			regulation after withdrawal of BPS	
-	Embryonic zebrafish exposure (2 to 120 hpf)	Thyroid	+	Delayed hatching Dose-dependent increase of T3 and T4 levels Upregulation of TH-regulating gene expression	Lee *et al., 2019*
-	Zebrafish embryos were exposed to concentrations of BPS of 0, 0.03, 0.3 and 3 mg/L until 6 dpf.	EAS	+	BPS decreased locomotor behavior and altered retinal structure partially by increasing oxidative stress and suppressing of levels of neurodevelopment genes (*α1-tubulin*, *elavl3*, *gap43*, *mbp*, *syn2a* and *gfap*).	Gu *et al.*, 2019
-	Oreochromis mossambicus juveniles and adults (exposure for 2, 4 and 12 weeks)	Steroidegenesis/estrogen Thyroid	+ +	Exposure to 100, 120 and 140 mg/L (juveniles) and 100, 125 and 150 mg/L (adults) Sign. increase of oestradiol in juvenile males. Sign decrease in adult males and female juveniles and female adults Significant decrease of testosterone in juvenile and adult males Sign. increase of E2/T ratio in male juveniles and adults Sign decrease of T3 and T4 in juveniles, while sign. increased in adults	Anjali *et al.*, 2019
-	Chicken eggs exposure (E4)	Estrogen	+/-	Increased embryonic mortality. Suspected atrophy of the ovaries. No clear effect in male (sample too small). Increase of gallbladder-somatic index.	Mentor *et al.*, 2020
-	Juvenile brown trout (2- and 8-weeks exposure)	Thyroid estrogen		Exposure via implantation of cholesterol pellets containing 2 and 20 mg/kg BPS resp. Sign. Increase of T3 at 20 mg/kg after 2 weeks exposure, no difference after 8 weeks No effect on T4 No effect at 2 mg/kg, sign. Increase at 20 mg/kg after 2 weeks, but only increasing trend at 8 weeks	Frenzilli *et al.* 2021
-	Zebrafish embryo's exposure from 2hpf to 240dpf	estrogen	+	Plasma VTG sign increased in females after exposure to 1µg/L of BPS, also sign. increased at 1 and 100 µg/L in the ovary	Qin *et al.*, 2021

				Lipid accumulation in ovaries leading to increase in full-grown stage oocytes and egg production Higher neutral lipid levels, impaired hatching and increased larval deformations in F1	
-	Embryonic exposure (2hpf until 120 hpf)	Steroidogenesis	+	LH: ↗, but not stat. sign. FSH: stat. sign. ↗ at 100* µg/L E2: stat. sign. ↗ at 1* and 100* µg/L GH: slightly ↗, but not stat; sign. At 100 µg/L (single conc. used): LH + ICI: ↙ at 100* µg/L compared to control, ↙ but not sig. compared to BPS LH + FAD: ↙, but not sign. FSH + ICI: ↗ at 100* µg/L compared to control, ↗ but not sig. compared to BPS FSH + FAD: ↗ at 100* µg/L compared to control, no stat. difference with BPS Kiss 1: dose dependent ↗, stat. sign at 100* µg/L Kiss2: dose dependent ↗, stat. sign at 100* µg/L Gnrh3: ↗, but not stat. sign. Fshβ: ↗, but not stat. sign. Lhβ: stat. sign. ↗ at 1* and 100 µg/L Anp: stat. sign. ↗ at 1* and 100 µg/L Ren: dose dependent ↗, stat. sign at 100* µg/L Pth1: dose dependent ↗, stat. sign at 100* µg/L Gh: dose dependent ↗, stat. sign at 100* µg/L Pr1: ↗, stat. sign. at 1* µg/l At 100 µg/L (single concentration used): lhβ + ICI: non-sign.↙ lhβ + FAD: ↙ at 100* µg/L compared to control, ↙ but not stat. different from BPS fshβ + ICI: ↙ at 100* µg/L compared to control, ↙ but not stat. different from BPS fshβ + FAD: ↙ at 100* µg/L compared to control and BPS Stat. sign. Upregulation of CYP19a1 (gonad) and CYP19a2 (brain) at 100* µg/L	Qiu *et al.*, 2021

				Stat. sign. ↗ of Hypothalamic GnrH3 neurons, no stat. sign. effect on Terminal Nerve GnrH3 ↙ in movement distances but not stat. sign.	
-	21d reproduction assay	Aromatase activity	+	Females: GSI and HSI sign. ↗ at 40 mg/L, sign. ↙ at 200 mg/L Regressed oocytes at 4 and 200 mg/L Sign. down-regulation vtg gene expression; no effect on Eα and Erβgene transcription Sign. ↗ of E2, TH and progesterone No effect on cortisol Males: GSI: no effect HSI: sign. ↗ at 200 mg/L No effect on testicular development Sign. upregulation vtg gene expression; sign. down-regulation of ERβ transcription; no effect on ERα gene transcription Sign. ↙ of T and 11-kT, sign. ↗ in TH Sign. ↗ E2/T ratio No effect on cortisol	Park *et al.*, 2022

Ji *et al.* (2013)

Adult zebrafish (AB type, 3-4 months old, six males and four females/replicate, 2 replicates per concentration or control) were exposed, after an acclimation period of > 2 weeks, to control, vehicle control (0.1% MeOH (v/v)), 0.5, 5 and 50 µg/L of BPS for 21 days following OECD test guideline 229 with minor modifications:

- 6 females and 4 males instead of 5 females and 5 males
- Fertilised eggs were collected after fish were exposed for 16d
- Semi static instead of flow through
- VTG level and gonadal histopathology were not reported.

pH and dissolved oxygen concentration during the whole exposure were not reported in the article.

No mortalities were seen at any treatment during the exposure period, water temperature of 25 ± 1°C, and the mean measured concentration were within the range of 80-110% of the nominal concentrations, therefore nominal concentrations are used.

Fish were kept under a photoperiod of 16h light/8 h dark and were fed twice a day with freshly hatched Artemia nauplii. Every 2 days the medium was renewed. After 16 days exposure of the fish, 30 eggs were randomly selected and were placed in clean water or exposed to the same concentration until 6 dpf. The effects on reproduction, sex steroid hormones, and transcription of the genes belonging to the hypothalamic-pituitary-gonad (HPG) axis were investigated. Following parameters were recorded after the exposure of the fish: total weight, snout-vent length, condition factor, brain-somatic index, hepatosomatic index and GSI.

4 males and 4 females were randomly selected for measurement of transcription of 21 genes representing key signalling pathways and functional processes of the HPG axis, one housekeeping gene (β-actin) was also measured.

The adverse effects on performances of the F1 generation were further examined with or without subsequent exposure to BPS. For this purpose, fertilised eggs were randomly collected on day 16 of fish exposure and placed in clean water or exposed to the same BPS concentrations as the adult fish until 6 day post-fertilization. Hatchability, time to hatch and malformations rates were determined from this F1 generation.

Reproduction:
Egg production and the gonadosomatic index in female fish were significantly decreased at ≥ 0.5 µg/L BPS.
The gonadosomatic index in males significantly decreased at 50 µg/L. A lower GSI value accompanied by an inhibition of egg production has been reported in fish exposed to estrogenic compounds (Van den Belt *et al.*, 2001).
Parental exposure to 50 µg/l and ≥5 µg/l BPS resulted in delayed and lower rates of hatching, even when they were hatched in clean water. Furthermore, parental exposure to BPS can be more important because there might be no excretion mechanism for BPS in the eggs as suggested for BPA (Takao *et al.*, 2008).

Plasma levels of 17β-estradiol (E2) were significantly increased in both male and female adult fish, respectively at ≥0.5µg/l and 50µg/L. Plasma testosterone (T) significantly decreased in males at 50µg/l, while no significant effect was seen in females. This was accompanied by up-regulation of the aromatase cyp19a and cyp19b genes (cyp19a and cyp19b catalyse the final step in the conversion of androgen to estrogen) and down-regulation of cyp17 and 17βhsd genes. However no significant difference was observed in gonadal transcription of CYP19a in females which may be explained by an increase of 17β-estradiol by another mechanisms (e.g., inhibition of E2 metabolism rather than enhancement of aromatase). Sulfotransferase may inactivate E2 by sulfonation. BPA, the structural analogue of BPS, showed that inhibition of estrogen sulfotransferase activity might result in an increase of estrogenicity (Zhang *et al.*, 2011). Furthermore, it was demonstrated that the sex hormones balance was altered and that males were more sensitive than females. The ratio E2/T was significantly increased at ≥0.5 µg/L and 50 µg/L BPS in male and female fish, respectively.

The change in sex steroid hormone levels may cause subsequent reproductive dysfunction by interfering with the regulatory mechanism of the HPG axis. It has been reported that steroidogenesis is a major target for endocrine disrupting chemicals including BPA (Sanderson, J. T., 2006).
The transcription of genes involved in the HPG axis was affected by exposure to BPS and also here males were more sensitive than females. Gnrh3, gnrhr1, and gnrhr2 genes were up regulated in male fish suggesting that BPS could modulate concentrations of GnRHs in fish, which could subsequently affect production of gonadotropin hormones. In vertebrates, gonadotropin-releasing hormone (GnRH) has a crucial role on the control of reproduction through HPG axis and regulates the synthesis and release of gonadotropin hormone. In Zebrafish two types of GnRH (GnRH2 and GnRH3) and four different GnRH receptors (GnRHR) exist. The up regulation of fshβ, lhβ, fshr, and lhr genes in male, observed in this present study, supports the fact that BPS can indirectly affect gonadotropin hormones.

F1 generation:
Continuous BPS exposure in the F1 embryos resulted in worse hatchability and increased malformation rates compared to those without BPS exposure. Cardiac oedema shortened tails and severe spinal kyphosis were observed after subsequent exposure to BPS.

Conclusion:
The observations showed that exposure to low, environmentally relevant levels of BPS can affect the feedback regulatory circuits of the HPG axis and impaired reproduction of adult fish and the development of offspring.

Comments eMSCA:

- There was no significant difference between the water and solvent control.
- From the additional information provided by the authors of this article, a dose-dependent significant decrease in egg production was observed: 19.36 at 0.5 µg/L, 15.99 at 5 µg/L, 12.69 at 50 µg/L vs 26.72 (solvent control) and 26.32 eggs/female/day in control)

Kinch *et al.* (2015)

Embryonic zebrafish (within 3hpf) were exposed to 0.0068 µM BPS (prepared in 0.08% MeOH) from 0-5 dpf. Zebra fish hypothalamic progenitor cells undergo neuronal differentiation
between 18–36 hpf, and neural development is complete by 48 hpf. At 5 dpf, zebrafish emerge as prey-seeking larva. BPS exposure resulted in a 240% increase in neuronal birth in the rostral hypothalamus at 24 hpf and a significant (160%) increase in locomotor bursting activity (similar to BPA), which was reduced by transient knockdown of AroB (aromatase B, the key enzyme for local estradiol synthesis), but not by treatment with 1 µM of the ER antagonist ICI indicating that BPS might not act via ERs (estrogen receptors). The results of this study show thus that both BPS and BPA influence hypothalamic development and may act through a similar AroB-mediated mechanism.

Ribera (2015)

After an acclimatisation period of 2 weeks, Juvenile brown trout (average weight of 100 f) were exposed for 2 and 8 weeks by implantation of cholesterol pellets containing resp. 2 mg/kg fish and 20 mg/kg fish of BPS. 14 tanks were filled with 10 fish per tank. Plasma levels of T3 and T4, VTG and cyp 1A induction in the liver (EROD activity) were measured.

No effect was seen on T3 and T4 in all treatment groups.

VTG slightly increased after exposure to 20 mg/kg BPS for 2 and 8 weeks but was not significantly different to control.

Cyp1A induction was lower at 2 mg/kg after 2 weeks exposure and after 8 weeks in the 20 mg/kg treatment. Cyp1a enzymes participate in the metabolization and clearance of toxicant compounds by increasing their solubility in water. Induction of Cyp1a levels is driven by the interaction of toxicant compounds with an aryl hydrocarbon receptor (AhR) present in the cytosol, which enhance the gene expression of Cyp1a and other enzymes.

Cano-Nicolau *et al.* (2016)

Besides the *in vivo* assays described underneath, authors performed *in vitro* studies which are described under OECD CF level2 studies above in this document.

Quantitative RT-PCR was used to monitor the expression levels of cyp19a 1b, a gene that is expressed in radial glial cells of the brain in zebrafish and serves as progenitors during the embryonic and adult neurogenesis. To analyze the induction and distribution of cyp19a 1b transcripts cyp19a 1b in situ hybridization was performed. For both approaches wildlife 1dpf embryos were exposed until 7dpf. For the quantification of the brain florescence of cyp19a 1b-GFP using the EASZY assay, 2h pf embryos were exposed until 4 dpf.

A 41-fold induction of Cyp19a 1b expression was detected after exposure to 1µM BPS compared to the solvent DMSO (1/10.000). This confirms the effect of BPS on the aromatase expression.

The results from the EASZY assay (cyp 19a 1b promotor activity) demonstrate a 6-fold induction of the GFP fluorescence in the brain of the transgenic zebrafish line after exposure to 1µM BPS, although it was not statistically significant.

Nevertheless, a correlation between the cyp19a 1b gene expression and the induction of the cyp 19a 1b promotor activity was observed.

Brain sections of 7dpf larva brains were analyzed for possible changes in the distribution of cyp19a 1b transcripts after BPS exposure. The cyp19a 1b transcripts distribution patterns in the whole brain were identical to EE2 and BPA which showed over-expression in posterior telencephalon, preoptic area and caudal hypothalamus, including the nucleus recesses posteriors were the induction was the strongest.

Lack of estrogen activity in the *in vitro* studies could indicate that estrogen activity of BPS demonstrated in the *in vivo* studies might be mediated trough another pathway than estrogen receptors.

Qiu *et al.* (2016)

Zebrafish embryos (2 hpf) were exposed to 100 µg/L of BPS. 0.005% DMSO was used as vehicle. There was no significant difference between the water and solvent control. Effects on BPS on GnRH3 neuron numbers in the terminal nerve (TN) and hypothalamus (HYPO), reproductive neuroendocinerelated genes (kiss1, kiss1r, gnrh3, lhβ, fshβ) and a biomarker for synaptic transmission (sv2) were examined after 25hpf. Furthermore, also potential signal transduction pathways, including ERs, THRs, and aromatase activity (AROM, converts androgen to estrogen) were examined. In order to do so embryos were exposed to 100 µg/l BPS for 120 hpf, in the presence of an ER (ICI 182780), THR (amiodarone hydrochloride) and AROM (fadrozole hydrochloride) antagonist.

Exposure to BPS had no effect on the survival rate of the embryos. However, a significant increase was seen in the number of HYPO-GnRH3 neurons, while no effect was observed in TN-GnRH3 neurons. BPS also up-regulated the expression of the reproduction-related genes kiss1/kiss1r (upstream regulator of GnRH neurons, also in fish), gnrh3 and erα.
BPS did not affect kiss2, kiss2r, fshβ and sv2 gene expression levels.

Study on the implication of ERs in the neuroendocrine system suggest that not erβ but erα mediates the effects of BPS as only a significant increase of erα was observed at 25 hpf.

Antagonists of ER, THR, and AROM blocked many of the effects of BPS on reproduction-related gene expression, providing evidence that those three pathways (partly) mediate the actions of BPS on the reproductive neuroendocrine system:

- Inhibition by ICI 182780 of stimulatory action of BPS on gnrH3 and fshβ, no significant effect on kisss1r, lhβ and erα. Only inhibition by amiodarone hydrochloride of the up-regulating effect of BPS on kiss1r and lhβ
- Inhibition by fadrozole hydrochloride of the up-regulating actions of BPS on gene expression of kiss1r, gnrh3, lhβ and erα.

Crump *et al.* (2016)

BPS was injected in the air cell of fertilized chicken eggs at concentrations of 0 (solvent control: ~ 1µg/L DMSO), 0.27, 0.91, 10.6, 52.8 and 207 µg/g egg. Effects on pipping success, development, hepatic messenger ribonucleic acid (mRNA) expression, thyroid hormone levels, and circulating bile acid were examined.

They observed a dose-dependent increase of BPS in whole-embryo homogenates. The concentrations in the embryos were, in all cases where BPS was detected (≥0.91 µg/g, much lower than the initial injected concentration). There was no treatment related effect on development.

At the highest dose a significant reduction in piping success (58%) was seen, as well as a significant decrease in embryonic weight and tarsus length.
A significant concentration-dependent increase in gallbladder size was already observed at concentrations ≥52.8 µg/g.

The liver of chicken embryos was exposed to 52.8 µg/g and 207 µg/g of BPS to examine the effects on genes involved with xenobiotic metabolism (Ugt1a9 and Sult1b1), lipid homeostasis (Acsl5 and Cyp7b1), heat-shock protein (Hsp90ab1) and the thyroid hormone pathway (Dio1). Significant upregulation of those 6 genes was seen at 52.8 µg/g. Although not significant, the same trend was seen at 207 µg/g compared to the solvent control DMSO.

Expression levels of 2 estrogen-responsive genes, apolipoprotein II and vitellogenin, were too low at the sampling time point assessed (i.e., pipping embryos) to quantify changes.
No effects were observed on free plasma thyroxine (T4) or bile acid concentrations.

Zhang *et al.* (2017)

In this study potential accumulation of BPS, whole-body thyroid hormones (THs), thyroid stimulating hormone (TSH) concentrations as well as transcriptional profiling of key genes related to the hypothalamic-pituitary-thyroid (HPT) axis were examined in Danio rerio (zebrafish) embryos. 2 hpf old embryos were exposed to BPS until 168 hpf at concentrations of 1, 3, 10, and 30 µg/L in the presence of 0.01% DMSO. Concentrations were chosen based on the environmental concentration.

Results were expressed in comparison to the solvent control, with concentrations of BPS below the detection limit.

No mortality, nor effects on growth and malformation rate were observed at the end of the study. A the highest concentration, however, a significant reduction in hatching rate was observed at 72 hpf (62.3*%), while this was restored at 96 hpf (>90%) [control, 1, 3, 10, and 30 µg/L : resp. 81.2 ± 2.65, 79.5 ± 4.04, 73.3 ± 3.76, 74.1 ± 3.2 and 62.3 ± 5.55* %, at 96hpf: resp. 94.5 ± 1.2, 93.3 ± 1.0, 90.8 ± 1.4, 91.2 ± 1.7 and 90.8 ± 1.9 %)].

It was noted that BPS accumulated in the embryos during the experiment (respectively 0.13, 0.36, 0.82, and 2.21 ng/g wet weight after exposure to 1, 3, 10, and 30 µg/L).

Exposure to BPS lead to a significantly decrease in whole-body Thyroxine (T4) and triiodothyronine (T3) levels at ≥ 10 and 30 µg/L respectively indicating that BPS can cause hypothyroidism.
On the other end, a significant increase of whole-body TSH was observed at 10 and 30 µg/L which might be the response of the pituitary gland to regulate the TH synthesis (negative feedback mechanism).

Furthermore, the mRNA expression of corticotrophin releasing hormone (crh) and thyroglobulin (tg) genes were dose-response dependent up-regulated at ≥ 10 µg/L. crh appears to simulate TSH secretion and might function as a common regulator of the HPT-axis. Upregulation of crh and increased TSH contents may promote TH synthesis and release to compensate for the decreased T4 levels in zebrafish larvae.

The transcription of genes involved in thyroid development (pax8) and synthesis (sodium/iodide symporter, slc5a5) were also significantly increased at the highest concentration. In addition, genes related to thyroid hormone metabolism (deiodinases, dio1, dio2 and uridine diphosphate glucuronosyltransferases, ugt1ab) were also significantly upregulated at 10 µg/L and higher concentration which might be responsible for the altered THs levels. By contrast, however, the transcript of transthyretin (ttr) was significantly downregulated at ≥ 3 µg/L, while the mRNA levels of thyroid hormone receptors (trα and trβ) and dio3 remained unchanged.

It can be concluded that BPS can interfere with the HPT-axis by altering the whole-body THs and TSH concentrations and by changing the expression profiling of key genes related to HPT axis.

Wang *et al.* (2017a)

The aim of this study was to develop an assay for detecting vitellogenin in guppy (*Poecilia reticulate*) caudal fin, as this one has a high capacity to regenerate, in order to reduce experimental animal killing. Furthermore, Guppy is considered an ideal model to study environmental estrogens for guppy is more sensitive to exogenous estrogens than zebrafish and rainbow trout

To validate the newly developed assay, the authors exposed 20 adult male guppies to 1, 10 and 100 µg/L BPS for 21 days. Results were compared to the solvent control (not specified which solvent is used). Vitellogenin has been then quantified in the caudal fin, but also in liver and in the whole organism to check the reliability of the method. Vitellogenin concentration increased significantly at all doses, the maximal being reached at 10 µg/L BPS in all tissues (fin, liver and whole animal).

Moreman *et al.* (2017)

In this study, the authors assessed the toxicity and estrogenicity of some bisphenols, including BPS, using wild-type or transgenic zebrafish.
First, the authors performed an acute toxicity fish assay, following the OECD TG 236. They exposed 20 embryos per group to 10, 20, 50, 100, 200 and 300 mg/L BPS from 1 to 96 hpf. Results were compared to the solvent control (0.01% ethanol). Larvae were assessed regularly, and mortality, hatching rate and abnormalities were recorded. BPS showed an acute toxicity at 96 hpf (LC50) of 199 ± 7.6 mg/L, and a delayed hatching at 72 hpf (EC50) of 155 ± 15 mg/L. BPS caused developmental effects only at very high exposure concentration (≥ 200 mg/L), showing cardiac edema, craniofacial abnormalities and spinal deformity.

Measurement of BPS uptake in the larvae, 3.34 ± 0.15 ng/larvae, allow the estimation of the bioconcentration factor to 0.067 ± 0.003.

The authors also exposed transgenic zebrafish expressing estrogen-responsive element linked to GFP (strain Tg (ERE: Gal4ff)(UAS:GFP)) to 10, 20 and 50 mg/L BPS from 1 to 120 hpf. BPS induced GFP expression, mainly in the heart (up to 2.7 and 10.8-fold more than in control at 20 and 50 mg/L, respectively), but also in the liver and the tail. The GFP expression was totally inhibited after addition of ICI 182,780, an estrogen receptor antagonist.

Le Fol *et al.* (2017)

The authors assessed the estrogenic activity of BPA, BPF and BPS in zebrafish, by testing the ERs transactivation potential *in vitro*, but also *in vivo* by quantifying the aromatase activity in the developing brain of embryos. The *in vitro* results are described above.
To determine the estrogenic potential of BPS *in vivo*, the authors used a zebrafish embryo assays (EASZY assay) based on the cyp19a1b-GFP transgenic line expressing the GFP under the control of the ER-regulated cyp19a1b aromatase gene in the brain. Newly fertilized zebrafish eggs were exposed from 0 to 4 days post fertilization (dpf) to 0, 0.25, 0.5, 1, 2.5, 5, 10, 20, 30 and 60 µM BPS. Results were compared to the solvent control (0.01% DMSO (v/v)). Then the GFP activity has been quantified. BPS induced a 4-fold GFP expression at 30*** (7500 µg/L) and 60*** µM (15 000 µg/L). Interestingly, contrary to BPA and BPF induced GFP expression, the co-exposure to ICI 1 µM, a known ER antagonist, was not able to block the effect of BPS, suggesting the involvement of an ER-independent pathway.

VTG was measured in 6-months old male zebrafish exposed for 7days to 0.1, 1 and 10µM of BPS. VTG was significantly induced at 0.1 and 1 µM ($p<0.001$). However, the highest concentration of 10 µM lead to a high mortality rate.

Comments e-MS:

It should be noted that co-exposure with ICI was not tested at the concentration reaching the highest activation level measured in the dose response curve (60 µM). Furthermore, the ratio BPS/ICI (30 µM BPS vs 1 µM ICI) was very high at the concentration tested (30 µM BPS). Therefore, it is not surprising that the inhibition (at very low concentration of ICI compared to BPS) was not sufficiently to inhibit the effect of BPS on aromatase.

Zhang *et al.* (2018b)

The authors of this study assessed the thyroid disrupting potential of BPA, BPF and BPS in several *in vitro* and *in vivo* assays. Results of *in vitro* assays are shown above.
To confirm the agonistic and antagonistic activity of BPS *in vivo*, the authors exposed *Pelophylax nigromaculatus* tadpoles at Gosner stage 27 to 0.01 to 10 µM of BPS in the presence or absence of T3 for 48 h (n= 12/group). Results were compared with the solvent control (1% DMSO). At the end of the experiment, tadpoles were sacrificed, and RNA extracted to perform qRT-PCR of six TH-response genes: TH receptor β (TRβ), TH-responsive basic leucine zipper transcription factor (TH/bZIP), basic transcription element binding protein (BTEB), type 3 iodothyronine deiodinase (DIO3), matrix metalloproteinase 2 (MMP2) and sonic hedgehog (SHH).
Higher concentrations (1-10 µM) of BPS up regulated some of these genes (TH/bZIP and BTEB) in absence of T3. Interestingly in presence of T3, low concentration of BPS (0.1 µM) promoted T3 actions on TRβ, TH/bZIP and BTEB transcription, whereas higher doses (1-10 µM) antagonized T3 actions on transcription of all the six genes tested, revealing a non-monotonic dose response.

Qiu *et al.* (2018b)

The aim of the study was to compare the effects of BPA, BPS and BPF on the immune system during zebrafish early development. Zebrafish embryos (n= 200 per group) were exposed to 0.1, 1, 10, 100 and 1000 µg/L from 4 to 120 hpf. 0.005% DMSO was used as vehicle. Results were compared with the vehicle control.
Exposure to environmentally relevant concentrations of 1, 10, 100 and 1000 µg/L BPS resulted in an early hatching, as the hatching rate at 48 and 54 hpf was higher than in the control. Interestingly the highest concentration (1000 µg/L) had no effect on hatching rate. Confirming the early hatching, the high doses reduced (≥ 100 µg/L) the body length of zebrafish in a dose-dependent manner. However, there were no difference in survival rate after chemicals exposure.

BPS high doses exposure increased the oxidative stress in zebrafish, showing an increase of ROS concentration, a higher NO content and the induction of expression for most cytokine genes.

The authors hypothesized that these observations were related to ER or nuclear factor κb. Indeed, exposure to 100 µg/L BPS for 120 h also increased significantly mRNA levels of erα and nf-κb in zebrafish. And co-exposure with the ER antagonist ICI 182 780 and NF-κb antagonist pyrrolidine dithiocarbamate (PDTC) significantly attenuated the stimulatory actions of BPS on gene expression of the cytokines il-1β, il-6 and tnfα.

Mu *et al.* (2018)

The authors exposed zebrafish embryos to different bisphenols including BPS to determine the developmental effects of the substances.
The authors first performed a high acute toxicity assay to determine the LC50 of the compounds. Embryos (1.5−1.7 h postfertilization) were exposed to 3, 6, 12.5, 25 and 50

mg/L BPS for 96 h. Acetone was used as vehicle at concentration of 0.1 mL/L, except for 12.5 mg/l and 25 mg/L of BPS where 0.125 mg/L and 0.25 mg/L acetone was used resp. Results were expressed compared with the solvent control. However, no significant mortality has been observed, even at the highest dose.
Zebrafish embryos were then exposed to 2.5, 12.5 and 25 mg/L for 96 h to assess developmental toxicity. None of these concentrations showed neither an influence on hatching, mobility of embryos, heart rate nor abnormality incidence.
As BPS is known to be estrogenic, the authors observed the effects of BPS exposure to estrogen receptors. However, neither ERα protein nor ERs-coding gene (esr1, esr2a, esr2b or vtg1) was induced or affected after 96 h of exposure.

***Herrero et al.* (2018)**

In this study *Chironimus riparius* larvae (early phases of fourth instar larvae) were exposed for 24h to a solvent control (0.05% DMSO) and 0.5, 5, 50 and 500 µg/L BPS, followed by a recovery in fresh medium of 24h. Transcriptional activity of EcR, ERR, E74, Vtg, cyp18a1 (involved in development and metamorphosis); hsp70 and hsp40 (heat shock); GAPDH; cyp4g, GPx, and GSTd3 (involved in biotransformation reactions); its2, rpL4, and rpL13 (involved in ribosomal biogenesis) were examined. Results were compared to the solvent control.

No significant mortality was noted during the experiment.

In general, 24h exposure to BPS caused **significant up-regulation** of most of the genes in a non-monotonic dose response manner. This was followed by a down-regulation when BPS was withdrawn, with the exception for cyp4g, GPx and GSTd3 where no remarkable difference was observed after withdrawal:

Table 79: Upregulation of genes after 24 exposures to BPS (Herrero *et al.*, 2018)

24h exposure to BPS			
	Significant upregulation (µg/L)	Maximum level expression of effect (µg/L)	Mean increase compared to control (fold)
EcR*	≥5	50	3.8
ERR	≥5	50	2
cyp18a1	0.5 and 50	50	2.5
hsp70	0.5 and 5	0.5	1.7
hsp40	≥5	50	2.5
Cyp4g	≥5	50	6.4
GPx	5 and 50	50	1.8
GSTd3	≥5	50	2.1

* a higher dose of BPA (3000µg/L) was needed to generate half the effect of BPS at 50µg/L

BPS upregulated also, although not significantly, E74 with 2.4-fold compared to control and reached his maximum at 50 µg/L.
Although activity was more or less unaltered, a down-regulation of *its2* (sign.), *rpL4* (sign.) and *rpl13* was observed at 0.5 µg/L with a 25% decrease compared to the control. After BPS removal *its2* and *rpL4* were repressed, while rpL13 increased up to 2-fold although not statistically significant.

Furthermore, BPS evoked slight non-significant upregulation of Vtg (max. at 50 µg/L) and had no remarkable effect on GAPDH.

It can be concluded that exposure to BPS impacted transcriptional activity of several genes leading to a potential effect on hormonal system and metabolism in insects.

Lee *et al.* (2019)

The aim of the study was to compare the thyroid disrupting effects of BPA, BPS, BPF and BPZ during zebrafish early development. Zebrafish embryos were exposed to 0.4, 2, 10 and 50 mg/L BPS from 4 to 120 hpf. <0.1% of DMSO was used as vehicle and results were compared with this vehicle control.

Exposure to BPS did not show any significant effect on the survival, hatchability, body length nor eyeball size. Time to hatch occurred a little bit later than in control, but the delay was only significant at the lowest dose. The embryos exposed to BPS showed also a higher malformation rate, but it was not significant due to high standard deviation.

Table 80: Effects of BPS during zebrafish early development (Lee *et al.*, 2019)

Dose (mg/L)	Time to hatch (d)	Malformation rate (%)
0	2.09 ± 0.11	0.0 ± 0.0
0.4	2.90 ± 0.12*	20.0 ± 23.1
2.0	2.51 ± 0.37	5.0 ± 10.0
10	2.38 ± 0.34	4.2 ± 8.3
50	2.51 ± 0.26	10.4 ± 12.5

* = $p < 0.05$

The level of T3 and T4 were dose-dependently increased after BPS exposure. At 50 mg/L, the level of T3 was significantly higher. The expression of TH regulating genes was also increased after BPS exposure, particularly thsβ, tshr, hhex, tpo, ttr and ugt1ab.

Gu *et al.* (2019)

Zebrafish embryos (1hpf) were exposed to concentrations of BPS of 0, 0.03, 0.3 and 3 mg/L until 6 dpf. Results were compared to the vehicle control (0.01% DMSO (v/v))

BPS decreased locomotor behaviour and altered retinal structure partially by increasing oxidative stress and suppressing the levels of neurodevelopment genes (*α1-tubulin*, *elavl3*, *gap43*, *mbp*, *syn2a* and *gfap*).

Anjali *et al.* (2019)

Oreochromis mossambicus juvenile and adult fish were exposed for 4-, 8- and 12-days resp. to 100, 120 and 140 mg/L BPS and 100, 125 and 150 mg/L resp. Serum levels of T3, T4 cortisol, E2 and T were measured.
Fish were divided in 10 groups prior to the experiment: juvenile fish with bw of 7±2g, adults with bw of 45±2g.
Juvenile male fish showed a significant time-dependent increase of E2 at all concentrations, while a significant decrease was seen in adult male fish: at 100 mg/L in a time-dependent manner at all periods of exposure. At 125 and 150 mg/L the decrease was significant from day 3 with a maximum after day 6 and 9.

A time-dependent significant decrease of E2 was shown at all exposure concentrations in both female juveniles and female adult fish.

Testosterone concentration decreased significantly in juvenile fish at 100 mg/L after 3 and 6 days, with a maximum after day 9. In the test group of 120 and 140 mg/l BPS, testosterone decreases significantly after all exposure periods. Also, in adults a time-dependent significant decrease was observed, with a maximum in the 125 and 150 mg/L test group. BPS may inhibit Leydig cell function and might lead to feminization of exposed males.

A clear time-dependent increase in the E2/T ratio was seen in both juvenile and adult male fish, up to 1.5. Changes in E2/T ratio can lead to incomplete or improper gonadal development.

Increased E2 level in male juveniles, decreased E2 level in female juveniles, male and female adults and altered E2/T ratio in juvenile and adult male fish after exposure to BPS point towards estrogen activity.

T3 decreased significantly at 100 mg/L BPS in juvenile fish, while at 120 mg/L and 140 mg/L an increase was observed from day 3 followed by a significant decrease at 6- and 9-day exposure groups. T4 decreased significantly at all exposure concentrations. BPS may affect thyroid development and thyroid hormones' homeostasis thereby causing enhanced thyroid hormone level. This decrease of T3 and T4 might be indicative of redirecting metabolic energy away from anabolic process which is essential to carry through life thereby triggering thyroid endocrine disruption
In adult fish T3 and T4 significantly increased in a time-dependent manner in all exposure groups. Increasing levels of T4 occur due to decrease in the conversion rate of T4 into T3

Cortisol (stress hormone) decreased at 100 mg/L in juveniles in all day treatments. At 120 mg/L no changes were recorded in the 3- and 6-day treatment, while in the 9-day treatment cortisol increased significantly. A significant and sharp increase was seen in juveniles exposed to 140 mg in the 6 and 9 d exposure period. In adults significant decrease was seen at 100 and 125 mg/L BPS. In the 150 mg/L group no changes were seen in the 4-day treatment while in the 8 and 12 day exposure group a significant increase of cortisol was observed. The reduced cortisol level could be considered as an adaptive response by way of maintaining low metabolic rate upon endocrine disruption.

Qiu *et al.* (2019)

The main part of this study concerns the exposure of red common carps (Cyprinus carpio) to 0, 0.1, 1, 10, 100 and 1000 µg/L BPS *in vivo* for 60 days. The study included an *in vitro* experiment, for which primary macrophages isolated from the fish head kidney were exposed to 0, 0.1, 1, 10, 100 and 1000 µg/L BPS for 6 hours. Results of the *in vitro* experiment are discussed above.

After exposure, the liver of carps exposed to BPS showed an induced immune response an upregulated immune response, showing an increased oxidative stress (increased ROS level) and pro-inflammatory effects (NO production, induction of inflammatory cytokine expression…).

Both *in vivo* and *in vitro* experiments showed a decreased gene expression of *pparγ* and *rxra*, whereas *nfκb* was significantly increased, confirming the implication of PPARγ.

Mentor *et al.* (2020)

The authors injected one single dose of BPA, BPF, BPAF or BPS (210 nmol/g egg) in 4-days old, fertilized chicken eggs (embryonic day 4 E4), to check the effect on reproductive organ development. Embryos were then dissected at E19, i.e., 2 days before hatching.

BPS exposure showed a high mortality *in ovo* compared to control (57% vs 24% in control). There was no effect on body weight nor liver-somatic indices. However, the gallbladder-somatic index was significantly increased by BPS.

On the 12 surviving embryos exposed to BPS, only 3 were males. None of them showed indication of feminization. But due to the low number of animals, it was not possible to conclude on the effect of BPS on testis feminization (BPA, BPF and BPAF induce feminizing effect in testis). In females, 2 (of 9) exhibited an ovary thinner than normal, revealing a possible atrophy (not significant).

Frenzilli *et al.* (2021)

Approximative 1 year old Juvenile brown trout (Salmo trutta) with an average weight of 132 +/- 25g were exposed for 2 of 8 weeks to BPS under flow-through conditions. BPS was administered via sustained-release cholesterol implants containing doses of 2 mg/kg or 20 mg/kg fish of BPS to attain a chronic exposure. Fish (10 per tank) were randomly distributed to 10 tanks after an acclimatisation period of 2 weeks. Thyroid hormones, glucose and VTG levels were measured.

Concentrations of BPS in the liver were significantly higher when compared to the control, both after 2 and 8 weeks. A positive correlation was seen between tissue concentration of BPS and treatment.

T3 levels increased significantly at 20 mg/kg after 2 weeks but no differences were observed in all exposure groups after 8 weeks. No effect was seen on T4 levels.

Because only T3 levels were affected after BPS exposure and not T4 levels, BPS might potentially act at tissue deiodinase activity.

No significant difference was observed in VTG at 2 mg/kg after exposure of BPS for 2 and 8 weeks. However, VTG significantly increased at 20 mg/kg after 2 weeks exposure. An increasing trend was seen after 8 weeks exposure, although there was no significant difference compared to control.

Glucose levels increased after exposure to BPS. Furthermore, a positive correlation was found between glucose level and BPS liver concentrations.

The above results indicate that BPS might affect thyroid function and alter glucose balans.

Qin *et al.* (2021)

To examine the effect of BPS on lipid metabolism, 2hpf zebrafish embryos were exposed to concentrations of 1 and 100 µg/l of BPS until 240 dpf. Results were compared to the solvent control (0.002% DMSO). For this purpose, VTG and lipids contents in plasma, liver and ovary were measured as well as the expression of genes involved in fatty acids synthesis and β-oxidation metabolism in ovaries of female zebrafish.

VTG promotes oocyte maturation after it is secreted from the yolk particles of the oocytes into the blood stream. Fatty acids are the fuel source for oocyte maturation and ovulation. These acids are formed through hydrolysis of triacylglycerol (TAG) by lipases. The source of primary energy is generated by B-oxidation of fatty acids in mitochondria which is regulated in oocytes by carnitine transferase (CPTs).

In an earlier study, authors demonstrated an increase in TAG levels and a mildly accelerated energy metabolism by stimulation of β-oxidation in male zebrafish liver, while no excessive fats were observed in the livers of females exposed to BPS.

Exposure to BPS did not cause an effect on total size, but a significant increase was observed on body weight and BMI at 1 and 100µg/L, and HSI and GSI were significantly increased at 100 µg/L of BPS in female fish.

VTG levels in plasma increased significantly at 1µg/L in plasma and in all test concentrations in ovary, but no changes in liver were observed. Total cholesterol followed the same trend.

Liver TAG levels significantly increased in all test concentrations while plasma TAG levels significantly decreased at 10 µg/L but significantly increased at 100µg/L. Increase in ovarian TAG levels was only significant at 1 µg/L of BPS.

Low density lipoprotein (LDL) levels significantly decreased in the liver but increased in the plasma and ovary at 1µg/L of BPS. Concentrations of high-density lipoprotein (HDL) increased only in plasma at 1µg/L of BPS.

Furthermore, significant lipid accumulation was seen in interstitial fibrosis tissue (IF). IF diffuses around the oocyte and is necessary for supplying nutrients to oocytes.

After exposure to 1 and 100µg/l of BPS, proportion of primary growth stage oocytes decreased significantly, while the % of full-grown stage oocytes increased significantly. Furthermore, significant increase was seen at all concentrations of the number of cortical follicle- and vitellogenin-stage oocytes. Atretic follicles and macrophages aggregates (which contribute to the resorption of atretic follicle material) were observed in ovaries before the normal reproductive cycle.

BPS exposure to 100 µg/L caused significant upregulation of acc, fasn, acsl1, and pparγ in the ovary and increase also the mRNA levels of acadm, acadl, and pparα. Furthermore, expression of fatty acid β-oxidation geneses hsl, cpt2 and hsd17β4 increased with increasing exposure of BPS.

Number of eggs/breeding tank/day were significantly increased after exposure to 1 and 100 µg/L of BPS.

In F1 embryos a significant increased mortality rate (87% survival in solvent control, 75% at 1 µg/l and 82% at 100 µg/L BPS) and a reduction in body length was observed at 1µg/L BPS. Furthermore, BPS affected hatching rate: sign. decreased at 60 and 72hpf at 1 µg/L and increased at 100 µg/L at 48hpf. Heart rate sign. increased at 48hpf but decreased at 120hpf in all treatments. Malformation rate increased at 100µg/L BPS. Also a sign. increase in total cholesterol and LDL was seen at 100µg/L BPS at 120 hpf. Furthermore, more visceral lipid accumulation was observed compared to solvent control.

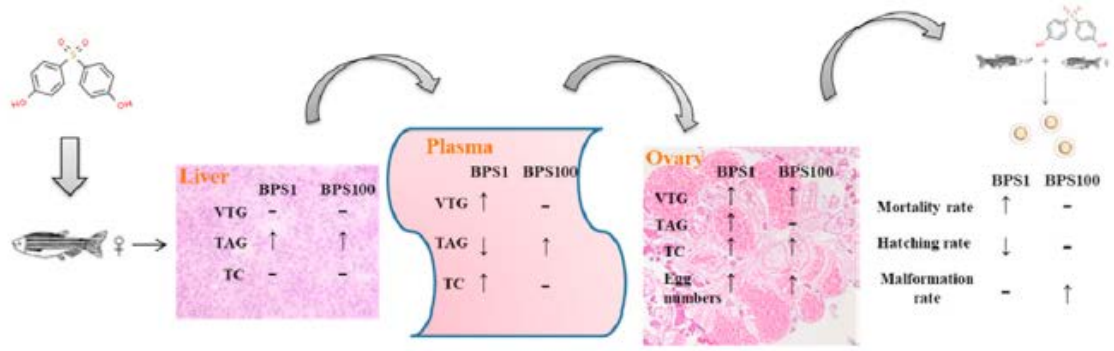


Figure 9: Graphical abstract from Qin *et al*., 2021

It can be concluded that the elevated VTG content, disrupted lipid metabolism and lipid accumulation in the ovary after exposure to BPS lead to a change in the early stage of normal female zebrafish spawning cycle and that maternal exposure to BPS causes excessive lipid content in F1 embryos affecting early development.

eMSCA Comment:

- The result of the number of eggs is doubtful as the number of eggs produced per female per reproduction day e.g., +/- 300 eggs/breeding tank/day at 100 µg/L BPS (2 replicates/treatment and 4 females/treatment) is unrealistically high.

- The results on oocyte maturation are unclear in this study as the increase in mature oocytes is expressed in percentage, not taking into account the atretic oocytes which were reported to increase in number.

Qiu *et al.* (2021)

After an exposure of 2 hpf-old zebrafish embryos to 1 and 100 µg/L of BPS until 120 hpf, effects on hatching rate, body length, motor behaviour, GnRH3-neurons, hormone levels, expression of neuroendocrine-related genes and ERs and Cyp19 were measured. 0.005% of DMSO was used as solvent control, but no statistically significant difference with the water control was observed. Therefore, results were compared to the water control.

BPS increased in a dose-dependent manner the hatching rate (statistically significant at 100* µg/L at 48hpf), while body length was dose-dependently decreased (statistically significant at 100* µg/L at 120 hpf).

The decrease in movement distance was not statistically significant.

The number of hypothalamic GnrH3 neurons increased statistically significantly at 100* µg/L, while only an increase (non-statistically significant) of number of Terminal Nerve GnrH3 was observed.

BPS exposure impacted the transcription of several genes:

Kiss 1, kiss2, ren, pth1, gh were dose dependently and stat. sign increased at 100* µg/L. Lhβ and Anp were statistically significantly increased at 1* and 100 µg/L and pr1 at 1* µg/L. Gnrh3 and Fshβ increased but not statistically significant. Addition of ICI resulted in a significant decrease of fshβ (100* µg/L), but not of lhβ when compared to the control. No statistically difference was seen with the BPS (100 µg/L). The same was observed for lhβ in addition of FAD. Adding FAD decreased fshβ statistically significant compared to the control and 100 µg/L BPS.

Furthermore, BPS induced a statistically significant upregulation of Cyp19a1 and Cyp19a2 at 100* µg/L.

BPS impaired hormone levels: FSH and E2 were statistically significantly increased resp. at 100* µg/L and ≥1* µg/L. The increase of LH and GH (slight) were not statistically significant. Neither ICI, nor FAD were able to impact the effect of BPS:
LH + ICI: ↙ at 100* µg/L compared to control, ↙ but not sig. compared to BPS
LH + FAD: ↙, but not sign.
FSH + ICI: ↗ at 100* µg/L compared to control, ↗ but not sig. compared to BPS
FSH + FAD: ↗ at 100* µg/L compared to control, no stat. difference with BPS

Park *et al.* (2022)

Adult zebrafish (3-4 months old) were exposed for 21 days to BPS at concentrations of 0 (solvent control: 01% DMSO), 8, 40 and 200 mg/L under semi-static conditions.

No mortality was observed throughout the exposure period.

GSI and HSI reflecting gonadal development or sexual maturation, sign. increased in females exposed to 40 mg/L of BPS. However GSI in females sign. decreased at 200 mg/L. In males no effect on GSI was observed, but HSI sign. increased at 200 mg/L.

For determination of the oocyte maturation authors divided oocytes in 3 stages: immature oocytes (incl. perinuclear oocytes and yolk vesicle oocytes), mature oocytes (incl. yolk globule stage oocytes) and regressed oocytes (observed oocyte degeneration). Compared to the control and the 8 µg/L exposure group where females mainly contained mature

oocytes, regressed oocytes were found at the 40 and 200 mg/L of BPS. However, no effect on testicular development was seen in male fish.

Sign. down-regulation of hepatic VTG gene expression in females was reported while a sign. increase was seen in males at 200 mg/L. No sign. effect was seen on hepatic ER α mRNA activation in males and females. However ERβ activation decreased significantly in males, while no ef. t was seen in females.

Whole body concentrations of progesterone and 17ß-estradiol (E2) levels were significantly, and dose dependently increased in females while testosterone and 11-ketotestosterone were significantly decreased in male fish. E2 increased significantly and dose-dependently in males. The ratio E2/T was sign. increased in male fish indicating aromatase activity.

The increase of hepatic VTG expression together with the increased E2 level in males might reduce spermatogenesis.

Furthermore, also thyroid hormones were sign. and dose dependently elevated in males and females. No effect was seen on cortisol levels in both males and females.

Wang et al. (2020b)

In this study adult male and female zebrafish (Danio rerio) of six months old were exposed to a blank control, a solvent control, and three concentrations of BPS (1, 10 and 100 µg/L).

In shoaling behavioural experiments, female shoaling was weakened, as the time ratio spent in the area close to other fish was significantly decreased ($p<0.05$), and the time ratio in the far area was significantly increased in the female 100 µg/L BPS group ($p<0.05$) compared to the solvent control.

Female fish from BPS-treated groups also showed altered external features. Depigmentation (a significant decrease in melanin) of the body colour was observed for the 1 µg/L BPS group ($p<0.05$) and for the 10 and 100 µg/L BPS groups (both $p<0.01$) when comparing to the solvent control group.

Histological examination of ovaries showed that the total proportion of late vitellogenin and spawning oocytes in ovaries was 28.12 in the solvent control and reduced to 11.46 (1 µg/L BPS), to 17.89 (10 µg/L BPS) and to 13.68 (100 µg/L BPS).

It was also clear that during mating behaviour, untreated males spent significantly less time close to the BPS-treated females (1, 10 and 100 µg/L BPS; $p<0.05$), when comparing with the solvent control group. The authors linked the lower attraction potential of BPS-treated females to the fact that untreated females had a higher proportion of late vitellogenin and spawning oocytes, and to a changed body colour pattern of BPS-treated females.

It can be concluded that BPS exposure altered behaviour, could impair survival capacity (search for food, feeding and hiding for predators) and that females exposed to BPS may have less chance to attract a male for mating due to their low social status in the population (due to abnormal body pigments, their weak shoaling ability and the presence of oocytes of lower quality). Exposure to BPS could thus be associated with the impairment of mating behaviour, and by extension be linked to the inability to find a suitable mate in order to produce offspring.

Conclusion: OECD CF level 3 data

Disruption of steroidogenesis (aromatase activity). Disturbed balance of sex steroid hormones. Increase of vitellogenin concentration (biomarker of estrogenic activity). Increased expression of ER and Cyp19-dependent genes.

Disturbed development (egg production, oocyte maturation, hatching).

Several indications that BPS can interfere with the HPG axis, disrupts neurogenesis in the brain and alters social behaviour.

Thyroid-related gene expression modified. Contradictory effects on thyroid hormone levels.

OECD Level 4: *in vivo* assays providing data on adverse effects on endocrine relevant endpoints

Table 81: Overview of endocrine disruption – environment (OECD Level 4, literature data)

Method (guideline	Short description of method	Endpoint	Result (*positive/negative/trend*)	Description of result (*positive/negative/trend*)	References
-	Fish Sexual development test	Estrogen Thyroid	+	Developmental toxicity, reproduction impairments, imbalance of steroid and thyroid hormones and induction of VTG in males	Naderi *et al.*, 2014
-	Reproduction effects on Caenorhabditis elegans (nematode) 4d exposure	Fertility Reproduction	+ +	Increase in embryonic lethality and decrease in fertility Reproductive gene response	Chen *et al.*, 2016
Compliant with OECD TG 210 and TG 230	Embryonic zebrafish exposure (2 hpf to 120 dpf) to assess transgenerational thyroid disruption	Thyroid	+	Decreased T4 and increased T3 in F0 females, but also in F1 unexposed embryos. F1 showed also delayed embryonic development and other neurological effects	Wei *et al.*, 2018

Fish data:

Naderi *et al.* (2014)

In a developmental toxicity study, 2hpf embryos of zebrafish (Danio rerio) were exposed to 0 (solvent control: 0.01% acetone), 0,1, 1, 10 and 100 µg/l of BPS for 75 days with daily renewal of 50% of the exposure solution.

Embryos were obtained from spawns of wild type adult zebra fish (age 4-5 months). Adult fish, acclimated for 2 weeks prior to the experiment, were kept in water with a pH of 7.0-7.4, a temperature of 28 ± 0.5 °C and a photoperiod of 14h light/10h dark. They were fed twice a day with artemia nauplii.

Approximately 7000 embryos were randomly distributed into the tanks, 3 replicates/exposure concentration. After the 75dpf exposure period, male and female fish were placed in separate tanks with clean water for an acclimation period of 2 weeks.

Subsequently male and females from the same treatment were randomly allocated to 31 tanks (2 males and 2 females/replicate) and acclimatised for 3 days. Number of eggs, hatching rate and time to hatch were daily recorded during the next 7days. At termination of the study, following parameters were examined: total length, body weight, GSI, HSI, sperm count and hormone levels (E2, T, VTG, T4 and T3). The hormone levels were analysed using the blood plasma method.

During exposure, the water temperature was 27 ± 2 °C, growth of fish (fish wet weight, blotted dry) between 238.33 and 271.33 mg; length (standard length) between 24.8 and 28.84 mm; sex ratio : 46.3% females : 30-70% from control group at termination of test; no significant effect of solvent control on mortality and a hatching rate >80% was seen. All values are represented as mean ± SEM. Dissolved oxygen concentration remained > 60% of the air saturation value throughout the exposure period.

Although not mentioned in the article, authors confirmed to the eMSCA that results of the water control and the solvent control were the same.

Significant mortality was observed at 100* µg/L (+/-30% mortality compared to +/-5% in the control group).

The sex ratio in adults (F_0) skewed towards females: resp. 58.8% and 66.7% were females at concentrations of 10 and 100 µg/l, compared to 46.3% in the solvent control. Exposure of teleost fish to oestrogen chemicals can lead to a shift in sex ratio towards females.

Body length was significantly lower in male fish compared to female fish and significantly lower in male fish at concentrations of 100 µg/L. This might be explained by the estrogenic activity of BPS.

The gonadosomatic index (GSI) was significantly lower in females at 100 µg/L, while a significant decrease in males was already seen at 10 µg/L.

The hepatosomatic index (HSI) was significantly higher in females treated with 10 and 100 µg/l, while a significant increase in males was only seen at 100 µg/L.

Egg production and sperm count decreased significantly in fish exposed ≥ 10 µg/L. BPS altered the hatching rate (decreased) and time to hatch (delayed) of embryos, even after fish were placed in clean water.

Plasma levels of 17β-estradiol (E2) were significantly increased in both male and female fish, resp. at 1 and 10 µg/L. Plasma testosterone (T) significantly decreased in males exposed to 10 and 100 µg/L, whereas in females no changes were observed. This confirms the alteration of sex hormones seen in the study of Ji *et al.* (2013) where these alterations were accompanied by up-regulation of the aromatase (cyp19a and cyp19b) genes and down-regulation of cyp17 and 17βhsd genes.

VTG was induced in females and males, resp. at 10 and 100 µg/l. This response could be triggered through exerting estrogenic effects. Increase of E2 can also lead to an induction of VTG.

A decrease of T3 and T4 levels was shown in males and females resp. at 10 and 100 µg/L. T4, which is primarily produced by the thyroid follicles in fish, is almost entirely converted in peripheral tissues by deiodinase (Dio1, 2 and 3) to the biological active T3. Reduction of plasma T4 contributes to a decrease in T3. Dio1 has an influence on iodine recovery and thyroid hormone degradation (Van der Geyten *et al.*, 2005). Dio 2 is an important enzyme in the conversion of T4 to active T3. Dio3 is a purely inactive enzyme. Further studies are needed to clarify the mechanism in decreased plasma T3 and T4 levels.

Conclusion: Sexual differentiation was influenced by altering the sex steroid hormones balance. In zebrafish, gonads differentiate towards ovary or testes after approx. 5-7 weeks post hatching (Maack and Segner, 2003; Spence *et al.*, 2008).

Adult fish, which experienced developmental exposure, demonstrated impaired reproductive success (reduced egg production and sperm count). Growth suppression in males might be explained by the estrogenic activity of BPS through interfering with somatotropic axis (growth hormone and insulin growth factors) as suggested for BPA, disruption of the thyroid hormones (interference with secretion of T3, T4) and VTG (by requiring a lot of energy and resulting in a shift in energy utilization from somatic growth to VTG production). VTG production can also lead to hyperplasia or hypertrophy of hepatocytes, which may be responsible for elevated values of HSI.

Wei *et al.* (2018)

The aim of this study was to assess the potential of long-term BPS exposure to disrupt the thyroid hormones in the next generation in zebrafish. The experiment was conducted in compliance with OECD TG 210 (fish, early-life stage toxicity test) and TG 230 (21-day fish assay). The authors exposed zebrafish to BPS 0, 1, 10 and 100 µg/L from 2 hpf to 120 dpf (F0) in the presence of 0.002% (v/v) DMSO. Results were expressed in comparison to the solvent control. After 120 days of exposure, 36 females and 36 males were sacrificed and analysed. Additionally, 36 females and 72 males were paired in clean water. The resulting eggs (F1) were then partly collected for subsequent experiments (<2 hpf) or cultured in BPS-free water until 96 hpf to assess developmental disorders.

Long-term exposure to BPS significantly decreased T4 plasma level and increased T3 level in all exposure group of F0 females, whereas only T3 was affected in males at 1 and 10 µg/L. Interestingly, the F1 eggs showed the same pattern as F0 females, i.e., decreased T4 level and increased T3 level.

Table 82: Effects of long-term exposure to BPS on T4 and T3 levels (Wei *et al.*, 2018)

Dose	F0 female (ng/mL)		F0 male (ng/mL)		F1 eggs (ng/g)	
(µg/L)	T4	T3	T4	T3	T4	T3
0	8.09 ± 1.08	1.01 ± 012	5.76 ± 0.63	1.35 ± 0.23	6.96 ± 0.84	0.43 ± 0.07
1	5.74 ± 0.87*	1.51 ± 0.14*	5.77 ± 0.76	1.67 ± 0.17*	5.10 ± 0.68*	0.73 ± 0.13*
10	5.46 ± 0.39*	1.56 ± 0.26*	6.29 ± 1.02	1.79± 0.20*	5.24 ± 1.12*	0.68 ± 0.11*
100	6.33 ± 0.58*	1.64 ± 0.25*	5.65 ± 0.35	1.24 ± 0.23	4.90 ± 0.92*	0.66 ± 0.14*

* = $p < 0.05$

The impact of parental BPS exposure had several effects in F1, including delayed development, reduced hatch rate and affected movement of embryos. Larvae were also affected showing decreased inflation of the swim bladder (involved in the transition embryo to larva), decreased motility (neurodevelopmental effect) and reduced pigmentation. All effects in embryos and larvae were correlated with mRNA levels. This confirms that the thyroid disruption can be transmitted to the next generation in zebrafish.

Non-fish data

Chen *et al.* (2016)

Caenorhabditis elegans larvae (nematode) were exposed for 4 days, encompassing the first larval stage until completion of the first day of adulthood (24h post L4-stage), to concentrations of 125, 250 and 500 µM, corresponding to mean internal doses of 0.21 µg/g and 0.39 µg/g resp. for the highest doses. Worms were exposed by dissolving the concentrations into the solvent and were then put on the plates. Results were compared with the solvent control (0.1% ethanl). The internal dose at the lowest concentration was beneath the sensitivity threshold of 0.1 µg/g. Those doses in C. elegans representing approx. the human internal physiological concentrations of BPS.

Exposure to BPS caused significant mortality among embryos at all doses tested, with a 5-fold increase at 500 µM. Furthermore, a slight increase in egg number was reported, although not statistically significant.

A significant decrease in brood size (total number of adults) was observed only at the lowest and highest dose. This decrease corresponds with the rate of embryonic mortality.

Germline nuclear loss (pachytene gaps) was significantly increased in all doses. Furthermore, a non-significant trend towards larger germline was seen. Despite the normal germline size, chromosome morphogenesis was abnormal. Chromosomes at the stage of diakinesis failed to condense properly: SYP-1 disassembly was altered as the oocytes progress through diakinesis and distinct chromosomal domains are established. This effect was dose dependent and followed embryonic mortality.

Germline apoptosis, partly mediated by the activation of the pCHK-1/CEP- 1(p53) axis in response to aberrant meiotic recombination was dose-dependently increased. Exposure to BPS points to a profound perturbation of the germ cell differentiation process. In contrast to BPA, BPS did not delay the kinetics of meiotic recombination in late pachytene.

Conclusion: OECD CF level 4 data

Developmental toxicity, reproduction impairments, imbalance of steroid and thyroid hormones and induction of VTG in males.

Disturbed balance of thyroid hormones that could be also transgenerational. Effect on neurodevelopment and hatching.

OECD Level 5: *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism

Table 83: Overview of endocrine disruption – environment (OECD Level 5, Registration dossier)

Method	Short description of method	Endpoint Result	Description of Result (*positive*/*negative*/*trend*)	References
Range finding study to ZEOGRT Non-GLP	OECD TG 210 (ELS)+OECD TG 234 (FSDT)+OECD TG 229 (FSTRA) Test conc: 2 replicates/conc. instead of 4 Increased number of test conc: 8 + control Nom. test conc: 0.0032, 0.010, 0.032, 0.1, 0.320, 1.0, 3.2 and 10.0 mg/L Growth assessment on study day 33/34	Growth, survival and reproduction	Growth: No effect on growth after 32d exposure, slight reduction at day 65 at 3.2 and 320 µg/l, non-treatment related and sign. Reduced at day 106 but not dose-related No effect on survival Fecundity: non-sign. decrease Jockheere_terpstra. Recalculation with Wilcoxon shows drastic and sign. decrease at 10 µg/L (by 70%) No sign. effect on sex ratio Low fertility was observed in all test groups (control below validation criterion of > 80%) probably due to individual fish (most pairs >90% fertilisation, 1-2 pairs with +/-50% fertilisation	Registration dossier: Unpublished study report, 2020

	(not 30 days after end of hatch)			
ZEOGRT (OECD TG 240- adapted for zebrafish)	Nom. Conc.: 0, 2, 10, 50, 250 and 1250 µg/L 4 replicates/conc.	Survival, hatching, growth, reproduction,	No effects on survival, hatching, growth and reproduction (fecundity/fertility) in F0 and on survival in F1. VTG: Not measured in F0, No sign. effect in F1 Gonad maturation: No significant effects on gonad maturation in F1 males, however, decrease in mature females Sex ratio: Trend towards feminisation Non-dose-dependent Sign. Effects on fecundity in F1 (at 2 and 50 µg/L) Although length (females and males) was significantly affected in F1 at ≥ 10µg/L after day 35, the effect was transient and no longer observed at day 65. Length and body weight of females did not statistically significantly differ from control at day 65 and at the end of the experiment. NOEC = 250 µg/L (male body length of F1 at end study (day 125-128) F2: statistically significant difference on start to hatch at 10 (94%), 250 (95%) and 1250 µg/L (94%).	REACH registration dossier: Unpublished study report, 2020

In the frame of Substance Evaluation under REACH, a **ZEOGRT** was performed according to OECD TG 240 (MEOGRT), adapted for zebrafish.

In first instance a **range finding study** was performed as recommended in the Substance Evaluation Decision to identify the growth LOEC as highest concentration for the definitive test.
The study protocol deviated from Annex 2 of this Decision with regard to the number of test concentrations, number of replicates and the test duration:

- The decision recommended to cover the concentration range from Naderi *et al.* (2014) and the high and mid concentrations from Ji *et al*. (2013): 320, 100, 32, 10, 3.2 µg/L. Instead 8 concentrations (0.0032, 0.010, 0.032, 0.1, 0.320, 1.0, 3.2 and 10.0 mg/L)+ control were used.
- Number of replicates/ test concentration: 2 instead of 4
- Test duration: OECD TG 210 (FELS) was followed by an OECD TG 234 (FSDT) and an OECD TG 229 (FSTRA) resulting in an exposure period of 148 days instead of 34 days to cover a full zebrafish generation.

No effect on growth and length was observed after 35 days. Although not dose-dependent, at day 65 significant difference in growth was seen at test concentration 3.2 and 320 µg/L. In all treatment groups a significantly reduced growth was reported at day 106 but not concentration-related and thus considered non-treatment related. The reduction is likely incidental due to small unequal sample size and the low replication.

No statistically significant effect observed on survival and sex ratio (resp. 32%, 30%, 41%, 33%, 37%, 37%, 40%, 50% and 39% M at 0, 0.0032, 0.010, 0.032, 0.1, 0.320, 1.0, 3.2 and 10.0 mg/L).

Fecundity decreased non-significantly between test day 111 and 128 at BPS concentrations of 3.2, 10 and 32 µg/L by 36, 70 and 50 % respectively. Statistical analysis was performed according to a Jonckheere-Terpstra test. It should be noted that the mean values of the treatment had a non-ordering hypothesis (non-monotonic) which makes this statistical method not fit for observing significant effects in this case. A recalculation using the Wilcoxon test showed, on the contrary, showed that fecundity was drastically and statistically significantly decreased (by 70%) at 10 µg/L. A 36 and 50% decrease in fecundity was also noted after exposure to 3.2 and 32 µg/L, showing repeatedly the non-monotonic effect on fecundity. All treatment groups showed low fertility and control fertility was below the validation criterion of >80%.

For the **definite test** +/- 30-week-old zebrafish (F0) were exposed to nominal concentrations of BPS of 2, 10, 50, 250 and 1250 µg/L for a total of 150 days. The exposure period of F0, F1 and F2 was 49-50 days, 125-128 days and 96h resp. 4 replicates per test concentration were used.

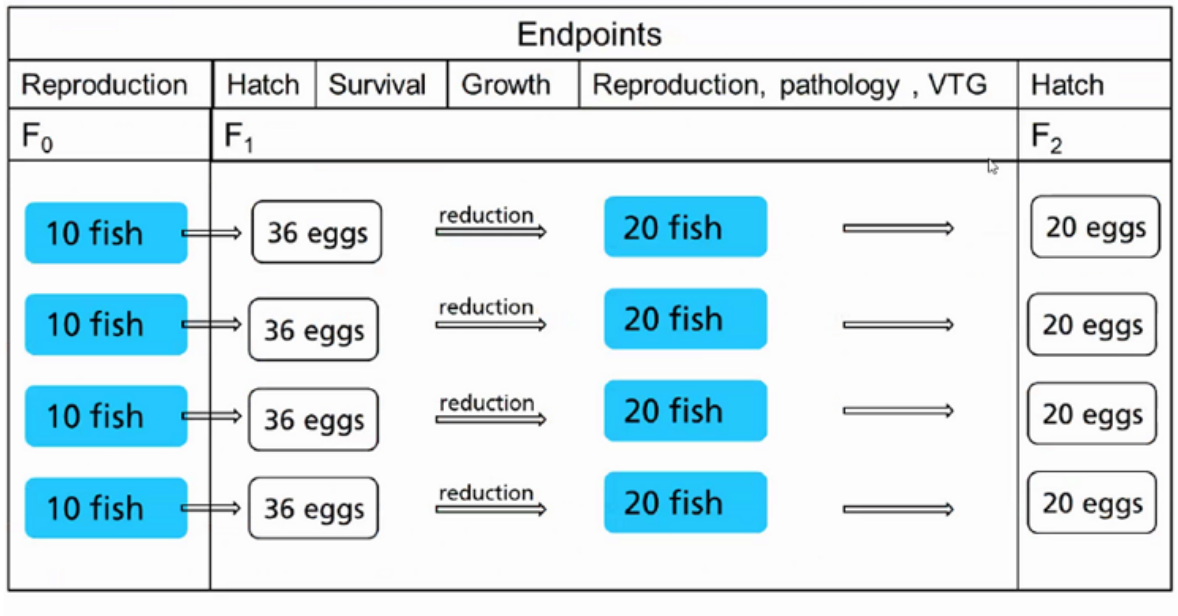


Figure 10: Overview of the methodology used during performance of the ZEOGRT (OECD TG 240 (MEOGRT), adapted for zebrafish)

To start the F0 generation, blocks were filled by randomly allocating the tanks with the highest per-female fecundity (established during pre-exposure) among the A replicates, followed by the group of second-highest spawners in the B replicates, etc. until all replicates were filled. 5 males and 5 females were allocated to each replicate.

During the 4th week of exposure 36 fertilised eggs of the F0 were collected/replicate to start the F1. After 35 dpf fish were reduced to 20 fish/replicate. Around Day 140 of total exposure (exposure day 118 of F1) 20 fertilized eggs were collected from corresponding F1 test groups and evenly distributed to the F2 replicates.

It was agreed upon that determination of VTG in F0 was not needed when a plausible biological link between adverse effects observed in F0 and/or F1 and an impact on VTG was seen in F1. Furthermore, measurement of 11-keto testosterone was considered of no added value as it would be unlikely to detect a statistical decrease when exposed to a weak estrogen substance.

Endpoints results:

Mean measured concentrations were found to be in the range of 88-99% of nominal concentration in the F0- generation, 91-107% in the F1-generation and 100-107% in the F2-generation.

Test acceptability criteria for controls/validity criteria (successful reproduction, post hatch survival of larvae, survival of juveniles and adults and sex ratio) were met.

No effects were seen on survival, growth and reproduction in F0 and on survival in F1.

Although length (females and males) was significantly affected in F1 at ≥ 10µg/L after day 35, the effect was transient and no longer observed at day 65. Length and body weight of females did not statistically significantly differ from control at day 65 and at the end of the experiment. The lowest NOEC of 250 µg/L was determined for male body length of F1 at the end of the study (day 125-128), which was significantly different from control at 1250 µg/L.

In the F2-generation a statistically significant difference was determined on start to hatch at 10 (94*%), 250 (95*%) and 1250 µg/L (94*%). However this effect is considered minor.

For F0 no effects on fecundity are observed (start age of fish: +/-30 weeks post fertilisation count of eggs/female reproduction day from day 1 to day 23).

In F1, however, a statistically significant difference with control was seen for fecundity at 2 and 50 µg/L (22 egg counts during days 97-118).

Fecundity decreased in F1 in a non-dose-dependent manner (resp. by 43, 21, 36, 21 and 7%). When using the Wilxocon test, a statistically significant difference with control was seen (one-sided), with respectively 8 and 9 eggs/ female reproduction day at 2 and 50 µg/L vs 14 eggs/female reproduction day in control.
Furthermore, fecundity in F1 was lower than in F0 due to the difference of age at start of exposure (F0 was much older than F1 when fecundity started) and thus may explain the difference in maturity and the time needed to synchronize and reproduce in F1.
No significant effect on sex ratio in F1 was demonstrated, although an increasing trend in females was observed not dose-related and within natural variation (30/70).

No effect on VTG in F1 males is observed. Some high VTG values in males were measured in control groups and showed an unusual high variability which was at least partially explained by cross-contamination from previously examined females.

Table 84: Percentage survival at different nominal concentrations of BPS

Endpoint (group means)			Nominal concentrations (µg/L)					
			0 Group 0	2 Group 1	10 Group 2	50 Group 3	250 Group 4	1250 Group 5
Survival	F0	Day 0 – sacrifice day 49-50	100%	100%	100%	100%	100%	100%
	F1	start – hatch (day5)	99%	100%	99%	99%	99%	100%
		Hatch (day5) – swim-up (day6)	100%	99%	100%	100%	99%	99%

		swim-up (day6) – day 35	83%	87%	87%	87%	82%	84%
		day 35 (reduction) – day 65	100%	100%	100%	100%	100%	100%
		day 65 – sacrifice days 125-128	100%	100%	99%	100%	100%	100%
		day 35 – sacrifice days 125-128	99%	99%	99%	100%	100%	98%
	F2	Start – hatch (day4)	100%	95%	94%[b]	95%	95%[b]	94%[b]
		hatch – swim-up (day4)	100%	100%	100%	100%	100%	100%

b = statistical significance p ≤0.05 when using Wilcoxon test (one-sided -)

Table 85: Growth at different nominal concentrations of BPS

Endpoint (group means)			Nominal concentrations (µg/L)					
			0	2	10	50	250	1250
Growth	F0	Male body weight at sacrifice day 50 [mg]	443	474	463	465	452	456
		Male body length at sacrifice day 49-50 [cm][f]	3.7	3.8	3.7	3.8	3.7	3.8
		Female body weight at sacrifice day 49-50 [mg]	415	389	396	410	415	368
		Female body length at sacrifice day 49-50 [cm][f]	3.5	3.5	3.5	3.5	3.5	3.5
	F1	Length day 35 [cm][e]	1.7	1.7	1.6[c, d]	1.6[c, d]	1.6[c]	1.7[c]
		Length day 65 [cm][e]	2.7	2.8	2.7	2.7	2.7	2.8
		Male body weight at sacrifice, days 125 – 128 [mg]	403	408	374	387	391	367
		Male body length at sacrifice, days 125 – 128 [cm][f]	3.7	3.6	3.6	3.5	3.6	**3.5[c]**
		Female body weight at sacrifice, days 125 – 128 [mg]	449	453	437	437	450	435
		Female body length at sacrifice, days 125 – 128 [cm][f]	3.6	3.6	3.6	3.6	3.6	3.6

c = statistical significance p ≤0.05 when using William's test (one-sided -)
d = statistical significance p ≤0.05 when using Dunnett test (one-sided -)
e = measured with computer software (image tool)
f = measured with metric ruler

Table 86: Reproduction at different nominal concentrations of BPS

Endpoint (group means)	Nominal concentrations (µg/L)

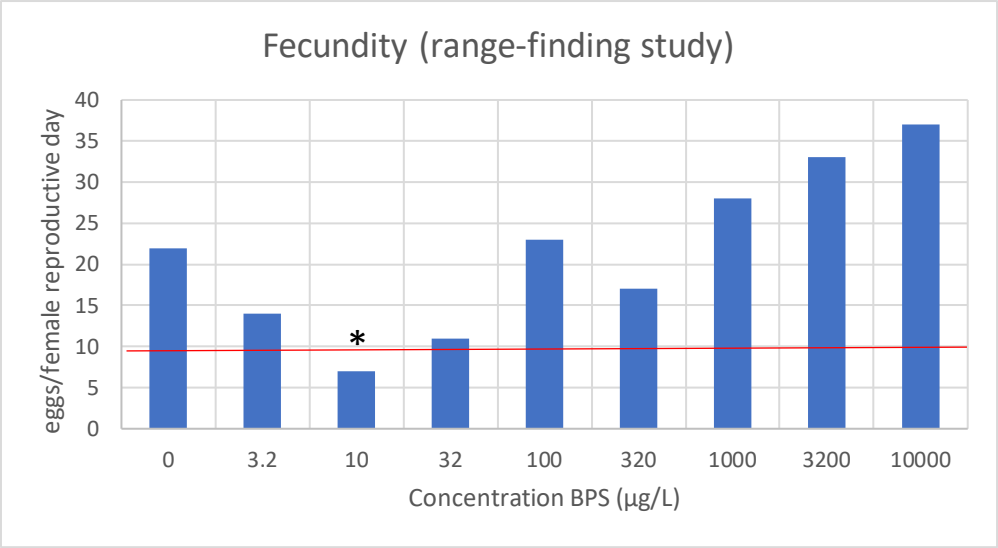
			0	2	10	50	250	1250
Reproduction (dev. + repro)	F0	% Fertility	92.2%	92.0%	91.6%	90.0%	92.4%	91.3%
		Fecundity (eggs/female reproductive day): count from day 1-23d=23d)	21	19	17	21	21	19
	F1	% Fertility	91.4%	90.5%	88.7%	90.8%	94.1%	93.5%
		Fecundity (eggs/female reproductive day): count from day 97-118= 22d)	14	8b	11	9b	11	13
		Sex ratio (males)	0.407	0.314	0.291	0.325	0.488	0.295
		Females % [SD]	59% [0.19]	68% [0.20]	71% [0.08]	67% [0.15]	51% [0.23]	70% [0.08]
		Males % [SD]	41% [0.19]	31% [0.20]	29% [0.08]	33% [0.15]	49% [0.23]	30% [0.08]
		Vitellogenin content Males [median/aquarium]	14.2	9.4	9.8	7.9	7.3	7.7
		Vitellogenin content Females [median/aquarium]	33475	43586	30561	30732	32071	27927
		Gonad Maturity Index (males)	3	3	3	3	3	3
		Gonad Maturity Index (females)	3	3	3	3	3	3

b = statistical significance p ≤0.05 when using Wilcoxon test (one-sided -)

eMSCA comment on ZEOGRT:

- Range finding study:

  - Fecundity: as the Jonckheere-Terpstra test not fit for purpose as mean values of the treatment had a non-ordering hypothesis (non-monotonic) the eMSCA performed a recalculation using Wilcoxon test



  - Fertility:

    Fertility (non-significantly) decreased in the range-finding study but also here the Jonckheere-Terpstra-test was applied.

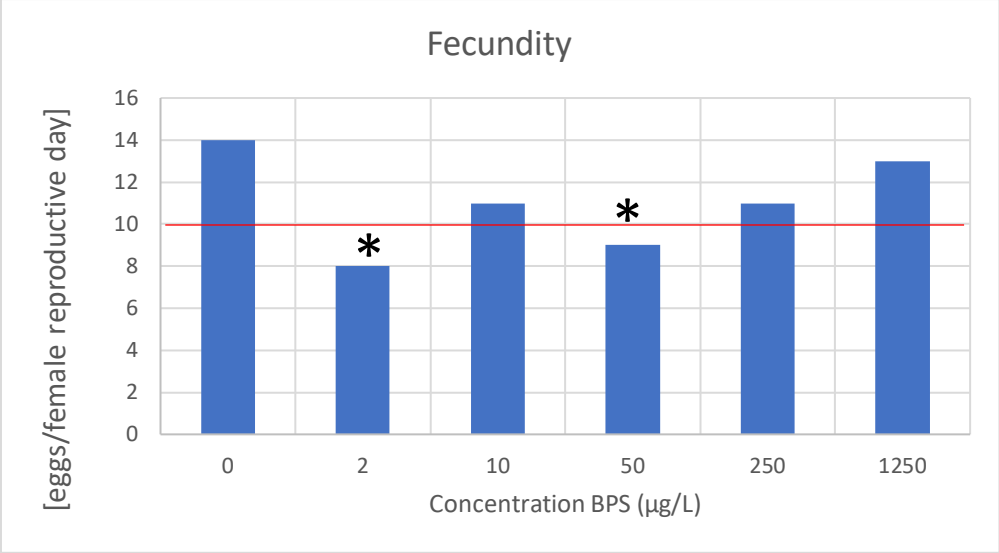
- Main study:

  - VTG:

    - A high variability in VTG data in the male controls was reported partially explained by cross-contamination from previously examined females. Levels of VTG differed however an order of magnitude of 3 between males and females. Furthermore, the use of median data put less weight on the outliners caused by cross contamination. Therefore, it can be concluded that no significant effect on VTG in F1 males is observed.

    - A higher sensitivity of VTG induction after exposure to estrogenic substances in adults compared to juveniles has been observed in several literature studies. This might be due to the immaturity of the juveniles leading to a different estrogen receptor expression or differences in biotransformation reaction rates of the substance (Kinnberg *et al.*, 2015). VTG was not measured in F0 in the ZEOGRT.

  - Fecundity:



  - Histological analysis:

    Calculation using Cochrane-Armitage trend analysis demonstrated a **dose-dependent decrease in the number of females with mature oocytes** at the end of the experiment (170 days) for all concentrations. Oocyte maturation was **significantly affected at 1250 µg/L** using Fisher exact test.

dose (µg/L)	**0**	**2**	**10**	**50**	**250**	**1250**
group	0	1	2	3	4	5
Proportion of mature (%)	14,893617	7,4074074	3,6363636	9,2592593	4,8780488	1,8181818
P-value (compared to control) [a]	NA	0,341	0,079	0,542	0,17	0,025

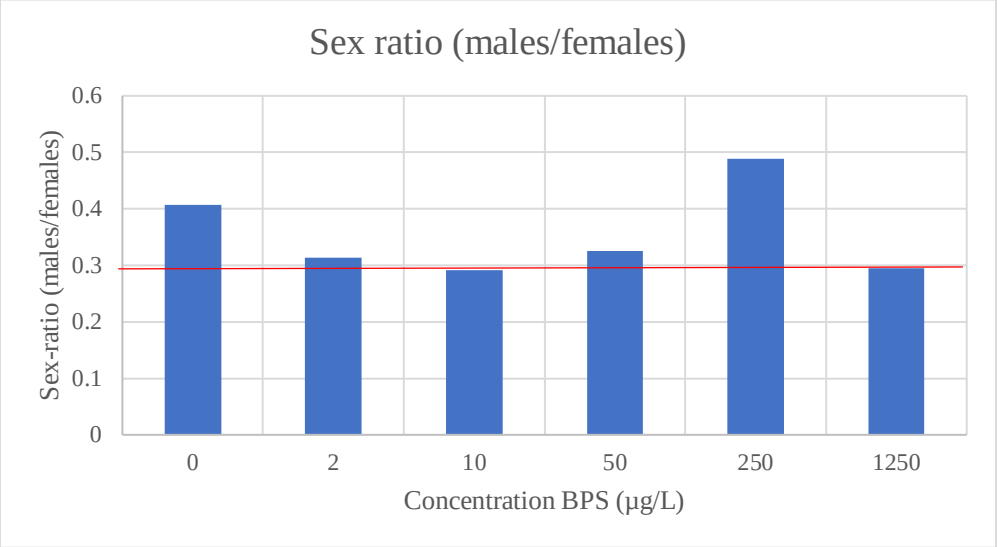
P-value (trend test) [b]	0,0311					

[a] Fisher exact test: test comparing each proportion individually (=1 test for each dose compared to control)

[b] Cochrane-Armitage test: trend test for proportions (=a single test to evaluate if there is a global trend)

- Sex ratio:

  - The sensitivity on the observations is impacted due to the **very low % of males in the control** (resp. 41% in the main study and 32% in the Range finding study).



Conclusion: OECD CF level 5 data

Significant decrease of male body length in F1
Significant decrease in fecundity in F1
Significant decrease in number of F1 females with mature oocytes
Trend towards feminisation
Significant mortality in F2 (although minor)

Other adverse effects

Catron *et al.* (2019) did not observe developmental toxicity in zebrafish embryos at 10 dpf after exposure to concentrations of BPS up to 45 µM. Neither did they observe effect on locomotory activity during a 20 min. testing period. They found however that microbial community structure in zebrafish were disrupted at all tested concentrations from 4.4 to 45 µM. Microbiota play important roles in health and disease. BPS exposure caused a shift in family level taxa: non detectable levels of the Neisseriaceae family and a higher relative abundance of Cryomorphaceae with increasing concentrations. Rheinheimera, Pseudomonas, and Leptothrix were significantly sensitive to BPS. Also, significant changes in BPS-resistant (Fluviicola, Pseudomonadaceae_unclassified, Flectobacillus, Aeromonadaceae_unclassified, and Delftia) and BPS-sensitive (Pseudomonas and Runella) taxa were identified. An inversely relationship was found between microbiota disruption on the one hand and developmental toxicity and estrogenicity on the other hand. In this respect estrogenic activity results of Mesnage *et al.*, 2017 were used (see OECD CF level 2, 1 estrogenic MoA).

After exposure BPS can be absorbed and accumulated in different organs of aquatic animals. Referring to the results of their earlier study performed in 2020 where it can be concluded that the highest accumulation of levels of BPS was found in the intestine, followed by brain and muscle, Wang *et al.* (2021) investigated the underlying mechanism by examining oxidative damage, inflammation and transcriptome profiles in zebrafish intestine as well as changes in microbial community structure. BPS significantly increased oxidative damage and inflammatory effects. They confirmed the disrupted microbial structure in fish intestine seen by Catron *et al*. (2019). BPS increased the relative abundance of potentially pathogenic bacteria (such as Flavobacterium, Pseudomonas, and Stenotrophomonas) which might indicate a potential new health hazard in zebrafish.

Social Behaviour

Table 87: Social behaviour in zebrafish

Method (guideline)	Short description of Method	Endpoint	Description of Result (*positive / negative / trend*)	References
-	Adult female zebrafish (9 months old): 120d exposure	Social behavior	Impact on object recognition, object placement, social recognition, Upregulation of E2-receptor expression (*esr2a* and *esr2b*)	Naderi *et al.*, 2020
-	Adult zebrafish (six months old)	Social behavior	Females: *Shoaling - Time ratio spent in the area close to other fish: sign. ↙ at 100* µg/L - Time ratio in far area: sign. ↗ at 100* µg/L *Anxiety: - Time spent at the bottom: sign. ↙ at 10* µg/L - Freezing frequency at the bottom: decreasing trend but not sign. Males: *Shoaling - Time ratio spent in the area close to other fish: sign. ↙ at 1**, 10* and 100* µg/L - Time ratio in far area: trend to increase but not sign. *Anxiety: - Time spent at the bottom: trend to increase but not sign. - Freezing frequency at the bottom: sign. ↗ at 10* µg/L Number of attacks of F and M: No sign. effect, but increased in males	Wang *et al.*, 2020b
-	Adult zebrafish (9 months old males and females): 75d exposure	Social behaviour	Impact on social behaviour (group preference, shoaling behaviour and social approaches) together with altered expression of gene expression of isotocin (it) and arginine vasotocin (avt)	Salahinejad *et al.*, 2020

			Co-exposure with E2: it: upregulated in males, downregulated in females avt: upregulated in males and females	
-	Maternal exposure (60d): 6-month-old male offspring	Social behaviour	Impact on social behaviour (inter-individual distance, excursions from shoal, group preference)	Salahinejad *et al.*, 2022
-	Embryonal exposure of zebrafish (2hpf until 120 hpf)	Social behaviour: Aromatase activity	Impact on social behaviour: inter individual distance and social preference (time spent near conspecifics) Co-exposure with ICI: ↗ anxiety and effects on social behaviour, while no reverse effect when co-exposure with FAD	Naderi *et al.*, 2022

Naderi *et al.* (2020)

In order to examine the effect on recognition memory, adult female zebrafish (9 months old) were exposed for 120d to environmental relevant concentrations of 1, 10 and 30 µg/L of BPS (nominal), as well as to 1 µg/L E2 (positive control) and 0.01% DMSO (solvent control). Results were compared to the solvent control. It is known that the impact of E2 on the sculpting of the fish brain and the behaviour (learning and memory) is more pronounced in female than in male fish.

An intact recognition memory is of great importance for successful navigation of physical and social environments and thus essential for appropriate predator approach/avoidance, reproductive advantages (e.g., mate choice) and foraging strategies. In this respect the study focused on the ERK/MAPK pathway, which connects the membrane receptors to numerous extracellular signals, cascading down the transcription factors which control learning and memory gene transcription.

Object Recognition (OR) memory was impacted at ≥10 µg/L, resulting in similar times spent with familiar and novel objects compared to the control and a difference in exploration ratio with less time spent with the novel object. No changes were observed after E2-treatment.

Object placement (OP memory), assessing the spatial memory of the fish, was improved at 1 µg/L and significantly impacted at 30 µg/L. OP memory was improved after exposure to E2. It is likely that improvement of OP is related to an elevation in Brain-derived neurotrophic factor (BDNF) levels.

At 30 µg/L fish social recognition (SR) was significantly affected as fish spent less time chance exploring unfamiliar species members. Furthermore, significantly less time was spent to investigate the novel stimulus fish at ≥10 µg/L. No changes were observed after E2-treatment.

Locomotory activity was not affected in this study.

The ratio of p-ERK1/2/total ERK1/2 and the phosphorylation level of cAMP Response Element-Binding Protein (CREB) were significantly decreased at 30 µg/L. CREB is a primary regulator of expression of genes associated with synapse re-modeling, synaptic plasticity and memory. The reduced phosphorylation of ERK at the highest dose might be a protective mechanism against harmful estrogenic stimulation. Phosphorylation levels were increased after exposure to E2 and 1 µg/L of BPS.

Activity of ERK1/2 in the brain, components of the ERK/MAP signaling cascade, is primarily associated with long term memory. *erk1* sign. reduced at the highest concentration of BPS. erk2 was sign. increased at 1 µg/L. No statistical change in the transcript abundance of creb1a was noted, while an up-regulation was seen after exposure to E2.

Significant up-regulation of expression of E2-receptor subtypes *esr2a* and *esr2b* was observed at 1 µg/L of BPS but was unchanged at higher concentrations. BPS may thus induce effects on the structure and function of the brain.

Glutamate receptors expression is essential for the increase in dendritic growth, spine formation and synaptic efficacy. *grm1b* and *grm5b* were sign. upregulated at 1µg/L while *grm5b* was sign. downregulated at ≥10 µg/L. Furthermore, grm*5a* was sign. downregulated at 30 µg/L. *Glur1a* and *glur1b* were sign. up regulated at 1 µg/l, glur3a was sign. downregulated at 30 µg/L. Grin1a was also sign. up-regulated at 1 µg/l while sign down-regulated at the highest conc.

Results of mRNA expression of synaptic activity-inducible immediate early genes (IEGs), an integral part of the synaptic plasticity and memory formation:

- *bndf*: sign. up-regulation at 1 µg/L of BPS and E2, while down-regulated ≥10 µg/L of BPS and with statistical significance at 30 µg/L of BPS
- *wnt3* and *c-fos:* sign. increase at 1 µg/L
- *npas4:* sign. up-regulation at 1 µg/L, although an apparent but non-sign. down-regulation was observed 30 µg/L

It can be concluded that exposure to BPS did improve OP memory at the lowest exposure concentration in the same magnitude as E2 by modulating glutamatergic/ERK/CREB signaling cascade. In contrast to E2, BPS did not impact OR and SR memory at 1µg/L implying that to a certain extent the underlying molecular mechanism is different. However, at higher concentrations of BPS OP, OR and SR memories were affected suggesting the effect of BPS on the recognition memory is biphasic in female fish.

Wang et al. (2020b)

In this study adult male and female zebrafish (Danio rerio) of six months old were exposed to a blank control, a solvent control, and three concentrations of BPS (1, 10 and 100 µg/L).

In shoaling behavioural experiments, female shoaling was weakened, as the time ratio spent in the area close to other fish was significantly decreased ($p<0.05$), and the time ratio in the far area was significantly increased in the female 100 µg/L BPS group ($p<0.05$) compared to the solvent control.

Female fish from BPS-treated groups also showed altered external features. Depigmentation (a significant decrease in melanin) of the body colour was observed for the 1 µg/L BPS group ($p<0.05$) and for the 10 and 100 µg/L BPS groups (both $p<0.01$) when comparing to the solvent control group.

Histological examination of ovaries showed that the total proportion of late vitellogenin and spawning oocytes in ovaries was 28.12 in the solvent control and reduced to 11.46 (1 µg/L BPS), to 17.89 (10 µg/L BPS) and to 13.68 (100 µg/L BPS).

It was also clear that during mating behaviour, untreated males spent significantly less time close to the BPS-treated females (1, 10 and 100 µg/L BPS; $p<0.05$), when comparing with the solvent control group. The authors linked the lower attraction potential of BPS-treated females to the fact that untreated females had a higher proportion of late vitellogenin and spawning oocytes, and to a changed body colour pattern of BPS-treated females.

It can be concluded that BPS exposure altered behaviour, could impair survival capacity (search for food, feeding and hiding for predators) and that females exposed to BPS may have less chance to attract a male for mating due to their low social status in the population

(due to abnormal body pigments, their weak shoaling ability and the presence of oocytes of lesser quality). Exposure to BPS could thus be associated with the impairment of mating behaviour, and by extension be linked to the inability to find a suitable mate in order to produce offspring.

Salahinejad *et al.* (2020)

Authors investigated the social behaviour (shoaling, social preference and locomotory activity) in zebrafish by exposing 9 months old male and female fish for 75 days to a solvent control (0.01% v/v), 1, 10 and 30 µg/L BPS and to 1 µg/L E2 (positive control). Results were compared to the solvent control.

They reported a statistically significant increase in inter-individual distances (decreased shoal cohesion) at 30 µg/L BPS and 1 µg/L of E2, while excursion behaviour increased non-significantly.

At all BPS concentrations and 1µg/L of E2, social preference for conspecifics (group preference) was statistically significantly and dose-dependently affected, as fish swam farther away from each other.

These changes in social behaviour were observed together with altered gene expression of isotocin (*it*) and arginine vasotocin (*avt*). Both are neuropeptides of the brain involved in regulation of non-reproductive social behaviours like group preferences, shoaling behaviour and social approaches, which are crucial for mating. BPS and E2 upregulated the transcription of the *it*-gene in a non-monotonic manner (sign. at 30 µg/L BPS) in males while it was downregulated in females (non-monotonic, sign. at 30 µg/L BPS). *itr*-expression was dose-dependently upregulated in males (sign. at 30 µg/L BPS and 1 µg/L E2) and non-significantly, not-dose dependently downregulated in females. Furthermore, BPS and E2 also altered the expression levels of *avt* in a dose-dependent manner (sign. at 30 µg/Lin males and females; sign. at 1 µg/L E2 in females only). While an up-regulation in the expression of *avt* gene was found in males and females, E2 and BPS decreased the transcript levels of AVT-receptors in both sexes. It is suggested that changes in IT and its receptors may be due to the estrogenic activity of BPS in males, as males have lower estrogen levels than females. In females, however, it might be due to a direct interference with the estrogen receptors or by a negative feedback mechanism. Furthermore, the authors concluded that the shift in balance from IT towards AVT is possibly the most important underlying mechanism for alteration of the social behaviour in zebrafish.

No effect on locomotory activity was observed.

Salahinejad *et al.* (2022)

In their experiment, Salahinejad *et al*. exposed 9 months old female zebrafish for 60 days to 1, 10 and 30 µg/L BPS and 1 µg/L E2 (positive control). 0.01% DMSO was used as solvent control. Results were compared to the solvent control.

After mating with non-exposed males, no BPS could be detected above the detection limit of ~0.25 µg/L in the collected eggs.

Maternal exposure to BPS affected the inter-individual distance, among shoal members of 6 months old male offspring, which was significantly decreased at 10 µg/L. Maternal treatment with 30 µg/L BPS also significantly increased the number of excursions that the offspring undertook from the shoal. Fish group preference was reduced compared to the control in a not dose-dependently manner and was only significant after maternal exposure to 1 µg/L BPS. Moreover, maternal exposure to 1 and 30 µg/L BPS and 1 µg/L E2 caused a significant decrease in the anxiety response as less time was spent at the bottom half of the tank by male offspring. Also here, no significant effect on locomotion was recorded.

Maternal exposure induced a non-dose-dependent increase in AVT-levels in the male offspring brain (only sign. at 1 µg/L BPS) while no significant effect was observed on IT and its receptors. *avp*-genes from the AVT receptor on the other hand were sign. upregulated at 1 and 10 µg/L BPS and non-sign. downregulated at 30 µg/L.

Naderi *et al.* (2022)

2hpf zebrafish embryos were exposed to BPS at 0.001, 0.01 and 0.1 µM (resp. ~0.25, 2.5 and 25 µg/L) until 5 dpf. 0.001% DMSO was used as solvent control. Results were compared to the solvent control. Authors studied thigmotaxis (anxiety assessed by tendency of animals to remain close to the walls of an arena), object recognition memory and social behaviour. The latter comprised social preference (time spent in a specific side) and social activity (number of valid contacts between 2 larvae and inter-fish distance). Furthermore whole-brain mRNA expression was analysed.

Exposure of zebrafish embryos to 0.001 µM (~0.25 µg/L) of BPS lead to thigmotaxis, an anxious behaviour where animals have the tendency to remain close to the walls of an arena.

Fish exposed to low and high concentrations (0.001 µM; 0.1 µM) showed signs associated with social deficits (e.g., decreased inter-fish distance), while fish exposed to a high concentration of 0.1 µM of BPS showed object recognition memory disorders (e.g. decrease in exploration ratio) at 21 days of age.

Co-exposure to the aromatase inhibitor fadrozole reversed the anxiogenic effects of BPS, except for object recognition memory, which was the only effect reversed by co-administration with the ER inhibitor ICI 182,780.

Moreover, high and low BPS concentrations had opposite effects on the expression of it-genes (sign. increased at 0.001µM, sign. decreased at 0.1 µM). Furthermore, 0.001µM BPS statistically sign. increased the expression of esr1 and esr2a genes, while expression of esr2b was statistically sign. reduced at mid and high dose. BPS induced a biphasic change in the mRNA expression levels of nkcc1, while the mRNA expression of kcc2 in the larval brain was up regulated after exposure to low BPS concentration. Furthermore, it was shown that E2 exposure up regulated the transcription of both genes in larval fish. At higher concentrations of BPS nkcc1 and kcc2b were down-regulated, probably due to the impact of BPS on sex steroid hormone levels or estrogen signaling. Those genes allow the excitatory/inhibitory switch of GABAergic neurons in the brain, a switch that is associated with neuronal developmental defects when affected. Moreover, Song *et al. (*2017) demonstrated that GABA (via $GABA_B$ receptors) regulates GnRH3 neurons in a developmentally dependent manner in zebrafish.

Conclusion

BPS influences hypothalamic development and may act through an Aromatase-mediated mechanism. However, estrogen mechanism cannot be excluded.

7.10.2. Endocrine disruption – Human health

OECD Level 3: *in vivo* assay providing data about selected endocrine mechanism(s)/pathway(s)

Table 88: Overview of endocrine disruption – human health (OECD Level 3, literature data)

Method	Short description of Method	Endpoint	Result (*positive / negative / trend*)	Description of Result (*positive / negative / trend*)	References
OECD TG 440	Uterotrophic assay (immature non-ovariectomised female rats) Subcutaneous route	Estrogen	+	Increase in absolute and relative uterine weight at 20 and 500 mg/kg, not at 100 mg/kg Weak estrogenic activity	Yamasaki *et al.*, 2004
OECD TG 440	Uterotrophic assay (immature non-ovariectomised female rats) Subcutaneous route	Estrogen	+	Estrogenic (logLED = 1.90 µmol/kg/day) and anti-estrogenic response (3.30 µmol/kg/day)	Akahori *et al.*, 2008
Similar to OECD TG 440	Uterotrophic assay (ovariectomized adult rats) Oral route	Estrogen	+	Increased uterine weight (wet and blotted)	Conley *et al.*, 2016

Yamasaki *et al.* (2004)

Besides the uterotrophic assay, the authors performed also a receptor binding assay with 14 chemicals (with BPS being one of these tested chemicals). For the description and the results of the *in vitro* study see "OECD CF level 2".

In the uterotrophic assay, immature 20day-old female rats were subcutaneously injected with individual test chemicals once daily for 3 consecutive days. Estrogenic activity of the test chemicals was examined based on the absolute and relative uterine weight after necropsy.

A vehicle control group was injected with olive oil alone, and a positive control group was injected with ethinyl estradiol after administration of olive oil. A group injected with the estrogen antagonist chemical tamoxifen at a dose of 1 mg/kg bw per day plus ethinyl estradiol was included to confirm the reliability of the study.

BPS was administered at doses of 20, 100, and 500 mg/kg bw. An apparent estrogenic effect was shown by increased absolute and relative uterine weight at 20 mg/kg bw ($p < 0.05$) and 500 mg/kg bw ($p < 0.01$), but not at 100 mg/kg bw. The outcome of this study is in agreement with the receptor binding assays results and indicates that BPS has a (weak but positive) estrogenic activity.

In similar treatments, ethinyl estradiol in olive oil was also subcutaneously injected into the back of the rats at a dose of 0.6 µg/kg bw per day for three consecutive days together with administration of the test substance.

In the treatments with ethinyl estradiol administered together with BPS, the low BPS treatment (20 mg/kg bw) showed significantly higher absolute and relative uterine weight compared to ethynyl estradiol only, whereas at the high BPS treatment (500 mg/kg bw) the absolute and relative uterine weights were significantly reduced. No significant effects were observed at the intermediate dose (100 mg/kg bw).

Akahori *et al.* (2008)

In a comparative study with 65 chemicals the relationship between the results of *in vitro* binding assay to human estrogen receptor α and *in vivo* immature rat uterotrophic assay was examined (see "OECD CF level 2" for the results of *in vitro* binding assay).

To evaluate estrogenic activity in the uterotrophic assay, immature non-ovariectomised 19-day old female rats were injected subcutaneous into their back with BPS for three consecutive days. Three doses were used (6 rats/group), with the highest dose sets as maximum tolerance dose based on results of a range-finding test.

Anti-estrogen activity was evaluated by using 17α-ethynylestradiol (0.6 µg/kg bw/day) co-administered with BPS. The vehicle control group treated with olive oil and the positive control group for estrogen activity (0.6 µg/kg bw/day 17α-ethynylestradiol) were concurrently run. The positive control group for anti-estrogenicity was treated with 1 mg/kg bw/day of tamoxifen co-administrated with 0.6 µg/kg bw/day 17α-ethynylestradiol.

Estrogenic activity was indicated when uterine weights were significantly increased, anti-estrogen activity when uterine weights were significantly decreased. The lowest dose showing a statistically significant effect (The lowest effective dose LED, µmol/kg/day) was used as a quantitative parameter.

Estrogenic and anti-estrogenic response was demonstrated for BPS. LogLED for estrogenic activity was 1.90 µmol/kg bw/day and 3.30 µmol/kg bw/day for anti-estrogenic activity.

Conley *et al.* (2016)

The aim of this study was to check the predictive capacity of *in vitro* data, comparing the estrogenic activity determined using *in vitro* ERα-mediated transcriptional activation reporter assay and *in vivo* rat uterotrophic assay. See above the "OECD level 2 assays" for the results of the *in vitro* reporter assay.

In vivo estrogenic activity was assessed using the uterotrophic assay. Ovariectomized adult rats were exposed orally to 0, 50, 100, 200, 400 and 800 mg/kg bw/d BPS (n = 6/dose). The wet uterus weight increased in a dose-dependent manner (31.6, 55.0, 63.0, 77.2, 139.5 and 156.7 mg, resp.) as well as the blotted uterus weight (25.4, 44.9, 54.8, 64.9, 107.2 and 121.4 mg, resp.). All results were significantly higher than the negative control ($p<0.05$). Dose-related histological changes, including increased epithelial and glandular cell height were also observed from 200 mg/kg bw/d).

This resulted in an agonist activity with an EC50 at 259.2 M (wet uterus), i.e., about 5 orders of magnitude lower than EE2 (relative potency = 8.04×10^{-4}).

Conclusion: OECD CF level 3 data

Estrogenic and anti-estrogenic activity, depending on the exposure dose.

OECD Level 4: *in vivo* assays providing data on adverse effects on endocrine relevant endpoints

Table 89: Overview of endocrine disruption – human health (OECD Level 4, literature data)

Method	Short Method description	Description of result (*positive*/*negative*/*trend*)	References
OECD TG 407	28-days repeated dose toxicity study including 2-weeks observation of reversibility	Mortality: 2 ♂ exposed to the highest dose (dilatation of cecum and signs of intestine haemorrhage at necropsy)	REACH registration dossier: Unpublished

	Rat / SD 6/sex/group (for main group) + 6/sex/group (for recovery group) Gavage GLP Doses: 0, 40, 200 and 1000 mg/kg bw/d for main groups 0, 200 and 1000 mg/kg bw/d for recovery groups Duration of exposure: 28d, daily Observation period: 2 w for recovery groups	Clinical signs: 1000 mg/kg bw/d : abdominal distension in 1 ♀ after 15 days and in 5 ♀ after 28 days (disappeared during the recovery) BW: lower value at 1000 mg/kg bw/d during the dosing period (sign in ♂). Recovered at the end of the recovery period BWG: sign. lower in both sexes for the exposure period and sign. reduce in ♂ for the recovery period <u>Animals necropsied at the end of dosing</u>: Gross pathology findings: 1000 mg/kg bw/d : dilatation of cecum in all animals Organ weight: few modification Histopathological findings observed in cecum (hyperplasia of mucosa, single cell necrosis of mucosal epithelium), liver (hypertrophy centrilobular), adrenals (hypertrophy of zona fasciculate), femur (increase spongy bone) and thymus (atrophy) <u>Animals necropsied at the end of recovery</u>: Gross pathology findings: dark red spots in the glandular stomach in 2 ♀ of the mid dose and in 2 ♀ of the high dose. Organ weight: few modifications Histopathological findings observed in cecum (hyperplasia of mucosa and single cell necrosis of mucosal epithelium), liver (microgranuloma) and femur (increase spongy bone)	study report, 1999
-	13-day repeated dose toxicity study Rat / strain not specified / male 5 males/group Diet No guideline followed No GLP Doses: 0, 0.1 and 1 % (± 0, 97 and 810 mg/kg bw/d)	Body weight: severely decreased at the highest dose (1%) Organ weight: lower kidneys and liver weights at highest dose Histopathology: adipose tissue atrophy and cytoplasmic basophilia of epithelium of the renal distal convoluted tubule at highest dose	REACH registration dossier Unpublished study report, 1973
OECD TG 408	90-day repeated dose toxicity study Rat / Wistar / males + females 10/sex/dose Gavage GLP Doses: 0, 100, 300 and 1000 mg/kg bw/d (in	Body weight sign. decreased in ♂ at the highest dose. The bwg (D 0–91) was significantly lower in ♂ in the mid and high dose level. Dilatation of cecum in all ♂ of the high dose and enlarged liver in 8 ♀ at the high dose level Organ weight: sign. changes observed in both sexes and sign. lower ♂ reproductive organ weight (testes and epididymides)	REACH registration, Unpublished study report, 2014

	males the highest dose was reduced to 600 mg/kg bw/d after 70 D)	Dilatation of cecum in all ♂ and ♀ at the highest dose + increase incidence of apoptosis Mammary glands: in ♂ : increased incidence of multifocal atrophy at the mid and high dose Uterus: increased incidence of squamous metaplasia	
OECD TG 414	Prenatal developmental toxicity study Rat / wistar 25 pregnant females/group Gavage GLP Doses: 0, 30, 100 and 300 mg/kg bw/d Duration of exposure: 10w for males and continued through premating, gestation, and lactation periods for females	<u>Dams</u>: Bw: no sign change (however bwg GD 6 – 19 and GD 8 - 10 were sign lower) Uterus weight: no effects Necropsy observation: no treatment-related effects Reproduction data (nb of dams with viable foetuses, corpora lutea, implantation sites, pre and post implantation loss, resorption): no effects <u>Offspring</u>: Sex ratio: unaffected Mean nb of live foetuses: no effects Bw: unaffected A few skeletal variations observed	REACH registration, Unpublished study report, 2014
OECD TG 421	Reproductive toxicity test Rats / Sprague-Dawley (SD) 12/sex/group Gavage GLP Doses: 0, 10, 60 and 300 mg/kg bw/d Exposure: 45 D for males (including 14 D of premating period) and 40 to 46 D for females (from premating, mating, gestation until lactation day 3)	<u>Parental generation:</u> Body weight: reduced at the highest dose in both sexes In ♂: sign. increase of relative pituitary and rela liver weights and sign. decrease of seminal vesicle weight In ♀: no sign. changes Distension of cecum (hyperplasia) observed in 1 male (♂) and 1 female (♀) in the mid dose level and in all ♂ and 4 ♀ at the highest dose level. In Liver, centrilobular hypertrophy of hepatocytes observed in 5 ♂ at 300 mg/kg bw/d Reproductive data: No effect on copulation index, parturition index, delivery index, nb of corpora lutea, gestation period The mean duration of oestrus cycle was sign. higher at the highest dose (5.57**d vs 4.08d in control group) and 5 ♀ exposed to 300 mg/kg bw/d exhibited a longer dioestrus period (vs 0 ♀ in control). Decreased mean nb of implantation sites at 300 mg/kg bw/d (10.7 vs 15.9 in control group) and sign. lower implantation index at 300 mg/kg bw/d (64.89** % vs 95.80 % in control group) Severe decrease of fertility index: 58.3 % at the highest dose vs 91.7 % in control group	REACH registration, Unpublished study report, 2000

		Offspring: Decreased mean nb of offspring at birth at 300 mg/kg bw/d (9.1 at 300 mg/kg bw/d vs 14.3 in control group) No abnormalities in external appearance and clinical signs nor bw, body weight gain (bwg), viability index, ano-genital distance (AGD)	
OECD TG 422	Range finding study preceding the EOGRTS, Similar to a combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test Rats / SD 10/sex/dose Gavage GLP Doses: 0, 30, 100 and 300 mg/kg bw/d Duration of exposure: 10 w for males and continued through premating, gestation, and lactation periods for females	F0 generation: Body weight lowered at the highest dose (-7 % in ♂ and -6 % in ♀ compared to the control group) Organ weight: sign. higher rel. kidneys weight in ♂ (+11.5 and +35 % resp. at 100 and 300 mg/kg bw/d) and sign higher rel. liver weight in ♂ at the highest dose (+11 %). In ♀, uterus weight modified at 300 mg/kg bw/d Increased incidence of cecum dilatation enlarged and enlarged liver in ♂ exposed to 300 mg/kg bw/d (confirmed by histopathology). Mammary gland affected in ♂ Reproductive data: Oestrus cycle: prolonged at the highest dose (5.16** vs 4.02 d in control group) Sign. lower mean nb. of implantation sites at 300 mg/kg bw/d (10.4** vs 15.8 in control group) % of post-implantation loss: sign. higher at 300 mg/kg bw/d (34.6* vs 3.6 % in control group) F1: Mean nb of pups delivered sign decreased at the highest dose (10.8** vs 15.2) Pups body weight sign. higher in ♂ of the low dose at PND 21 (+6.6 % compared to the control group)	REACH registration, Unpublished study report, 2017
-	Range-finding study preceding the EOGRTS, similar to a 28-day repeated dose toxicity study Rat / SD 5/sex/dose Gavage No guideline followed Not GLP Doses: 0, 100, 300 and 600 mg/kg bw/d	Body weight gain sign. lower at the highest dose in ♂ Final body weight lower at the mid and high dose levels (-9 and -12 % resp. at 300 and 600 mg/kg bw/d) A few changes observed in kidneys, adrenals, liver, prostate and sem. ves. weight, Enlarged kidneys observed in 4 ♂ exposed to 600 mg/kg bw/d and in 3 ♂ exposed to 300 mg/bw/d. Moreover, cecum dilatation was noted in 2 ♂ of the highest dose. Mammary glands affected.	REACH registration, Unpublished study report, 2017
-	28-days rat exposure	Increased oxidative stress biomarkers Decreased testosterone concentration	Ullah *et al.*, 2016

		Decreased number of spermatids and thinning of epithelial cells of the seminiferous tubules	
-	CD-1 mice exposed during lactation period (GD 9 to LD 20)	Alteration of lactating mammary gland structure Decrease 17β-estradiol maternal serum level[9] Change nursing behaviour of mothers and pups	LaPlante *et al.*, 2017
-	Neonatal male and female CD-1 mice exposure from birth to PND 60 Assessment of reproductive function of males and females	Sperm count and mobility reduced Affected spermatogenesis progression Increased serum levels of estradiol[9] and testosterone Subfertility	Shi *et al.*, 2017
-	Gestational exposure of sheep from GD 30 to 100	Placental endocrine dysfunction Reduced progesterone release Impaired trophoblast cell fusion	Gingrich *et al.*, 2018
-	Neonatal female rats' exposure (PND 1 to 10)	Delayed puberty onset Altered oestrous cyclicity Reduced uteri weight Changed hormones concentration in blood (increased testosterone and estradiol[9], reduced progesterone, LH and FSH) Increased number of follicles in the ovaries	Ahsan *et al.*, 2018
-	4 weeks exposure to very low doses BPS	Effect on ovaries (folliculogenesis) and oocytes quality Effect on fertilization rate	Nevoral *et al.*, 2018
-	28-days rat exposure	Decreased testosterone concentration Thinner epithelial cells of the seminiferous tubules	Ullah *et al.*, 2018a
-	Prenatal exposure CD1 mice (GD 10 to 17).	Accelerated mammary gland development Changed mammary gland morphology	Tucker *et al.*, 2018
-	Prenatal exposure CD1 mice (GD 9 to GD 20)	Changed morphology of mammary glands Changed expression of ERα and PR in mammary tissue	Kolla *et al.*, 2018
-	Chronic exposure (from PND 23 for 48 weeks)	Reduced testis, seminal vesicles, epididymis and prostate weights Reduced testosterone, LH and FSH concentrations in plasma, increased estradiolconcentration[9] Reduced mobile sperm Reduced spermatogonia, spermatocytes and spermatids	Ullah *et al.*, 2018b

		Reduced epithelial height	
-	Prenatal exposure of CD1-mice (GD 11 to birth) – Males	Reduced sperm count at PND 60 Low sperm motility Disrupted germ cell development Increase E2 level[9], trend decrease T level Increased cell death in developing testis. Affected mRNA expression	Shi *et al.*, 2018
-	Prenatal exposure of CD1-mice (GD 11 to birth) – Females	Affected onset of puberty and oestrous cyclicity. Decreased fertility Increased T level Affected folliculogenesis	Shi *et al.*, 2019a
-	Prenatal exposure of male CD-1 mice (from GD 9 to PND 2 or 20)	Changed morphology of the mammary gland in adult males	Kolla *et al.*, 2019
-	Prenatal exposure of CD1-mice (GD 7 to birth) F3 females	Affected onset of puberty and oestrous cyclicity Slight effects on fertility and 17β-estradiol level[9]	Shi *et al.*, 2019b
-	Prenatal exposure of CD1-mice (GD 7 to birth) F3 males	Decreased sperm count and disrupted spermatogenesis Decreased testosterone level Disruption of steroidogenesis enzymes	Shi *et al.*, 2019c
-	Prenatal exposure of Sprague-Dawley rats (GD 1 to 21) – Effects on male progeny	Reduced seminal vesicle weight Increases level of stress markers in testis tissues and affected histology of testis Decreased sperm count and disrupted spermatogenesis, decreased sperm motility Decreased levels of testosterone, LH and FSH, increased estradiol level[9]	Ullah *et al.*, 2019a
-	28-days male rat exposure	Increased oxidative stress biomarkers and DNA damaged in sperm Reduced daily sperm production. Non-significant decrease of sperm motility	Ullah *et al.*, 2019b
-	Prenatal/Lactation exposure of Wistar rats (GD1 to weaning)	In dams, decreased testosterone, trend lower estradiol and thyroid hormones. In pups, decreased progesterone in both males and females, decreased testosterone in males, increased estradiol in females[9].	Da Silva *et al.*, 2019

		Affected structure of brown adipose tissue in females. Increased anxiety-behaviour in males	
-	Perinatal exposure of CD-1 mice (GD9 to PND2) – Sensitization of female pups to estrogen challenge	Slight effect on mammary gland morphology. No sensitization observed after estrogen challenge	Kolla and Vandenberg, 2019
-	Adult female C57BL/6 mice exposure for 10 weeks	No effect on body weight nor glucose homeostasis Increased expression of PPARγ target genes	Gao *et al.*, 2020
	Prenatal exposure of ICR mice (GD 12 to 15) Effects on oocyte maturation in F1 and F2 embryos	In F1, disturbed folliculogenesis. No effect on the number of oocytes. Epigenetic modification. In F2, litter size, BW and sex ratio not affected, but slight effect on oocyte maturation.	Zhang *et al.*, 2020b
-	28-days female rat exposure	Reduced ovary and uterus weight Increased oxidative stress biomarkers Increased level of testosterone decreased levels of estradiol[9], LH, FSH and progesterone. Affected folliculogenesis (decreased number of corpora luteum and antral follicles, increased number of atretic follicles)	Ijaz *et al.*, 2020
	Perinatal exposure of CD-1 mice (PND 1 to 3) Effects on oocyte maturation	No effect on total number of oocytes, but increase of primordial follicles, resp. decrease of cysts.	Liu *et al.*, 2021
	Chronic exposure (from PND 23 for 48 weeks) at low dose	Reduced testis, seminal vesicles, epididymis and prostate weights. Reduced GSI. Reduced testosterone, LH and FSH concentrations in plasma, increased estradiol[9] concentration Reduced mobile sperm and daily sperm production Reduced spermatogonia, spermatocytes and spermatids Reduced epithelial height	Ullah *et al.*, 2021

[9] When tested, the detected levels of estradiol were below or at the limit of sensitivity of the use assay for controls and treated groups. Therefore the eMSCA considers with caution the reliability of these values.

28-day repeated dose toxicity study including 2-weeks of recovery period (REACH registration dossier: Unpublished study report, 1999)

Test type

Similar to OECD TG 407

GLP

Test substance

- 4,4'-sulphonyldiphenol
- *Degree of purity :* confidential

Test animals

- *Species/strain/sex :* rat / SD / male + female
- *No. of animals per sex per dose :* 6/sex/group for main groups + 6/sex/group for recovery groups
- *Age and weight at the study initiation :* 6 w old; 206-224 g for males and 156-180 g for females

Administration / exposure

- *route of administration :* oral, gavage
- *duration of test/exposure period :* 28 days
- *frequency of exposure :* daily
- *doses/concentration levels :* main groups : 0, 40, 200 and 1000 mg/kg bw/d
  recovery groups : 0, 200 and 1000 mg/kg bw/d
- *post exposure observation period :* 2 weeks for the recovery groups
- *vehicle :* 0.5 % aqueous solution of methylcellulose

Results and discussion

- *mortality :* 2 males, exposed to the highest dose, died (1 of the main group (at D13) and 1 of the recovery group (at D21)). The necropsy of these animals revealed a dilatation of the cecum and signs of haemorrhage in the intestinal tract.
- *description of clinical signs :* an abdominal distension was observed at the highest dose in 1 female after 15 D and in 5 females after 28 D. During the recovery period, this effect disappeared.
- *body weight and body weight changes :* significant lower bw value at the highest dose were noted in males during the dosing period. After 14 D of the recovery period, this change was not significant.

Table 90: Body weight data during dosing and recovery period (in grams)

	Males				Females			
Dose level (mg/kg bw/d)	0	40	200	1000	0	40	200	1000
Exposure period								
D 1	217	214	215	215	165	165	166	168
D 14	330	324	325	281**	216	216	210	206
D 28	409	402	401	337**	258	256	244	240
BWG (1 – 28)	192	187	186	122**	93	91	78*	72**

Recovery period								
D 1	398	/	411	330**	258	/	250	242
D 10	435	/	445	384*	279	/	271	259
D 14	457	/	465	416	286	/	278	269
BWG (1 – 14)	59	/	54	86**	28	/	28	27

* : p<0.05 ; ** : p<0.01

- *food consumption :* significant decrease at 1000 mg/kg bw/d in both sexes (more marked in males)
- *haematological findings :* significant decrease of RBC, haemoglobin and haematocrit were observed in both sexes at the highest dose level. Moreover, a significant reduced prothrombin time was observed in females.

  In all dose levels, a significant lower value of mean corpuscular haemoglobin concentration was observed in females.

Table 91: Haematological findings during dosing and recovery period

		Males				Females			
Dose level (mg/kg bw/d)		0	40	200	1000	0	40	200	1000
Dosing period	RBC (10^4/mm³)	770	764	763	687*	773	766	776	705**
	Hb (g/dL)	15.9	15.9	15.8	14.3**	16.2	15.9	15.9	13.9**
	Ht (%)	47	47	46	42**	47	47	47	42**
	MCHC (%)	34.1	33.9	34.1	33.7	34.5	34.0*	33.9*	33.6*
	PT (sec)	12.9	13.4	13.6	12.9	12.0	12.2	12.0	11.4*
Recovery period	RBC (10^4/mm³)	804	/	793	735**	809	/	801	762
	Hb (g/dL)	16.0	/	15.9	15.3	16.2	/	15.8	15.2**
	Ht (%)	47	/	47	45	47	/	47	45
	MCHC (%)	33.8	/	33.9	33.8	34.1	/	33.3*	33.4
	PT (sec)	13.5	/	13.2	11.8**	11.9	/	11.6	11.7

* : p<0.05 ; ** : p<0.01

- *clinical biochemistry findings :* in males, a significant higher ALP activity and a significant lower LDH activity were noted at the highest dose level. Whereas, in females, a significant increase of tot prot, albumin and calcium and a significant decrease of total cholesterol were observed.

Table 92: Blood chemical findings during dosing and recovery period

		Males				Females			
Dose level (mg/kg bw/d)		0	40	200	1000	0	40	200	1000
Dosing period	GOT (IU/L)	44	47	55	56	64	59	57	52
	LDH (IU/L)	43	40	39	25**	27	25	27	28
	ALP (IU/L)	307	298	289	424*	205	209	197	222
	Tot. prot. (g/dL)	6.3	6.1	6.1	6.4	6.2	6.2	6.3	7.2**
	Albumin (g/dL)	3.7	3.7	3.6	3.8	3.7	3.8	3.8	4.2**
	Tot. chol. (mg/dL)	65	61	64	25**	85	64	71	42**
	Ca (mg/dL)	9.4	9.3	9.4	9.7	9.5	9.4	9.5	10.1**
Recovery period	GOT (IU/L)	43	/	43	24*	60	/	57	55
	LDH (IU/L)	53	/	40	55	23	/	26	23

	ALP (IU/L)	249	/	260	271	152	/	144	142
	Tot. prot. (g/dL)	6.4	/	6.4	6.1	6.8	/	6.5	6.7
	Albumin (g/dL)	3.7	/	3.8	3.7	4.0	/	3.9	4.0
	Tot. chol. (mg/dL)	72	/	73	69	86	/	75	100
	Ca (mg/dL)	9.1	/	9.1	9.1	9.4	/	9.2	9.3

* : p<0.05 ; ** : p<0.01

- *gross pathology findings :*
  - cases necropsied at the end of the dosing period : a dilatation of cecum was observed in all animals of the highest dose level (5 out of 5 males and 6 out of 6 females at 1000 mg/kg bw/d vs 0 out of 6 males and 0 out of 6 females in control group and in the low and mid dose groups).
  - cases necropsied at the end of the recovery period : dark red spots in the glandular stomach were observed in 2 females of the mid dose level and 2 females of the high dose level. No abnormalities were seen in males.
- *Organ weight :* few changes were observed (see table 95 and 96)
  - cases necropsied at the end of the dosing period :

Table 93: Absolute and relative organ weights data

		males				females			
Dose level (mg/kg bw/d)		0	40	200	1000	0	40	200	1000
Nb of animals examined		6	6	6	6	6	6	6	6
FBW (g)		389	369	364	311**	234	235	223	218
Adrenals	Abs.(mg)	70	71	66	101**	72	74	65	74
	Rela	18	19	18	33**	31	32	29	34
Brain	Abs. (g)	2.07	2.06	2.07	1.99	1.90	1.89	1.86	1.82
	Rela	0.53	0.56	0.57	0.64**	0.81	0.80	0.84	0.84
Heart	Abs. (g)	1.30	1.18	1.21	0.99**	0.84	0.87	0.79	0.81
	Rela	0.34	0.32	0.33	0.32	0.36	0.37	0.36	0.37
Kidneys	Abs. (g)	2.79	2.68	3.09	2.76	1.84	1.76	1.73	1.83
	Rela	0.72	0.73	0.85**	0.89**	0.79	0.75	0.77	0.84
Liver	Abs. (g)	11.98	11.35	11.14	10.94	7.23	6.83	6.99	8.46
	Rela	3.07	3.07	3.06	3.54**	3.09	2.90	3.14	3.89**
Lung	Abs. (g)	1.33	1.28	1.33	1.13*	1.09	1.06	1.04	0.92**
	Rela	0.34	0.35	0.37	0.36	0.47	0.45	0.47	0.42**
Thymus	Abs. (mg)	438	428	493	252**	475	521	441	259**
	Rela	113	117	135	82	203	221	199	119**
Testes	Abs. (g)	3.07	2.94	3.06	2.96	-	-	-	-
	Rela	0.79	0.81	0.84	0.96**	-	-	-	-
Ovaries	Abs. (mg)	-	-	-	-	86.1	92.0	85.1	76.5
	Rela	-	-	-	-	36.7	38.9	38.1	34.9

* : p<0.05 ; ** : p<0.01

  - cases necropsied at the end of the recovery period :

Table 94: Absolute and relative organ weight data

	Males			Females		
Dose level (mg/kg bw/d)	0	200	1000	0	200	1000
Nb of animal examined	6	6	6	6	6	6

FBW (g)		425	432	376*	265	257	246
Adrenals	Abs.(mg)	59	60	80**	71	69	75
	Rela	14	14	21**	27	27	31
Brain	Abs. (g)	2.05	2.05	1.99	1.89	1.95	1.88
	Rela	0.48	0.48	0.53*	0.71	0.76	0.77
Heart	Abs. (g)	1.33	1.38	1.29	0.88	0.90	0.90
	Rela	0.31	0.32	0.34*	0.33	0.35	0.37*
Kidneys	Abs. (g)	2.76	3.21**	2.71	1.84	1.86	1.98
	Rela	0.65	0.75**	0.72*	0.70	0.73	0.81
Liver	Abs. (g)	12.86	13.32	11.03	7.40	7.25	7.95
	Rela	3.02	3.07	2.93	2.79	2.82	3.21*
Lung	Abs. (g)	1.30	1.37	1.24	1.05	1.06	1.00
	Rela	0.31	0.32	0.33*	0.40	0.41	0.41
Thymus	Abs. (mg)	434	415	336	407	342	335
	Rela	103	96	91	154	134	134
Testes	Abs. (g)	3.29	3.20	3.15	-	-	-
	Rela	0.78	0.74	0.84	-	-	-
Ovaries	Abs. (mg)	-	-	-	77.0	75.4	70.4
	Rela	-	-	-	29.1	29.4	28.7

* : p<0.05 ; ** : p<0.01

- *histopathology findings :* few significant changes were observed in animals at the end of dosing period (see table 97). These changes were not present at the end of recovery period (see table 98).
    - cases necropsied at the end of the dosing period :

Table 95: Histopathological findings at the end of the dosing period

			Males				Females			
Dose level (mg/kg bw/d)			0	40	200	1000	0	40	200	1000
cecum	Hyperplasia mucosa	Inc.	0/6	0/6	6*/6	5*/5	0/6	0/6	5**/6	6**/6
		grade 1	/	/	2	2	/	/	4	5
		grade 2	/	/	4	3	/	/	1	1
	Single cell necrosis, mucosal epithelium	Inc.	0/6	0/6	4*/6	5*/5	0/6	0/6	4*/6	4*/6
		grade 1	/	/	3	2	/	/	4	1
		grade 2	/	/	1	3	/	/	/	2
		grade 3	/	/	/	/	/	/	/	1
liver	Hypertrophy centrilobular		0/6	0/6	0/6	3*/5 (3 slight)	0/6	0/6	0/6	3/6 (3 slight)
adrenals	Hypertrophy, zona fasciculata		0/6	0/6	0/6	4*/5 (4 slight)	0/6	0/6	0/6	0/6
thymus	atrophy		0/6	0/6	0/6	4**/5 (4 slight)	0/6	0/6	0/6	4*/6 (4 slight)
femur	Increase spongy bone		0/6	0/6	0/6	5**/5 (5 slight)	0/6	0/6	0/6	4*/6 (4 slight)

P : present ; grade 1 : slight ; grade 2 : mild ; grade 3 : moderate ; * : p<0.05 ; ** : p<0.01

- *cases necropsied at the end of the recovery period :*

Table 96: Histopathological findings at the end of the recovery period

			Males			Females		
Dose level (mg/kg bw/d)			0	200	1000	0	200	1000
cecum	Hyperplasia, mucosa	Inc.	0/6	1/6	3/5	0/6	1/6	1/6
		grade 1	/	/	1	/	/	1
		grade 2	/	1	2	/	1	/
	Single cell necrosis, mucosal epithelium	Inc.	0/6	1/6	3/5	0/6	0/6	0/6
		grade 1	/	1	2	/	/	/
		grade 2	/	/	1	/	/	/
liver	microgranuloma		0/6	0/6	2/5 (2 slight)	2/6	/	2/6
femur	Increase spongy bone		0/6	0/6	1/5 (1 slight)	0/6	0/6	4*/6 (4 slight)

P : present ; grade 1 : slight ; grade 2 : mild ; * : p<0.05

13-day repeated dose toxicity study (REACH registration dosser : Unpublished study report, 1973)

Test type

No guideline followed

No GLP

Only short abstract available

Test substance

- 4,4'-sulphonyldiphenol
- *Degree of purity :* confidential

Test animals

- *Species/strain/sex :* rat / not specified / male
- *No. of animals per sex per dose :* 5 males/dose

Administration/exposure

- *route of administration :* diet
- *duration of test/exposure period :* 13 days
- *frequency of test/exposure period :* daily
- *doses/concentration levels :* 0, 0.1 and 1 % (approx. 0, 97 and 810 mg/kg bw/d)
- *vehicle:* 1 % corn oil

Results and discussion

- *mortality :* no effects
- *clinical signs :* no effects
- *body weight and body weight changes :* greatly depressed at the highest dose (no more information available)
- *haematological findings:* slight increase in RBC count, haemoglobin concentration and haematocrit were observed at the highest dose. The slight increase in haemoglobin concentration was already observed at the low dose level. (no more information available)

- *clinical biochemistry findings:* a lower aspartate aminotransferase value was noted at 1 % (no more information available)
- *gross pathology findings:* an adipose tissue atrophy was noted in 1 male exposed to 0.1 % and in all 5 males exposed to 1 %.
- *organ weight :* lower absolute liver and kidney weights were observed at the highest dose (no more information available).
- *histopathology findings:* adipose tissue atrophy and cytoplasmatic basophilia of epithelium of the renal distal convoluted tubule were noted at 1 % (no more information available).

90-day repeated dose toxicity study (REACH registration dossier : Unpublished study report, 2014)

Test type

According to OECD TG 408

GLP

Test substance

- 4,4'-sulphonyldiphenol
- *Degree of purity :* confidential

Test animals

- *Species/strain/sex :* rat / Wistar / males + females
- *No. of animals per sex per dose :* 10/sex/dose
- *Age and weight at the study initiation :* approx. 42 d

Administration/exposure

- *route of administration :* gavage
- *duration of test/exposure period :* 90 days
- *frequency of exposure :* daily
- *doses/concentration levels :* 0, 100, 300 and 1000 mg/kg bw/d (for males, the highest dose changed to 600 mg/kg bw/d onwards 70 days)
- *vehicle:* 1 % CMC

Results and discussion

- *mortality and time to death :* no animal died
- *description, severity, time of onset and duration of clinical signs :* soft and discoloured faeces and salivation were noted in all animals of the mid and high dose.
- *body weight and body weight changes :* a lower bw was observed in male at the mid and highest dose level. This decreased was significant at the highest dose.

Table 97: Body weight and body weight gain data (in g)

	Males				Females			
Dose level (mg/kg bw/d)	0	100	300	1000/600	0	100	300	1000
D 0	158.4	157.1	158.1	158.2	126.1	127.0	126.0	126.7
D 7	203.3	199.9	198.0	189.9**	143.7	147.0	144.0	142.7

D 42	351.4	343.8	326.0	294.3**	208.4	204.7	202.8	205.2
D 91	417.1	400.7	377.3	334.7**	237.3	231.7	225.0	222.5
BWG (D 0-91)	258.7	243.6	219.2*	176.4**	111.2	104.6	99.0	95.9

* : p < 0.05 ; ** : p < 0.01

- *food consumption :* lower food consumption was observed in males at 1000 mg/kg bw/d (-18 % from days 7 to 63). After the reduction of the dose, the food consumption was within the usual range.
- *sensory activity, grip strength and motor activity assessments :* no test substance related effects were observed
- *ophthalmologic findings :* no effects
- *haematological findings :* RBC counts and haemoglobin values were decreased at the highest dose in both sexes. Additionally, lower haematocrit value and mean corpuscular haemoglobin concentration were observed in females at 1000 mg/kg bw/d. In males, higher mean corpuscular volume value, relative reticulocyte counts and neutrophiles and lower WBC counts were noted at the highest dose.

Table 98: Haematological findings (examined at the end of the administration period)

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000/600	0	100	300	1000
RBC (tera/L)	8.71	8.83	8.46	8.08**	7.89	7.82	7.76	7.45**
Hb (mmol/L)	9.0	9.0	8.8	8.6**	8.8	8.6	8.5	8.0**
Ht (L/L)	0.427	0.426	0.420	0.412	0.408	0.406	0.402	0.380**
MCV (fL)	49.1	48.2	49.6	51.0**	51.8	52.0	51.8	51.0
MCHC (mmol/L)	21.05	21.17	20.97	20.95	21.62	21.28	21.24*	21.07**
RET (%)	1.5	1.2	1.6	1.9*	1.8	2.0	2.2	2.3
WBC (giga/L)	5.51	5.11	4.59*	4.28**	4.09	4.35	3.85	3.56

* : p < 0.05 ; ** : p < 0.01

- *clinical biochemistry findings:* some modification were noted (see table 101)

Table 99: Enzyme data (examined at the end of the administration period)

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000/600	0	100	300	1000
ALT (µkat/l)	0.68	0.80	0.91**	0.92	0.58	0.63	0.58	0.79
AST (µkat/l)	1.63	1.42	1.77	1.81	1.38	1.54	1.36	1.19
ALP (µkat/l)	1.25	1.43	1.40	1.41	0.66	0.55	0.69	1.01*
GGT_C (nkat/l)	0	0	0	0	0	0	0	0
Chol (mmol/L)	1.85	1.65	1.23**	1.03**	1.62	1.56	1.30	1.33
Trig (mmol/L)	0.97	1.53**	1.48**	2.32**	0.72	0.81	0.79	0.99

* : p < 0.05 ; ** : p < 0.01

- *gross pathology findings :* Dilatation of cecum was noted in all males at the highest dose level. While the liver was enlarged bw/d in 8 females out of 10 exposed to 1000 mg/kg. An uterus dilatation was observed in 3 females at 300 mg/kg bw/d.

- *organ weight :* reproductive organs weights was significantly decreased in males at 1000 mg/kg bw/d (epididymides and testes (also at the mid dose level). Significant lower brain and thymus weights were observed in both sexes at the highest dose whereas a higher adrenal glands weight was noted.

Table 100: Organ weight (relative weight in %)

		Males				Females			
Dose level (mg/kg bw/d)		0	100	300	1000/600	0	100	300	1000
FBW (g)		394.02	376.95	356.6**	311.89**	221.72	214.72	207.95	205.6
Adrenal glands (g)	Abs	64.5	59.1	63.7	90.1**	65.6	64.5	74.6	80.4**
	Rela	0.016	0.016	0.018	0.029**	0.03	0.03	0.036*	0.039**
Brain (g)	Abs	2.212	2.098**	2.074**	2.084**	2.007	1.992	1.99	1.913*
	Rela	0.565	0.564	0.583	0.675**	0.91	0.931	0.962	0.932
Heart (g)	Abs	1.115	1.039	1.026*	0.958**	0.752	0.739	0.755	0.763
	Rela	0.284	0.277	0.288	0.309*	0.341	0.344	0.364	0.371*
Kidneys (g)	Abs	2.507	2.646	2.762	2.485	1.5	1.489	1.584	1.644*
	Rela	0.636	0.702*	0.775**	0.795**	0.679	0.695	0.765*	0.799**
Liver (g)	Abs	8.936	8.402	8.415	8.347	5.106	5.39	5.688	7.043**
	Rela	2.269	2.226	2.359	2.676**	2.297	2.502	2.75**	3.433**
Spleen (g)	Abs	0.628	0.585	0.535**	0.595	0.44	0.447	0.465	0.454
	Rela	0.16	0.156	0.15	0.19**	0.198	0.209	0.224**	0.22*
Thymus (mg)	Abs	327.5	269.4	271.3	226.1**	303.2	292.4	245.3	222.7**
	Rela	0.084	0.071	0.076	0.073	0.136	0.136	0.118	0.108*
Epididymides (g)	Abs	1.209	1.16	1.126	1.072**	-	-	-	-
	Rela	0.308	0.31	0.316	0.346**	-	-	-	-
Testes (g)	Abs	3.914	3.862	3.636*	3.592*	-	-	-	-
	Rela	0.999	1.035	1.021	1.162**	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	104.7	104.0	106.9	126.9
	Rela	-	-	-	-	0.047	0.048	0.052	0.061*
Uterus (g)	Abs	-	-	-	-	0.724	0.864	1.284	0.648
	Rela	-	-	-	-	0.332	0.41	0.615	0.315

* : p < 0.05 ; ** : p < 0.01

- *histopathology findings:* effects were revealed in few organs.

Table 101: Histopathological findings

			Males				Females			
Dose level (mg/kg bw/d)		Grade	0	100	300	1000/600	0	100	300	1000
Adrenal cortex	Hypertrophy/ hyperplasia	Inc	0/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10
Cecum	Dilatation	Inc	0/10	0/10	0/10	10/10	0/10	0/10	1/10	10/10
	Parasite(s) in lumen	Inc	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	Increased apoptosis	Inc	0/10	3/10	4/10	7/10	0/10	1/10	4/10	7/10
Kidneys	Mineralisation, medulla	Inc	0/10	7/10	9/10	6/10	5/10	NE	NE	3/10
		1	/	4	6	6	3			2
		2	/	3	2	/	2			1
		3	/	/	1	/	/			/

	Tubules, basophilic	Inc	8/10	8/10	9/10	8/10	2/10	NE	NE	3/10
Liver	Centrilobular hypertrophy	Inc	0/10	0/10	0/10	0/10	0/10	2/10	5/10	10/10
		1	/	/	/	/	/	1	1	/
		2	/	/	/	/	/	1	3	/
		3	/	/	/	/	/	/	1	10
	Hyperplasia, bile duct	Inc	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
	Cellular alteration	Inc	0/10	1/10	0/10	2/10	1/10	1/10	1/10	6/10
Mammary gland	Atrophy multifocal	Inc	0/10	0/10	7/10	10/10	0/10	NE	NE	0/10
		1	/	/	7	/	/			/
		2	/	/	/	4	/			/
		3	/	/	/	2	/			/
		4	/	/	/	3	/			/
		5	/	/	/	1	/			/
Spleen	Haematopoiesis extramedullary	Inc	0/10	0/10	0/10	8/10	2/10	1/10	4/10	10/10
		1	/	/	/	5	2	1	4	3
		2	/	/	/	3	/	/	/	7
Uterus	Squamous metaplasia	Inc	-	-	-	-	0/10	2/10	2/10	5/10
		1	-	-	-	-	/	2	2	4
		2	-	-	-	-	/	/	/	1
	Dilatation of horn(s)	Inc	-	-	-	-	0/10	0/10	3/10	0/10

P : present ; grade 1 : minimal ; grade 2 : slight ; grade 3 : moderate ; grade 4 : marked (severe) ; grade 5 : massive (extreme)

Prenatal developmental toxicity study (REACH registration dossier : Unpublished study report, 2014)

Test type

According to OECD TG 414

GLP

Test substance

- 4,4'-sulphonyldiphenol
- *Degree of purity :* confidential

Test animals

- *Species/strain/sex :* rat / Wistar / pregnant female
- *No. of animals per sex per dose :* 25 pregnant females/dose

Administration/exposure

- *Route of administration :* gavage
- *duration of test/exposure period :* GD 6 – 19 (sacrifice of the animals at GD 20)
- *doses/concentration levels :* 0, 30, 100 and 300 mg/kg bw/d
- *vehicle:* 1 % carboxymethylcellulose

Results and discussion

For dams :

- *mortality :* no mortality observed
- *clinical observations :* at the highest dose level, 7 out of 25 females exhibited excessive salivation after exposure.

- *body weight data :* bwg calculated for the entire treatment period (GD 6 – 19) was significantly reduced at the highest dose. The corrected body weight gain (terminal body weight on GD 20 minus uterus weight minus body weight on GD 6) was reduced at the highest dose (40.9, 43.7, 40.0 and 36.9 g respectively at 0, 30, 100 and 300 mg/kg bw/d).

Table 102: Body weight and body weight gain data (in g)

Dose level (mg/kg bw/d)	0	30	100	300
GD 0	164.9	167.5	168.7	165.6
GD 6	195.9	199.1	199.2	198.3
GD 15	239.3	243.5	240.6	236.1
GD 20	295.9	302.4	297.8	291.0
GD 8 - 10	9.6	9.3	9.4	6.8*
GD 6 - 19	85.2	89.8	84.3	78.6*
GD 0 - 20	131.0	134.9	129.1	125.4
Corrected bwg	40.9	43.7	40.0	36.9

* p<0.05 ; Only pregnant dams with scheduled sacrifice (GD 20) were used for the calculation of bw.
1 female of the highest dose was excluded as this rat was not pregnant

- *food consumption :* lower food consumption observed during the entire treatment period at 300 mg/kg bw/d (-8 % compared to control group).
- *organ weight data :* no substance related effect for the mean gravid uterus weight

Table 103: Mean gravid uterine weight and net maternal bwg (in g)

Dose level (mg/kg bw/d)	0	30	100	300
Gravid uterus (in g)	59.1	59.6	58.7	55.8
Carcass	236.8	242.8	239.1	235.2
Terminal bw minus uterine weight	236.8	242.8	239.1	235.2

- *reproduction data :*
  - number of corpora lutea : no effects
  - number of implantation sites : no effects
  - number of pre- and post-implantation loss : no effects
  - number of resorptions and viable foetuses : no effects

Table 104: Reproduction data

Dose level (mg/kg bw/d)	0	30	100	300
Nb of females mated	25	25	25	25
Conception rate (%)	100	100	100	96 (24/25)
Nb of females aborted	0	0	0	0
Nb of dams with viable foetuses	25	25	25	24
Mean nb. of corpora lutea	11.5	11.8	11.7	11.4
Mean nb. of implantation sites	11.1	11.0	11.1	10.8
Mean pre implantation loss (%)	3.6	6.1	5.4	5.3

Mean post implantation loss (%)	4.7	3.9	3.9	6.3
Mean early resorption	0.5	0.4	0.4	0.6
Mean late resorption	0.0	0.0	0.1	0.0
Mean total resorption	0.5	0.4	0.5	0.7
Nb of dead foetuses	0	0	0	0
Mean live foetuses (females/males)	10.6 (5.2/5.4)	10.6 (5.0/5.6)	10.6 (6.0/4.6)	10.1 (4.8/5.3)

- *necropsy findings :* no substance-related necropsy findings (1 female exhibited a diaphragmatic hernia (females which failed to be pregnant), another one had dilated renal pelvis and another one had hemometra)
- *placenta weight :* no effects (mean placental weight of all viable foetuses : 0.45, 0.46, 0.45 and 0.47 g respectively at 0, 30, 100 and 300 mg/kg bw/d)

For foetuses :

- *mean number of live pups (litter size):*

Table 105: Pups data

Dose level (mg/kg bw/d)	0	30	100	300
Nb of litter evaluated	25	25	25	24
Nb of live foetuses evaluated	264	265	265	243
Mean nb of live foetuses	10.6	10.6	10.6	10.1
Nb of dead foetuses evaluated	0	0	0	0

- *sex ratio :* no effect (48.9/51.1, 47.2/52.8, 56.6/43.4 and 47.3/52.7 % of females/males resp. at 0, 30, 100 and 300 mg/kg bw/d)
- *mean litter or pup weight by sex and with sexes combined :* the mean foetal weight was comparable to control group

Table 106: Mean foetal weight (in g)

Dose level (mg/kg bw/d)	0	30	100	300
Foetal weight of all viable foetuses	3.6	3.6	3.4	3.4
Foetal weight of male foetuses	3.6	3.7	3.5	3.5
Foetal weight of female foetuses	3.5	3.5	3.4	3.3

- *external, soft tissue and skeletal malformations and other relevant alterations :*
  - *external examination :*
    - *malformations :* 1 foetus at 100 mg/kg bw/d presented multiple external malformations (misshapen head and absent face (anophthalmia, astomia, anotia)).
    - *variations :* no external variation were found
  - *soft tissue examination :*
    - *malformations :* no soft tissue malformations were recorded
    - *variations :* effects were observed such as dilated renal pelvis, dilated ureter, however these effects were also noted in control groups.

Table 107: Total soft tissue variations

Dose level (mg/kg bw/d)	0	30	100	300
Nb of litter	25	25	25	24
Nb of foetuses	127	128	125	116
Foetal incidence : nb (%)	7 (5.5)	5 (3.9)	12 (9.6)	10 (8.6)
Litter incidence : nb (%)	7 (28)	4 (16)	9 (36)	9 (38)
Mean affected foetuses/litter	6.1	4.1	9.1	8.5

- *Skeletal examination :*
  - *malformations :* effects were observed in all groups. These effects affected the skull, sternum and forelimbs.

Table 108: Total skeletal malformations

Dose level (mg/kg bw/d)	0	30	100	300
Nb of litter	25	25	25	24
Nb of foetuses	137	137	140	127
Foetal incidence : nb (%)	1 (0.7)	0.0	3 (2.1)	5 (3.9)
Litter incidence : nb (%)	1 (4.0)	0.0	3 (12)	5 (21)
Mean affected foetuses/litter	0.7	0.0	2.8	4.3*

* p<0.05

0 mg/kg bw/d :1 female exhibit shortened humerus

30 mg/kg bw/d : none

100 mg/kg bw/d : 1 male with multiple skeletal malformations, 1 male with shortened scapula, 1 female with shortened humerus

300 mg/kg bw/d : 2 male exhibited malpositioned and bipartite sternebra, 1 male with shortened humerus, 1 female with misshapen basisphenoid and 1 female with misshapen tuberositas deltoidea

- *variations :* effects were recorded in all groups

Table 109: Skeletal variations data

Dose level (mg/kg bw/d)	0	30	100	300	HCD mean % (range)
Nb of litter	25	25	25	24	/
Nb of foetuses	137	137	140	127	/
Total skeletal variations					
Foetal incidence : nb (%)	136 (99)	135 (99)	139 (99)	127 (100)	/
Litter incidence : nb (%)	25 (100)	25 (100)	25 (100)	24 (100)	/
Mean affected foetuses/litter	99.2	98.3	98.7	100.0	/
Incidence of significant increased foetal skeletal variations (mean % of affected foetus/litter)					
Incomplete ossification of supraoccipital (unchanged cartilage)	34.1	35.2	37.6	45.2*	43.5 (10.3 – 64.3)
Dumbbell ossification of thoracic centrum (unchanged cartilage)	0.7	3.0	0.0	5.6**	6.9 (0.0 – 14.5)

Unossified sternebra (unchanged cartilage)	1.5	5.0	4.6	11.0**	8.2 (2.6 – 20.7)
Incomplete ossification of pubis (cartilage present)	0.0	0.8	2.0*	1.7	0.3 (0.0 – 2.4)
Incomplete ossification of ischium (cartilage present)	0.0	0.0	2.0*	1.7	0.2 (0.0 – 0.8)

* : p<0.05 ; ** : p<0.01

Reproductive toxicity screening assay (REACH registration dossier : Unpublished, 2000)

Test type

According to OECD TG 421

GLP

Test substance

- 4,4'-sulphonyldiphenol
- *Degree of purity :* see confidential annex

Test animals

- *Species/strain/sex :* rat / SD / male + female
- *No. of animals per sex per dose :* 12/sex/dose
- *Age and weight at the study initiation :* 9 w old

329 – 374 g in males and 206 – 251 g in females

Administration/exposure

- *Route of administration :* gavage
- *duration of test/exposure period :*
  - males : a total of 45 days (14 d of pre-mating period, through mating period to the day before necropsy)
  - females : a total of 40 to 46 days (14 d before mating, mating through gestation and until LD 3) (females without delivery were exposed until D 25 after confirmation of copulation)
- *frequency of exposure :* daily
- *doses/concentration levels :* 0, 10, 60 and 300 mg/kg bw/d
- *vehicle :* 0.5 % aqueous sodium CMC solution with 0.1 % Tween 80

Results and discussion

For P adults :

- *clinical observations :* 300 mg/kg bw/d : excessive salivation was noted immediately before or immediately after administration in 7 males and 1 female. However, all of them recovered ± 30 min after administration.
- *body weight data for P animals selected for mating :* a lower bwg was observed at the highest dose in both sexes. Moreover, a significantly decrease bw was noted in females at day 4 of lactation was observed at the mid dose level.

Table 110: Body weight data (g)

Dose level (mg/kg bw/d)			0	10	60	300
Males		Nb of animals examined	12	12	12	12
		D 0	351.9	354.3	352.5	354.2
		D 3	373.8	373.5	373.7	357.5*
		D 14	435.9	435.9	437.9	404.5**
		D 42	511.8	514.5	523.5	486.0
		BWG D 0-42	159.9	160.2	171.0	131.8
Females	Premating	Nb of animals examined	12	12	12	12
		D 0	229.1	228.4	228.2	230.3
		D 14	264.2	263.8	262.3	251.7
		BWG D 0-14	35.1	35.4	34.1	21.4**
	Gestation	Nb of animals examined	11	11	12	7
		D 0	272.8	277.1	266.8	264.4
		D 20	436.5	433.1	418.6	390.4**
		BWG D 0-20	163.6	156.0	151.8	126.0**
	Lactation	Nb of animals examined	11	11	12	7
		D 0	325.5	327.5	314.3	316.0
		D 4	360.1	354.1	333.0*	338.6
		BWG D 0-4	34.5	26.5	18.7	22.6

* : p < 0.05 ; ** : p < 0.01

- *precoital interval (number of days until mating and number of oestrus periods until mating) and reproductive performance :* extended cycles were noted at the highest dose. Five females showed a dioestrus period of 6 to 10 days and 4 out of these 5 females did not conceive.

Table 111: Reproductive performance

Dose level (mg/kg bw/d)	0	10	60	300
Mean duration of oestrus cycle in days	4.08	4.01	4.14	5.57**
Incidence of females with irregular oestrus cycle	0/12	0/12	1/12	5/12*
Copulation index in %	100.00	100.00	100.00	100.00
Fertility index in % (nb. of pregnant females/nb. of copulated females)	91.7 (11/12)	91.7 (11/12)	100.00 (12/12)	58.3 (7/12)

* : p < 0.05 ; ** : p < 0.01

- *number of implantations, corpora lutea, litter size :* a significant decrease in the implantation index was observed at the highest dose. Furthermore, a lower total number of offspring was observed at this dose level.

Table 112: Delivery data

Dose level (mg/kg bw/d)	0	10	60	300
Nb of animals examined	11	11	12	7
Gestation length (d)	22.9	23.0	22.8	22.9
Mean nb of corpora lutea	16.6	15.9	17.3	15.7

Mean nb of implantation sites	15.9	13.3	14.8	10.7
Mean nb of offspring	14.3	12.5	13.5	9.1
Implantation index (%)	95.80	80.84	86.15	64.89**
Delivery index (%)	90.03	94.60	91.22	89.57
Gestation index (%)	100.00	100.00	100.00	100.00

** : p < 0.01

- *effect on sperm :* not examined
- *necropsy findings :* distension of the cecum was noted in 1 male and 1 female at 60 mg/kg bw/d and in all males (12) and 4 females at 300 mg/kg bw/d
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :* changes in organ weights were observed at 300 mg/kg bw/d (see table 115)

Table 113: Organ weight

	Males				Females			
Dose level (mg/kg bw/d)	0	10	60	300	0	10	60	300
FBW (g)	513.4	517.3	526.7	488.1	360.1	354.1	333.0*	338.6
Liver (g)	16.373	16.246	16.803	17.439	15.289	14.114	14.490	14.393
Liver rel.	3.185	3.135	3.185	3.562**	4.247	3.989	4.359	4.246
Pituitary (mg)	14.92	13.60	15.12	16.68	21.18	21.64	21.45	21.07
Pituitary rela ($x10^{-3}$)	2.89	2.63	2.88	3.43**	5.90	6.14	6.45	6.21
Thymus (mg)	289.6	336.3	332.1	254.5	263.1	312.7	253.5	221.7
Epididymis (g)	1.355	1.292	1.328	1.292	-	-	-	-
Prostate (g)	0.723	0.746	0.777	0.708	-	-	-	-
Sem. ves. (g)	2.825	2.718	2.860	2.428**	-	-	-	-
Sem. ves. rela	0.552	0.531	0.546	0.498	-	-	-	-
Testes (g)	3.559	3.480	3.554	3.503	-	-	-	-
Ovaries (mg)	-	-	-	-	110.35	116.02	114.86	105.63
Uterus (g)	-	-	-	-	0.691	0.683	0.713	0.700

* : p < 0.05 ; ** : p < 0.01

- *histopathological findings:* changes observed in cecum and liver at both sexes (see table 116)

Table 114: Incidence of histopathological findings

		Males				Females			
Dose level (mg/kg bw/d)		0	10	60	300	0	10	60	300
Cecum	Diffuse hyperplasia, mucosal epithelium	0/12	/	0/1	11/12**	0/1	/	1/1	4/4
	Single cell necrosis, absorptive epithelium	0/12	/	0/1	5/12*	0/1	/	0/1	1/4
Liver	Extramedullary haematopoiesis	2/12	2/12	3/12	2/12	6/12	6/12	7/12	5/12
	Centrilobular hypertrophy, hepatocytes	0/12	0/12	0/12	5/12*	0/12	0/12	0/12	3/12

* : p < 0.05 ; ** : p < 0.01

For offspring :

- *mean number of live pups (litter size) :* lower total mean number of offspring at birth, mean number of live offspring at birth and mean number of live offspring on lactation day 4 were noted at 300 mg/kg bw/d.

Table 115: Mean number of offspring

Dose level (mg/kg bw/d)	0	10	60	300
Mean nb offspring at birth	14.3	12.5	13.5	9.1
Mean nb of live offspring at birth	14.2	12.5	13.4	9.1
Mean nb of live offspring at D 4	14.1	12.4	13.3	9.1

- *body weight data (in g) :* no significant change

Table 116: Pups body weight data (in g)

Dose level (mg/kg bw/d)		0	10	60	300
Males	PND 0	7.4	7.5	7.3	7.8
	PND 4	12.0	12.4	12.1	14.1
Females	PND 0	6.9	7.0	6.9	7.3
	PND 4	11.7	11.7	11.5	13.3

- *viability index (pups surviving 4 days/total births) :* no significant difference in viability index of PND 4

Table 117: Viability index

Dose level (mg/kg bw/d)	0	10	60	300
Viability index at D 0 (%)	99.35	100.00	99.48	100.00
Viability index at D 4 (%)	99.30	95.45	99.48	100.00

- *external, soft tissue and skeletal malformations and other relevant alterations :* no abnormalities were observed in dead offspring on LD 0 to 4 and live offspring on LD 4.
- *anogenital distance :* no effects

Table 118: Anogenital distance in mm (D 4 after birth)

Dose level (mg/kg bw/d)	0	10	60	300
Males	5.03	4.97	4.71	5.19
Females	2.42	2.26	2.27	2.44

- *necropsy :* no abnormalities were observed in either group

Range finding study preceding the EOGRTS, similar to a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (REACH registration, Unpublished study report, 2017)

Test type

Range finding study

Similar to OECD TG 422

GLP

Test substance

- 4,4'-sulphonyldiphenol
- *Degree of purity :* see confidential annex

Test animals

- *Species/strain/sex :* rat / SD / both sexes
- *No. of animals per sex per dose :* 10/sex/dose
- *Age and weight at the study initiation :* approx. 9 w for males and 10 w for females

Administration / exposure

- *Route of administration :* gavage
- *duration and frequency of test/exposure period :* daily
    - Males : 10 w
    - Females : from premating period until lactation period (until one day before necropsy)
- *doses/concentration levels :* 0, 30, 100 and 300 mg/kg bw/d
- *vehicle:* CMC

Results and discussion

For P:

- *time of death during the study and whether animals survived to termination :* no premature death was observed
- *clinical observations: description, severity, time of onset and duration :* increased incidence of excessive salivation was observed at the highest dose level
- *body weight data :* bw was reduced at 300 mg/kg bw/d (-7 % in males and -6 % in females compared to the control group)
- *haematological and clinical biochemistry findings if available :* no effects (no more information available)
- *toxic response data by sex and dose including indices of mating, fertility, gestation, birth, viability and lactation; indicate the numbers used in calculating the indices :* Females exhibited prolonged oestrus. Pregnant females had a significantly lower average number of implantation sites. Moreover, post-implantation loss was significantly higher and 2 out of 8 pregnant females had complete intrauterine litter losses.

Table 119: Reproductive data

Dose level (in mg/kg bw/d)	0	30	100	300
Mean duration of oestrus cycle (d)	4.02	3.97	4.01	5.16**
Fertility index (%)	100	90	100	80[10]

[10] Note that this specific effect was initially mentioned to be 60% at the highest dose tested in the CLH dossier and on IUCLID, but during the consultation it was noted that this effect size was

Mean nb of implantation sites	15.8	15.0	15.5	10.4**
Females without implantation sites	0	0	0	2
% of post-implantation loss	3.6	5.2	6.5	34.6*
Mean duration of gestation (d)	22	22.1	22	22
Tot. nb of pups delivered	152	127	145	65
Nb of stillborn	2	1	3	3
Mean nb of pups delivered	15.2	14.1	14.5	10.8**
Mean perinatal loss (%)	1.3	0.6	2	5.3

* : p<0.05 ; ** : p<0.01

- *effects on sperm :* not examined
- *necropsy findings :* Males exposed to 300 mg/kg bw/d exhibited dilatation of cecum (3 males out of 10), enlarged and discoloration of kidneys (9 males out of 10 for enlarge and 8 males out of 10 for discoloration) and enlarged liver (3 males out of 10).
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :* higher kidneys weight in males at the mid and high doses (+11.5 and 35 % resp. at 100 and 300 mg/kg bw/d) and higher liver weight in males at the highest dose (+11 %).

Table 120: Organ weight data (FBW in g and organ weight in %)

	Males				Females			
Dose level (in mg/kg bw/d)	0	30	100	300	0	30	100	300
FBW	548.6	530.2	546.3	497.5*	304.5	301.8	292.6	286.6
Adrenals	0.014	0.014	0.014	0.015	0.027	0.029	0.03	0.03
Kidneys	0.662	0.692	0.748**	1.013**	0.722	0.731	0.762	0.752
Liver	2.375	2.378	2.519	2.668**	2.846	2.938	3.297[A]	2.927
Prostate	0.302	0.303	0.278	0.297	-	-	-	-
Sem. ves.	0.357	0.366	0.336	0.348	-	-	-	-
Testes	0.685	0.663	0.663	0.734	-	-	-	-
Ovaries	-	-	-	-	0.035	0.035	0.037	0.034
Uterus	-	-	-	-	0.197	0.224	0.224	0.307B

* p<0.05 ; ** : p<0.01

[A] : S.d : 0.154, 0.395, 0.677 and 0.189, respectively at 0, 30, 100 and 300 mg/kg bw/d

[B] : S.d : 0.026, 0.088, 0.099 and 0.152, respectively at 0, 30, 100 and 300 mg/kg bw/d

- *histopathological findings:*

incorrect and should be changed into 80% fertility at the highest dose tested. The information in IUCLID was updated to this regard as well (ECHA dissemination website). The eMSCA was unable to verify the exact number as it had no access to the underlying study report. However, the eMSCA wants to highlight that, either way, a dose-dependent decrease in fertility index is observed in this study

Table 121: Incidence of microscopic data

		Males				Females			
Dose level (in mg/kg bw/d)		0	30	100	300	0	30	100	300
Cecum	Dilatation	0	0	0	3	0	0	0	0
	Thickening of wall	0	0	5	9	0	0	0	0
	Increased apoptosis	0	0	3	9	0	0	0	0
Kidneys	Degeneration/regeneration	0	0	6	10	0	0	0	0
	mineralisation	0	0	2	2	1	0	0	4
	Tubular distension	0	0	5	10	0	0	0	0
Liver	Infiltration lymphoid	10	1	2	10	10	0	0	10
	Multifocal necrosis	1	0	1	1	0	0	0	0
Mammary gland	Diffuse atrophy	0	0	0	10	0	0	0	0

For F1 pups/litters (per dose):

- *clinical signs :* no effects were observed
- *mean number of live pups (litter size) :*

Table 122: Live pups data

Dose level (in mg/kg bw/d)	0	30	100	300
Tot. nb of pups delivered	152	127	145	65
Nb of stillborn	2	1	3	3
Mean nb of pups delivered	15.2	14.1	14.5	10.8**
Mean perinatal loss (%)	1.3	0.6	2	5.3

** : $p<0.01$

- *sex ratio :* no information available
- *viability index (pups surviving 4 days/total births) :* no mortality occurred (no more information available)
- *survival index at weaning* : no information available
- *mean litter or pup weight by sex and with sexes combined :* a significant bw increase (+6.6 %) was observed in male pups of the low dose at PND 21
- *external, soft tissue and skeletal malformations and other relevant alterations :* no effects (no more information available)

Range finding study preceding the EOGRTS, similar to a 28-day repeated dose toxicity study (REACH registration, Unpublished study report , 2017)

Test type

Preliminary study

No guideline followed

No GLP

Test substance

- 4,4'-sulphonyldiphenol

- *Degree of purity :* confidential

Test animals

- *Species/strain/sex :* rat / SD / both sexes
- *No. of animals per sex per dose :* 5/sex/dose
- *Age and weight at the study initiation :* approx. 63 D for males and 62 D for females

Administration/exposure

- *Route of administration :* gavage
- *duration and frequency of test/exposure period :* 28 d, daily
- *doses/concentration levels :* 0, 100, 300 and 600 mg/kg bw/d
- *vehicle:* 0.5 % CMC

Results and discussion

- *clinical observations :* excessive salivation (no more information available)
- *body weight data :* sign. lower bwg value was noted in males exposed to the highest dose
- *haematological and clinical biochemistry findings if available :* no information available
- *necropsy findings :* Changes were observed in males. Enlarged kidneys were noted in 3 males exposed to 300 mg/kg bw/d and in 4 males exposed to 600 mg/kg bw/d and dilatation of cecum was observed in 2 males of the highest dose.
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :* Males exhibited a significantly decrease of FBW (-9 and -12 % respectively at 300 and 600 mg/kg bw/d, compared to control). Moreover, few relative organ weights were modified. The relative kidneys weight was higher at the mid and high doses in both sexes (+33 % and +12 % resp. in males and females, compared to the control group). The relative adrenals weight was higher in males (+18 and +39 % resp. at 300 and 600 mg/kg bw/d, compared to the control group). The relative liver weight was significantly increased in females (+9 and +12 % resp. at 300 and 600 mg/kg bw/d, compared to the control group). The relative prostate and seminal vesicles weights were also modified at the highest dose (-15 % for prostate and -16 % for seminal vesicles).

  No more information available
- *histopathological findings: nature and severity :* few changes :
  - In 2, 5 and 5 males exposed respectively to 100, 300 and 600 mg/kg bw/d, minimal to moderate tubular degeneration/regeneration in kidneys was noted. Moreover, tubular hypertrophy was observed in 5 males of the highest dose (moderate hypertrophy), in 5 males of the mid dose (minimal hypertrophy) and in 1 male of the low dose (minimal hypertrophy).
  - Minimal hypertrophy/hyperplasia in the adrenal cortex was observed in 3 males exposed to 600 mg/kg bw/d.

- Centrilobular hypertrophy of the liver was noted in 1 males of the low dose (minimal hypertrophy), in 4 males of the mid dose (slight hypertrophy) and in all animals of the high dose (moderate hypertrophy in males and slight in females).
- 5 males and 2 females of the highest dose exhibited dilatation of the cecum.
- 3 males exposed to 300 mg/kg bw/d and 4 males exposed to 600 mg/kg bw/d exhibited diffuse atrophy of the mammary gland.

Ullah *et al.* (2016)

The aim of the study was to assess the effect of BPS on the rat male reproductive system *in vitro* and *in vivo* (see *in vitro* description above). Adult male rats (n = 6 per group) have been exposed to 0, 1, 5, 25 and 50 µg/kg bw/day BPS for 28 consecutive days. On 29th day, animals were killed, blood was collected and different tissues (testis and epididymis) were dissected out. The level of antioxidant enzymes activity (catalase CAT, peroxidase POD, superoxide dismutase SOD), reactive oxygen species (ROS) and testosterone have been measured.

BPS exposure had no effect on body weight nor testis weight.

After exposure to BPS, the amount of ROS was significantly increased in the testicular tissues, whereas SOD, POD and CAT activities were significantly reduced. This discrepancy regarding the *in vitro* results could be explained by the activation of these antioxidant enzymes as a defence mechanism against the ROS increase in the animals.

Sub-chronic exposure to 50 µg/kg bw/day of BPS resulted in a significant decrease of testosterone concentration in the plasma (3.97 vs 4.87 ng/mL in the control) and in the testis (27.36 vs 42.22 ng/g tissue). Histopathological analysis of the testes showed a dose-dependent reduction of the epithelial height of the seminiferous tubules (70.60, 69.73, 65.20, 59.66 and 59.20 µm after exposure to 0, 1, 5, 25 and 50 µg/kg bw/day, respectively). Finally, in the animals exposed to 50 µg/kg bw/day showed a reduction of the number of spermatocytes (69.58 vs 74.08 in the control) and spermatid (241.00 vs 248.32 in the control). However, this reduction was not statistically significant. The reductions of testosterone concentration, epithelial height of the seminiferous tubules and sperm count are indicative of an anti-androgenic potential of BPS.

LaPlante *et al.* (2017)

The authors investigated the effects of BPS on the lactating mammary gland. Female CD-1 mice were exposed to 0, 2 or 200 µg/kg bw/d via food from pregnancy day 9 until lactational day 2 (early lactation) or 20 (late lactation). The sample size was different in each group: at LD 2, there were 5, 8 and 9 mice exposed to 0, 2 and 200 µg/kg bw/d, respectively; at LD 21, there were 14, 17 and 15 dams exposed to 0, 2 and 200 µg/kg bw/d, respectively.

Whole-mount mammary glands were examined at both stages. At LD 2, BPS-treated mice showed less dense mammary glands, with more epithelial structures, whereas they were denser at LD 21. This non-significant trend suggests that BPS exposure might slightly increase the amount of functional mammary tissue in early lactation (LD 2) but induce early involution toward the end of the lactation period. This was confirmed by looking at the volume fraction of lobules and the lobule size. Indeed, at LD 2 no change was observed on volume fraction whereas the lobule size was slightly increased (23 to 30 % after in BPS-treated mice). In contrast, the volume fraction of lobules on LD 21 was significantly reduced at 200 µg/kg bw/d, and the lobules size was slightly reduced (8 to 24%).

BPS alters also the expression of prolactin receptors and ERα in mammary glands specifically. ERβ and progesterone receptor were not detected in mammary gland tissue. BPS-exposed dams had also lower serum concentrations of 17β-oestradiol.

The authors also observed if BPS induced proliferation of the mammary alveolar buds. Normally proliferation is very low during lactation, and BPS exposure was not sufficient to induce this.

Nursing behaviour was affected at LD 14, dams spending more time in nursing when exposed to BPS than the controls. The pups behaviour was also affected. In general, the growth and development of the pups was delayed after BPS exposure.

Shi *et al.* (2017)

The aim of this study was to assess the effect of BPA, BPE and BPS on the reproductive function of CD-1 mice. Neonates mice (n = max 10 pups/litters, 5-6 litters per group) were treated with vehicle control, 50 µg/kg bw (low dose) or 10 mg/kg bw BPS every 3 days by sub-cutaneous injections. At PND 60 half of the pup males and females were euthanized, their body and reproductive organs weighted and examined. The remaining animals (n = 10-18/group) were then individually mated with unexposed mice. The reproductive functions were then investigated (fertility, gestational length, pup numbers and weights, pups sex ratio). The mated animals were then euthanized at PND 90 and examined as the previous ones.

In males, BPS exposure did not affect the body and testis weights at PND 60 and PND 90. However at PND 60 the sperm counts were significantly reduced. When mated with normal CD-1 females, all females became pregnant and had normal gestational days, litter size, ratio of pup's sex and pup weights. However, BPS treated males tended to need longer time to successfully mate (> 10 days for 4 animals of 12 vs 1 to 4 days in controls), suggesting that they are sub fertile. The development of spermatocytes was partly affected, but not disturbed, after exposure to high dose BPS. Hormone level analysis showed a significant increase of serum E2 and testosterone levels after low and high dose BPS exposure.

Table 123: Sperm count and sperm motility at different doses of BPS (Shi *et al.*, 2017)

Dose BPS	Sperm count at PND 60	Sperm motility at PND 60
0	6.4 ± 0.2 x 10^6/ml	76.8 ± 1.2 %
50 µg/kg bw	2.5 ± 0.2 x 10^6/ml**	67.2 ± 1.7 %*
10 mg/kg bw	3.8 ± 0.3 x 10^6/ml**	63.1 ± 2.1 %**

* = $p < 0.05$, ** = $p < 0.001$

In females, BPS did not affect the body and testis weights at PND 60 and PND 90, and had no impact on the onset of puberty. As for the male, after mating, all female became pregnant, showing no effect on reproductive function. However, here also the BPS treated females tended to need more time to successfully being pregnant, suggesting subfertility (> 10 days for 3 animals of 10). Serum E2 was also significantly increased after both high and low-dose BPS exposure, whereas the testosterone level was only higher at high dose.

Gingrich *et al.* (2018)

The authors assessed the effect of BPS exposure at an environmentally relevant dose on placenta of sheep. Pregnant ewes received daily injections of 0.5 mg/kg BPS from GD30 to GD 100 (n = 7 for BPS, n = 8 for control). Maternal serum samples were collected in all pregnant females every 2 weeks for hormonal, protein and biochemical analyses, including

measuring levels of pregnancy-associated glycoprotein 1 (PAG1), pregnancy-specific protein B (PSPB) and progesterone. After a 20 day period without injection (recovery), the ewes were euthanized and their placenta weighted and analysed.

Exposure to BPS daily from GD 30 to 100 resulted in reduced maternal serum concentrations of trophoblastic proteins PAG1 and PSPB. The reductions were significant from GD75 and GD60, respectively, but were partially recovered after stopping the BPS injections. The physiological increase in progesterone level tended to be lower after BPS exposure. No morphological nor histological change has been observed. Trophoblast cell fusion was affected, showing less e-cadherin level and reduced mRNA expression of genes involved in this process.
The authors also demonstrated that BPS is able to transfer to foetus. Indeed, one hour after a single injection of 0.5 mg/kg bw in 2 ewes, foetuses contained 4.9 and 10.6 ng/mL BPS.

Ahsan *et al.* (2018)

The authors compared the effects of BPA and BPS on the development of female reproductive system in rats. They exposed female neonates (n = 15/group) received daily subcutaneous injections of BPS (0, 0.5, 5 and 50 mg/kg bw/day) or from PND 1 to PND 10. The animals have been maintained and examined until PND 75. 8 animals per group were then killed on the morning of the next oestrus cycle. The remaining females (5 per group) were then breeded with healthy males to assess the impact on fertility.

In the group exposed to the highest BPS dose (50 mg/kg bw/d), a significant increase of the body weight has been observed on PND 30 ($p<0.05$), PND 45 and PND 60 ($p<0.01$). The puberty onset was also significantly delayed in this group.

Exposure to 5 and 50 mg/kg bw/d BPS significantly decreased the absolute ovarian weight ($p<0.05$ and $p<0.01$). In the highest dose group, the relative uterus weight was also significantly decreased ($p<0.001$). The relative liver also weight increased dose dependently.

Table 124: Body weight and weight of organs at different doses of BPS (Ahsan *et al.*, 2018)

Dose (mg/kg bw/d)	Body weight at PND 60 (g)	Absolute paired ovarian weight (mg)	Relative uterus weight (%)	Relative liver weight (%)
0	159.4 ± 1.70	129.8 ± 1.50	1.63 ± 0.02	3.07
0.5	161.4 ± 4.19	122.2 ± 4.54	1.55 ± 0.05	3.41
5	162.4 ± 1.43	119.6 ± 1.08*	1.55 ± 0.01	3.61
50	173.6 ± 2.77**	115.6 ± 1.08**	1.35 ± 0.03***	3.87

* = $p < 0.05$, ** = $p < 0.01$, *** = $p <0.001$

BPS impacted also the oestrous cycle and the folliculogenesis in the rats, showing a decreased number of corpus luteum and antral follicles after exposure to 5 and 50 mg/kg bw/d. On the contrary there was significantly more atretic follicles in the 50 mg/kg bw/d BPS group.

Exposure to BPS also changed the levels of hormones in plasma, showing a significant reduction of LH and FSH levels at 50 mg/kg bw/d. The level of progesterone was also strongly reduced in this group, whereas the level of testosterone was higher ($P<0.05$). The oestradiol level was not affected.

Table 125: Hormone levels at different doses of BPS (Ahsan *et al*., 2018)

Dose (mg/kg bw/d)	LH (ng/mL)	FSH (mIU/mL)	Estradiol (pg/mL)	Testosterone (ng/mL)	Progesterone (ng/mL
0	2.65 ± 0.05	4.69 ± 011	22.93 ± 1.60	0.36 ± 0.09	3.59 ± 013
0.5	2.34 ± 0.13	4.54 ± 0.07	18.91 ± 1.38	0.34 ± 0.06	2.46 ± 0.65
5	2.30 ± 0.03	4.47 ± 0.06	22.24 ± 1.51	0.39 ± 0.11	2.52 ± 0.90
50	2.21 ± 0.03*	4.19 ± 0.05*	25.23 ± 0.49	0.79 ± 0.05*	0.88 ± 0.14*

* = $p < 0.05$

High BPS dose neonatal exposure (50 mg/kg bw/d) affected also the fertility of the female rats, as only 3 of 5 females were able to conceive after mating. Moreover, the number of pups born per female was significantly reduced (5.33 vs 8.80 in control).

Nevoral *et al.* (2018)

The aim of the study was to evaluate the impact of low-dose BPS on the ovaries and oocytes in mice. Four-week old mice were exposed to 0, 0.001, 0.1, 10 and 100 µg/kg bw/day BPS for 4 weeks.

A significant dose-dependent decrease of the ovarian volume and weight has been recorded, mainly due to the reduction of the corpus luteum volume and the decrease of number and volume of antral follicles. These observations were correlated with a significant decrease of 17β-estradiol concentration in plasm after 10 and 100 µg/kg bw/day BPS exposure.

The oocytes were also affected after exposure to BPS, showing an increased incidence of spindle malformation and abnormal chromosome alignment. The fertilization rate was decreased at 10 µg/kg bw/day but increased at 100 µg/kg bw/day, both significantly.

Ullah *et al.* (2018a)

The aim of the study was to compare the effect of several bisphenols, i.e., BPA, BPS, BPB and BPF on the rat male reproductive system *in vitro* and *in vivo* (see *in vitro* description above). The experiment is similar to the study of the same group published in 2016 (Ullah *et al.*, 2016), but the doses used *in vivo* were higher than in the previous experiment. Adult male rats (n = 7 per group) have been exposed orally to 0, 5, 25 and 50 mg/kg bw/day BPS for 28 consecutive days. On 29th day, animals were killed, blood was collected, and different tissues (testis and epididymis) were dissected out. The level of antioxidant enzymes activity (catalase CAT, peroxidase POD, superoxide dismutase SOD), reactive oxygen species (ROS) and testosterone have been measured.

BPS exposure led to a slight, non-significant body weight decrease (33, 25, 26 and 28 g, at 0, 5, 25 and 50 mg/kg bw/d resp.), as well as testis weight decrease (2.19, 1.98 and 2.01 g at 0, 25 and 50 mg/kg bw/d resp.).

After exposure to BPS, SOD, POD, and CAT activities were clearly (but not always significantly) reduced, whereas the LPO and ROS levels were clearly increased (sign. at 50 mg/kg bw/d) in the testicular tissues. Total amount of protein was significantly decreased at all doses.

Sub-chronic exposure to BPS resulted in a clear decrease of testosterone concentration in the plasma (5.90, 4.39, 4.45 and 3.93 ng/mL at 0, 5, 25 and 50 mg/kg bw/d resp.) and in the testis (54.27, 45.82, 47.65 and 44.71 ng/g tissue at 0, 5, 25 and 50 mg/kg bw/d resp.). The results at 50 mg/kg bw/d were significant, as the decrease in the testis at 5 mg/kg bw/d. Histopathological analysis of the testes showed a dose-dependent reduction

of the epithelial height of the seminiferous tubules (71.48, 69.03, 64.22 and 58.64 µm after exposure to 0, 5, 25 and 50 mg/kg bw/day, resp.). The reductions of testosterone concentration and epithelial height of the seminiferous tubules are indicative of an anti-androgenic potential of BPS.

Tucker *et al.* (2018)

The authors analysed the impact of foetal exposure to diverse bisphenols (BPA, BPAF and BPS) on the mammary gland of the offspring. Pregnant mice (n = 11 or 12 per group) were gavaged twice daily with 0, 0.05, 0.5 or 5 mg/kg bw/d BPS from GD 10 to 17. Litters were then culled to 10 pups at PND 3 (minimum 5 females). Puberty onset an oestrous cyclicity were monitored. Randomly chosen pups females (1 per litter) were then weighed and euthanized at PND 20, 28, 35 and 56, and 3, 8 and 14 months. The authors determined then their hormones concentration in the serum and examined their mammary gland development.

Prenatal exposure to BPS did not influence the body weight of the female offspring, nor the pubertal onset and first oestrous timing. However the mammary gland developed more rapidly after exposure to BPS, showing a significantly dose-dependent higher terminal end buds (TEB) count and ratio TEB:Length at PND 20. The mammary epithelial branching was also significantly more dense after 0.05 and 5 mg/kg bw/d BPS foetal exposure. This earlier mammary gland development was also seen at PND 35. The control group caught up the accelerated development at PND 56.

Mammary glands were also evaluated in adult mice exposed prenatally to BPS, showing increased TEB. Inflammation, neoplasia and non-neoplastic lesions were also more frequently observed after BPS exposure in utero.

The mice exposed prenatally to mid- and high-doses of BPS showed significantly serum estradiol concentration at PND 20, prior to vaginal opening. This was then no more significant. The progesterone concentration was strongly up-regulated at PND 56 in the mid- and high-doses group. There was no clear pattern regarding testosterone concentration and DHEA was not affected.

Finally, the authors did not observed significant changes in steroid receptor expression at 14 months.

Kolla *et al.* (2018)

The aim of this study was to assess the impact of BPS low-dose in utero exposure on the mammary gland morphology of mice before and during puberty, and at adulthood. Pregnant female CD-1 mice were exposed orally via water to 0, 2 or 200 µg/kg bw/day BPS. After birth, litters were culled to 10 pups on PND 1. One female from each litter was sacrificed at PND 24 (before puberty), PND 32-35 (after vaginal opening), and at week 9 (adulthood), i.e. n = 20 in control group, n = 14 in low-dose group and n = 10 in high dose group. Whole mounts mammary glands were then collected and analysed.

Whereas no change has been observed during puberty, the mammary glands of mice exposed in utero to BPS were more developed, showing significant increase in total TEB area and average TEB size. At adulthood also, significantly more alveolar buds were observed in mice exposed to 200 µg/kg bw/day BPS, whereas the females exposed to 2 µg/kg bw/day BPS had more terminal ends. Both groups showed more total epithelium in the mammary gland, and revealed the presence of TEB-like structures (similar to the TEB found in pubertal mammary gland).

Cell proliferation was assessed in the mammary tissue via the expression of Ki67, a marker of proliferation. At PND 24, the mice exposed to BPS exhibited lower Ki67, whereas the expression of this marker was slightly increased during puberty and significantly increased

during adulthood (only for the mice exposed to 200 µg/kg bw/day). This proliferation was consistent with histological examination, the mice exposed to BPS in utero showing significantly higher rate of intraductal hyperplasia and duct with epithelial cells within the lumen.

The authors also analysed the expression oestrogen receptor α (ERα) and progesterone receptor (PR) in mammary tissues. Whereas no difference was observed before puberty (PND 24), ERα and PR expression was significantly increased during puberty (PND 32-35). At adulthood, the ERα expression was decreased (no effect on PR).

Ullah *et al.* (2018b)

The aim of the study was to compare the effect of several bisphenols, i.e. BPA, BPS, BPB and BPF on the rat male reproductive system *in vivo* (see *in vitro* description above) after chronic low-dose exposure. The experiment is similar to the study of the same group published in 2016 (Ullah *et al.*, 2016), but the exposure period was prolonged to 48 weeks.

Adult male rats (n = 7 per group) have been exposed to 0, 5, 25 and 50 µg/L BPS from PND 23 and for 48 weeks, daily via water. Animals were then killed, blood was collected and reproductive tissues (testis, epididymis, seminal vesicle and prostate) were dissected out. The level of antioxidant enzymes activity (catalase CAT, peroxidase POD, superoxide dismutase SOD), reactive oxygen species (ROS) have been measured. Sperm motility and viability was also examined, and the plasma level of some hormones has been determined (testosterone, oestrogen, LH and FSH).

BPS exposure leaded to a significant body weight increase at 50 µg/kg bw/d (548.80 g vs. 541.11 g in the control group). The absolute paired testis weight was dose-dependently decreasing, but not significantly, as well as the absolute epididymis and relative prostate weights. Both absolute and relative weights of seminal vesicle were also dose-dependently decreasing, the highest dose being in both cases significantly lighter.

Table 126: Body weight and weight of reproductive organs at different doses of BPS (Ullah *et al*., 2018b)

Dose (µg/L)	Final body weight (g)	Absolute paired testis weight (g)	Absolute paired epididymis weight (g)	Absolute seminal vesicle weight (g)	Relative seminal vesicle weight (mg/g)	Relative prostate weight (mg/g)
0	541.11 ± 2.02	3.68 ± 0.08	1.44 ± 0.03	1.90 ± 0.04	3.55 ± 0.04	2.71 ± 0.05
5	510.20 ± 2.35	3.55 ± 0.04	1.43 ± 0.05	1.87 ± 0.02	3.49 ± 0.02	2.67 ± 0.03
25	538.60 ± 0.50	3.51 ± 0.05	1.42 ± 0.04	1.83 ± 0.03*	3.42 ± 0.04	2.68 ± 0.04
50	548.80 ± 2.28*	3.50 ± 0.03	1.41 ± 0.02	1.79 ± 0.03**	3.32 ± 0.03**	2.64 ± 0.03

* = $p < 0.05$, ** = $p < 0.01$

After chronic exposure to BPS, SOD, POD and CAT activities were dose-dependently reduced (significantly at highest doses), whereas the LPO and ROS levels were dose-dependently increased (significantly at 50 µg/L) in the testicular tissues.

Table 127: Antioxidant enzymes activity and reactive oxygen species at different doses of BPS (Ullah *et al*., 2018b)

Dose (µg/L)	CAT (U/mg protein)	SOD (U/mg protein)	POD (U/mg protein)	LPO (U/mg protein)	ROS (U/mg protein)
0	7.47 ± 0.15	32.34 ± 0.29	6.04 ± 015	7.72 ± 0.24	98.70 ± 0.29
5	7.08 ± 0.26	32.59 ± 0.17	5.62 ± 0.09	7.45 ± 0.10	98.84 ± 0.40
25	6.46 ± 0.20	31.63 ± 0.16	5.45 ± 0.09*	7.56 ± 0.08	105.4 ± 1.37

50	6.36 ± 0.16*	30.57 ± 0.15	5.44 ± 0.11**	8.60 ± 0.03**	121.5 ± 3.28***

* = p < 0.05, ** = p < 0.01, *** = p <0.001

Chronic exposure to BPS resulted in a clear dose-dependent increase of estradiol and decrease of testosterone, LH and FSH concentrations in the plasma.

Table 128: Hormone levels at different doses of BPS (Ullah *et al.*, 2018b)

Dose (µg/L)	Testosterone (ng/mL)	Estradiol (pg/mL)	LH (ng/mL)	FSH (mIU/mL)
0	12.02 ± 0.98	2.81 ± 0.33	1.79 ± 0.07	0.79 ± 0.07
5	11.39 ± 0.11	3.43 ± 0.31	1.63 ± 0.06	0.74 ± 0.03
25	10.31 ± 0.63*	3.82 ± 0.16	1.56 ± 0.06	0.60 ± 0.02
50	9.45 ± 0.033***	4.39 ± 0.29**	1.49 ± 0.02	0.58 ± 0.03*

* = p < 0.05, ** = p < 0.01, *** = p <0.001

Sperm parameters was also affected after chronic BPS exposure. Motile sperms and daily sperm production was dose-dependently reduced, as the numbers of spermatogonia, spermatocytes and spermatids. Finally, histopathological analysis of the testes showed a dose-dependent reduction of the epithelial height of the seminiferous tubules. This confirms the estrogenic and anti-androgenic effects of BPS and its potential effect on the male reproductive system of mammals.

Table 129: Effects on sperm at different doses of BPS (Ullah *et al.*, 2018b)

Dose (µg/kg bw/d)	Motile sperms (%)	Daily sperm production (x 10^6)	Spermatogonia (n)	Spermatocytes (n)	Spermatids (n)	Epithelial height (µm)
0	79.56 ± 0.54	53.34 ± 0.6	65.66 ± 0.62	77.10 ± 1.06	257.26 ± 1.79	71.22 ± 1.90
5	78.12 ± 0.51	52.24 ± 0.5	63.40 ± 1.05	74.74 ± 1.30	250.04 ± 2.77	66.26 ± 2.65
25	75.27 ± 1.10*	50.32 ± 0.8	63.64 ± 1.15	73.84 ± 1.23	248.32 ± 2.52	64.44 ± 1.87
50	74.28 ± 0.74***	48.22 ± 0.5**	61.58 ± 0.87*	72.12 ± 1.24*	244.02 ± 2.01***	61.96 ± 2.72*

* = p < 0.05, ** = p < 0.01, *** = p <0.001

Shi *et al.* (2018)

The aim of this study was to assess the effect of BPA, BPE and BPS on the reproductive function of male CD-1 mice after prenatal exposure. Pregnant mice (n = 5/group) were exposed orally to 0, 0.5, 20 or 50 µg/kg bw/day of BPS from GD11 until birth. At delivery, pups were sexed and the size of each litter was adjusted to 10 pups with 5 males and 5 females. At PND 12 or PND 60, 2-3 male pups were euthanized. Testes were prepared for analysis (mRNA expression and histology), blood collected for hormones level quantification, and caudal epididymis collected for sperm counts.

At PND 60, BPS prenatal exposure did not affect body weight nor testis weight. However the mean sperm counts were significantly reduces to 66% and 55% after exposure to 0.5 and 20 µg/kg bw/day (compared to the control). Interestingly the highest dose did not affect sperm counts. Sperm motility was decreased to about 60% relative to control after exposure to 0.5 µg/kg bw/day BPS (no effect in other groups). Further analysis showed an altered spermatogenesis, resulting in decreased number of Stage VII and an increased number of Stage VIII spermatocytes.

At PND 60, BPS exposure affects also the steroid hormones level, showing a significantly increase of serum estradiol-17β after exposure to 50 µg/kg bw/day (trend increase at 20 µg/kg bw/day). The level of testosterone showed a decreasing trend (but not significant).

At PND 12, neonatal testis exhibited increased apoptosis after exposure to BPS at all doses. The testis showed higher number of cell deaths, but also modified expression of apoptotic factors, oxidative stress responding genes and epigenetic-related factors. This indicates that prenatal exposure to BPS causes spermatogenic defects in the developing testis that lead to eventual reduction of sperm production and quality in adults.

Shi *et al.* (2019a)

The aim of this study was to assess the effect of BPA, BPE and BPS on the reproductive function of female CD-1 mice after prenatal exposure. Pregnant mice (n = 5/group) were exposed orally to 0, 0.5, 20 or 50 µg/kg bw/day of BPS from GD 11 until birth. At delivery, pups were sexed and the size of each litter was adjusted to 10 pups with 5 males and 5 females. One female per litter was euthanized at PND 4. The remaining female pups were examined for vaginal opening (indicative of puberty onset) and oestrous cyclicity. A 3, 6, and 9 months, female pups of each litter were mated with normal CD-1 males. Standard pregnancy parameters were recorded. The female pups were euthanized 2 weeks after parturition. Ovaries were prepared for analysis (mRNA expression and histology) and blood collected for hormones level quantification.

After prenatal exposure to 0.5 µg/kg bw/day of BPS, the vaginal opening was detected 1-2 days earlier than controls, whereas 20µg/kg bw/day exposure delayed it for 2.5 days. No effect was observed at the highest dose. However all doses resulted in irregular oestrous cycles, with several days in oestrus and dioestrus phases.

The mice were then mated at 3, 6 or 9 months. Whereas the controls and 3 months groups needed 2-5 days to show the vaginal plug, it took much longer in older mice. After 9 months, exposed mice showed lower mating success (equivalent of fertility index)

Table 130: Effects on time to vaginal plug and mating at different doses of BPS (Shi *et al*., 2019a)

Months	Time to vaginal plug in F1 females (days)				Successful mating in F1 females (%)			
	Control	0.5 µg/kg bw/d	20 µg/kg bw/d	50 µg/kg bw/d	Control	0.5 µg/kg bw/d	20 µg/kg bw/d	50 µg/kg bw/d
3	2.3 ± 0.8	3.3 ± 0.8	2.3 ± 0.8	2.8 ± 0.6	100.0	100.0	100.0	100.0
6	3.5 ± 0.5	13.5 ± 0.6	15.0 ± 5.4	7.6 ± 1.9	100.0	75.0	75.0	100.0
9	3.0 ± 0.4	17.7 ± 3.4	21.1 ± 3.8	19.7 ± 6.5	100.0	66.7	40.0	40.0

Pregnancy parameters were then observed. At 9 months, some pregnant females of all doses exposed groups exhibited parturition and nursing issues, resulting in total litter loss within 24h. A reduction of number of pups/litter at 9 months. This was also observed in controls, but less drastically than in exposed groups. The reduction was even significant in mice exposed to 20 µg/kg bw/day. However, the pup weight and sex ratio was not affected.

Table 131: Effects on number of F2 pups/litter at different doses of BPS (Shi *et al*., 2019a)

Months	Number of F2 pups/litter			
	Control	0.5 µg/kg bw/d	20 µg/kg bw/d	50 µg/kg bw/d
3	14.3 ± 1.3	12.0 ± 1.5	12.8 ± 1.7	11.0 ± 1.7
6	9.8 ± 1.7	7.8 ± 3.2	7.0 ± 2.4	12.3 ± 0.8

9	7.3 ± 0.6	2.0 ± 0.9	1.3 ± 1.3*	2.0 ± 2.0

* = $p < 0.05$

BPS exposure affects also the steroid hormones level, showing a significantly increase of serum testosterone after exposure to 50 µg/kg bw/day (trend increase at 0.5 and 20 µg/kg bw/day) at 9 months. 17β-estradiol was not affected.

The authors also assessed the effect of BPS exposure on the development of the follicles in PND 4 mice. Normally germ cell nests break apart to release individual oocytes and form primordial follicles after birth, a process mostly complete by PND 4 in mice. However, after 0.5 and 20 µg/kg bw/day of BPS exposure, significantly more germ cells remained in nest compared with control ovaries without affecting the number of primordial follicles. Moreover the number of primary follicles was significantly decreased in all exposed groups. Even if not significant, the number of secondary follicles showed a decreasing trend also. This shows that BPS exposure affects the ovaries follicles development, but also disrupts the oestrous cyclicity and decreases fertility.

Kolla *et al.* (2019)

The aim of this study was to assess the impact of BPS low-dose in utero exposure on the mammary gland morphology of male mice in foetus (E16), just before puberty (PND 24), and at adulthood (week 9). In a first experiment, pregnant CD-1 mice were exposed orally (on wafers) to 0, 2 or 200 µg/kg bw/day BPS. Some dams were euthanized at pregnancy day 16 to allow E16 foetal mammary glands examination. After birth, litters were culled to 10 pups on PND 1. One male from each litter was sacrificed at PND 24 (before puberty), the other males were euthanized at week 9 (adulthood), i.e. n = 20 in control group, n = 14 in low-dose group and n = 10 in high dose group. Whole mounts mammary glands were then collected and analysed.

Only modest effects were observed in embryonic males, such as more space between the cells, but no difference was observed on the expression of ERα nor AR in the E16 mammary gland. Prior to puberty, left mammary glands of males exposed to 200 µg/kg bw/days BPS showed significantly smaller ductal trees, indicating a possible suppression of growth of the ductal tree prior to puberty. However at adulthood, similarly to BPA, males exposed to 2 or 200 µg/kg bw/day had significantly larger epithelial trees. This suggests that the effects of BPS on growth of the mammary gland are most striking after puberty.

The authors performed then a second experiment to evaluate if BPS-treated males have a heightened sensitivity to hormones by performing a oestrogen challenge at puberty. Here pregnant CD-1 mice were exposed orally (pipet) from GD 9 to PND 2 to 0, 2, 200 or 2000 µg BPS/kg bw/day. Then at PND21, 2 males from each litter were selected randomly. One received vehicle (corn oil) and the other an oestrogen challenge (1 µg EE2/kg bw/day) for 10 days.

BPS did not affect the Anogenital index nor the seminal vesicle weight, two hormone-sensitive outcomes. However, the oestrogen challenge revealed a dose-specific effect on the response of the male mammary glands (increase of branching points, TEBs and ductal area at 200 and 2000 µg/kg bw/day, but decrease at 2 µg/kg bw/days). These two experiments provide evidence that exposure to BPS during early development disrupts male mammary gland morphology.

Shi *et al.* (2019b)

The aim of this study was to assess the transgenerational effects of BPA, BPE and BPS on the reproductive function of female CD-1 mice after prenatal exposure. F0 pregnant mice (n = 5/group) were exposed orally to 0, 0.5 or 50 µg/kg bw/day of BPS from GD 7 until birth. To generate F2 generation, F1 offspring were bred to other F1 mice from the same treatment group but different litter at 6-7 weeks. Similar breeding was done with F2 mice

to generate the F3 generation. One F3 female per litter was euthanized at PND 4. The remaining F3 female pups were examined for vaginal opening (indicative of puberty onset) and oestrous cyclicity. At 3, 6, and 9 months, female pups of each litter were mated with normal CD-1 males. Standard pregnancy parameters were recorded. The female pups were euthanized 2 weeks after parturition. Ovaries were prepared for analysis (mRNA expression and histology) and blood collected for hormones level quantification.

After prenatal exposure of F1 to BPS, F3 females showed a statistically earlier puberty onset. They showed also irregular oestrous cyclicity (not statistically significant). When bred, these F3 mice needed more time to receive the plug. Reduction of successful mating was observed at 9 month. Some mice also did not maintain the pregnancy at 6 and 9 months. Additionally parturition and/or nursing issues were observed at 9 month. Number of F4 pups, their body weight or sex ratio was not affected. No effect was observed on folliculogenesis.

Serum E2 level was increased after prenatal F1 exposure in F3 females at 3, 6 and 9 months (only significant in mice exposed to 50 µg/kg bw/day at 6 months).

Shi *et al.* (2019c)

The aim of this study was to assess the transgenerational effects of BPA, BPE and BPS on the reproductive function of male CD-1 mice after prenatal exposure. F0 pregnant mice (n = 5/group) were exposed orally to 0, 0.5 or 50 µg/kg bw/day of BPS from GD 7 until birth. To generate F2 generation, F1 offspring were bred to other F1 mice from the same treatment group but different litter at 6-7 weeks. Similar breeding was done with F2 mice to generate the F3 generation. One F3 male per litter was euthanized at PND 6 or PND 60. Testes were prepared for analysis (mRNA expression and histology). At PND 60, body and paired testes weight were recorded, blood collected for hormones level quantification and sperm counted.

After prenatal exposure of F1 to BPS, F3 males showed a statistically reduced body weight. Their sperm counts were significantly reduced, showing 40 and 48 % sperm in comparison with the control group, at 0.5 and 50 µg/kg bw/day, resp. Sperm motility was also significantly reduced at 0.5 µg/kg bw/day (but not at higher dose). The spermatogenesis was also disturbed, showing significantly more testis tubules in stages I-VI, and significantly less tubules in stage IX. Again, this effect was only observed at 0.5 µg/kg bw/day and not at 50 µg/kg bw/day.

Serum testosterone level was significantly decreased after prenatal F1 exposure in F3 males at 50 µg/kg bw/day. However, in adult testis, the expression of steroidogenesis hormones Star and Cyp19a1 were significantly higher at both doses. The authors also observed that the prenatal exposure in F0 affected in F3 the expression of several enzymes involved in epigenetic, such as DNA methyltransferases and histone methyltransferases.

Ullah *et al.* (2019a)

The aim of the study was to compare the effect of several bisphenols, i.e. BPA, BPS, BPB and BPF on the rat male reproductive system after prenatal exposure. Pregnant Sprague-Dawley rats (n = 8 per group) have been exposed from GD 1 until delivery to 0, 5, 25 and 50 µg/L bisphenols in drinking water. On PND 16, 2 males per litter were sacrificed for early development study (organs weight). The other pups (n = 8 per group) were euthanized at PND 80, blood was collected and reproductive tissues (testis, epididymis, seminal vesicle and prostate) were dissected out. The level of antioxidant enzymes activity (catalase CAT, peroxidase POD, superoxide dismutase SOD), reactive oxygen species (ROS) have been measured in testis tissues. Sperm motility and viability was also examined, and the plasma level of some hormones has been determined (testosterone, oestrogen, LH and FSH).

Prenatal exposure to BPS had no effect on dams nor litter parameters (including AGD or nipple retention). No significant effect was also observed in young males at PND 16 on organ or body weight. However at PND 80, the body weight was significantly higher and the weight of seminal vesicle significantly reduced in rats exposed to 50 µg/L BPS.

Table 132: Effects on seminal vesicle and prostate weight at different doses of BPS (Ullah *et al*., 2019)

Dose (µg/L)	PND80 Body weight (g)	Seminal vesicle weight (g)	Prostate weight (g)
0	192.26 ± 0.70	1.17 ± 0.01	0.53 ± 0.05
5	204.35 ± 3.98	1.16 ± 0.01	0.54 ± 0.02
25	200.37 ± 7.09	1.14 ± 0.02	0.47 ± 0.04
50	210.41 ±6.31*	1.13 ± 0.06*	0.48 ± 0.03

* = $p < 0.05$

After prenatal to BPS, SOD, POD and CAT activities were dose-dependently reduced (significantly at highest doses), whereas the LPO and ROS levels were dose-dependently increased (significantly at 50 µg/L) in the testicular tissues.

Table 133: Effects on antioxidant enzyme activities and reactive oxygen species at different doses of BPS (Ullah *et al*., 2019)

Dose (µg/L)	CAT (U/mg protein)	SOD (U/mg protein)	POD (U/mg protein)	LPO (U/mg protein)	ROS (U/mg protein)
0	8.28 ± 0.25	33.35 ± 0.29	7.26 ± 0.25	8.35 ± 0.31	95.71 ± 2.54
5	7.28 ± 0.48	33.61 ± 0.17	6.44 ± 019	8.09 ± 0.35	95.85 ± 2.30
25	6.86 ± 0.37	32.65 ± 0.16	6.27 ± 0.22*	8.37 ± 0.49	102.25 ± 2.80
50	6.56 ± 0.46*	31.58 ± 0.15**	6.25 ± 0.20*	10.01 ± 0.25**	118.86 ± 7.06**

* = $p < 0.05$, ** = $p < 0.01$

Sperm parameters was also affected after chronic BPS exposure. Motile sperms and daily sperm production was dose-dependently reduced, as the numbers of spermatogonia, spermatocytes and spermatids. Finally, histopathological analysis of the testes showed a dose-dependent reduction of the epithelial height of the seminiferous tubules. This confirms the oestrogenic and anti-androgenic effects of BPS and its potential effect on the male reproductive system of mammals.

Table 134: Effects on sperm at different doses of BPS (Ullah *et al*., 2019)

Dose (µg/L)	Daily sperm production (x 10^6)	Caput/carpus epididymis sperm number (x 10^6/organ)	Spermatogonia (n)	Spermatocytes (n)	Spermatids (n)	Motile sperm (%)
0	73.37 ± 0.6	303.17 ± 1.39	64.66 ± 0.61	76.11 ± 1.05	256.25 ± 1.77	79.56 ± 0.54
5	63.28 ± 1.5	295.57 ± 1.57	62.40 ± 1.04	73.72 ± 1.31	251.06 ± 2.75	78.12 ± 0.51
25	62.33 ± 0.2	293.43 ± 1.79*	62.62 ± 1.14	72.83 ± 1.22	247.31 ± 2.51	75.27 ± 1.10*
50	61.26 ± 0.6*	293.19 ± 1.92*	60.55 ± 0.85*	71.11 ± 1.23*	243.01 ± 2.03**	74.28 ± 0.74**

* = $p < 0.05$, ** = $p < 0.01$

The prenatal exposure to BPS also strongly affected the morphology of testicular cells, mainly on the seminiferous tubules. On the other side, the caput and cauda epididymis did not show any histological changes.

Table 135: Effects on testicular cells at different doses of BPS (Ullah *et al.*, 2019)

Dose (µg/L)	Area of seminiferous tubule (%)	Area of interstitial space (%)	Area of lumen (%)	Area of epithelium (%)	Seminiferous tubule diameter (µm)	Seminiferous tubule epithelial height (µm)
0	88.12 ± 0.4	14.17 ± 0.5	15.65 ± 0.1	84.56 ± 0.3	226.32 ± 2.8	59.03 ± 0.2
5	86.37 ± 0.2	13.17 ± 0.3	15.67 ± 0.1	84.54 ± 0.8	226.24 ± 0.3	58.47 ± 0.1
25	84.74 ± 0.5**	12.95 ± 0.5	15.37 ± 0.3	84.72 ± 0.7	224.17 ± 0.4	58.09 ± 0.6
50	83.86 ± 0.5***	12.15 ± 0.2*	15.04 ± 0.1***	84.72 ± 0.1	222.32 ± 0.5*	61.14 ± 0.3**

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Prenatal exposure to BPS resulted in a clear dose-dependent increase of estradiol and decrease of testosterone, LH and FSH concentrations in the plasma.

Table 136: Effects on hormone levels at different doses of BPS (Ullah *et al.*, 2019)

Dose (µg/L)	Testosterone (ng/mL)	Estradiol (pg/mL)	LH (ng/mL)	FSH (mIU/mL)
0	4.82 ± 0.04	1.27 ± 0.09	1.67 ± 0.06	1.41 ± 0.19
5	4.39 ± 0.55	1.43 ± 0.03	1.57 ± 0.08	0.90 ± 0.14
25	3.71 ± 0.28	2.54 ± 0.48	1.46 ± 0.03	0.96 ± 0.24
50	3.45 ± 0.43*	3.79 ± 0.27***	1.21 ± 0.02***	0.53 ± 0.05**

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Ullah *et al.* (2019b)

The authors assessed the effects of BPA and its analogues BPB, BPF and BPS on SD rats sperm *in vitro* and in vivo, with a particular focus on oxidative stress and DNA damage.

Fresh rat sperm has been incubated *in vitro* for 2 hours with 0, 1, 10 and 100 ng/mL of bisphenol. Exposure to 100 µg/L increased significantly the SOD and LPO activities, as well as the level of ROS ($p < 0.05$). At lower concentrations, a slight but not significant increase was also observed, excepted for ROS level at 1 ng/mL, showing a slightly lower concentration. A comet assay performed on the sperm revealed an increased DNA fragmentation in spermatozoa nuclei exposed to 100 µg/L BPS. No difference has been observed at lower concentration.

Sprague-Dawley adult male rats (n = 7/group) were then exposed *in vivo* to 0, 5, 25 and 50 mg/kg bw/day BPS (gavage) for 28-days, similarly to OECD Test Guideline. The comet assay has been repeated with the sperm of these rats and showed a significant increase of DNA damage in rats exposed to the highest dose. Additionally the daily sperm production was significantly reduced after exposure to 50 mg/kg/day ($p<0.05$). The sperm motility decreased dose-dependently, but not significantly in rats exposed to BPS (87.8, 85.1, 84.9 and 83.7%, respectively).

This study shows that BPS has the potential to damage DNA, induce oxidative stress and affect sperm production and quality in rat.

Da Silva *et al.* (2019)

The authors assessed the effects of BPS on lipid metabolism and behaviour in Wistar rats pups exposed during the pregnancy and lactation.

Pregnant dams were treated by gavage with 0, 10 or 50 µg/kg bw/d BPS from GD1 and until weaning (n = 10 dams/dose). At birth, litters were adjusted to 8 pups per dam (4 males and 4 females). At weaning, the dam and 1 animal of each sex per litter was

euthanized (i.e., 10 per dose per sex). The others were maintained until PND180 (i.e., 30 per dose per sex).

BPS exposure did not affect the body weight of dams. At the highest dose, the level of testosterone in plasma was significantly reduced (0.5* vs 0.8 ng/mL in control). Estradiol level trends to be lower in low dose group (57.7 vs 76.6 pg/mL in control), as well as the thyroid hormones (Total T3: 62.5, 55.6 and 46.4 ng/dL, Free T4: 0.74, 0.65 and 0.57 ng/dL after exposure to 0, 10 and 50 µg/kg bw/d, respectively).

In pups also, no effect on body weight was observed at weaning, nor in adulthood. However, BPS exposure affected the structure of brown adipose tissue in females, but not in males. On the other hand, males exposed to BPS showed an increased anxiety-like behaviour, whereas females' behaviour was not affected.

Finally in pups, the changes observed on plasma hormonal or biochemical parameters were not consistent between weaning and PND180; the progesterone level trend was lower in both males and females, with a significant decrease at PND180 in females exposed to 10 µg/kg bw/d BPS (25.1 vs 45.9 mg/mL). Testosterone level trends to be lower in males (5.3, 4.9 and 4.6 ng/mL at PND180), whereas Estradiol level trends to be higher in females (89.9, 65.2 and 103.0 pg/mL at PND180).

This study shows that BPS affects the hormone levels and behaviour of progeny exposed during the pregnancy and the lactation.

Kolla and Vandenberg (2019)

The aim of the study was to see if BPS perinatal exposure sensitizes the animal to subsequent oestrogen exposure, particularly the effect on the mammary gland morphology. CD-1 mice dams were orally exposed to 0, 2, 200 or 2000 µg/kg bw/d BPS from GD9 to PND2, and then female pups were challenged with 1 µg ethinyl estradiol/kg bw/d or with vehicle from PND21 to PND30 (n =7-10 per group with or without challenge).

A slight significant decrease of the ductal area of mammary gland was observed only after gestational exposure to 2 µg/kg bw/d BPS. There was no effect of peripubertal ethinyl estradiol challenge. Neither BPS nor estradiol exposure affected the terminal buds of mammary gland. Expression of ER gene was enhanced only after exposure to 200 µg/kg bw/d BPS, and the expression of PR gene was not affected. Whereas BPS did not affect the timing of vaginal opening, this endpoint was shortened after ethinyl estradiol challenge (from PND 27.6 to PND 25.2), but not significantly.

Therefore, BPS seems not to sensitize the female to an estrogenic challenge administered during the peripubertal period.

Gao *et al.* (2020)

The aim of this study was to investigate *in vitro* the activity of BPA and BPS on PPARγ pathway in human macrophages (see results above), and to confirm this interaction *in vivo* in mice.

The *in vitro* results showed that BPS could activate PPARγ and upregulate its target genes in human macrophages. The authors then wanted to see if PPARγ pathway-related effects could be accordingly observed *in vivo*. Therefore, they exposed for 10 weeks adult C57BL/6 female mice to 0, 50 or 5000 µg/kg bw/day BPS via drinking water (n = 6 mice/group). BPS exposure had no effect on the mouse body weight. Additionally, a glucose tolerance test was conducted, but no difference was observed. The authors concluded that BPS, as BPA, has no effect on the glucose metabolism homeostasis.

Then the authors assessed the expression of some PPARγ-target genes in mouse liver after exposure to BPS. Contrary to *in vitro* data, BPS did not enhance the expression of PPARγ mRNA nor CD36 mRNA. However, a significant increase of FABP4 was observed at high dose. LXRα expression was also significantly higher at both doses (dose-dependent).

Finally, the authors observed that BPS exposure significantly disturbed the metabolic profile.

Zhang *et al.* (2020b)

The authors investigated the effect of maternal exposure to BPS on the oocyte's maturation in F1 and F2 mice. ICR mice were exposed orally to 0, 2 or 10 µg/kg bw/d BPS from GD 12 to 15, i.e., a critical stage for the oocyte development in female foetuses. Ovaries from GD 15 foetuses, PND 3 and PND 21 female offspring were collected and analysed for oocytes maturation. F1 females were mated with unexposed F1 males to produce F2, whom ovaries were also collected and analysed at PND 3 and 21.

The authors observed a clear disturbance of oocyte maturation. At GD 15, the BPS treated foetuses showed a significant accelerated meiosis. This was confirmed at PND 3 and PND 21 with significant increase of primordial follicles, even if the total number of oocytes were not changed. This reduced significantly the fertilization and embryo development (rate of 2-cell embryos: 88.3, 67.6 and 55.8 % at 0, 2 and 10 µg BPS/kg bw/d, resp.). Similar results were observed in 4-cells embryos (85.5, 68.9 and 47.0% at 0, 2 and 10 µg BPS/kg bw/d, resp.) and blastocyst rate (76.9, 65.6 and 56.1% at 0, 2 and 10 µg BPS/kg bw/d, resp.). Some epigenetic modifications were also observed.

In F2, the litter size, body weight nor sex ratio were not affected. However, some slight effects on oocyte maturation were also observed at PND 3.

Ijaz *et al.* (2020)

The aim of this study was to assess the effect of BPA analogues BPB, BPF and BPS on the female reproductive health of rats.

Healthy adult female Sprague-Dawley rats were exposed through intraperitoneal injections to 0, 0.05, 0.5., 5 and 50 mg/kg bw/d BPS for 28 days (n=10/group). The exposed rats showed a slightly increased weight gain than the control (not significant) but ovary and uterine weights were significantly reduced (ovaries: 0.184, 0.174, 0.164, 0.145 and 0.122* g; uterus (absolute): 256.4, 244.2*, 240.6*, 236.0* and 215.0* mg; uterus (relative): 1.40, 1.59, 1.63, 1.63* and 1.40* mg/g, after exposure to 0.05, 5 and 50 mg/kg bw/d, respectively).

Analysis revealed an enhanced oxidative stress in ovarian tissues after BPS exposure, with a decrease of CAT and SOD activities (CAT: 1.90, 1.20, 0.97, 0.89 and 0.41* U/mg protein, SOD: 6.51, 4.56*, 5.03, 4.94 and 4.84* U/mg protein) and important increase of ROS level: 2.79, 3.47, 4.09*, 4.22* and 4.74* U/min tissue).

The level of hormones in the plasma was also strongly affected after BPS exposure, showing a strong testosterone increase (0.59* and 1.22* ng/mL in rats exposed to 5 and 50 mg/kg vs 0.34 ng/mL in the control), a strong estradiol decrease (1.55* and 1.05* pg/mL in rats exposed to 5 and 50 mg/kg vs 1.90 pg/mL in the control). The plasma hormone level decreased also significantly for progesterone (0.26*, 0.41* and 0.37* ng/mL in rats exposed to 0.5., 5 and 50 mg/kg vs 0.56 ng/mL in the control), LH (2.23* and 2.01* ng/mL in rats exposed to 5 and 50 mg/kg vs 2.64 ng/mL in the control) and FSH (4.24* and 3.90* mIU/mL in rats exposed to 5 and 50 mg/kg vs 4.68 mIU/mL in the control).

Finally BPS exposure strongly affects folliculogenesis, showing a strong decrease of corpus luteum (12.6, 11.6, 10.8*, 8.0*, and 6.1*) and antral follicles (10.6, 11.4, 11.2, 8.8* and 6.4*), whereas the number of atretic follicles is significantly increased (2.2, 2.8, 3.0, 3.8* and 6.4*, after exposure to 0.05, 5 and 50 mg/kg bw/d, respectively). The diameter of antral follicle was also significantly increased at all doses.

This study confirms that pre-pubertal exposure to BPS can induce oxidative stress, disturb the hormones production and affects the ovarian folliculogenesis in rats.

Liu *et al.* (2021)

The authors exposed CD-1 mice (intraperitoneal injection) to 0, 2 or 10 µg/kg bw/d BPS from PND 1 to PND 3 to investigate the effects on oocyte maturation. At PND 3, ovaries were collected and scored for the number of oocytes in or already developed in primordial follicles.

BPS exposure did not affect the total number of oocytes but accelerated the development of primordial follicles in a dose-dependent manner (33.55, 49.02 and 52.35 % at 0, 2 or 10 µg BPS /kg bw/d, resp) with a corresponding reduction of percentage of oocytes in cysts. Further analyses *in vitro* using specific antagonists on cultured ovaries showed that this abnormal germ cell cyst breakdown depended on ERs and on c-Jun N-terminal kinases (JNK) phosphorylation.

In PND 21 mice exposed to 10 µg BPS /kg bw/d, nb of primary follicles were significantly increased (132.0 vs 68.7 in control group), whereas a significant reduction was observed for secondary (94.7 vs 148.3) and antral follicles (53.7 vs 120.3). The number of primordial follicles was also reduced, but non-significantly. Similar trend was observed at 2 µg BPS/kg bw/d.

Ullah *et al.* (2021)

The aim of the study was to assess the effect of chronic exposure of BPS on the rat male reproductive system *in vivo*. The experiment is similar to the study of the same group published in 2018 (Ullah *et al.*, 2018b), but including a lower dose and including histopathological data to confirm the impact of BPS on male reproductive system.

Adult male rats (n = 10 per group) have been exposed to 0, 0.5, 5 and 50 µg/L BPS from PND 23 and for 48 weeks, daily via water. Animals were then killed, blood was collected, and reproductive tissues (testis, epididymis, seminal vesicle and prostate) were dissected out. Histopathological analysis of the testicular tissues have been performed. The level of antioxidant enzymes activity (catalase CAT, peroxidase POD, superoxide dismutase SOD), reactive oxygen species (ROS) have been measured. Sperm motility and viability was also examined, and the plasma level of some hormones has been determined (testosterone, oestrogen, LH and FSH).

Results were almost similar to the ones observed in Ullah *et al* (2018b), showing a significant higher body weight at highest dose, but lower relative epididymis weight and seminal vesicle weight (absolute and relative). This is reflected in a lower Gonadosomatic index (GSI, 0.64* at high dose vs 0.68 in control group). As in the previous study, BPS exposure increased the oxidative stress, with significant decrease of CAT and POD activities whereas LPO and ROS level increased significantly. Contrary to the study from 2018, the level of SOD activity was also significantly decreased in high dose group.

Level of hormones was also affected by BPS exposure in a dose dependent-manner, with a significant decrease of testosterone (12.36, 11.77, 10.56** and 9.76***) and a significant increase of estradiol (2.71, 3.52, 3.69* and 4.11**, at 0, 0.5, 5 and 25 µg/L BPS, respectively). Contrary to the previous study (Ullah *et al.*, 2018b), the level of LH and FSH were significantly decreased at the highest dose (LH: 1.45* vs 1.84 ng/mL, FSH 0.48 vs 0.79* mIU/mL in the control group). As it was previously reported, sperm motility and daily sperm production were significantly reduced at high dose, and the spermatogenesis was strongly affected. Effect of BPS on the testicular tissues has been confirmed histologically.

Conclusion: OECD CF level 4 data

Fertility concern (e.g., fertility index) in OECD TG 421 and 422 tests and other literature studies (see above).

Disturbed balance of sex hormones. Generally showing a decrease of testosterone and progesterone levels. Estradiol seems also increased, but the levels detected were below or at the limit of sensitivity of the used assay for controls and treated groups. Therefore, the eMSCA considers with caution the reliability of the estradiol level values.

Some adverse effects are observed on organs linked to the endocrine system in the OECD TG 408 (but not in OECD TG 414 nor 407), and in several other literature studies (testis, seminal vesicle, ovaries, adrenal glands, uterus…).

Change in mammary gland morphology observed in OECD TG 408 and TG 422 (in males only), as well as in several literature studies (females and males).

Effects on male reproduction observed in several studies, including reduction of sperm count and motility, weight of reproductive organs (testis, seminal vesicles, prostate), and testis histopathology.

Effects on female reproduction observed in OECD TG 421 and TG 422, and several literature studies, including affected puberty onset, folliculogenesis and disturbance of oestrous cyclicity.

OECD Level 5: *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism

Table 137: Overview of endocrine disruption – human health (OECD Level 5, Registration dossier)

Method	Result (*positive* / *negative* / *trend*)	Reference
Extended-one-generation reproductive toxicity study (EOGRTS) with F2, developmental neurotoxicity (DNT) (cohorts 2A and 2B) and developmental immunotoxicity (DIT) (cohort 3) Rats / SD F0 generation: 24/sex/dose F1 generation: 20/sex/dose for cohort 1A, 24/sex/dose for cohort 1B, 10/sex/dose for cohorts 2A, 2B and 3 Gavage Following OECD TG 443 GLP Doses: 0, 20, 60 and 180 mg/kg bw/d Duration of exposure: Minimum 10 w after the beginning of exposure, males and females from the same dose group were mated. Shortly before weaning of the F1 pups, the	Parental generation: Bw: sign. higher only in ♀ during the in-life period (D 7 and 14 in the mid dose) ♂ reproductive data: sign. reduction in % of motile sperm in all tested dose group (88, 84*, 85* and 86* %, resp at 0, 20, 60 and 180 mg/kg bw/d) ♀ reproductive data: mean duration of oestrus cycle : sign. increase at 180 mg/kg bw/d (4.1* d vs 3.9d in control) Mean nb of implantation site: reduced at the highest dose (14.3 vs 15.3 in control) Mean nb of post-implantation loss sign. affected (1.5** vs 0.5 in control) Necropsy: enlarged cecum and changes in kidneys observed in ♂ at 180 mg/kg bw/d F1 pups: Sign. lower tot. nb. of liveborn pups (285* at 180 mg/kg bw/d vs 340 in control) and sign. higher nb of stillborn pups (8* at 180 mg/kg bw/d vs 2 in control) Cohort 1A: Final body weight (FBW): slightly reduced at 180 mg/kg bw/d in ♂	REACH registration dossier, Unpublished study report, 2020

F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups. Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after weaning, placed into cohorts.	BW: in ♀, body weight was sign. higher at D14 and D28 in the mid and high dose groups Necropsy: adrenal glands, kidneys, liver, spleen, thymus and prostate showed a significant deviation in absolute (abs.) or rela value An atrophy of the mammary gland was noted in ♂ of the highest dose <u>Cohort 1B</u>: higher mean duration of oestrus cycle at 180 mg/kg bw/d (4.1[11] d vs 3.9 d in control) Lower mean nb of implantation sites and sign. higher incidence of post-implantation loss at 180 mg/kg bw/d Necropsy: FBW slightly reduced in ♂ at the highest dose and a few organ weights modified <u>Cohort 2A</u>: Auditory startle response, home cage observations, open field observations, sensorimotor tests/reflexes, motor activity measurements and learning and memory tests: unaffected <u>Cohort 2B</u>: Necropsy examination: no effects observed <u>Cohort 3</u>: Clinical and bw examination: unaffected Necropsy examination: sign lower rela thymus weight at 180 mg/kg bw/d T-cell dependent antibody response: slight change in the low and mid dose groups in ♀ <u>F2 pups</u>: Decrease tot. nb of pups delivered at the highest dose	

<u>Extended-one generation reproductive toxicity study (REACH registration dossier: Unpublished study report, 2019)</u>

Test type

OECD TG 443

GLP

Test substance

- BPS

[11] RAC (2020): Note that this specific effect was initially mentioned to be 4.5 days at the highest dose tested in the CLH dossier and on IUCLID, but during the PC was noted that this effect size was incorrect and should be changed into 4.1 days. The information in IUCLID was updated to this regard as well (ECHA dissemination website consulted on 09-09-2020)

- *Degree of purity:* see confidential annex

Test animals

- *Species/strain/sex:* rat / SD / both sexes
- *No. of animals per sex per dose:*
    - F0 generation parental animals: 24/sex/dose
    - F1 rearing animals, cohort 1A (reproductive PND 90): 20/sex/dose (1 male and 1 female pup/litter)
    - F1 rearing animals, cohort 1B (F1 generation parental animals): 24/sex/dose (1 male and 1 female pup/litter)
    - F1 rearing animals, cohort 2A (neurotoxicity PND 75-90): 10/sex/dose (1 male or 1 female pup/litter)
    - F1 rearing animals, cohort 2B (neurotoxicity PND 22): 10/sex/dose (1 male or 1 female pup/litter)
    - F1 rearing animals, cohort 3 (immunotoxicity): 10/sex/dose (1 male or 1 female pup/litter)
    - Number of samples for thyroid hormones (PND 4): 10/sex/dose
    - Number of samples for thyroid hormones and pathology (PND 22): 10/sex/dose
- *Age and weight at the study initiation:* 5 weeks (for females) and 6 weeks old (for males)

Administration/exposure

- *route of administration:* gavage
- *duration and frequency of test/exposure period:* Daily, minimum 10 weeks after the beginning of exposure, males and females from the same dose group were mated. Shortly before weaning of the F1 pups, the F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups.

  Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after weaning, placed into cohorts.
    - Cohort 1A were sacrificed approximately at 13 weeks old.
    - Cohort 1B were selected to produce F2 pups: minimum 10 weeks after assignment of the F1 parental animals, the males and females were mated. As for the F0 generation, F1 males were sacrificed shortly before weaning and F1 females shortly after the weaning.
    - Cohort 2A were selected to examine neurotoxicity parameters and were sacrificed approximately at 11 weeks old.
    - Cohort 2B were selected to examine neurotoxicity parameters and were sacrificed approximately at 3 weeks old.
    - Cohort 3 were selected to examine immunotoxicity parameters and were sacrificed approximately at 8-9 weeks old.

Pups, which were not chosen for the cohorts or for blood sampling on PND 4 and 22, were sacrificed after standardization or weaning.
All pups were macroscopically examined and only animals with notable findings or abnormalities were further evaluated.

- *doses/concentration levels:* 0, 20, 60 and 180 mg/kg bw/d
- *vehicle:* 0.5% CMC

Results and discussion

For P adults:

- *number of animals at the start of the test:* 24/sex/dose
- *time of death during the study and whether animals survived to termination:* 1 female of the low dose group was sacrificed moribund on D 63, due to clinical signs (piloerection, encrusted nose, unsteady gait, hypothermia, poor general condition, laboured respiration, pale skin). The necropsy of this animal revealed findings consistent with a gavage error.
- *clinical observations:* transient salivation was observed immediately after dosing in 13 males and 6 females of the highest dose during the first weeks of exposure. However, the maternal care was not affected during gestation and lactation periods.
- *water consumption:* significantly higher at the highest dose in both sexes
- *food consumption:* significantly increased in females exposed to 180 mg/kg bw/d during premating period and during GD 14-20 (up to 36 % and 8 %, respectively).

Table 138: Mean food consumption per animal and per day (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
In-life period D 0-69	27.7	28.3	29.2	29.5	16.9	17.4	18.5	19.8**
GD 0-20	-	-	-	-	23.4	24.2	24.3	24.2
LD 1-21	-	-	-	-	64.5	63.9	66.9	65.1

**: $p<0.01$

- *body weight data:*

Table 139: Body weight data in males (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
In-life period	D 0	188.4	187.9	188.6	190.0
	D 21	350.9	347.2	357.1	358.8
	D 42	433.4	426.7	435.5	434.4
	D 63	490.9	482.5	487.1	479.1
Parental period	W 0	492.5	487.2	488.4	478.7
	W 2	523.0	513.9	520.1	506.9
	W 5	544.1	535.9	563.7	551.7

Table 140: Body weight data in females (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
In-life period	D 0	115.3	115.9	116.5	115.3
	D 7	141.3	144.8	148.3*	145.2
	D 14	162.8	169.9	172.4*	169.6
	D 21	184.5	192.6	193.3	190.7
	D 42	225.5	234.0	235.2	233.2
	D 63	249.1	259.0	258.1	256.4
Gestation period	GD 0	256.2	262.9	261.9	257.5
	GD 14	323.6	329.9	329.5	323.4
	GD 20	402.4	403.6	405.6	398.0
Lactation period	LD 0	300.8	310.9	310.2	304.7
	LD 10	336.2	337.4	344.2	340.9
	LD 21	316.6	324.8	327.8	322.5

*: $p<0.05$

- *haematological and clinical biochemistry findings:* significant change was only noted for MCH in males (1.07, 1.06, 1.09 and 1.11* fmol resp. at 0, 20, 60 and 180 mg/kg bw/d). Enzymes were not affected

Table 141: Enzymes data

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
ALT (µkat/l)	0.72	0.73	0.74	2.60[A]	0.77	0.70	0.72	0.81
AST (µkat/l)	1.99	2.12	1.97	12.29[B]	1.78	1.70	1.91	1.93
ALP (µkat/l)	1.32	1.36	1.24	1.45	1.21	1.21	1.10	1.48
GGT_C (nkat/l)	25	25	25	25	25	25	25	25

[A:] S.d for ALT : 0.13, 0.12, 0.13 and 5.68 in males, respectively at 0, 20, 60 and 180 mg/kg bw/d

[B]: S.d for AST : 0.45, 0.50, 0.63 and 32.80 in males, respectively at 0, 20, 60 and 180 mg/kg bw/d

- *thyroid hormones:* no significant changes were observed
  - *T4:* 56.15, 51.97, 54.31 and 53.04 nmol/l in males and 35.24, 37.40, 36.21 and 33.43 nmol/L in females, respectively at 0, 20, 60 and 180 mg/kg bw/d.
  - *TSH:* 8.47, 9.40, 9.05 and 8.65 µg/l in males and 5.50, 5.21, 5.54 and 4.52 µg/l in females, respectively at 0, 20, 60 and 180 mg/kg bw/d.
- *effects on sperm:*
  - *% of motile sperm:* 88, 84*, 85* and 86* % respectively at 0, 20, 60 and 180 mg/kg bw/d.
  - *Tot. spermatids/gram testis:* 100 in control vs 104 Mio/g at the highest dose
  - *Tot. sperms/gram cauda epididymis:* 732 in control vs 728 Mio/g at the highest dose
  - *% of abnormal sperms:* 5.6 in control vs 5.5 % at the highest dose
- *fertility data for males:*
  - *male mating index:* 100 % for all tested and control groups

- *male fertility index (number of males with females pregnant/number of males placed with females):* 96 (23/24), 91 (21/23), 100 (24/24) and 96 % (23/24) resp. at 0, 20, 60 and 180 mg/kg bw/d.

- *female reproduction data:*

Table 142: Female reproduction and delivery data

Dose level (in mg/kg bw/d)	0	20	60	180
Females mated	24	23	24	24
Female mating index (in %)	100	100	100	100
Mean mating day until DPC 0	2.0	2.3	2.2	2.3
Female fertility index (in %)	96	91	100	96
Nb. of females with liveborn pups	23	21	24	23
Nb. of females with stillborn pups	2	4	2	4
Nb. of females with all stillborn	0	0	0	0

- *number of P females cycling normally and cycle length:* mean duration of oestrus cycle : 3.9, 3.9, 3.9 and 4.1* d resp. at 0, 20, 60 and 180 mg/kg bw/d
  Mean number of days in stage: prooestrus : 2.21 d at 180 mg/kg bw/d vs 4.67 d in control
  Mean number of days in stage: oestrus : 5.17 d at 180 mg/kg bw/d vs 5.12 d in control
  Mean number of days in stage: metoestrus : 5.87 d at 180 mg/kg bw/d vs 5.83 d in control
  Mean number of days in stage: dioestrus : 9.04 d at 180 mg/kg bw/d vs 6.33 d in control
  At 20 mg/kg bw/d, 1 female exhibited a mean of cycle length of 5.3 days, and one other female had a mean cycle length of 4.0 days however this female showed 1 oestrus cycle with a dioestrus period of 9 days.
  At 180 mg/kg bw/d, 2 females exhibited a mean cycle length of 4.7 and 5.0 days. This last one had one cycle with a dioestrus period of 5 days.
- *duration of gestation (calculated from day 0 of pregnancy):* 22.0 d in all tested and control groups.
- *number of implantations, corpora lutea, litter size*
  - *Total number of implantation sites:* 353, 310, 357 and 328 resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Mean number of implantation sites:* 15.3, 14.8, 14.9 and 14.3 resp. at 0, 20, 60 and 180 mg/kg bw/d
- *number of pre- and post-implantation loss*
  - *Total number of post implantation loss:* 11, 16, 32 and 35 resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Mean number of post implantation loss:* 0.5, 0.8, 1.3* and 1.5** resp. at 0, 20, 60 and 180 mg/kg bw/d

- *Mean % of post implantation loss:* 3.1, 5.9, 9.4* and 10.5** % resp. at 0, 20, 60 and 180 mg/kg bw/d

- *necropsy findings:* Enlarged cecum was observed in 3 males of the highest dose and enlarged kidneys was noted in 6 males of the highest dose. Other findings were observed individually or equally distributed.
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Table 143: Organ weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (in g)		521.575	514.408	521.35	507.229	272.125	278.826	275.429	273.408
Adrenal glands	Abs (mg)	54.0	55.958	58.75*	60.625*	80.208	70.391	77.208	71.625
	Rela	0.01	0.011	0.011*	0.012**	0.029	0.025	0.028	0.026
Brain	Abs (g)	2.293	2.27	2.238	2.256	2.018	2.03	2.036	2.043
	Rela	0.443	0.448	0.431	0.449	0.745	0.732	0.741	0.751
Heart	Abs (g)	1.667	1.708	1.698	1.718	1.145	1.178	1.198	1.22
	Rela	0.32	0.334	0.327	0.338	0.422	0.424	0.435	0.448
Kidneys	Abs (g)	3.543	3.391	3.673	4.124**	2.083	2.135	2.137	2.148
	Rela	0.68	0.663	0.705	0.817**	0.767	0.768	0.776	0.787
Liver	Abs (g)	12.572	13.298	13.003	12.46	8.08	8.259	8.348	8.695
	Rela	2.413	2.575	2.491	2.455	2.968	2.964	3.029	3.181**
Pituitary gland	Abs (mg)	13.542	13.583	13.875	14.417	14.375	15.13	14.833	14.625
	Rela	0.003	0.003	0.003	0.003	0.005	0.005	0.005	0.005
Spleen	Abs (g)	0.776	0.814	0.828	0.766	0.536	0.564	0.583	0.531
	Rela	0.15	0.159	0.159	0.152	0.197	0.204	0.213	0.195
Thymus	Abs (mg)	250.167	283.375	283.292 *	233.708	239.167	224.391	233.625	218.875
	Rela	0.048	0.056	0.054*	0.046	0.088	0.081	0.085	0.08
Thyroid glands	Abs (mg)	24.833	25.625	24.875	25.625	15.625	18.13	17.125	16.542
	Rela	0.005	0.005	0.005	0.005	0.006	0.007	0.006	0.006
Cauda epididymis	Abs (g)	0.548	0.559	0.552	0.537	-	-	-	-
	Rela	0.106	0.111	0.106	0.107	-	-	-	-
Epididymis	Abs (g)	1.304	1.328	1.3	1.3	-	-	-	-
	Rela	0.252	0.263	0.25	0.258	-	-	-	-
Prostate	Abs (g)	1.478	1.423	1.454	1.335	-	-	-	-
	Rela	0.286	0.281	0.281	0.266	-	-	-	-
Sem. ves.	Abs (g)	1.924	1.788	1.847	1.834	-	-	-	-
	Rela	0.37	0.352	0.355	0.365	-	-	-	-
Testes	Abs (g)	3.642	3.739	3.53	3.659	-	-	-	-
	Rela	0.703	0.739	0.679	0.726	-	-	-	-
Ovaries	Abs (mg)	-	-	-	-	102.667	111.13	106.0	104.042
	Rela	-	-	-	-	0.038	0.04	0.039	0.038
Uterus	Abs (g)	-	-	-	-	0.728	0.709	0.75	0.737
	Rela	-	-	-	-	0.268	0.255	0.274	0.273

*: p<0.05 ; ** : p<0.01

histopathological findings: nature and severity:

Table 144: Incidence of microscopic findings

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
Kidneys									
Nb of animals examined		24	24	24	24	20	4	3	21
Mineralisation, medulla	Inc.	0	0	1	21	14	2	1	15
	Grade 1			1	11				
	Grade 2				7				
	Grade 3				1				
	Grade 4				2				
Nuclear crowding	Inc.	0	0	0	22	0	0	0	0
	Grade 1				11				
	Grade 2				8				
	Grade 3				3				
Dilatation, tubular	Inc.	0	0	0	13	0	0	0	0
	Grade 1				7				
	Grade 2				6				

For F1 pups/litters:

- *mean number of live pups (litter size):*

Table 145: Litter data

Dose level (in mg/kg bw/d)	0	20	60	180
Tot. nb of pups delivered	342	294	325	293
Mean nb of pups delivered	14.9	14.0	13.5	12.7
Nb of litters	23	21	24	23
Nb of liveborn	340	289	322	285*
Nb of stillborn	2	5	3	8*

*: $p<0.05$

At 20 mg/kg bw/d, 3 females had a lower number of live pups (1 with 9 live pups (0 dead pups), 1 with 3 live pups (0 dead pups) and 1 with 5 live pups (1 dead pups)).

At 60 mg/kg bw/d, 3 females had a lower number of live pups (2 with 9 live pups (0 dead pups) and 1 with 5 live pups (1 dead pups).

At 180 mg/kg bw/d, 3 females had a lower number of live pups (2 with 9 live pups and 1 with 3 live pups).

Table 146: Mean number of live pups/litters

Dose level (in mg/kg bw/d)	0	20	60	180
D1	14.7	13.5	13.4	12.4
D4 (pre-culling)	14.6	13.3	13.3	12.3
D4 (post-culling)	10.0	9.4	9.7	9.6
D21	10.0	9.4	9.7	9.6

- *sex ratio:* 53.8/46.2, 51.6/48.4, 46.9/53.1 and 47.7/52.3 % of live males/live females at day 0, resp. at 0, 20, 60 and 180 mg/kg bw/d

  50.2/49.8, 50.3/49.7, 47.4/52.6 and 49.1/50.9 % of live males/live females at day 21, resp. at 0, 20, 60 and 180 mg/kg bw/d
- *clinical observations:* no test related effects were observed
- *viability index:*
  - *viability index (pups surviving days 0 to 4 (pre-culling)):* 99 (336), 97 (280), 99 (319) and 99 % (283) resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *lactation index (pups surviving days 4 (post-culling) to 21):* 100 (229), 100 (197), 100 (232), 100 % (220) resp. at 0, 20, 60 and 180 mg/kg bw/d
- *mean litter or pup weight by sex and with sexes combined:*

Table 147: Pup body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
D 1	Males	7.1	7.4	7.7*	7.7[A]
	Females	6.7	7.0	7.2*	7.3*
	M + F	6.9	7.2	7.5*	7.5*
D 4 (post-culling)	Males	10.5	10.9	11.5*	11.4*
	Females	9.9	10.3	10.9*	10.9*
	M + F	10.2	10.6	11.2*	11.2*
D 21	Males	54.0	56.8	57.4*	55.7
	Females	52.0	54.3	54.8*	53.7
	M + F	53.0	55.5	56.0*	54.7

*: p<0.05
[A]: S.d : 0.52, 0.76, 0.74 and 0.76

- *thyroid hormones:*

Table 148: Thyroid hormones data

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
PND 4								
T4 (nmol/l)	27.47	23.48	25.49	25.64	24.54	28.17	24.80	24.85
TSH (µg/l)	3.87	3.67	3.79	3.98	4.15	3.54**	3.85	3.95*
PND 22								
T4 (nmol/l)	51.13	54.45	55.55	57.49	56.09	55.98	49.75	51.07
TSH (µg/l)	4.35	4.56	5.38	4.17	4.35	4.48	4.39	4.59

*: p<0.05 ; ** : p<0.01

- *necropsy observations:*
  - *number of pups evaluated:* 152, 122, 154 and 125 resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *total pup incidence:* 2 (2 with empty stomach), 2 (1 with empty stomach and 1 with post mortem autolysis), 2 (1 with haemorrhagic testis and 1 with

eye(s) discoloured) and 1 (post-mortem autolysis) resp. at 0, 20, 60 and 180 mg/kg bw/d

- *anogenital distance:*

Table 149: Mean anogenital distance on D 1 (in mm)

Dose level (in mg/kg bw/d)	0	20	60	180
Male	3.59	3.67	3.61	3.63
Female	1.72	1.78	1.78	1.79

- *sexual maturation:*

Table 150: Vaginal opening: number of pups reaching criteria / number of tested (cumulated value)

Dose level (in mg/kg bw/d)	0	20	60	180
D 28	0/64	0/64	0/64	4/64
D 35	59/64	57/64	60/63	59/63
D 40	64/64	64/64	63/63	63/63
Mean days to reach criterion	32.3	32.2*	32.3	31.8
BW at day reaching criterion (in g)	105.8	112.6*	113.4*	109.9

*: $p<0.05$

Table 151: Preputial separation: number of pups reaching criteria / number of tested (cumulated value)

Dose level (in mg/kg bw/d)	0	20	60	180
D 38	0/64	1/64	2/64	0/64
D 45	54/64	54/64	55/64	50/64
D 55	64/64	64/64	64/64	64/64
Mean days to reach criterion	43.1	43.0	42.5	43.3
BW at day reaching criterion (in g)	221.1	222.8	223.3	222.5

- *presence of areolas/nipples:*
  - % of pups reaching criteria on PND 13: 66, 72, 68 and 64 % resp. at 0, 20, 60 and 180 mg/kg bw/d
  - % of pups reaching criteria on PND 20: 0 % at all tested and control groups. No nipples/areolae were detected in any male pups.

For cohort 1A:

- *number of animals at the start of the test:* 20/sex/dose
- *time of death during the study and whether animals survived to termination:* 1 female of the highest dose was found dead on study day 0. Necropsy revealed a slight fibrinous inflammation in the lung, focal hyperplasia in the mammary gland and an atrophic uterus.
- *clinical observations:* transient salivation was observed immediately after dosing at the highest dose in both sexes (12 males out of 20 and 14 females out of 20)

- *water consumption:* significantly higher in females at the highest dose level
- *food consumption:* significantly higher in females exposed to 180 mg/kg bw/d (up to 21% compared to control).

Table 152: Mean food consumption (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
Mean D 0-56	26.2	26.0	26.9	26.4	17.2	17.6	18.3	19.7*

*: p<0.05

- *body weight data:*

Table 153: Body weight data (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
D 0	86.8	86.8	85.5	85.9	77.5	78.4	79.9	78.2
D 14	208.0	203.0	204.9	203.7	149.0	153.4	159.6*	159.6*
D 21	266.4	262.7	263.9	254.0	173.9	176.1	184.2	183.2
D 28	326.7	322.0	323.5	315.5	193.5	196.0	207.3*	207.1*
D 42	408.4	404.1	408.4	394.1	226.9	228.4	237.6	241.0
D 63	488.3	490.6	472.9	459.2	264.1	256.3	265.1	277.0

*: p <0.05

- *haematological and clinical biochemistry findings if available :* Males exposed to the highest dose exhibited significant haematological changes such as higher HGB (8.7, 8.6, 8.7 and 9.0* mmol/L resp. at 0, 20, 60 and 180 mg/kg bw/d) and higher HQT (34.2, 34.3, 34.0 and 37.4* sec resp. at 0, 20, 60 and 180 mg/kg bw/d).

  Females exposed to the highest dose showed significant changes such as higher tot. prot (65.99, 65.02, 68.41 and 70.15* g/L resp. at 0, 20, 60 and 180 mg/kg bw/d), higher albumin (40.40, 40.23, 40.99 and 42.83* g/l resp. at 0, 20, 60 and 180 mg/kg bw/d).
- *thyroid hormones:*

Table 154: Thyroid hormones

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
T4 (nmol/l)	72.40	64.16	63.47	60.64	38.39	37.84	38.58	39.88
TSH (µg/l)	9.61	7.08	8.61	6.48	3.23	3.64	3.49	3.93

- *Lymphocyte subpopulations in spleen at PND 90:* No changes in the lymphocyte subpopulation cell counts in the spleen were observed (B-lymphocytes, T-lymphocytes, CD4-T-lymphocytes, CD8-T-lymphocytes and NK were examined)
- *effects on sperm*

- *% of motile sperm:* 84, 83, 84 and 83 % resp. at 0, 20, 60 and 180 mg/kg bw/d
- *Tot. spermatids/gram testis:* 106 in control vs 107 Mio/g at the highest dose
- *Tot. sperms/gram cauda epididymis:* 794 in control vs 846 Mio/g at the highest dose
- *% of abnormal sperms:* 5.2 in control vs 5.6 % at the highest dose

- *number of F1 females cycling normally and cycle length:* mean oestrus cycle duration was of 4.1 d in all tested and control groups.
- *necropsy findings:* all findings occurred either individually or were biological equally distributed.
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Table 155: Organ weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (g)		455.095	449.15	452.61	433.11	242.17	240.59	248.375	251.237
Adrenal glands	Abs (mg)	65.0	63.2	63.6	70.5	69.05	69.15	71.5	76.737
	Rela	0.014	0.014	0.014	0.016**	0.029	0.029	0.029	0.031
Axillary lymph nodes	Abs (mg)	130.4	118.3	107.7	105.8	68.4	70.5	71.8	73.6
	Rela	0.028	0.026	0.024	0.025	0.028	0.03	0.029	0.029
Brain	Abs (g)	2.219	2.175	2.199	2.196	2.041	2.013	2.029	2.012
	Rela	0.491	0.49	0.486	0.51	0.851	0.841	0.821	0.803
Heart	Abs (g)	1.586	1.535	1.515	1.489	0.975	0.924	0.946	0.99
	Rela	0.349	0.343	0.335	0.344	0.403	0.385	0.382	0.394
Kidneys	Abs (g)	3.224	3.137	3.335	3.599**	1.797	1.791	1.86	1.91
	Rela	0.712	0.701	0.737	0.832**	0.745	0.745	0.747	0.759
Liver	Abs (g)	13.032	13.349	12.923	11.265**	6.828	6.725	6.906	7.238
	Rela	2.863	2.973	2.858	2.601**	2.814	2.794	2.78	2.88
Mesenteric lymph nodes	Abs (g)	298.4	318.7	291.6	322.9	236.5	221.1	257.4	244.9
	Rela	0.065	0.07	0.066	0.075	0.098	0.096	0.105	0.098
Pituitary gland	Abs (mg)	13.0	13.05	13.05	13.3	13.55	13.75	14.25	14.947
	Rela	0.003	0.003	0.003	0.003	0.006	0.006	0.006	0.006
Spleen	Abs (g)	0.876	0.817	0.801*	0.726**	0.524	0.494	0.529	0.502
	Rela	0.194	0.182	0.177*	0.168**	0.216	0.206	0.213	0.2
Thymus	Abs (mg)	435.7	418.45	435.35	350.85*	354.05	356.75	381.8	355.158
	Rela	0.095	0.094	0.096	0.08	0.146	0.148	0.154	0.142

Thyroid glands	Abs (mg)	24.5	26.15	23.75	23.45	16.7	17.35	15.8	16.526
	Rela	0.005	0.006	0.005	0.005	0.007	0.007	0.006	0.007
Cauda epididymis	Abs (g)	0.493	0.49	0.472	0.482	-	-	-	-
	Rela	0.109	0.11	0.104	0.111	-	-	-	-
Epididymis	Abs (g)	1.167	1.159	1.14	1.151	-	-	-	-
	Rela	0.258	0.26	0.252	0.266	-	-	-	-
Prostate	Abs (g)	1.163	1.118	1.053*	1.046**	-	-	-	-
	Rela	0.257	0.252	0.233	0.242	-	-	-	-
Sem. ves.	Abs (g)	1.353	1.254	1.285	1.258	-	-	-	-
	Rela	0.3	0.282	0.284	0.291	-	-	-	-
Testes	Abs (g)	3.655	3.56	3.69	3.63	-	-	-	-
	Rela	0.807	0.801	0.818	0.842	-	-	-	-
Ovaries	Abs (mg)	-	-	-	-	82.2	82.9	88.2	86.222
	Rela	-	-	-	-	0.034	0.034	0.036	0.034
Uterus	Abs (g)	-	-	-	-	0.707	0.709	0.716	0.823
	Rela	-	-	-	-	0.294	0.299	0.288	0.328

*: p<0.05 ; ** : p<0.01

- *histopathological findings: nature and severity:*

Table 156: Incidence of microscopic findings

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
Kidneys									
Nb. of animals examined		20	20	20	20	20	1	0	20
Mineralisation, medulla	Inc.	0	0	1	7	17	0	/	13
	Grade 1			1	2				
	Grade 2				2				
	Grade 3				3				
Nuclear crowding	Inc.	1	1	/	6	0	0	/	0
	Grade 1	1	1		5				
	Grade 2				1				
Dilatation, tubular	Inc.	0	0	2	7	0	0	/	0
	Grade 1			2	4				
	Grade 2				3				
Mammary gland									
Nb. of examined		20	18	20	20	20	/	/	20
Atrophy	Inc.	1	0	2	7	0	/	/	0

- *Differential ovarian follicle count:*
  - *Number of primordial follicles:* mean value of 398.60 at 180 mg/kg bw/d vs 335.10 in control group (absolute values : 7972 at 180 mg/kg bw/d vs 6702 in control group)

- *Number of growing follicles:* mean value of 11.95 at 180 mg/kg bw/d vs 11 in control group (absolute value : 239 at 180 mg/kg bw/d vs 220 in control group)

For cohort 1B:

- *number of animals at the start of the test and mating:* 24/sex/dose
- *time of death during the study and whether animals survived to termination:* 1 female of the mid dose group was found dead on premating D3, histopathological examination of this animal was not performed.
- *clinical observations:* excessive salivation was observed immediately after exposure at the highest dose group (11 males and 9 females during the in-life period and 10 females during gestation period).
- *water consumption:* significantly higher in females of the highest dose
- *food consumption:* significantly higher in females exposed to 180 mg/kg bw/d during D 0-70.

Table 157: Mean food consumption (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	80
D 0-70	26.9	26.6	27.4	27.1	17.7	17.7	19.1	19.9*
GD 0-20	-	-	-	-	25.7	24.9	25.6	26.1
LD 1-21	-	-	-	-	71.6	70.8	72.3	67.1

*: p<0.05

- *body weight data:*

Table 158: Male body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
In-life period	D 0	79.8	80.1	82.3	78.9
	D 14	190.0	179.2	177.6*	173.7**
	D 21	253.9	250.3	260.4	248.4
	D 49	422.2	416.4	437.7	405.5
	D 70	489.2	481.8	502.7	466.8
Parental period	W 0	503.0	498.2	517.7	479.7
	W 5	564.3	559.2	579.7	541.6

*: p<0.05 ; ** : p<0.01

Table 159: Female body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
In-life period	D 0	73.4	71.5	75.4	73.6
	D 21	170.9	170.7	185.8**	184.7**
	D 49	237.6	233.0	252.7*	258.4**
	D 70	265.6	260.9	280.9	284.4*
Gestation period	GD 0	276.8	270.5	292.2	291.4
	GD 14	345.9	335.1	356.3	355.7
	GD 20	426.0	412.3	436.5	415.7
Lactation period	LD 0	330.6	323.8	343.8	341.3

	LD 10	359.3	350.6	371.4	367.0
	LD 21	342.3	332.9	356.6	353.0

*: p<0.05 ; ** : p<0.01

- *male fertility data:*

Table 160: Male fertility data

Dose level (in mg/kg bw/d)	0	20	60	180
Number of males placed with females	24	24	23	24
Mating index (in %)	100	100	100	100
Number of males with pregnant females	24	24	22	23
Fertility index (in %)	100	100	96	96

- *female fertility data:*

Table 161: Female reproduction and delivery data

Dose level (in mg/kg bw/d)	0	20	60	180
Females mated	24	24	23	24
Female mating index (in %)	100	100	100	100
Mean mating day until DPC 0	3.0	2.4	2.5	3.0
Female fertility index (in %)	100	100	96	96
Number of females with liveborn pups	24	24	21	21
Number of females with stillborn pups	6	2	2	6
Number of females with all stillborn	0	0	0	0

- *number of P and F1 females cycling normally and cycle length:* mean duration of oestrus cycle : 3.9, 4.0, 4.0 and 4.1[12] d resp. at 0, 20, 60 and 180 mg/kg bw/d (S.d : 0.29, 0.16, 0.13 and 1.51, resp. at 0, 20, 60 and 180 mg/kg bw/d)
  Mean number of days in stage: prooestrus : 4.71, 2.83, 2.22 and 1.25 d, resp. at 0, 20, 60 and 180 mg/kg bw/d
  Mean number of days in stage: oestrus : 5.42, 5.21, 5.35 and 4.625 d, resp. at 0, 20, 60 and 180 mg/kg bw/d
  Mean number of days in stage: metoestrus : 6.0, 6.0, 6.3 and 5.875 d, resp. at 0, 20, 60 and 180 mg/kg bw/d
  Mean number of days in stage: dioestrus : 6.83, 8.375, 9.17 and 11.21 d, resp. at 0, 20, 60 and 180 mg/kg bw/d
- *duration of gestation (calculated from day 0 of pregnancy):* 22.0, 21.9, 22.0 and 22.0 d resp. at 0, 20, 60 and 180 mg/kg bw/d
- *number of implantations, corpora lutea, litter size*

[12] RAC (2020): Note that this specific effect was initially mentioned to be 4.5 days at the highest dose tested in the CLH dossier and on IUCLID, but during the PC was noted that this effect size was incorrect and should be changed into 4.1 days. The information in IUCLID was updated to this regard as well (ECHA dissemination website consulted on 09-09-2020)

- *Total number of implantation sites:* 364, 350, 338 and 316 resp. at 0, 20, 60 and 180 mg/kg bw/d
- *Mean number of implantation sites:* 15.2, 14.6, 15.4 and 13.7 resp. at 0, 20, 60 and 180 mg/kg bw/d

- *number of pre- and post-implantation loss*
  - *Total number of post implantation loss:* 22, 18, 25 and 76 resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Mean number of post implantation loss:* 0.9, 0.8, 1.1 and 3.3** resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Mean % of post implantation loss:* 6.4, 5.3, 11.1 and 24.6** % resp. at 0, 20, 60 and 180 mg/kg bw/d
- *necropsy findings:* in males, enlarged kidneys were observed in 1 males of the mid dose and in 10 males of the highest dose.
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals*

Table 162: Organ weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (g)		536.054	530.863	548.279	510.363	291.842	284.588	304.817*	308.2
Adrenal glands	Abs (mg)	59.792	62.625	67.708**	64.708	76.708	72.292	77.435	80.083
	Rela	0.011	0.012	0.012*	0.013**	0.026	0.026	0.025	0.026
Kidneys	Abs (g)	3.375	3.43	3.807**	4.252**	2.158	2.115	2.212	2.31*
	Rela	0.632	0.649	0.696**	0.832**	0.741	0.746	0.726	0.752
Liver	Abs (g)	14.813	15.395	14.677	13.272*	9.455	9.326	9.5	9.716
	Rela	2.758	2.902	2.669	2.6*	3.237	3.28	3.119	3.175
Pituitary gland	Abs (mg)	12.917	13.042	12.958	13.167	15.542	15.25	15.714	15.542
	Rela	0.002	0.002	0.002	0.003	0.005	0.005	0.005	0.005
Cauda epididymis	Abs (g)	0.542	0.544	0.535	0.525	-	-	-	-
	Rela	0.102	0.103	0.098	0.104	-	-	-	-
Epididymis	Abs (g)	1.324	1.347	1.319	1.308	-	-	-	-
	Rela	0.248	0.255	0.242	0.258	-	-	-	-
Prostate	Abs (g)	1.557	1.489	1.47	1.398	-	-	-	-
	Rela	0.293	0.283	0.269	0.274	-	-	-	-
Sem. ves.	Abs (g)	1.808	1.725	1.813	1.74	-	-	-	-
	Rela	0.34	0.327	0.332	0.342	-	-	-	-
Testes	Abs (g)	3.853	3.954	3.874	3.857	-	-	-	-
	Rela	0.723	0.75	0.712	0.764	-	-	-	-

Ovaries	Abs (mg)	-	-	-	-	109.542	109.5	113.217	106.833
	Rela	-	-	-	-	0.038	0.039	0.037	0.035
Uterus	Abs (g)	-	-	-	-	0.747	0.669	1.666	0.778
	Rela	-	-	-	-	0.255	0.235	0.538	0.255

*: p<0.05 ; ** : p<0.01

- *histopathological findings: nature and severity:* An atrophy of the mammary gland was noted in 1 male of each group (control and tested groups).

For F2 pups/litters of the cohorts 1B:

- *mean number of live pups (litter size):*

Table 163: Litter data

Dose level (in mg/kg bw/d)	0	20	60	180
Tot. nb of pups delivered	342	332	313	240
Mean nb of pups delivered	14.3	13.8	14.9	11.4**
Nb of litters	24	24	21	21
Nb of liveborn pups	336	330	311	234
Nb of stillborn pups	6	2	2	6

**: p<0.01

Table 164: Mean number of live pups/litters

Dose level (in mg/kg bw/d)	0	20	60	180
D 1	13.8	13.8	14.6	11.0
D 4 (preculling)	13.7	13.7	14.5	11.0
D 4 (postculling)	9.8	9.8	10.0	8.7
D 21	9.8	9.8	10.0	8.6

- *sex ratio (at day 0):* 52.7/47.3, 52.1/47.9, 47.3/52.7 and 48.7/51.3 % of live males/live females resp. at 0, 20, 60 and 180 mg/kg bw/d
- *viability index (pups surviving 4 days/total births):*
  - *viability index (pups surviving days 0 to 4 (pre-culling)):* 98 (329), 100 (329), 98 (304) and 99 % (232) resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *lactation index (pups surviving days 4 (post-culling) to 21):* 100 (236), 100 (235), 100 (209) and 99 % (181) resp. at 0, 20, 60 and 180 mg/kg bw/d
- *mean litter or pup weight by sex and with sexes combined:*

Table 165: Pup body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
D 1	Males	7.5	7.5	7.4	8.0
	Females	7.2	7.0	7.0	7.7*
	M + F	7.4	7.2	7.2	7.9
D 4 (post-culling)	Males	1.3	11.0	11.0	12.3
	Females	10.9	10.5	10.5	11.8
	M + F	11.1	10.7	10.8	12.1

D 21	Males	59.1	58.9	58.6	61.1
	Females	56.9	56.2	56.5	58.3
	M + F	58.1	57.5	57.5	60.0

*: $p<0.05$

- *necropsy observation:*
  - *number of pups evaluated:* 336, 330, 309 and 239 pups resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *pup incidence* : 9 (5 with dilated renal pelvis, 3 with post mortem autolysis, 1 with small testis), 3 (2 with dilated renal pelvis, 1 with post mortem autolysis), 4 (2 with dilated renal pelvis, 1 with post mortem autolysis, 1 with empty stomach) and 3 (2 with dilated renal pelvis, 1 with empty stomach) affected pups resp. at 0, 20, 60 and 180 mg/kg bw/d
- *mean organ weight (males + females):*
  - *Brain:* 1.502, 1.496, 1.488 and 1.514 g resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Thymus:* 0.246, 0.229, 0.219 and 0.228 g resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Spleen:* 0.201, 0.223, 0.196 and 0.222 g resp. at 0, 20, 60 and 180 mg/kg bw/d
- *anogenital distance:*

Table 166: Mean anogenital distance on D 1 (in mm)

Dose level (in mg/kg bw/d)	0	20	60	180
Male	3.44	3.49	3.42	3.56
Female	1.67	1.67	1.65	1.71

- *presence of areolas/nipples:*
  - % of pups reaching criteria on PND 13: 79, 76, 77 and 66 % resp. at 0, 20, 60 and 180 mg/kg bw/d
  - % of pups reaching criteria on PND 21: 0.0 % at all tested and control groups

For cohort 2A:

- *number of animals at the start of the test:* 10/sex/dose
- *time of death during the study and whether animals survived to termination:* no mortality observed during the study period.
- *clinical observations:* excessive salivation was observed immediately after exposure in 1 female and in 3 males exposed to 180 mg/kg bw/d
- *body weight data:*

Table 167: Body weight data for cohort 2A

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180

D 0	102.5	101.9	104.2	101.0	89.0	87.3	93.4	95.3
D 21	288.0	281.6	297.4	294.1	187.3	185.3	193.6	199.1
D 42	402.5	399.6	413.3	408.2	235.8	235.2	237.8	253.0

- *startle response examination at PND 24:*
  - *Mean max. ampl. (block 1-5) :* 265.9, 188.4*, 229.8 and 272.1 in males and 218.0, 214.2, 221.6 and 225.4 in females, resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Mean latency (block 1-5):* 19.4, 20.3, 19.6 and 19.6 msec in males and 20.7, 19.8, 21.1 and 19.2 msec in females, resp. at 0, 20, 60 and 180 mg/kg bw/d
- *FOB examination at D 75:*
  - Home cage observations: animals did not exhibit tremors, convulsions, abnormal movements. 1 females of the control group, 2 males and 3 females of the mid dose and 2 males of high dose were sitting or lying and not walking during the observation.
  - Open field observations: animals did not exhibit resistance against handling, salivation, nasal discharge, lacrimation, abnormal eyes/pupil size, abnormal posture, abnormal respiration, tremors, convulsions, abnormal movements/stereotypy. 1 males of the control group and 1 males of the highest dose were not walking during the observation.
  - Sensorimotor tests/reflexes: animals did show reactions during the examination the approach and touch responses. Moreover, no abnormal reactions were detected during the examination of audition, pinna reflex, coordination of movements, behaviour during handling and pain perception.

Table 168: Sensorimotor tests/reflexes

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
Rearing (N)	8	8	6	6	12	13	12	13
GS F (Newton)	9.7	10.2	10.5	10.3	7.8	8.0	7.4	8.6
GS H (Newton)	5.7	5.4	6.1	6.3	4.4	4.3	4.6	4.7
FST (cm)	12.6	11.6	12.8	12.0	10.1	11.4	10.4	11.1

- *Motor activity at D 75:* Sum of the interr. 1-12 was of 2811.8, 2951.3, 2495.9 and 2487.7 in males and of 3731.3, 3685.1, 3389.9 and 3227.5 in females, resp. at 0, 20, 60 and 180 mg/kg bw/d
- *Rearing at D 75:* Sum of the interr. 1-12 was of 528.4, 573.6, 477.3 and 482.2 and of 609.5, 584.6, 535.3 and 461.2 in females, resp. at 0, 20, 60 and 180 mg/kg bw/d
- *Morris water maze:* no difference observed in the distance to and the time spent in the target quadrant between control and treated groups.

Table 169: Morris water maze data: learning on PND 60

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
Mean cumul. Distance (in cm)								
D 1	109939.4	120616.2	113809.1	119463.9	93981.0	140207.3	123301.7	154551.5
D 2	44195.3	36789.1	44854.6	46090.5	48406.2	43069.4	36504.2	41593.1
D 3	26804.5	23618.1	16086.4	24773.5	36996.6	55256.4	35006.3	34114.6
D 4	23282.4	30615.9	30908.4	41351.6	47550.5	32484.5	26717.0	31356.4
Median latency time (in ms)								
D 1	41232.0	37162.8	36791.3	45872.0	33985.3	42142.3	39123.8	69668.3
D 2	11431.0	11992.3	10572.0	12863.8	19182.0	17311.3	15481.8	15442.8
D 3	12551.3	10222.3	7784.3	8281.0	10582.8	11709.8	11652.0	13753.3
D 4	9971.5	9332.0	8992.3	8651.3	16452.5	8160.8	10001.5	14222.0

Table 170: Morris water maze data: relearning on PND 67

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
Mean cumul. Distance (in cm)								
D 6	39305.2	67564.2	33109.5	42331.4	41815.2	41719.3	43567.1	33506.2
D 7	25605.5	26582.4	35749.7	31374.5	24911.5	20785.0	26228.4	29318.7
D 8	18368.6	22044.8	23446.0	19910.2	33169.2	28938.8	21488.0	32905.8
D 9	17520.7	24455.7	16109.0	25269.0	26111.0	23675.0	17311.8	28368.1
Median latency time (in ms)								
D 6	13411.3	14649.0	10961.8	12582.8	11321.8	10982.5	14851.8	12472.5
D 7	8241.8	9132.8	12343.0	8802.0	11372.8	8384.0	9132.8	9992.8
D 8	7662.0	6284.3	6271.5	8811.5	12952.0	12582.8	10461.8	11802.5
D 9	6172.3	10982.5	7150.8	5191.0	11011.3	7872.5	6854.3	10361.3

- *necropsy findings:* no treatment related effects were noted
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Table 171: Brain weight data for cohort 2A

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (in g)		394.72	394.66	408.31	402.59	236.19	227.32	233.66	248.19
Brain	Abs (g)	2.262	2.166	2.223	2.242	2.047	2.02	2.033	2.077
	Rela	0.579	0.55	0.546	0.557	0.871	0.897	0.872	0.842

- *Length and width of brain:*
  - *Length:* 2.20, 2.17, 2.21 and 2.22 cm in males and 2.12, 2.12, 2.13 and 2.13 cm in females, resp. at 0, 20, 60 and 180 mg/kg bw/d

- *Width:* 1.62, 1.61, 1.63 and 1.60 cm in males and 1.58, 1.58, 1.57 and 1.59 cm in females, resp. at 0, 20, 60 and 180 mg/kg bw/d

- *histopathological findings: nature and severity:* no treatment related effects were observed

For cohort 2B:

- *necropsy findings:* no abnormalities observed
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Table 172: Brain weight data for cohort 2B

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (in g)		59.88	57.64	60.29	60.71	56.1	57.39	58.25	59.61
Brain	Abs (g)	1.828	1.783	1.855	1.819	1.757	1.74	1.751	1.801
	Rela	3.063	3.109	3.087	3.004	3.146	3.041	3.016	3.023

- *Length and width of brain:*
  - *Length:* 1.95, 1.91, 1.94 and 1.95 cm in males and 1.91, 1.91, 1.92 and 1.92 cm in females resp. at 0, 20, 60 and 180 mg/kg bw/d
  - *Width:* 1.53, 1.53, 1.53 and 1.55 cm in males and 1.51, 1.52, 1.52 and 1.51 cm in females, resp. at 0, 20, 60 and 180 mg/kg bw/d
- *histopathological findings: nature and severity:* no abnormalities observed

For cohort 3:

- *number of animals at the start of the test:* 10/sex/dose
- *clinical observations:* no effects were observed
- *time of death during the study and whether animals survived to termination:* one female of the lowest dose was found dead on study day 18
- *body weight data:*

Table 173: Body weight data (in g) for cohort 3

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
D 0	100.2	100.6	105.9	98.7	91.2	93.1	88.8	92.9
D 14	214.5	219.2	228.4	219.3	160.8	160.8	161.1	173.2[A]
D 28	328.8	339.9	344.1	344.6	203.7	204.8	202.4	217.4[B]

[A]: S.d : 15.7, 12.9, 21.5 and 14.2, respectively at 0, 20, 60 and 180 mg/kg bw/d
[B]: S.d : 23.3, 13.7, 25.1 and 15.3, respectively at 0, 20, 60 and 180 mg/kg bw/d

- *T-cell dependent antibody response (SRBC) at D 63:*
  - *Males:* 3738, 3727, 4414 and 3599 U/ml resp. at 0, 20, 60 and 180 mg/kg bw/d (positive control : 927 U/ml)

- *Females:* 13647, 8239, 9598 and 14555 U/ml resp. at 0, 20, 60 and 180 mg/kg bw/d (positive control : 1546 U/ml)

- *necropsy findings:* no treatment related effects were observed.
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Table 174: Organ weight data for cohort 3

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (g)		332.29	345.04	345.59	349.43	198.92	200.711	198.8	211.29
Spleen	Abs (g)	0.717	0.705	0.668	0.677	0.465	0.479	0.416	0.478
	Rela	0.217	0.205	0.193	0.193	0.233	0.239	0.211	0.227
Thymus	Abs (mg)	620.4	602.6	645.7	530.1	478.1	467.222	488.1	486.6
	Rela	0.187	0.176	0.187	0.152*	0.239	0.231	0.247	0.231

*: p<0.05

- *histopathological findings: nature and severity:* examination not performed

For pups not selected for cohorts:

- *necropsy findings:* no treatment related effects were observed.
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Table 175: Organ weight data for pups not selected for cohorts

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (g)		56.91	58.94	62.79	61.83	56.35	60.05	60.32	57.09
Brain	Abs (g)	1.554	1.567	1.628**	1.596	1.543	1.564	1.512	1.522
	Rela	2.742	2.672	2.606	2.585	2.747	2.617	2.513**	2.677
Spleen	Abs (g)	0.238	0.293	0.284	0.285	0.242	0.267	0.276	0.266
	Rela	0.416	0.495**	0.453	0.462	0.429	0.447	0.457	0.465
Thymus	Abs (mg)	228.6	284.2**	267.2*	247.8	260.7	265.1	273.5	258.5
	Rela	0.402	0.483**	0.425	0.401	0.465	0.44	0.543	0.454

*: p<0.05 ; ** : p<0.01

- *histopathological findings: nature and severity:* no treatment related effects were noted.

Conclusion: OECD CF level 5 data

Effects on female reproduction observed in parental generation and F1B cohort, including prolongation of oestrus cycles (shortened prooestrus and prolonged dioestrous periods), and decrease of implantation sites.

Effects on male reproduction observed in parental generation and F1A cohort, showing reduction of sperm motility.

Strong developmental effect observed in F1 and F2 pups, showing significant decrease of post-implantation loss, resulting in lower number of pups. Increased pup weight observed in F1 and F2 generations. Some marginal neuro- and immuno-developmental effects also observed F2A and F2B cohorts.

Some adverse effects are observed on organs linked to the endocrine system (kidneys, adrenal glands, thymus).

Atrophy of male mammary gland morphology observed in F1A.

7.10.3. Conclusion on endocrine disrupting properties (combined / separate)

1) Human health:

Endocrine Mode of Action
Endocrine activity

Bisphenols are known to target many endocrine pathways. Consistent *in vivo* and *in vitro* evidence is available on steroidogenesis and in particular on estrogenic activity.

- *Estrogenic activity*

In vitro ER binding assays demonstrate that BPS is capable of binding to the oestrogen receptor, with IC50 ranging from 5.8 to 105 µM depending on the cell line used (rat and human). Several *in vitro* literature studies using different cell cultures showed a weak increase in the estrogenic activity (ER reporter gene assays, proliferative assays and ER-regulated gene expression assays). *In vivo*, the increase in uterine weight, observed in all rodent uterotrophic assays, is a parameter diagnostic of estrogenicity.

Vitellogenin, a biomarker of estrogenic activity in fish, was induced in embryonic and adult male zebrafish. Literature data also reported a change in steroidal hormone balance with decreased testosterone and increased estradiol levels and an increased E2/T ratio in zebrafish.

BPS exhibits estrogenic activity.

- *Steroidogenesis*

In a range of *in vitro* assays investigating steroidogenesis following exposure with BPS, a clear trend towards decreased testosterone was observed. Furthermore, an increase in testis aromatase expression was observed in several studies following exposure to BPS. Several, but not all, *in vivo* studies, showed decrease in serum testosterone level in rodents.

Moreover, the impact on the synthesis of steroid hormones (decrease of testosterone and increase of oestrogen) was clearly shown in *in vivo* studies with zebrafish. These findings were accompanied by an increased expression of genes involved in steroidogenesis and specifically in aromatase (CYP19a, CYP19b in testis and brain resp.).

BPS is shown to affect steroidogenesis

Adverse effects

BPS consistently affects the oestrous cyclicity in female rodents, at different windows of exposure. All the available studies show irregular cycles, linked in most of them to a prolongation of the dioestrus phase. The disturbance of oestrous cycle is considered as EATS (estrogenic, androgenic, thyroidal, and steroidogenic)-mediated.

In addition, effects that are sensitive to, but not diagnostic of, EATS (as potentially linked to other Modes of Action) were also reported regarding rodent female reproduction. A statistically significant decrease of the number of embryo implantation sites was observed in reproductive toxicity studies, resulting in decreased fertility and number of pups.

Other developmental and male reproductive adverse effects were observed in the available rodent studies supporting the endocrine disrupting properties of BPS. These include EATS-mediated effects such as reduced sperm count and motility at low doses and a high incidence of male rodent mammary gland multifocal atrophy. Additionally, adverse effects sensitive to, but not diagnostic of, EATS were observed including dose-dependent increased post-implantation loss in reproductive toxicity studies and higher adrenal glands weight, in particular in males, in several independent studies.

These adverse effects have been observed at doses showing neither maternal toxicity nor severe general toxicity. Moreover, since oestrogen signalling is critical to reproductive success in all vertebrates including mammals, it is assumed that the observed adverse effects on fertility through disruption of oestrogen signalling in rodents are relevant to humans.

The complexity of the effects sensitive to, but not diagnostic of, EATS observed following exposure to BPS suggests the interaction of multiple MoAs to produce the observed effects, increasing the concern for human health. For example, the consistent effects on the mammary gland in males in two rodent species provides an indication of hormonal disturbance and may have influence on e.g. human breast tumour development.

Plausible link between adverse effects and endocrine activity

Considering the results of all available experimental studies, there is strong evidence that the adverse effects on fertility in females are due to the estrogenic activity of BPS. The increase in uterus weight (as seen in all three uterotrophic assays) is a strong diagnostic parameter for estrogenicity. Furthermore, the prolongation of the oestrous cycle was consistently observed in the majority of the studies. In addition, the number of implantation sites was decreased in three reproductive studies, resulting in a decrease of both fertility and number of pups. All of these parameters are considered as either EATS-mediated or sensitive to EATS modalities (OECD GD 150, 2018). The different effects of BPS, in particular on the female reproductive system, can be plausibly linked to the estrogenic activity of the substance and could therefore explain the adverse impacts seen on fertility endpoints.

Other modes of action than those involving estrogenic activity and/or signalling pathways are likely. For example, altered testosterone production is probably linked to adverse effects on the male reproductive system or the male mammary gland. Despite the fact that these data give further indications of the endocrine activity of BPS, they were considered as supportive adverse human health effects.

In conclusion, the effects on the female reproductive organs and functional parameters are consistent with an estrogenic mode of action of BPS. While considering that effects on the oestrous cycle are EATS-mediated (OECD Guidance Document 150), the causal link between the endocrine activity and the adverse effects is demonstrated. These adverse

effects have been observed at doses showing neither maternal toxicity nor general toxicity. There is strong evidence that the **adverse effects on fertility and sexual function are plausibly linked to the estrogenic activity of the substance. BPS is therefore an endocrine disruptor according to the WHO/IPCS definition (WHO/IPCS, 2002) with regard to human health.**

2) Environment:

Endocrine activity

See endocrine activity above on human health

Adverse effects

There is evidence in literature that BPS affects sperm count and sex ratio in zebrafish (*Danio rerio*) after exposure in the µg/L range. In a ZEOGRT (OECD TG 240 adapted for zebrafish), the findings on sex ratio were not significant. However, a similar trend towards feminisation was observed with the number of males close to or even below natural variation at low concentrations. These EATS-mediated effects were observed at concentrations below general toxicity.

In addition, effects that are sensitive to, but not diagnostic of, EATS (as potentially linked to other Modes of Action) were also reported regarding reproductive effects: reduced fecundity, reduced hatching rate and altered oocyte maturation in fish.

Other important adverse effects on brain neurogenesis and behaviour were identified in fish. Experimental data on zebrafish demonstrated that these effects depend on BPS-induced changes in aromatase activity.

Effects on apical endpoints such as fecundity and altered sex ratio are considered to impair population stability and recruitment. Therefore, these effects are to be considered population relevant for the environment.

Plausible link between adverse effects and endocrine activity

Based on the weight of evidence approach and considering the results of all available studies there is evidence that the adverse effects of BPS on sperm count and sex ratio in zebrafish are due to the estrogenic activity and to disrupted steroidogenesis.

Skewed sex ratio is recognised as an EATS-mediated effect. Altered gametogenesis as reduced sperm counts has been also observed. Based on the existing knowledge in mammals and the similarities with fish gametogenesis, reduced sperm count is considered as EATS-mediated also in fish. The estrogenic activity of BPS is demonstrated in mammals and is further evidenced by vitellogenin induction in fish. Altered steroidogenesis may lead to the observed decreased sperm counts and altered oocyte maturation which, in turn, may lead to impaired hatchability of the eggs. Increased aromatase activity is consistently observed and is clearly responsible for effects on fish brain and behaviour. Impaired social behaviour may also result in reduced reproduction.

There is a large degree of conservation of the endocrine system, implying large commonalities between non-mammalian and mammalian vertebrate species in regard to hormones, enzymes and receptors involved in the EATS modalities. All mammalian data provide substantial evidence that BPS can disrupt particularly estrogenic pathways. Therefore, those data were also considered in the Weight of Evidence approach for the assessment of the ED properties in the environment and thus wildlife species.

Considering all relevant and reliable information in a weight of evidence approach, it is concluded that BPS is an endocrine disruptor according to the WHO/IPCS definition (WHO/IPCS, 2002) with regard to environment.

3) Conclusion

Based on all available scientific evidence, it can be concluded that BPS fulfils the WHO/IPCS (2002)[13] definition of an endocrine disruptor:

- It shows clear reproductive adverse effect in rodents and fish. The reproductive endocrine system is highly conserved not only between mammals, but also between mammals and other vertebrates like fish.

- It has endocrine modes of action: clear estrogenic mode of action and alteration of steroidogenesis.

- The adverse effects, including the recognised EAS-mediated effects (e.g., on oestrous cycle and sex ratio) and effects sensitive, but not diagnostic of EAS (e.g. fecundity, fertility, implantation sites and number of pups), are a consequence of the endocrine mode of action.

The scientific assessment performed demonstrates that **BPS causes serious effects to the environment and human health due to its endocrine disrupting properties, which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of the REACH Regulation.**

7.11. PBT and vPvB assessment

1) Persistence

Table 176: Overview of the P and vP criteria

A substance fulfils the persistence criterion (P) in any of the following situations:	***A substance fulfils the "very persistent" criterion (vP) in any of the following situations:***
(a) the degradation half-life in marine water is higher than 60 days. *(b) the degradation half-life in fresh or estuarine water is higher than 40 days.* *(c) the degradation half-life in marine sediment is higher than 180 days.* *(d) the degradation half-life in fresh or estuarine water sediment is higher than 120*	*(a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days.* *(b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days.* *(c) the degradation half-life in soil is higher than 180 days.*

[13] An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny or (sub)populations.

days. *(e) the degradation half-life in soil is higher than 120 days.*	

Based on the results of the available screening tests (OECD TG 301C; enhanced OECD 301B and modified Zahn-Wellens (equivalent or similar to 302B) it can be concluded that BPS is not readily biodegradable.
BPS degraded resp. 0% after 28d and 32% after 59 d in the ready tests (OECD TG 301). Furthermore, only 47% of BPS was degraded after 14d (36% after 7d) in an inherent study equivalent or similar to OECD TG 302 B (modified Zahn-Wellens) and can thus be considered as non-inherently degradable (<70% biodegradation within 7 days; ECHA guidance IR/CSA chapter R.11).

No aerobic degradation was seen in seawater, resp. 0% degradation after 22 days under aerobic conditions) and after 60d in a TOC Handai and sea river die-away simulation study (Danzl *et al.*, 2009). However, BPS degraded (60%) under anaerobic conditions at ca. day 80 in a TOC Handai method assay (Ike *et al.*, 2006).

Rapid degradation was observed in soil, resp. with DT50 of 1.1 d (Registration dossier, Choi and Lee, 2017a) and 2.8d (Cao *et al*., 2020).

Conclusion: **Screens as P / vP**

Results from ready biodegradation studies show that BPS is not readily degradable and therefore fulfils the screening criteria for persistency indicating P and vP properties.

No degradation was observed in aerobic seawater after 60d.

Because no degradation half-life was reported for river and seawater-degradation no conclusion can be drawn on P/vP based on the currently available data.

2) Bioaccumulation

Table 177: Overview of the B and vB criteria

A substance fulfils the bioaccumulation criterion (B) when the bioconcentration factor in aquatic species is higher than 2000.	*A substance fulfils the "very bioaccumulative" criterion (vB) when the bioconcentration factor in aquatic species is higher than 5000.*

The substance has a measured log Kow of 1.2. The experimentally derived BCF was determined to be <2.2. Therefore, no aquatic bioaccumulation is expected.

Conclusion: **Not B / vB**

3) Toxicity

Table 178: Overview of the T criteria

A substance fulfils the toxicity criterion (T) in any of the following situations:
(a) the long-term no-observed effect concentration (NOEC) or EC10 for marine or freshwater organisms is less than 0.01 mg/L.

(b) the substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) according to Regulation EC No 1272/2008.

(c) there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Regulation EC No 1272/2008.

Classification and labelling: covered by index number 604-098-00-1 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances)[14]: Repr. 1B, H360FD. Therefore, the substance fulfills the T-criterion

Conclusion: **T**

4) Overall conclusion

As BPS is not B, the substance is not considered as a potential PBT or vPvB.

7.12. Exposure assessment

7.12.1. Human health

eMSCA did only perform an exposure assessment for workers.

7.12.1.1. Worker

7.12.1.1.1. Exposure assessment performed by the eMSCA

The eMSCA has used the ECETOC Targeted Risk Assessment Model (ECETOC TRA, version 3) in order to perform the exposure assessment for the Substance. Some parameters were taken from a worst-case perspective by the eMSCA, which is sometimes stricter than what the Registrants indicate in their assessment.

Table 181 gives an overview of the physicochemical properties and reference values that the eMSCA used as input values into the model.

Table 179: Physicochemical properties and reference values used as input values for the ECETOC TRA tool

Physicochemical properties	
Molecular weight	250.27 g/mol
Vapour pressure	6.29E-10 hPa
Water solubility	715 mg/L

[14] COMMISSION DELEGATED REGULATION (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (18th ATP)

Partition coefficient octanol-water (Log K_{ow})	1.2
Biodegradability test result	Not biodegradable
Partition coefficient organic carbon (K_{oc})	661
Reference values	
Reference value long-term inhalation workers	1.4 mg/m^3
Reference value long-term dermal workers	2 mg/kg/day

7.12.1.1.1.1. Exposure Scenario 1 - Manufacture of the substance

Manufacture of the substance is described by PROCs 1, 8b, 9, 15 and 28[15]. Respiratory protection (90%) is indicated by the Registrants for the PROC 8b and PROC 28 contributing scenarios. This process takes place in an industrial setting. Indoor use with good general ventilation, but no use of LEV (Local Exhaust Ventilation) is indicated by the Registrants. Gloves with efficiency 90% was indicated by the Registrants for all PROCs, except for PROC 1.

The eMSCA used a worst-case approach (duration of activity longer than 4 hours/day, use of the substance indoors with good general ventilation, no use of respiratory protection, use of gloves with efficiency 80%).

[15] For more elaborate descriptions on the PROCs: ECHA Guidance Chapter R.12 on Use description (https://echa.europa.eu/documents/10162/13632/information_requirements_r12_en.pdf/ea8fa5a6-6ba1-47f4-9e47-c7216e180197)

Table 180: Exposure values for manufacture of the substance, estimated by the eMSCA

Industrial				
Contributing scenario	Assessment parameters	Solid (dustiness)	Long-term Inhalative Exposure Estimate (mg/m^3)	Long-term Dermal Exposure Estimate (mg/kg/day)
PROC 1	Duration of activity > 4 hours/day, indoors with good general ventilation (no LEV), respiratory protection not used, substance not used in a preparation, glove efficiency is 80%	No	0,073	0,00686
PROC 8b		Yes (medium)	0,7	2,74
PROC 9		Yes (medium)	3,5	1,37
PROC 15		Yes (medium)	0,35	0,0686
PROC 8a*		Yes (medium)	3,5	2,74

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

7.12.1.1.1.2. Exposure Scenario 2 - Use as monomer for manufacture of PESU

Use as monomer for manufacture of PESU is described by PROCs 1, 8b, 15 and 28. Respiratory protection is indicated by the Registrants for the PROC 8b (90%) and PROC 28 (95%) contributing scenarios. This process takes place in an industrial setting. Indoor use with good general ventilation, but no use of LEV is indicated by the Registrants. Gloves with efficiency 90% was indicated by the Registrants for all PROCs, except for PROC 1.

The eMSCA used a worst-case approach (duration of activity longer than 4 hours/day, use of the substance indoors with good general ventilation, no use of respiratory protection, use of gloves with efficiency 80%).

Table 181: Exposure values for use as monomer for manufacture of PESU, estimated by the eMSCA

Industrial				
Contributing scenario	Assessment parameters	Solid (dustiness)	Long-term Inhalative Exposure Estimate (mg/m^3)	Long-term Dermal Exposure Estimate (mg/kg/day)
PROC 1	Duration of activity > 4 hours/day, indoors with good general ventilation (no LEV), respiratory protection not used, substance not used in a preparation, glove efficiency is 80%	No	0,073	0,00686
PROC 8b		Yes (medium)	0,7	2,74
PROC 15		Yes (medium)	0,35	0,0686
PROC 8a*		Yes (medium)	3,5	2,74

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

7.12.1.1.1.3. Exposure Scenario 3 - Use as monomer in the production of synthetic tanning agents (Syntans)

Use as monomer in the production of synthetic tanning agents (Syntans) is described by PROCs 1, 8a, 8b, 9 and 15. Respiratory protection (95%) is indicated by the Registrants for the last PROC 8b and the last PROC 8a contributing scenario. This process takes place in an industrial setting. Indoor use with good general ventilation was indicated by the Registrants in general. Indoor use with LEV was reported for the first PROC 8b contributing scenario, and outdoor use of the substance was reported for the second PROC 8b contributing scenario. Gloves with efficiency 80% was indicated by the Registrants for all PROCs, except for PROC 1.

The eMSCA used a worst-case approach (duration of activity longer than 4 hours/day, use of the substance indoors with good general ventilation, no use of respiratory protection, use of gloves with efficiency 80%).

Table 182: Exposure values for use as monomer in the production of synthetic tanning agents (Syntans), estimated by the eMSCA

Industrial					
Contributing scenario	Assessment parameters	Solid (dustiness)	Substance in preparation	Long-term Inhalative Exposure Estimate (mg/m^3)	Long-term Dermal Exposure Estimate (mg/kg/day)
PROC 1	Duration of activity > 4 hours/day, indoors with good general ventilation (no LEV), respiratory protection not used, glove efficiency is 80%	Yes (medium)	No	0,007	0,00686
PROC 8b		No	<1%	0,073	0,274
PROC 8b		No	<1%	0,073	0,274
PROC 8b		Yes (medium)	1-5%	0,14	0,549
PROC 9		No	<1%	0,073	0,137
PROC 9		Yes (medium)	1-5%	0,7	0,274
PROC 8a*		No	<1%	0,073	0,274
PROC 8a*		Yes (medium)	1-5%	0,7	0,549
PROC 15		No	No	0,73	0,0686

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

7.12.1.1.1.4. Exposure Scenario 4 - Use of Syntans for tanning in leather production

Use of Syntans for tanning in leather production is described by PROCs 3, 8a, 8b, 13 and 21. The Registrants report that respiratory protection is not used, for none of the scenarios. This process takes place in an industrial setting. Indoor use with good general ventilation, but no use of LEV is indicated by the Registrants. Gloves with efficiency 95% was indicated by the Registrants for all PROCs, except for PROCs 3 and 21.

The eMSCA used a worst-case approach (duration of activity longer than 4 hours/day, use of the substance indoors with good general ventilation, no use of respiratory protection, use of gloves with efficiency 80%).

Table 183: Exposure values for use of Syntans for tanning in leather production, estimated by the eMSCA

Industrial					
Contributing scenario	Assessment parameters	Solid (dustiness)	Substance in preparation	Long-term Inhalative Exposure Estimate (mg/m^3)	Long-term Dermal Exposure Estimate (mg/kg/day)
PROC 3	Duration of activity > 4 hours/day, indoors with good general ventilation (no LEV), respiratory protection not used, glove efficiency is 80%	No	< 1%	0,073	0,0137
PROC 8b		No	< 1%	0,073	0,274
PROC 8b		Yes (medium)	1-5%	0,14	0,549
PROC 8b		No	< 1%	0,073	0,274
PROC 13		No	< 1%	0,073	0,274
PROC 21		Yes (low)	< 1%	0,07	0,0566
PROC 8a*		No	< 1%	0,073	0,274
PROC 8a*		Yes (medium)	1-5%	0,7	0,549

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

7.12.1.1.1.5. Exposure Scenario 5 - Use of leather articles

Use of leather articles is described by PROC 21. The Registrants report that respiratory protection is not used. This process takes place in a professional setting. Indoor use, but no use of LEV is indicated by the Registrants. No use of gloves was reported by the Registrants.

As the Registrants already used a worst-case approach (duration of activity longer than 4 hours/day, use of the substance indoors, no use of respiratory protection, no use of gloves), the eMSCA decided to use the approach provided by the Registrants for this particular Exposure Scenario.

Table 184: Exposure values for use of leather articles, estimated by the eMSCA

Industrial					
Contributing scenario	Assessment parameters	Solid (dustiness)	Substance in preparation	Long-term Inhalative Exposure Estimate (mg/m^3)	Long-term Dermal Exposure Estimate (mg/kg/day)
PROC 21	Duration of activity > 4 hours/day, indoors (no LEV), respiratory protection not used, gloves not used	Yes (low)	< 1%	0,3	0,283

7.12.1.1.2. Literature data on exposure

- From urine samples from 17 **French** cashiers and 15 controls (non-occupationally exposed workers) it can be concluded that the general population is exposed to BPS and that frequent contact with thermal paper can be responsible for an increase of BPS in urine of cashiers (Ndaw *et al.*, 2018). Geometric mean concentrations of total BPS found in controls were 0.72 µg/L/0.52 µg/g creatinine and 2.48 µg/L/2.12 µg/g creatinine in cashiers.

- Urine and blood from 29 adults working in a hazardous waste incinerator in **Spain** were examined for the presence of 8 bisphenols (BPA, BPS, BPF, BPB, BPAF, BPZ, BPE and BPAP). Besides BPA and traces from BPB, no other bisphenols were detected neither in the urine nor in the blood (González et al., 2019).

- BPS was found in urine of cashiers and non-cashiers from North Carolina after handling of thermal receipts, as well as in their serum (Thayer *et al*.,2016). Thermal receipts were categorised based on their main analyte detected into BPA, BPS and BPSIP receipts. Each receipt contained 1–2% by weight of the paper of BPA, BPS, or BPSIP.

Table 185: BPS found in urine of cashiers and non-cashiers by Thayer *et al*. (2016)

Type of receipt	**Total BPS Urine (2011-2013)**		
	Cashiers		Non-cashiers (n=21)
	Pre-shift µg/g creatinine (geometric mean)	Post-shift µg/g creatinine (geometric mean)	µg/g creatinine (geometric mean)
BPA receipts	0.31 (n=33)	0.25 (n=33)	Na
BPS receipts	0.23 (n=31)	0.54 (n=31)	Na
BPSIP*[1] receipts	0.38 (n=12)	0.28 (n=12)	Na
BPS in urine			0.41

Table 186: BPS found in serum of cashiers by Thayer *et al.* (2016)

Type of receipt	Total BPS in serum (2011-2013)	
	Cashiers	
	Pre-shift (number >LOD)	Post-shift (number >LOD)
BPA receipts	9/33 (27.3%)	5/33 (15.2%)
BPS receipts	5/32 (15.6%)	13/32 (40.6%) *[2]
BPSIP*[1] receipts	2/12 (16.7%)	1/12 (8.3%)

*[1]BPSIP: 4-hydroxyphenyl 4-isoprooxyphenylsulfone, also called D-8
*[2]p = 0.02, significant difference between pre-shift and post-shift.
Serum LOD BPS: 0.002–0.01 ng/mL

7.12.1.2. Consumer

Literature data

Exposure of consumers to BPS can occur via different types of articles and products (food and food contact material, personal care products, paper products like currency ticket and airplane tickets, thermal paper, textiles, dental sealants, medicinal material and medicines) and indirectly via environmental contamination routes like indoor dust, water, sediment and sewage sludge.

Furthermore, it can be concluded that the major source of human exposure to BPS is via thermal paper (Liao *et al.*, 2012b; Pivnenko *et al.*, 2015) and in a lesser extent via food and personal care products. A negative correlation was found between BPA and BPS in thermal paper, meaning that thermal paper receipts containing high concentrations of BPS contained low or non-detectable concentrations of BPA and vice versa (Liao *et al.*, 2012b; Pivnenko *et al.*, 2015). A clear substitution of BPA by BPS is seen in the receipts containing low or non-detectable BPA. In addition, the total volume of thermal paper in the EU increased with 11,74% from 2014 to 2017 as well as the volume share of BPS in thermal paper. Moreover, keeping in mind the migrate potential of BPS from thermal receipts to other (paper) products and thus care should be taken for the implication on the recycling of paper products.

1. Human biomonitoring

Several biomonitoring studies are available assessing the presence of BPS in **human urines** from general populations in the United States, China, India, Japan, Korea, Kuwait, Malaysia, Vietnam (Liao *et al.*, 2012a), USA (Lehmler *et al*., 2018) and Saudi Arabia (Asimakopoulos *et al.*, 2016), from people living near a BPAF manufacturing plant in South China (Yang *et al.*, 2014), from adults in USA-Atlanta (Zhou *et al.*, 2014), from children in China (Yao *et al.*, 2018), from diabetes patient in China (Duan *et al.*, 2018), and in pregnant women in Australia (Heffernan *et al.*, 2016), China (Wan *et al.*, 2018; Zhang *et al.*, 2020a) and USA (Ihde *et al.*, 2018) and Israel (Machtinger et al., 2018)].

Some of the literature studies examined **urine** samples in Europe (Gyllenhammar *et al.*, 2017; Philips *et al.*, 2018; Larsson *et al*., 2017; Tkalec *et al*., 2021; Ndaw *et al*., 2018; Sakhi *et al.*, 2018; Husøy *et al*., 2019; Balicco *et al.*, 2019; Frederiksen *et al*., 2020 and Hartmann, 2021).

The study results from cashiers and non-cashiers in US North Carolina (Thayer *et al.*, 2016) and in France (Ndaw *et al.*, 2018) are reported in section 7.12.1.1. workers.

Presence of BPS in **blood** serum was measured by Thayer *et al. (*2016), Jin *et al*. (2018), Ihde *et al. (* 2018), Tan *et al*. (2019), Zhang *et al*. (2020a) and Li *et al*. (2020). Gély *et al*. (2021) and Zhang *et al.* 2020a reported values of BPS in cord blood/cord serum and amniotic fluid as well.

Furthermore, some studies examined the presence of BPS in **human placenta** (Overmeire *et al*., 2019), in **follicular fluid** (Amar *et al*., 2020) and migration of BPS from reusable plastic bottles into artificial **saliva** (Banaderakshan *et al*., 2022).

It should be noted that most of the articles don't describe the form of BPS (free, conjugated or total form) that was measured (in biological samples). When information was available this was explicitly added for the literature studies examining European human biological samples.

General population

Europe:

- Gyllenhammar *et al*. (2017), examined the urine concentrations of BPS in first-time mothers (n=178) in **Sweden** (Uppsala) from 2009 to 2014. Ammonium acetate and glucuronidase was added before the analysis. Lower concentrations of BPS (0.11 ng/L, mean value, density adjusted) were detected in comparison to what was found by Yang *et al*. (2014), and Zhou *et al*. (2014), in China and the U.S. resp. Also, no statistically significant temporal trend was observed for BPS, but such trends could be missed when single spot urine sample are taken, and a relative short period is examined.

- In the Larsson *et al*. (2017) study, concentrations of BPS in urine (from 113 **Swedish** preschool children with age ranging between 40 and 58 months) ranged between 20-33 000 ng/L of and 30-50 000 ng/L for unadjusted and density adjusted levels resp. (geom mean conc. of resp. 190 and 200 ng/L, median conc of 170 and 160 µg/L). Before analysing the samples, they were treated with ammonium acetate and glucorinidase. In this study samples were collected between March and May 2015 from a subset of 28 of the 100 selected preschools from six areas of Stockholm municipality. Furthermore, a time trend was studied by analysing the spot urine samples from longitudinal birth cohort BAMSE (Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology) collected between 1998 and 2000 from children in Stockholm. However due to the large number of samples below the LOD in 1998-2000 it was not possible to make a trend evaluation for BPS. Daily intake of BPS via dust was calculated to be 0.0004 µg/kg bw/d (geom. mean).

- Sakhi *et al*. (2018), collected urine samples from mothers and their children in spring 2012 in **Norway** and analysed them for the presence of environmental phenols. BPS was detected most frequently in the urine samples with a detection frequency of 42% in mothers with a Specific Gravity adjusted mean and geometric mean concentration of 0.18 ng/mL and 0.11 ng/mL resp. and of 48% in children with a Specific Gravity adjusted mean and geometric mean concentration of 0.45 ng/mL and 0.16 ng/mL resp.

- Bisphenol and phthalate concentrations were measured in first trimester spot urine samples of pregnant women (median gestational age of 12.9 weeks) in the **Netherlands** from February 2004 and July 2005 (Philips *et al*., 2018). Urine samples were deconjugated with β-glucuronidase before extraction using a liquid-liquid extraction method. Median urinary BPS concentration was 0.35 ng/mL with an interquartile range of 0.17 ng/mL and 1.03 ng/mL. 29.5% of the values were below the LOD (0.05 ng/mL).

- In the frame of a Norwegian biomonitoring study (part of the EU project EuroMix) levels of phenols and phthalates were measured between September 2016 and November 2017 in urine from **Norwegian** adults (Husøy *et al*., 2019). Samples were deconjugated using β-glucoronidase in ammonium acetate buffer. The detection rate of BPS in urine was 29% in all participants (n=144) and the Specific Gravity adjusted mean concentration and geometric mean were resp. 0.36 ng/mL and 0.19 ng/mL, with min 0.04 ng/mL and max 12.74 ng/mL.

- In the 'Esteban cross-sectional study', urine from 500 **French** children and 900 adults (age between 6 and 74 years) was monitored between April 2014 and March 2016 (Balicco *et al*., 2019). BPS was detected in almost all samples (resp. 99.9% and 100% for 'total BPS' and 'free BPS') and quantified in resp. in 99.9% and 56.2% of the samples. The geometric mean of the 'total BPS' impregnation was 0.444 µg/L (0.362-0.545 µg/L) and 0.442 µg/g creatinine (0.359-0.544 µg/g creatinine). The geometric mean for free BPS was not calculated due to a censoring rate > 40 %. The impregnation was higher in children than in adults. Although the conclusion of the causality of such cross-linking study should be treated with caution, concentrations in children were found to increase with the consumption of pre-packaged fish and a less regular ventilation of the dwelling, while in adults the increase was found to be due to the consumption of pre-packaged foods. (See https://www.hbm4eu.eu/hbm4eu-substances/bisphenols/)

- Van Overmeire et al. (2019) detected levels of BPS in human placenta samples ranging from 0.8 to 1.3 ng/g. 71 samples were collected in 2014-2015 in the Hospital Oost-Limburg in Genk (Belgium) and analysed by ultra- high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method.

- Urine and blood from 29 adults working in a hazardous waste incinerator in **Spain** were examined for the presence of 8 bisphenols (BPA, BPS, BPF, BPB, BPAF, BPZ, BPE and BPAP). Besides BPA and traces from BPB, no other bisphenols were detected neither in the urine nor in the blood (González *et al.*, 2019).

- Frederiksen *et al*. (2020) investigated amongst others exposure to BPS by monitoring of 100 urine samples in 2009, 2013 and 2017. The urine samples were collected from **Danish** men with a mean age of 20 years (range 18 – 30 years) and were deconjugated before analysis. The authors observed significant increase of the urinary median osmolality adjusted concentration of BPS over the study period. In 2009 following values were obtained: 0.11 ng/mL (median); 0.21 ng/mL (75 percentile); 0.76 ng/mL (95 percentile), 4.62 ng/mL (max.), while in 2017: 0.18 ng/mL (median); 0.39 ng/mL (75 percentile); 2.78 ng/mL (95 percentile), 36 ng/mL (max.). Interestingly, the urinary median osmolality adjusted concentration of BPA significantly decreased with 57% in from 2009 to 2017.

- Amar *et al*. (2020) detected levels of BPS glucuronide in follicular fluid samples collected from 59 **French** women who underwent in vitro fertilisation procedure. The concentration ranged from 0.5 to 12.6 nM, with an average of 4.4 ± 1.4 nM (1.9 ± 0.6 ng/mL). BPS glucuronide was detected in 11 of the 59 samples.

- Tkalec *et al*. (2021) studied the presence of selected bisphenols, parabens and triclosan in first morning void from 246 children (6-9 years) and adolescents (11-15 years) in a rural region in **Slovenia**. Glucuronide and sulfate metabolites were deconjugated using β -glucuronidase/arylsulfatase. As a result of this study BPS was found in 27% of children samples and in 35% of samples taken from the adolescents (geom means of 0.30 and 0.36 µg/L, respectively). The highest measured concentration of BPS was 23 µg/L in adolescents. The results obtained indicated also that despite of the effort put in substitution of BPA, this substance was still found in the highest concentrations throughout the studied population (99% of kids and 100% of adolescents with respective geom means: 2.1 and 1.9 µg/L).

- In an **Austrian** children survey, a total of 85 elementary school children aged 6-10 years (45 girls and 40 boys) were examined for the presence of 130 compounds, amongst others bisphenol S (Hartmann, 2021). The study demonstrated an increase of exposure to BPA alternatives like BPS. BPS was found in urine with concentrations up to 4.2 µg/L (4.5 µg/g creatinine).

- In a study conducted by Gély *et al*. (2021), the cord blood was collected in 44 pregnant women in **France** between June 2014 and October 2015. BPS-G (glucuronide) was

determined in almost half of the cord plasma samples with concentration ranges nd-0.586 ng/mL.

- Banaderakhshan *et al*. (2022) examined the migration of BPS from reusable plastic bottles into artificial saliva by using HPLC-MS/MS. Bottles from five different brands were collected in **Austrian** stores during the summer of 2020. Depending on the type of bottle, BPS was detected in the saliva in concentrations between <LOQ (0.001 µg/L,) and 0.070 µg/L.

Outside Europe:

- BPS was analysed in human urine samples from general populations of the United States, China, India, Japan, Korea, Kuwait, Malaysia and Vietnam (Liao *et al*., 2012a). In total, 315 urine samples, collected from the populations of the abovementioned countries, were analysed for the presence of total BPS (free plus conjugated) concentrations by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Urinary bisphenol A concentration has been used in the estimation of a human exposure dose. Given the similarity in molecular structure between BPS and bisphenol A, the human exposure to BPS has been estimated based on a model reported for bisphenol A. Estimated daily intake (EDI; µg/day) = urinary BPS concentration (µg/L) x urine excretion rate (L/day). The urine output varies by age and sex and is reported to be about 1.2-2 L/day for adults. Values for daily urine excretion rate used in the exposure assessment were adopted from the reference values given by the International Commission on Radiological Protection (ICRP). According to the ICRP, gender difference in urine excretion rate is observed for adults (≥ 20 years), and the rates are 1.6 and 1.2 L/day for men and women, respectively. The reported ICRP urine excretion rates for 5-, 10-, and 15-year-old individuals were 0.5, 0.7, and 1.2 L/day, respectively, for both male and female subjects, which were used for individuals of ages 2 to ≤ 5 years, 6-10 years, and 11-19 years, in the calculations. Data analysis was performed with Origin 7.5 or SPSS 17.0. Concentrations below the limit of quantitation (LOQ) were substituted with a value equal to LOQ divided by 2 or the square root of 2 for the calculation of arithmetic mean and geometric mean (GM), respectively. Differences between groups were compared by a one-way analysis of variance (ANOVA) and the Tukey test. Values of $p < 0.05$ denoted significance.

  BPS was detected in 81% of the urine samples analysed at concentrations ranging from below the LOQ of 0.02 ng/mL to 21 ng/mL (geometric mean: 0.168 ng/mL). This study is considered the first study to report the occurrence of BPS in human urine.

  Relatively higher concentrations and detection frequencies of BPS in urine samples from Japan and the U.S. were in accordance with the tendency to replace bisphenol A with BPS in recent years. Japan has phased out bisphenol A in thermal receipt papers since 2001 and made an effort to develop alternatives, including BPS. A major manufacturer of thermal receipt papers in the U.S. announced replacement of bisphenol A with BPS in 2006. The urinary BPS concentration varied among countries, and the highest geometric mean concentration of BPS was found in urine samples from Japan, followed by the United States, China, Kuwait, and Vietnam. There were no significant differences in BPS concentrations between genders (male versus female), or among age groups (≤ 19, 20-29, 30-39, 40-49, and ≥50) or races (Caucasian versus Asian).

  The mean and median estimated daily intakes were reported to be 0.93 and 0.248 µg/d, respectively, for all eight countries together. The highest estimates were obtained for Japan (3.47 and 1.67 µg/d, resp.), followed by the US (1.48 and 0.316 µg/d, resp.). The authors mention that several uncertainties exist in the assessment of exposure to BPS based on the measured urinary concentrations. The pharmacokinetics of BPS are not well understood, and it has been assumed that BPS

and BPA behave similarly in the human body. The discussion of the association of BPS concentrations with demographic features was tempered by the small sample size from individual countries. The author indicated that studies with large sample sizes are needed.

- Zhou *et al.* (2014), examined 100 urine samples of non-occupational exposed U.S. adults (Atlanta) between 2009 and 2012. Urine samples were analysed by high performance liquid chromatography isotope dilution tandem mass spectrometry and BPS was found in 78% of the samples with a median concentration of 0.13 ng/mL (range between <LOD (0.03 ng/mL) and 12.3 ng/mL). In the frame of the NHANES (National Health and Nutrition Examination Survey) urine samples of adults (n=1808) and children (868) collected in 2013-2014 in the US were analysed for their presence of BPS (Lehmler *et al*., 2018). BPS was quantified using on-line SPE−HPLC−MS/MS. Median levels of BPS were 0.37 µg/L for adults (detection frequency of 89.4%) and 0.29 µg/L for children. LOD was 0.1 µg/L.

- The relation between 57 xenobiotics (including BPS) and oxidative stress was examined by analysis of their concentrations in 130 urine samples from a Saudi Arabian population (Asimakopoulos *et al*., 2016) by using liquid chromatography tandem mass spectrometry (LC/MS/MS). Of the bisphenols, BPS was predominantly found in the urine with a detection rate of 100% and a mean concentration of 13,3 ng/mL. Those concentrations were higher than those of BPA (mean 5,71 ng/mL). Furthermore, BPS showed a strong correlation with the oxidative stress biomarker, 8-hydroxy-2′-deoxyguanosine (8OHDG Ln values demonstrated on a CR-adjusted basis): BPS (r=0.30, p=0.0005) leading to the conclusion that BPS might contribute to the induction of oxidative stress.

- Concentrations of free BPS ranging from <LOQ and 0.058 ng/mL creatinine (0.022 ng/mL- geom mean), and total BPS ranging from <LOQ to 7.046 ng/mL creatinine (0.028 ng/mL, geom mean) were detected in the urine of people from South China (n=94), aged between 26 and 84 years and living near a BPAF manufacturing plant (Yang *et al*., 2014). Measurement was performed by using liquid chromatography coupled to mass spectrometry (LC–MS/MS). Free BPS was detected in 9.4% of the samples, while total BPS in 40.4%.

- BPS was measured in the urine of pregnant women (gestation age: 14-18 weeks) in Australia using automated online SPE-LC-QTRAP-MS/MS method (Heffernan *et al*., 2016). BPS was detected in 10% of the samples in a concentration range <LOR - 8.1 ng/mL. BPS was found at trace levels of 0.09 ng/mL in the average blank (n=4). The limit of reporting (LOR) was calculated as 10 times the signal-to-noise in low-level spiked synthetic urine, or three times the average blank for compounds presenting procedural blanks – whichever gave the highest value. LOD of BPS was determined to be 0.067 ng/mL.

- Duan *et al*., 2018 collected spot urine samples between May 2016 to June 2017 from controls and type 2 diabetes mellitus cases (T2DM) in China. BPS had a detection rate of 58% in total (n=502) and 47.8% and 68.1% for controls (n=251) and T2DM (n=251) resp. Creatinine-corrected concentrations of the urine samples resulted in median concentrations ranging from not detected to 0.248 µg/g creatinine in controls. A median concentration of 0.199 µg/g creatinine (ranging from not detected to 0.563 µg/g creatinine) was measured in T2DM cases. BPS concentrations were significantly associated with T2DM (log transformed and categorical statistical models).

- Urine was sampled from 40 Chinese school children (8-11 years of age) and analysed using ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry contained 0.25 to 50 ng/mL of BPS (Yao *et al*., 2018).

- BPS was found in human plasma from Chinese adults with mean concentrations of 0.15 ng/mL (Jin *et al*., 2018). Mass fractions in plasma (mean of 0.78) indicate a strong partitioning of BPS to the plasma fraction.

- BPS was found in a concentration of 0.17 µg/L (geometric mean, specific gravity adjusted) in urine of pregnant Chinese woman at admission to labour (Wan *et al*., 2018). The substance was detected in 93.7% of the spot urine samples (n=985). An increase of BPS in the urine with 1 ln-unit caused an increase of the pregnancy duration of 0.72d. Furthermore, when stratified for foetal sex, a significant correlation was found between a ln-unit increase of BPS in maternal urine and increased gestational age and increased odds of later term birth. Although not statistically significant, a trend was seen between the maternal urinary BPS concentration and decreasing birth weight in girls. No effects were seen on birth length.

- A novel mass spectrometric (MS) method was run by Ihde *et al*. (2018) on 30 paired maternal urine and foetal cord blood samples from mothers (New Jersey, USA) undergoing an elective Caesarean section. 60% of the mothers tested positive for BPS. BPS was not detected in the cord blood.

- Machtinger *et al*. (2018) investigated urine of 50 pregnant women (mean patient age: 34.4 ± 6.2 years; mean delivery week: 38 ± 1.1) in Israel in order to characterise exposure to selected phthalates, bisphenols and other chemicals in personal care products and to evaluate associations between prenatal exposure to endocrine disruptors. The samples were collected on the same day or the day before scheduled elective caesarean section or upon admission to the delivery room. Additionally, questionnaires regarding patients' consumer habits during pregnancy were filled in. The study indicated that the BPS could have been found in 27% of the samples with a specific gravity adjusted concentration of 0.4 µg/L (90th percentile) and 0.7 µg/L (95th percentile).

- The presence of 6 bisphenols and 10 phthalates in urine samples of men before and after cardiac surgery was investigated by Shang *et al*. (2019). They collected urine samples from the Jewish General Hospital (Montreal, Canada) and subsequently exposed mouse models with the measured concentrations in those urine samples. The concentration of BPS measured in the urine sample before surgery ranged from 0.17-1.8 µg/gm creatinine (median 0.29 µg/gm creatinine); 12h post-surgery from 0.17-1.0 µg/gm creatinine (median 0.27 µg/gm creatinine) and 24h post-surgery from 0.16-0.28 µg/gm creatinine (median 0.19 µg/gm creatinine). No increase in BPS was found suggesting that BPS was not present in medical devices used in cardiac surgery in Canada as of May 2018.

- BPS concentrations were measured in urine from healthy 22–37-year-old participants (n=33) from Singapore and ranged < detection limit (0.007 ng/mL) and 1.93 ng/mL (specific gravity adjusted), with a geom. mean of 0.077 ng/ml (Liu *et al*., 2019a). The detection frequency of BPS was 94%. For BPS analysis, urine samples were deconjugated with β-glucuronidase before extraction using a liquid-liquid extraction method.

- Blood samples collected from a hospital in China were analysed for BPA, BPF, BPS, BPB, BPAF, BPAP and BPZ by Tan *et al*. (2019) using pre-column derivatisation with high-performance liquid chromatography and tandem mass spectrometry. BPS had a detection frequency of 25%. Measured geom mean concentration was 0.06 ng/ml (<LOD~0.79 ng/mL).

- Zhang *et al*., 2020a examined concentrations of BPS in urine, serum and amniotic fluid samples from April 2017 to August 2017 collected from 106 pregnant women originating from an e-waste dismantling area in South China. BPS was found with a detection rate (n=106) of 30% in maternal serum and 63% in cord serum. BPS

detection rate in 15 pairs was resp. 27% (maternal serum), 80% (cord serum), 100% (maternal urine) and 67% (amniotic fluid). BPS was detected at geometric mean concentration of 0.05 ng/mL in maternal urine, 0.01 ng/mL in maternal serum, 0.03 ng/mL in cord serum and 0.02 ng/mL in amniotic fluid.

- BPS was one of the most frequently detected of 10 bisphenols identified in serum of 181 pregnant women from China, with a detection rate of 72.4% and a concentration of 0.113 ng/mL (Li *et al*., 2020).

2. Human exposure to BPS via articles and products

Several literature studies demonstrate the occurrence of BPS in different types of articles and products in Europe and worldwide (food and food contact material, thermal paper and other paper products, textiles, personal care products, dental sealants, medicinal material and medicines).

Concentrations in food and food contact material

BPS has a European SML (specific migration limit) of 0,05 mg/kg for plastic materials and articles intended to come into contact with food (European Commission, 2011). After evaluating of the new toxicity data (EOGRT with DNT and DIT cohorts, toxicokinetic study in rats) introduced following the substance evaluation under REACH Regulation (EC)1907/2006, EFSA concluded that the current SML is still appropriate (EFSA, 2020).

Some data on the presence of BPS in food and food contact material in Europe are available. Other studies were conducted in USA, Canada and China.

Europe:

- Different types of canned vegetables and their supernatant liquid were analysed for the presence of BPA, BPS and 2,2'-biphenol (BP). Cans, protected with an inner layer of epoxy lacquers, were collected from different manufacturers. Up to 36.1 ng/g of BPS was found in the food and up to 175 ng/ml in the liquid (Viñas *et al*., 2010). Furthermore, migration of BPS was tested using food simulants according to Council Directive 85/572/EEC. Levels of BPS increased when acetic acid was used as simulant as well as temperature and contact time increased. Concentrations were up to 0.52 ng/mL (water, 25°C), 3.54 ng/mL (water, 80°C), 2.17 ng/mL (3% HAc, 25°C) and 5.36 ng/mL (3% HAc, 80°C) after 240h.

- However, Gallart-Ayla *et al.* (2011) did not detect BPS in canned soft drinks collected in supermarkets in Barcelona (**Spain**) in July 2009. Analysis was done by on-line solid phase extraction fast liquid chromatography-tandem mass spectrometry.

- In Simoneau *et al*. (2011), the release of BPS from polyether sulphone baby bottles was found to be below the detection limit of the analytical methods used (0.1 µg/kg for the HPLC- and 0.3 µg/kg for the UPLC-MS method). Bottles were collected in the USA and 11 **European countries**: Belgium, Bulgaria, Germany, Denmark, Spain, France, Italy, Lithuania, Malta, Poland and United Kingdom).

- Canned and non-canned beverages purchased on the **Belgian** market between April and December 2014 did not contain detectable levels of BPS (Reguiero and Wenzl, 2015). LOD of non-alcoholic and alcoholic beverages was 17.6 ng/L and 19.4 ng/L resp., the LOQ resp. 58.6 and 64.6 ng/L.

- García-Córcoles *et al.* (2018) examined the presence of seven bisphenols in 15 ready-to-eat plastic packed baby foods (powdered milk, cereals with milk, juices, yoghurt and homogenised fruit, meat and fish) from different brands in Granada, **Spain**. BPS was found the most abundant of the bisphenols with concentrations between 11.7 and

49.2 ng/g (LOD: 0.3 ng/g, LOQ: 1 ng/g). BPS was detected and quantified in 5 of the 15 samples.

- Levels of BPS were detected in food and beverage collected on a **Dutch** market (Van Leeuwen *et al.*, 2019). Concentrations in Tuna brine (juice accompanying the meat in the can), wiener sausage brine, carbonated soft drinks and apple juice were resp. 0.2 ng/mL, 0.1 ng/mL, <0.0007 and <0.001 ng/mL. BPS was lost during sample preparation in tuna meat, Wiener sausage, Corn, Tomato soup, pineapple and tomato puree.

- BPA-free reusable drinking bottles from five different brands were collected in Austrian stores during the summer of 2020 (Vienna) (Banaderakhshan *et al.*, 2022). Most of the bottles were made of Tritan and for usage by children and adults. Migration experiment shows that BPS was detected in each leaching sample at levels between 0.013 µg/Land 0.26 µg/L (20°C) and 0.010 µg/L and 0.065 µg/L (60°C).

Outside Europe:

- One study (Liao and Kannan, 2013) reports concentrations of several bisphenol analogues (including BPA, BPF, and BPS) in foodstuffs collected in 2008, 2011 and 2012 from Albany, NY, USA, using HPLC-MS/MS. Foodstuffs were divided into nine categories : beverages, dairy products, fats and oils, fish and seafood, cereals, meat and meat products, fruits, vegetables, and “others”. Concentrations of BPS were in general 1-2 orders of magnitude lower than those of BPA and BPF. BPS contributed <10% in the sum of the concentrations of the analysed bisphenols in the 9 food categories. BPS was most frequently (43.1%) and with the highest concentrations (in a geometric mean conc of 0.609 mg/g, 95th percentile of 0.780 mg/g) found in meat and meat products.

Table 187: BPS concentrations in foodstuffs (Liao and Kannan, 2013)

BPS conc.	Beverages (n = 31)	Dairy Products (n = 29)	Fats and Oils (n = 5)	Fish and Seafood (n = 23)	Cereals and Cereal Products (n = 48)	Meat and Meat Products (n = 51)	Fruits Including Canned Fruits (n = 20)	Vegetables Including Canned Vegetables (n = 45)	Others (n = 15)	All (n = 267)
Mean (mg/g)	0.007	0.040	0.005	0.021	0.013	0.609	0.009	0.018	0.005	0.130
95th percentile (mg/g)	0.005	0.020	0.005	0.081	0.036	0.780	0.023	0.057	0.007	0.076
Frequency (%)	3.23	13.8	0	26.1	14.6	43.1	25.0	22.2	6.67	20.9

- Another study (Liao and Kannan, 2014b) analysed 289 food samples collected in 2012 from nine cities in China. Significant positive correlations were found between BPA and BPS which suggest co-occurrence and similarity in sources in foods. BPS was most dominantly found in fish and seafood (frequency of 72.7%) in mean concentrations of 0.564 ng/g. The highest concentration was however found in meat and meat products with a mean of 2.16 ng/g and a frequency of 30%. The EDI (estimated daily dietary intake was estimated to be 9.55 ng/kg bw/day by adult males and 9.56 ng/kg bw/day by female adults.

Table 188: BPS concentrations in food samples (Liao and Kannan, 2014b)

BPS conc	Cereals and cereal products (n = 39)	Meat and meat products (n = 20)	Fish and seafood (n = 11)	Eggs (n = 11)	Milk and milk products (n = 17)	Bean products (n = 27)	Fruits (n = 20)	Vegetables (n = 42)	Cookies /Snacks (n = 26)	Beverages (n = 4)	Cooking oils (n = 11)	Condiments (n = 48)	Others (n = 13)	All (n = 289)
Mean (ng/g)	0.042	2.16	0.564	0.005	0.012	0.054	0.011	0.644	0.065	0.005	0.014	0.020	0.009	0.287
Median (ng/g)	0.005	0.005	0.145	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Range (ng/g)	n.d.–1.11	n.d.–42.3	n.d.–4.25	n.d.	n.d.–0.110	n.d.–1.13	15.0	n.d.–24.8	n.d.–0.624	n.d.	n.d.–0.108	n.d.–0.245	n.d.–0.034	n.d.–42.3
95th percentile	0.078	2.60	2.53	0.005	0.036	0.104	0.040	0.270	0.302	0.005	0.056	0.115	0.027	0.241
Frequency (%)	23.1	30.0	72.7	0	11.8	14.8	n.d.–0.073	31.0	34.6	0	9.1	16.7	15.4	22.5

- From a review of literature studies from 2010 to 2017 it can be concluded that in the US and China food is the dominant source for human exposure to BPS, while the contribution of personal care product usage is low (Wu *et al*., 2018).

- A novel liquid chromatography tandem-mass spectrometry (LC-MS/MS) was used by Hwang *et al*. (2018) to measure the migration of 8 bisphenols (BPA, BPS, BPF, BPB, BPP, BPAF, BPAP and BPZ) from 11 types of food contact materials. 234 articles were sampled from online kitchenware market and departments stores in Korea. No bisphenols were detected in any of the samples.

- Zhou *et al*., 2019 examined the contamination of BPS in foodstuffs. 379 samples were collected in the Zhejiang Province (China) and divided in twelve categories:

Table 189: BPS concentrations in foodstuffs (Zhou *et al*., 2019)

Category	Frequency of BPS (%)	Mean/median concentration of BPS (µg/kg or µg/L)
Water	30.3	0.02/0.0
Beverages	23.9	0.01/0.0
Rice	25.8	0.11/0.0
Wheat flower	-	-
Shellfish	100.0	2.1/0.5
Fish	100.0	9.2/3.9
Meat	75.0	9.0/2.3
Vegetables	62.5	0.5/0.4
Canned cereal	-	-
Canned fish	50.0	0.3/0.1
Canned meat	13.3	0.05/0.0
Others (edible oil, egg, honey, …)	7.3	0.05/0.0

- After analysis of 23 dairy products from a local market in China, BPS was detected in milk beverages (0.4 µg/kg +/-6.1) and yogurt (0.-4-0.5 µg/kg) (Cheng *et al*., 2017).

- Cao *et al*. (2019) did not detect BPS in canned food composite samples from a Canadian total diet study. BPS was however detected in nine food composite samples prepared from meat and meat products with concentration ranging between 1.2 and 35 ng/g.

- Zhang *et al.* (2019) examined the presence of BPS in soy-bean milk and pacifiers collected on the Chinese market. Pacifiers were soaked in hot water to investigate the solution behaviour of BPS and 7 other bisphenols. BPS concentrations were <LOQ in soy-bean milk, but could be found in pacifiers and their soaking solution with concentrations of resp. 2.34 µg/kg and 0.417 µg/kg resp.

Concentrations in leather and textiles

- In the study of Xue *et al.* (2017) investigating 77 textiles for babies (socks, fabric nappies, blankets, and bodysuits), concentrations of BPS ranging between 0.7 and 394 ng/g were reported.

- Li and Kannan (2018) examined concentrations and profiles of 23 endocrine-disrupting chemicals, including BPS, in 74 pantyhose samples collected from 6 countries. This study revealed median concentrations of 1430 ng/g of BPS in almost all of the samples investigated (100% and 96% of the samples, respectively). Moreover, it was found that pantyhose made in Japan and China with 21–50% Spandex contained the highest concentrations of BPS (2.2 mg/g). Consequently, the authors calculated dermal exposure doses of 45 900 pg/kg bw/d.

- In the study of Wang *et al*. (2019b), BPS was found in 43% of the samples of collected garments (n=93). Used clothes (n=49) were selected from wardrobes of 38 families in 3 Chinese cities, while new clothes (44) were purchased from online retailers and local stores in Tianjin (China). BPS concentration in the textiles ranged from <0.53 to 536 ng/g with a median and mean concentration of resp. 7.38 and 44.0 ng/g. Used clothes contained less BPS than new clothes (5.67 vs 12.3 ng/g, median concentration). Furthermore, very high concentrations of BPS were found in new textiles made of polyester and Spandex (536 ng/g).

- Smit and Zoon published on their website figures of residual BPS in syntans used for leather retanning, which revealed concentrations of BPS ranging from >20 up to >28400 mg/kg (https://www.smitzoon.com/en/sustainability/chemicals-bisphenol/). The lowest detection limit was 20 mg/kg.

Concentrations in personal care products

- After BPA (13%), BPS was the second most frequently detected of the bisphenols (11%) analysed in 117 samples of personal care products collected from retail stores in China and US during 2012-2013. The highest detection rate was found in makeup (18.8%). The low detection frequency and concentrations of BPS in the analysed products suggest that personal care products are not the most dominant source of exposure to BPS in those countries (Liao and Kannan, 2014a).

Table 190: BPS concentrations in personal care products (Liao and Kannan, 2014a)

Type of personal care product		Body washes (n=31)	Hair care products (n=32)	Make up (n=32)	Sanitary products (n=9)	Skin lotions (n=107)	Toilet soaps (n=3)
BPS	GM (ng/g)	0.364	0.354	0.504	0.369	0.437	0.495
	DR (%)	3.2	0	18.8	11.1	14	0.972

GM: geometric mean
DR: detection rate

- Lu *et al*., 2018b collected 150 samples of 11 categories of personal care products in retail stores and supermarkets in Guangzhou and Shenzen (China) in September 2015. BPS had a detection frequency of 33.3% when total of personal care products is considered.

Table 191: BPS concentrations in personal care products (Lu *et al*., 2018b)

Category		Tooth pastes (n=28)	Shampoos (n=15)	Face cleansers (n=18)	Bath gels (n=21)	Hand saitizers (n=11)	Sunscreens (n=12)	Body lotions (n=8)	Lipsticks (n=2)	Hand lotions (n=17)	Hair gels (n=7)	Masks (n=11)	TOTAL
BPS	Median (ng/g)	3.22	3.22	3.22	-	-	-	-	-	-	-	-	-
	Mean (ng/g)	2.6	2.79	1.79	-	-	1.07	0.40	-	0.19	-	-	1.11
	Max. (ng/g)	8.45	3.22	3.22	-	-	3.22	3.22	-	3.22	-	-	8.45
	Min. (ng/g)	-	-	-	-	-	-	-	-	-	-	-	-
	DR %	75.0	86.7	55.6	-		33.3	12.5	-	5.9	-	-	33.3

DR: detection rate
LOD=1.9ng/g; LOQ=6.4 ng/g

They also estimated the intake and uptake of BPS from personal care products by dermal contact. The daily dermal uptake was estimated by considering a dermal absorption rate of 10% (similar to BPA).

Table 192: Estimated dermal intake and uptake of BPS from personal care products (Lu *et al*., 2018b)

Category		Tooth pastes (n=28)	Shampoos (n=15)	Face cleansers (n=18)	Bath gels (n=21)	Hand saitizers (n=11)	Sunscreens (n=12)	Body lotions (n=8)	Lipsticks (n=2)	Hand lotions (n=17)	Hair gels (n=7)	Masks (n=11)	TOTAL
BPS	EDI (ng/kg bw/d)	6.29×10^{-3}	6.28×10^{-3}	1.28×10^{-3}	0	0	5.38×10^{-2}	6.15×10^{-2}	0	6.83×10^{-3}	0	0	0.13
	EDU (ng/kg bw/d)	6.29×10^{-4}	6.28×10^{-4}	1.28×10^{-5}	0	0	5.38×10^{-3}	6.15×10^{-3}	0	6.83×10^{-4}	0	0	1.35×10^{-2}

EDI= Estimated dermal intake
EDU= Estimated dermal uptake

- Gao and Kannan (2020) examined 77 feminine hygiene products from Albany, USA. The products were divided into 7 categories and 24 chemicals were measured of which 8 bisphenols (BPA, BPF, BPP, BPS, BPZ, BPAP, BPAF and BPB). BPF, BPA and BPS were the major bisphenols found in these products.
  The highest detection frequency for BPS was in wipes (17%) and panty liners (15%), but the highest concentration was found in bacterial creams and solutions with a mean of 0.18 ng/g.

Table 193: BPS concentrations and dermal absorption doses in feminine hygiene products (Gao and Kannan, 2020)

Category		Pads (n=18)	Panty liners (n=13)	Tampons (n=12)	Wipes (n=12)	Bacterial creams and solutions (n=14)	Deodorant sprays (n=4)	Powders (n=4)
BPS Conc	Mean (ng/g)	<LOD	0.12	0.02	0.05	0.18	<LOD	<LOD
	Median (ng/g)	<LOD	<LOD	<LOD	<LOD	0.06	<LOD	<LOD
	Range (ng/g)	<LOD	<LOD-1.34	<LOD-0.22	<LOD-0.62	<LOD-0.08	<LOD	<LOD
	DF (%)	0	15	8	17	8	0	0
Dermal absorption dose	Median (ng/kg bw/d)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Maximum (ng/kg bw/d)	<0.001	0.006	0.003	<0.001	<0.001	<0.001	<0.001

DF: detection frequencies
LOQ in the range of 0.1-0.4 ng/g

Concentration in dental sealants

- BPS was not detected in dental sealants collected between June and August 2015 on the US market, originating from the US, Korea, **Liechtenstein, The Netherlands** and **Greece** (Xue *et al*., 2018). However, authors measured BPS in the only analysed dental sealant sample from Japanese origin, with a concentration of 21.5 ng/g. The detection frequency was 1.4%.

Concentrations in Medicinal material

- Shang *et al*., 2019 couldn't detect BPS in urine from Canadian men (pre- and post-surgery) suggesting that BPS is not present in medicinal device used in cardiac surgery in Canada as of May 2018 (see also human biomonitoring).

- Zhang *et al*., 2019 determined concentration of 8 BPs via UHPLC–MS/MS in sodium chloride injection and glucose injection purchased at the Liaoning Province Tumour

Hospital in China. BPS concentration were <LOQ in sodium chloride, but 0.051 µg/L in the glucose injections.

Concentrations in medicines

- BPS was detected in samples of commonly used over-the-counter medicines manufactured in China, including western medicines and Chinese patent medicines for adults and children which were collected from local drugstores in China (n=28) and 8 Chinese families in the USA (n=58) in June 2014 (Jia *et al*., 2021). BPS had a frequency of resp. 14% and 19% in paediatric and adult medicines, with a mean concentration of resp. 0.21 and 0.07 ng/g).

Table 194: BPS concentrations and frequencies in different medicines (Jia *et al*., 2021)

Category		Paediatric medicines (n=58)	Adult medicines (n=28)	Total medicines (n=86)
BPS	Frequency (%)	14	19	17
	Max. (ng/g)	4.93	1.13	4.93
	95th	0.43	0.46	0.47
	Median (ng/g)	n.d.	n.d.	n.d.
	Mean (ng/g)	0.21	0.07	0.11

n. d=not detected

Point assessment and probabilistic assessment of EDIs for BPS in different age categories were:

Table 195: Point and Probabilistic estimation of EDIs for BPS in OTC medicines (Jia *et al*., 2021)

		<1 year old	1-3 years old	Male	Female
Point estimation calculated with measured concentrations					
BPS	Mean (µg/kg bw/d)	0.77	0.33	-	0.01
	Median (µg/kg bw/d)	-	-	-	-
	95th	3.96	1.24	0.04	0.04
Probabilistic estimation from the Monte Carlo simulation					
BPS	Mean (µg/kg bw/d)	0.39	0.41	0.02	0.02
	Median (µg/kg	0.10	0.08	0.01	0.01

	bw/d)				
	95th	0.83	0.81	0.04	0.05

Concentrations in thermal Paper and other paper products

In Europe:

- Thermal paper receipts like cashier receipts, ATM receipts, parking tickets, bus tickets etc. were collected in the period between September 2013 and January 2014 in **Switzerland** (Goldinger *et al*., 2015). BPS occurred only in 4 out of 124 samples. Concentrations ranged between 8.3 and 12.6 mg/g (mean: 10.2 mg/g).

- BPS was found in 31 out of 50 samples of thermal paper receipts collected on the **Italian** market from 2015-2016. Concentrations were determined by liquid chromatography to tandem fluorescence and ultraviolet detection and ranged between <LOQ to 357.989 µg/100 mg paper with a mean concentration of 41.97 µg/100 mg paper (Russo *et al*., 2017).

- Molina-Molina *et al.* (2019) collected 112 thermal paper receipts in 2017 from Brazil (n=22), **Spain** (43) and **France** (47) and analysed them for the presence of BPA, BPS and BPF as well as for their estrogenic and anti-androgenic activity. In 9.1, 4.6 and 21.3% of the samples, BPS was detected in the receipts from Brazil, Spain and France resp. and in concentrations between <0.03 and 13.29 mg/g.

Table 196: BPS concentrations and detection in thermal paper receipts from different countries (Molina-Molina *et al*., 2019)

Country samples	% Detected	Geometric mean (mg/g)	Range (mg/g)
Brazil (n=22)	9.1	-	<0.03-8.933
France (n=47)	21.3	10.235	<0.03-12.56
Spain (n=43)	4.6	-	<0.03-13.29

- Furthermore, it should be mentioned that the total volume of thermal paper in the **EU** increased with 11,74% in 2017 (4195 Ton) compared to 2014 (3754 Ton). The volume share of BPS also increased from 150 Ton, representing 4% of the total volume of thermal paper of 2014 to 397 Ton, representing 9% of the total volume of 2017 (ECHA, 2018).

  In the meanwhile, the volume of BPS used in thermal paper manufactured and placed on the market in the **EU** is tripled from 64 kTon in 2014 to 187 kTon in 2019, whereby the substance became the main developer used in thermal paper in 2019. If a 75% switch of BPA-based thermal paper to BPS is considered, it is estimated that 307 kTon BPS will be used as developer in thermal paper in 2022, representing a growth of 376% for the period of 2014-2022 and a total share in thermal paper consumed in the EU market of 61%. (ECHA, 2020).

- BPS was detected in the majority of the nine samples of thermal papers (cash receipts) collected in 2020 in the most common supermarkets and service providers in Vienna (**Austria**) (Banaderakhshan *et al*., 2022). Concentrations of BPS were analysed using

HPLC-MS/MS and were in the range of <LOQ (0.01 µg/L) and 38 µg/g. BPS and BPA were the most common bisphenols present in the examined samples.

Outside Europe:

- From the Liao *et al*. (2012b) study, it can be concluded that the major human exposure source to BPS among the analysed paper types comes from handling of thermal receipt papers (>88%).

  Between 2010 and 2011, thermal paper receipts (n=111), currency bills (n=52) and other paper products (n=105) were collected. Thermal paper samples were obtained from the USA, Japan, Korea, and Vietnam; currency bills from the USA, Canada, Czech Republic, Russia, Turkey, Australia, Brazil, Egypt, South Africa, China, India, Japan, Korea, Kuwait, Malaysia, Philippines, Singapore, Thailand, Vietnam, and United Arab Emirates and other papers mainly from Albany (New York, USA).
  "Other paper products" were further divided in 14 categories.

  BPS was present in all thermal paper receipts (100%) with concentrations ranging from 0.0000138 to 22 mg/g (geometric mean 0.181 mg/g) which is in the same order of magnitude as BPA found in *Liao et al.*, 2011a. Furthermore, a negative correlation was observed between BPS and BPA in those receipts: thermal paper receipts containing high concentrations of BPS contained low or non-detectable concentrations of BPA and vice versa.

  Because of the structural similarity with BPA and the similar concentrations of BPA found in thermal receipts, migration of BPS from thermal receipts to paper currencies is also assumed every time a receipt is placed near the currency in a cash register or wallet and thus considered an important source of BPS presence in paper currency. The same can be also the case when those thermal receipts come in contact with other products, what should be kept in mind when thermal receipt papers are recycled together with other paper products.

  The highest concentrations of BPS in other paper products were found in airplane luggage tags, boarding passes and mailing envelopes. Most probably due to a similarity in thermal printing process used.

Table 197: BPS concentrations in various paper products (Liao *et al*., 2012b)

Paper type		BPS concentration			
		Number of samples	Geometric mean	range	Detection rate (%)
			mg/g	**mg/g**	
Thermal receipts		111	0.181	0.0000138–22.0	100
			µg/g	**µg/g**	
Paper currencies		52	0.0290	<LOQ* –4.04	94
Other paper products	Flyers (e.g., advertisement brochures, store coupons, gift cards, bus schedule)	30	0.0099	<LOQ–5.41	80
	magazines	5	0.0003	<LOQ–0.0045	40
	tickets (e.g., train and bus tickets)	4	2.33	0.183–5.93	100
	mailing envelopes	5	1.33	0.476–8.08	100
	newspapers	8	0.0007	<LOQ–0.840	25
	food contact papers (e.g., fast-food wrappers, paper cups, paper plates)	12	0.0001	<LOQ–0.0117	8.3
	food cartons (e.g.,	7	0.0048	<LOQ–0.143	57

	pizza paperboards, food buckets, snack food boxes)				
	airplane boarding passes	4	3.00	1.51–8.38	100
	airplane luggage tags	4	3.56	2.19–5.58	100
	printing paper (i.e., regular copy paper)	3	0.0001	<LOQ	0
	business cards	6	0.0005	<LOQ–0.0730	33
	facial tissue	8	0.0004	<LOQ–1.53	25
	kitchen rolls (paper towels)	3	0.0001	<LOQ	0
	toilet paper	6	0.0001	<LOQ–0.0009	17

*LOQ: 0.1 ng/g

Considering a body weight of 70 kg, the median and 95th daily intake for the general population and occupational exposure for thermal paper receipts was estimated to be 4.18 and 11.0 ng/kg bw/day and 312 and 821 ng/kg bw/day respectively.

Estimated daily intake of BPS via dermal absorption after handling of the different types of paper:

Table 198: Estimated daily intake of BPS via dermal absorption after handling of paper products (Liao *et al.*, 2012b)

Paper type		Estimated daily intake (ng/day, geometric mean)	
		General population	Occupational exposure
Thermal receipts		10.5	787
Paper currencies		0.0017	0.0168
Other paper products	flyers (e.g., advertisement brochures, store coupons, gift cards, bus schedule)	0.0014	0.0014
	magazines	0.0001	0.0001
	tickets (e.g., train and bus tickets)	0.339	0.339
	mailing envelopes	0.194	0.194
	newspapers	0.0002	0.0002
	food contact papers (e.g., fast-food wrappers, paper cups, paper plates)	0.00002	0.00002
	food cartons (e.g., pizza paperboards, food buckets, snack food boxes)	0.0007	0.0007
	airplane boarding passes	0.435	0.435
	airplane luggage tags	0.518	0.518
	printing paper (i.e., regular copy paper)	0.00001	0.00001
	business cards	0.00007	0.00007
	facial tissue	0.0001	0.0001
	kitchen rolls (paper towels)	0.00002	0.00002
	toilet paper	0.00003	0.00003
Total exposure		12.0	789

Raw paper materials

- Samples of 6 different basic raw materials used for the production of paper packages including food and hygiene packages, collected in **Europe** in 2015, were analysed for the presence of BPA and its bisphenol analogues (Jurek and Leitner, 2018). Packaging products can be divided in virgin fibre samples (coated board, cellulose rolled and board primary bleached) and recycled samples (test liner white, coated recycled and recycled bleached). BPS was found in all samples in concentrations ranging from 0.0000019 to 0.000099 mg/g, with much higher concentrations in recycled samples (51-99 µg/kg) compared to virgin samples (0.11-13 µg/kg). A specific migration limit for BPS of 50 µg/kg exists for plastic food contact materials. Although 100% migration is not likely to occur, concentrations of BPS between 0.002 and 1.1 µg/kg (virgin samples: 0.002-0.23 µg/kg; recycled: 0.31-1.1µg/kg) are calculated in such case.

Recycling

- In the analysis of household wastepaper and board from **Denmark** by Pivnenko *et al.* (2015), it is suggested that the BPS is the main substitute for BPA in thermal receipts.

  The highest concentrations of BPS were found in thermal paper receipts (210 µg/g) although in much lower concentrations than BPA (8300µg/g). BPS was found in 70% of the samples demonstrating the spreading in use or through wastepaper recycling. No significant difference was seen between source-segregated wastepaper intended for recycling and the mixed (residual) waste paper. Nevertheless in an earlier study performed by the Danish EPA (Miljøstyrelsen, 2011), BPS was identified in just 25% of the thermal paper receipts.

  The second highest concentration (1.3 µg/g) was found in corrugated boxes, but in much lower concentrations than in paper receipts suggesting the spreading of BPS through paper recycling or the use of epoxy glues, in which BPS can be employed as a curing agent. Furthermore it can be concluded that BPA was substituted by BPS because all samples with no or low BPA concentrations showed high BPS concentrations, confirming the negative correlation between BPA and BPS concentrations found by Liao *et al*., 2011b.

  Based on the high affinity of BPS to both the water and solid phase (potential to retain in paper fibres when waste paper is recycled) prediction of the behaviour of BPS in a recycling process is difficult. But, if BPS is removed from the paper matrix via the water used in the paper recycling process it might end up in the environment. Although this is expected to be in very small concentrations due to the optimalisation of paper production methods (recirculation of the water) it might be an important exposure source when considering the persistency of BPS.

Table 199: BPS concentrations in various paper products (Pivnenko *et al*., 2015)

Household wastepaper	Source segregated		Non-sorted	
	% of waste paper	BPS (µg/g dm)	% of waste paper	BPS (µg/g dm)
Receipts	0.005	210	0.5	170
Corrugated boxes (shipping, food, non-food)	15.7	0.55-1.3	9.5	0.92-9.1
Newspapers	22.5	<LOD	11.2	<LOD
Flyers (glued, non-glued)	43.2	<LOD-0.10	24.2	0.16-0.27

Office paper	3.6	0.33	8.5	0.54
Envelopes	0.6	0.2	3.2	0.43
Folding boxes (food, non-food)	3.4	0.38-0.45	16.4	0.13-0.45
Magazines (glued, non-glued)	9.3	<LOD-0.075	7.2	<LOD
Beverage cartons	0.1	<LOD	15.1	<LOD
Books	1.5	0.46	4.3	0.56

Concentrations of BPS in thermal paper and paper products ranged from <LOD (0.7µg/g) to 8100 µg/g, with a median concentration of 7800 ng/g. There was no difference between recycled and virgin printer paper : <LOD. Also the concentration of BPS in non-carbon copy paper was below the LOD.

7.12.2. Environment

7.12.2.1. Aquatic compartment (incl. sediment)

Environmental biomonitoring data

Ruus *et al*., 2014 detected BPS in plankton (0.24-4.83 ng/g), bird eggs (not detected-44.2 ng/g), polychaetes (0.06-2.35 ng/g), fish (<0.5-20.5 ng/g), prawns (1.34-2.87 ng/g) and mussels (<0.3-1.89 ng/g) of an urban fjord (Inner Oslofjord) in **Norway** in the frame of the "Environmental Contaminants in an Urban Fjord"-programme. However in a follow up study of 2016 and 2018, BPS was not detected resp. in cod liver neither in blood and eggs from the herring gull resp. (Ruus *et al.* 2017; Ruus *et al.*, 2019 resp.).

BPS was also detected in the Artic in seabird eggs of black-legged kittiwake and glaucous gull as well as in artic char muscle. 10 eggs of seabirds and 10 muscle of fishes were examined. Samples were randomly collected in 2013 and 2014 in Kongsfjorden (island of the Svalbard Archipelago). The measured concentrations in Artic scar were between <0.3-1.3 ng/g, while concentrations in seabird eggs were between <0.3 and 1.1 ng/g ww (Lucia *et al.*, 2016).

The presence of ten bisphenols, among which BPS, in the northern pike (*Esox lucius*) were analysed by Tian *et al.*, 2019. Fish were collected in late May to early June 2014 and 2015 from the St. Lawrence River, Canada, 4 km upstream (n =12) and 4 km downstream (n = 14) of the point of discharge of a major primary WWTP. BPS was not detected in the muscle tissues.

Zhu *et al*. (2019) determined the concentrations of 45 substances in urine samples of various bovine breeds. 183 samples were collected in rural and agricultural areas (without point sources in the vicinity) in China, India and US between March and November 2018. Bovines from China were housed permanently in shelters and fed with commercial food while those from India and US were allowed to graze in open pastured/grassland and fed with a combination of grain and grass. The detection frequency of BPS for the urine was 77%, 82% and 100% resp. with a median concentrations resp. <LOQ (ND-3.7ng/mL), <LOQ (ND-4.0ng/mL) and 0.40 ng/mL (<LOQ-1.7). LOQs for the 8 measured bisphenols (BPA, BPAF, BPAP, BPS, BPF, BPP, BPZ and BPB) was between 0.12 and 1.2 ng/mL.

Liao and Kannan (2019) collected eleven mollusks species between 2006 and 2015 from coastal areas of five cities located along the Bohai Sea (China). Concentrations of 8 bisphenols and 5 benzophenones were determined in 186 samples. BPS was detected in

<5% of the samples. Concentrations of BPS ranged between not detected and 4.68 ng/g dw, with a geometric mean and median value of 0.146 and 0.141 ng/g dw resp.

Wild-caught marine organisms were gathered from fisherman in the Pearl River Estuary in South China and comprised shellfish (n=11) and fish (n=10) (Zhao *et al*., 2019). Concentration of BPS in the marine organisms ranged between not detected and 328 ng/g, with a median concentration of 1.28 ng/g.

Occurrence in the environment:

The environmental occurrence of BPS is merely examined in samples from the US and Asian countries (China, Japan, Korea, India). Data for Europe are scarce. Furthermore, due to the difference in production, uses and sources of discharge in the different countries, different concentrations and detection frequencies of BPS are observed.

From a review of the literature between 2010 and 2017, it can be concluded that BPS is found in several environmental media including water, sediment, sludge, indoor dust and air but generally in lower concentrations than BPA, although concentrations are almost comparable in aquatic environment (Wu *et al.*, 2018).

- **Surface water**

  - In **Europe**, BPS was found in the river Meuse at a concentration up to 3 µg/L (Kienhuis and Geerdink, 2000)

  - Several results are available for the Taihu lake (China). Jin and Zhu (2016) reported a mean concentration of BPS of 6.0 ng/L in samples of 2013, which is comparable to those found by Liu *et al.* (2017), in samples from 2016 (6.4 ng/L). Concentrations in Wang *et al.*, 2017b, from samplings from 2015 were much higher with mean concentration of 27.6 ng/L. This is due to the inclusion of measurement of BPS in the SPM (suspended particulate matter). Mean contribution of the SPM-bound bisphenols to the total water concentrations was in the range of 2.52–53.6%. The detection frequency of BPS in those 3 studies was 100%.

  - From samples taken in 2013-2014, Yamazaki *et al.* (2015), reported concentrations of BPS up to 42 ng/L in Korean and 135 ng/L in Chinese rivers. They detected extremely high concentrations in Indian rivers (ND-6840 ng/L). Furthermore river samples from 2016-2017 collected in South China contained up to 65.6µg/L of BPS (Huang *et al.*, 2018).

    In seawater from Tokyo Bay (Japan) concentrations of BPS were in the range of ND to 15 ng/L (mean 8.5 ng/L) (Yamazaki *et al.*, 2015).

  - Seawater samples were collected from the Pearl River Estuary in South China by Zhao *et al.* (2019). All samples contained BPS (100% detection frequency). The median concentration in the aqueous phase was 10.3 ng/L (1.60-59.8 ng/L), 1.6 ng/L in suspended particle matter (3.30-343 ng/L) and 12.3 ng/L in total aquatic phase (3.14-121 ng/L).

  - Zhao *et al*. (2021) evaluated the occurrence of Bisphenols in marine organisms (13 species; n = 74), as well as in seawater (n = 15) from the East China Sea. BPS concentration in seawater was 3.7 ± 2.8 ng/L (mean).

- **Sediment**

  - Liao *et al.* (2012d), analysed sediments from the industrialised areas in the US, Japan and Korea:

Table 200: BPS concentrations in sediments from different countries (Liao *et al.*, 2012d)

BPS concentration	Sampling year	Mean (ng/g dw)	Range (ng/g dw)	detection rate (%)
US (n=82)	1998-2012	0.21	ND-4.65	15.9
Japan (n=56	2012	0.42	ND-4.46	46.4
Korea (N=34)	2008	61.4	ND-1970	29.4
All (n=117)		12.37	ND-1970	28.5

- An increase of the concentrations of BPS is seen in those sediments from 2000 to 2012 probably due to the increased concentrations in waste water treatment discharges and land-applied biosolids (Choi and Lee, 2017b). Concentrations of BPS were found in sediment as high as 1970 ng/g (Choi and Lee, 2017a).

- However Huang *et al.* (2018), recorded lower BPS concentrations in Chinese river sediment samples collected between 2016 and 2017: up to 45.4 ng/g (mean 7.25 ng/g, 100% detection frequency).

- **Sludge**

  - Ruan *et al.* (2015), examined sewage sludge from waste water treatment plants in 15 cities in China. BPS was identified in 12 out of 15 sludge samples with an EEQ (estradiol equivalence quantities) between 0.00013 and 0.12 pg E/g dw.

    EEQ (ng E2/g dw)=$(EC20_{E2})$/(EC20sample) x (sample dilution factor)

  - In his review Chen *et al.* (2016), reported median concentrations of BPS in sludge from waste water treatment plants in China, Korea and the US : 5.3 ng/g, 3.8 ng/g and 5.8 ng/g resp.

    Review Chen *et al.* (2016) (incl more details from Song *et al.*, 2014, Lee *et al.*, 2015 and Yu *et al.*2015):

Table 201: BPS concentrations in sludge from different countries (Chen *et al.*, 2016)

	Sampling period		BPS concentration in sludge (ng/g)		
		Detection rate (%)	Median	Mean	Range
China (n=52)	2010-2011	82.7	4.3	3.02	0.17-110
Korea (n=40)	2011	70.0	3.8	44.9	ND-523
USA (n=76)	2006-2007	84	5.8	34.5	<1.79–1480

- Removal capacity/efficacy in WTP

Removal capacity/efficacy in WTP was determined in several literature studies, although little information is available for Europe.

- The mean concentration of eight bisphenols, including BPS (aqueous and suspended particulate matter combined) were measured in 5 Indian STPs by Karthikraj and Kannan K (2017). Concentrations of BPS were found in the influent, effluent and sludge of the STPs (measured concentrations of 14.7, 2.4 and 185.7 ng/L resp.). From the high sludge concentration it can be concluded that BPS has a potential to absorb to particulate matter with a higher affinity than Bisphenol A. Removal efficiency was calculated to be 77.7% (mean; range 69.6-96.7%).

- Wang *et al*. (2019a) reviewed the occurrence and removal mechanisms of BPA and its analogues, including BPS, in municipal WWTPs from several countries/regions. The removal capacity of BPS in full-scale municipal WWTPs ranged between 3.6% to 100.0% (average 81.2%) which indicate a good removal performances of municipal WWTPs. Based on the relatively low Log Kow of BPS it is suggested that biodegradation is likely to be an important route of removal. The concentration of BPS in the sewage sludge were in the range of not detected-600 ng/g with an average concentration of 31.13 ng/g dw.

  In this study, findings from Česen *et al.* (2018), for BPS in five municipal/industrial WWTPs in **Slovenia** were included. The mean concentration in the influent waters was 21.3 ng/l was, while in the effluent it was <LOD, concluding a 100% removal capacity.

  Also, findings from Sun *et al.* (2017) were included in this review. BPS was detected in the influent and the sludge of seven waste-water treatment plants in Xiamen (China) with a medium concentration of 48.0 ng/L and 1.01 µg/kg resp. The concentration of BPS was below the detection limit in the effluent. Total mass loads of BPS in the influent were 56.2 g with an adsorbed mass value of 1,19 g. After waste-water treatment mass load in the effluent and sludge was 0.703 g and 0.259 g resp. The removal efficiency of BPS was 98.3% and based on the mass balance analysis it can be concluded that mass loss was through biodegradation.

- BPS was one of the most abundant bisphenols found in the influent, primary effluent and final effluent of 2 WWTPs in Albany, New York (USA) (Xue and Kannan, 2019). The detection rate was 44% in the influent (raw wastewater) with concentrations ranging from MLQ-707 ng/L. BPS was not detected in the suspended particulate matter phase of the wastewater influent in one WWTP, but had a detection rate of 6.3% in the 2nd WWTP. The geometric mean concentration in the sludge was between 7.76 and 15.8 ng/g dw, with detection rate of 77% and 73% resp. Mean removal efficiency of BPS in WWTP after primary treatment was between 6.4 and 24% and -11 and 1.1% after secondary treatment. No BPS was detected in ash of incinerated sludge, demonstrating that this is a good removal method for BPS.

7.12.2.2. Terrestrial compartment

7.12.2.3. Atmospheric compartment

- <u>Concentrations in indoor dust/air</u>

<u>In Europe</u>:

- BPS was found in indoor dust samples taken from 12 countries (China, Colombia, **Greece**, India, Japan, Kuwait, Pakistan, **Romania**, Saudi Arabia, South Korea, U.S., and Vietnam). Samples were collected from homes (n=284) and from other microenvironments (n=104, laboratories, offices, cars, air conditioner, and e-waste

workshop). BPS was detected in all samples from Romania (n=23) and 85% of the samples from Greece (n=28). The mean concentrations found in in house dust in Greece and Romania were 1500 and 380 ng/g respectively (Wang *et al*., 2015).

Table 202: BPS concentrations in house dust samples (Wang *et al*., 2015)

Country	China	Colombia	**Greece**	India	Japan	South Korea	Kuwait	Pakistan	**Romania**	Saudi Arabia	US	Vietnam
Concentration BPS found in house dust (ng/g, mean)	<2	3.7	**1500**	12	440	8,8	38	10	**380**	110	2.1	28

- Larsson *et al.* (2017) examined the presence of phthalates, non-phthalate plasticizers and bisphenols in dust from 100 preschools from six areas of Stockholm (**Sweden**). Samples were collected in two stages : 30 preschool samples between February and April 2015 and 70 additional preschool samples between September and November 2015. BPS was detected in concentrations ranging from <LOD (0.12 µg/g) to 22 µg/g dust, with a geometric mean of 0.26 µg/g. In 80% of the samples, concentrations were above the limit of detection.

- Giovanoulis *et al.* (2019) selected 20 **Swedish** preschools (area of Stockholm) from the 100 preschools sampled in the previous study from Larsson *et al*. (2017). Dust samples were collected during January to February 2018 and results were compared with those of the previous study. The detection frequency of BPS increased from 80% in 2015 to 95% in 2018 as well as the median concentration in the dust, which increased with 93% from 0.255 µg/g to 0.626 µg/g. The median estimated daily intake from ingestion of preshool dust is 1.32 and 2.20 for intermediate and high exposure scenario resp. (mean EDI: 1.18 and 1.97 ng/kg bw/d resp.).

- Dueñas-Mas *et al.* (2019) detected BPS in samples collected from public environments (electronic shops, clothing shops, sport clothing shop, decoration shop, bazaars and a cafeteria, n=10) in 2018 in **Spain** by using SUPRASs (simultaneous extraction/clean-up method based on the use of supramolecular solvents). BPS was found in a concentration range between the detection limit (1 ng/g) to 736 ng/g, with a mean and median concentration of 290 ng/g and 193 ng/g resp. BPS was detected in 70 percent of the samples (n=10).

<u>Outside Europe:</u>

- BPS has been detected in all samples of indoor dust (n=156) from the United States, China, Japan, and Korea at concentrations ranging from 0.0008 to 26.6 µg/g (0.34 µg/g geometric mean) (Liao *et al*., 2012c).

- In the study of Xue *et al*. (2016), BPS was found in the vapor phase of indoor air samples from parking garages, auto repair shops, cars, barber shops, public places, homes, labs and offices in Albany (USA). Furthermore, it was the most frequently detected of the eight bisphenols analysed in the vapor phase, with a detection rate of 26.5%. However, based on the vapour pressure and the predicted particle-gas partition coefficient (kp) a preferential portioning of BPS into the particulate phase was to be expected. The higher concentrations in the vapor phase might be due to an artifact of sampling or other factors such as sources of release or temperature related. Concentrations in bulk air (sum of particulate and vapor phase concentrations) ranged from <MLOQ (Method LOQ) to 0.94 ng/m3, with a mean of 0.07 ng/m3. The daily intake of BPS (total, geometric mean) was estimated to be 0.39 ng/day for infants,

0.60 ng/day for toddlers, 0.89 ng/day for children, 1.24 ng/day for teenagers and 1.13 ng/day for adults.

- To measure the concentration of BPS in indoor dust, Liu *et al*. (2019a) took samples in Singaporean houses during November 2017 (n=32). BPS was detected in all dust samples ranging from 153 to 6491 ng/g with a geom.mean of 713 ng/g dust. BPS was detected in all samples.

7.12.3. Combined exposure assessment

NA

7.13. Risk characterisation

eMSCA performed a risk characterisation only for workers.

7.13.1. Human health

7.13.1.1. Worker

7.13.1.1.1. Exposure assessment performed by the eMSCA

Based on the Registrants' exposure calculations and the DNELs proposed by the Registrants, RCRs (risk characterisation ratios) < 1 have been obtained for every exposure scenario. The eMSCA also performed its own exposure assessment. This risk characterisation is based on the eMSCA's exposure estimates. The RCRs > 1 are presented in bold.

7.13.1.1.1.1. Exposure Scenario 1 - Manufacture of the substance

Table 203: Risk characterization ratios for manufacture of the substance, estimated by the eMSCA

Industrial			
Contributing scenario	RCR Long-term Inhalation	RCR Long-term Dermal	RCR Combined
PROC 1	0,0521	0,00343	0,0556
PROC 8b	0,5	**1,37**	**1,87**
PROC 9	**2,5**	0,686	**3,19**
PROC 15	0,25	0,0343	0,284
PROC 8a*	**2,5**	**1,37**	**3,87**

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

If a worst-case scenario is applied; PROCs 8b, 9 and 28 result in combined RCRs[16] > 1. This illustrates the importance of using respiratory protection (90%) for PROCs 8b and 28,

[16] According to the ECHA Guidance, a combined risk characterisation ratio (RCR) can be derived for dermal and inhalation exposure, if systemic effects are relevant.

ECHA Guidance Chapter R.14 on Occupational exposure assessment
(https://echa.europa.eu/documents/10162/17224/information_requirements_r14_en.pdf/bb14b581-f7ef-4587-a171-17bf4b332378)

as reported by the Registrants. Also, gloves with efficiency 90% need to be used for all PROCs (except for PROC 1, which covers a closed system with no risk of exposure), as reported by the Registrants. Gloves with a lower efficiency result in RCRs higher than 1. Lower durations of activity for these scenarios are also reported by the Registrants.

Conclusion: The risk management measures presented by the Registrants are sufficient for Exposure Scenario 1.

7.13.1.1.1.2. Exposure Scenario 2 - Use as monomer for manufacture of PESU

Table 204: Risk characterization ratios for use as monomer for manufacture of PESU, estimated by the eMSCA

Industrial			
Contributing scenario	RCR Long-term Inhalation	RCR Long-term Dermal	RCR Combined
PROC 1	0,0521	0,00343	0,0556
PROC 8b	0,5	**1,37**	**1,87**
PROC 15	0,25	0,0343	0,284
PROC 8a*	**2,5**	**1,37**	**3,87**

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

If a worst-case scenario is applied; PROCs 8b and 28 result in combined RCRs > 1. This illustrates the importance of using respiratory protection for PROCs 8b (90%) and 28 (95%), as reported by the Registrants. Also, gloves with efficiency 90% need to be used for all PROCs (except for PROC 1, which covers a closed system with no risk of exposure), as reported by the Registrants. Gloves with a lower efficiency result in RCRs higher than 1. Lower durations of activity for these scenarios are also reported by the Registrants.

Conclusion: The risk management measures presented by the Registrants are sufficient for Exposure Scenario 2.

7.13.1.1.1.3. Exposure Scenario 3 - Use as monomer in the production of synthetic tanning agents (Syntans)

Table 205: Risk characterization ratios for use as monomer in the production of synthetic tanning agents (Syntans), estimated by the eMSCA

Industrial			
Contributing scenario	RCR Long-term Inhalation	RCR Long-term Dermal	RCR Combined
PROC 1	0,005	0,00343	0,00843
PROC 8b	0,0521	0,137	0,189
PROC 8b	0,0521	0,137	0,189
PROC 8b	0,1	0,274	0,374
PROC 9	0,0521	0,0686	0,121
PROC 9	0,5	0,137	0,637
PROC 8a*	0,0521	0,137	0,189

PROC 8a*	0,5	0,274	0,774
PROC 15	0,521	0,0343	0,556

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

If a worst-case scenario is applied, all the contributing scenarios result in combined RCRs < 1. The Registrants have indicated even stricter risk management measures, such as respiratory protection (95%) for the last PROC 8b and the last PROC 8a contributing scenario, and outdoor use of the substance for the second PROC 8b contributing scenario. As the worst-case scenario applied by the eMSCA does not result in high RCRs, also the risk management measures indicated by the Registrants will not lead to combined RCRs which are higher than one.

Conclusion: The risk management measures presented by the Registrants are sufficient for Exposure Scenario 3.

7.13.1.1.1.4. Exposure Scenario 4 - Use of Syntans for tanning in leather production

Table 206: Risk characterization ratios for use of Syntans for tanning in leather production, estimated by the eMSCA

Industrial			
Contributing scenario	RCR Long-term Inhalation	RCR Long-term Dermal	RCR Combined
PROC 3	0,0521	0,00686	0,059
PROC 8b	0,0521	0,137	0,189
PROC 8b	0,1	0,274	0,374
PROC 8b	0,0521	0,137	0,189
PROC 13	0,0521	0,137	0,189
PROC 21	0,05	0,0283	0,0783
PROC 8a*	0,0521	0,137	0,189
PROC 8a*	0,5	0,274	0,774

*PROC 8a is representing PROC 28 (input in ECETOC TRA model by the eMSCA)

If a worst-case scenario is applied, all the contributing scenarios result in combined RCRs < 1. The Registrants have indicated even stricter risk management measures, such as gloves with efficiency 95% for all PROCs, except for PROCs 3 and 21. Regarding inhalation, the Registrants however report that respiratory protection is not used. As the worst-case scenario applied by the eMSCA does not result in high RCRs, also the risk management measures indicated by the Registrants will not lead to combined RCRs which are higher than one.

Conclusion: The risk management measures presented by the Registrants are sufficient for Exposure Scenario 4.

7.13.1.1.1.5. Exposure Scenario 5 - Use of leather articles

Table 207: Risk characterization ratios for use of leather articles, estimated by the eMSCA

Industrial

Contributing scenario	RCR Long-term Inhalation	RCR Long-term Dermal	RCR Combined
PROC 21	0,214	0,141	0,356

If the worst-case scenario (provided by the Registrants) is applied; PROC 21 results in a combined RCR < 1. Use of respiratory protection or gloves is not needed for this Exposure Scenario.

Conclusion: The risk management measures presented by the Registrants are sufficient for Exposure Scenario 5.

7.14. References

References containing specific information on **BPS** are printed in **bold.**

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7.15. Abbreviations

♂: males

♀: females

* : p<0.05

** : p<0.01

Abs. : Absolute

AC50 : half-life activity concentration

AhR : Aryl hydrocarbon receptor

ALP : alkaline phosphatase

ALT : alanine aminotransferase

AM: amiodarone

ANOVA : analysis of variance

ApoII : Apolipoprotein II

Approx. : approximately

AR : androgen receptor

Arob: aromatase B

AROM : aromatase

AST : aspartate aminotransferase

ATE : Acute Toxicity Estimate

ATP : adaptation to technical and scientific progress

AUC: area under the curve

AVP : Arginine Vasopressin (gene)

AVT : Arginine vasotocin (hormone)

BAF : bioaccumulation factor

BCF : bioconcentration factor

BDNF :brain-derived neurotrophic factor

BE CA: Belgian competent authority

BLYES : Bioluminescent Yeast reporter assay

BECA : Belgian Competent Authority

BP : 2,2'-Biphenol

BPA : bisphenol A, 4,4′-isopropylidenediphenol

BPA carboxylic acid : 2,2-bis-(4-hydroxyphenyl)-1-propionic acid

BPAF : bisphenol AF, 4,4'-(hexafluoroisopropylidene) diphenol

BPA glucuronide : bisphenol A β-D-glucuronide, 4-[1-(4-hydroxyphenyl)-1-methylethyl]phenyl β-Dglucopyranosiduronic Acid

BPA ol : 2,2-bis-(4-hydroxyphenyl)-1-propanol

BPA-R : Bisphenol A targeted receptor

BPAP : bisphenol AP, 1,1-bis(4-hydroxyphenyl)-1-phenylethaneBPA quinone : 4,5-bisphenol-o-quinone

BPB : bisphenol B, 4,4'-(1-methylpropylidene)diphenol

BPBP : bisphenol BP, 4,4′-(diphenylmethylene)diphenol

BPC : bisphenol C, 4,4′-isopropylidenedi-o-cresol

BPC2 : bisphenol C2, Bis(4-hydroxyphenyl)-2,2-dichloroethylene

BPE : bisphenol E, 4,4′-ethylidenediphenol

BPF : Bisphenol F, 4,4'-Methylenediphenol

BPG : bisphenol G, 4,4′-isopropylidenedi(2-isopropylphenol)

BPM : bisphenol M, 4,4'-(1,3-phenylene-bis(1-methylethylidene))diphenol

BPP : bisphenol P, 4,4′-(1,4-phenylenediisopropylidene)diphenolBPPH: bisphenol PH, 5,5′-isopropylidenedi-2-biphenylol

BPS : Bisphenol S; 4,4'-sulphonyldiphenol

BPS-DAE : 4, 4'-Diallyloxy diphenyl sulfone

BPS-G : bisphenol S-glucuronide

BPSIP : 4-hydroxyphenyl 4-isoprooxyphenylsulfone, also called D-8

BPSM1 : 4-(4-hydroxybenzenesulfonyl)-benzene-1,2-diol

BPTMC: bisphenol TMC, 4,4'-(3,3,5-trimethylcyclohexane-1,1-diyl)diphenol

BPZ: bisphenol Z, 4,4′-cyclohexylidenediphenol

BW : body weight

BWG : body weight gain

Ca : calcium

Ca. circa

CAR : constitutive androstane receptor

Cat. : category

CAT: catalase

Chol : cholesterol

CLP: classification, labelling and packaging

CMC : carboxymethylcellulose

CMR : carcinogen, mutagen and reprotoxic

Conc. : concentration

CoRAP : community rolling action plan

CPT : carnitine transferase

Cre : creatinine

CREB : cAMP Response Element-Binding Protein

Crh : corticotrophin releasing hormone

CSA : chemical safety assessment

Cumul : cumulative

Cyp18a1 : Cytochrome P450 Family 18 Subfamily a Member 1

Cyp19a1: Cytochrome P450 Family 19 Subfamily a Member 1

Cyp19b: Cytochrome P450 Family 19 Subfamily b

Cyp4g : Cytochrome P450 Family 4 Subfamily G

d : day

DE CA : German competent authority

DEX : dexamethasone

DFO : double first order

DHEA : dehydroandrosterone

DHT : dihydrotestosterone

DIT : developmental immunotoxicity

DMSO : dimethyl sulfoxide

DNT : developmental neurotoxicity

DOC : dissolved organic carbon

DPC : day post-coitum

Dpf : day post-fertilisation

Dph : days post hatching

DPP : day post-partum

DPM : disintegration per minute

DS : dossier submitter

DT50 : degradation half-life

Dw : dry weight

E2 : 17β-estradiol

E74 : early ecdysone-inducible gene

EATS : estrogenic, androgenic, thyroidal and steroidogenic

EC : effect concentration

E2: estradiol

EC10 : concentration producing effect in 10% of the test organisms

EC50 : concentration producing effect in 50% of the test organisms

ECETOC : European Centre for Ecotoxicology and Toxicology of Chemicals

ECHA : European Chemicals Agency

EcR : ecdysone receptor

ED: endocrine disruptor

EDI : estimated daily intake

EDU : estimated daily uptake

EE : estradiol equivalent

EE2: ethinylestradiol

EEC : European Economic Community

EEF : estradiol equivalency factor

EEQ : estradiol equivalence quantities

EFSA : European Food Safety Authority

Emax : maximum efficiency

EMPA: Eidgenössische Materialprüfungs- und Forschungsanstalt (Swiss Federal Laboratories for Materials Testing and Technology)

eMSCA: evaluation member state competent authority

ENV: environment

EOGRTS : extended one generation reproductive toxicity study

EPA : Environmental Protection Agency

Epith. : epithelium

ErC50 : concentration affecting growth rate in 50% of the test organisms

ER : estrogen receptor

ERE : estrogen response element

ERK : extracellular signal-regulated kinase

EROD : Ethoxyresorufin-O-deethylase

ERRγ : oestrogen related receptor gamma

ERTA: estrogen receptor transcriptional activation

ESI-MS: electrospray Ionisation Mass Spectroscopy

EU : European union

e-waste: electronic waste

f : female

F: fertility

FBW : final body weight

FELS : Fish Early Life Stage

F.i.: for instance

FOB : functional observational battery

Foc : fraction of sediment

FRST-50 : forest-50

FSDT : Fish Sexual Development Test

FSH : Follicle Stimulating Hormone

Fshr : Fsh receptor gene

FSTRA : Fish Short Term Reproduction Assay

g : gram

G15 : (3aS*,4R*,9bR*)-4-(6-bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-3H-cyclopenta[c] quinolone

GABA : gamma-aminobutyric acid

GAPDH : glyceraldehyde 3-phosphate dehydrogenase

GC-MS : Gas Chromatography coupled to Mass Spectrometry

GD : gestational day

Geom. : geometric

GFP : green fluorescent protein

GGT : gamma-glutamyltransferase

GH: growth hormone

GI : gastro-intestinal

GLP : good laboratory practice

GnRH : gonadotropin-releasing hormone

GOT : glutamic oxaloacetic transaminase

GPMT : guinea pig maximisation test

GPx : glutathione peroxidase

GR : glucocorticoid receptor

GSI : gonadosomatic index

GSTd3 : glutathione S-transferase D3

h : hour

hAR : human androgen receptor

Hb : haemoglobin

HCD : historical control data

HDL: high density lipoprotein

hERα : human estrogen receptor α

hERβ : human estrogen receptor β

HGB or HB : haemoglobin

HGPRT : hypoxanthine-guanine phosphoribosyl transferase gene in human cells

HH: human health

Hpf : hour post-fertilisation

HPG : hypothalamic-pituitary-gonad

HPLC: high-performance liquid chromatography

HPT : hypothalamic-pituitary-thyroid

hPXR : human pregnane receptor

HSI : hepatosomatic index

hsp40 : 40 kDa heat-shock protein

hsp70 : 70 kDa heat-shock protein

Ht : haematocrit

HYPO : hypothalamus

IC50 : concentration with 50% inhibition

ICI : ICI 182,780; fulvestrant (CAS 129453-61-8)

ICRP : International Commission on Radiological Protection

ID : intra dermal

IF : interstitial fibrosis tissue

Inc : incidence

Incl. : including

Interr. : interrval

IPCS : International Programme on Chemical Safety

Irrit : irritation

ISO : International Organization for Standardization

It : isotocin

Its2 : Internal transcribed spacer 2

JNK: c-Jun-N-terminal kinase

kg : kilogram

KI : klimisch index

L : liter

LBD : ligand binding domain

LC50: lethal concentration causing 50% death

LC–MS/MS : liquid chromatography coupled to mass spectrometry

LC-QTOF-MS : Liquid chromatography–Quadrupole Time of Flight Mass spectrometry

LD: lactation day

LDH : lactate deshydrogenase

LDL: low density lipoprotein

LED: lowest effective dose

LEV : local exhaust ventilation

LH : luteinizing hormone

LLNA : local lymph node assay

LOD : limit of detection

LOEC: lowest observed effect concentration

LOQ : limit of quantification

LOR : limit of reporting

LPO : lipid peroxidation

M : male

Max : maximum

MAPK : mitogen-activated protein kinase

MBP : 4,4'-(4-methylpent-1-ene-2,4-diyl)diphenol

MCF : Michigan Cancer Foundation

MCHC : mean corpuscular haemoglobin concentration

MCV : mean corpuscular volume

Meas. : measured

MeOH : methanol

MEOGRT: Medaka Extended One Generation Reproduction Test

Met. Act. : metabolic activation

Min : minimum

mg: milligram

MITI : Ministry of International Trade and Industry, Japan

mL millilitre

MLOQ : Method limit of quantification

MoA : mode of action

MPC : maximum plasma concentration

MPP : 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole dihydrochloride

MR : mineralocorticoid receptor

mRNA : messenger ribonucleic acid

mRXR : mouse retinoid X receptor

MS : mass spectrometry

MSC: member state committee

MTT : 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

MW : molecular weight

NA : not applicable

Nb : number

NC : not classified

ND or n.d.: not detected

NER: non-extractable residues

ng : nanogram

NHANES : National Health and Nutrition Examination Survey

NMDR : non-monotonic dose response

NO : nitric oxide

NOAEL: No observed adverse effect level

NOEC: No observed adverse effect concentration

NOErC : No observed adverse effect concentration for growth rate

Nom. : nominal

N.t. : not tested

NY : New York

NZW : New Zealand White

OBBPS : Tetrabromobisphenol S bis-(2,3-dibromopropyl ether)

Obs. : observation

OC : organic Carbon

OECD : Organisation for Economic Co-operation and Development

OECSEH : Office of Environmental Chemicals Safety Environmental Health Bureau

OP : object placement

OR : Object Recognition

Org. : organic

P : present

PBT : Persistent bioaccumulative and toxic

PC50 : response that is 50% of the maximal positive control response

PCA : protein-fragmentation complementation assay

PDTC : pyrrolidine dithiocarbamate

PEC: Predicted environmental concentration

PESU: polyethersulfone

pg : picogram

PHTTP : 4-[2-phenyl-5,7-bis(trifluoromethyl) pyrazolo[1,5-a] pyrimidin-3-yl]phenol

PMA : Phorbol 12-myristate 13-acetate

PND : post-natal day

PNEC: Predicted no effect concentration

POD : peroxidase

PPAR : peroxisome proliferator-activated receptor

PPARγ : peroxisome proliferator-activated receptor gamma

PR : progesterone receptor

PRL : prolactin

PROC : process category

PSF-51 : Purdue Student Organic Farm-51

Pt : prothrombin time

PXR: pregnane X receptor

QSAR: Quantitative structure-activity relationship

R1881 : 17R-methyl-^{3}H]methyltrienolone

RA : relative activity

RAC : risk assessment committee

RAR : retinoic acid receptor

RBA: relative binding affinity

RBC : red blood cell

RCR : risk characteriasation ratio

REACH : registration evaluation and authorization of chemicals

REC10 : 10% relative effective concentration

REC50 : 50% relative effective concentration

Rel. : reliability

Rela. : relative

REP : relative potency to E2

Repr. : reprotoxic

Resp. : respectively

RET : reticulocyte

RIC50 : 50% relative inhibitory concentration

RMOA: risk management option analysis

RNA : ribonucleic acid

ROI: register of intentions

ROR : retinoic acid receptor (RAR)-related orphan receptor

ROS : reactive oxygen species

ROSI : rosiglitazone

RPF : relative potency factors

rpL4 : ribosomal protein L4

rpL13 : ribosomal protein L13

RPP : relative proliferative potency

RPT3 : relative potency in comparison with T3

RT-PCR : real time polymerase chain reaction

RXR : retinoid X receptor

RUC : rat uterine cytosolic

SAR : structure activity relationship

S.d. : standard deviation

SD : Sprague-Dawley

SDA: sea die-away

Sec : secondary

Sem. Ves. : seminal vesicle

SEM: standard error of mean

Sev: substance evaluation

SFO : single first order

SI : Simulation index

Sign. : significant

SML: specific migration limit

SOD : superoxide dismutase

Sp. : species

SPE-HPLC-MS/MS: solid-phase extraction (SPE)−high-performance liquid chromatography (HPLC) −mass spectrometry (MS)/MS

SPE-LC-QTRAP-MS/MS solid phase extraction-liquid chromatography (SPE-LC) Quadrupole-Linear Ion Trap Mass Spectrometry (QTRAP-MS) /MS

SPM: suspended particulate matter

SR : social recognition

St. dev. : Standard deviation

Stat : statistically

STP : sewage treatment plant

STTA : stably transfected transcriptional activation assay

SVHC: substance of very high concern

T: testosterone

T2DM : type 2 diabetes mellitus

T3 : triiodothyronine

T4 : thyroxine

TAG : triacylglycerol

TBBPA : tetrabromobisphenol A, 4,4′-(propane-2,2-diyl)bis(2,6-dibromophenol)

TBBPS : tetrabromobisphenol S

TBT : tributyltin

TCBPA : tetrachloro bisphenol A, 2,2-bis-(3,5-dichloro-4-hydroxyphenyl)propane, 2,2′,6,6′-tetrachloro-4,4′-isopropylidenediphenol

TEB : terminal end buds

TMBPA : tetramethyl bisphenol A, 4,4′-isopropylidenedi-2,6-xylol, 2,2′,6,6′-tetramethyl-4,4′-isopropylidenediphenol

tg : thyroglobulin

TG : test guideline

TH : thyroid hormone

THR : thyroid hormone receptor

TN : terminal nerve

TOC : total organic carbon

TOF : time-of-flight

Tot : total

Tot. chol. : total cholesterol

Tot. prot. : total protein

TR : thyroid receptor

TRα : thyroid receptor alfa

TRβ : thyroid receptor beta

TR-LBD : thyroid receptor ligand binding domain

Trig : triglyceride

TSH : thyroid stimulating hormone

Ttr : transcript of transthyretin

TTR : thyroid hormone transporter

TWA : time-weighted average

µg : microgram

UHPLC–MS/MS : Ultra High Performance liquid chromatography tandem mass spectrometry

UHPLC-TOF : ultra-high performance liquid chromatography mass spectrometry

US : United States

USA : United States of America

UV: ultraviolet

vB : very bioaccumulative

VDR: vitamin D receptor

vP : very persistent

Vs : versus

Vtg : vitellogenin (gene)

VTG: vitellogenin (protein)

WBC : white blood cell

WHO : World Health Organisation

WI: wistar

WS : water solubility

ww : weight by weight

WWTP : Waste water treatment plant

YAS : Yeast androgen screen

YES : Yeast estrogen screen

ZEOGRT: Zebrafish Extended One Generation Reproduction Test

ZFL: zebrafish liver