CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Chemical name: 4,4'-methylenediphenol; bisphenol F

EC Number: 210-658-2

CAS Number: 620-92-8

Index Number: -

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CONTENTS

1 IDENTITY OF THE SUBSTANCE 1

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 1
1.2 COMPOSITION OF THE SUBSTANCE 2

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING 4

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 4

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 6

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 6

5 IDENTIFIED USES 6

6 DATA SOURCES 7

7 PHYSICOCHEMICAL PROPERTIES 7

8 EVALUATION OF PHYSICAL HAZARDS 8

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 8

9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S) 11

10 EVALUATION OF HEALTH HAZARDS 13

10.1 ACUTE TOXICITY - ORAL ROUTE 13
10.2 ACUTE TOXICITY - DERMAL ROUTE 13
10.3 ACUTE TOXICITY - INHALATION ROUTE 13
10.4 SKIN CORROSION/IRRITATION 13
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 13
10.6 RESPIRATORY SENSITISATION 13
10.7 SKIN SENSITISATION 13
10.8 GERM CELL MUTAGENICITY 13
10.9 CARCINOGENICITY 13
10.10 REPRODUCTIVE TOXICITY 14
10.10.1 Adverse effects on sexual function and fertility 14
10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility 35
10.10.3 Hypothesis for read-across 51
10.10.4 Comparison with the CLP criteria 72
10.10.5 Adverse effects on development 73
10.10.6 Short summary and overall relevance of the provided information on adverse effects on development 75
10.10.7 Comparison with the CLP criteria 76
10.10.8 Adverse effects on or via lactation 77
10.10.9 Short summary and overall relevance of the provided information on effects on or via lactation . 78
10.10.10 Comparison with the CLP criteria 78
10.10.11 Conclusion on classification and labelling for reproductive toxicity 78
10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE 78
10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE 78
10.13 ASPIRATION HAZARD 79

11 EVALUATION OF ENVIRONMENTAL HAZARDS 79

12 EVALUATION OF ADDITIONAL HAZARDS 79

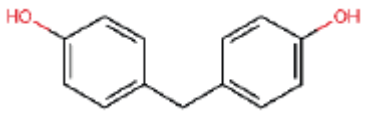
13 ADDITIONAL LABELLING 79

14 REFERENCES 79

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	4,4'-Dihydroxydiphenylmethane 4,4'-Methylenediphenol 4-[(4-Hydroxyphenyl)methyl]phenol
Other names (usual name, trade name, abbreviation)	Bisphenol F BPF
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	210-658-2
EC name (if available and appropriate)	4,4'-methylenediphenol
CAS number (if available)	620-92-8
Other identity code (if available)	-
Molecular formula	$C_{13}H_{12}O_2$
Structural formula	
SMILES notation (if available)	C(C1=CC=C(O)C=C1)C2=CC=C(O)C=C2
Molecular weight or molecular weight range	200.24 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	-

1.2 Composition of the substance

The substance has not been registered by a company in the EEA.

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
4,4'-methylenediphenol	Not relevant (mono-constituent substance)	Not included in Annex VI	Skin Sens. 1, Skin Irrit. 2, Eye Dam. 1, Eye Irrit. 2, STOT SE 3 (respiratory tract/not provided), Aquatic Chronic 2 and 3, and Not classified.[1]

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No information				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No information					

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Other information provided in the studies	The studies in which the test substance is used
Bisphenol F	> 99.3% [^{3}H]BPF and > 98% unlabelled BPF	CAS: 620-92-8	Cabaton et al., 2006
Bisphenol F	unknown	Chemical structure: illustrated	Lee et al., 2022a
Bisphenol F	98% BPF and 99% internal standard $^{13}C_{12}$-BPF	Cat#: B47006; Lot#: 05712ME; Sigma-Aldrich, St. Louis, MO, USA	Gingrich et al., 2019
Bisphenol F	98%	CAS: 620-92-8	Lee et al., 2022b
Bisphenol F	unknown	CAS / Chemical name: unknown	Fatai and Aribidesi, 2022
Bisphenol F	>99%	CAS: 620-92-8	Li et al., 2022
Bisphenol F	100%	CAS: 620-92-8	Higashihara et al., 2007
Bisphenol F	99%	CAS / Chemical name: unknown	Ullah et al., 2018a
Bisphenol F	99%	Chemical name: 4,4´-methylenediphenol	Ullah et al., 2019c
Bisphenol F	99%	CAS / Chemical name: unknown	Ullah et al., 2019b

[1] Number of Aggregated notifications: 8

Bisphenol F	unknown	CAS / Chemical name: unknown	Ullah et al., 2018b
Bisphenol F	unknown	CAS / Chemical name: unknown	Ullah et al., 2019a
Bisphenol F	unknown	CAS: 620-92-8	Gao et al., 2022
Bisphenol F	99%	CAS / Chemical name: unknown	Ijaz et al., 2020
Bisphenol F	unknown	CAS / Chemical name: unknown	Nevoral et al., 2021
Bisphenol F	99.9%	CAS: 620-92-8	Yamasaki et al., 2003
Bisphenol F	98%	CAS: 620-92-8	Stroheker et al., 2003
Bisphenol F	99.9%	CAS: 620-92-8	Yamasaki et al., 2004
Bisphenol F	99.9%	CAS / Chemical name: unknown	Yamasaki et al., 2002
Bisphenol F	99%	CAS: 620-92-8	Mu et al., 2022
Bisphenol F	98%	CAS: 620-92-8	Yang et al., 2017
Bisphenol F	>98%.	CAS: 620-92-8	Mentor et al., 2020
Bisphenol F	unknown	CAS / Chemical name: unknown	Chen et al., 2022
Bisphenol F	unknown	CAS / Chemical name: unknown	Benson et al., 2020
Bisphenol F	unknown	CAS / Chemical name: unknown	Ješeta et al., 2022
Bisphenol F	unknown	Chemical name: bis(4-hydroxyphenyl)methane	Castellini et al., 2021
Bisphenol F	>99%	CAS / Chemical name: unknown	Desdoits-Lethimonier et al., 2017

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6. For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	604-RST-VW-Y	4,4'-methylenediphenol; bisphenol F	210-658-2	620-92-8	Repr. 1B	H360F	GHS08 Dgr	H360F			

Table 7: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	Harmonised classification proposed	**Yes**
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

4,4'-Methylenediphenol (herein referred to as BPF) has not previously been discussed and/or agreed by the TC C&L (Dir. 67/548/EEC) and is not included in CLP Annex VI.

This classification proposal is supported by read-across from 4,4'-isopropylidenediphenol (bisphenol A, BPA, EC 201-245-8, CAS 80-05-7) which is a structural analogue to BPF (see read-across justification in section 10.10.3). BPA has a harmonised classification as Repr. 1B, H360F; STOT SE 3, H335; Eye Dam. 1, H318; Skin Sens. 1, H317; Aquatic Acute 1, H400; Aquatic Chronic 1, H410.
The hazard class evaluated in this proposal for BPF is reproductive toxicity.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

BPF is considered to fulfil the criteria for classification as toxic to reproduction (Repr. 1B, H360F). Therefore, a harmonised classification is justified according to Article 36(1)(d) of the CLP Regulation.

5 IDENTIFIED USES

There is no registration of BPF according to the REACH regulation. Information on uses is, therefore, from other publicly available sources. BPF is used as a monomer in polycarbonate and epoxy resins (intermediate use). For example, the epoxy resin BFDGE (Bisphenol F Diglycidyl Ether) is made from BPF, and BPF is also a component in NOGE (novolac glycidyl ethers) prepared from formaldehyde, phenol and epichlorohydrin. Applications that include the use of BPF-derived epoxy resins are coatings, lacquers, pipe linings, industrial floors, road and bridge deck toppings, adhesives, plastics, water pipes, dental sealants, and food packaging. Epoxies, that are based on BPF, have lower viscosity and greater resistance than those based on BPA (e.g., BADGE). The use of BFDGE and NOGE in food contact materials and articles (made of any type of plastics or covered by surface coatings and adhesives) is prohibited since 2005[2]. BPF has also been identified as a realistic replacement option for BPA in thermal paper. According to the PubChem database, the use of BPF as an additive in thermal paper appears in newer patents (year >2012) compared to previous ones. BPF has also been identified as a component in synthans for leather tanning processes. Although not intentionally used, BPF was identified as an unwanted reaction by-product with concentrations in synthans of 0.1-1%.

Other BPF derivates have been identified by ECHA but information on these derivates does not provide additional evidence to the CLH-proposal and, therefore, they will not be taken into consideration.

[2] COMMISSION REGULATION (EC) No 1895/2005 of 18 November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food. L 302/28.

6 DATA SOURCES

BPF is not registered according to the REACH regulation and, hence, there are no toxicity studies available in ECHA's registration database. Information on reproductive toxicity following BPF exposure was therefore gathered from scientific studies in the open literature (PubMed: biomedical and life sciences literature database, https://pubmed.ncbi.nlm.nih.gov/). A systematic review methodology was used to retrieve studies that were published up to the date of 31 May 2022 (Wiklund and Beronius, 2022). Thereafter, ad-hoc searches in the open literature were performed for studies published between 31 May 2002 and 31 December 2022.

The current CLH-proposal on reproductive toxicity is based on a weight of evidence of substance specific data on BPF and cover *in vivo* rodent and non-rodent studies as well as *ex vivo* studies and human studies (section 10.10.1). For the *in vivo* studies on rodents, a klimisch score has been assigned to assess the studies' reliability[3]. A short summary of the studies and their results on reproductive toxicity in males and females are provided in section 10.10.2.

To strengthen the weight of evidence, the CLH-proposal is further supported by evidence of read-across from BPA to BPF (section 10.10.3). The read-across is based on information from comparative studies following BPF and BPA exposure using the same data source as aforesaid, and from the RAC opinion proposing harmonised classification and labelling at Community level of BPA (ECHA, 2014).

7 PHYSICOCHEMICAL PROPERTIES

Table 8. Summary of physicochemical properties

Property	**Value**	**Reference[4]**	**Comment (e.g., measured or estimated)**
Physical state at 20°C and 101,3 kPa	No data found		
Melting/freezing point	162.5 (160 - 163) °C	PubChem	
Boiling point	No data found		
Relative density	No data found		
Vapour pressure (Pa at 25 °C)	4.96 x 10^{-5}	Danish (Q)SAR database	Predicted
Surface tension	No data found		
Water solubility (g/L)	0.543 0.612	Danish (Q)SAR database Danish (Q)SAR database	Predicted Predicted
Partition coefficient n-octanol/water (Log Kow)	2.91 3.06	Danish (Q)SAR database Danish (Q)SAR database	Measured Predicted
Flash point	No data found		
Flammability	No data found		
Explosive properties	No data found		
Self-ignition temperature	No data found		
Oxidising properties	No data found		
Granulometry	No data found		
Stability in organic solvents and identity of relevant degradation products	No data found		
Dissociation constant (pKa)	9.7	Danish (Q)SAR database	Predicted
Viscosity	No data found		

[3] The reliability assessment was based on a method described by Wiklund and Beronius (2022).

[4] Danish (Q)SAR Database, Division of Diet, Disease Prevention and Toxicology, National Food Institute, Technical University of Denmark, http://qsar.food.dtu.dk.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH-proposal.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

BPF has no registered data and, for this reason, information on toxicokinetics has been retrieved from scientific studies in the open literature (PubMed, https://pubmed.ncbi.nlm.nih.gov/). The proposed harmonised classification of BPF as toxic for reproduction is further supported by read-across from BPA (Section 10.10.3).

Table 9: Summary table of toxicokinetic studies

<table>
<tr><th>Method</th><th>Results
The results are presented as mean values.</th><th>Reference</th></tr>
<tr><td>Method: Metabolic Balance and Tissue Distribution
Species, strain, sex, no/group: 16 Sprague-Dawley female rats (8-10 weeks old).
Test substance, dose levels, duration of exposure: Oral (gavage) with a single dose of 7 (LD) or 100 (HD) mg/kg bw of tritium-labelled BPF ([^{3}H]BPF), adjusted with unlabelled BPF for the pregnant group, and dissolved in propylene glycol (1 mL per rat).
Purity: > 99.3% [^{3}H]BPF and > 98% unlabelled BPF (CAS 620-92-8).
Treatment groups:
NP-LD = 4 low-dosed nonpregnant females,
NP-HD = 4 high-dosed nonpregnant females,
P-LD = 4 low-dosed pregnant females (dosed on GD17),
P-HD = 4 high-dosed pregnant females (dosed on GD17).
Sampling: After dosing, the females were kept in metabolic cages. Urine and faeces were collected once every 24-h over a 96-h period. 96-h after dosing, the females were euthanized by exsanguination after cervical dislocation.
GLP / OECD TGs not stated.</td><td>Distribution
Mean percentage of radioactive BPF (in relation to the administered dose, %) and calculated mean BPF concentrations (ng/g; ppb) are reported in the table below.
The main radioactivity was found in urine (42 – 53%) and faeces (14 – 18%), followed by the digestive tract (8 – 10%) and the carcass (5 – 8%).
<table>
<tr><th>Body compartment</th><th>NP-LD (n=4)</th><th>P-LD (n=4)</th><th>NP-HD (n=4)</th><th>P-HD (n=4)</th></tr>
<tr><td>%</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Urine</td><td>42.85</td><td>44.39</td><td>43.80</td><td>53.72</td></tr>
<tr><td>Faeces</td><td>14.31</td><td>18.99</td><td>18.29</td><td>18.96</td></tr>
<tr><td>Digestive tract</td><td>8.51</td><td>8.14</td><td>9.95</td><td>10.49</td></tr>
<tr><td>Tissues</td><td>0.53</td><td>0.85</td><td>0.46</td><td>0.75</td></tr>
<tr><td>Liver</td><td>0.48</td><td>0.75</td><td>0.43</td><td>0.54</td></tr>
<tr><td>Carcasses</td><td>8.05</td><td>8.38</td><td>5.97</td><td>6.69</td></tr>
<tr><td>ng/g</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Liver</td><td>832.5</td><td>679.0</td><td>13072.6</td><td>7558.0</td></tr>
<tr><td>Kidney</td><td>319.3</td><td>268.3</td><td>4571.2</td><td>3535.3</td></tr>
<tr><td>Brain</td><td>159.9</td><td>88.0</td><td>1411.7</td><td>499.4</td></tr>
<tr><td>Muscle</td><td>160.6</td><td>121.3</td><td>1506.4</td><td>778.8</td></tr>
<tr><td>Fat</td><td>105.1</td><td>85.8</td><td>1743.7</td><td>500.4</td></tr>
<tr><td>Blood</td><td>91.1</td><td>137.1</td><td>2540.1</td><td>1343.9</td></tr>
</table>
Distribution of Intrauterine Compartment
Mean percentage of radioactive BPF (in relation to the administered dose, %) and calculated mean BPF concentrations (ng/g; ppb) are reported in the table below.
In the P-LD and P-HD foetuses, the main radioactivity was found in the body (71% resp. 69%), and less amount was found in the head (23% resp. 25%) and liver (6% resp. 6%).
<table>
<tr><th>Body compartment</th><th>NP-LD</th><th>P-LD</th><th>NP-HD</th><th>P-HD</th></tr>
<tr><td>%</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Uterus</td><td><0.01</td><td>0.07</td><td><0.01</td><td>0.18</td></tr>
<tr><td>Fetuses + Placenta</td><td>-</td><td>1.32</td><td>-</td><td>0.91</td></tr>
</table></td><td>Cabaton et al., 2006</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results
The results are presented as mean values.</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td colspan="5">ng/g</td></tr>
<tr><td>Ovary</td><td>196.9</td><td>133.7</td><td>2257.6</td><td>1179.8</td></tr>
<tr><td>Uterus</td><td>217.8</td><td>197.4</td><td>3025.5</td><td>6486.8</td></tr>
<tr><td>Vagina</td><td>449.1</td><td>209.5</td><td>4145.3</td><td>3838.2</td></tr>
<tr><td>Placenta</td><td>-</td><td>241.8</td><td>-</td><td>2141.8</td></tr>
<tr><td>Amniotic Fluid</td><td>-</td><td>195.1</td><td>-</td><td>2691.0</td></tr>
<tr><td>Foetuses</td><td>-</td><td>193.9</td><td>-</td><td>2892.6</td></tr>
</table>
Excretion
Urinary excretion reached a maximum amount of BPF-radioactivity at the start of the study period (0-24h), whereas faecal excretion reached a maximum amount at 48 – 72 h. At 96 h, radioactivity (8-10%) was still recovered in the contents of the digestive tract.

Profiling of metabolites
In the radiochromatographic analysis of urine from females in the P-HD, unchanged BPF was detected with a retention time of 32.5 min.

The major metabolite of BPF (>50% of urine radioactivity) had a retention time of 22.9 min and was hydrolysed by sulfatase but not β-glucuronidase. At least five other unknown radioactive peaks were detected. The parent compound accounted for less than 25% of the radioactivity present in urine.
</td><td></td></tr>
<tr><td>Method: Biliary Excretion
Species, strain, sex, no/group: 4 Sprague-Dawley female rats (16 weeks old).
Test substance, dose levels, duration of exposure: Females were gavaged with a single dose of 1.5 mg/kg bw of [^{3}H]BPF dissolved in 1 mL of propylene glycol.
Sampling: The females were anesthetized, and the bile duct was cannulated with a catheter. Bile collection began 2-h after dosing and collected every 30 min over a 6.5-h period. After 6.5-h, the animals were euthanized by exsanguination.

GLP / OECD TGs not stated.</td><td>Excretion
More than 46% of the measured radioactivity (in relation to the administered dose) was recovered 6-h after bile collection started.</td><td>Cabaton et al., 2006</td></tr>
<tr><td>Method: Pharmacokinetic study
Species, strain, sex, no/group: 5 male and 5 female Sprague-Dawley rats. Age of the animals is not stated.
Test substance, dose levels, duration of exposure: Rats were orally given a dose of 200 mg/kg bw BPF dissolved in corn oil at a 20% concentration.
Sampling: Blood collected: 0.25,</td><td>Distribution
<table>
<tr><th></th><th>Male (n=5)</th><th>Female (n=5)</th></tr>
<tr><td>C_{max} plasma (ng/ml)</td><td>12 552</td><td>16 101</td></tr>
<tr><td>Tmax (h)</td><td>0.25</td><td>0.25</td></tr>
<tr><td>T ½ (h)</td><td>9.02</td><td>21.7</td></tr>
<tr><td>AUC (mg*h/ml)</td><td>15 112</td><td>23 377</td></tr>
<tr><td>Cl (ml/h/kg)</td><td>14 642</td><td>9 581</td></tr>
</table>
</td><td>Lee et al., 2022a</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results
The results are presented as mean values.</th><th>Reference</th></tr>
<tr><td>0.5, 1, 3, 6, 8, 12, 24, 48, and 72-h.

GLP / OECD TGs not stated.</td><td></td><td></td></tr>
<tr><td>Method: Toxicokinetic study
Species, strain, sex, no/group: 6 Polypay x Dorset cross-bred female sheep (at 2nd or 3rd parity).
Test substance, dose levels, duration of exposure: All females underwent foetal catheterization surgery (placing a catheter in the foetal descending aorta and inferior vena cava) at GD 114.8 ± 0.8 days. After recovery, 3 females were injected with a single s.c. dose of 1.5 mg/kg bw of a mixture of BPA, BPS, and BPF (0.5 mg/kg bw per chemical) dissolved in corn oil.
Purity: 98% BPF and 99% internal standard $^{13}C_{12}$-BPF.
Sampling: Maternal plasma: 0, 1, 3, 5, 9, 24, 36, and 48-h.
Maternal urine: 0, 9 and 24-h.
Foetal plasma: 0, 3, 9, 24, 36, 48, and 72-h.
Foetal urine: 72-h.
Amniotic fluid: 0 and 72-h.

GLP / OECD TGs not stated.

Comparative study.</td><td>Distribution<table><tr><td></td><td>Female (n=3)</td></tr><tr><td>Maternal</td><td></td></tr><tr><td>C_{max} plasma (ng/ml)</td><td>48.8</td></tr><tr><td>T ½ (h)</td><td>7.7</td></tr><tr><td>AUC (mg*h/ml)</td><td>0.40</td></tr><tr><td>C urine (ng/ml at t = 9 h)</td><td>3.9</td></tr><tr><td>C urine (ng/ml at t = 24 h)</td><td>18.7</td></tr><tr><td>$Cl_{total\ body}$ (ml/h)</td><td>103.8</td></tr><tr><td>Foetal</td><td></td></tr><tr><td>C_{max} plasma (ng/ml)</td><td>20.9</td></tr><tr><td>T ½ (h)</td><td>14.2</td></tr><tr><td>AUC (mg*h/ml)</td><td>0.21</td></tr><tr><td>C urine (ng/ml at t = 72 h)</td><td>13.8</td></tr><tr><td>C amniotic fluid (ng/ml at t = 72 h)</td><td>16.4</td></tr></table></td><td>Gingrich et al., 2019</td></tr>
<tr><td>Method: Review

BPF data based on 6 studies.

GLP / OECD TGs not stated.

Comparative study.</td><td>Metabolism<table><tr><td>In vitro metabolites</td></tr><tr><td>• HepG2 cell line: BPF sulfate.
• Human hepatocytes: BPF glucuronide and BPF sulfate.
• Human intestinal LS174T cells: BPF glucuronide.
• Human liver microsomes: glutathione conjugates forming BPF quinone.
• Human liver microsomes: 4-(hydroxymethyl)-phenol.</td></tr><tr><td>In vivo metabolites</td></tr><tr><td>• Rat (urine): BPF sulfate, oxidative metabolites (hydroxylated BPF occurring on both the ortho and meta positions of the phenol ring), BPF dimer (lower polarity than BPF).</td></tr><tr><td>In vitro enzymes</td></tr><tr><td>• UGT1A10 mainly responsible for BPF glucuronidation in humans (higher BPF biotransformation observed in intestine than in liver).</td></tr></table></td><td>Skledar and Masic, 2016</td></tr>
</table>

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Cabaton et al., 2006 performed two analyses to study the distribution, metabolism and excretion of radiolabelled BPF ([^{3}H]BPF) *in vivo*, namely *Metabolic balance and tissue distribution* and *Biliary excretion*. Both radiolabelled BPF (purity >99.3%; specific activity of 300.36 MBq/mmol) and unlabelled BPF (purity >98%; CAS number 620-92-8) were dissolved in propylene glycol (1 mL per animal).
In the former analysis, 16 Sprague-Dawley female rats (8-10 weeks old) were divided into 4 groups. One low dose and one high dose of non-pregnant females (NP-LD and NP-HD) and one low dose and one high dose of pregnant females (P-LD and P-HD). The females were orally given a single dose of 7 or 100 mg/kg bw of [^{3}H]BPF, adjusted with unlabelled BPF for the pregnant group, and dissolved in propylene glycol. The pregnant females were dosed on gestational day 17. After dosing, the females were kept in metabolic cages. Urine and faeces were collected once every 24-h over a 96-h period. 96-h after dosing, the females were euthanatized by exsanguination after cervical dislocation.
In the latter analysis, 4 Sprague-Dawley female rats (16 weeks old) were orally given a single dose of 1.5 mg/kg bw of [^{3}H]BPF dissolved in 1 mL of propylene glycol. The females were anesthetized, and the bile duct was cannulated with a catheter. Bile collection began 2-h after dosing and collected every 30 min over a 6.5-h period. After 6.5-h, the animals were euthanatized by exsanguination.
Radioactivity was measured using a scintillation analyser.

Results are presented in means.

Most of the radioactivity was detected in urine (42 – 53%) and to a much lesser extent in faeces (14 – 18%). In urine, BPF was detected as unchanged (not metabolised) and as metabolites. At least six different metabolites were detected, suggesting multiple metabolic pathways. The major metabolite (>50% of urine radioactivity) was hydrolysed by sulfatase but not β-glucuronidase, indicating a metabolic formation of a sulfate conjugate of BPF. Excretion of BPF in urine gradually decreased over the 96-h period but remained quantitatively significant up to the end of the sample collection, suggesting an enterohepatic cycling of BPF and its metabolites. In the biliary excretion analysis, 46% of the distributed radioactivity was excreted in bile over a 6-h period after initial dosing.

Radioactivity was also detected in the carcass (5 – 8%) and in tissues, of which most of the radioactivity was detected in the liver (0.4 – 0.7%). When expressed as ng/g (ppb), the largest amount of mean BPF concentrations were found in the liver (679 – 13072 ng/g), followed by the kidneys (268 – 4571 ng/g), vagina (209 – 4145 ng/g), and in brain, muscle, fat, ovary, uterus and blood (ranging from 85 to 2257 ng/g).

Results from the pregnant females, showed that radioactivity was detected in the uterus, placenta, amniotic fluid, and in the foetuses (0.9 – 1.3%). In the P-LD group, BPF concentrations were of the same magnitude in the placenta, amniotic fluid and in the foetus (241.8, 195.1 respectively 193.9 ng/g). This was also true for P-HD (2141.8, 2691.0 respectively 2892.6 ng/g).

In summary, BPF was distributed to most organs of the female rat. In pregnant rats, BPF was also distributed to the uterus, placenta, amniotic fluid and the foetus, suggesting that BPF passes the placental barrier. Excretion of BPF was mainly through urine and smaller amounts were found in faeces.

Lee et al., 2022a performed a pharmacokinetic study on 5 male and 5 female Sprague-Dawley rats orally administered with a single dose of 200 mg/kg bw BPF. When receiving the purchased BPF, the substance displayed properties of crystal foam, with low solubility and dispersibility in polar solvents. It was therefore sieved through a 100-mesh screen and processed into powder form and later dispersed in corn oil at a 20% concentration. Blood samples were collected at different time points (0.25, 0.5, 1, 3, 6, 8, 12, 24, 48, and 72-h). Concentrations of BPF were analysed using a LC/MS/MS. No information was provided on the age of the animals or the CAS number. Results are presented in means.

The maximum plasma concentration of BPF was similar in females and males (C_{max} 16 101 ng/ml resp. 12 552 ng/ml). The elimination rate was, however, lower (AUC 23 377 mg*h/ml and Cl 9 581 ml/h/kg) and the half-life was higher ($t_{½}$ 21.7 h) in females than in males (AUC 15 112 mg*h/ml; Cl 14 642 ml/h/kg; $t_{½}$ 9.02 h), suggesting that BPF persists longer in females than in males.

In summary, BPF seems to persist for a longer period in the body of females than in males.

Gingrich et al., 2019 aimed to study the toxicokinetics in 6 Polypay x Dorset cross-bred female sheep (at 2nd or 3rd parity). On gestational day 114.8 (± 0.8 days), all females underwent foetal catheterization surgery by placing a catheter in the foetal descending aorta and inferior vena cava. After recovery, the females were subcutaneously injected with a single dose of 1.5 mg/kg bw of a mixture of BPA, BPS, and BPF (0.5 mg/kg bw per chemical), at a dosing volume of 1.9 ± 0.1 ml. The substances were dissolved in corn oil. The three bisphenols had a purity of ≥ 98%. Information on CAS number was not provided. Sampling of maternal and foetal plasma and urine, and amniotic fluid occurred at different timepoints (see table above). The chemicals were analysed using a HPLC-MS/MS. Subcutaneous injection was used as the exposure route to avoid confounding factors from ruminal metabolism. Results are presented in means.

The maximum concentration of BPF in maternal plasma (C_{max} 48.8 ng/ml) was twice as high as the concentration in foetal plasma (C_{max} 20.9 ng/ml) and almost 3 times higher than the concentration in the amnionic sac (16.4 ng/ml at 72 h). The rate of elimination was also lower in dams than in foetuses (AUC 0.40 resp. 0.21 mg*h/ml). Despite this, the half-life of BPF in foetuses was twice as long than in dams ($t_{½}$ 14.2 h resp. 7.7 h).

In summary, concentrations of BPF were detected in both maternal and foetal body compartments (including the amnionic sac), suggesting that BPF crosses the placenta reaching the foetus, in which BPF seems to persist for a longer period of time than in dams.

Skledar and Masic, 2016 has summarised in a review a literature search of the *in vivo* and *in vitro* studies on the metabolism of BPF.

Metabolism: BPF sulfate (BPF-S) has been shown to be the main metabolite detected in urine in *in vivo* studies on rats. *In vitro* systems of HepG2 cell lines confirm the findings of the *in vivo* studies. However, both BPF-S and BPF-G (glucuronide) were formed in human hepatocytes. In human intestinal LS174T cells, the main metabolite was BPF-G metabolised by the intestinal UGT enzyme (uridine 5′-diphospho-glucuronosyltransferases) UGT1A10, which is in agreement with the higher BPF biotransformation observed in intestine than in liver.

Several oxidative metabolites of BPF have also been detected in *in vitro systems*. Among them, was hydroxylated BPF metabolites (i.e., ortho- and meta-hydroxylated) as well as dihydroxylated BPF, dihydroxybenzophenone, 4-(hydroxymethyl)-phenol and BPF dimer. Additionally, two glutathione conjugates have been detected.

ED activity: *in vitro assays* reveal estrogenic and anti-androgenic activities of BPF. In contrast, hydroxylated BPF metabolites (i.e., ortho- and meta-hydroxylated) did not show any oestrogen activity. The dihydroxybenzophenone showed similar oestrogen activity in transcriptional activation assays of HepG2cells as BPF. However, BPF showed higher affinity for ERα, whereas dihydroxybenzophenone showed higher affinity for ERβ. 4-(hydroxymethyl)-phenol had no oestrogen activity. The metabolites, ortho- and meta-hydroxylated BPF, dihydroxybenzophenone, and 4-[hydroxymethyl]-phenol) were without activity on androgen receptors.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated in this CLH-proposal.

10.2 Acute toxicity - dermal route

Not evaluated in this CLH-proposal.

10.3 Acute toxicity - inhalation route

Not evaluated in this CLH-proposal.

10.4 Skin corrosion/irritation

Not evaluated in this CLH-proposal.

10.5 Serious eye damage/eye irritation

Not evaluated in this CLH-proposal.

10.6 Respiratory sensitisation

Not evaluated in this CLH-proposal.

10.7 Skin sensitisation

Not evaluated in this CLH-proposal.

10.8 Germ cell mutagenicity

Not evaluated in this CLH-proposal.

10.9 Carcinogenicity

Not evaluated in this CLH-proposal.

10.10 Reproductive toxicity

BPF has no registered data on reproductive toxicity. For this reason, substance specific data has been retrieved from scientific studies in the open literature (PubMed, https://pubmed.ncbi.nlm.nih.gov/). The following section on reproductive toxicity is solely based on data on BPF and cover *in vivo* rodent and non-rodent studies as well as *in vitro* studies and epidemiological human studies. The data is divided into male and female fertility and presented according to the OECD Conceptual Framework (CF)[5] Levels.

The proposed harmonised classification of BPF as toxic for reproduction is further supported by read-across from BPA (Section 10.10.3).

10.10.1 Adverse effects on sexual function and fertility

Table 10: Summary table of animal studies on adverse effects on sexual function and fertility (OECD CF Level 4).

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, dose levels, duration of exposure**	**Results** The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	**Reference**
The following *in vivo* studies were obtained from publicly available scientific journals and concerns **male reproduction**.				
Reproduction/ developmental Toxicity Screening Test. OECD TG 421 (2016). GLP facility but not within the scope of GLP regulation.	**Rat** (Sprague-Dawley). Total no = 120 (12 animals / sex / group). Age at start of treatment: ~8 weeks old.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 98%. Dose levels: 1, 5, 20 and 100 mg/kg bw dissolved in 4 ml/kg of corn oil. Control group received only corn oil. Exposure: Oral gavage, daily for 2 weeks prior to mating and throughout the day before sacrifice in males (total 62 days) and through lactation day (LD) 13 in females (total at least 41 days). Dose selection rational: Based on results from the 28-day RDT by Higashihara et al., 2007.	**General clinical signs** No mortality observed in males. BPF-related salivation was observed in all males at 100 mg/kg bw. **Body weight and food consumption** No treatment-related mean body weight alterations observed in males. Food consumption was not affected. **Reproductive performance** No BPF treatment-related changes observed in precoital time, mating index, fertility index, or fecundity (pregnancy) index. **Reproductive organ weight** ↑ in absolute and relative weight of Cowper's gland ($p<0.01$ at 100 mg/kg bw/day). **Histo(patho)logical analysis** No findings. **Biochemical analysis (serum)** No changes. **Hormone analysis (serum)** No changes in T4 levels.	Lee et al., 2022b Klimisch: *Reliable without restriction*

[5] OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. Part of the guidance document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (originally published in 2012 and updated in 2018). https://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
28-day RDT study. GLP/ OECD TGs not stated.	**Rat** (Wistar). Total no = 160 (8 groups with 10 males and 80 females to match the males). Weight at start of treatment: 180-200 g.	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: unknown. Dose levels: 10, 30 and 50 mg/kg bw. Dilution unknown. Control group received 0.5ml normal saline. Exposure: Oral cannula, daily for 28 days. Recovery groups (n=4 a´10 males): one control, one low dose, one medium dose and one high dose group, were observed for additional 28 days after the 28-day treatment period. Females were made sexually receptive by inducing subcutaneous administration of 10 µg/100g bw of oestradiol benzoate and 0.5 mg/100g bw of progesterone 48 and 4 h respectively before mating.	Mortality and body weight during the treatment period were not reported. **Reproductive performance** ↓ fertility success (-20%, -20% and -30% at 10, 30 and 50 mg/kg bw)[6]. ↓ fertility index (-11%, -11% and -12.5% at 10, 30 and 50 mg/kg bw)[7]. **Sexual performance indices** ↓ motivation to mate (-20.5% at 50 mg/kg bw). ↑ mount latency (55.6% at 50 mg/kg bw). ↓ mount frequency (-128.0% at 50 mg/kg bw). ↑ intromission latencies (35.5% at 50 mg/kg bw). ↓ intromission frequency (-72.0% at 50 mg/kg bw). ↑ ejaculation latency (50.7% at 50 mg/kg bw). ↓ ejaculation frequency (-146.3% at 50 mg/kg bw). ↓ post ejaculation interval (-35.8% at 50 mg/kg bw). The observed effects on sexual performance indices were dose-response related and $p<0.05$ at all three dose levels. The effects were not reversed during the 28-day recovery period. **Histo(patho)logical analysis** ↓ sperm motility (-34% at 50 mg/kg bw), viability (-39% at 50 mg/kg bw), morphology (-43% at 50 mg/kg bw) and sperm count (-20% at 50 mg/kg bw). All parameters were dose-response related and $p<0.05$ at all three dose levels. **Hormone analysis (plasma)** ↓ levels of testosterone ($p<0.05$ at all dose levels). ↓ levels of FSH and LH ($p<0.05$ at 30 and 50 mg/kg bw). ↑ levels of oestrogen and prolactine ($p<0.05$ at 30 and 50 mg/kg bw).	Fatai and Aribidesi, 2022 Klimisch: *Reliable with restriction*
21-day RDT study.	**Rat** (Sprague-	Substance: Bisphenol F. CAS: 620-92-8.	**General clinical signs** No mortality was observed.	Li et al 2022

[6] Fertility success = number of pregnant females/number paired x 100.

[7] Fertility index = number of pregnant females/number mated x 100.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
GLP/ OECD TGs not stated.	Dawley). Total no = 32 (4 groups with 8 males). Age at start of treatment: 5 weeks old.	Purity: >99%. Dose levels: 1, 10 and 100 mg/kg bw dissolved in 1 ml/kg of corn oil. Control group received only corn oil. Exposure: Oral gavage, daily for 21 days (from PND 35 to PND 56).	**Body weight** No difference in body weight. **Reproductive organ weight** No difference in absolute or relative testicular or epididymal weight. **Histo(patho)logical analysis** ↓ sperm count in cauda epididymis ($p<0.01$ at 10 mg/kg bw, and $p<0.05$ at 1 and 100 mg/kg bw, exact numbers not reported). ↓ Leydig cell size and cytoplasmic size and cytoplasm/nucleus ratio ($p<0.001$ at 100 mg/kg). **Hormone analysis (serum)** ↓ levels of testosterone ($p<0.01$ at 1 and 10 mg/kg bw, and $p<0.05$ at 100 mg/kg bw). No difference in levels of LH, FSH or E_2.	Klimisch: *Reliable with restriction*
28-day RDT study. GLP. Enhanced OECD TG 407 (1999, 2000)	**Rat** (Sprague-Dawley). Total no = 80 (4 groups with 10 males and 4 groups with 10 females). Age at start of treatment: 8 weeks old.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 100%. Dose levels: 20, 100 and 500 mg/kg bw dissolved in 10 ml/kg of olive oil. Control group received only olive oil. Exposure: Oral gavage, daily for 28 days. Dose selection rational: In a preliminary test, rats were orally gavaged with BPF for 14 days. At 500 and 1000 mg/kg bw, rats had decreased body weights, haematological and biochemical abnormalities and organ weight changes.	**General clinical signs** At 500 mg/kg bw, ↓ spontaneous locomotion, stained lower abdomen, staining around anus, white turbid urine, and soft stool were observed. Mortality was not reported. **Body weight** Only males at 500 mg/kg had ↓ body weight, starting from day 12 (-14% on day 28, $p<0.01$). **Reproductive organ weight** No difference observed in absolute testis weight. ↑ relative weight in all treated groups (16% at 500 mg/kg, $p<0.05$, dose-response). **Histo(patho)logical analysis** No dose-response related histopathological changes observed including no abnormal spermatological findings (i.e., sperm morphology and sperm count). **Hormone analysis (serum)** At 500 mg/kg bw, ↓ T3 levels and ↑ in T4 levels (-18% and 18%, both $p<0.05$). No difference in TSH levels. Testosterone, oestradiol, LH or FSH were not analysed. **Biochemical analysis (plasma)**	Higashihara et al., 2007 Klimisch: *Reliable with restriction*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
			↓ total cholesterol levels (-16% and -22%, p<0.05 at 100 and 500 mg/kg bw, dose-response related).	
28-day RDT study. GLP/ OECD TGs not stated. Comparative study.	**Rat** (Sprague-Dawley). Total no = 91 (13 groups with 7 males/ group.) Age at start of treatment: 70-80 days (~ 10-12 weeks old).	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: 99%. Dose levels: 5, 25, and 50 mg/kg bw. Stock solution diluted with saline (concentration of ethanol < 0.1-0.5%). No information on what the control group received. Exposure: Oral (type of administration is unknown), daily. Animals were euthanised on Day 29.	Mortality was not reported. **Body weight** No difference observed in body weight gain after end of treatment. **Reproductive organ weight** No difference observed in right or left testis weight. **Histo(patho)logical analysis** Testicular tissues revealed a thin epithelium and ↓ number of secondary spermatocytes in all treated groups (no counting of sperms was presented in the study). At 50 mg/kg bw, there were few and irregular seminiferous tubules and no elongated spermatids in the lumen. No difference was observed in arrangement or shape of the seminiferous tubules or in morphometry of caput and cauda epididymis regions. ↓ epithelial height of testis (-17% at 50 mg/kg bw, p<0.01, dose-response). **Hormone analysis (plasma and testis)** ↓ plasma levels of testosterone (-32% at 5 and 50 mg/kg bw, p<0.05). ↓ intra-testicular tissue levels of testosterone (-22% at 50 mg/kg bw, p<0.01 at all dose levels). LH, FSH and oestradiol were not examined. **Antioxidant enzyme analysis (testis)** ↓ levels of CAT (-15% at 50 mg/kg bw, p<0.01, dose-response) and POD (-17% at 50 mg/kg bw, p<0.01). ↑ levels of LPO (15% at 50 mg/kg bw, p<0.001) and ROS (50% at 50 mg/kg bw, p<0.01), both dose-response.	Ullah et al., 2018a Klimisch: *Not reliable*
28-day RDT study. GLP not stated. Enhanced OECD TG 407.	**Rat** (Sprague-Dawley). Total no = 91 (13 groups with 7 adult males /	Substance: Bisphenol F. CAS: unknown. Chemical name: 4,4´-methylenediphenol. Purity: 99%. Dose levels: 5, 25, and 50	Mortality and body weight were not reported. **Histo(patho)logical analysis** No difference observed in sperm motility. ↓ in daily sperm production (19th stage spermatids) at 50 mg/kg (p<0.05, no exact	Ullah et al., 2019c Klimisch: *Reliable with restriction*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
Comparative study.	group). Age at start of treatment: 70-80 days.	mg/kg/day in 2 mL water. Stock solutions of 2 g/L of BPF in ethanol were diluted with media/saline just before use. Control group received 2-mL water containing 0.1% ethanol. Exposure: Oral (gavage), daily.	numbers presented). **Comet assay - DNA damage (testis)** ↑ in number of comets/100 cells, tail moment (mm), and tail DNA (%) at 50 mg/kg (27-33%, p<0.05).	
28-day RDT study. GLP/ OECD TGs not stated.	**Rat** (Sprague-Dawley). Total no = 42 (6 groups with 7 males/ group). Age at start of treatment: 80-90 days.	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: 99%. Dose levels: 1, 5, 25, 50, and 100 mg/kg bw. Stock solution diluted with saline (concentration of ethanol < 0.1%). Control group received saline. Exposure: Oral (type of administration is unknown), daily.	No mortality was observed. **Body weight** No difference observed in body weight gain after end of treatment. **Reproductive organ weight** No difference observed in right or left testis weight. **Histo(patho)logical analysis** Testicular tissues revealed a thin epithelium and ↓ number of secondary spermatocytes in all treated groups (no counting of sperms was presented in the study). At higher doses, few tubules and few elongated spermatids in the lumen was observed (non-stat sign). No difference in morphometry of caput and cauda epididymis regions was observed. **Hormone analysis (plasma and testis)** ↓ plasma levels of testosterone (-47% at 100 mg/kg bw, p<0.01), LH (-29% at 100 mg/kg bw, p<0.001) and FSH (-52% at 100 mg/kg bw, p<0.001), all with dose-response. ↓ intra-testicular tissue levels of testosterone (-18% at 100 mg/kg bw, p<0.01). Oestradiol was not analysed. **Antioxidant enzyme analysis (testis)** ↓ levels of CAT (-28% at 100 mg/kg bw, p<0.01, dose-response) and POD (-17% at 100 mg/kg bw, p<0.05). ↑ levels of LPO (19% at 100 mg/kg bw, p<0.05) and ROS (231% at 100 mg/kg bw, p<0.01).	Ullah et al., 2019b Klimisch: *Not reliable*
48-week RDT	**Rat** (Sprague-	Substance: Bisphenol F.	Mortality was not reported.	Ullah et al.,

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
study. GLP not stated. Exposure duration according to OECD TG 452. Comparative study.	Dawley). 7 males/ group. Age at start of treatment: 22 days (~ 3 weeks old).	CAS / Chemical name: unknown. Purity: unknown. Dose levels: 5, 25 and 50 µg/L. Stock solution diluted with water (concentration of ethanol < 0.1%). Control group received water containing 0.1% ethanol. Exposure: Oral (drinking water), daily.	**Body weight** No changes in body weight gain. ↑ final body at 50 µg/L (<2%, p<0.05). **Reproductive organ weight** ↓ GSI (-7% at 50 µg/L, p<0.05), relative epididymis weight (-4% at 50 µg/L, p<0.01), relative prostate weight (-3% at 50 µg/L, non-stat sign), absolute paired testis weight (-5% at 50 µg/L, non-stat sign), relative seminal vesicle weight (-7% at 50 µg/L, p<0.01), and epithelial height of testis (-15% at 50 µg/L, p<0.01), all were dose-response. ↓ absolute paired epididymis weight (-1% at 50 µg/L, non-stat sign), absolute prostate weight (-3% at 50 µg/L, non-stat sign) and absolute seminal vesicle weight (-4% at 25 µg/L, p<0.05; and -2% at 50 µg/L, p<0.01). **Histo(patho)logical analysis** A dose-response related ↓ was observed in number of: - daily sperm production; 53 x 10^6 (ctl), 52 x 10^6 (5 µg/L), 50 x 10^6 (25 µg/L) and 48 x 10^6 (-9% at 50 µg/L, p<0.05), - spermatogonia (-7% at 50 µg/L, p<0.05), spermatocytes (-7% at 50 µg/L, p<0.05) and spermatids (-5% at 50 µg/L, p<0.01) in seminiferous tubules, - sperms in caput epididymis; 303 x 10^6/g (ctl), 295 x 10^6/g (5 µg/L), 293 x 10^6/g (25 µg/L, p<0.05) and 288 x 10^6/g (-5% at 50 µg/L, p<0.01), - sperms in cauda epididymis; 598 x 10^6/g (ctl), 592 x 10^6/g (5 µg/L), 589 x 10^6/g (25 µg/L) and 583 x 10^6/g (-3% at 50 µg/L, p<0.05), as well as in, - sperm motility (-5% at 25 µg/L, p<0.05; and -6% at 50 µg/L, p<0.01) and, - viable sperms (-2% at 50 µg/L, non-stat sign). **Hormone analysis (plasma)** ↓ levels of testosterone (-21% at 50 µg/L, p<0.001), LH (-17% at 50 µg/L, p<0.05) and FSH (-25% at 50 µg/L, p<0.05), all were dose-response. ↑ levels of oestradiol (59% at 50 µg/L,	2018b Klimisch: *Not assignable*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
			p<0.01, dose-response). No analysis was performed on testicular tissue. **Antioxidant enzyme analysis (testis)** ↓ levels of CAT, SOD and POD (-17%, -6% and -10% at 50 µg/L, all with p<0.01 and all with dose-response). ↑ levels of LPO (11% at 50 µg/L, p<0.01) and ROS (23% at 50 µg/L, p<0.001), both were dose-response.	
Prenatal toxicity study GLP/ OECD TGs not stated. Comparative study.	**Rat** (Sprague-Dawley). Total no = 104 (13 groups with 8 pregnant females/ group). Age: at start of mating: 80-90 days (~ 11-13 weeks old).	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: unknown. Dose levels: 5, 25 and 50 µg/L. Stock solution diluted with water (concentration of ethanol < 0.5%). Control group received water containing 0.1–0.5% ethanol. Exposure: Oral (drinking water), daily. Exposure during pregnancy from GD 1 to PND 1. PND 16: 2 male pups/litter were euthanised for further investigations. PND 80: 8 male pups/group were euthanised for further investigations.	Mortality was not reported. **Dams** No external clinical signs observed. ↑ body weight gain between GD 1-21 (4% at 50 µg/L, non-stat sign). **Litter size** ↓ litter size (-10% at 50 µg/L, non-stat sign but dose-response). **Body weight (male offspring)** At birth, ↓ body weight (ctl 5.3g; low dose 5.11g; mid dose 4.96g; and high dose 4.46g, non-stat sign but dose-response), whereas at *PND 16* there was no difference. At *PND 80*, ↑ body weight (ctl 192g; low dose 190g; mid dose 204g; and high dose 210g, p<0.05 at 50 µg/L, dose-response). **Reproductive organ weight (male offspring)** On *PND 16*, no difference in absolute weight of testis, prostate, epididymis, seminal vesicle, adrenals, bulbourethral gland or bulbocavernosus muscles. On *PND 80*, no difference was observed in absolute weight of testis, epididymis, prostate, or adrenals. ↓ seminal vesicle weight (-5% at 50 µg/L, p<0.05). **Endocrine pathology (male offspring)** No difference in anogenital distance on PND 1, nipple retention on PND 14, or puberty onset on PND 80. **Histo(patho)logical analysis** On *PND 80*, ↓ number of spermatogonia, spermatocytes and spermatids in seminiferous tubules (-5% to -7% at 50 µg/L, p<0.05 - <0.01), all with a dose-	Ullah et al., 2019a Klimisch: *Not reliable*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
			response. ↓ sperm motility (-6% at 50 µg/L, p<0.01) and viable sperms (-2%, non-stat sign), both with a dose-response. ↓ number of daily sperm production (-16% at 50 µg/L, p<0.05), and number of sperms in caput/carpus epididymis (-3% at 50 µg/L, p<0.05) and cauda epididymis (-2% at 50 µg/L, non-stat sign), all with a dose-response. ↑ sperm transit time (days) in the caput/corpus and cauda epididymis (non-stat sign), both with a dose-response. **Histomorphometry (testis)** On *PND 80*, ↓ area of seminiferous tubule (-5% at 50 µg/L, p<0.001), interstitial space (-15% at 50 µg/L, p<0.05) and lumen (-4% at 50 µg/L, p<0.001), and of seminiferous tubule diameter (-1% at 50 µg/L, p<0.05), all with a dose-response. No difference in area of epithelium. ↑ seminiferous tubule epithelial height (3% at 50 µg/L, p<0.05). ↓ in caput epididymis histology, i.e., lumen diameter (µm), epithelial height (µm), area of epithelium (%) and lumen (%). None of the parameters were stat sign but with a dose-response. No difference in tubular diameter (µm). ↓ in cauda epididymis histology, i.e., lumen diameter (µm), epithelial height (µm), and area of epithelium (%). Area of lumen (%) was increased (dose-response related). None of the parameters were stat sign but with a dose-response. No difference in tubular diameter (µm). **Hormone analysis (plasma)** On *PND 80*, ↓ levels of testosterone (-31% at 50 µg/L, p<0.05) and LH (-27% at 50 µg/L, p<0.001), both with a dose-response. ↓ levels of FSH (-61% at 50 µg/L, p<0.01). ↑ levels of oestradiol (158% at 50 µg/L, p<0.001, dose-response). **Antioxidant enzyme analysis (testis)** On *PND 80*, ↓ levels of CAT (-35% at 50 µg/L, p<0.05), SOD (-6% at 50 µg/L, p<0.01) and POD (-14% at 50 µg/L, p<0.05), all with a dose-response. ↑ levels of LPO and ROS (20% and 24% at	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
			50 µg/L, both p<0.01, dose-response).	
28-day RDT study. GLP/ OECD TGs not stated. Comparative study.	**Mouse** (Kunming). Total no = 16 (2 groups with 8 males) Age at start of treatment: 8 weeks old.	Substance: Bisphenol F. CAS: 620-92-8. Purity: unknown. Dose level: 25 mg/kg bw dissolved in corn oil. Control group received only corn oil. Exposure: Oral gavage, daily for 28 days.	Mortality was not reported. **Body weight, water and food intake** No differences observed during treatment. **Reproductive organ weight** No difference in absolute or relative testis weight. **Histo(patho)logical analysis** ↑ sperm deformity rate (p<0.05), ↓ sperm motility (p<0.05) and ↓ sperm count (p<0.01). ↓ proportion of seminiferous tubules at stage VII–VIII (p<0.001). ↑ gap of spermatogenic cell in the seminiferous tubule and ↓ mature sperm number in the lumen. ↓ thickness of germinal epithelium and atrophy of some seminiferous tubules. ↓ enzyme activities of LDH and SDH (p<0.05-0.01). Sperm morphology revealed acrosome loss and cervical/tail folding. **Hormone analysis (serum)** ↓ levels of testosterone (p<0.05). No difference in levels of LH, FSH, E_2 or free/unbound testosterone. **Hormone analysis (testis)** ↓ levels of testosterone (p<0.05). No difference in levels of E_2. **Biochemical analysis** ↓ levels of total cholesterol in Leydig cells, but not in serum or liver.	Gao et al., 2022 Klimisch: *Reliable with restriction*
The following *in vivo* studies were obtained from publicly available scientific journals and concerns **female reproduction**.				
Reproduction/ developmental Toxicity Screening Test. OECD TG 421 (2016). GLP facility but not within the scope of GLP	**Rat** (Sprague-Dawley). Total no = 120 (12 animals / sex / group). Age at start of treatment: ~8 weeks	Substance: Bisphenol F. CAS: 620-92-8. Purity: 98%. Dose levels: 1, 5, 20 and 100 mg/kg bw dissolved in 4 ml/kg of corn oil. Control group received only corn oil. Exposure: Oral gavage, daily	**General clinical signs** Mortality observed in one female in the 20 mg/kg bw group, that was nonpregnant and sacrificed unscheduled on day 27. BPF-related salivation was observed in all females at 100 mg/kg bw. **Body weight and food consumption** Body weights were stat sign ↓ in females at 100 mg/kg bw during gestation and lactation (p<0.05-0.01). Weight gain was	Lee et al., 2022b Klimisch: *Reliable without restriction*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
regulation.	old.	for 2 weeks prior to mating and throughout the day before sacrifice in males (total 62 days) and through lactation day (LD) 13 in females (total at least 41 days). Dose selection rational: Based on results from the 28-day RDT by Higashihara et al., 2007.	stat sign ↓ during pre-mating day 7 and gestational day 20 ($p<0.01$). During lactation, body weight changes were rebounded. Food consumption was stat sign ↓ during the whole treatment period (-28% and -24% at pre-mating day 7 resp. 14, -22%, -20%, -18% at GD 7, 14 resp. 20, -15% and -10% at LD 4 resp. 13). **Reproductive performance** No BPF treatment-related changes observed in precoital time, mating index, fertility index, or fecundity (pregnancy) index. **Reproductive organ weight** Stat sign ↓ in absolute left ovary weight ($p<0.05$) at 100 mg/kg bw. **Histo(patho)logical analysis** Dose-response related ↑ incidence of vaginal mucification (minimal to slight severity) were observed in all treated groups; 1, 2, 2, 3, and 8 females in the 0, 1, 5, 20, and 100 mg/kg bw. Stat sign ↓ in implantation sites at 100 mg/kg bw ($p<0.01$). This was confirmed by ↓ number of pups born ($p<0.01$). The length and regularity of the oestrous cycle was not changed. **Biochemical analysis (serum)** Stat sign ↑ GGT and TBIL at 100 mg/kg bw ($p<0.05$). **Hormone analysis (serum)** No changes in T4 levels.	
28-day RDT study. Employing OECD TG 407 (2008). GLP not stated. Comparative study.	**Rat** (Sprague-Dawley). Total no = 10 adult females/ group. Age at start of treatment: at post-weaning (mean body weight 90g	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: 99%. Dose levels: 50 and 500 µg/kg + 5 and 50 mg/kg. Dilution unknown. Control group received 1 ml/kg saline. Exposure: Intraperitoneal, daily. Euthanised in the oestrous	Mortality was not reported. **Body weight** No difference observed in body weight gain after end of treatment. **Reproductive organ weight** ↓ paired ovarian weight and GSI (-34% resp. -42% at 50 mg/kg, $p<0.05$), dose-response. ↓ absolute and relative uteri weight (-11% resp. -22% at 50 mg/kg, $p<0.01$). **Histo(patho)logical analysis**	Ijaz et al., 2020 Klimisch: *Not reliable*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
	~ PND 30).	phase.	↓ number of corpus luteum (-54% at 50 mg/kg, p<0.001, dose-response) and antral follicles (-23% at 50 mg/kg, p<0.001). ↑ number of atretic follicles (182% at 50 mg/kg, p<0.001, dose-response). No differences in preovulatory follicles. **Morphology** ↑ diameter of corpus luteum and antral follicle (29% resp. 55% at 50 mg/kg, p<0.001). ↑ height of granulosa (2% at 50 mg/kg, p<0.01) and theca (19% at 50 mg/kg, non-stat sign but dose-response). **Hormone analysis (plasma)** ↑ levels of testosterone (173% at 50 mg/kg, p<0.001). ↓ levels of LH (-16% at 50 mg/kg, p<0.001), FSH (-17% at 50 mg/kg, p<0.01) and oestradiol (-19% at 50 mg/kg, p<0.05), all were dose-response. ↓ progesterone (-32% at 50 mg/kg, p<0.01). **Biochemical analysis (ovarian)** ↓ levels of CAT (-72% at 50 mg/kg, p<0.01, dose-response) and SOD (-23% at 50 mg/kg, non-stat sign). ↑ levels of ROS (25% at 50 mg/kg, non-stat sign), TBARS (25% at 50 mg/kg, p<0.05) and POD (30%, non-stat sign).	
28-day RDT study. GLP. Enhanced OECD TG 407 assay (1999, 2000)	**Rat** (Sprague-Dawley). Total no = 80 (4 groups with 10 males and 4 groups with 10 females). Age at start of treatment: 8 weeks old.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 100%. Dose levels: 20, 100, 500 mg/kg dissolved in 10 ml/kg of olive oil. Control group received only olive oil. Exposure: Oral gavage, daily for 28 days.	Mortality was not reported. **Body weight** From day 17, all treated female groups had ↓ mean body weights (p<0.05). On day 28, females at 500 mg/kg had a mean body weight of -13% (p<0.01). **Reproductive organ weight** No dose-response related difference in relative uterus or ovary weights. **Histo(patho)logical analysis** No abnormal oestrous cycles were detected.	Higashihara et al., 2007 Klimisch: *Reliable with restriction*
Neonatal sub-chronic study. GLP/ OECD TGs not	**Mouse** (ICR). Total no = 6-8 litters.	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: unknown.	**Histo(patho)logical analysis** No effect on primary, preantral or antral follicles in adult ovaries at PND 60 (3-4 females examined). No stat sign difference for DES either.	Nevoral et al., 2021 Klimisch:

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, dose levels, duration of exposure**	**Results** The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	**Reference**
stated.	Age: F0 females were 6-7 weeks old at start of study and used for producing F1 generation offspring.	Dose levels: 0.2 and 20 ng/g bw/day (0.375 ng/mL and 37.5 ng/mL). Control group received 0.1% ethanol in sterile tap water. Positive control group received 0.2 ng/g bw/day diethylstilbestrol (DES). Exposure: nursing dams (4-5 dams/group) treated via drinking water from PND 0 (day of delivery) to PND 15. Ovaries were isolated on PND 60 (adult females).		*Reliable with restriction*

Table 11. Summary table of animal studies on adverse effects on sexual function and fertility **(OECD CF Level 3).**

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, dose levels, duration of exposure**	**Results** The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	**Reference**
Hershberger assay. GLP. OECD TGs not stated. Comparative study.	**Rat** (Wistar Han), castrated males. Total no = 6 males/ group. Age at start of treatment: 56 days old.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 99.9%. Dose levels: 50, 200 and 1000 mg/kg dissolved in 2 ml/kg of olive oil. Control group received only olive oil. Exposure: Oral (gavage), daily for 10 days.	**General observations** ↓ body weight (-7% at 1000 mg/kg, $p<0.05$) and ↓ spontaneous locomotion at 1000 mg/kg. **Reproductive organ weight** Slight ↑ in relative ventral prostate, seminal vesicle, and Cowper's gland at 1000 mg/kg, but non-stat sign. The ↑ was not modified by co-administration with testosterone propionate (s.c. 0.2 mg/kg).	Yamasaki et al., 2003 Klimisch: *Reliable with restriction*
Uterotrophic assay. GLP/ OECD TGs not stated. Comparative study.	**Rat** (Wistar). Total no = 8 females/ group (uterotrophic) and 4 females/ group (vaginal cornification assay). Age at start of treatment: 22 days old. Ovariectomy at week 6 of age.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 98%. *Immature females*: Dose levels: 25, 50, 100 and 200 mg/kg bw dissolved in PEG, and only 100 mg/kg bw in the vaginal cornification assay. Co-administration with 45 µg/kg bw of 17β-E2. *Ovariectomized females*: Dose levels: 100 mg/kg bw dissolved in PEG. Co-administration with 100 µg/kg bw of 17β-E2. Control group received PEG only. Exposure: Oral gavage, daily for 4 days.	Mortality and body weight was not reported. **Uterotrophic assay** In immature females, ↑ relative wet and dry uterine weight (exact numbers not reported, $p<0.05$, both dose-response related). Co-administration with 17β-E2 did not modify the increase. In ovariectomized females, no difference was observed in wet or dry uterine weight nor after co-administration with 17β-E2. **Vaginal cornification** In immature females, ↑ vaginal cornification (exact numbers not reported, $p<0.05$). ↑ cornification after co-administration with 17β-E2. In ovariectomized females, no difference was observed in cornification, only a non-stat sign ↑ after co-administration with 17β-E2.	Stroheker et al., 2003 Klimisch: *Reliable with restriction*
Uterotrophic assay. GLP. OECD TGs not stated.	**Rat** (Sprague-Dawley). Total no = 6 immature females/ group Age at start of treatment: 20 days old.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 99.9%. Dose levels: 100, 300, 1000 mg/kg dissolved in 4 ml/kg of olive oil. Co-administration with ethynyl estradiol (CAS 57-63-6) in olive oil injected subcutaneously into the back at a dose of 0.6 µg/kg.	Mortality was not reported. **Body weight** ↓ in body weight with increasing dose levels (-4% at 1000 mg/kg, non-stat sign). **Reproductive organ weight** ↑ in absolute and relative wet uterine weight (336% resp. 353% at 1000 mg/kg, $p<0.01$, dose-response). ↑ in absolute and relative blotted uterine weight (280% resp. 294% at 1000 mg/kg,	Yamasaki et al., 2004 Klimisch: *Reliable with restriction*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
		Control group received only olive oil. Positive control group received ethynyl estradiol of 0.6 µg/kg. Negative control group received the oestrogen-antagonist tamoxifen of 1 mg/kg per day plus ethynyl estradiol. Exposure: sub-cutaneous, daily for 3 days.	p<0.01, dose-response). A slight ↓ in uterine weight was observed after co-administration with ethynyl estradiol but far from the results of the negative control group.	
Uterotrophic assay. GLP/ OECD TGs not stated. Comparative study.	**Rat** (Sprague-Dawley). Total no = 6 immature females/ group Age at start of treatment: 20 days old.	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: 99.9%. Dose levels: 2, 20, 200 mg/kg bw dissolved in 4 ml/kg of olive oil. Control group received only olive oil. Exposure: sub-cutaneous, daily for 3 days.	Mortality was not reported. **Reproductive organ weight** ↑ absolute blotted uterine weight (59% at 200 mg/kg, p<0.01).	Yamasaki et al., 2002 Klimisch: *Not reliable*

Table 12. Summary table of *non-rodent* studies on adverse effects on sexual function and fertility (OECD CF Level 3).

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
The following *in vivo* studies were obtained from publicly available scientific journals and concerns **male reproduction**.				
21-day short-term fecundity assay. GLP/ OECD TGs not stated.	**Zebrafish** (Wild-type, AB) Age: from embryonic stage of F0 generation. F0 and F1 generation. Cross-mating: NWT male and female zebrafish.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 99%. Dose levels: 0.5, 5 and 50 µg/L dissolved in a final acetone concentration of 0.005 mL/L. Control group receiving the solvent acetone. Blank control group also included.	**Reproductive performance** *Internal mating:* ↓ total spawning numbers (-72.2%, -54.5%, and -33.8% at 0.5, 5 and 50 µg/L, p<0.001, dose-response) and↓ total fertilised embryos. ↓ embryo number of each clutch at 5 and 50 µg/L, p<0.05 (exact numbers not reported). At 50 µg/L, ↓ fertility rate and spawning times (p=0.019 resp. p=0.022, exact numbers not reported). *Cross-mating:* Treated males: ↓ total number of fertilised embryos and fertility rate at 50 µg/L (both p<0.05, exact numbers not reported). No change in spawning numbers or spawning time. **Gonad examination** No change in GSI. **Histo(patho)logical analysis (gonads)** ↓ number of spermatozoa at 50 µg/L (non-stat sign, exact numbers not reported). **Hormone analysis (gonads)** ↓ oestradiol (17β-E2) and 11-K testosterone in testis at 50 µg/L (p<0.05, exact numbers not reported).	Mu et al., 2022 Klimisch: *Not reliable*
21-day short-term fecundity assay. Employing OECD TG 229 and 230. GLP not stated.	**Zebrafish** (Danio rerio, AB). Age: F0 males and females were 4,5 months old at start of study. F0 and F1 generation.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 98%. Dose levels: 0.001, 0.01, 0.1 and 1 mg/L dissolved in dimethyl sulfoxide (DMSO) into a water tank. Control group received 0.01% (v/v) DMSO. No blank control groups included.	No mortalities and no changes in body weight or length. **Reproductive performance** ↓ hatching rate of embryos in all treated groups (p<0.05 at 1 mg/L, exact numbers not reported). **Gonad examination** ↓ GSI in all treated groups (-27% at 1 mg/L, p<0.05). **Histo(patho)logical analysis (gonads)** ↓ number of spermatogonia and of spermatocytes, and ↑ number of spermatids, and an enlargement of interstitial space at 1 mg/L (no counting of sperms was done to	Yang et al., 2017 Klimisch: *Reliable with restriction*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
			present the magnitude of decrease). **Hormone analysis (gonads)** ↓ testosterone levels ($p<0.05$ at 0.01, 0.1 and 1 mg/L, dose-response, exact numbers not reported). ↑ oestradiol levels (17β-E2) in all treated groups ($p<0.05$ at 0.1 and 1 mg/L, exact numbers not reported).	
In ovo study. GLP/ OECD TGs not stated. Comparative study.	**Chicken** (Gallus gallus domesticus). Total no = 47.	Substance: Bisphenol F. CAS: 620-92-8. Purity: >98%. Dose level: 210 nmol/g egg (42 µg/g egg) dissolved in dimethyl sulfoxide (DMSO). Control group (embryos) received DMSO only. Exposure: Bolus dose in ovo. Chicken embryos dissected on day (E) 19 i.e., 2 days before hatching.	**Mortality** 25 of 47 embryos died (53%). **Sex distribution** The remaining embryos was 13 females and 9 males (22 in total). **Gross morphology and histo(patho)logy** 1 of 9 males had ovotestis (i.e., feminised gonads) in the gross morphology. This was confirmed in the histopathological analysis were the (left) ovotestis showed thickened ovary-like cortex with high columnar epithelium and numerous oocyte-like cells and a medulla containing lacunae (similar to those in the ovarian medulla) and testicular cords appeared irregular. Remaining 8 males were not classified as having ovotestis in the gross morphology but they had >10 oocyte-like cells in testis in the histological analysis.	Mentor et al., 2020 Klimisch: *Reliable with restriction*
The following *in vivo* studies were obtained from publicly available scientific journals and concerns **female reproduction**.				
21-day short-term fecundity assay. GLP/ OECD TGs not stated.	**Zebrafish** (Wild-type, AB) Age: from embryonic stage of F0 generation. F0 and F1 generation. Cross-mating: NWT male and female zebrafish.	Substance: Bisphenol F. CAS: 620-92-8 Purity: 99%. Dose levels: 0.5, 5 and 50 µg/L dissolved in a final acetone concentration of 0.005 mL/L. Control group receiving the solvent acetone. Blank control group also included.	**Reproductive performance** *Internal mating*: ↓ total spawning number (-72.2%, -54.5%, and -33.8% at 0.5, 5 and 50 µg/L, $p<0.001$, dose-response) and ↓ total fertilised embryos. ↓ embryo number of each clutch at 5 and 50 µg/L, $p<0.05$ (exact numbers not reported). At 50 µg/L, ↓ fertility rate and spawning times ($p=0.019$ resp. $p=0.022$, exact numbers not reported). *Cross-mating:* Treated females: ↓ total number of spawning and fertilised embryos at 5 and 50 µg/L (both $p<0.01$, exact numbers not reported). No change in spawning times or fertility rate.	Mu et al., 2022 Klimisch: *Not reliable*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
			Gonad examination No change in GSI. **Histo(patho)logical analysis** Loose ovary structure, interstitial oedema, oocyte necrosis, separation of the follicular membrane from the yolk, a dose-dependent increase in the degree of lesions and a significant dissolution of ovum was observed. There was no clear trend in the number of ovarian follicles in the different developing stages. **Hormone analysis (gonads)** ↓ 17β-E2 and 11-KT in ovaries at 50 µg/L ($p<0.05$).	
21-day short-term fecundity assay. Employing OECD TG 229 and 230. GLP not stated.	**Zebrafish** (Danio rerio, AB). Age: F0 males and females were 4,5 months old at start of study. F0 and F1 generation.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 98%. Dose levels: 0.001, 0.01, 0.1 and 1 mg/L (= 1, 10, 100 and 1000 µg/L) dissolved in dimethyl sulfoxide (DMSO) into a water tank. Control group received 0.01% (v/v) DMSO. No blank control groups included.	No mortalities and no changes in body weight or length. **Reproductive performance** ↓ egg production and hatching rate of embryos in all treated groups ($p<0.05$ at 1 mg/L, exact numbers not reported). **Gonad examination** ↓ GSI in all treated groups (-21% at 1 mg/L, $p<0.05$). **Histo(patho)logical analysis** There was no clear trend in the number of ovarian follicles in the different developing stages. **Hormone analysis (gonads)** At 1 mg/L, ↓ testosterone levels in ovaries and ↑ 17β-E2 levels (both $p<0.05$, exact numbers not reported).	Yang et al., 2017 Klimisch: *Reliable with restriction*
4-day sub-acute in ovo study. GLP/ OECD TGs not stated. Comparative study.	**Chicken** (Gallus gallus domesticus). Total no = 47.	Substance: Bisphenol F. CAS: 620-92-8. Purity: >98%. Dose levels: 210 nmol/g egg (42 µg/g egg) dissolved in dimethyl sulfoxide (DMSO). Control group (embryos) received DMSO only. Exposure: Bolus dose in ovo. Chicken embryos dissected on day (E) 19 i.e., 2 days before hatching.	**Mortality** 25 of 47 embryos died (53%). **Sex distribution** The remaining embryos were 13 females and 9 males (22 in total). **Histo(patho)logical analysis** No changes in ovaries or Mullerian ducts in females.	Mentor et al., 2020 Klimisch: *Reliable with restriction*

Table 13. Summary table of *ex vivo* studies on adverse effects on sexual function and fertility (OECD CF Level 2).

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
The following *ex vivo* studies were obtained from publicly available scientific journals and concerns **male reproduction.**				
Testicular tissues from 7 sacrificed Sprague-Dawley rats. GLP/ OECD TGs not stated. Comparative study.	**Rat** (Sprague-Dawley). Age: 70-80 days old.	Substance: Bisphenol F. CAS: unknown. Chemical name: bis(4-hydroxyphenyl)methane. Purity: 99%. Exposure: 2 h incubation. Dose levels: 1, 10, and 100 ng/ml.	**Hormone analysis (testis)** ↓ levels of testosterone (-23% at 100 ng/ml, non-stat sign, dose-response related). **Antioxidant enzyme analysis (testis)** ↑ levels of ROS ($p<0.001$ at 10 ng/ml).	Ullah et al., 2018a Klimisch: *Not reliable*
Testicular tissues from 36 sacrificed Sprague-Dawley rats. GLP/ OECD TGs not stated.	**Rat** (Sprague-Dawley). Age: 80-90 days old.	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: 99%. Exposure: 2 h incubation. Dose levels: 1, 10, 25, 50, and 100 ng/ml. Stock solution diluted with saline (concentration of ethanol < 0.1%).	**Hormone analysis (testis)** ↓ levels of testosterone (-10% at 100 ng/ml, non-stat sign). **Antioxidant enzyme analysis (testis)** At 100 ng/ml, ↓ levels of CAT (-20%) and POD (-3%), both non-stat sign. ↑ levels of SOD (40%, non-stat sign), LPO (51%, $p<0.01$) and ROS (43%, $p<0.001$).	Ullah et al., 2019b Klimisch: *Not reliable*
Spermatozoa from 26 sacrificed Sprague-Dawley rats. GLP not stated. Enhanced OECD TG 407. Comparative study.	**Rat** (Sprague-Dawley). Age: 70-80 days old.	Substance: Bisphenol F. CAS: unknown. Chemical name: 4,4'-methylenediphenol. Purity: 99%. Exposure: 2 h incubation. Dose levels: 0, 1, 10, and 100 ng/mL diluted in culture media.	**Antioxidant enzyme analysis (testis)** At 100 ng/ml, ↑ levels of SOD ($p<0.01$), ROS ($p<0.05$), and TBARS ($p<0.01$). Exact numbers not reported. **Comet assay** At 100 ng/ml, ↑ number of comets/100 cells (13%, $p<0.05$), tail moment (µm; 27%, $p<0.05$), and tail DNA (20%, $p<0.05$).	Ullah et al., 2019c Klimisch: *Reliable with restriction*
The following *ex vivo* studies were obtained from publicly available scientific journals and concerns **female reproduction.**				
Neonatal sub-chronic study. GLP/ OECD TGs not stated.	**Mouse** (ICR). Total no = 6-8 litters. Age: F0 females were 6-7 weeks old at start of study and	Substance: Bisphenol F. CAS / Chemical name: unknown. Purity: unknown. Dose levels: 0.2 and 20 ng/g bw/day (0.375 ng/mL and 37.5 ng/mL). Control group received 0.1%	**Histo(patho)logical analysis** No trend observed in number of GV oocytes obtained per female, nor in maturation (assessed by germinal vesicle breakdown and maturation rate). **Immunocytochemistry** Matured oocytes showed an ↑ in occurrence of spindle abnormalities at 20 ng/g ($p<0.01$, exact number not reported), accompanied	Nevoral et al., 2021 Klimisch: *Reliable with restriction*

	used for producing F1 generation offspring.	ethanol in sterile tap water. Exposure: nursing dams (4-5 dams/group) treated via drinking water from PND 0 (day of delivery) to PND 15. Immature oocytes (GV stage) were isolated from 8-10-weeks old females (~ PND 56 – 70) and used as such or further cultured to matured MII oocytes.	by ↑ chromosome misalignment (non-stat sign). ↓ demthylation of histone H3 on lysine K27 (H3K27me2, a chromatin repressive marker) at 0.2 and 20 ng/g (both $p<0.05$, exact number not reported).	

Table 14: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
The following epidemiological studies were obtained from publicly available scientific journals and concerns both **male** and **female fertility**.				
Cross-sectional study Comparative study.	Urinary levels of bisphenol F. CAS / Chemical name: unknown.	984 Chinese men were recruited from an infertility clinic between March and June 2013. Age: mean 32.0 ± 5.4 years. The men provided urinary samples (2 spot urine samples for BPF measurements), semen samples and a questionnaire on demographic characteristics lifestyles, occupational exposure and medical history. Multivariate logistic or linear regression models for average or quartiles of urinary bisphenol concentrations and continuous or having below WHO reference values of semen quality parameters.	**Urinary BPF levels** 1st sample: median 0.56 µg/g creatinine-adjusted (p25-p75: 0.20-1.55). 2nd sample: median 0.55 µg/g creatinine-adjusted (p25-p75: 0.19-1.74). **Effect of BPF on semen quality** ↑ quartiles of urinary BPF levels associated with ↓ progressive motility at the 4th quartile, OR = 0.71 [95% CI = 0.47, 1.06], p for trend 0.05, non-dose response related. ↑ quartiles of urinary BPF levels associated with ↑ percentage of sperm abnormal heads at the 4th quartile, Regression coefficient = 1.58 [95% CI = 0.24, 3.39], p for trend 0.04, dose-response related.	Chen et al., 2022
Cross-sectional study Comparative study.	Urinary levels of bisphenol F. CAS / Chemical name: unknown.	556 Danish men were recruited from a Fetal Programming of Semen Quality (FEPOS) cohort between 2017-2019. Age: 18-20 years. The men underwent a clinical examination and provided a urine sample for BPF measurements, semen samples and a questionnaire on lifestyle factors. Negative binomial regression model was used to estimate crude and adjusted ratios for continuous or quartiles of urinary bisphenol concentrations and semen quality parameters.	**Urinary BPF levels** 1st sample: median 0.14 ng/mL creatinine-adjusted (p5-p95: <LOD-2.44). **Effect of BPF on semen quality** No effects seen that are significant and dose-response related.	Benson et al., 2020
Prospective case-control study Comparative study.	Urinary and seminal plasma levels of bisphenol F. CAS / Chemical	8 men with normozoospermia and 8 men after surgical vasectomy with azoospermia from the Czech Republic were recruited between January	**Urinary BPF levels** 1st sample: All samples were <LOD, except one in the normozoospermia group (0.8 ng/mL) and one in the vasectomy group (1.94 ng/mL).	Ješeta et al., 2022

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	name: unknown.	2020 and December 2021. Age: 22-41 years (mean 32.4). The men provided urine samples and semen samples. The Welch's t-test and the Mann–Whitney U-test were used to test for differences in ratios between men with normozoospermia (control group) and men with vasectomy.	**Seminal plasma BPF levels** 1st sample: All samples were <LOD, except one in the vasectomy group (0.991 ng/mL).	
The following *ex vivo* studies were obtained from publicly available scientific journals and concerns both **male** and **female fertility**.				
In vitro study. Comparative study.	Bisphenol F. Chemical name: bis(4-hydroxyphenyl)methane. Purity: unknown.	Spermatozoa from **human donors**. Age: 25 – 30 years. Exposure: 4 h incubation. Dose levels: 10, 100, 300 and 400 µM diluted in DMSO. 7 replicates/donor.	**Sperm parameters at 400µM** ↓ Sperm motility: ~ -50% ↓ Sperm viability: ~ -15% ↓ Sperm mitochondrial membrane potential (ΔΨm): ~ -45% ↑ Sperm mitochondrial generation of superoxide anion (MRS %): ~40% All dose-response related.	Castellini et al., 2021
In vitro study. Comparative study.	Bisphenol F. CAS / Chemical name: unknown. Purity: 99%.	Testis from 3-5 **human donors** (prostate cancer patients who had no anti-androgen treatment). Age: 46.7 ± 4.6 years. Exposure: 24 and 48 h incubation. Dose levels: 10^{-9} - 10^{-5} µM diluted in DMSO.	**Hormone analysis (testis)** ↓ testosterone levels (stat sign) Most anti-androgenic at 10^{-6} M at both time points (24-h and 48-h; -23.4% resp. -44.9%).	Desdoits-Lethimonier et al., 2017

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

BPF has no REACH registered data on reproductive toxicity. For this reason, substance specific data on BPF was therefore gathered from scientific studies from the open literature (PubMed, https://pubmed.ncbi.nlm.nih.gov/). The current CLH-proposal is based on a weight of evidence of reproductive toxicity of BPF and cover *in vivo* rodent and non-rodent studies as well as *ex vivo* studies and human studies. The data is divided into male and female fertility and presented according to the OECD Conceptual Framework (CF) Levels.

To strengthen the weight of evidence, the proposed harmonised classification of BPF is further supported by read-across from BPA on reproductive toxicity from comparative studies and from ECHA's RAC opinion proposing a harmonised classification and labelling of BPA, 2014 (section 10.10.3). The justification for read-across is based on the structural similarity, toxicokinetic patterns as well as biological and toxicological profile of the substances.

A Klimisch score has been assigned to the main *in vivo* studies on rodents to assess their reliability[8]. Studies with a Klimisch as 'not reliable' or 'not assignable' were also included in a weight of evidence as the results from these studies were in line with the studies having a Klimisch as 'reliable with/without restrictions'.

RODENT STUDIES ON MALE FERTILITY (OECD CF LEVEL 4)

In the **Reproduction/developmental Toxicity Screening** Test (OECD TG 421, 2016) by **Lee and co-workers** (**2022b**), Sprague-Dawley rats received BPF via oral gavage at ~8 weeks of age at dose levels of 1, 5, 20 and 100 mg/kg bw/day dissolved in 4 ml/kg corn oil. The treatment period lasted from 2 weeks prior to mating and throughout the day before sacrifice in males (total 62 days) and through lactation (LD 13) in females (total at least 41 days). The control group received only corn oil. Each group (=5) contained 12 animals/sex. 1:1 mating for 2 weeks. Mating was confirmed by vaginal smears. The study was conducted at a GLP facility but not within the scope of the GLP regulation. Dose selection rational was based on results from the 28-day RDT study by Higashihara et al., 2007 (see below).

No mortality was observed in males. BPF-related salivation was observed in all males at 100 mg/kg bw.
No treatment-related mean body weight alterations or changes in food consumption was observed.

The measurement of reproductive performance revealed no BPF treatment-related changes in precoital time, mating index, fertility index, or fecundity (pregnancy) index.

The measurement of reproductive organ weights revealed a statistically significant increase ($p<0.01$) in absolute and relative weight of Cowper's gland when comparing the control group with the highest dose group of 100 mg/kg bw/day (absolute weight: 0.12g versus 0.15g, and relative weight 0.023g versus 0.030g). No other changes were found in weight of testis, epididymis, seminal vesicles, prostate, glans penis, or levator anibulbocavernosus. Also, there were no macroscopic or histo(patho)logical findings.

The clinical biochemical analysis revealed no alternations in blood chemical parameters (GLU, TP, ALB, A/G, GLO, AST, ALT, TBIL, GGT, ALP, TCHOL, TG, PL, or T4 levels).

In the **28-day oral repeated dose toxicity study (Fatai and Aribidesi, 2022)**, Wistar rats received BPF via oral administration (cannula) at dose levels of 10, 30 and 50 mg/kg bw (dilution not stated). The control group received 0.5 ml normal saline. Each group (=4) contained 10 animals/sex. Each male was matched with a female, that was made sexually receptive by inducing a subcutaneous injection of 10 µg/100g bw of oestradiol benzoate and 0.5 mg/100g bw of progesterone at 48 h and 4 h respectively before mating. Weight at start of treatment was 180-200 g (age not stated).
In addition, four recovery groups were established for the control and the three dose groups (10 animals/sex/group). The males were observed for additional 28 days after the 28-day treatment period.
No information was provided about the type of Wistar rat strain, vehicle given to the treated groups, age of

[8] The reliability assessment was based on a method described by Wiklund and Beronius 2022.

the animals at start of treatment, IUPAC nomenclature, purity, or CAS number. Also, no information was provided about mortality or body weight changes during treatment.

Reproductive performance, measured as fertility success, i.e., males that mated (-11%, -11% and -22% at 10, 30 and 50 mg/kg bw) and fertility index, i.e., females conceived (-2.8%, -2.8% and -2.8% at 10, 30 and 50 mg/kg bw), was decreased.

Sexual performance indices of males were statistically significantly altered ($p<0.05$ at all dose levels) in a dose-response related manner. At the highest dose level (50 mg/kg bw) the following magnitude of alterations were observed; a decrease of -20.5% in motivation to mate, -128.0% in mount frequency, -72.0% in intromission frequency, -146.3% in ejaculation frequency, -35.8% in post ejaculation interval, and an increase of 55.6% in mount latency, 35.5% in intromission latencies, and 50.7% in ejaculation latency. The effects were not reversed during the 28-day recovery period.

Histo(patho)logy of epididymis revealed a statistically significant ($p<0.05$ at all dose levels) and dose-response related decrease in sperm motility (-34% at 50 mg/kg bw), viability (-39% at 50 mg/kg bw), sperm count (-20% at 50 mg/kg bw) and abnormal morphology of sperm / sperm defects (-43% at 50 mg/kg bw).

Hormone analysis of plasma revealed a decrease in levels of testosterone ($p<0.05$ at all dose levels), FSH and LH ($p<0.05$ at 30 and 50 mg/kg bw), and an increase in levels of oestrogen and prolactine ($p<0.05$ at 30 and 50 mg/kg bw).

In the **21-day oral repeated dose toxicity study (Li et al., 2022)**, Sprague-Dawley rats received BPF as oral gavage at 5 weeks of age (from PND 35 to PND 56) at dose levels of 1, 10 and 100 mg/kg bw dissolved in 1 ml/kg of corn oil. The control group received corn oil only. Each group contained 8 males.

No mortality or treatment-related mean body weight alterations was observed.

The measurement of reproductive organ weights revealed no differences in absolute or relative testicular or epididymal weight. However, the histo(patho)logy revealed a statistically significant decrease in sperm count of cauda epididymis ($p<0.01$ at 10 mg/kg bw, and $p<0.05$ at 1 and 100 mg/kg bw, exact numbers not reported). There was also a statistical relationship between BPF exposure and a significant decrease in Leydig cell size, cytoplasmic size and cytoplasm/nucleus ratio ($p<0.001$ at 100 mg/kg).

The hormone analysis (serum) revealed a decrease in levels of testosterone ($p<0.01$ at 1 and 10 mg/kg bw, and $p<0.05$ at 100 mg/kg bw) but not in levels of LH, FSH or E_2.

In the **28-day oral repeated dose toxicity study (Higashihara et al., 2007)**, Sprague-Dawley rats received BPF as oral gavage at 8 weeks of age at dose levels of 20, 100 and 500 mg/kg bw dissolved in 10 ml/kg of olive oil. The control group received olive oil only. Each group contained 10 animals/sex. Males were necropsied on day 29. The dose selection rational was based on a preliminary study (unpublished), in which decreased body weights, haematological and biochemical abnormalities and organ weight changes were observed in Sprague-Dawley rats after receiving BPF as oral gavage for 14 days at dose levels of 500 and 1000 mg/kg bw.

In the present study, the general clinical signs at 500 mg/kg bw included decreased spontaneous locomotion, stained lower abdomen, staining around anus, white turbid urine and soft stool. These males also had lower mean body weights than the controls starting from day 12 (-14% on day 28, $p<0.01$).

The measurement of reproductive organ weights revealed no changes in organ weight (i.e., testes, epididymis, ventral prostate, dorsolateral prostate and seminal vesicles) nor were there any abnormal histo(patho)logical findings (including any abnormal spermatological findings i.e., sperm morphology and sperm count) in the aforementioned organs. The statistically significant increase in mean relative testis weight at 500 mg/kg bw (16% ↑) was probably attributed to the decrease in body weight (mean: -14%).

The hormone analysis revealed a statistically significant ($p<0.05$) and dose-response related decrease in serum T3 levels and a statistically significant ($p<0.05$) increase in T4 levels (-18% respectively 18% at 500 mg/kg bw). No difference in TSH levels. No other hormones e.g., testosterone, oestradiol, LH or FSH were analysed.

The clinical biochemical analysis revealed a statistically significant ($p<0.05$) and dose-response related

decrease in levels of total cholesterol in plasma (-16% and -22% at 100 respectively 500 mg/kg bw).

In the **28-day comparative oral repeated dose toxicity study (Ullah et al., 2018a)**, Sprague-Dawley rats received BPF orally at 70-80 days of age (~10-11 weeks) at dose levels of 5, 25, and 50 mg/kg bw dissolved in saline. Each group contained 7 males. No information was provided about the vehicle given to the control group, type of oral administration, IUPAC nomenclature or CAS number.

There were no changes in body weight gain.

The measurement of reproductive organ weights revealed no difference in testis weight. However, the histo(patho)logy revealed a thin epithelium of testis that was decreased in height (-17% at 50 mg/kg bw, $p<0.01$, dose-response). At 50 mg/kg bw, there were few and irregular seminiferous tubules and no elongated spermatids in the lumen (exact numbers not reported). A reduced number of secondary spermatocytes was also observed in all treated groups. No difference was observed in arrangement and shape of the seminiferous tubules or in morphometry of caput and cauda epididymis regions (diameter, height and shape).

The hormone analysis revealed a decrease in testosterone levels in plasma (-32% at 50 mg/kg bw, $p<0.05$) and in intra-testicular tissue (-22% at 50 mg/kg bw, $p<0.01$ at all dose levels). Other hormones e.g., LH, FSH and oestradiol were not examined.

The antioxidant enzyme analysis of testicular tissue homogenates (left testis and left epididymis) revealed decreased levels of catalase, CAT (-15% at 50 mg/kg bw, $p<0.01$, dose-response) and peroxidase, POD (-17% at 50 mg/kg bw, $p<0.01$). A dose-response related increase was observed in levels of lipid peroxidation, LOP (15% at 50 mg/kg bw, $p<0.001$) and reactive oxygen species, ROS (50% at 50 mg/kg bw, $p<0.01$).

In the **28-day comparative oral repeated dose toxicity study (Ullah et al., 2019c)**, Sprague-Dawley rats received BPF as oral gavage at 70-80 days of age (~10-11 weeks) at dose levels of 5, 25, and 50 mg/kg dissolved in 2 mL water. The control group received 2-mL water containing 0.1% ethanol only. Each group contained 7 males. No information was provided about the CAS number.

The histo(patho)logy of testis revealed a decrease in in daily sperm production (19^{th} stage spermatids) at 50 mg/kg ($p<0.05$, exact numbers not reported), but no difference in sperm motility.

The comet assay on DNA damage revealed an increase in numbers of comets/100 cells, tail moment (mm), and tail DNA (%) at 50 mg/kg (27-33%, $p<0.05$).

In the **28-day oral repeated dose toxicity study (Ullah et al., 2019b)**, Sprague-Dawley rats received BPF orally at 80-90 days of age (~11-12 weeks) at dose levels of 1, 5, 25, 50, and 100 mg/kg bw dissolved in saline. The control group received saline only. Each group contained 7 males. No information was provided on the type of oral administration, IUPAC nomenclature or CAS number.

Neither mortality nor changes in body weight gain was observed.

The measurement of reproductive organ weights revealed no difference in testis weight. However, the histo(patho)logy of testicular tissues revealed a thin epithelium and a reduced number of secondary spermatocytes in all treated groups. At higher doses, few tubules and few elongated spermatids in the lumen was observed. The effects were non-statistically significant. No difference in morphometry of caput and cauda epididymis regions (diameter, height, and shape) was observed.

The hormone analysis revealed a dose-response related decrease in testosterone levels in plasma (-47% at 100 mg/kg bw, $p<0.01$) and a decrease in testosterone levels in intra-testicular tissue (-18% at 100 mg/kg bw, $p<0.01$). A dose-response related decrease in levels of LH (-29% at 100 mg/kg bw, $p<0.001$) and FSH (-52% at 100 mg/kg bw, $p<0.001$) was also observed in plasma. Oestradiol was not analysed.

The antioxidant enzyme analysis of intra-testicular tissue revealed a decrease in levels of catalase, CAT (-28% at 100 mg/kg bw, $p<0.01$, dose-response related) and peroxidase, POD (-17% at 100 mg/kg bw, $p<0.05$). An increase was observed in levels of lipid peroxidation, LPO (19% at 100 mg/kg bw, $p<0.05$) and reactive oxygen species, ROS (231% at 100 mg/kg bw, $p<0.01$).

In the **48-week comparative oral repeated dose toxicity study (Ullah et al., 2018b)**, Sprague-Dawley rats received BPF orally via drinking water at ~3 weeks of age at dose levels of 5, 25 and 50 µg/L. The control group received water only. Each group contained 7 males. No information was provided on the IUPAC nomenclature, or CAS number.

No changes in body weight gain but an increase in final body was observed at 50 µg/L (<2%, $p<0.05$).

The measurement of reproductive organ weights revealed a dose-response related decrease in gonadosomatic index, GSI (-7% at 50 µg/L, $p<0.05$), relative epididymis (-4% at 50 µg/L, $p<0.01$), relative seminal vesicle weight (-7% at 50 µg/L, $p<0.01$), and relative prostate weight (-3% at 50 µg/L, non-statistically significant). Absolute seminal vesicle weight (-2% at 50 µg/L, $p<0.01$), absolute paired epididymis weight (-1% at 50 µg/L, non-statistically significant), absolute prostate weight (-3% at 50 µg/L, non-statistically significant), and absolute paired testis weight (-5% at 50 µg/L, non-statistically significant) were also decreased. The histomorphometry showed a dose-response related decrease in epithelial height of testis (-15% at 50 µg/L, $p<0.01$).

A dose-response related decrease in number of daily sperm production of spermatids (-9% at 50 µg/L, $p<0.05$) in testicular tissues and of sperms in caput (-5% at 50 µg/L, $p<0.01$) and cauda epididymis (-3% at 50 µg/L, $p<0.05$) as well as of spermatogonia (-7% at 50 µg/L, $p<0.05$), spermatocytes (-7% at 50 µg/L, $p<0.05$) and spermatids (-5% at 50 µg/L, $p<0.01$) in seminiferous tubules was observed. There was also a dose-response related decrease in sperm motility (-5% at 25 µg/L, $p<0.05$; and -6% at 50 µg/L, $p<0.01$) and in viable sperms (-2% at 50 µg/L, non-statistically significant).

The hormone analysis of plasma revealed a dose-response related decrease in levels of testosterone (-21% at 50 µg/L, $p<0.001$), LH (-17% at 50 µg/L, $p<0.05$) and FSH (-25% at 50 µg/L, $p<0.05$), whereas plasma levels of oestradiol showed a dose-response related increase (59% at 50 µg/L, $p<0.01$). No analysis performed on testicular tissue.

The antioxidant enzyme analysis of testicular tissue homogenates showed a dose-response related decrease in levels of catalase, superoxide dismutase and peroxidase (-17% for CAT, -6% for SOD and -10% for POD at 50 µg/L, all with $p<0.01$), whereas an increase was observed in levels of lipid peroxidation (11% at 50 µg/L, $p<0.01$) and reactive oxygen species (23% at 50 µg/L, $p<0.001$).

In a **comparative prenatal toxicity study (Ullah et al., 2019a)**, pregnant female Sprague-Dawley rats received BPF orally via drinking water at 80-90 days of age (~11-13 weeks) during gestation (GD 1 to PND 1) at dose levels of 0, 5, 25 and 50 µg/L. The control group received water only. Each group contained 8 pregnant females. Anogenital distance (AGD) was measured in male pups on PND 1 under an ocular stereomicroscope. On PND 14, pups were examined for the number of nipple retention (NR). On PND 16, 2 male pups/litter were euthanised for determining body weight and weight of reproductive organs (testis, prostate, epididymis, seminal vesicle, bulbourethral gland and bulbocavernosusmuscles) and other organs (adrenals, fat pad, liver). On PND 80, 8 male pups/group were euthanised for determining body weight, weight of reproductive organs (testis, prostate, epididymis, and seminal vesicle), weight of other organs (kidney, adrenals, fat pad, liver), puberty onset, histo(patho)logy of reproductive organs, hormone analysis and antioxidant analysis. Preputial separation was checked daily from day 35 till the pubertal day 1. No information was provided on the IUPAC nomenclature, or CAS number.

There were no external clinical signs or any significant difference in body weight gain among the dams in the treated groups during gestation (GD 1-21). A dose-response related decrease (non-statistically significant) in litter size was observed (10% ↓ at 50 µg/L).

The male offspring showed a decrease in birth weight (ctl 5.3g; low dose 5.11g; mid dose 4.96g; and high dose 4.46g, non-statistically significant), whereas at PND 16 there was no difference. At PND 80, an increase in body weight was observed (ctl 192g; low dose 190g; mid dose 204g; and high dose 210g, $p<0.05$ at 50 µg/L).

The measurement of reproductive organ weights revealed no statistically significant difference in weight on PND 16. On PND 80, only a decrease in seminal vesicle weight was observed (-5% at 50 µg/L, $p<0.05$).

No difference in AGD (PND 1), NR (PND 14) or preputial separation (PND 35 - pubertal day 1). The anogenital index (i.e., normalising the data by dividing anogenital distance by the cube root of pup weight) was not performed.

The histo(patho)logy of testis on PND 80 revealed a dose-response related decrease in number of spermatogonia, spermatocytes and spermatids in the seminiferous tubules (-5% to -7% at 50 µg/L, $p<0.05$ - <0.01). The same trend was observed for sperm motility (-6% at 50 µg/L, $p<0.01$) and viable sperms (-2%, non-statistically significant) as well as for the number of daily sperm production (-16% at 50 µg/L, $p<0.05$) and sperms in caput/carpus epididymis (-3% at 50 µg/L, $p<0.05$) and cauda epididymis (-2% at 50 µg/L, non-statistically significant). A dose-response related increase sperm transit time (days) in the caput/corpus and cauda epididymis was observed (non-statistically significant).

The histo(patho)logy of testis on PND 80 revealed a dose-response related decrease in area of seminiferous tubule (-5% at 50 µg/L, $p<0.001$), interstitial space (-15% at 50 µg/L, $p<0.05$) and lumen (-4% at 50 µg/L, $p<0.001$), and of seminiferous tubule diameter (-1% at 50 µg/L, $p<0.05$). No difference in area of epithelium. On the contrary, an increase in seminiferous tubule epithelial height was observed (3% at 50 µg/L, $p<0.05$). Further, in the histo(patho)logy of caput and cauda epididymis, a dose-response related decrease in lumen diameter, epithelial height, area of epithelium and lumen was observed (but non-statistically significant). No difference in tubular diameter.

The hormone analysis in blood plasma on PND 80 revealed a dose-response related decrease in levels of testosterone (-31% at 50 µg/L, $p<0.05$) and LH (-27% at 50 µg/L, $p<0.001$). FSH levels were also statistically significantly decreased (-61% at 50 µg/L, $p<0.01$), but not dose-response related. Levels of oestradiol were increased (158% at 50 µg/L, $p<0.001$, dose-response related).

The antioxidant enzyme analysis in testicular tissue homogenates on PND 80 revealed a dose-response related decrease in levels of catalase, CAT (-35% at 50 µg/L, $p<0.05$), superoxide dismutase, SOD (-6% at 50 µg/L, $p<0.01$) and peroxidase, POD (-14% at 50 µg/L, $p<0.05$), whereas an increase was observed in levels of lipid peroxidation, LPO, and reactive oxygen species, ROS (20% and 24% at 50 µg/L, both $p<0.01$, both dose-response related).

In the **28-day comparative oral repeated dose toxicity study (Gao et al., 2022)**, Kunming mice received BPF as oral gavage at 8 weeks of age at dose levels of 25 mg/kg bw dissolved in corn oil. The control group received corn oil only. Each group contained 8 males. No information was provided about the purity or the amount of corn oil.

There were no differences in body weight or in water and food intake during the treatment period. Mortality was not reported.

The measurement of reproductive organ weights revealed no difference in testis weight. However, the histo(patho)logy revealed a statistically significant increase in sperm deformity rate ($p<0.05$), and a statistically significant decrease in sperm motility ($p<0.05$) and sperm count ($p<0.01$). In the seminiferous tubule, there was a reduced thickness of germinal epithelium, an increased gap of spermatogenic cells and higher frequency of atrophy. The number of mature sperm in the lumen was reduced and the proportion of seminiferous tubules at stage VII–VIII was statistically significantly decreased ($p<0.001$). The sperm morphology revealed acrosome loss and cervical/tail folding.

Hormone analysis of serum and testis revealed a statistically significant decrease in levels of testosterone ($p<0.05$). No difference was observed in levels of LH, FSH or free/unbound testosterone in serum, or levels of E_2 in serum or testis.

Biochemical analysis revealed a decrease in levels of total cholesterol in Leydig cells, but not in serum or liver. Activities of testicular marker enzymes were also measured to further evaluate the disturbance of the spermatogenesis. A statistically significant decrease in levels of lactate dehydrogenase (LDH, $p<0.05$) and succinate dehydrogenase (SDH, $p<0.01$) was observed, but no change in acid phosphatase (ACP) or alkaline phosphatase (AKP).

RODENT STUDIES ON MALE FERTILITY (OECD CF LEVEL 3)

In a **comparative Hershberger assay (Yamasaki et al., 2003)**, castrated Wistar Han rats received BPF via oral gavage, daily for 10 days, at 56 days of age at dose levels of 50, 200 and 1000 mg/kg dissolved in 2 ml/kg of olive oil. The control group received only olive oil. Each group contained 6 males.

General observations of the males included lower body weights at the end of treatment (-7% at 1000 mg/kg, $p<0.05$) and decreased spontaneous locomotion at 1000 mg/kg. Mortality was not reported.

The measurement of reproductive organ weights revealed a slight increase in relative ventral prostate, seminal vesicle, and Cowper's gland at 1000 mg/kg, but none was statistically significant. The effect was also not modified after co-administration with testosterone propionate (s.c. 0.2 mg/kg), suggesting that BPF has no androgenic (or anti-androgenic) activity.

NON-RODENT STUDIES ON MALE FERTILITY (OECD CF LEVEL 3)

In the **21-day short-term fecundity assay** (**Mu et al., 2022**), wild-type zebrafish received BPF via water tank starting from embryonic stage of F0 generation at dose levels of 0.5, 5 and 50 µg/L. Both F0 and F1 generations were examined.

From the reproductive performance of internal mating, there was a dose-response related reduction in total number of spawning and fertilised embryos (spawning numbers: -72.2%, -54.5%, and -33.8% at 0.5, 5 and 50 µg/L, $p<0.001$). At 5 and 50 µg/L, the mean embryo number of each clutch was significantly reduced ($p<0.05$), and at 50 µg/L, a significant decrease in fertilised rate ($p=0.019$) and spawning times ($p=0.022$) was observed (exact numbers not reported). From the cross-mating of treated males and untreated females, a reduced number of fertilised embryos and fertility rate (both $p<0.05$ at 50 µg/L) was observed (exact numbers not reported). No change in spawning numbers or spawning time.

There was no difference in gonadosomatic index (GSI).

Despite the reduced fertility rate, the histo(patho)logy at 150 days of age revealed no major changes in the different stages of spermatogenesis (only a non-statistically significant decrease in number of spermatozoa at 50 µg/L but only 5 fish were examined. Exact numbers not reported).

In the hormone analysis of gonads (testis), a decrease in levels of 11-keto testosterone but also in estradiol (17β-E2) was observed ($p<0.05$ at 50 µg/L).

In the **21-day short-term fecundity assay** (**Yang et al., 2017**), Danio rerio zebrafish received BPF via water tank at 4,5 months of age at dose levels of 1, 10, 100 and 1000 µg/L (1 mg/L). Both F0 and F1 generations were examined.

No mortalities and no changes in body weight or length in F0 generation. The gonadosomatic index (GSI) was decreased in all treated groups (-27% at 1 mg/L, $p<0.05$).

The histo(patho)logy revealed a decrease in number of spermatogonia and spermatocytes, and an increase in number of spermatids and enlargement of interstitial space at 1000 µg/L (no counting of sperms was done to present the magnitude of decrease).

A decrease in the fecundity of parental fish was explained by a decrease hatching rate of embryos (and egg production) in all treated groups ($p<0.05$ at 1000 µg/L, exact numbers not reported).

The hormone analysis of gonads (testis) revealed a dose-response related decrease in testosterone levels ($p<0.05$ at 10, 100 and 1000 µg/L) and increased estradiol levels (17β-E2) in all treated groups ($p<0.05$ at 100 and 1000 µg/L). The increase in oestradiol levels coincided with a statistically significant and dose-response related induction of vtg1 gene expression in the male liver ($p<0.05$ at 10, 100 and 1000 µg/L). Exact numbers not reported.

In the **comparative in ovo study** (**Mentor et al., 2020**), 47 chicken eggs (gallus gallus domesticus) were injected with a bolus dose of 42 µg BPF/g egg into the air sac of the fertilized eggs on embryonic day 4. Chicken embryos were dissected on day 19 i.e., 2 days before hatching.

The number of dead embryos was 25 of 47 (i.e., 53%). The sex distribution of the remaining embryos (22)

was 13 females and 9 males.

The gross morphology revealed feminised gonads (1 of 9 males had ovotestis, non-statistically significant). This was confirmed in the histo(patho)logical analysis were the (left) ovotestis showed thickened ovary-like cortex with high columnar epithelium and numerous oocyte-like cells and a medulla containing lacunae (similar to those in the ovarian medulla) and testicular cords appeared irregular. Still, many of the testes that were not classified as ovotestis by gross morphology showed mild histological changes (i.e., 8 of 9 males had >10 oocyte-like cells in testis).

EX VIVO STUDIES ON MALE FERTILITY (OECD CF LEVEL 2)

In the *in vitro* studies by **Ullah and co-workers (2018a, 2019b and 2019c)**, testicular tissues were collected from sacrificed Sprague-Dawley rats (70 – 90 days old). The tissues were treated with BPF (99% purity) and incubated for 2 h.

In **Ullah et al., 2018a**, tissues from 7 male rats were exposed to 1, 10, and 100 ng/ml BPF. The results revealed a dose-response related decrease in testosterone levels (-23% at 100 ng/ml, non-stat sign) and a statistically significant increase in levels of reactive oxygen species, ROS ($p<0.001$ at 10 ng/ml).

In **Ullah et al., 2019b**, tissues from 36 male rats were exposed to 1, 10, 25, 50, and 100 ng/ml BPF. Again, the testosterone levels were decreased at 100 ng/ml (-10%, non-stat sign). A more extensive biochemical analysis revealed a decrease at 100 ng/ml in levels of catalase, CAT (-20%, non-stat sign) and peroxidase, POD (-3%, non-stat sign) and an increase in levels of sodium dismutase, SOD (40%, non-stat sign), lipid peroxidation, LPO (51%, $p<0.01$) and reactive oxygen species, ROS (43%, $p<0.001$).

In **Ullah et al., 2019c**, spermatozoa from 26 male rats were exposed to 1, 10, and 100 ng/mL BPF. An increase was observed in levels of sodium dismutase, SOD ($p<0.01$), reactive oxygen species, ROS ($p<0.05$) and thiobarbituric acid reactive substance, TBARS ($p<0.01$) at 100 ng/ml. Exact numbers not reported. The comet assay revealed an increase in number of comets/100 cells (13%, $p<0.05$), tail moment (µm; 27%, $p<0.05$), and tail DNA (20%, $p<0.05$) at 100 ng/ml.

HUMAN STUDIES ON MALE FERTILITY

In a **comparative epidemiological cross-sectional study** (**Chen et al., 2022**), 984 Chinese men (mean 32.0 ± 5.4 years old) were recruited from an infertility clinic between March and June 2013. The men provided urinary samples (2 spot urine samples for BPF measurements), semen samples and a questionnaire on demographic characteristics lifestyles, occupational exposure and medical history. Multivariate logistic or linear regression models was used to estimate the relationship between the average or quartiles of urinary bisphenol concentrations and continuous or having below WHO reference values of semen quality parameters.

Median creatinine-adjusted urinary BPF levels ranged from 0.55 – 0.56 µg/g (p25 = 0.19 – 0.20; p75 = 1.55 – 1.74). The statistical analyses revealed an association between increased quartiles of urinary BPF levels and decreased progressive motility at the 4th quartile. OR = 0.71 [95% CI = 0.47, 1.06], p for trend 0.05, non-dose response related. Also, an association between increased quartiles of urinary BPF levels and increased percentage of sperm abnormal heads at the 4th quartile. Regression coefficient = 1.58 [95% CI = 0.24, 3.39], p for trend 0.04, dose-response related.

In another **comparative epidemiological cross-sectional study** (**Benson et al., 2020**), 556 Danish men (18-20 years old) were recruited from a Fetal Programming of Semen Quality (FEPOS) cohort between 2017-2019. The men underwent a clinical examination and provided a urine sample for BPF measurements, semen sample and a questionnaire on lifestyle factors. Negative binomial regression model was used to estimate crude and adjusted ratios for continuous or quartiles of urinary bisphenol concentrations and semen quality parameters.

Median creatinine-adjusted urinary BPF level was 0.14 ng/mL (p5 – p95 = <LOD – 2.44). The statistical analyses revealed no association (that was statistically significant, and dose-response related) between urinary BPF levels and semen quality.

In a **comparative epidemiological prospective case-control study** by **Ješeta and co-workers (2022)**, 8 men with normozoospermia and 8 men after surgical vasectomy with azoospermia (22 – 41 years old) from the Czech Republic were recruited between January 2020 and December 2021. The men provided urine samples and semen samples. The Welch's t-test and the Mann–Whitney U-test were used to test for differences in ratios between men with normozoospermia (control group) and men with vasectomy (patient group).

Specific gravity-adjusted urinary BPF levels were below LOD except for one man in the control group (0.810 ng/mL) and one patient (1.94 ng/mL). BPF levels in seminal plasma were also below LOD except for in one patient (0.991 ng/mL). No further investigation on BPF was therefore conducted.

In the two following in vitro studies, **Castellini and co-workers (2021)** exposed human spermatozoa from male donors (aged 25 – 30 years) to 10, 100, 300 and 400 µM of BPF during 4-h of incubation, and **Desdoits-Lethimonier and co-workers (2017)** exposed human spermatozoa from donors of prostate cancer patients (mean age 46.7 ± 4.6 years) to 10^{-9} - 10^{-5} µM of BPF during 24-h and 48-h of incubation.

In the former in vitro study, there was a dose-response related decrease in sperm motility, viability and mitochondrial membrane potential (-50%, -15% respectively -45% at 400 µM, non-statistically significant). The mitochondrial generation of superoxide anion (MRS %) was increased by 40% at 400 µM (dose-response related, non-statistically significant).

In the latter in vitro study, there was a statistically significant decrease in testosterone levels at 10^{-6} M at both time points (24-h and 48-h; -23.4% respectively -44.9%).

SUMMARY OF BPF ON MALE REPRODUCTION

The majority of the scientific studies on experimental rodent animals shows that exposure to BPF affects male sexual function and fertility by statistically significantly altering reproductive performance (i.e., decreased fertility success, fertility index) and sexual performance (i.e., decreased motivation to mate, mount frequency, intromission frequency, ejaculation frequency, post ejaculation interval). Histo(patho)logical analysis of epididymis revealed negative changes in sperm parameters (i.e., decreased motility, viability and count) as well as abnormal morphology of sperm and of seminiferous tubule (i.e., decreased area, lumen and diameter, acrosome loss and cervical/tail folding), and decreased Leydig cell size and cytoplasmic size and cytoplasm/nucleus ratio.

These adverse effects are supported by statistically significant alterations in levels of cholesterol and hormones in plasma following exposure to BPF in these animals (i.e., decreased levels of cholesterol, testosterone, FSH and LH, and increased levels of oestrogen and prolactine). Furthermore, BPF also causes cytotoxicity in the intra-testicular tissues (i.e., decreased levels of catalase, superoxide dismutase and peroxidase, and increased levels of lipid peroxidation and reactive oxygen species).

The *ex vivo* assays on male reproduction, show that BPF causes oxidative stress in rat testicular tissues and in spermatozoa by increasing the levels of reactive oxygen species. The substance also leads to a statistically significant increase in DNA fragmentation in the spermatozoa, suggesting a damage to the DNA. The alterations in hormone levels and oxidative stress parameters as well as DNA damage confirms the results observed in the *in vivo* studies.

Findings from the two non-rodent studies on zebrafish revealed a statistically significant reduction in reproduction (i.e., reduced fertility rate, spawning times, total number of spawnings and fertilised embryos, embryo number of each clutch, and hatching rate of embryos). Cross-mating of treated males with untreated females revealed a statistically significant reduced total number of fertilised embryos and fertility rate.

At higher BPF concentrations (i.e., 1000 µg/L), the spermatogenesis was altered (i.e., decreased number of spermatogonia and spermatocytes, and increased number of spermatids) and gonadosomatic index (GSI, a good indicator for reproductive activity) was statistically significantly decreased. A statistically significant decrease in levels of testosterone in the gonads was observed following BPF exposure, whereas the levels of estradiol was only increased at higher BPF concentrations (≥ 100 µg/L) compared to a decrease in estradiol levels at lower BPF concentrations (≤ 50 µg/L). The increase in estradiol levels coincided with a statistically significant and dose-response related induction of vtg1 gene expression in the male liver.

The *in ovo* study on chicken eggs examined the effect of BPF on the development of reproductive organs that are oestrogen-regulated, without the influence of maternal toxicity. Here, BPF exposure was associated with testicular feminisation (so called ovotestis).

The two epidemiological cross-sectional studies reveal diverging results. The reason for this could be due to the differences in age of the recruited participants, the fertility status of the participants, the concentration levels of BPF found in urine, the analytical instrument, the sampling and the statical methods. The median BPF urine concentration in the study by Chen and co-workers was 0.55 – 0.56 µg/g and was associated with decreased progressive motility and increased percentage of sperm abnormal heads, compared to 0.14 ng/mL in the study by Benson and co-workers who found no associations.

In the two *in vitro* studies on human spermatozoa, BPF exposure was associated with decreased sperm motility, viability, mitochondrial membrane potential and testosterone levels, supporting the study by Chen and co-workers.

The strength of the findings lies on the same type of effect observed consistently across these studies, which include different types of study designs i.e., reproduction/developmental toxicity screening study, 28-day study and 48-week repeated dose toxicity studies, and a prenatal toxicity study, and some of which are supported by hormone and biochemical analysis as well as *in vitro* assays. The observed adverse effects on male sexual function and fertility in rodents are further supported by the those observed in the non-rodent studies. Unfortunately, no conclusions can be drawn from the two epidemiological studies show as they show inconsistent results between themselves.

The less pronounced effects on spermatogenesis observed in the three 28-day studies could be due to the lack of covering a complete cycle of the spermatogenesis. This is achieved in the 90-day study, which is, in this

respect, regarded as superior to the 28-day study. Also, the longer exposure duration in the 48-week study could explain the more severe reproductive effects observed in this study. Still, the results from the 28-day studies give an indication of a disturbed spermatogenesis, which were confirmed in the 48-week study.

The study by Higashihara and co-workers (2007) showed no alterations on male sexual function and fertility. The reason for this is not clear, but the vehicle used to dissolve BPF was olive oil, compared to saline or water used as vehicle in the other studies. The volume of olive oil solution was 10 ml/kg. Unfortunately, the study was not a comparative study in which BPA was also studied, but the high solution of olive oil is more than twice as high as the one recommended in, for example, OECD TG 417[9] and 443[10]. Using an oily vehicle while the substance is water soluble may decrease the bioavailability of the substance. BPF is moderately soluble in water (0.54 g/L) and relatively hydrophilic (Log Kow 2.91-3.06). In the study by Lee and co-workers (2022b), BPF was dissolved in 4 ml/kg corn oil. In this study, the only reproductive effect observed on male fertility was a statistically significant increase in absolute and relative weight of Cowper's gland. Yamasaki and co-workers (2003) also used olive oil as a solution for BPF but at a much lower concentration (2 ml/kg). In this comparative Hershberger assay, the slight increase in weight in three out of five male sex organs was not sufficient to conclude that BPF is acting through an (anti-)androgenic pathway. Still, the exposure to BPA caused the same effects as BPF and at the same magnitude and/or trend of effect. In the study by Li and co-workers, they used 1 ml/kg corn oil. The results revealed a statistically significant decrease in sperm count of cauda epididymis from a dose level of 1 mg/kg bw up to 100 mg/kg bw but no effect on testicular or epididymal organ weight.

A possible mode of action is described in section 10.10.3.

[9] OECD TG 417 (§28): Volumes of vehicles used for more lipophilic test substances might start at 4 mL/kg body weight. For repeated dosing, when daily fasting would be contraindicated, lower dose volumes (e.g., 2-4 mL/kg body weight) should be considered.

[10] OECD TG 443 (§31): When the test chemical is administered by gavage, the volume of liquid administered at one time should not normally exceed 1 mL/100 g body weight (0.4 mL/100 g body weight; 4 ml/kg) is the maximum for oil, e.g., corn oil).

RODENT STUDIES ON FEMALE FERTILITY (OECD CF LEVEL 4)

In the **Reproduction/developmental Toxicity Screening** Test (OECD TG 421, 2016) by **Lee and co-workers** (**2022b**), Sprague- Dawley rats received BPF via oral gavage at ~8 weeks of age at dose levels of 1, 5, 20 and 100 mg/kg bw/day dissolved in 4 ml/kg corn oil. The treatment period lasted from 2 weeks prior to mating and throughout the day before sacrifice in males (total 62 days) and through lactation (LD 13) in females (total at least 41 days). The control group received only corn oil. Each group (=5) contained 12 animals/sex. 1:1 mating for 2 weeks. Mating was confirmed by vaginal smears. The study was conducted at a GLP facility but not within the scope of GLP regulation. Dose selection rational was based on results from the 28-day RDT by Higashihara et al., 2007 (see below).

Mortality was observed in one female in the 20 mg/kg bw group, that was nonpregnant and sacrificed on day 27. BPF-related salivation was observed in all females at 100 mg/kg bw.

Body weight and weight gain were statistically significantly decreased in females at 100 mg/kg bw during gestation and lactation ($p<0.05$-0.01), respectively during pre-mating on day 7 and on gestational day (GD) 20 ($p<0.01$). During lactation, body weight changes were rebounded.

Food consumption was statistically significantly decreased during the whole treatment period (-28% and -24% at pre-mating day 7 resp. 14; -22%, -20%, -18% at GD 7, 14 resp. 20; and -15% and -10% at LD 4 resp. 13).

The measurement of reproductive performance revealed no BPF treatment-related changes in precoital time, mating index, fertility index, or fecundity (pregnancy) index. The length and regularity of the oestrous cycle was not changed.

The measurement of reproductive organ weights revealed a statistically significant decrease ($p<0.05$) in absolute left ovary weight at 100 mg/kg bw. A non-statistically significant decrease was observed for absolute right ovary weight (0.052, 0.053, 0.053, 0.048 and 0.045 g at 0, 1, 5, 20 respectively 100 mg/kg bw). No changes were observed in uterus/cervix organ weights. The histo(patho)logy revealed a dose-response related increased incidence of vaginal mucification (minimal to slight severity) in all treated groups; 1, 2, 2, 3 and 8 females at 0, 1, 5, 20 respectively 100 mg/kg bw. Also, a statistically significant decrease ($p<0.01$) in implantation sites at 100 mg/kg bw was observed and was confirmed by a reduced number of pups born ($p<0.01$).

The clinical biochemical analysis revealed a statistically significant increase ($p<0.05$) in GGT and TBIL at 100 mg/kg bw. No other alternations in GLU, TP, ALB, A/G, GLO, AST, ALT, TBIL, GGT, ALP, TCHOL, TG, PL was observed, nor were the any changes in T4 levels.

In the **28-day comparative intraperitoneal repeated dose toxicity study (Ijaz et al., 2020**), Sprague-Dawley rats received BPF intraperitoneally at post-weaning (mean body weight 90g) at dose levels of 50 µg/kg, 500 µg/kg, 5 mg/kg and 50 mg/kg. The control group received 1 ml/kg saline. Each group contained 10 females. No information was provided on the CAS number. The study employed OECD 407.

No differences in body weight gain were observed among the treated groups at the end of treatment.

The measurement of reproductive organ weights revealed a dose-response related decrease in paired ovarian weight ($p<0.05$ at 50 mg/kg), in gonadosomatic index, GSI ($p<0.05$ at 50 mg/kg) and in relative uteri weight ($p<0.05$ at 500 µg/kg and $p<0.01$ at 5 and 50 mg/kg). A decrease was also observed for absolute uteri weight ($p<0.001$ at 50 mg/kg).

The histo(patho)logy revealed a dose-response related decrease in number of corpus luteum ($p<0.001$ at 5 and 50 mg/kg), a dose-response related increase in number of atretic follicles ($p<0.001$ at 5 and 50 mg/kg), and a decrease in number of antral follicles ($p<0.001$ at 50 mg/kg). No differences in preovulatory follicles. The morphology revealed an increase in diameter of corpus luteum ($p<0.001$ at 50 mg/kg) and antral follicle ($p<0.001$ at all dose levels). An increase in height was observed for granulosa cells ($p<0.05$ at 500 µg/kg and 5 mg/kg and $p<0.001$ at 50 mg/kg) and for theca cells (19% at 50 mg/kg, non-statistically significant but dose-response related).

The hormone analysis of blood plasma revealed an increase testosterone ($p<0.05$ at 5 mg/kg and $p<0.001$ at 50 mg/kg). A dose-response related decrease was observed for LH ($p<0.01$ at 5 mg/kg and $p<0.001$ at 50 mg/kg), FSH ($p<0.05$ at 5 mg/kg and $p<0.01$ at 50 mg/kg) and oestradiol ($p<0.05$ at 50 mg/kg). Likewise,

progesterone was also decreased ($p<0.001$ at 5 mg/kg and $p<0.01$ at 50 mg/kg).

The biochemical analysis of ovarian tissue homogenates revealed a decrease in levels of catalase, CAT ($p<0.05$ at 5 mg/kg and $p<0.01$ at 50 mg/kg, dose-response related) and superoxide dismutase, SOD ($p<0.05$ at 5 mg/kg). Levels of reactive oxygen species, ROS ($p<0.001$ at 500 µg/kg and 5 mg/kg) and thiobarbituric acid reactive substance, TBARS ($p<0.05$ at 50 mg/kg) were increased but a dose-response related increase was only observed in levels of peroxidase, POD (non-statistically significant).

In the **28-day oral repeated dose toxicity study (Higashihara et al., 2007)**, Sprague-Dawley rats received BPF as oral gavage at 8 weeks of age at dose levels of 20, 100 and 500 mg/kg bw dissolved in 10ml/kg of olive oil. The control group received olive oil only. Each group contained 10 animals/sex. The dose selection rational was based on a preliminary study (unpublished), in which decreased body weights, haematological and biochemical abnormalities and organ weight changes were observed in Sprague-Dawley rats after receiving BPF as oral gavage for 14 days at dose levels of 500 and 1000 mg/kg bw. Females were necropsied after having been dosed for at least 29 days and sacrificed on days 30–34 to allow them to be sacrificed in the dioestrus stage.

The clinical observations revealed a stained nose, mouth, anus, and lower abdomen at 100 and 500 mg/kg. Also, white turbid and reddish urine, and decreased spontaneous locomotion was observed at 500 mg/kg.

Mean body weight of the low dose group (20 mg/kg bw) was lower than the control group from day 17 and continued to decrease throughout the treatment period ($p<0.01$-0.05). Similar trend was observed for the mid and high dose group (100 and 500 mg/kg bw) but starting from day 8. At the end of the treatment period, the low, mid and high dose group had -11%, -15% resp. -13% lower mean body weights than the control group.

The measurements of reproductive organ weights were reported in the study as relative to body weight. There were no differences in relative uterus and ovary weights among the treated groups, not even when calculating the absolute uterus and ovary weights using the mean body weight as reported on day 28 (calculated by dossier submitter). No histo(patho)logy was reported in the study. The histology of vaginal smears revealed no abnormal oestrous cycles (assessed daily from day 22 until the day of sacrifice).

The hormone analysis revealed a decrease in serum T3 levels ($p<0.05$ at 500 mg/kg bw, -15% vs control) and an increase in T4 levels ($p<0.05$ at 20, 100 and 500 mg/kg bw) and in TSH levels (54% at 500 mk/kg bw vs control, non-statistically significant but dose-response related). No other hormones such as testosterone, oestradiol, LH or FSH were analysed.

In the **15-day neonatal sub-chronic study (Nevoral et al., 2021)**, nursing dams of ICR mice received BPF via drinking water at 6-7 weeks of age at dose levels of 0.2 and 20 ng/g bw/day (0.375 ng/mL and 37.5 ng/mL). The control group received 0.1% ethanol in sterile tap water. Each group contained 4-5 dams. The F1 generation offspring contained 6-8 litters. Female pups were bred to 60 days and oocytes were collected. No information was provided on the IUPAC nomenclature, CAS number or purity.

No effect on weight gain of dams during the nursing period. The histo(patho)logy of primary, preantral or antral follicles in adult ovaries on PND 60 revealed no effects.

RODENT STUDIES ON FEMALE FERTILITY (OECD CF LEVEL 3)

In the **comparative uterotrophic assay** (**Stroheker et al., 2003**), Wistar rats were divided into 2 groups: immature and ovariectomized females. The females were also included in a vaginal cornification assay. BPF was given daily via oral administration (gavage) for 4 days at 22 days of age.
Immature females received BPF at dose levels of 25, 50, 100 and 200 mg/kg bw dissolved in PEG (10 females/dose group. In another experiment, females received one of the following; the vehicle, 45 µg/kg bw/day of 17β-E2, 100 mg/kg bw/day of BPF, or 45 µg/kg bw/day of 17β-E2 co-administered with 100 mg/kg bw/day of BPF (8 females/dose group).
Ovariectomized females received one of the following; the vehicle, 100 µg/kg bw/day of 17β-E2, 100 mg/kg bw/day of BPF dissolved in PEG, or 100 µg/kg bw/day of 17β-E2 co-administered with 100 mg/kg bw/day of BPF (8 females/dose group). Ovariectomy occurred at 6 weeks of age.
The co-administration was used to observe an additive or antagonist estrogenic effect of BPF.

Mortality and body weight were not reported.

The uterotrophic assay of immature females revealed a dose-response related increase in both relative wet ($p<0.05$ at 100 and 200 mg/kg) and dry ($p<0.05$ at 200 mg/kg) uterine weight. Co-administration with 17β-E2 did not modify the increase. The vaginal cornification assay of immature females revealed an increase in cornification at 100 mg/kg ($p<0.05$). Co-administration with 17β-E2 increased the cornification reaching 100% (no percentage reported on the increase prior to co-administration in the study).

The uterotrophic assay of ovariectomized rats revealed no alterations in uterine weight. Co-administration with 17β-E2 did not modify the effect. The vaginal cornification assay of ovariectomized rats revealed a slight but non-statistically significant increase (due to high variability in data) in cornification.

In the **uterotrophic assay (Yamasaki et al., 2004)**, Sprague- Dawley female rats received BPF via subcutaneously injected at 20 days of age for three days at dose levels of 100, 300, 1000 mg/kg dissolved in 4 ml/kg of olive oil. The control group received olive oil only. Positive control group received ethynyl estradiol of 0.6 µg/kg. Negative control group received the estrogen-antagonist tamoxifen of 1 mg/kg per day plus ethynyl estradiol. Some of the treated females also received co-administration with ethynyl estradiol in olive oil injected subcutaneously into the back at a dose of 0.6 µg/kg. Each group contained 6 immature females.

Mortality was not reported. Body weight reduced with increasing dose levels (-4% at 1000 mg/kg, non-statistically significant).

The measurement of reproductive organ weights revealed a dose-response related increase in absolute and relative wet uterine weight (336% resp. 353% at 1000 mg/kg, $p<0.01$), as well as in absolute and relative blotted uterine weight (280% resp. 294% at 1000 mg/kg, $p<0.01$). Similar results were observed for the positive control group. A slight decrease in uterine weight was observed after co-administration with ethynyl estradiol, but far from the results of the negative control group.

In the **comparative uterotrophic assay (Yamasaki et al., 2002)**, Sprague- Dawley female rats received BPF via subcutaneously injected at 20-22 days of age for three days at dose levels of 2, 20, 200 mg/kg bw dissolved in 4 ml/kg of olive oil. The control group received olive oil only. Each group contained 6 immature females. No information was provided on the IUPAC nomenclature and the CAS number seems to be incorrectly stated.

Mortality and body weight were not reported.

The measurement of reproductive organ weights revealed an increase in absolute blotted uterine weight (59% at 200 mg/kg, $p<0.01$).

NON-RODENT STUDIES ON FEMALE FERTILITY (OECD CF LEVEL 3)

In the **21-day short-term fecundity assay** (**Mu et al., 2022**), wild-type zebrafish received BPF via water tank starting from embryonic stage of F0 generation at dose levels of 0.5, 5 and 50 µg/L. Both F0 and F1 generations were examined.

From the reproductive performance of internal mating, there was a dose-response related reduction in total number of spawning and fertilised embryos (spawning number: -72.2%, -54.5% and -33.8% at 0.5, 5 and 50 µg/L, $p<0.001$). At 5 and 50 µg/L, the mean embryo number of each clutch was significantly reduced ($p<0.05$), and at 50 µg/L, a significant decrease in fertilised rate ($p=0.019$) and spawning times ($p=0.022$) was observed (exact numbers not reported).

From the cross-mating of treated females and untreated males, a reduced number of spawning and fertilised embryos at 5 and 50 µg/L (both $p<0.01$, exact numbers not reported). No change in spawning times or fertility rate.

There was no difference in gonadosomatic index (GSI).

The histo(patho)logy at 150 days of age revealed a loose ovary structure, interstitial oedema, oocyte necrosis, separation of the follicular membrane from the yolk, a dose-dependent increase in the degree of lesions and a significant dissolution of ovum. Significant effects in the number of ovarian follicles in the different

developing stages was observed but without any clear dose-response relationship.

In the hormone analysis of gonads, a decrease in levels of estradiol (17β-E2), 11-keto testosterone and VTG was observed ($p<0.05$ at 50 µg/L).

In the **21-day short-term fecundity assay in zebrafish** (**Yang et al., 2017**), Danio rerio zebrafish received BPF via water tank at 4,5 months of age at dose levels of 1, 10, 100 and 1000 µg/L. Both F0 and F1 generations were examined.

No mortalities and no changes in body weight or length. The gonadosomatic index (GSI) was decreased in all treated groups (-21% at 1 mg/L, $p<0.05$).

A decrease in the fecundity of parental fish was explained by a decrease in egg production and hatching rate of embryos in all treated groups ($p<0.05$ at 1 mg/L, exact numbers not reported).

The histo(patho)logy of ovaries revealed a higher proportion of pre-vitellogenic stage I oocytes, and lower proportions of pre-vitellogenic stage II, vitellogenic III, and post-vitellogenic IV oocytes at 1 mg/L.

The hormone analysis of gonads (ovaries) revealed a decrease in testosterone levels and an increase estradiol levels at 1 mg/L (both $p<0.05$). Exact numbers not reported.

In the **comparative in ovo study** (**Mentor et al., 2020**), 47 chicken eggs (gallus gallus domesticus) were injected with a bolus dose of 42 µg BPF/g egg into the air sac of the fertilized eggs on embryonic day 4. Chicken embryos were dissected on day 19 i.e., 2 days before hatching.

The number of dead embryos was 25 of 47 (i.e., 53%).

The measurement of reproductive organ weights revealed no differences in ovaries or Mullerian duct between the dose groups.

EX VIVO STUDIES ON FEMALE FERTILITY (OECD CF LEVEL 2)

In a neonatal sub-chronic study **by Nevoral and co-workers (2021)**, ICR female mice (6-7 weeks old at start of the study) were used for producing F1 generation offspring (total no = 6-8 litters). Exposure to BPF was during nursing of the dams (4-5 dams/group) via drinking water from PND 0 (day of delivery) to PND 15. The dose levels were 0.2 and 20 ng/g bw/day (0.375 ng/mL and 37.5 ng/mL). The control group received 0.1% ethanol in sterile tap water.

Immature oocytes (GV stage) were isolated from 8-10-weeks old F1 females (~ PND 56 – 70) and used as such or further cultured to matured MII oocytes.

The histo(patho)logy revealed no dose-response relationship between levels of BPF and the number of GV oocytes obtained per female, nor in maturation (assessed by germinal vesicle breakdown and maturation rate).

The immunocytochemistry, on the other hand, revealed an increase in occurrence of spindle abnormalities in matured oocytes at 20 ng/g ($p<0.01$, exact number not reported), accompanied by an increase in chromosome misalignment (non-stat sign). A decrease in demethylation of histone H3 on lysine K27 (H3K27me2, a chromatin repressive marker) at 0.2 and 20 ng/g (both $p<0.05$, exact number not reported) was also observed.

SUMMARY OF BPF ON FEMALE REPRODUCTION

In two (one reproduction/developmental toxicity screening study and one 28-day repeated dose toxicity study) of the three studies on rats, exposure to BPF showed adverse effects on female sexual function and fertility by statistically significantly altering the reproductive organ weights (i.e., decreased ovaries, uteri and gonadosomatic index) and folliculogenesis (i.e., decreased number of corpus luteum and antral follicles, and increased number of atretic follicles, as well as morphological alterations of the follicles i.e., increased diameter of corpus luteum and antral follicles, and increased height of granulosa cells theca cells). Histo(patho)logical analysis also revealed a dose-response related increased incidence in vaginal mucification and a statistically significant decrease of implantation sites, which was confirmed by a statistically significant reduced number of pups born.

In the abovementioned 28-day study, the adverse effects observed are supported by findings of statistically significant alterations in plasma hormone levels (i.e., increased levels of testosterone, and decreased levels of LH, FSH, oestradiol and progesterone) and cytotoxicity in the ovarian tissues (i.e., decreased levels of catalase and superoxide dismutase, and increased levels of reactive oxygen species and thiobarbituric acid reactive substance).

Furthermore, the three uterotrophic assays revealed a disturbance of the hypothalamic-pituitary-ovarian axis (i.e., dose-response related and statistically significant increase in absolute and relative wet, dry and blotted uterine weight, as well as a statistically significant increase in vaginal cornification).

Findings from the two non-rodent studies on zebrafish revealed a statistically significant reduction in reproduction (i.e., reduced fertility rate, spawning times, total number of spawnings and fertilised embryos, embryo number of each clutch, egg production, and hatching rate of embryos). Cross-mating of treated females with untreated males revealed a statistically significant reduced total number of spawning and fertilised embryos.

The two non-rodent studies, using different exposure windows and dose levels of BPF, also revealed histo(patho)logical findings of the female gonads (i.e., loose ovary structure, interstitial oedema, oocyte necrosis, separation of the follicular membrane from the yolk, a dose-dependent increase in the degree of lesions and a significant dissolution of ovum, as well as a shift in the proportion of oocytes in the different developing stages with less viable oocytes leading to a statistically significant decrease in gonadosomatic index). Additional findings included a statistically significant decrease in levels of testosterone in the gonads, and an increase in levels of estradiol at higher BPF concentrations (≥ 100 µg/L) compared to a decrease in estradiol levels at lower BPF concentrations (≤ 50 µg/L).

As discussed previously in the summary of BPF on male reproduction, the reason for why the 28-day repeated dose toxicity study by Higashihara and co-workers (2007) did not find an adverse effect related to BPF exposure is not clear. One major difference, compared to the other studies, is that the authors used 10 ml/kg olive oil as the vehicle to dissolve BPF. In comparison, in the reproduction/developmental toxicity screening study (Lee et al., 2022b) and in two of the uterotrophic assays (Yamasaki et al., 2002 and 2004), BPF was dissolved in only 4 ml/kg of an oily vehicle.

Another issue to discuss is the 28-day repeated dose toxicity study by Ijaz and co-workers (2020), who used an intraperitoneal route of administration. In this scenario, BPF is directly transported to the systemic circulation instead of being transported via first-pass metabolism. However, the findings (at least on the reproductive organ weights) are supported by those observed in the reproduction/developmental toxicity screening study.

In a fourth study on mice, dams were exposed during lactation to very low concentrations of BPF in drinking water (the highest dose group received 20 ng/g bw/day corresponding to 0.02 mg/kg bw/day). BPF showed no effects on the number of oocytes in the dams (Nevoral et al., 2021). However, in their *in vitro* assay, immature oocytes (GV stage) were isolated from the 8-10-weeks old F1 females (~ PND 56 – 70) and used as such or further cultured to mature oocytes. The immunocytochemistry revealed an increase in occurrence of spindle abnormalities in the matured oocytes accompanied by an increase in chromosome misalignment.

In a non-rodent *in ovo* study on chicken eggs, which examines the effect of substances on the development of reproductive organs that are oestrogen-regulated without the influence of maternal toxicity, BPF had no effect on the ovaries or on the Müllerian ducts. The only effect observed was on male chickens (i.e.,

increased incidence of ovotestis). According to the authors, the reason for this may be due to the low concentration of BPF injected into the eggs (42 µg/g egg) compared to previous studies on the analogue BPA (200 µg/g egg), showing an induction of Müllerian duct abnormalities in embryos of quail. This suggests that in the chicken embryo testis feminization may be a more sensitive endpoint.

A possible mode of action is described in section 10.10.3.

10.10.3 Hypothesis for read-across

According to the CLP regulation (Annex I, paragraph 1.1.1.3), a weight of evidence bearing on the determination of hazard may consider information from the application of the category approach (grouping, read-across), among others. In the current CLH-proposal, the substance 4,4'-isopropylidenediphenol (EC/CAS = 201-245-8 / 80-05-7; herein referred to as BPA) has been used in a read-across approach to strengthen the weight of evidence of a harmonised classification of BPF as toxic for reproduction. BPA has a harmonised classification for the following hazard classes: Repr. 1B (H360F), STOT SE 3, Eye Dam. 1, Skin Sens. 1, Aquatic Acute 1 and Aquatic Chronic 1. The substance has also been identified as a SVHC based on its properties as toxic for reproduction (Article 57c) and its endocrine disrupting properties for both human health and the environment (Article 57f).

The hypothesis for the read-across is based on structural similarities, physicochemical properties, and biological and toxicological properties between the target chemical BPF and the source chemical BPA. Below follows a number of tables with data from comparative studies of the target substance BPF and the source substance BPA that support the justification for read-across for reproductive toxicity (Table 15-21). Also, a comparison is made between the main findings reported in the RAC opinion for BPA related to male and female fertility (2014) and the findings reported for BPF in the current CLH-proposal.

Structure and physiochemical properties

BPF and BPA are members of the same chemical group, i.e., bisphenols. They share a common chemical structure of two phenols linked at the 4,4' carbons. For BPF, the two phenols are linked by a methylene group whereas for BPA the bridging carbon has two methyl substituents. The molecular weight of BPF (200.24 g/mol) is very close to that of BPA (228.29 g/mol), with a Tanimoto coefficient of 0.85. Both substances are moderately soluble in water (0.54 g/L respectively 0.3 g/L), and relatively hydrophilic (Log Kow 2.91 respectively 3.4).

Other bisphenols and BPF derivates have been identified by ECHA. Information on these substances is not considered to provide additional evidence to the CLH-proposal, except for the arguments presented for BPA, and therefore these substances will not be taken into consideration.

Toxicokinetic profile

In a comparative experimental sheep study following subcutaneous administration of BPF and BPA, both substances were able to circulate in maternal plasma and cross the placental barrier reaching the plasma and urine of the foetus as well as the amniotic sac. While the maximum concentrations and half-lives of BPF and BPA are similar in maternal plasma, the rate of maternal clearance, amount of maternal and foetal urinary excretion, half-life in foetuses and amount transported across the placenta are somewhat different between the two bisphenols.

In vitro and *ex vivo* studies reveal a similar pattern in metabolism between BPF and BPA. Both substances are metabolised to a glucuronide conjugate and a sulphate conjugate. The two substances also form oxidative metabolites, among others, suggesting that they have several metabolic pathways in common.

Cellular signalling pathways

Several comparative *in vitro* assays, covering much of the cellular signalling pathways (i.e., receptor binding, transcriptional activation and cell proliferation), demonstrate an oestrogen agonistic activity and less antagonistic activity at the same magnitude for both BPF and BPA (Table 17). BPF seemed to be unable to activate PXR, whereas BPA was a weak agonistic activator. Neither BPF nor BPA showed PXR antagonistic activity.

Reproductive toxicity

In 2014, **RAC adopted their opinion** on a harmonised classification and labelling for BPA as Repr. 1B (H360F) based on adverse effects on male and female sexual function and fertility. Below is a comparison between the main findings reported in the RAC opinion related to male and female fertility following BPA exposure (Table 20) and the findings reported for BPF in the current CLH-proposal. Adding to the justification for read across on reproductive toxicity, is also data from the comparative studies of BPF and BPA.

Male fertility

Several non-guideline rodent studies show an association between oral, subcutaneous and intraperitoneal **BPA** exposure and adverse effects on male fertility. The effects observed were decreased testes, prostate, epididymis and seminal vesicle weight, whereas ventral prostate weight was increased. Histo(patho)logical analysis revealed a decrease in daily sperm production, sperm count and sperm motility. There were also abnormalities in seminiferous tubules including lack of a spermatogenic cycle, decreased number of Leydig cells, presence of multinucleated giant cells in the lumen and degeneration of epithelium. The hormonal analysis showed a decrease in levels of testosterone and luteinizing hormone (LH) in serum, and of testosterone in testis (Leydig cells). Regarding sexual maturation there was a delay in testicular descent.

Many of the abovementioned adverse effects are similar to those reported in the studies of the current CLH-proposal for BPF. Exposure to **BPF** in rodents caused a decrease in reproductive performance (i.e., decreased fertility success, fertility index) and in sexual performance (i.e., decreased motivation to mate, mount frequency, intromission frequency, ejaculation frequency, post ejaculation interval). Histo(patho)logical analysis of epididymis revealed negative changes in sperm parameters (i.e., decreased motility, viability and count, and altered morphology) as well as morphological alterations of seminiferous tubule (i.e., decreased area, lumen and diameter). These findings were supported by alterations in steroidogenesis in these animals (i.e., decreased levels of cholesterol, testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and increased levels of oestrogen and prolactine). Further, BPF also caused cytotoxicity in the intra-testicular tissues (i.e., decreased levels of catalase, superoxide dismutase and peroxidase, and increased levels of lipid peroxidation and reactive oxygen species).

Female fertility

Several guideline and non-guideline rodent studies also show effects on female fertility following oral or subcutaneous **BPA** exposure. The effects observed were decreased absolute and relative ovary weight as well as benign cysts and severe lesions in the ovaries. Histo(patho)logical analysis also revealed alterations of the oocyte development (i.e., meiotic abnormalities leading to aneuploidy) and of the folliculogenesis (i.e., increased depletion of corpus luteum and antral follicles). In the uterus, endometrial hyperplasia or atypical hyperplasia and even more malignant invasions (squamous metaplasia or polyps) were present. Irregular oestrous cycles were also observed, mostly characterized by prolonged oestrus. The hormonal analysis revealed a decrease in luteinizing hormone (LH) levels in plasma.

These findings coincide with some of those reported in the rodent studies following **BPF** exposure and presented in the current CLH-proposal. The effects include alterations of reproductive organ weights (i.e., decreased ovaries, uteri and gonadosomatic index) and folliculogenesis (i.e., decreased number of corpus luteum and antral follicles and increased number of atretic follicles) as well as morphological alterations of the follicles (i.e., increased diameter of corpus luteum and antral follicles and increased height of granulosa cells theca cells). Histo(patho)logical analysis also revealed a dose-response related increased incidence in vaginal mucification. The adverse effects are supported by alterations in plasma hormone levels (i.e., increased levels of testosterone and decreased levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestradiol and progesterone) and cytotoxicity in the ovarian tissues (i.e., decreased levels of catalase and superoxide dismutase and increased levels of reactive oxygen species and thiobarbituric acid reactive substance).

Reproductive capacity

Exposure to **BPA** was also reported to be associated with fewer pregnancies and implantation sites, reduced fertility (less females giving birth), litters/pair, litter size and live pups per litter at birth, and increased number of resorptions. Rodent studies on **BPF** also showed a statistically significant decrease in implantation sites, which was confirmed by a reduced number of pups born, reduced litter size, litter weight and survival at weaning. This finding was supported by a study on zebrafish, in which decreased egg production, hatching rate of embryos, and survival rate of larvae were observed.

In **comparative experimental animal studies** (Table 18), exposure to BPF and BPA show similar effects and magnitude of effect on male and female sexual function and fertility in the highest dose group as compared to the control group. Changes in reproductive organ weight, from exposure to BPF and BPA, range from no changes (in comparison to the control group) to a dose-response related decrease in epididymis, prostate, paired testis and seminal vesicles in males, and in paired ovaries and uterus in females; some of which were statistically significant in the highest dose group. In the hershberger assay, neither BPF nor BPA show androgenic or anti-androgenic activities that in turn mediate biological functions such as the spermatogenesis. The results show that the weight of two androgen-dependent tissues (i.e., relative ventral prostate and seminal vesicle) were increased but not statistically significantly and co-administration with testosterone propionate, to detect antiandrogens, had no modifying effect. In the uterotrophic assays, on the other hand, both BPF and BPA show a statistically significant increase in absolute blotted uterine weight, but only BPF show a statistically significant dose-response related increase in relative dry and wet uterine weight. Also, both BPF and BPA resulted in a statistically significant increase in vaginal cornification, which was further increased by co-administration with 17β-E2; suggesting an oestrogen agonistic activity that in turn mediate biological functions such as the spermatogenesis.

The histo(patho)logical findings in the comparative studies on males reveal a statistically significant decrease in daily sperm production, in number of spermatogonia, spermatocytes and spermatids in seminiferous tubules, in number of sperms in epididymis and in motility after exposure to BPF and BPA. In females, both substances statistically significantly decreased the number of corpus luteum and antral follicles, whereas the number of atretic follicles were statistically significantly increased. Morphological changes of the seminiferous tubules and the follicles were also observed in males respectively females following exposure to BPF and BPA.

Similar pattern between BPA and BPF was also observed in alterations of hormone levels in plasma and gonads (i.e., decreased levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) and increased oestrogen levels) and in alterations of oxidative stress parameters in the gonads. These findings are supported by the results of *ex vivo* assays, which also revealed DNA damage of spermatozoa following exposure to BPF and BPA (Table 19). A decrease in testosterone and an increase in oestrogen may lead to decreased sperm quality and/or quantity in adult males resulting in decreased fertility (OECD AOP 67). Also, the presence of oxidative stress in the gonads can have a direct negative effect on fertility.

Mode of action

RAC concluded in their opinion that **BPA** may exert its toxic effects on the female gonads either directly by causing hyperplasia or hypertrophy, or indirectly via effects on the HPO-axis by causing negative feedback at the hypothalamic-pituitary level through reduced gonadotropin release and reduced luteinizing hormone (LH) secretion.

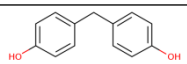
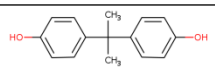
In the current CLH-proposal, **BPF** demonstrate an oestrogen agonistic activity that supports the results from the uterotrophic studies, suggesting an endocrine mechanism of action. The decrease in levels of cholesterol, testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) and an increase in levels of oestrogen in plasma and/or the gonads indicate that there is an effect on the HPG-axis. The induction of the formation of reactive oxygen species (ROS) and lipid peroxidation (LPO) and the decrease in antioxidants (i.e., catalase and sodium dismutase, CAT and SOD) does suggest that BPF can have a direct cytotoxic effect on the gonads.

Human data

The two epidemiological cross-sectional studies reported in Table 21 show comparable results between BPF and BPA, despite the two studies showing conflicting results between themselves; one study found an association between changes in sperm parameters (e.g., decreased progressive motility, sperm concentration and sperm count and increased abnormal heads) and urine concentration levels of BPF and BPA, whereas the other study could not find an association - neither for BPF nor for BPA. In a prospective case-control study of men with either normozoospermia or azoospermia, no statistical analysis could be performed due to a high number of analytical values of BPF below the limit of detection.

In *in vitro* studies of human spermatozoa, both BPF and BPA show a decrease in sperm motility, viability, and mitochondrial membrane potential as well as a decrease in testosterone levels in human testis, although the effects are more pronounced for BPA.

Table 15. Summary of structure and physiochemical properties of BPF and BPA.

	BPF		**BPA**	
	Data	Reference[11]	Data	Reference[11]
EC name	4,4'-methylenediphenol		4,4'-isopropylidenediphenol	
EC / CAS	210-658-2 / 620-92-8		201-245-8 / 80-05-7	
REACH registered	Not registered		≥ 1 000 000 tpa	
Structure				
Molecular formula	$C_{13}H_{12}O_2$		$C_{15}H_{16}O_2$	
Molecular weight	200.24 g/mol		228.29 g/mol	
Tanimoto coefficient (vs BPA)	0.85	NTP RR 4, Oct 2017		
Physical state (20°C and 101,3 kPa)	No data found		Solid	ECHA website
Water solubility (g/L)	0.543 (predicted) 0.612 (predicted)	WSKOW v1.42 WATERNT v1.01	0.173 (predicted) 0.146 (predicted) 0.12 (measured) 0.3 (measured)	WSKOW v1.42 WATERNT v1.01 Dorn et al., 1987 OECD 105, REACH Registration Dossier (ECHA)
Dissociation constant (pKa)	9.7 (predicted)	ACDLabs	9.7 (predicted) 11.3	ACDLabs TG not specified, REACH Registration Dossier (ECHA)
Partition coefficient n-octanol/water (Log Kow)	2.91 (measured) 3.06 (predicted)	Hansch et al., 1995 KOWWIN v1.68	3.32 (measured) 3.64 (predicted) 3.4 (measured)	Hansch et al., 1995 KOWWIN v1.68 OECD 107, REACH Registration Dossier (ECHA)
Partition coefficient n-octanol/water (Log D, pH interval 4-9)	2.85 min – 2.93 max (predicted)	ACDLabs	3.38 min – 3.46 max (predicted)	ACDLabs
Melting/freezing point	160 - 163 °C	PubChem	154 - 156.5 °C (measured)	ASTM D4493, REACH Registration Dossier (ECHA)
Vapour pressure (Pa at 25 °C)	4.96 x 10^{-5} (predicted)	MPBPVP v1.43	3.03 x 10-5 (predicted) 4.12 x 10^{-9} (measured)	MPBPVP v1.43 OECD 104, REACH Registration Dossier (ECHA)

[11] Information with references to Dorn et al., 1987, Hansch et al., 1995, WSKOW v1.42, WATERNT v1.01, KOWWIN v1.68, ACDLabs and MPBPVP v1.43 were obtained from the Danish (Q)SAR Database, Division of Diet, Disease Prevention and Toxicology, National Food Institute, Technical University of Denmark, http://qsar.food.dtu.dk.

Table 16. Summary table of comparative studies relating to toxicokinetics.

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Method: Toxicokinetic study
Species, strain, sex, no/group: 6 Polypay x Dorset cross-bred female sheep (at 2nd or 3rd parity).
Test substance, dose levels, duration of exposure: All females underwent foetal catheterization surgery (placing a catheter in the foetal descending aorta and inferior vena cava) at GD 114.8 ± 0.8 days.
After recovery, 3 females were injected with a single s.c. dose of 1.5 mg/kg bw of a mixture of BPA, BPS, and BPF (0.5 mg/kg bw per chemical) dissolved in corn oil.
Purity: 98% BPF and >99% BPA. 99% internal standard $^{13}C_{12}$-BPF and $^{13}C_{12}$-BPA.
Sampling: Maternal plasma: 0, 1, 3, 5, 9, 24, 36, and 48 h.
Maternal urine: 0, 9 and 24 h.
Foetal plasma: 0, 3, 9, 24, 36, 48, and 72 h.
Foetal urine: 72 h.
Amniotic fluid: 0 and 72 h.
Comparative study (BPA, BPS, BPF).
GLP and OECD TGs not stated.</td><td>Distribution<table><tr><th></th><th>BPF (n=3</th><th>BPA (n=3)</th></tr><tr><td>Maternal</td><td></td><td></td></tr><tr><td>C_{max} plasma (ng/ml)</td><td>48.8</td><td>66.7</td></tr><tr><td>T ½ (h)</td><td>7.7</td><td>5.3</td></tr><tr><td>AUC (mg*h/ml)</td><td>0.40</td><td>0.81</td></tr><tr><td>C urine (ng/ml at t = 9 h)</td><td>3.9</td><td>1 300</td></tr><tr><td>C urine (ng/ml at t = 24 h)</td><td>18.7</td><td>298</td></tr><tr><td>$Cl_{total\ body}$ (ml/h)</td><td>103.8</td><td>45.4</td></tr><tr><td>Foetal</td><td></td><td></td></tr><tr><td>C_{max} plasma (ng/ml)</td><td>20.9</td><td>87.6</td></tr><tr><td>T ½ (h)</td><td>14.2</td><td>52.0</td></tr><tr><td>AUC (mg*h/ml)</td><td>0.21</td><td>3.9</td></tr><tr><td>C urine (ng/ml at t = 72 h)</td><td>13.8</td><td>144</td></tr><tr><td>C amniotic fluid (ng/ml at t = 72 h)</td><td>16.4</td><td>119</td></tr></table></td><td>Gingrich et al., 2019</td></tr>
<tr><td>Method: Review
BPF based on 6 studies.
BPA based on 32 studies.
GLP and OECD TGs not stated.</td><td>Metabolism<table><tr><th>BPF</th><th>BPA</th></tr><tr><td colspan="2">In vitro metabolites</td></tr><tr><td>• HepG2 cell line: BPF sulfate.
• Human hepatocytes: BPF glucuronide and BPF sulfate.
• Human intestinal LS174T cells: BPF glucuronide.
• Human liver microsomes: glutathione conjugates forming BPF quinone.
• Human liver microsomes: 4-(hydroxymethyl)-phenol.</td><td>• Human/rat hepatic or intestinal microsomes: BPA glucuronide.
• Human skin explants: BPA glucuronide and BPA sulfate.
• Hepatocytes from rats, monkeys, humans: BPA glucuronide and BPA sulfate.
• HepG2 and MCF7 cell lines: BPA glucuronide and BPA sulfate.
• Mouse HC11 cell line: BPA glucuronide and BPA sulfate.
• Mice /rat liver microsomes, S9 fraction: BPA glucuronide, ortho-hydroxylated BPA,</td></tr></table></td><td>Skledar and Masic, 2016</td></tr>
</table>

Method	Results		Reference
		isopropyl-hydroxyphenol -4,4'-(1-hydroxypropane-2,2-diyl)diphenol, glutathione conjugates, BPA dimers, MBP. • Peroxidase activation system: BPA ortho-quinone. • Human liver microsomes: hydroxylated BPA, hydroxycumyl alcohol, GSH conjugate of BPA, GSH conjugate of 4-isopropyl phenol, GSH conjugate of hydroxylated BPA.	
	Ex vivo metabolites		
	• Rat (urine): BPF sulfate, oxidative metabolites (hydroxylated BPF occurring on both the ortho and meta positions of the phenol ring), BPF dimer (lower polarity than BPF).	• Rat (urine): BPA glucuronide • Mouse (urine, liver, GI tract): BPA glucuronide • Human: BPA glucuronide • Rat and Mouse (faeces): Hydroxylated BPA • Rat, Mouse (faeces, urine): BPA sulfate • Mouse (urine): Glucuronide of 4,4'-(1-hydroxypropane-2,2-diyl)diphenol • Rat: BPA ortho-quinone	
	In vitro enzymes		
	• UGT1A10 mainly responsible for BPF glucuronidation in humans (higher BPF biotransformation observed in intestine than in liver).	• UGT2A1 and UGT1A10 mainly responsible for BPA glucuronidation in the respiratory system and intestine, whereas UGT1A1 is important for glucuronidation in breast tissue. • SULT1A1 is the main enzyme responsible for BPA sulfation.	

Table 17. Summary table of *in vitro* assays of hormone-related activities relevant for toxicity of sexual development and reproduction, including comparative studies (OECD CF level 2). Some of the results were qualitatively reported and therefore indicated with ~.

Assay	Endpoint	Species, model	Parameter	Effect of BPF	Effect of BPA	References
Oestrogen receptors						
Competitive receptor-binding assay	HELN-hERα and HELN-hERβ cells	Human, Cervical cancer cell line (HeLa)	IC50 (ERα)	2182 ± 87 nM	839 ± 270 nM	Molina-Molina et al., 2013
			RBA (ERα)	0.005 %	0.014 %	
			IC50 (ERβ)	1452 ± 261 nM	401 ± 126 nM	
			RBA (ERβ)	0.014 %	0.052 %	
Competitive receptor-binding assay	NR-LBD of ERα, β and γ	Human, cDNA of NRs	IC50 (ERα)	5250 ± 497 nM	1080 ± 84 nM	Liu et al., 2019
			IC50 (ERβ)	3590 ± 251 nM	1000 ± 90.5 nM	
			IC50 (ERRγ)	150 ± 11 nM	4.98 ± 0.79 nM	
Competitive receptor-binding assay	Uterine cytosol ER	Rat, Uteri from ovariectomised Sprague Dawley rats	IC50 (ER)	95.0 ± 5 µM	11.7 ± 6.4 µM	Blair et al., 2000
			RBA (to E2)	0.0009%	0.008%	
Competitive receptor-binding assay	Two yeast hybrid system with / without rat S9 mix	Yeast, (Strain Y190)	Relative β-galactosidase activity	~0.7 (without S9), ~0.8 (with S9)	~0.4 (without S9), ~0.5 (with S9)	Hashimoto et al., 2001
Competitive receptor-binding assay [Fluorescence polarization method]	hrERα and hERβ	Human recombinant oestrogen receptor and an intrinsically fluorescent non-steroid oestrogen	Inhibition	~90%	~80%	
Transactivation assay [Luciferase activity]	Two hybrid system with the hERα and coactivator TIF2	Yeast, (Strain Y190)	β-galactosidase activity	~700 at 10µM	*Not analysed*	Zheng et al., 2016
Transactivation assay [Luciferase activity]	HELN-hERα and -hERβ cells	Human, Cervical cancer cell line (HeLa)	EC50 (ERα)	1.73 ± 0.46 µM	0.41 ± 0.11 µM	Molina-Molina et al., 2013
			EC50 (ERβ)	1.43 ± 0.19 µM	0.52 ± 0.09 µM	
Transactivation assay	MELN cells	Human, Breast cancer cell lines (MCF-7)	EC50	0.98 ± 0.05 µM	0.47 ± 0.03 µM	
Transactivation assay	HepG2-hERα and -	Human,	EC50 (Erα)	2.39 µM	*Not analysed*	Cabaton et al., 2009

Assay	Endpoint	Species, model	Parameter	Effect of BPF	Effect of BPA	References
[Luciferase activity]	hERβ	Liver cancer cell line (HepG2)	EC50 (ERβ)	6.04 µM	*Not analysed*	
			Inhibition (ERα)	~65% at 10^{-5}M	*Not analysed*	
			Inhibition (ERβ)	~118% at 10^{-5}M	*Not analysed*	
Transactivation assay [Luciferase activity]	CHO-K1 cells	Hamster, Ovary CHO-K1 cells	REC50 (ERα)	0.32× µM	0.33 µM	Kojima et al., 2019
			REC50 (ERβ)	0.24 µM	0.12 µM	
			RIC50 (ERα)	*No effect/inactive*	*No effect/inactive*	
			RIC50 (ERβ)	*No effect/inactive*	*No effect/inactive*	
Bioluminescence estrogen assay	BLYES- hERα	Yeast, Saccharomyces cerevisiae, (Strain BLYES)	EC50	4.01 x 10^3 nM	4.1 x 10^3 nM	Ruan et al., 2015
Cell proliferation [E-screen bioassay]	MCF-7 cells	Human, Breast cancer cell lines (MCF-7)	EC50	1.01 µM	0.47 µM	Molina-Molina et al., 2013
Cell proliferation [E-screen bioassay]	MCF-7 BUS cells	Human, Breast cancer cell lines (MCF-7)	Cell growth	Up to 4-fold increase	Up to 4-fold increase	Hashimoto et al., 2001
Androgen receptors						
Transactivation assay [Green fluorescent protein, yEGFP]	Recombinant yeasts - hAR	Recombinant yeasts with cDNA of hAR (Strain Saccharomyces cerevisiae)	EC50 (AR)	*No effect/inactive*	*No effect/inactive*	Roelofs et al., 2015
			IC50 (AR)	20 mM	39 mM	
Competitive receptor-binding assay	NR-LBD of AR	Human, cDNA of NRs	IC50 (AR)	>10 µM	*No effect/inactive*	Liu et al., 2019
Transactivation assay	PALM cells	Human, Prostate cancer cell lines (PC3)	EC50 (AR)	*No effect/inactive*	55.38 ± 6.46 µM	Molina-Molina et al., 2013
			IC50 (AR)	6.98 ± 0.15 µM	0.92 ± 0.02 µM	
Transactivation assay [Luciferase activity]	CHO-K1 cells	Hamster, Ovary CHO-K1 cells	RIC50	4.5 µM	2.2 µM	Kojima et al., 2019
Transactivation assay [Luciferase activity]	MDA-kb2 cells	Human, breast cancer cell line (MDA-kb2) an AR+ derived from the MDA-MB453 cell line, stably transfected with the MMTV-	Inhibition	~41% at 10^{-5}M	*Not analysed*	Cabaton et al., 2009

Assay	Endpoint	Species, model	Parameter	Effect of BPF	Effect of BPA	References
		neo-luc plasmid.				
Cell proliferation [E-screen bioassay]	MCF-7 AR1 cells	Human, Breast cancer cell lines (MCF-7)	IC50 (AR)	*No effect/inactive*	10 µM	Molina-Molina et al., 2013
Pregnane X receptor						
Competitive receptor-binding assay	NR-LBD of PXRα, β, and γ	Human, cDNA of NRs	IC50 (PXRα, β, γ)	*No effect/inactive*	*No effect/inactive*	Liu et al., 2019
			IC50 (PXR)	>10 µM	5460 ± 403 nM	
Transactivation assay	*HG5LN-PXR*	Human, Cervical cancer cell line (HeLa)	EC50 (PXR)	*No effect/inactive*	17.72 ± 2.43 µM	Molina-Molina et al., 2013
			IC50 (PXR)	*No effect/inactive*	*No effect/inactive*	
Transactivation assay [Luciferase activity]	COS-7 cells	Simian, Kidney COS-7 cells	REC50	*No effect/inactive*	*No effect/inactive*	Kojima et al., 2019

Table 18. Summary table of comparative *in vivo* studies on sexual function and fertility following exposure to BPF and BPA (OECD CF level 3 - 4).

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.</th><th>Reference</th></tr>
<tr><td colspan="3">The following in vivo studies were obtained from publicly available scientific journals and concerns both male and female reproduction.</td></tr>
<tr><td>28-day sub-chronic study.
Rat (Sprague- Dawley), adult, males.
Exposure: oral, daily.
BPF: 5, 25, and 50 mg/kg bw.
BPA: same doses as BPF.</td><td>
<table>
<tr><th>Bisphenol</th><th>BPF</th><th>BPA</th></tr>
<tr><td>Body weight</td><td>No difference to the control.</td><td>No difference to the control.</td></tr>
<tr><td>Testis weight</td><td>No difference to the control.</td><td>No difference to the control.</td></tr>
<tr><td>Histo(patho)logy of sperms (testis)</td><td>↓ secondary spermatocytes,
no elongated spermatids in lumen.</td><td>No difference to the control.</td></tr>
<tr><td>Morphology (testis)</td><td>↓ area of seminiferous tubules,
↑ area of interstitium (trend),
↓ and irregular seminiferous tubules,
↓ epithelial height (HD**; trend).</td><td>↓ area of seminiferous tubules (trend),
↑ area of interstitium (trend),
↓ epithelial height (HD*; trend).</td></tr>
<tr><td>Hormone analysis (plasma)</td><td>32% ↓ T (HD*)</td><td>34% ↓ T (HD*)</td></tr>
<tr><td>Hormone analysis (testis)</td><td>22% ↓ T (HD**)</td><td>23% ↓ T (HD**; trend)</td></tr>
<tr><td>Antioxidant enzyme analysis (testis)</td><td>↓ CAT (HD**; trend)</td><td>↓ CAT (MD**)</td></tr>
<tr><td></td><td>↓ SOD</td><td>↓ SOD</td></tr>
<tr><td></td><td>↓ POD (HD**)</td><td>↓ POD (HD***; trend)</td></tr>
<tr><td></td><td>↑ LPO (HD***; trend)</td><td>↑ LPO (HD*)</td></tr>
<tr><td></td><td>↑ ROS (HD**; trend)</td><td>↑ ROS (HD***)</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship
T = testosterone
CAT = catalase; SOD = superoxide dismutase; POD = peroxidase; LPO = lipid peroxidation; ROS = reactive oxygen species
</td><td>Ullah et al., 2018a
Klimisch:
Not reliable</td></tr>
<tr><td>48-week chronic toxicity study.
Rat (Sprague- Dawley), adult males.
Exposure: oral (drinking water), daily.</td><td>
<table>
<tr><th>Bisphenol</th><th>BPF</th><th>BPA</th></tr>
<tr><td>Body weight</td><td>No difference to the control, except ↑ in final body at HD* (< 2%).</td><td>No difference to the control, except ↑ in final body at HD* (< 2%).</td></tr>
<tr><td>Gonadosomatic index</td><td>7% ↓ at HD*</td><td>7% ↓ at HD*</td></tr>
<tr><td>Reproductive organ weight</td><td>Epididymis</td><td>Epididymis</td></tr>
</table>
</td><td>Ullah et al., 2018b
Klimisch:
Not</td></tr>
</table>

Study, species, strain, exposure route, test substance, dose levels	**Results** The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.			**Reference**
BPF: 5, 25, and 50 µg/L. BPA: same doses as BPF.		Abs: **1%** ↓ at HD Rel: **4%** ↓ at HD** (*trend*)	Abs: **3%** ↓ at HD Rel: **4%** ↓ at HD** (*trend*)	*assignable*
		Prostate Rel: **3%** ↓ at HD (*trend*)	Prostate Rel: **2%** ↓ at HD (*trend*)	
		Paired testis Abs: **5%** ↓ at HD (*trend*)	Paired testis Abs: **5%** ↓ at HD (*trend*)	
		Seminal vesicle Abs: **2%** ↓ at HD** Rel: **7%** ↓ at HD** (*trend*)	Seminal vesicle Abs: **6%** ↓ at HD** (*trend*) Rel: **7%** ↓ at HD** (*trend*)	
	Histo(patho)logy of sperms (testis)	**9%** ↓ in daily sperm production at HD** (*trend*)	**9%** ↓ in daily sperm production at HD** (*trend*)	
		Spermatogonia: **7%** ↓ at HD* (*trend*), Spermatocytes: **7%** ↓ at HD* (*trend*), Spermatids: **5%** ↓ at HD** (*trend*), in seminiferous tubules.	Spermatogonia: **7%** ↓ at HD*, Spermatocytes: **6%** ↓ at HD* (*trend*), Spermatids: **4%** ↓ at HD** (*trend*), in seminiferous tubules.	
		5% ↓ in caput epididymis sperms at HD** (*trend*)	**3%** ↓ in caput epididymis sperms at MD* and HD**	
		3% ↓ in cauda epididymis sperms at HD* (*trend*)	**3%** ↓ in cauda epididymis sperms at HD* (*trend*)	
		6% ↓ in **s**perm motility at HD** (*trend*)	**3%** ↓ in sperm motility at HD*	
		2% ↓ in viable sperms at HD (*trend*)	**2%** ↓ in viable sperms at HD (*trend*)	
	Morphology (testis)	**15%** ↓ in epithelial height at HD** (*trend*)	**14%** ↓ in epithelial height at HD* (*trend*)	
	Hormone analysis (plasma)	**21%** ↓ **T** (HD***; *trend*)	**19%** ↓ **T** (HD**; *trend*)	
		59% ↑ **E** (HD**; *trend*)	**49%** ↑ **E** (HD*; *trend*)	
		17% ↓ **LH** (HD*; *trend*)	**15%** ↓ **LH** (HD*; *trend*)	
		25% ↓ **FSH** (HD*; *trend*)	**25%** ↓ **FSH** (HD*; *trend*)	
	Antioxidant enzyme analysis (testis)	↓ **CAT** (HD**; *trend*)	↓ **CAT** (HD**; *trend*)	
		↓ **SOD** (HD**; *trend*)	↓ **SOD** (HD**; *trend*)	
		↓ **POD** (HD**; *trend*)	↓ **POD** (HD**; *trend*)	
		↑ **LPO** (HD**)	↑ **LPO** (HD**)	
		↑ **ROS** (HD***; *trend*)	↑ **ROS** (HD***; *trend*)	
	Significance: *p<0.05, **p>0.01, ***p>0.001 HD = high dose. Only statistical significance for the high dose group is reported in the table. *trend* = dose-response relationship T = testosterone; E = oestradiol; LH = luteinizing hormone; FSH = follicle stimulating hormone			

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.</th><th>Reference</th></tr>
<tr><td></td><td>CAT = catalase; SOD = superoxide dismutase; POD = peroxidase; LPO = lipid peroxidation; ROS = reactive oxygen species</td><td></td></tr>
<tr><td>Prenatal toxicity study

Rat (Sprague- Dawley), male offspring.

Exposure: oral (drinking water), daily.

BPF: 5, 25, and 50 µg/L.

BPA: same doses as BPF.</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Body weight of dams</td><td>4% ↑ body weight gain between GD 1-20 at HD (trend).</td><td>No difference to the control.</td></tr>
<tr><td>Litter size</td><td>10% ↓ at HD (trend)</td><td>No difference to the control.</td></tr>
<tr><td rowspan="3">Body weights</td><td>Birth weight: 16% ↓ at HD (trend)</td><td>Birth weight: No difference to the control.</td></tr>
<tr><td>PND 16: No difference.</td><td>PND 16: 6% ↑ at HD (trend)</td></tr>
<tr><td>PND 80: 8% ↑ at HD*</td><td>PND 80: 8% ↑ at HD* (trend)</td></tr>
<tr><td rowspan="2">Reproductive organ weight</td><td>PND 16
7% ↑ in absolute prostate at HD (trend).
No difference in absolute right/left testis, epididymis, seminal vesicle, bulbourethral gland or bulbocavernosus muscles.</td><td>PND 16
4% ↓ in absolute right testis weight at HD (trend)
No difference in absolute left testis, prostate, epididymis, seminal vesicle, bulbourethral gland or bulbocavernosus muscles.</td></tr>
<tr><td>PND 80
3% ↓ in absolute seminal vesicle weight at HD*.
No difference in absolute left/right testis, left/right epididymis, or prostate weight.</td><td>PND 80
5% ↓ in absolute seminal vesicle weight at HD* (trend).
No difference in absolute left/right testis, left/right epididymis, or prostate weight.</td></tr>
<tr><td rowspan="6">Histo(patho)logy of sperms (testis) on PND 80</td><td>16% ↓ in daily sperm production at HD* (trend)</td><td>16% ↓ in daily sperm production at HD*</td></tr>
<tr><td>3% ↓ in caput epididymis sperms at HD* (trend)</td><td><3% ↓ in caput epididymis sperms at HD*</td></tr>
<tr><td>2% ↓ in cauda epididymis sperms at HD (trend)</td><td>2% ↓ in cauda epididymis sperms at HD (trend)</td></tr>
<tr><td>Spermatogonia: 7% ↓ at HD* (trend), Spermatocytes: 7% ↓ at HD* (trend),
Spermatids: 5% ↓ at HD** (trend), in seminiferous tubules.</td><td>Spermatogonia: 7% ↓ at HD*, Spermatocytes: 6% ↓ at HD* (trend),
Spermatids: 4% ↓ at HD** (trend), in seminiferous tubules.</td></tr>
<tr><td>6% ↓ in sperm motility at HD** (trend)</td><td>3% ↓ in sperm motility at HD*</td></tr>
<tr><td>2% ↓ in viable sperms at HD (trend)</td><td>2% ↓ in viable sperms at HD (trend)</td></tr>
<tr><td rowspan="2">Morphology (testis)</td><td>5% ↓ area of seminiferous tubules at HD*** (trend)</td><td>4% ↓ area of seminiferous tubules at HD*** (trend)</td></tr>
<tr><td>15% ↓ area of interstitial space at HD* (trend)</td><td>16% ↓ area of interstitial space at HD* (trend)</td></tr>
</table>
</td><td>Ullah et al., 2019a

Klimisch:

Not reliable</td></tr>
</table>

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td></td><td>4% ↓ area of lumen at HD*** (trend)</td><td>4% ↓ area of lumen at HD*** (trend)</td></tr>
<tr><td rowspan="4">Hormone analysis (plasma) on PND 80</td><td>21% ↓ T (HD*; trend)</td><td>30% ↓ T (HD*; trend)</td></tr>
<tr><td>158% ↑ E (HD***; trend)</td><td>183% ↑ E (HD***)</td></tr>
<tr><td>27% ↓ LH (HD***; trend)</td><td>30% ↓ LH (HD***; trend)</td></tr>
<tr><td>60% ↓ FSH (HD**)</td><td>60% ↓ FSH (HD**)</td></tr>
<tr><td rowspan="5">Antioxidant enzyme analysis (testis) on PND 80</td><td>↓ CAT (HD*; trend)</td><td>↓ CAT (HD**; trend)</td></tr>
<tr><td>↓ SOD (HD**; trend)</td><td>↓ SOD (HD**)</td></tr>
<tr><td>↓ POD (HD*; trend)</td><td>↓ POD (HD**; trend)</td></tr>
<tr><td>↑ LPO (HD**)</td><td>↑ LPO (HD**)</td></tr>
<tr><td>↑ ROS (HD**; trend)</td><td>↑ ROS (HD**; trend)</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship
T = testosterone; E = oestradiol; LH = luteinizing hormone; FSH = follicle stimulating hormone
CAT = catalase; SOD = superoxide dismutase; POD = peroxidase; LPO = lipid peroxidation; ROS = reactive oxygen species
</td><td></td></tr>
<tr><td>28-day sub-chronic study.
Enhanced OECD TG 407.

Spermatozoa from 91 sacrificed rats (Sprague- Dawley), age: 70-80 days.

Exposure: oral (gavage), daily.

Bisphenol F: 4,4’-methylenediphenol.

Purity: 99%.

Dose levels: 5, 25, and 50 mg/kg bw/day.

3 replicates/sample.

BPA: same doses as BPF.</td><td>
<table>
<tr><td colspan="2">Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td colspan="2">Motility (spermatocytes)</td><td>↓ (trend)</td><td>↓ (no trend)</td></tr>
<tr><td colspan="2">Daily sperm production (19th stage spermatids)</td><td>↓ (HD*)</td><td>↓ (HD*)</td></tr>
<tr><td rowspan="3">DNA damage (comet assay)</td><td>No of comets/100 cells</td><td>27% ↑ (HD*)</td><td>30% ↑ (HD*)</td></tr>
<tr><td>Tail moment (µm)</td><td>30% ↑ (HD*)</td><td>30% ↑ (HD*)</td></tr>
<tr><td>Tail DNA (%)</td><td>33% ↑ (HD*)</td><td>39% ↑ (HD*)</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship.
Exact numbers not reported for Daily sperm production in the scientific article.
</td><td>Ullah et al., 2019c

Klimisch:

Reliable with restriction</td></tr>
<tr><td>28-day sub-chronic study.

Rat (Sprague- Dawley), adult</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Body weight</td><td>No difference to the control.</td><td>No difference to the control.</td></tr>
</table>
</td><td>Ijaz et al., 2020</td></tr>
</table>

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.</th><th>Reference</th></tr>
<tr><td>females.
Exposure: intraperitoneal, daily.
BPF: 50 and 500 µg/kg + 5 and 50 mg/kg.
BPA: same doses as BPF.</td><td>
<table>
<tr><td>GSI</td><td>42% ↓ at HD* (trend)</td><td>40% ↓ at HD* (trend)</td></tr>
<tr><td rowspan="2">Organ weight</td><td>Paired ovaries
Abs: 64% ↓ at HD* (trend)</td><td>Paired ovaries
Abs: 33% ↓ at HD* (trend)</td></tr>
<tr><td>Uterus
Abs: 11% ↓ at HD***
Rel: 22% ↓ at HD** (trend)</td><td>Uterus
Abs: 15% ↓ at HD*** (trend)
Rel: 21% ↓ at HD**</td></tr>
<tr><td rowspan="4">Histo(patho)logy (ovaries)</td><td>54% ↓ in corpus luteum at HD*** (trend)</td><td>49% ↓ in corpus luteum at HD*** (trend)</td></tr>
<tr><td>182% ↑ in atretic follicles at HD***</td><td>209% ↑ in atretic follicles at HD***</td></tr>
<tr><td>22% ↓ in antral follicles at HD***</td><td>46% ↓ in antral follicles at HD***</td></tr>
<tr><td>No difference to the control in preovulatory follicles.</td><td>No difference to the control in preovulatory follicles.</td></tr>
<tr><td>Morphology (ovaries)</td><td>↑ diameter of corpus luteum (HD***),
↑ diameter of antral follicle (HD***),
↑ height of granulosa (HD**)</td><td>↑ diameter of corpus luteum (HD***),
↑ diameter of antral follicle (HD**),
↑ height of granulosa</td></tr>
<tr><td rowspan="5">Hormone analysis (plasma)</td><td>173% ↑ T (HD***)</td><td>365% ↑ T (HD***; trend)</td></tr>
<tr><td>18% ↓ E (HD*; trend)</td><td>41% ↓ E (HD*)</td></tr>
<tr><td>16% ↓ LH (HD***; trend)</td><td>16% ↓ LH (HD***; trend)</td></tr>
<tr><td>17% ↓ FSH (HD**)</td><td>10% ↓ FSH (HD*; trend)</td></tr>
<tr><td>42% ↓ progesterone (HD**)</td><td>45% ↓ progesterone (HD***)</td></tr>
<tr><td rowspan="4">Antioxidant enzyme analysis (ovaries)</td><td>↓ CAT (HD**; trend)</td><td>↓ CAT (HD**)</td></tr>
<tr><td>↓ SOD</td><td>↓ SOD (HD*)</td></tr>
<tr><td>↑ LPO (HD*)</td><td>↑ LPO (HD*)</td></tr>
<tr><td>↑ ROS</td><td>↑ ROS (HD***)</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship
T = testosterone; E = oestradiol; LH = luteinizing hormone; FSH = follicle stimulating hormone
CAT = catalase; SOD = superoxide dismutase; LPO = lipid peroxidation; ROS = reactive oxygen species
</td><td>Klimisch:
Not reliable</td></tr>
<tr><td>Uterotrophic assay.
Rat (Wistar rats), immature females or ovariectomized females.
Exposure: oral gavage, daily.</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Immature females</td><td>↑ in relative wet uterine weight at HD* (trend),
↑ in relative dry uterine weight at HD* (trend),</td><td>No difference to the control in relative wet or dry uterine weight.</td></tr>
</table>
</td><td>Stroheker et al., 2003
Klimisch:
Reliable with</td></tr>
</table>

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.</th><th>Reference</th></tr>
<tr><td>BPF: 25, 50, 100 and 200 mg/kg bw in immature females, and only 100 mg/kg bw in ovariectomized females and for the vaginal cornification assay.
BPA: the same doses as BPF.</td><td>
<table>
<tr><td></td><td>Co-administration with 17β-E2 (45 µg/kg bw) did not modify the increase.</td><td></td></tr>
<tr><td></td><td>↑ in vaginal cornification at HD*,
Co-administration with 17β-E2 (45 µg/kg bw) increased cornification.</td><td>↑ in vaginal cornification at HD*,
Co-administration with 17β-E2 (45 µg/kg bw) increased cornification.</td></tr>
<tr><td>Ovariectomized females</td><td>↑ in relative wet uterine weight,
↑ in relative dry uterine weight,
Co-administration with 17β-E2 (100 µg/kg bw) did not modify the increase.</td><td>No data shown.</td></tr>
<tr><td></td><td>↑ in vaginal cornification,
Co-administration with 17β-E2 (100 µg/kg bw) increased cornification.</td><td>↑ in vaginal cornification,
Co-administration with 17β-E2 (100 µg/kg bw) increased cornification.</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship.
</td><td>*restriction*</td></tr>
<tr><td>Uterotrophic assay.
Rat (Sprague- Dawley), immature females.
Exposure: sub-cutaneous, daily.
BPF: 2, 20, 200 mg/kg bw.
BPA: same doses as BPF.</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Immature females</td><td>59% ↑ in absolute blotted uterine weight at HD**</td><td>97% ↑ in absolute blotted uterine weight at HD** (trend)</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship.
</td><td>Yamasaki et al., 2002
Klimisch:
Not reliable</td></tr>
<tr><td>Hershberger assay.
Rat (Wistar Han), castrated males.
Exposure: oral (gavage), daily.
BPF: 50, 200 and 1000 mg/kg.
BPA: 50, 200 and 600 mg/kg.</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Castrated males</td><td>9% ↑ in relative ventral prostate,
10% ↑ in seminal vesicle,
16% ↑ in Cowper’s gland,
Co-administration with testosterone propionate (s.c. 0.2 mg/kg) did not modify the increase.</td><td>7% ↑ in relative ventral prostate,
18% ↑ in seminal vesicle (trend),
23% ↑ in glans penis at HD** (trend),
Co-administration with testosterone propionate (s.c. 0.2 mg/kg) did not modify the increase.</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship.
</td><td>Yamasaki et al., 2003
Klimisch:
Reliable with restriction</td></tr>
<tr><td>In ovo study.</td><td></td><td>Mentor et al.,</td></tr>
</table>

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.</th><th>Reference</th></tr>
<tr><td>Chicken (Gallus gallus domesticus), males and females

Exposure: Bolus dose in ovo.

BPF: 210 nmol/g egg (42 µg/g egg).

BPA: 210 nmol/g egg (48 µg/g egg).</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Mortality</td><td>25 of 47 embryos died (53% **).</td><td>5 of 27 embryos died (19%).</td></tr>
<tr><td>Sex distribution of the remaining embryos</td><td>13 females and 9 males (22 in total).</td><td>6 females and 16 males (22 in total).</td></tr>
<tr><td>Reproductive organs</td><td>1 of 9 males had ovotestis (i.e., feminised gonads, 11%).</td><td>3 of 16 males had ovotestis (i.e., feminised gonads, 18.7%).</td></tr>
<tr><td rowspan="2">Histo(patho)logy (left testis)</td><td>8 of 9 males had >10 oocyte-like cells in testis.</td><td>9 of 16 males had >10 oocyte-like cells in testis.</td></tr>
<tr><td>No effect on ovaries or Mullerian ducts in females.</td><td></td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship.
</td><td>2020

Klimisch:

Reliable with restriction</td></tr>
</table>

Table 19. Summary table of comparative *ex vivo* studies of BPF and BPA relevant for toxicity on sexual function and fertility (OECD CF level 2).

<table>
<tr><th>Type of study/data, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect between the control group and the highest dose group. In case of statistically significant differences, the highest dose group (HD) is reported with asterisks.</th><th>Reference</th></tr>
<tr><td colspan="3">The following ex vivo studies were obtained from publicly available scientific journals.</td></tr>
<tr><td>Bisphenol F: bis(4-hydroxyphenyl)methane.
Purity: 99%.
Testicular tissues from 7 sacrificed Sprague-Dawley rats.
Age: 70-80 days.
Exposure: 2 h incubation.
Dose levels: 1, 10, and 100 ng/ml.
BPA: same doses as BPF.</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Hormone analysis (testis)</td><td>23% ↓ T (trend)</td><td>23% ↓ T (trend)</td></tr>
<tr><td>Antioxidant enzyme analysis (testis)</td><td>↑ ROS (HD**)</td><td>↑ ROS</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship
T = testosterone
CAT = catalase; SOD = superoxide dismutase; POD = peroxidase; LPO = lipid peroxidation; ROS = reactive oxygen species
</td><td>Ullah et al., 2018a</td></tr>
<tr><td>Bisphenol F: 4,4’-methylenediphenol.
Purity: 99%.
Spermatozoa from 26 sacrificed Sprague-Dawley rats.
Age: 70-80 days.
Exposure: 2 h incubation.
Dose levels: 0, 1, 10, and 100 ng/mL diluted in culture media.
BPA: same doses as BPF.</td><td>
<table>
<tr><td colspan="2">Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td colspan="2" rowspan="3">Antioxidant enzyme analysis (spermatozoa)</td><td>↑ SOD (HD**)</td><td>↑ SOD (HD*)</td></tr>
<tr><td>↑ ROS (HD*)</td><td>↑ ROS (HD*)</td></tr>
<tr><td>↑ TBARS (HD**)</td><td>↑ TBARS (HD**)</td></tr>
<tr><td rowspan="3">DNA damage (comet assay)</td><td>No of comets/100 cells</td><td>13% ↑ (HD*)</td><td>12% ↑ (HD*)</td></tr>
<tr><td>Tail moment (µm)</td><td>27% ↑ (HD*)</td><td>33% ↑ (HD*)</td></tr>
<tr><td>Tail DNA (%)</td><td>20% ↑ (HD*)</td><td>19% ↑ (HD*)</td></tr>
</table>
</td><td>Ullah et al., 2019c</td></tr>
</table>

Table 20. Information on adverse effects on male sexual function and fertility gathered from Table 2 in the RAC opinion on a harmonised classification and labelling for BPA (2014).

Study, species, strain, exposure route, test substance, dose levels	**Results** The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect.	**Reference**
The following studies concerns **male reproduction**.		
Rats (Sprague Dawley and Alderley park, derived from Wistar). Exposure: Oral, during GD 6 – GD 21. Dose levels: 20 µg/kg, 100 µg/kg bw, 50 mg/kg.	Observations made in adults (90 days): Significant ↓ sperm production at 50 mg/kg, only observed in Alderley park rats.	Tinwell et al., 2002
Mice (ddY). Exposure: Oral (gavage), during GD 10 – GD 17. Dose levels: 0, 1, 10 and 100 mg/kg bw/day.	Observations made at 60 and 120 days (adults): Histological abnormalities in seminiferous tubules.	Iida et al., 2002
Rats (Sprague Dawley). Exposure: Oral, during GD 6 – PND 20. Dose levels: 4 - 40 and 400 mg/kg bw/day.	Observations made at PND 63 and PND 252: Significant ↑ in plasma testosterone concentrations from 4 mg/kg, with no alterations of LH or FSH.	Watanabe et al., 2003
Mice (ICR). Exposure: Oral, during embryonic/foetal life and during lactation. Dose levels: 5 or 10 µg BPA/mL in drinking water.	Observations made in 4-weeks old pups: ↑ thiobarbituric acid-reactive substances in testis. ↓ wet weight of testis.	Kabuto et al., 2004
Rats (Long-Evans), females. Exposure: Oral (gavage), during GD 12 – PND 21. Dose levels: 0 - 2.4 µg/kg bw/day.	Observations made at 90-day old: ↓ testis and seminal vesicles weight. Unchanged prostate weight. ↓ specific Leydig cells testosterone production.	Akingbemi et al., 2004
Mice (CF-1). Exposure: Oral, during GD 14 – GD 18. Dose levels: 0 - 10µg/ kg bw/day.	Abnormal growth of the prostate; primitive prostate gland duct epithelial proliferation was found at birth.	Timms et al., 2005
Rats (Holtzman). Exposure: Oral, during GD 12 – PND 21. Dose levels: 1.2 – 2.4 µg/kg bw/day.	F1, F2, F3: ↓ litter size. significant ↑ post-implantation loss in F3. ↓ sperm count and sperm motility at both doses. ↑ copulation delay. ↓ expression profile of testicular Erβ.	Salian et al., 2009c
Rats (Long-Evans), weanling males. Exposure: Oral (gavage), during PND 21 – PND 35. Dose levels: 0 - 2.4 µg/kg bw/day.	Observations made at PND 35: ↓ serum LH and testosterone levels.	Akingbemi et al., 2004
Rats (Sprague Dawley). Exposure: Oral (gavage), during PND 23 – PND 30. Dose level: 40 µg/kg bw/day.	Observations made at pubertal and adult age: ↓ testosterone levels in juveniles, lasting until adulthood. ↓ sexual performances in adult animals.	Della Seta et al., 2006

Study, species, strain, exposure route, test substance, dose levels	**Results** The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect.	**Reference**
Rats (Sprague Dawley). Exposure: Oral (gavage), during PND 23 – PND 53. Dose levels: 100 mg/kg bw/day.	Observations made at PND 53: Only 66.7% of the treated rats reached a complete preputial separation. No significant effects were seen on the testis, epididymis or adrenal weight but morphological changes or differences in testicular histology was observed.	Tan et al., 2003
Rats (Long-Evans), weanling males. Exposure: Oral (gavage), during PND 21 – PND 90. Dose levels: 0 - 2.4 µg/kg bw/day.	Observations made at 91days: No effect on the body weight or the testis weight. ↓ in sex hormone levels, specifically testosterone produced by the Leydig cells.	Akingbemi et al., 2004
Rats (Wistar). Exposure: Oral, during PND 45 – PND 90. Dose levels: 0.2 – 20 µg/kg bw/day.	Observations at adult age: Significant ↓ in relative weights of testis and epididymis. Significant ↑ in relative weight of ventral prostate. Significant ↓ in epididymal sperm motility and sperm count. Effects on levels of enzymes related to oxidative stress.	Chitra et al., 2003
Rats (Sprague-Dawley). Exposure: Oral, from Day 6 to adult age (11 weeks). Dose levels: 0.02, 0.2, 2.0, 20 and 200 mg/kg bw/day.	No significant effect on the sperm production.	Sakaue et al., 2001
Rats (Sprague-Dawley), pregnant females. Exposure: Oral (gavage), during GD 6 - PND 90. Dose levels: 2.5, 8.0, 25, 80, 260, 840, 2700, 100 000, and 300 000 µg/kg bw/day.	Testicular descent was delayed. ↑ incidence of seminiferous tubule giant cells. ↑ T3 level at PND 15 and ↓ cholesterol levels at PND 90. Trend: first ↑ serum testosterone levels from 2.5 - 25 µg/kg bw/day: then ↓ serum testosterone levels from 80 µg/kg bw/day - 300 mg/kg. ↓ in weight of epidydimal fat pad. Dose dependent ↓ of seminal vesicle weight. ↓ ventral prostate weight.	NTCR, 2013
Mice. Exposure: Oral, from 10 weeks before mating until adulthood. Dose levels: 0.0007-0.003, 0.015-0.062, 0.22-0.73, 4.1-15.4, 37.6-167.2 and 434-1823 mg/kg bw/day.	Effect on reproductive organ weights, on daily sperm production and epididymal sperm concentration.	Tyl et al., 2008
Rat Exposure: Oral, from 10 weeks before mating until adulthood. Dose levels: 0.018, 0.18, 1.8, 30, 300 and 3500 ppm.	No effect on reproduction.	Tyl et al., 2002
Mice (CD-1). Exposure: Oral (diet). Dose levels: 300 or 325, 600 or 650 and 1200 or 1300 mg/kg for males or females respectively.	Significantly ↓ left testis/epididymis weights. Significantly ↓ seminal vesicle weight. ↓ proportion of motile sperm. No histological changes observed in male reproductive organs.	NTP, 1985b

Table 21. Human epidemiological studies and *ex vivo* studies conducted on human biological samples.

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect.</th><th>Reference</th></tr>
<tr><td colspan="3">The following epidemiological studies were obtained from publicly available scientific journals and concerns male reproduction.</td></tr>
<tr><td>Cross-sectional study.

984 Chinese men (32.0 ± 5.4 years old) were recruited from an infertility clinic between March and June 2013.

The men provided urinary samples (2 spot urine samples for BPF measurements), semen samples and a questionnaire on demographic characteristics lifestyles, occupational exposure and medical history.</td><td><table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Urinary creatinine adjusted levels (average).</td><td>0.69 µg/g
(83% of samples > LOD)</td><td>1.81 µg/g
(85-87% of samples > LOD)</td></tr>
<tr><td>Multivariate logistic or linear regression models for average or quartiles of urinary bisphenol concentrations and continuous or having below WHO reference values of semen quality parameters.</td><td>↓ progressive motility (p trend 0.05),
↑ abnormal heads (p trend 0.04),</td><td>↓ sperm concentration (p trend 0.04),
↓ total sperm count (p trend 0.01),
↓ progressive motility (p trend <0.01),
↓ total motility (p trend <0.01),
↓ linearity (p trend 0.04).</td></tr>
</table></td><td>Chen et al., 2022</td></tr>
<tr><td>Cross-sectional study

556 Danish men were recruited from a Fetal Programming of Semen Quality (FEPOS) cohort between 2017-2019.

Age: 18-20 years.

The men underwent a clinical examination and provided a urine sample for BPF measurements, semen samples and a questionnaire on lifestyle factors.</td><td><table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Urinary creatinine adjusted levels (average).</td><td>0.14 ng/mL
(92% of samples > LOD)</td><td>1.30 ng/ml
(95% of samples > LOD)</td></tr>
<tr><td>Negative binomial regression model was used to estimate crude and adjusted ratios for continuous or quartiles of urinary bisphenol concentrations and semen quality parameters.</td><td>No effects seen that were significant and dose-response related.</td><td>No effects seen that were significant and dose-response related.</td></tr>
</table></td><td>Benson et al., 2020</td></tr>
<tr><td>Prospective case-control study

16 Czech Republican men (8 men with normozoospermia and 8 men after surgical vasectomy with azoospermia) were recruited between January 2020 and December 2021.

Age: mean 32.4 years (22-41).

The men provided urine and semen samples.</td><td><table>
<tr><td></td><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td rowspan="2">Normozoospermia group:</td><td>Urinary specific gravity adjusted levels.</td><td>0.8 ng/mL (1 sample)
(7/8 samples < LOD)</td><td>0.022 - 0.335 ng/mL</td></tr>
<tr><td>Seminal plasma levels.</td><td>8/8 samples < LOD</td><td>0.006 - 0.090 ng/mL</td></tr>
<tr><td rowspan="2">Vasectomy group:</td><td>Urinary specific gravity adjusted levels.</td><td>1.94 ng/mL (1 sample)
(7/8 samples < LOD)</td><td><0.017 - 2.503 ng/mL</td></tr>
<tr><td>Seminal plasma levels.</td><td>0.99 ng/mL (1 sample)
(7/8 samples < LOD)</td><td>0.007 - 33.683 ng/mL</td></tr>
<tr><td colspan="2">The Welch’s t-test and the Mann–Whitney U-test were used to test for differences in ratios between men with normozoospermia (control group) and men with vasectomy.</td><td>No statistical analysis performed due to the high number of measurements < LOD.</td><td>Non-significant difference in ratios between urinary and seminal fluid levels in the two groups.</td></tr>
</table></td><td>Ješeta et al., 2022</td></tr>
</table>

<table>
<tr><th>Study,
species, strain, exposure route, test substance, dose levels</th><th>Results
The percentages are reported as an increase ↑ or decreased ↓ in mean values of an effect.</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td colspan="3">The following ex vivo studies were obtained from publicly available scientific journals and concerns male reproduction.</td></tr>
<tr><td>Bisphenol F: bis(4-hydroxyphenyl)methane.
Purity: unknown.
Spermatozoa from human donors.
Age: 25 – 30 years.
Exposure: 4 h incubation.
Dose levels: 10, 100, 300, 400 µM diluted in DMSO.
7 replicates/donor.
BPA: 400 µM.</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Sperm motility</td><td>~ 50% ↓ (trend)</td><td>100% ↓ (HD**)</td></tr>
<tr><td>Sperm viability</td><td>~ 15% ↓ (trend)</td><td>~ 85% ↓ (HD*)</td></tr>
<tr><td>Sperm mitochondrial membrane potential (Δψm)</td><td>~ 45% ↓ (trend)</td><td>~ 95% ↓ (HD*)</td></tr>
<tr><td>Sperm mitochondrial generation of superoxide anion (MRS %):</td><td>~ 40% ↑ (trend)</td><td>~ 85% ↑ (HD*)</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.
trend = dose-response relationship.
~ = approximately; the numbers are obtained from graphs and not reported as exact numbers in the scientific article.</td><td>Castellini et al., 2021</td></tr>
<tr><td>Bisphenol F.
Purity: 99%.
Testis from 3-5 human donors (prostate cancer patients who had no anti-androgen treatment).
Age: 46.7 ± 4.6 years.
Exposure: 24 and 48 h incubation.
Dose levels: 10^{-9} - 10^{-5} µM diluted in DMSO.
Comparative study.
BPA: same doses as BPF.</td><td>
<table>
<tr><td>Bisphenol</td><td>BPF</td><td>BPA</td></tr>
<tr><td>Hormone analysis (testis)</td><td>↓ T (HD*)
Most anti-androgenic at 10^{-6} M at both time points (24 and 48 h; −23.4 resp. −44.9%).</td><td>↓ T (HD**)
Most anti-androgenic at 10^{-5} M at both time points (24 and 48 h; −28.7 resp. −39.2%).</td></tr>
</table>
Significance: *p<0.05, **p>0.01, ***p>0.001
HD = high dose. Only statistical significance for the high dose group is reported in the table.

T = testosterone.</td><td>Desdoits-Lethimonier et al., 2017</td></tr>
</table>

10.10.4 Comparison with the CLP criteria

Based on a weight of evidence assessment including read-across from BPA, BPF fulfils the criteria of Category 1B as it exhibits adverse effects on male and female sexual function and fertility in absence of marked general toxicity.

In experimental male animal studies, reduced sexual and reproductive performance was supported by altered histo(patho)logical and morphological findings of male reproductive organs. Fewer studies were available on females. These, however, did report decreased reproductive organ weights and altered histo(patho)logical and morphological findings of the follicles. Furthermore, BPF demonstrate an oestrogen agonistic activity that supports the results from the uterotrophic studies, suggesting an endocrine mechanism of action. The decrease in levels of cholesterol, testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) and an increase in levels of oestrogen in plasma and/or the gonads indicate that there might be an effect on the HPG-axis. The results also demonstrate alterations of the male and female reproductive system across experimental animal species (rat, mouse, chicken, and zebrafish).

BPF is a structural analogue to BPA. The similarities in chemical structure and in physiological and toxicological properties justifies read-across from BPA. Hence, the weight of evidence based on substance specific data and read-across from BPA on the effects observed on male and female sexual function and fertility are considered as clear evidence and there is no indication that raises doubt on the relevance of this effect for humans.

Classification in Repr. 1A is not appropriate as it should be based on human data and currently the human data on BPF (and BPA as pointed out in the RAC opinion from 2014) is not considered robust enough to justify classification in category 1A, but instead the results are used in a weight of evidence.

Classification in Repr. 2 is not appropriate as the evidence for adverse effects on sexual function and fertility from existing experimental data on BPF and the read-across from BPA are considered as *clear* evidence and not as *some* evidence.

10.10.5 Adverse effects on development

Table 22: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
Reproduction /developmental Toxicity Screening Test. OECD TG 421 (2016). GLP facility but not within the scope of GLP regulation.	**Rat** (Sprague-Dawley). Total no = 120 (12 animals / sex / group). Age at start of treatment: ~8 weeks old.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 98%. Dose levels: 1, 5, 20 and 100 mg/kg bw dissolved in 4 ml/kg of corn oil. Control group received only corn oil. Exposure: Oral gavage, daily for 2 weeks prior to mating and throughout the day before sacrifice in males (total 62 days) and through lactation day (LD) 13 in females (total at least 41 days). Dose selection rational: Based on results from the 28-day RDT by Higashihara et al., 2007.	**General clinical signs (F0)** No mortality in males. Mortality observed in one female in the 20 mg/kg bw group, that was nonpregnant and sacrificed on GD 27. BPF-related salivation was observed in all males and females at 100 mg/kg bw. **Body weight and food consumption (F0)** In females, body weights were stat sign ↓ at 100 mg/kg bw during gestation and lactation ($p<0.05$-0.01). Weight gain was stat sign ↓ during pre-mating day 7 and gestational day 20 ($p<0.01$). During lactation, body weight changes were rebounded. In females, food consumption was stat sign ↓ during the whole treatment period (-28% and -24% at pre-mating day 7 resp. 14, -22%, -20%, -18% at GD 7, 14 resp. 20, -15% and -10% at LD 4 resp. 13). No treatment-related alterations in body weight or food consumption were observed in males. **F1 offspring** No BPF treatment-related changes observed in clinical observation or external examination. No changes in body weights or anogenital distance. At 100 mg/kg bw, one male had traces of two nipples. No changes in serum T4 levels.	Lee et al., 2022b Klimisch: *Reliable without restriction*
28-day RDT study. GLP/ OECD TGs not stated.	**Rat** (Wistar). Total no = 160 (8 groups with 10 males and 80 females to	Substance: Bisphenol F. CAS: unknown. Purity: unknown. Dose levels: 10, 30 and 50 mg/kg bw. Dilution unknown. Control group received 0.5ml normal saline.	**General clinical signs (F0)** Mortality and body weight during the treatment period were not reported. **F1 offspring** ↓ mean litter size (9.0, 9.0, 8.9 and 8.9 at ctl, 10, 30 and 50 mg/kg bw, non-stat sign). ↓ mean litter weight (6.03g, 5.92g,	Fatai and Aribidesi, 2022 Klimisch: *Reliable with restriction*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
	match the males). Weight at start of treatment: 180-200 g.	Exposure: Oral cannula, daily for 28 days. Recovery groups (n=4 a´10 males): one control, one low dose, one medium dose and one high dose group, were observed for additional 28 days after the 28-day treatment period. Females were made sexually receptive by inducing subcutaneous administration of 10 µg/100g bw of oestradiol benzoate and 0.5 mg/100g bw of progesterone 48 and 4 h respectively before mating.	5.90g and 5.92g at ctl, 10, 30 and 50 mg/kg bw, non-stat sign). ↓ survival at weaning (95%, 89%, 88% and 82% at ctl, 10, 30 and 50 mg/kg bw, non-stat sign).	

Table 23: Summary table of human data on adverse effects on development

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data.				

Table 24: Summary table of other studies relevant for developmental toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
21-day short-term fecundity assay. GLP/ OECD TGs not stated.	**Zebrafish** (Wild-type, AB) Age: from embryonic stage of F0 generation. F0 and F1 generation. Cross-mating: NWT male and female zebrafish.	Substance: Bisphenol F. CAS: 620-92-8. Purity: 99%. Dose levels: 0.5, 5 and 50 µg/L dissolved in a final acetone concentration of 0.005 mL/L. Control group receiving the solvent acetone. Blank control group also included.	**F1 offspring** ↓ heart rate ($p<0.01$ at all doses, non-dose response), ↓ body length ($p<0.01$ at 5 and 50 µg/L) and ↓ spontaneous movement ($p<0.05$ at 50 µg/L). No effect on the survival rate or hatchability.	Mu et al., 2022 Klimisch: *Not reliable*
21-day short-term fecundity assay.	**Zebrafish** (Danio rerio, AB). Age: F0	Substance: Bisphenol F. CAS: 620-92-8.	**F1 offspring** ↓ egg production, hatching rate of embryos, and survival rate of larvae in all treated	Yang et al., 2017

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	Reference
Employing OECD TG 229 and 230. GLP not stated.	males and females were 4,5 months old at start of study. F0 and F1 generation.	Purity: 98%. Dose levels: 0.001, 0.01, 0.1 and 1 mg/L dissolved in dimethyl sulfoxide (DMSO) into a water tank. Control group received 0.01% (v/v) DMSO. No blank control groups included.	groups ($p<0.05$ at 1 mg/L). At 1 mg/L, the offspring showed developmental malformations of embryos and larvae including pericardial oedema, tail malformation, trunk curvature.	Klimisch: *Reliable with restriction*

10.10.6 Short summary and overall relevance of the provided information on adverse effects on development

The data on BPF is based on scientific studies from the open literature. A klimisch score has been assigned to each study to assess their reliability[12].

RODENT STUDIES ON FEMALE FERTILITY (OECD CF LEVEL 4)

In the **Reproduction/developmental Toxicity Screening** Test (OECD TG 421, 2016) by **Lee and co-workers (2022b)**, Sprague- Dawley rats received BPF via oral gavage at ~8 weeks of age at dose levels of 1, 5, 20 and 100 mg/kg bw/day dissolved in 4 ml/kg corn oil. The treatment period lasted from 2 weeks prior to mating and throughout the day before sacrifice in males (total 62 days) and through lactation (LD 13) in females (total at least 41 days). The control group received only corn oil. Each group (=5) contained 12 animals/sex. 1:1 mating for 2 weeks. Mating was confirmed by vaginal smears and mating-proven females were monitored twice a day for signs of parturition, including abortion, premature delivery, and difficult or prolonged parturition from GD 21. Dams were allowed to have their litter, and then the duration of gestation, number of live/dead pups, sex of pups, body weight of each of the live pups, and external abnormalities were observed. On PND 0, 4, 7, and 13, pup mortality, general clinical signs, and body weight were observed. Delivery index (% of dams with live pups) and viability index (% of pup survival rate from birth to PND 13) were calculated. Both pup body weight and anogenital distance were measured on the same day (PND 4), and the anogenital distance was normalized based on the cube root of body weight. In addition, the number of nipples in all male pups was counted on PND 12. Serum levels of thyroxine (T4) were measured in whole blood of parental males, females, and their pups at necropsy. The study was conducted at a GLP facility but not within the scope of GLP regulation. Dose selection rational was based on results from the 28-day RDT by Higashihara et al., 2007 (see list of references).

F0 generation: Mortality was observed in one female in the 20 mg/kg bw group, that was nonpregnant and sacrificed on day 27. No mortality was observed in males. Both females and males had BPF-related salivation at 100 mg/kg bw. Body weights were statistically significantly decreased in females at 100 mg/kg bw during gestation and lactation ($p<0.05$-0.01). Weight gain was statistically significantly decreased during pre-mating day 7 and gestational day 20 ($p<0.01$). During lactation, body weight changes were rebounded. Food consumption was statistically significantly decreased during the whole treatment period (-28% and -24% at pre-mating day 7 resp. 14, -22%, -20%, -18% at GD 7, 14 resp. 20, -15% and -10% at LD 4 resp. 13). No treatment-related mean body weight alterations or changes in food consumption was observed in males.

[12] The reliability assessment was based on a method described by Wiklund and Beronius 2022.

F1 generation: In the offspring, there were no BPF treatment-related changes observed in the clinical observation or external examination. Also, there were no changes in body weights or anogenital distance. At 100 mg/kg bw, only one male had traces of two nipples. No changes in serum T4 levels.

In the **28-day oral repeated dose toxicity study (Fatai and Aribidesi, 2022)**, Wistar rats received BPF via oral administration (cannula) at dose levels of 10, 30 and 50 mg/kg bw (dilution not stated). The control group received 0.5 ml normal saline. Each group (=4) contained 10 animals/sex. Each male was matched with a female, that was made sexually receptive by inducing a subcutaneous injection of 10 µg/100g bw of oestradiol benzoate and 0.5 mg/100g bw of progesterone at 48 and 4 h respectively before mating. Weight at start of treatment was 180-200 g (age not stated).
In addition, four recovery groups were established for the control and the three dose groups (10 animals/sex/group). The males were observed for additional 28 days after the 28-day treatment period.
No information was provided about the type of Wistar rat strain, vehicle given to the treated groups, age of the animals at start of treatment, IUPAC nomenclature, purity, or CAS number. Also, no information was provided about mortality or body weight changes during treatment.

In the F1 offspring, the mean litter size, weight and survival at weaning were all non-statistically significant, even though a dose-response related decrease was observed for survival at weaning (95%, 89%, 88% and 82% at ctl, 10, 30 and 50 mg/kg bw).

NON-RODENT STUDIES ON FEMALE FERTILITY (OECD CF LEVEL 3)

In one of the non-rodent studies, **Mu and co-workers** (**2022**) performed a **21-day short-term fecundity assay** on wild-type AB zebrafish. The fish received BPF via tank water from embryonic stage of F0 generation at dose levels of 0.5, 5 and 50 µg/L. Exposed F0 and F1 generations were examined.

The F1 offspring showed decreased heart rate in treated groups ($p<0.01$), reduced body length ($p<0.01$ at 5 and 50 µg/L) and reduced spontaneous movement ($p<0.05$ at 50 µg/L). No effect on the survival rate and hatchability.

In the other **21-day short-term fecundity assay** on Danio rerio zebrafish **Yang and co-workers** (**2017**), exposed the fish to BPF via water tank at 4,5 months of age at dose levels of 1, 10, 100 and 1000 µg/L (1 mg/L). Both F0 and F1 generations were examined.

In the F1 offspring, the survival rate of larvae was decreased in all treated groups ($p<0.05$ at 1 mg/L, exact numbers not reported) and the histo(patho)logical analysis revealed developmental malformations of the embryos and larvae i.e., pericardial oedema, tail malformation and trunk curvature at 1 mg/L.

SUMMARY OF BPF ON DEVELOPMENT

No clear developmental effects following BPF exposure in rodent offspring were observed in the reproduction/developmental oral toxicity screening study (Klimisch score 1 ‘reliable without restriction’) or the 28-day repeated dose subcutaneous toxicity study (Klimisch score 2 ‘reliable with restriction’). Only the latter study showed a dose-response related decrease (non-statistically significant) in survival rate at weaning, but no information was provided on the body weight of the dams.

The two studies on zebrafish showed decreased heart rate, developmental malformations such as pericardial oedema, tail malformation and trunk curvature, and reduced spontaneous movement. Only one of studies showed a decrease in survival rate, but at higher dose levels than the other study.

10.10.7 Comparison with the CLP criteria

The criteria for classification as reproductive toxicant for adverse effects on development of offspring following BPF exposure are not considered to be met because of diverging results and lack of consistency in the results of the few studies available. Differences between the studies in design, dose

levels and strain, among others, exist. Hence, more data is considered necessary to provide clear or some evidence of an effect on development.

Specifically, a decrease in survival rate in offspring was observed in one of the two rodent studies (28-day repeated dose subcutaneous toxicity study on Wistar rats). This was also observed at a high dose level in one of the two 21-day short-term fecundity assays in Danio rerio zebrafish. The same effect was, however, not observed in the Reproduction/ developmental oral Toxicity Screening Test in Sprague- Dawley rats or in the other 21-day short-term fecundity assay in wild-type AB zebrafish. Effects on development, other than survival rate, were not observed in the two rodent studies, whereas the two 21-day short-term fecundity assays did show other effects, but the results were not consistent between the two studies. Therefore, on the basis of available data, no classification for effects on development is proposed.

10.10.8 Adverse effects on or via lactation

Table 25: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, dose levels, duration of exposure**	**Results** The results are presented as mean values in comparison to the data of the control group, unless otherwise stated.	**Reference**
Neonatal sub-chronic study. GLP/ OECD TGs not stated.	**Mouse** (ICR). Total no = 6-8 litters. Age: F0 females were 6-7 weeks old at start of study and used for producing F1 generation offspring.	Substance: Bisphenol F. CAS: unknown. Purity: unknown. Dose levels: 0.2 and 20 ng/g bw/day (0.375 ng/mL and 37.5 ng/mL). Control group received 0.1% ethanol in sterile tap water. Positive control group received 0.2 ng/g bw/day diethylstilbestrol (DES). Exposure: nursing dams (4-5 dams/group) treated via drinking water from PND 0 (day of delivery) to PND 15.	**Litter weight** No effect on litter weight or litter weight gain during the nursing period (PND 0-21). **Endocrine parameters** No stat sign difference in anogenital distance on PND 21 (6-7 female offspring examined) nor vaginal opening (recorded on the day of pubertal onset in 10-11 female offspring examined). No stat sign difference for DES either.	Nevoral et al., 2021 Klimisch: 2 *Reliable with restriction*

Table 26: Summary table of human data on effects on or via lactation

Type of data/report	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data.				

Table 27: Summary table of other studies relevant for effects on or via lactation

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data.				

10.10.9 Short summary and overall relevance of the provided information on effects on or via lactation

In the **15-day neonatal sub-chronic study (Nevoral et al., 2021)**, nursing dams of ICR mice received BPF via drinking water at 6-7 weeks of age at dose levels of 0.2 and 20 ng/g bw/day (0.375 ng/mL and 37.5 ng/mL). The control group received 0.1% ethanol in sterile tap water. Each group contained 4-5 dams. The F1 generation offspring contained 6-8 litters. Female pups were bred to 60 days and oocytes were collected. On PND 21, anogenital distance was measured as the distance from the superior edge of the external genitalia to the inferior edge of the anus. No information was provided on the IUPAC nomenclature, CAS number or purity of the substance.

No effect on weight gain of dams during the nursing period or litter weight was observed.

The endocrine parameters (to detect androgenic and oestrogenic activity) revealed no difference in anogenital distance (AGD) on PND 21 (6-7 female offspring examined) nor on vaginal opening (recorded on the day of pubertal onset in 10-11 female offspring) between the dose groups.

SUMMARY OF BPF VIA LACTATION

No effects on mice pups were observed in the 15-day neonatal sub-chronic study. However, the dams in the highest dose group received very low concentrations of BPF via drinking water (20 ng/kg bw/day corresponding to 0.02 mg/kg bw/day) compared to the highest dose group in other rodent studies reported in this CLH-proposal (50 – 500 mg/kg bw/day).

Also, the reporting of anogenital distance should preferably include the anogenital distance divided by the cubic root of the body weight. Still, the results are in line with those observed in the reproduction/developmental Toxicity Screening study (Lee et al., 2022b), where no effects were observed on anogenital distance on PND 4 in rat offspring following exposure to BPF.

The current results are not sufficient to conclude on an association between BPF and adverse effects on or via lactation.

10.10.10 Comparison with the CLP criteria

The criteria for classification as reproductive toxicant for adverse effects on or via lactation following BPF exposure are not considered to be met based on the available 15-day neonatal sub-chronic study showing no results via lactation. Therefore, on the basis of available data, no classification for effects via lactation is proposed.

10.10.11 Conclusion on classification and labelling for reproductive toxicity

Classification of BPF for adverse effects on sexual function and fertility as Repr. 1B H360F is warranted. No classification for adverse effects on developmental toxicity or for adverse effects on or via lactation is warranted.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH-proposal.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this CLH-proposal.

10.13 Aspiration hazard

Not evaluated in this CLH-proposal.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH-proposal.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH-proposal.

13 ADDITIONAL LABELLING

Not relevant.

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