Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

4,4'-sulphonyldiphenol; bisphenol S

EC Number: 201-250-5

CAS Number: 80-09-1

CLH-O-0000006929-56-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC

Adopted

10 December 2020

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CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

4,4’-sulphonyldiphenol; bisphenol S

EC Number: 201-250-5

CAS Number: 80-09-1

Index Number: NA

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Version number: 2 **Date: October 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON; 4,4'-SULPHONYLDIPHENOL; BISPHENOL S

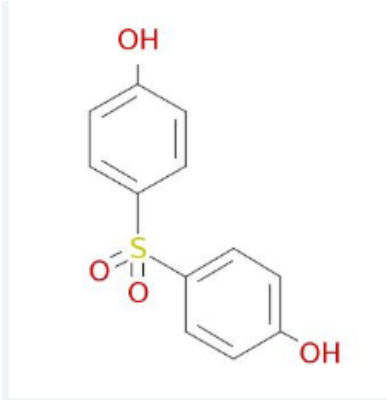
CONTENTS

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-hydroxybenzenesulfonyl)phenol
Other names (usual name, trade name, abbreviation)	Bisphenol S 1,1'-Sulfonylbis[4-hydroxybenzene] 4,4'-Bisphenol S 4,4'-Dihydroxydiphenyl sulfone 4,4'-Sulfonylbisphenol 4,4'-Sulfonyldiphenol 4-Hydroxyphenyl sulfone Bis(4-hydroxyphenyl) sulfone Bis(hydroxyphenyl)sulfone BIS(HYDROXYPHENYL)SULPHONE Bis(p-hydroxyphenyl) sulfone BPS 1 Dihydroxydiphenyl sulfone DIHYDROXYDIPHENYLSULPHONE Diphone C p,p'-Dihydroxydiphenyl sulfone Phenol, 4,4'-sulfonylbis- (9CI) Phenol, 4,4'-sulfonyldi- (6CI, 8CI) Phenol, sulfonylbis- Phenol, sulfonyldi- PHENOL, SULPHONYLBIS PHENOL, SULPHONYLDI Sulfonyldiphenol- SULPHONYLDIPHENOL
EC number (if available and appropriate)	201-250-5
EC name (if available and appropriate)	4,4’-sulphonyldiphenol
CAS number (if available)	80-09-1
Molecular formula	$C_{12}H_{10}O_4S$

Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	250.27
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)	≥ 99.7 %

1.2 Composition of the substance

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 3.1 (CLP)	Current self-classification and labelling (CLP)
4,4'-sulphonyldiphenol EC n° : 201-250-5	≥ 99.7 -100.0 % (W/W)	NA	Self clasification in the public REACH registration dossiers: - Repr. 2, H361 - Not classified

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 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
See confidential Annex				The impurities do not contribute to the classification

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 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Labelling**			**Specific Conc. Limits, M-factors**	**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 submitters proposal	TBD	4,4’-sulphonyldiphenol; bisphenol S	201-250-5	80-09-1	Repr. 1B	H360FD	GHS08 Dgr	H360FD			
Resulting Annex VI entry if agreed by RAC and COM	TBD	4,4’-sulphonyldiphenol; bisphenol S	201-250-5	80-09-1	Repr. 1B	H360FD	GHS08 Dgr	H360FD			

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public 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	Repr. 1B, H360FD	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'-sulphonyldiphenol (BPS) is a chemical substance which is registered under the REACH Regulation (1907/2006/EC). The substance is not listed in annex VI of CLP and classification and labelling was not previously discussed by the TC C&L.

The C&L inventory contains several different self classifications for this substance (C&L Inventory, 9/7/2019) :

- Repr. 2, H361 (fertility)
- Not classified
- Skin Irrit. 2, H315
- Eye Irrit. 2, H319
- STOT SE 3, H335
- Aquatic chronic 3, H412

RAC general comment

Bisphenol S is a structural analogue and functional replacement of bisphenol A (Figure 1). In 2014, bisphenol A was classified as **Repr. 1B; H360F** by RAC (ECHA, 2014). Despite the structural and functional similarities between the substances, no comparison to the hazard profile of bisphenol A has been made for the purpose of this opinion on bisphenol S, which relies on its own data and not on read across.

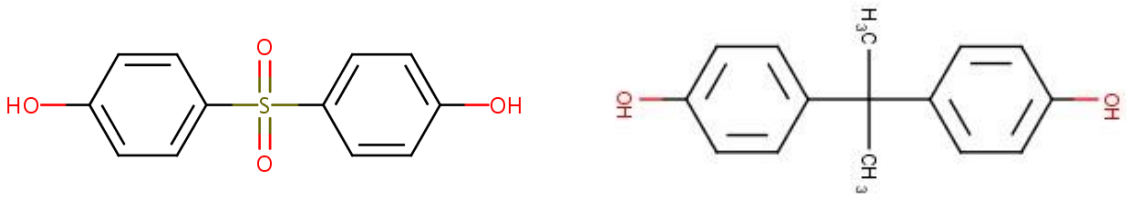


Figure 1. Structural formulas of **bisphenol S (left)** and **bisphenol A (right)** as retrieved from the ECHA dissemination website.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No justification is needed. The substance is self classified as a reprotoxicant.

Currently 4,4'- sulphonyldiphenol is self-classified as Repr. 2 in 2 out of the 3 public REACH registration dossiers. However based on the available data classification as Repr. 1B H360FD is warranted.

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

[B.] Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Disagreement by DS with current self-classification

5 IDENTIFIED USES

The substance is used in articles, by professional workers, in formulation or re-packaging at industrial sites and in manufacturing.

No public registered data are available indicating whether or in which chemical products the substance might be used.

However 4,4'- sulphonyldiphenol is reported in the literature (Molina-Molina *et al.*, 2013) to be used in high temperature resistant thermoplastic polymers :

- In BPS-based epoxy resins and

- as monomer in the production of cyclic carbonates and sulfonated poly(ether ketone ether sulfone)

Some other uses are reported in the same article:

- chemical additive in pesticides, dyestuffs, colour-fast agents, leather tanning agents, dye dispersants and fiber improvers.

- as a developer in dyes for thermal paper (as alternative to Bisphenol A or BPA).

Finally, 4,4'-sulphonyldiphenol has been detected in canned food and in paper products and currency bills.

6 DATA SOURCES

Registration dossier (last modification : 20-Mar-2019 ; consultation by the DS : 24-June-2019; https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/14986/1)

C&L inventory : consulted by the DS : 9/7/2019

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	A fine white odourless powder	- Anonymous 1, 2012	value used for Chemical Safety Assessment (CSA) : solid at 20° C and 101.3 kPa

Property	Value	Reference	Comment (e.g. measured or estimated)
		- Anonymous 2, 2009 -GESTIS Substance database, 2008	
Melting/freezing point	245-248° C	Beilstein, 2007 BGIA Gestis Stoffdatenbank, 2008	Beilstein covers a melting point of 245 - 248° C as a range of 8 independent entries. GESTIS gives 242 -247° C as value for the melting point. The range of values cited in Beilstein was taken as key value. Reliability 2
Boiling point	Not applicable	Study report, 2010 Anonymous 3	OECD TG 103, dynamic method decomposition at 315° C The boiling point of the test item could not be determined, because at a temperature of 315° C a continuously increasing pressure was observed. This is presumably caused by a limited stability and a thermal change of the test item. Reliability 1
Relative density	-1.37 g/cm³ at 15° C -1.37 g/cm³ at 15° C -1.4 g/cm³ at 15° C	- Annaheim, 2007 - Yaws, Carl L., 2009 - Beilstein, 2007	Measured Reliability 2
Vapour pressure	Negligible 6.29E-10 hPa at 25° C	Neely WB & Howard PH, 1995	The melting point of the substance is between 200°C and 300° C The calculated value of vapour pressure at 25° C (MPBPWIN v1.42) is quite low as expected
Surface tension	Not applicable		Based on chemical structure, no surface activity is predicted.
Water solubility	- 1.1 g/L at 20° C - 505 mg/L at 25° C - 1774 mg/L at 25° C - ca. 1.1 g/L at 20° C	- BGIA Gestis Stoffdatenbank, 2008 - Meylan WM *et al.*, 1996 - BASF AG, 2007 - Clairant, 2004	- Measured (Key study, Reliability 2), Value used for CSA: 1.1 g/L at 20 °C - Calculated (Reliability 2) - Calculated (Reliability 2) - Measured (Reliability 4)
Partition coefficient n-octanol/water	-log Pow = 1.2 at 23° C, pH 6.2 -log Pow = 1.65 at 25° C	- Study report, 2010 Anonymous 4 - Peer review database, 2007	- OECD TG 117 Measured (key study, Reliability 2) - EPIWin calculation KOWWIN v1.67 (Reliability 2) - Model calculation (Reliability 2)

Property	Value	Reference	Comment (e.g. measured or estimated)
	-log Pow = 1.65 at 25° C -log Pow = 1.65 at 25° C	Anonymous 5 - Meylan WM, & Howard PH., 1995 - Peer review database, 2009 Anonymous 6	- Calculation (Reliability 2)
Flash point	Not applicable		The substance is a solid.
Flammability	- The substance is not a highly flammable solid. - No selfheating up to 350° C. - Combustible solid	- Study report, 2009 Anonymous 7 - 2006, anonymous 8 - BGIA Gestis Stoffdatenbank, 2008	- EU Method A.10 (Key study, reliability 2) - according to VDI 2263 (Reliability 2) - measured (Reliability 2) Value used for CSA: Non flammable solid. Based on chemical structure pyrophoric properties and flammability in contact with water are not to be expected. The substance or mixture does not need to be classified as self-reactive as the heat of decomposition is less than 300 J/g. The substance or mixture does not need to be classified as self-heating as the onset temperature is greater than 220 °C in the Grewer Oven test (screening test). The substance or mixture does not need to be classified as an organic peroxide as by definition based on their chemical structure the substance is no organic peroxide.
Explosive properties	Non explosive		Value used for CSA: non explosive There are no chemical groups associated with explosive properties present in the molecule
Self-ignition temperature	- No Self ignition up to 350° C - ≥ 400 °C at 1013 hPa	- study report, 2006 Anonymous 9 - Clairant, 2004	- EU Method A.16 (Reliability 2) - According to DIN 51 794 (Reliability 4) The substance is a solid and self-heating of the substance up to 400° C is excluded.
Oxidising properties	No oxidising properties	Study report, 2010 Anonymous 10	EU Method A.17 (Reliability 1) Value used for CSA: Oxidising: no The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested is lower than the maximum burning rate of the reference mixture
Granulometry	- Particles <100 µm approximate 55 %, - Particles <10	Study report, 2009 Anonymous 11	OECD TG 110

Property	Value	Reference	Comment (e.g. measured or estimated)
	µm approximate 1.8 %, - Particles <4 µm approximate 0.4 %		
Stability in organic solvents and identity of relevant degradation products	Not applicable		The stability of the substance is not considered as critical.
Dissociation constant	- pKa=8 at 20° C - pKa=7.93 at 25°C - pKa=8.14 at 25°C	- Study report, 2009 Anonymous 2 - BASF SE, 2008	- OECD TG 112 (Key study, reliability 2) - SPARC calculation (Reliability 2)
Viscosity	Not applicable		Substance is a solid at 20° C and atm. pressure

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not evaluated in this dossier

10.2 Acute toxicity - dermal route

Not evaluated in this dossier

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier

10.4 Skin corrosion/irritation

Not evaluated in this dossier

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier

10.6 Respiratory sensitisation

Not evaluated in this dossier

10.7 Skin sensitisation

Not evaluated in this dossier

10.8 Germ cell mutagenicity

Not evaluated in this dossier

10.9 Carcinogenicity

Not evaluated in this dossier

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reproductive toxicity test Rats / Spargue-Dawley (SD) 12/sex/group	4,4'-sulphonyldiphenol Vehicle : 0.5 % aqueous sodium CMC solution with 0.1 % Tween 80	<u>Parental generation :</u> Clinical signs : excessive salivation at 300 mg/kg bw/d Body weight (bw) : reduced at the highest dose	Anonymous 12, 2000

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Gavage Following OECD TG 421 GLP	Doses : 0, 10, 60 and 300 mg/kg bw/d Exposure : a total (tot.) of 45 D for males (including 14 D of premating period, through mating to the day before necropsy) and a total of 40 to 46 D for females (from premating, mating, gestation until lactation day (LD) 3) (females without delivery were exposed until D 25 after confirmation of copulation)	in both sexes (see table 9) Gross necropsy findings : distension of cecum observed in 1 male (♂) and 1 female (♀) in the mid dose level and in all ♂ and 4 ♀ at the highest dose level Organ weight : in ♂ : sign. increase of relative (rel.) pituitary and rel. liver weights and sign. decrease of seminal vesicle weight (see table 12) In ♀ : no sign. changes at the highest dose observed Histopathology : cecum : sign. increased incidence of hyperplasia of the mucosal epithelium (epith.) (in 11 ♂) and sign. higher incidence of single cell necrosis (in 5 ♂) at the highest dose Liver : centrilobular hypertrophy of hepatocytes observed in 5 ♂ at 300 mg/kg bw/d Reproductive data : copulation index, parturition index, delivery index, number (nb) of corpora lutea, gestation period : no effects The mean duration of oestrus cycle was sign. higher at the highest dose (5.57**d vs 4.08d in control group) and 5 ♀ exposed to 300 mg/kg bw/d exhibited a longer dioestrus period (vs 0 ♀ in control). Decreased mean nb of implantation sites at 300 mg/kg bw/d (10.7 vs 15.9 in control group) and sign. lower implantation index at 300 mg/kg bw/d (64.89** % vs 95.80 % in control group) Severe decrease of fertility index : 58.3 % at the highest dose vs 91.7 % in control group <u>Offspring :</u> Decreased mean nb of offspring at birth at 300 mg/kg bw/d (9.1 at 300 mg/kg bw/d vs 14.3 in control group) No abnormalities in external appearance and clinical signs nor bw, body weight gain (bwg), viability index, ano-genital distance (AGD)	
Extended-one-generation reproductive toxicity study (EOGRTS) with F2, developmental neurotoxicity (DNT) (cohorts 2A and 2B) and developmental	4,4'-sulphonyldiphenol Vehicle : 0.5 % CMC Doses : 0, 20, 60 and 180 mg/kg bw/d Duration of exposure :	<u>Parental generation :</u> Bw : sign. higher only in ♀ during the in-life period (D 7 and 14 in the mid dose) ♂ reproductive data : sign. reduction in % of motile sperm in all tested dose group (88, 84*, 85* and 86* %, respectively at 0, 20, 60 and	Anonymous 13, 2019

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
immunotoxicity (DIT) (cohort 3)Rats / SD F0 generation : 24/sex/dose F1 generation : 20/sex/dose for cohort 1A, 24/sex/dose for cohort 1B, 10/sex/dose for cohorts 2A, 2B and 3 Gavage Following OECD TG 443 GLP	Minimum 10 w after the beginning of exposure, males and females from the same dose group were mated. Shortly before weaning of the F1 pups, the F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups. Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after weaning, placed into cohorts.	180 mg/kg bw/d) ♀ reproductive data : mean duration of oestrus cycle : sign. increase at 180 mg/kg bw/d (4.1* d vs 3.9d in control) Mean nb of implantation site : reduced at the highest dose (14.3 vs 15.3 in control) Mean nb of post-implantation loss sign. affected (1.5** vs 0.5 in control) Necropsy : enlarged cecum and changes in kidneys observed in ♂ at 180 mg/kg bw/d <u>F1 pups :</u> Sign. lower tot. nb. of liveborn pups (285* at 180 mg/kg bw/d vs 340 in control) and sign. higher nb of stillborn pups (8* at 180 mg/kg bw/d vs 2 in control) <u>Cohort 1A :</u> Final body weight (FBW) : slightly reduced at 180 mg/kg bw/d in ♂ BW : in ♀, body weight was sign. higher at D14 and D28 in the mid and high dose groups Necropsy : adrenal glands, kidneys, liver, spleen, thymus and prostate showed a significant deviation in absolute (abs.) or rel. value An atrophy of the mammary gland was noted in ♂ of the highest dose <u>Cohort 1B :</u> higher mean duration of oestrus cycle at 180 mg/kg bw/d (4.5 d vs 3.9 d in control) Lower mean nb of implantation sites and sign. higher incidence of post-implantation loss at 180 mg/kg bw/d Necropsy : FBW slightly reduced in ♂ at the highest dose and a few organ weights modified <u>Cohort 2A :</u> Auditory startle response, home cage observations, open field observations, sensorimotor tests/reflexes, motor activity measurements and learning and memory tests : unaffected <u>Cohor 2B :</u> Necropsy examination : no effects observed <u>Cohort 3 :</u>	

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Clinical and bw examination : unaffected Necropsy examination : sign lower rel. thymus weight at 180 mg/kg bw/d T-cell dependent antibody response : slight change in the low and mid dose groups in ♀ F2 pups : Decrease tot. nb of pups delivered at the highest dose	
Range finding study preceding the EOGRTS, Similar to a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test Rats / SD 10/sex/dose Gavage Similar to OECD TG 422 GLP	4,4'-sulphonyldiphenol Vehicle : CMC Doses : 0, 30, 100 and 300 mg/kg bw/d Duration of exposure : 10 w for males and continued through premating, gestation, and lactation periods for females	F0 generation : Mortality : no premature death Clinical signs : excessive salivation observed at the highest dose Bw : lowered at the highest dose (-7 % in ♂ and -6 % in ♀ compared to the control group) Haematology and clinical biochemistry : no effects (no further information available) Gross pathological findings : increased incidence of cecum dilatation, enlarged and discoloration of kidneys and enlarged liver in ♂ exposed to 300 mg/kg bw/d Organ weight : sign. higher rel. kidneys weight in ♂ (+11.5 and +35 % respectively at 100 and 300 mg/kg bw/d) and sign higher rel. liver weight in ♂ at the highest dose (+11 %). In ♀, uterus weight modified at 300 mg/kg bw/d Histopathology : changes observed in liver in both sexes. Furthermore, mammary gland and cecum also affected in ♂ Reproductive data : Oestrus cycle : prolonged at the highest dose (5.16** vs 4.02 d in control group) Sign. lower mean nb. of implantation sites at 300 mg/kg bw/d (10.4** vs 15.8 in control group) % of post-implantation loss : sign. higher at 300 mg/kg bw/d (34.6* vs 3.6 % in control group) F1 : Mean nb of pups delivered sign decreased at the highest dose (10.8** vs 15.2) Pups bw sign. higher in ♂ of the low dose at PND 21 (+6.6 % compared to the control group) (no further information available) Gross pathological findings : no effects	Anonymous 14, 2017

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		observed (no further information available)	
Range finding study preceding the EOGRTS, similar to a 28 D repeated dose toxicity study Rat / SD 5/sex/dose Gavage No guideline followed GLP : no	4,4'-sulphonyldiphenol Vehicle : CMC Dose : 0, 100, 300 and 600 mg/kg bw/d Duration of exposure : 28 D	Clinical signs : excessive salivation BWG : sign. lower at the highest dose in ♂ (no further information available) FBW : lower at the mid and high dose levels (-9 and -12 % respectively at 300 and 600 mg/kg bw/d) Gross necropsy findings : enlarged kidneys observed in 4 ♂ exposed to 600 mg/kg bw/d and in 3 ♂ exposed to 300 mg/bw/d. Moreover, cecum dilatation was noted in 2 ♂ of the highest dose. Organ weight : a few changes observed in kidneys, adrenals, liver, prostate and sem. ves. Histopathology : a few changes observed in kidneys, adrenal glands, liver, cecum and mammary gland.	Anonymous 15, 2017
28-days repeated dose toxicity study including 2-weeks observation of reversibility Rat / SD 6/sex/group (for main group) + 6/sex/group (for recovery group) Gavage Similar to OECD TG 407 GLP	4,4'-sulphonyldiphenol Vehicle : 0.5 % aqueous solution of methylcellulose Conc. : 0, 40, 200 and 1000 mg/kg bw/d for main groups 0, 200 and 1000 mg/kg bw/d for recovery groups Duration of exposure : 28 d, daily Observation period : 2 w for recovery groups	Mortality : 2 ♂ exposed to the highest dose (dilatation of cecum and signs of intestine haemorrhage at necropsy) Clinical signs : 1000 mg/kg bw/d : abdominal distension in 1 ♀ after 15 days and in 5 ♀ after 28 days (this effect disappeared during the recovery period) BW : lower value at 1000 mg/kg bw/d during the dosing period (sign in ♂). Recovered at the end of the recovery period BWG : sign. lower in both sexes for the exposure period and sign. reduce in ♂ for the recovery period <u>Animals necropsied at the end of dosing period :</u> Gross pathology findings : 1000 mg/kg bw/d : dilatation of cecum in all animals Organ weight : few modification (see table 29) Histopathological findings observed in cecum (hyperplasia of mucosa, single cell necrosis of mucosal epithelium), liver (hypertrophy centrilobular), adrenals (hypertrophy of zona fasciculate), femur (increase spongy bone) and thymus (atrophy) <u>Animals necropsied at the end of recovery period :</u> Gross pathology findings : dark red spots in the glandular stomach in 2 ♀ of the mid dose	Anonymous 16, 1999

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		and in 2 ♀ of the high dose. Organ weight : few modifications Histopathological findings observed in cecum (hyperplasia of mucosa and single cell necrosis of mucosal epithelium), liver (microgranuloma) and femur (increase spongy bone)	
90-day repeated dose toxicity study Rat / Wistar / males + females 10/sex/dose Gavage Following OECD TG 408 GLP	4,4'-sulphonyldiphenol Vehicle : 1 % CMC Conc. : 0, 100, 300 and 1000 mg/kg bw/d (in males the highest dose was changed to 600 mg/kg bw/d after 70 D) Duration of exposure : 90 days	Mortality : no animals died Clinical signs : soft and discoloured faeces and salivation in all animals exposed to the mid and high doses. Bw : sign. decreased in ♂ at the highest dose. The bwg (D 0–91) was significantly lower in ♂ in the mid and high dose level. Gross necropsy findings : dilatation of cecum in all ♂ of the high dose Enlarged liver in 8 ♀ at the high dose level Organ weight : sign. changes observed in both sexes (see table 34) Sign. lower ♂ reproductive organ weight (testes and epididymides) Histopathology : few changes (see table 35) Dilatation of cecum in all ♂ and ♀ at the highest dose + increase incidence of apoptosis Mammary glands : in ♂ : increased incidence of multifocal atrophy at the mid and high dose Uterus : increased incidence of squamous metaplasia	Anonymous 17, 2014
13-day repeated dose toxicity study Rat / strain not specified / male 5 males/group Diet No guideline followed No GLP	4,4'-sulphonyldiphenol Vehicle : 1 % corn oil Doses : 0, 0.1 and 1 % (± 0, 97 and 810 mg/kg bw/d) Duration of exposure : 13 D	Bw : severely decreased at the highest dose (no further information available) Organ weight : lower kidneys and liver weights at 1 % (no further information available) Histopathology : adipose tissue atrophy and cytoplasmic basophilia of epithelium of the renal distal convoluted tubule at 1% (no further information available)	Anonymous 18, 1973

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

No human data and other studies available

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

In a reproductive toxicity study (Anonymous 12, 2000), following OECD TG 421, groups of 12 male and 12 female rats were given 4,4'-sulphonyldiphenol via gavage at concentrations of 0, 10, 60 or 300 mg/kg bw/d. Males were exposed for a total of 45 days including 14 days of pre-mating period, through mating period to the day before necropsy. While, females were exposed for a total of 40 to 46 days (from mating period through gestation to lactation day 3).

Excessive salivation was observed just before or immediately after administration of the test substance in 7 males and 1 female exposed to the highest dose, however all of them recovered within 30 minutes after administration. The food consumption was examined during the premating period (day 3, 7, 14), during the gestation and during the lactation. A statistically significant decrease of food consumption was only observed at day 3 of the premating period in males and females at the high dose group (24.3 mg/kg bw/d vs 30.7 mg/kg bw/d in control group and 14.8 mg/kg bw/d vs 20.2 mg/kg bw/d in control group, respectively in males and females). The body weight was analyzed in males and females at the same time as food consumption examination. A statistically significant decreased body weight was noted in females at the highest dose at the end of the gestation period (see table 9).

Table 9 : body weight data (in g)

Dose level (mg/kg bw/d)			0	10	60	300
Males		Nb. of animals examined	12	12	12	12
		D 0	351.9	354.3	352.5	354.2
		D 3	373.8	373.5	373.7	357.5*
		D 14	435.9	435.9	437.9	404.5**
		D 42	511.8	514.5	523.5	486.0
		BWG D 0-42	159.9	160.2	171.0	131.8
Females	Premating period	Nb. of animals examined	12	12	12	12
		D 0	229.1	228.4	228.2	230.3
		D 14	264.2	263.8	262.3	251.7
		BWG D 0-14	35.1	35.4	34.1	21.4**
	Gestation period	Nb. of animals examined	11	11	12	7
		D 0	272.8	277.1	266.8	264.4
		D 20	436.5	433.1	418.6	390.4**
		BWG D 0-20	163.6	156.0	151.8	126.0**
	Lactation period	Nb. of animals examined	11	11	12	7
		D 0	325.5	327.5	314.3	316.0
		D 4	360.1	354.1	333.0*	338.6
		BWG D 0-4	34.5	26.5	18.7	22.6

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

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

Table 10 : reproductive performance

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

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

Table 11 : female reproduction delivery data

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

** : p<0.01

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

Table 12 : organ weights

	Males				Females			
Dose level (mg/kg bw/d)	0	10	60	300	0	10	60	300
FBW (g)	513.4	517.3	526.7	488.1	360.1	354.1	333.0*	338.6
Pituitary (mg)	14.92	13.60	15.12	16.68	21.18	21.64	21.45	21.07
Pituitary rel. ($*10^{-3}$)	2.89	2.63	2.88	4.43**	5.90	6.14	6.45	6.21
Thymus (mg)	289.6	336.3	332.1	254.5	263.1	312.7	253.5	221.7
Liver (g)	16.373	16.246	16.803	17.439	15.289	14.114	14.490	14.393
Liver rel.	3.185	3.135	3.185	3.562**	4.247	3.989	4.359	4.246
Testes (g)	3.559	3.480	3.554	3.503	-	-	-	-
Prostate (g)	0.723	0.746	0.777	0.708	-	-	-	-
Sem. ves. (g)	2.825	2.718	2.860	2.428**	-	-	-	-
Epididymis (g)	1.355	1.292	1.328	1.292	-	-	-	-
Ovaries (mg)	-	-	-	-	110.35	116.02	114.86	105.63
Uterus (g)	-	-	-	-	0.691	0.683	0.713	0.700

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

Table 13 : incidence of histopathological findings

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

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

Regarding offspring examination, see section 10.10.5.

In an extended-one generation reproductive toxicity study (Anonymous 13, 2019), performed following OECD TG 443, groups of male and female rats were given 4,4'-sulphonyldiphenol at a concentration of 0, 20, 60 and 180 mg/kg bw/d.

For the F0 parental generation, minimum 10 weeks after the beginning of exposure, 24 males and 24 females from the same dose group were mated. Shortly before weaning of the F1 pups, the F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups. Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after weaning, placed into cohorts.

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

F0 parental and F1 pups (before weaning) :

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

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

Regarding female reproduction parameters, the mean oestrus cycle duration was significantly increased at the highest dose (see table 14). At 20 mg/kg bw/d, 1 female exhibited a mean oestrus length of 5.3 days and one other female had a mean cycle length of 4.0 days; however, this last female showed an oestrus cycle with a dioestrus period of 9 days. Two females exposed to 180 mg/kg bw/d exhibited a mean cycle length of 4.7 and 5.0 days. This last one had one cycle with a dioestrus period of 5 days. Fertility index (96, 91, 100 and 96 % respectively at 0, 20, 60 and 180 mg/kg bw/d) and duration of gestation (22.0 days in all groups) were not affected. However, a decreasing trend in the mean number of implantation sites was noted (15.3, 14.8, 14.9 and 14.3 respectively at 0, 20, 60 and 180 mg/kg bw/d).

Table 14 : oestrus cycle data

Dose level (in mg/kg bw/d)	0	20	60	180
Mean day from oestrus to oestrus (in day)	3.9	3.9	3.9	4.1*
Mean number of days in stage : prooestrus[A]	4.7	3.5	3.8	2.2
Mean number of days in stage : oestrus[A]	5.1	5.1	5.0	5.2
Mean number of days in stage : metoestrus[A]	5.8	6.0	5.8	5.9
Mean number of days in stage : dioestrus[A]	6.3	7.4	7.7	9.0

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

At necropsy, enlarged cecum and enlarged kidneys were observed in males of the highest dose (respectively in 3 males and in 6 males). Absolute and relative adrenal glands, kidneys and thymus weights were significantly modified in males and relative liver weight was significantly higher in females (see table 15).

Table 15 : Modified organ weights and microscopic findings

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

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

Regarding offspring examination, the mean number of F1 pups per dam was lower at the mid and high doses. Moreover, the mean pup body weight was significantly higher at the 2 highest dose levels. For more information see section 10.10.5.

Cohort 1A :

1 female of the highest dose was found dead on study day 0 (necropsy revealed a slight fibrinous inflammation in the lung, focal hyperplasia in the mammary gland and an atrophic uterus). 12 males and 14 females of the highest dose exhibited transient salivation immediately after dosing. Body weight was significantly higher in females exposed to 60 and 180 mg/kg bw/d at D 14 and 28 (see table 16).

Table 16 : body weight data (in g)

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

* : $p<0.05$

Regarding reproduction parameters, sperm was examined and did not show any modification. In females, during the 2 weeks of observation, oestrus cycle was of 4.1 days in all groups, but showed prolonged dioestrus stage, as in the other cohorts observed (see table 17).

Table 17 : oestrus cycle data

Dose level (in mg/kg bw/d)	0	20	60	180
Mean day from oestrus to oestrus (in day)	4.1	4.1	4.1	4.1
Mean number of days in stage : prooestrus[A]	2.2	2.0	2.2	1.3
Mean number of days in stage : oestrus[A]	3.5	3.6	3.5	3.2
Mean number of days in stage : metoestrus[A]	3.8	3.9	3.6	4.1
Mean number of days in stage : dioestrus[A]	4.5	4.6	4.8	5.4

* : $p<0.05$

[A] : Oestrus cycle data was generated during the last 3 weeks prior to mating. These mean were calculated by the DS

At the end of the exposure period (approximately 90 days), animals were sacrificed and necropsied. No macroscopic dose related findings were observed. Some organs exhibited weight differences (see table 18). As in the F0 parental generation histopathological changes were observed in kidneys (medullar mineralization, nuclear crowding and tubular dilatation). Moreover, an increased incidence in atrophy of mammary gland was observed at the highest dose (in 1, 0, 2 and 7 males respectively at 0, 20, 60 and 180 mg/kg bw/d).

Table 18 : organ weight changes

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

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

Cohort 1B :

During the study period, 1 female of the mid dose group was found dead on premating D 3 (histopathological examination not performed). Clinical observation revealed excessive salivation immediately after exposure to the test substance in 11 males and 9 females during the in-life period and in 10 females during gestation period. Significant body weight changes was observed in both sexes during the in-life period (see table 19 and 20).

Table 19: male body weight data (in g)

Dose level (in mg/kg bw/d)		0	20	60	180
In-life period	D 0	79.8	80.1	82.3	78.9
	D 14	190.0	179.2	177.6*	173.7**
	D 21	253.9	250.3	260.4	248.4
	D 49	422.2	416.4	437.7	405.5
	D 70	489.2	481.8	502.7	466.8
Parental period	W 0	503.0	498.2	517.7	479.7
	W 5	564.3	559.2	579.7	541.6

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

Table 20: female body weight data (in g)

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

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

Regarding reproduction data, fertility index was not affected (100, 100, 96, 96 % respectively at 0, 20, 60 and 180 mg/kg bw/d). However, a slightly reduced number of females with liveborn pups was observed at 60 and 180 mg/kg bw/d. The oestrus cycle was also modified at the highest dose level. Furthermore, the mean number of implantation sites tended to decrease at 180 mg/kg bw/d. (see table 21)

Table 21 : fertility data

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

Statistical examination was not performed regarding the mean day of oestrus's stage ; ** : $p<0.01$

[A] : Oestrus cycle data was generated during the last 3 weeks prior to mating. These mean were calculated by the DS

Shortly before weaning, parental animals were sacrificed. Necropsy revealed enlarged kidneys in 1 male of the mid dose and in 10 males of the highest dose. 3 organs showed weight modifications (see table 22). All other weight parameters did not show significant differences. Regarding the histopathological examination, an atrophy of the mammary gland was only noted in 1 male of each group.

Table 22 : organ weight data

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

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

[A] : S.d : 24.228, 20.968, 15.011 and 30.56, respectively at 0, 20, 60 and 180 mg/kg bw/d

Regarding offspring examination, the incidence of post-implantation loss and the number of liveborn pups were affected (for more information see section 10.10.5)

Cohort 2A :

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

Cohort 2B :

Necropsy examination did not reveal abnormalities.

Cohort 3 :

One female of the lowest dose was found dead on the study day 18. During the exposure period, clinical observation and body weight examination were not affected. No indication of toxicity of reproduction observed in this cohort. For developmental toxicity of the immune system, see section 10.10.5.

In a reproduction/developmental toxicity screening study *(*Anonymous 14, 2017*)*, performed as a range finding study preceding the EOGRTS, groups of 10 male and 10 female rats were given 4,4'-sulphonyldiphenol at a concentration of 0, 30, 100 and 300 mg/kg bw/d. Animals were exposed for 10 w (for males : 6 w of premating period, 2 w of mating period, and 4 w of post mating period ; and for females : 6 w of premating period, 2 w of mating period, and continued through gestation and lactation periods).

No premature death occurred during the exposure period. At the highest dose, animals exhibited excessive salivation and a lower bw value (-7 % and -6 % respectively in males and females, compared to the control group).

Reproductive data were examined and revealed effects at the highest dose. Females exhibited prolonged oestrus cycle and pregnant females had a significantly lower mean number of implantation sites.

Table 23 : reproductive data

Dose level (in mg/kg bw/d)	0	30	100	300
Mean duration of oestrus cycle (d)	4.02	3.97	4.01	5.16**
Fertility index (%)	100	90	100	60
Mean nb of implantation sites	15.8	15.0	15.5	10.4**
Females without implantation sites	0	0	0	2
Mean duration of gestation (d)	22	22.1	22	22

* p<0.05 ; ** : p<0.01

Necropsy revealed treatment-related effects. Macroscopic examination revealed changes in males. Cecum was dilated in 3 males exposed to 300 mg/kg bw/d. Moreover, kidneys were discolored in 8 males and enlarged in 9 males of the highest dose and liver was enlarged in 1, 2 and 3 males respectively at 30, 100 and 300 mg/kg bw/d). Significant higher relative kidneys weight was observed in males (+ 11.5 and + 35 % respectively at 100 and 300 mg/kg bw/d) and significant higher relative liver weight was noted in males at the highest dose (+ 11 %) (see table 24). Furthermore, uterus weight was increased in females exposed to 300 mg/kg bw/d. Microscopic examination revealed also changes in these organs as well as in mammary gland in males (see table 25).

Table 24 : Organ weight data (in g)

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

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

Table 25 : microscopic data

		Males				Females			
Dose level (in mg/kg bw/d)		0	30	100	300	0	30	100	300
Cecum	Dilatation	0	0	0	3	0	0	0	0
	Thickening of wall	0	0	5	9	0	0	0	0
	Increased apoptosis	0	0	3	9	0	0	0	0
Kidneys	Degeneration/regeneration	0	0	6	10	0	0	0	0
	mineralization	0	0	2	2	1	0	0	4
	Tubular distension	0	0	5	10	0	0	0	0
Liver	Infiltration lymphoid	10	1	2	10	10	0	0	10

	Multifocal necrosis	1	0	1	1	0	0	0	0
Mammary gland	Diffuse atrophy	0	0	0	10	0	0	0	0

Pups were recorded and examined. At the highest dose, the total number of pups delivered was reducedand the mean number of pups delivered was significantly lower. Fore more information see section 10.10.5

In a 28-day repeated dose toxicity study (Anonymous 15, 2017), performed as a range finding study preceding the EOGRTS, 5 male and 5 female rats were given 0, 100, 300 or 600 mg/kg bw/d of 4,4'-sulphonyldiphenol. Animals were exposed daily during 28 days.

A significantly lower bwg value was noted in males of the highest dose level (no further information available).

At the end of the exposure period, animals were sacrificed and examined. Males exhibited a significantly decreased FBW (-9 and -12 % respectively at 300 and 600 mg/kg bw/d, compared to control). Enlarged kidneys were noted in 3 males exposed to 300 mg/kg bw/d and in 4 males exposed to 600 mg/kg bw/d. The relative kidneys weight was higher at the mid and high dose in both sexes (+33 % and +12 % respectively in males and females, compared to the control group). Microscopic examination revealed also changes in kidneys. In 2, 5 and 5 males exposed respectively to 100, 300 and 600 mg/kg bw/d, minimal to moderate tubular degeneration/regeneration in kidneys was noted. Moreover, tubular hypertrophy was observed in 5 males of the highest dose (moderate hypertrophy), in 5 males of the mid dose (minimal hypertrophy) and in 1 male of the low dose (minimal hypertrophy). The relative adrenals weight was higher in males (+18 and +39 % respectively at 300 and 600 mg/kg bw/d, compared to the control group) and minimal hypertrophy/hyperplasia in the adrenal cortex was observed in 3 males exposed to 600 mg/kg bw/d. Liver exhibited also changes. The relative liver weight was increased in females (+9 and +12 % respectively at 300 and 600 mg/kg bw/d, compared to the control group). Centrilobular hypertrophy of the liver was noted in 1 males of the low dose (minimal hypertrophy), in 4 males of the mid dose (slight hypertrophy) and in all animals of the high dose (moderate hypertrophy in males and slight in females) (no further information available).

Examination of the reproductive organs revealed lower relative prostate and seminal vesicles weight at the highest dose (-15 % for prostate and -16 % for seminal vesicles). 3 males exposed to 300 mg/kg bw/d and 4 males exposed to 600 mg/kg bw/d exhibited diffuse atrophy of the mammary gland (no further information available).

In a 28-day repeated dose toxicity including 2-week observation of reversibility (Anonymous 16, 1999), performed similarly to OECD TG 407, main groups of 6 male and 6 female rats were given diets containing 4,4'-sulphonyldiphenol at a concentration of 0, 40, 200 and 1000 mg/kg bw/d during 28 days. Additionally of these main groups, recovery groups of 6 male and 6 female rats were given diets containing 4,4'-sulphonyldiphenol at a concentration of 0, 200 and 1000 mg/kg bw/d during 28 days and thereafter were observed during 2w.

During the dosing period, 2 males exposed to the highest dose died (1 after 13 D and 1 after 21 D). The clinical sign examination revealed an abdominal distension at the highest dose group in 1 female after 15 days of exposure and in 5 females after 28 days of exposure. During the recovery period, this effect disappeared.

Significant lower body weight values were recorded at the highest dose level in males during the dosing period. This significant reduction disappeared during the recovery period. In females, only a slight decrease was observed. However, the body weight gain (D 1–28) was significantly reduced at the highest dose in both sexes. (see table 26)

Table 26 : body weight data during dosing and recovery period (in g)

	Males				Females			
Dose level (mg/kg bw/d)	0	40	200	1000	0	40	200	1000
Exposure period								
D 1	217	214	215	215	165	165	166	168
D 14	330	324	325	281**	216	216	210	206
D 28	409	402	401	337**	258	256	244	240
BWG (1 – 28)	192	187	186	122**	93	91	78*	72**
Recovery period								
D 1	398	/	411	330**	258	/	250	242
D 14	457	/	465	416	286	/	278	269
BWG (1 – 14)	59	/	54	86**	28	/	58	27

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

Examination of the haematological and clinical chemistry parameters revealed significant changes (see table 27 and 28). Significant decrease of RBC, haemoglobin and haematocrit were observed in both sexes at the highest dose level and a significant reduced prothrombine time was noted in females. Furthermore, in males, a significant higher ALP activity and a significant lower LDH activity were noted at the highest dose level. Whereas, in females, a significant increase of total protein, albumin and calcium and a significant decrease of total cholesterol was observed.

Table 27 : haematological findings

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

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

Table 28 : blood chemical findings

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

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

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

Regarding the organ weight examination, animals necropsied at the end of dosing period showed, at 1000 mg/kg bw/d, significant changes of absolute thymus and lung weights in both sexes and significant changes of absolute heart and adrenal glands weights in males (See table 29). While, animals necropsied at the end of recovery period exhibited, at 1000 mg/kg bw/d, significant higher absolute adrenal glands weight in males (59, 60 and 80** mg respectively at 0, 200 and 1000 mg/kg bw/d), significant higher relative kidneys weight in males and females (0.70/0.65, 0.73/0.75** and 0.81**/0.72* respectively in females/males at 0, 200 and 1000 mg/kg bw/d), significant higher relative liver weight in females (2.79, 2.82 and 3.21* respectively at 0, 200 and 1000 mg/kg bw/d).

Table 29 : absolute and relative organ weights data of animals necropsied at the end of dosing period

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

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

Weight (absolute and/or relative) of adrenals, liver and thymus were modified. These organs were also affected at the histological level. Furthermore, cecum examination revealed a hyperplasia of the mucosa and single cell necrosis of the mucosal epithelium at 200 and 1000 mg/kg bw/d. Whereas, the histopathological examination of animals necropsied at the end of the recovery period revealed significant changes in femur (increase spongy bone in 4* females at 1000 mg/kg bw/d), in spleen (significant increase of extramedullary haematopoiesis). Moreover, hyperplasia of cecum mucosa was observed in 3 males and in 1 female at the highest dose level (vs in 0 males and in 0 females in control group). (See table 30)

Table 30 : histopathological findings in animals necropsied at the end of the dosing period

			Males				Females			
Dose level			0	40	200	1000	0	40	200	1000
Main groups										
Cecum	Hyperplasia mucosa	P	0/6	0/6	6*/6	5*/5	0/6	0/6	5**/6	6**/6
		Slight			2	2			4	5
		Mild			4	3			1	1
	Single cell necrosis, mucosal epithelium	P	0/6	0/6	4*/6	5*/5	0/6	0/6	4*/6	4*/6
		Slight			3	2			4	1
		Mild			1	3				2
		Moderate								1
Liver	Hypertrophy centrilobular	P	0/6	0/6	0/6	3*/5	0/6	0/6	0/6	3/6
		Slight				3				3
Adrenals	Hypertrophy, zona fasciculata	P	0/6	0/6	0/6	4*/5	0/6	0/6	0/6	0/6
		Slight				4				
Thymus	atrophy	P	0/6	0/6	0/6	4**/5	0/6	0/6	0/6	4*/6
		Slight				4				4
Femur	Increase spongy bone	P	0/6	0/6	0/6	5**/5	0/6	0/6	0/6	4*/6
		Slight				5				4
Spleen	Haematopoeisis, extramedullary	P	6/6	6/6	6/6	5/5	0/6	0/6	6/6	6/6
		Slight	6	5	5	2			5	4
		Mild		1	1	2			1	2
		Moderate				1				
Recovery groups										
Cecum	Hyperplasia, mucosa	P	0/6	/	1/6	3/5	0/6	/	1/6	1/6
		Slight				1				1
		Mild			1	2			1	
Femur	Increase spongy bone	P	0/6	/	0/6	1/5	0/6	/	0/6	4*/6
		Slight				1				4
Spleen	Haematopoeisis extramedullary	P	6/6	/	6/6	5**/5	6/6	/	6/6	6*/6
		Slight	3		2		5		5	1
		Mild	3		4		1		1	3
		Moderate				5				2

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

In a 90-day repeated dose toxicity study similar to OECD TG 408 (Anonymous 17, 2014), groups of 10 male and 10 female rats were exposed via gavage to 4,4'-sulphonyldiphenol at a concentration of 0, 100, 300 or 1000 mg/kg bw/d. The group of male rats receiving 1000 mg/kg bw/d was changed to 600 mg/kg bw/d onwards 70 days.

The animals of the 2 highest dose presented soft and discoloured faeces and excessive salivation. The body weight decreased in males at the mid dose level and was significantly lower in males at the highest dose. Furthermore, the body weight gain reduced significantly in males at the 2 highest dose levels. (See table 31)

Table 31 : body weight and body weight gain examination (in g)

	Males				Females			
Dose level (mg/kg bw/d)	0	100	300	1000/600	0	100	300	1000
D 0	158.4	157.1	158.1	158.2	126.1	127.0	126.0	126.7
D 42	351.4	343.8	326.0	293.3**	208.4	204.7	202.8	205.2
D 91	417.1	400.7	377.3	334.7**	237.3	231.7	225.0	222.5
BWG (D 0-91)	258.7	243.6	219.2*	176.4**	111.2	104.6	99.0	95.9

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

Blood examination revealed significant haematological changes and significant modification of enzymes. RBC and haemoglobin were significantly lower at the highest dose in both sexes. Other changes were observed however not in both sexes. (see table 32 and 33)

Table 32 : haematological findings (examined at the end of the administration period)

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

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

Table 33 : enzyme data (examined at the end of the administration period)

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

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

[A] : S.d : 0.12, 0.47, 0.16 and 0.46, respectively at 0, 100, 300 and 1000/600 mg/kg bw/d

At necropsy, a dilatation of cecum was observed in all males exposed to 1000 mg/kg bw/d of 4,4'-sulphonyldiphenol, while the liver was enlarged in 8 females out of 10 at this dose level. An uterus dilatation was also observed in 3 females at the mid dose level. After this external examination, the organ weight was recorded and revealed a significant reduction of the male reproductive organs. Moreover, significant lower brain and thymus weights were observed in both sexes at the highest dose whereas a higher adrenal glands weight was noted. (See table 34)

Table 34 : organ weight (relative weight in %)

		Males				Females			
Dose level (mg/kg bw/d)		0	100	300	1000/600	0	100	300	1000
FBW (g)		394.02	376.95	356.6**	311.89**	221.72	214.72	207.95	205.6
Adrenal glands (g)	Abs	64.5	59.1	63.7	90.1**	65.6	64.5	74.6	80.4**
	Rel	0.016	0.016	0.018	0.029**	0.03	0.03	0.036*	0.039**
Brain (g)	Abs	2.212	2.098**	2.074**	2.084**	2.007	1.992	1.99	1.913*
	Rel	0.565	0.564	0.583	0.675**	0.91	0.931	0.962	0.932
Heart (g)	Abs	1.115	1.039	1.026*	0.958**	0.752	0.739	0.755	0.763
	Rel	0.284	0.277	0.288	0.309*	0.341	0.344	0.364	0.371*
Kidneys (g)	Abs	2.507	2.646	2.762	2.485	1.5	1.489	1.584	1.644*
	Rel	0.636	0.702*	0.775**	0.795**	0.679	0.695	0.765*	0.799**
Liver (g)	Abs	8.936	8.402	8.415	8.347	5.106	5.39	5.688	7.043**
	Rel	2.269	2.226	2.359	2.676**	2.297	2.502	2.75**	3.433**
Spleen (g)	Abs	0.628	0.585	0.535**	0.595	0.44	0.447	0.465	0.454
	Rel	0.16	0.156	0.15	0.19**	0.198	0.209	0.224**	0.22*
Thymus (mg)	Abs	327.5	269.4	271.3	226.1**	303.2	292.4	245.3	222.7**
	Rel	0.084	0.071	0.076	0.073	0.136	0.136	0.118	0.108*
Epididymides (g)	Abs	1.209	1.16	1.126	1.072**	-	-	-	-
	Rel	0.308	0.31	0.316	0.346**	-	-	-	-
Testes (g)	Abs	3.914	3.862	3.636*	3.592*	-	-	-	-
	Rel	0.999	1.035	1.021	1.162**	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	104.7	104.0	106.9	126.9
	Rel	-	-	-	-	0.047	0.048	0.052	0.061*
Uterus (g)	Abs	-	-	-	-	0.724	0.864	1.284	0.648
	Rel	-	-	-	-	0.332	0.41	0.615	0.315

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

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

Table 35 : incidence of histopathological findings

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

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

In a 13-day repeated dose toxicity study not performed according to an OECD guideline (Anonymous 18, 1973), groups of 5 male rats were given diets containing 4,4'- sulphonyldiphenol at a concentration of 0, 0.1 or 1 % (corresponding to 0, 97 and 810 mg/kg bw/d).

Body weight was severely depressed at the highest dose level (no further information available). Blood examination revealed a slight increase in RBC count, haemoglobin concentration and haematocrit and a lower aspartate aminotransferase value at the highest dose. Moreover, a slight increase in haemoglobin concentration was already observed at the low dose level (no further information available).

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

10.10.3 Comparison with the CLP criteria

Criteria for Category 1	Criteria for category 2
"Known or presumed human reproductive toxicant Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human (category 1A) or from animal data (category 1B). Category 1A : known human reproductive toxicant. The classification is largely based on evidence from humans Category 1B : presumed human reproductive toxicant. The classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate."	"Suspected human reproductive toxicant Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

Since no human studies are available for effects on fertility, classification in Repr. 1A for fertility is not appropriate.

4,4'-sulphonyldiphenol consistently and severely disturbed reproductive parameters such as the number of implantation sites and the oestrus cycle.

The mean number of implantation sites was reduced by 33 % in females exposed to 300 mg/kg bw/d in the reproductive toxicity study (Anonymous 12, 2000) (10.7 vs 15.9 in the control group). The implantation index at the same dose level, in this study, was 64.89** % vs 95.80 % in the controls. The same reduction in the mean number of implantation sites was also observed in the combined repeated dose toxicity study with the reproduction/developmental screening test (Anonymous 14, 2017), with 10.4** vs 15.8 at 300 and 0 mg/kg bw/d, respectively.

In the EOGRTS (Anonymous 13, 2019), the number of implantation sites was moderately modified only in the cohort 1B (13.7 vs 15.2 at 180 and 0 mg/kg bw/d, respectively). However, the DS wants to highlight that this effect appeared at a much lower dose than in Anonymous 12 (2000) and in Anonymous 14 (2017).

Regarding the oestrus cycle, at 300 mg/kg bw/d, a significant increase in the mean duration of the oestrus cycle (5.57 ** vs 4.08 days in the control group) was observed in the reproductive toxicity test (Anonymous

12, 2000). Furthermore, in the same study, 5 females out of 12 showed a prolonged dioestrus period (6-10 days) and 4 out of 5 did not conceive at all, leading to a severely reduced fertility index in the highest dose group (58.3 % vs 91.7 % in the control group).

In the EOGRTS (Anonymous 13, 2019), the mean duration of the oestrus cycle in the parental generation was significantly increased at 180 mg/kg bw/d (4.1* vs 3.9 days in the control group). Also, as observed in Anonymous 12 (2000), the mean dioestrus period was increased in the highest dose group (9.0 at 180 mg/kg bw/d vs 6.3 days in the controls). The cohort 1B exhibited the same trend as the mean duration of the oestrus cycle was increased at the highest dose (4.5 d vs 3.9 d in the controls) and the mean number of days in dioestrus was also prolonged (11.8 d vs 6.8 d at 180 mg/kg bw/d and in controls, respectively). The mean duration of oestrus cycle was not prolonged during the short observation period of cohort 1A, but the same trend prolongation in dioestrus was also observed (5.4 vs 4.3 days at 180 mg/kg bw/d and in the control group, respectively).

Furthermore, in Anonymous 14 (2017), a significant increase in the mean duration of oestrus cycle was observed at the highest dose (5.16** d at 300 mg/kg bw/d vs 4.02 d in controls).

All these severe effects cannot be explained by maternal toxicity as general condition of the animals was unaffected by the treatment in all studies. Animals only exhibited excessive salivation just before or immediately after exposure to the test substance in all studies. Moreover, body weight examination showed only slight variations during the premating and mating periods that cannot be accounted for these effects.

Severe decreased number of implantation sites and severe higher oestrus duration were observed in three different studies. These effects were more pronounced in the reproductive toxicity test (Anonymous 12, 2000) and in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 14, 2017). In the EOGRTS (Anonymous 13, 2019), the highest tested dose was only 180 mg/kb bw/d (compared to 300 mg/kg bw/d in Anonymous 12 (2000) and Anonymous 14 (2017)). At this top dose, nearly absent general toxicity was observed. The DS wants to highlight that females exposed to 180 mg/kg bw/d exhibited already significant fertility effects which would be more pronounced if the study would have been dosed higher as it is the case in the Anonymous 12 (2000) and Anonymous 14 (2017).

According to the CLP criteria a classification as Repr. 1B for adverse effects on sexual function and fertility is warranted based on the above mentioned severe effects observed in the available studies, which cannot be related to a general toxicity.

10.10.4 Adverse effects on development

Table 36: Summary table of animal studies on adverse effects on development

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study rat / wistar 25 pregnant females/group Gavage Following OECD TG 414 GLP	4,4'-sulphonyldiphenol Vehicle : 1 % CMC Dose : 0, 30, 100 and 300 mg/kg bw/d Exposure : gestational day	<u>Dams :</u> Clinical signs : excessive salivation noted in 7 out of 25 females exposed to 300 mg/kg bw/d Bw : no sign change (however bwg GD 6 – 19 and GD 8 - 10 were sign lower) Uterus weight : no effects Necropsy observation : no treatment-related effects Reproduction data (nb of dams with viable foetuses,	Anonymous 19, 2014

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	(GD) 6 to 19 Sacrificed at GD 20	corpora lutea, implantation sites, pre and post implantation loss, resorption) : no effects <u>Offsprings :</u> Sex ratio : unaffected Mean nb of live foetuses : no effects Bw : unaffected A few skeletal variations observed	
Reproductive toxicity test Rats / Spargue-Dawley (SD) 12/sex/group Gavage Following OECD TG 421 GLP	4,4'-sulphonyldiphenol Vehicle : 0.5 % aqueous sodium CMC solution with 0.1 % Tween 80 Doses : 0, 10, 60 and 300 mg/kg bw/d Exposure : a total (tot.) of 45 D for males (including 14 D of premating period, through mating to the day before necropsy) and a total of 40 to 46 D for females (from premating, mating, gestation until lactation day (LD) 3) (females without delivery were exposed until D 25 after confirmation of copulation)	<u>Parental generation :</u> Clinical signs : excessive salivation at 300 mg/kg bw/d Bw : reduced at the highest dose in both sexes (see table 9) Gross necropsy findings : distension of cecum observed in 1 male (♂) and 1 female (♀) in the mid dose level and in all ♂ and 4 ♀ at the highest dose level Organ weight : in ♂ : sign. increase of relative (rel.) pituitary and rel. liver weights and sign. decrease of seminal vesicle weight In ♀ : no sign. changes at the highest dose observed Histopathology : cecum : sign. increased incidence of hyperplasia of the mucosal epithelium (in 11 ♂) and sign. higher incidence of single cell necrosis (in 5 ♂) at the highest dose Liver : centrilobular hypertrophy of hepatocytes observed in 5 ♂ at 300 mg/kg bw/d <u>Offspring :</u> Decreased mean nb of offspring at birth at 300 mg/kg bw/d (9.1 at 300 mg/kg bw/d vs 14.3 in control group) No abnormalities in external appearance and clinical signs nor bw, bwg, viability index, ano-genital distance (AGD)	Anonymous 12, 2000
EOGRTS with F2, DNT (cohorts 2A and 2B) and DIT (cohort 3)Rats / SD F0 generation : 24/sex/dose F1 generation : 20/sex/dose for cohort 1A, 24/sex/dose for cohort 1B, 10/sex/dose for cohorts 2A, 2B and 3 Gavage Following OECD TG 443	4,4'-sulphonyldiphenol Vehicle : 0.5 % CMC Doses : 0, 20, 60 and 180 mg/kg bw/d Duration of exposure : Minimum 10 w after the beginning of	<u>Parental generation :</u> Bw : sign. higher only in ♀ during the in-life period Mean nb of implantation site : reduced at the highest dose (14.3 vs 15.3 in control) Mean nb of post-implantation loss sign. affected (1.5** vs 0.5 in control) Necropsy : enlarged cecum and changes in kidneys observed in ♂ at 180 mg/kg bw/d <u>F1 pups :</u> Sign. lower tot. nb. of liveborn (285* at 180 mg/kg	Anonymous 13, 2019

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
GLP	exposure, males and females from the same dose group were mated. Shortly before weaning of the F1 pups, the F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups. Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after weaning, placed into cohorts.	bw/d vs 340 in control) and sign. higher nb of stillborn (8* at 180 mg/kg bw/d vs 2 in control) <u>Cohort 1A :</u> FBW : slightly reduced at 180 mg/kg bw/d in ♂ BW : in ♀, body weight was sign. higher at D14 and D28 in the mid and high dose groups Necropsy : adrenal glands, kidneys, liver, spleen, thymus and prostate showed a significant deviation in abs. or rel. value An atrophy of the mammary gland was noted in ♂ of the highest dose <u>Cohort 1B :</u> Lower mean nb of implantation sites and sign. higher incidence of post-implantation loss at 180 mg/kg bw/d Necropsy : FBW slightly reduced in ♂ at the highest dose and a few organ weights modified <u>Cohort 2A :</u> Auditory startle response, home cage observations, open field observations, sensorimotor tests/reflexes, motor activity measurements and learning and memory tests : unaffected <u>Cohort 2B :</u> Necropsy examination : no effects observed <u>Cohort 3 :</u> Clinical and bw examination : unaffected Necropsy : sign lower rel. thymus weight at 180 mg/kg bw/d T-cell dependent antibody response : slight change in the low and mid dose in ♀ <u>F2 pups :</u> Decrease tot. nb of pups delivered at the highest dose	
Range finding study preceding the EOGRTS, Similar to a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test Rats / SD 10/sex/dose Gavage Similar to OECD TG 422	4,4'-sulphonyldiphenol Vehicle : CMC Doses : 0, 30, 100 and 300 mg/kg bw/d Duration of exposure : 10w for males and continued through premating, gestation, and	<u>F0 generation :</u> Mortality : no premature death Clinical signs : excessive salivation observed at the highest dose Bw : lowered at the highest dose (-7 % in ♂ and -6 % in ♀ compared to the control group) Haematology and clinical biochemistry : no effects (no further information available) Gross pathological findings : increased incidence of cecum dilatation, enlarged and discoloration of kidneys and enlarged liver in ♂ exposed to 300 mg/kg bw/d	Anonymous 14, 2017

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
GLP	lactation periods for females	Organ weight : sign. higher rel. kidneys weight in ♂ (+11.5 and +35 % respectively at 100 and 300 mg/kg bw/d) and sign higher rel. liver weight in ♂ at the highest dose (+11 %). In ♀, uterus weight modified at 300 mg/kg bw/d Histopathology : changes observed in kidneys in both sexes. Furthermore, mammary gland and cecum also affected in ♂ % of post-implantation loss : sign. higher at 300 mg/kg bw/d (34.6* vs 3.6 % in control group) F1 : Mean nb of pups delivered sign decreased at the highest dose (10.8** vs 15.2) Pups bw sign. higher in ♂ of the low dose at PND 21 (+6.6 % compared to the control group) (no further information available) Gross pathological findings : no effects observed (no further information available)	

No human data or other studies available

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

In a prenatal developmental toxicity study following OECD TG 414 (Anonymous 19, 2014), groups of 25 pregnant rats were given 4,4'-sulphonyldiphenol via gavage at a concentration of 0, 30, 100 or 300 mg/kg bw/d. The animals were exposed to the test substance from gestational day 6 to 19 and were sacrificed at gestational day 20.

No mortality was noted during the exposure period. Furthermore, the clinical examination revealed only an increased incidence of females with excessive salivation. However, the maternal care was not affected. No significant body weight change was observed during the dosing period. However, the body weight gain value for GD 6 – 19 and GD 8 - 10 were significantly decreased at the highest dose (see table 37).

Table 37 : body weight data (in g)

Dose level (mg/kg bw/d)	0	30	100	300
GD 0	164.9	167.5	168.7	165.6
GD 6	195.9	199.1	199.2	198.3
GD 15	239.3	243.5	240.6	236.1
GD 20	295.9	302.4	297.8	291.0
GD 8 – 10	9.6	9.3	9.4	6.8*
GD 6 – 19	85.2	89.8	84.3	78.6*
Corrected bwg (terminal bw on GD 20 minus uterus weight minus bw on GD 6)	40.9	43.7	40.0	36.9

* : $p<0.05$; only pregnant dams with scheduled sacrifice (GD 20) were used for the calculation of bw. 1 female of the highest dose was excluded as this rat was not pregnant.

For dams, no test substance-related changes were observed for the gravid uterus weight (59.1, 59.6, 58.7 and 55.8 g respectively at 0, 30, 100 and 300 mg/kg bw/d), neither for the necropsy observation nor for the reproduction data parameters (see table 38).

Table 38 : reproductive data

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

The foetus examination revealed no differences in sex distribution, placental weight and foetal body weight compared to the control group (see table 39). Skeletal variations were observed in all doses. At 300 mg/kg bw/d, these variations were significant however comprised within the range of the historical control data (see table 40). No treatment-related external malformation and variation nor soft tissue malformation and variation were observed.

Table 39 : sex ratio and mean foetal weight (in g)

Dose level (mg/kg bw/d)	0	30	100	300
Sex ratio (in % females/males)	48.9/51.1	47.2/52.8	56.6/43.4	47.3/52.7
Mean placental weight	0.45	0.46	0.45	0.47
Mean weight of all viable foetuses	3.6	3.6	3.4	3.4
Mean weight of male foetuses	3.6	3.7	3.5	3.5
Mean weight of female foetuses	3.5	3.5	3.4	3.3

Table 40 : skeletal variations data

Dose level (mg/kg bw/d)	0	30	100	300	HCD mean % (range)
Nb of litter	25	25	25	24	/
Nb of foetuses	137	137	140	127	/
Tot. skeletal variations					
Foetal incidence : nb (%)	136 (99)	135 (99)	139 (99)	127 (100)	/
Litter incidence : nb (%)	25 (100)	25 (100)	25 (100)	24 (100)	/
Mean affected foetuses/litter	99.2	98.3	98.7	100.0	/
Incidence of significant increased foetal skeletal variations (mean % of affected foetus/litter)					
Incomplete ossification of supraoccipital (unchanged cartilage)	34.1	35.2	37.6	45.2*	43.5 (10.3 – 64.3)
Dumbbell ossification of thoracic centrum (unchanged cartilage)	0.7	3.0	0.0	5.6**	6.9 (0.0 – 14.5)
Unossified sternebra (unchanged cartilage)	1.5	5.0	4.6	11.0**	8.2 (2.6 – 20.7)
Incomplete ossification of pubis (cartilage present)	0.0	0.8	2.0*	1.7	0.3 (0.0 – 2.4)
Incomplete ossification of ischium (cartilage present)	0.0	0.0	2.0*	1.7	0.2 (0.0 – 0.8)

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

In a reproductive toxicity study (Anonymous 12, 2000), following OECD TG 421, groups of 12 male and 12 female rats were given 4,4'-sulphonyldiphenol via gavage at concentrations of 0, 10, 60 or 300 mg/kg bw/d. Males were exposed for a total of 45 days including 14 days of pre-mating period, through mating period to the day before necropsy. While, females were exposed for a total of 40 to 46 days (from mating period through gestation to lactation day 3).

Excessive salivation was observed just before or immediately after administration of the test substance, however all animals recovered within 30 minutes after administration. A statistically significant decrease of food consumption was only observed at day 3 of the premating period in males and females at the high dose group (24.3 mg/kg bw/d vs 30.7 mg/kg bw/d in control group and 14.8 mg/kg bw/d vs 20.2 mg/kg bw/d in control group, respectively in males and females). Furthermore, a statistically significant decreased body weight was noted in females at the highest dose at the end of the gestation period (see table 9 in section 10.10.2).

At necropsy, distension of the cecum was observed. The cecum examination revealed a significant increased incidence of diffuse hyperplasia of the mucosal epithelium and of single cell necrosis in males at 300 mg/kg bw/d. At the highest dose, the relative liver weight increased and a centrilobular hypertrophy of hepatocytes was observed in 5 males of the highest dose. In males and females, a tendency to decreased thymus weight was detected. Furthermore, in males, an increased relative pituitary weight and a decreased absolute seminal vesicle weight were observed. For more detail, see section 10.10.2.

Regarding offspring examination, a tendency to decrease in the total number of offspring at birth, number of live offspring at birth and number of live offspring on day 4 of lactation were observed in the highest dose group (see table 41). However, no significant difference in live birth index and viability index were noted (at PND 0 : 99.35, 100.00, 99.48 and 100.00 % respectively at 0, 10, 60 and 300 mg/kg bw/d and at PND 4 : 99.30, 95.45, 99.48 and 100.0 % respectively at 0, 10, 60 and 300 mg/kg bw/d). External examination did not reveal any clinical signs, body weight change (see table 42), nor anogenital distance modification. Furthermore, no abnormalities were observed in dead offspring on lactation day 0 to 4 and

live offspring on lactation day 4 in each group.

Table 41 : mean number of pups

Dose level (mg/kg bw/d)	0	10	60	300
Tot nb of offspring at birth	14.3	12.5	13.5	9.1
Tot nb of live offspring at birth	14.2	12.5	13.4	9.1
Nb of live offspring at PND 4	14.1	12.4	13.3	9.1

Table 42 : pups body weight data (in g)

Dose level (mg/kg bw/d)		0	10	60	300
Males	PND 0	7.4	7.5	7.3	7.8
	PND 4	12.0	12.4	12.1	14.1
Females	PND 0	6.9	7.0	6.9	7.3
	PND 4	11.7	11.7	11.5	13.3

In an extended-one generation reproductive toxicity study (Anonymous 13, 2019), performed following OECD TG 443, groups of male and female rats were given 4,4'-sulphonyldiphenol at a concentration of 0, 20, 60 and 180 mg/kg bw/d.

For the F0 parental generation, minimum 10 weeks after the beginning of exposure, 24 males and 24 females from the same dose group were mated. Shortly before weaning of the F1 pups, the F0 males were sacrificed whereas, the F0 females were sacrificed after weaning of the F1 pups. Before weaning of the F1 pups on PND 21, 74 animals/sex/group were randomly selected and, after weaning, placed into cohorts.

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

F0 parental and F1 pups (before weaning) :

Regarding the F0 parental generation, 1 female of the low dose group was sacrificed on study day 63 due to poor general condition. Thirteen males and 6 females exposed to 180 mg/kg bw/d exhibited transient salivation during the first weeks of exposure. However, the maternal care was not affected during gestation and lactation periods. Furthermore, a higher significant body weight value was only observed in females of the mid dose group at D 7 and 14 of the premating period.

The mean number of post-implantation loss and the mean percentage of post implantation loss were significantly higher in the mid and high dose groups (see table 43). The average litter size was dose-dependently reduced (14.9, 14.0, 13.5 and 12.7 pups/dam respectively at 0, 20, 60 and 180 mg/kg bw/d).

Table 43 : post implantation data

Dose level (in mg/kg bw/d)	0	20	60	180
Mean number of post-implantation loss	0.5	0.8	1.3*	1.5**
Mean % of post-implantation loss	3.1	5.9	9.4*	10.5**

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

At necropsy, enlarged cecum and enlarged kidneys were observed in males of the highest dose (respectively in 3 males and in 6 males). Absolute and relative adrenal glands, kidneys and thymus weights were significantly modified in males and relative liver weight was significantly higher in females. For more detail, see section 10.10.2

Regarding offspring examination, due to the higher resorptions, the mean number of F1 pups per dam was lower in all tested groups and was dose related (14.9, 14.0, 13.5, 12.7 respectively at 0, 20, 60 and 180 mg/kg bw/d). Furthermore, the number of liveborn pups was significantly reduced at the highest dose (340, 289, 322 and 285* pups respectively at 0, 20, 60 and 180 mg/kg bw/d) and the number of stillborn pups was also significantly increased at the highest dose (2, 5, 3 and 8* pups respectively at 0, 20, 60 and 180 mg/kg bw/d). Moreover, the mean pup body weight was significantly higher at the 2 highest dose levels (see table 44). Sex ratio, clinical observations, viability index, anogenital distance, vaginal opening, preputial separation and presence of areolas/nipples were not modified by exposure to the test substance.

Table 44 : pup body weight data (in g)

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

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

Cohort 1A :

1 female of the highest dose was found dead on study day 0 (necropsy revealed a slight fibrinous inflammation in the lung, focal hyperplasia in the mammary gland and an atrophic uterus). 12 males and 14 females of the highest dose exhibited transient salivation immediately after dosing. Body weight was significantly higher in females exposed to 60 and 180 mg/kg bw/d at D 14 and 28 (see table 16).

At the end of the exposure period (approximately 90 days), animals were sacrificed and necropsied. No macroscopic dose related findings were observed. Some organs exhibited weight differences (see table 18). As in the F0 parental generation histopathological changes were observed in kidneys (medullar mineralization, nuclear crowding and tubular dilatation). Moreover, an increased incidence in atrophy of

mammary gland was observed at the highest dose (in 1, 0, 2 and 7 males respectively at 0, 20, 60 and 180 mg/kg bw/d).

Cohort 1B :

During the study period, 1 female of the mid dose group was found dead on premating D 3 (histopathological examination not performed). Clinical observation revealed excessive salivation immediately after exposure to the test substance in 11 males and 9 females during the in-life period and in 10 females during gestation period. Significant body weight changes was observed in both sexes during the in-life period (see table 19 and 20).

Regarding female reproduction data, the mean number of implantation sites tended to decrease at 180 mg/kg bw/d and the mean number of post-implantation loss was significantly increased at the highest dose. (see table 45)

Table 45 : female reproduction data

Dose level (in mg/kg bw/d)	0	20	60	180
Nb of females with liveborn pups	24	24	21	21
Nb of females with stillborn pups	6	2	2	6
Mean nb of implantation sites	15.2	14.6	15.2	13.7
Tot. nb of post-implantation loss	22	18	25	76
Mean nb of post-implantation loss	0.9	0.8	1.1	3.3**
% of post-implantation loss	6.4	5.3	11.1	24.6**
Duration of gestation (in day)	22.0	21.9	22.0	22.0
Mean nb of pups delivered	14.3	13.8	14.9	11.4**

** : $p<0.01$

Shortly before weaning, parental animals were sacrificed. Necropsy revealed enlarged kidneys in 1 male of the mid dose and in 10 males of the highest dose. 3 organs showed weight modifications (adrenal glands, kidneys and liver see table 22). All other weight parameters did not show significant differences. Regarding the histopathological examination, an atrophy of the mammary gland was only noted in 1 male of each group.

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

Cohort 2A : See section 10.10.2

Cohort 2B : See section 10.10.2

Cohort 3 :

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

In a reproduction/developmental toxicity screening study *(*Anonymous 14, 2017*)*, performed as a range finding study preceding the EOGRTS, groups of 10 male and 10 female rats were given 4,4'-sulphonyldiphenol at a concentration of 0, 30, 100 and 300 mg/kg bw/d. Animals were exposed for 10 w (for males : 6 w of premating period, 2 w of mating period, and 4 w of post mating period ; and for females : 6 w of premating period, 2 w of mating period, and continued through gestation and lactation periods).

No premature death occurred during the exposure period. At the highest dose, animals exhibited excessive salivation and a lower bw value (-7 % and -6 % respectively in males and females, compared to the control group).

Reproductive data were examined and revealed effects at the highest dose. Post-implantation loss was significantly higher and 2 out of 8 pregnant females had complete intrauterine litter losses. These effects resulted in a significantly lower litter size.

Table 46 : reproductive data

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

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

Necropsy revealed treatment-related effects. Uterus weight was increased in females exposed to 300 mg/kg bw/d (0.197, 0.224, 0.224 and 0.307, respectively at 0, 30, 100 and 300 mg/kg bw/d). Microscopic examination revealed also changes in these organs as well as in mammary gland in males. For more detail, see section 10.10.2

Pups were recorded and examined. At the highest dose, the total number of pups delivered was reduced (152, 127, 145 and 65 pups respectively at 0, 30, 100 and 300 mg/kg bw/d) and the mean number of pups delivered was significantly lower (15.2, 14.1, 14.5 and 10.8** respectively at 0, 30, 100 and 300 mg/kg bw/d). At PND 21, a higher body weight value was noted in male pups of the low dose group (+ 6.6 % compared to the control group). Necropsy did not reveal gross pathological findings (no further information available).

10.10.6 Comparison with the CLP criteria

Criteria for Category 1	Criteria for category 2
"Known or presumed human reproductive toxicant Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human (category 1A) or from animal data (category 1B). Category 1A : known human reproductive toxicant. The classification is largely based on evidence from humans Category 1B : presumed human reproductive toxicant. The classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate."	"Suspected human reproductive toxicant Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

Since no human studies are available for effects on development, classification in Repr. 1A for development is not appropriate.

In the prenatal developmental toxicity study (Anonymous 19, 2014), the mean percentage of post-implantation loss was increased at the highest dose group (4.7, 3.9, 3.9 and 6.3 %, respectively at 0, 30, 100 and 300 mg/kg bw/d).

In the combined repeated dose toxicity study with reproduction/developmental toxicity screening test (Anonymous 14, 2017), the mean percentage of post-implantation loss was greatly affected by the treatment at 300 mg/kg bw/d (3.6, 5.2, 6.5 and 34.6* % at 0, 30, 100 and 300 mg/kg bw/d).

Moreover, in the parental generation of the EOGRTS (Anonymous 13, 2019), the mean number of post-implantation loss was dose-dependently increased and significant at the two highest doses (0.5, 0.8, 1.3* and 1.5** respectively at 0, 20, 60 and 180 mg/kg bw/d). This resulted in a higher percentage of post-implantation loss (3.1, 5.9, 9.4* and 10.5** % respectively at 0, 20, 60 and 180 mg/kg bw/d). As in the P

generation, the cohort 1B showed a significant increased incidence of post-implantation loss (0.9, 0.8, 1.1 and 3.3** at 0, 20, 60 and 180 mg/kg bw/d, respectively). Therefore, the mean percentage of post-implantation loss was raised accordingly at the mid-dose and significantly increased at the highest dose (6.4, 5.3, 11.1 and 24.6** % at 0, 20, 60 and 180 mg/kg bw/d). The DS wants to highlight that effects were already observed at 180 mg/kg bw/d. These effects would be again more pronounced if the study would have been dosed higher, as it is the case in the combined repeated dose toxicity study with reproduction/developmental toxicity screening test.

Post-implantation loss was increased by treatment and cannot be explained by maternal toxicity as general condition of the animals was unaffected by the treatment. Animals only exhibited excessive salivation just before or immediately after exposure to the test substance and maternal care was unaffected. Moreover, body weight examination showed variations that cannot be accounted for these effects.

According to the CLP criteria a classification as Repr. 1B for adverse effects on development is warranted based clear evidence of an adverse effect on development in the absence of toxic effect. Severe higher incidence of post-implantation loss were observed in two different studies, which cannot be related to a general toxicity.

10.10.7 Adverse effects on or via lactation

No animal studies, no human data and no other studies available

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

No information available

10.10.9 Comparison with the CLP criteria

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10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the available information, a classification as Repr. 1B H360FD is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

No human data on reproductive toxicity is included in the CLH dossier.

Animal data

Adverse effects on sexual function and fertility

The potential of bisphenol S to adversely affect sexual function and fertility was investigated in a reproduction/developmental toxicity screening test and an extended-one generation reproductive toxicity study (EOGRTS), including a range-finding study (in the

form of a combined repeated-dose toxicity study with the reproduction/developmental screening test) preceding the EOGRTS. Furthermore, relevant parameters related to sexual function and fertility were investigated in three additional repeated-dose toxicity studies with varying duration of exposure. All studies were conducted in rats exposed via the oral route, either via gavage or via diet.

In a 13-day repeated dose toxicity study (no guideline; non-GLP; Anonymous 18, 1973), bisphenol S was administered orally via diet to groups of 5 male rats (strain not specified), dosed at 0, 0.1 and 1% (corresponding to 0, 97 and 810 mg/kg bw/day). No treatment-related mortalities occurred. In the highest dose, toxicity signs included lower terminal body weights, reduced absolute kidney and liver weights, adipose tissue atrophy. Some kidney histopathological effects were also observed.

In a 28-day repeated dose toxicity study (similar to OECD TG 407; GLP; Anonymous 16, 1999), bisphenol S was administered orally via gavage to groups of 6 Sprague/Dawley rats/sex/dose at doses of 0, 40, 200 and 1000 mg/kg bw/day. Furthermore, groups of 6 Sprague/Dawley rats/sex/dose were given doses of 0, 200 and 1000 mg/kg bw/day for 28 days and followed for an additional 2 weeks to observe recovery. Two males in the high-dose group died during treatment. Terminal body weight was decreased in males (m) at the highest dose compared to controls (-20%), but for females (f) the terminal body weight remained unaffected. In the high dose group, weight increases of the liver (m/f; relative to bw), the kidneys (m; relative to bw), the adrenals (m; absolute and relative to bw), and the thymus (absolute to bw (m) and absolute and relative to bw (f)) were observed right after treatment, as well as slight histopathological effects in the cecum (m/f), the liver (m/f), the adrenals (m), the thymus (m/f), and the femur (m/f).

In a 28-day repeated dose toxicity study (similar to OECD TG 407; non-GLP; Anonymous 15, 2017) performed as a range-finding study for the EOGRTS, bisphenol S was administered orally via gavage to groups of 5 Sprague/Dawley rats/sex/dose at doses of 0, 100, 300 and 600 mg/kg bw/day. No treatment-related mortalities occurred. Toxicity at the high dose consisted of salivation (m/f), and slight reductions in terminal body weight (males -12%; females unaffected) as compared to controls. A few weight changes of the kidneys (m/f; relative to bw), adrenals (m; relative to bw), liver (f; relative to bw), prostate, seminal vesicles, and mammary gland (m), and weight decreases of the prostate (relative to bw) and the seminal vesicles (relative to bw), were observed. Some histopathological effects were observed in the kidneys (m), the adrenals (m), the liver (m/f), the cecum (m/f), and the mammary gland (m).

In a 90-day repeated dose toxicity study (OECD TG 408; GLP; Anonymous 17, 2014), bisphenol S was administered orally via gavage to groups of 10 Wistar rats/sex/dose at doses of 0, 100, 300 and 1000 mg/kg bw/day (changed from 1000 to 600 mg/kg bw/day for males after 70 days). No treatment-related mortalities occurred. Toxic effects included changes in faeces appearance and salivation (immediately after dosing, recovery within 30 minutes) in all animals in the mid- and high doses tested (m/f), decreased terminal body weights in the high dose (males -21%; females unaffected), histopathological changes (dilation of the cecum (m), enlarged liver (f), kidney mineralisation (m/f), extramedullary haematopoiesis of the spleen (m/f), uterus dilation), and changes in absolute and/or relative organ weights of several organs (e.g. kidneys, liver, adrenals, spleen, epididymis, testes). Furthermore, males in the mid- and high dose groups showed an increased incidence of multifocal mammary gland atrophy. Females showed an increased incidence of squamous metaplasia in all treated groups compared to controls.

In a reproduction/developmental toxicity screening test (OECD TG 421; GLP; Anonymous 12, 2000), bisphenol S was administered orally via gavage to groups of 12 Sprague/Dawley rats/sex/dose at doses of 0, 10, 60 or 300 mg/kg bw/day for 45 days (m) or 40-46 days (f). No treatment-related mortalities occurred. Parental toxicity at the high dose consisted of salivation right after dosing (m/f), and slight reductions in body weight as compared to controls at several time-points during pre-/post-mating and gestation (m/f) (treatment day 14: f -5%; m -7%). No statistical significance in body weight reduction was reached at any of the other days, apart from gestation day (GD) 20 when body weight of the females was 10% lower than the controls. This was not attributed to maternal toxicity, since no difference compared to controls in body weight was found on lactation day 0. In the high dose males, weight changes of the liver (increased relative to bw), the pituitary (increased relative to bw), the seminal vesicles (increased absolute weight), as well as slight histopathological changes in the liver and the cecum were observed. Females of the high dose group showed a prolonged mean oestrus cycle (5.57 days at 300 mg/kg bw/day compared to 4.08 days in controls), and an irregular oestrus cycle (5/12 at 300 mg/kg bw/day compared to 0/12 in controls). Most of the females that had a continued dioestrus phase, were not fertilised and consequently the fertilisation index was reduced to 58% (7/12). Furthermore, a decrease in mean number of implantation sites (10.7 compared to 15.9 in controls) and a statistically significantly decreased implantation index of 65% were noted at 300 mg/kg bw/day. Information on the number of pups at birth and the mean number of liveborn pups is summarised in the developmental toxicity section.

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422; GLP; Anonymous 14, 2017) performed as a range-finding study for the EOGRTS, bisphenol S was administered orally via gavage to groups of 10 Sprague/Dawley rats/sex/dose at doses of 0, 30, 100 or 300 mg/kg bw/day for 6 weeks of premating period (m/f), 2 weeks of mating period (m/f) and 4 weeks post mating period (m) or continued through gestation and lactation (f). No treatment-related mortalities occurred. Parental toxicity included slightly decreased terminal body weights (-7% (m) and -6% (f)), compared to controls at the high dose, and slight toxicity of the liver (m), the kidney (m), the cecum (m), and the mammary gland (m) at 300 mg/kg bw/day, compared to the control group. An increased number of females showed a prolonged mean oestrus cycle (5.16 days at 300 mg/kg bw/day compared to 4.02 days in controls), an increased absolute uterus terminal weight, and a fertility index of 80%[1] at the highest dose. Furthermore, a statistically significant decrease in mean number of implantation sites was noted in pregnant females at 300 mg/kg bw/day (10.4 compared to 15.8 in controls). Two out of eight females had complete intrauterine litter losses at the highest dose tested. Information on the number of pups at birth, the mean number of liveborn pups, and post-implantation loss is summarised in the developmental toxicity section below.

In an extended-one generation reproductive toxicity study (EOGRTS; OECD TG 443; GLP; Anonymous 13, 2019) with inclusion of cohorts 1A and 1B (with extension of cohort 1B to produce the F2 generation) and cohorts 2A/2B and 3, bisphenol S was administered orally

[1] Note that this specific effect was initially mentioned to be 60% at the highest dose tested in the CLH dossier and in IUCLID, but during the consultation it was noted that this effect size was incorrect and should be changed into 80% fertility at the highest dose tested. The information in IUCLID was updated to this regard as well (ECHA dissemination website consulted on 18-08-2020). The corrected value has been used in the RAC assessment; see 'Assessment and comparison with the classification criteria'.

via gavage to groups of 10-24 Sprague/Dawley rats (m/f) at doses of 0, 20, 60 or 180 mg/kg bw/day.

F0 animals

In females, body weights were significantly increased compared to controls at some timepoints (treatment day 7 and 14) at the mid dose (60 mg/kg bw/day), whereas body weights did not differ from controls in males in any of the treated groups. Water- (m/f) and food consumption (f) were significantly increased at 180 mg/kg bw/day. In the high dose males (180 mg/kg bw/day), very slight toxicity was observed in the form of enlarged cecum and changes in kidney weights. A statistically significant reduction in percentage of sperm motility was apparent in males from all treated groups; however, this reduction did not increase with increasing dose (84, 85 and 86% for the 20, 60 and 180 mg/kg bw/day dosed groups compared to 88% in controls). An increased number of females showed a prolonged mean oestrus cycle (4.1 days at 180 mg/kg bw/day compared to 3.9 days in controls), and some females showed an irregular oestrus cycle pattern at 20 and 180 mg/kg bw/day. The mean number of days in the prooestrus, oestrus, metoestrus and dioestrous stage (data generated during the last 3 weeks prior to mating), changed dose-dependently from 4.7, 5.1, 5.8, and 6.3, respectively, in controls to 2.2, 5.2, 5.9, and 9.0, respectively, in the 180 mg/kg bw/day dosed group. Fertility index was unaffected. A decrease in mean number of implantation sites was also noted in pregnant females at 180 mg/kg bw/day (14.3 compared to 15.3 in controls). Other parameters were unaffected. For information on developmental toxicity, see the developmental toxicity section.

F1 animals

Cohort 1A

Males had slightly decreased mean terminal body weight at 180 mg/kg bw/day compared to controls (-6%, not statistically significant), whereas females from the mid- and high dose groups had increased mean body weights at several timepoints (+7% for both doses at both PND14 and PND28) as well as an increased mean food consumption (+21%) at 180 mg/kg bw/day. In males, but not in females, absolute and/or relative weights of the adrenal glands, kidneys, liver, spleen, thymus, and prostate were statistically significantly altered compared to controls. Furthermore, in the high dose males an increased incidence in atrophy of the mammary gland was observed. Other parameters in treated animals (e.g. sperm parameters, mean oestrus cycle, ovarian follicle count) were unaffected. However, females in the high dosed group showed a prolonged dioestrus stage, as the mean number of days in the proestrus, oestrus, metoestrus, and dioestrus stage, measured during the last 3 weeks prior to mating, changed dose-dependently from 2.2, 3.5, 3.8, and 4.5, respectively, in controls to 1.3, 3.2, 4.1, and 5.4, respectively, at 180 mg/kg bw/day.

Cohort 1B

Terminal body weights for males were slightly lower (-5%) and for females slightly higher (+6%) at the highest dose tested; not statistically significant. In males, absolute and/or relative weights of the adrenals, kidneys, and the liver were statistically significantly altered compared to controls. In females, the absolute (but not relative) weight of the

kidneys was statistically significantly altered compared to controls. An increased number of females showed a prolonged mean oestrus cycle (4.1[2] ± 1.51 days at 180 mg/kg bw/day compared to 3.9 ± 0.29 days in controls). Females in the high dose group showed a prolonged dioestrus stage, as the mean number of days in the proestrus, oestrus, metoestrus, and dioestrus stage (data generated during the last 3 weeks prior to mating), changed dose-dependently from 4.7, 5.4, 6.0, and 6.8, respectively, in controls to 1.3, 4.6, 5.9, and 11.2, respectively, at 180 mg/kg bw/day. Furthermore, a decrease in mean number of implantation sites was noted in pregnant females at 180 mg/kg bw/day (13.7 compared to 15.2 in controls). Other parameters were unaffected. For information on developmental toxicity, see the developmental toxicity section below.

Cohort 2A/2B

For information on developmental toxicity, see the developmental toxicity section.

Cohort 3

For information on developmental toxicity, see the developmental toxicity section.

F2 animals

For information on developmental toxicity, see the developmental toxicity section.

The dossier submitter (DS) noted that the highest dose of the EOGRTS (i.e. 180 mg/kg bw/day) was relatively low compared to the highest doses of the two reproductive/developmental screening test (i.e. 300 mg/kg bw/day), and that hardly any general toxicity was seen in the animals of the EOGRTS, including the high dose group. However, at the high dose some slight effects on reproductive parameters were observed, which, if the high dose selected would have been higher would have been more pronounced, according to the DS.

Conclusion by the DS

Based on treatment-related adverse effects on fertility, reproduction and pregnancy outcome (i.e. decreased number of implantation sites and prolonged oestrus cycle) in three different studies in animals, seen together with only limited general toxicity, the DS concluded that bisphenol S meets the criteria for classification for adverse effects on sexual function and fertility as Repr. 1B; H360F.

Adverse effects on development

The potential of bisphenol S to adversely affect development was investigated in a prenatal developmental toxicity study, a reproduction/developmental screening test, an EOGRTS, and a range-finding study (in the form of a combined repeated-dose toxicity study with the reproduction/developmental screening test) preceding the EOGRTS. All studies were conducted in rats exposed via oral gavage.

In a prenatal developmental toxicity study (OECD TG 414; GLP; Anonymous 19, 2014), bisphenol S was administered orally via gavage to groups of 25 pregnant female Wistar rats/dose at doses of 0, 30, 100 or 300 mg/kg bw/day for 14 days during GD 6 to 19. No treatment-related mortalities occurred. No absolute changes in body weights were noted between groups at any timepoint. There was no effect on mean percentage post-

[2] This value was originally given as 4.5 in the CLH report, but later changed into 4.1 based on comments received during PC. The corrected value was used in the RAC assessment.

implantation loss (4.7, 3.9, 3.9, and 6.3% at 0, 30, 100 and 300 mg/kg bw/day, respectively) or other parameters. In the offspring there were no effects on body weights, sex ratio, or mean number of live foetuses. No relevant abnormalities were observed regarding external, soft tissue or skeletal malformations or variations.

In a reproduction/developmental toxicity screening test (OECD TG 421; GLP; Anonymous 12, 2000), bisphenol S was administered orally via gavage to groups of 12 Sprague/Dawley rats/sex/dose at doses of 0, 10, 60 or 300 mg/kg bw/day for 45 days (males) or 40-46 days (females). No treatment-related mortalities occurred. Parental toxicity at the high dose consisted of salivation right after dosing (m/f), slight reductions in body weight as compared to controls at several timepoints during pre-/post-mating and gestation (m/f) (treatment day 14: females -5%; males -7%; no statistical significance was reached at any of the other days, apart from GD 20 when body weight of the females was 10% lower than the controls; this was not attributed to maternal toxicity, since no difference was found on body weight on Lactation day 0). In the high dose males, weight changes of the liver (increased relative to bw), the pituitary (increased relative to bw), the seminal vesicles (increased absolute weight), as well as slight histopathological changes in the liver and the cecum were observed. The mean number of pups at birth (9.1 compared to 14.3 in controls), the mean number of liveborn pups at birth (9.1 compared to 14.2 in controls) and the mean number of live offspring at PND4 (9.1 compared to 14.1 in controls) was lower at 300 mg/kg bw/day. The offspring in the treated groups did not show any deviations from controls based on external appearance and clinical signs, body weight changes, viability index or anogenital distance.

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422; GLP; Anonymous 14, 2017) performed as a range-finding study for the EOGRTS, bisphenol S was administered orally via gavage to groups of 10 Sprague/Dawley rats/sex/dose at concentrations of 0, 30, 100 or 300 mg/kg bw/day for 6 weeks of premating period (m/f), 2 weeks of mating period (m/f) and 4 weeks post mating period (m) or continued through gestation and lactation (f). No treatment-related mortalities occurred. Parental toxicity included slightly decreased terminal body weights (-7% and -6% for males and females respectively, compared to controls) at the high dose, and slight toxicity of the liver (m), the kidney (m), the cecum (m), and the mammary gland (m) at 300 mg/kg bw/day compared to the control group. The total number of pups delivered was reduced and the mean number of pups delivered was statistically lower. The post-implantation loss in the high dose group was statistically significantly increased (34.6% and 3.6% for the animals in the high dose and control group, respectively). In the offspring, the mean number of pups delivered was significantly decreased (10.8 and 15.2 for the animals in the high dose and control group, respectively). Other parameters were unaffected. Information regarding fertility is summarised in the fertility section above.

In an extended-one generation reproductive toxicity study (OECD TG 443; GLP; Anonymous 13, 2019) with inclusion of cohorts 1A and 1B (with extension of cohort 1B to produce the F2 generation) and cohorts 2A/2B and 3, bisphenol S was administered orally via gavage to groups of 10-24 Sprague/Dawley rats (m/f) at concentrations of 0, 20, 60 or 180 mg/kg bw/day.

F0 animals

In females, body weights were significantly increased compared to controls at some

timepoints (treatment day 7 and 14) at the mid dose (60 mg/kg bw/day), whereas body weights did not differ from controls in males from all treated groups. Water- (m/f) and food consumption (f) were significantly increased at 180 mg/kg bw/day. In the high-dose males (180 mg/kg bw/day), very slight toxicity was observed in the form of enlarged cecum and changes in kidney weights. The mean number of post-implantation loss in high dose group and the mid dose group was significantly increased (1.5 and 1.3 pups/dam, respectively, compared to 0.5 pups/dam in controls) and the mean percentage post-implantation loss was 3.1, 5.9, 9.4*, and 10.5**% at 0, 20, 60 and 180 mg/kg bw/day, respectively. Litter size showed a non-statistically decreased trend (14.9, 14.0, 13.5, and 12.7 at 0, 20, 60 and 180 mg/kg bw/day, respectively). Other parameters were unaffected. Information regarding fertility is summarised in the fertility section above.

F1 animals

The total number of pups delivered was 342, 294, 325, and 293 at 0, 20, 60, and 180 mg/kg bw/day, respectively. There were statistically significant changes in the total number of live pups (285 live pups at 180 mg/kg bw/day compared to 340, 289, 322 at 0, 20, and 60 mg/kg bw/day, respectively) and the number of stillborn pups (8 stillborn pups at 180 mg/kg bw/day compared to 2, 5 and 3 at 0, 20, and 60 mg/kg bw/day, respectively). Furthermore, the mean body weight in F1 pups at PND1 (+9%) and PND4 (+10%) was statistically significantly increased compared to controls at the mid- and high dose tested, and stayed pronounced until PND21 in the mid-dose group. T4 levels were unchanged in both sexes, but TSH was decreased in females in all treated groups at PND4 (range of -15 to -5%; reaching statistical significance at 20 and 180 mg/kg bw/day only), but not at PND22. Changes in TSH were not apparent in males.

Cohort 1A

Males had a slightly decreased mean terminal body weight at 180 mg/kg bw/day compared to controls (-6%, not statistically significant), whereas females from the mid- and high dose tested had an increased mean body weight at several timepoints (+7% for both doses at both PND14 and PND28) as well as an increased mean food consumption (+21%) at 180 mg/kg bw/day. In males, but not in females, absolute and/or relative weights of the adrenal glands, kidneys, liver, spleen, thymus, and prostate were statistically significantly altered from controls. Furthermore, in the high dose males an increased incidence in atrophy of the mammary gland was observed. Other parameters (e.g. thyroid hormones) were unaffected. Information regarding fertility is summarised in the fertility section above.

Cohort 1B

Terminal body weights for males were slightly lower (-5%) and for females slightly higher (+6%) at the highest dose tested (not statistically significant). In males, absolute and/or relative weights of the adrenals, kidneys, and the liver were statistically significantly altered from controls. In females, the absolute (but not relative) weight of the kidneys was statistically significantly altered from controls. At 180 mg/kg bw/day, the mean number of post-implantation loss in this group was significantly increased (3.3 compared to 0.9, 0.8 and 1.1 at 0, 20 and 60 mg/kg bw/day, respectively). The mean percentage implantation loss was 6.4, 5.3, 11.1* and 24.6**% at 0, 20, 60 and 180 mg/kg bw/day, respectively. Consequently, litter size for the F2 generation was statistically significantly affected at the highest dose group (11.4 at 180 mg/kg bw/day compared to 14.3, 13.8 and 14.9 at 0, 20 and 60 mg/kg bw/day, respectively). Other parameters were

unaffected. Information regarding fertility is summarised in the fertility section above.

Cohort 2A/2B

There was trend in increased body weights in males (from PND 21 onwards) and females (from PND 0 onwards). There were some effects observed regarding brain morphometry in the 180 mg/kg bw/day dosed group in cohort 2A. Statistically significant results were noted on the left nucleus caudatus width in males (10% reduced) and females (9% increased). Moreover, the corpus callosum width was statistically significantly reduced (-17%) in males at this dose. All other parameters were unaffected.

Cohort 3

Clinical signs, and food intake were unaffected. In females, body weights were statistically significantly increased at PND14 and PND28 in the high dose group. Also in males a non-statistically significantly trend in increased bw was observed at 60 and 180 mg/kg bw/day at PND14, and at all treated dose-groups at PND28. Relative thymus weight, but not absolute thymus weight, was significantly decreased in males at the highest dose compared to controls (-19%). Furthermore, in the females of the low- and mid dose, there was a slight decrease in T-cell dependent antibody response to sheep red blood cells (SRBC) compared to controls (13647 ± 12787, 8239 ± 5678, 9598 ± 8936, and 14555 ± 11711 U/mL at 0, 20, 60, and 180 mg/kg bw/day respectively).

F2 animals

The total number of pups delivered was 342, 332, 313, and 240 at 0, 20, 60 and 180 mg/kg bw/day, respectively. Furthermore, the number of liveborn pups was decreased (although not reaching statistical significance) to 234 at 180 mg/kg bw/day compared to 336, 330 and 311 at 0, 20 and 60 mg/kg bw/day, respectively. No effect was seen on the number of stillborn pups. The mean number of pups delivered was statistically significantly reduced at the highest dose tested (14.3, 13.8, 14.9, and 11.4 at 0, 20, 60, and 180 mg/kg bw/day, respectively). There was a trend in increased bw of the pups, reaching statistical significance in female pups (+7%) at PND1. Other parameters (e.g. sex ratio, anogenital distance) were unaffected.

The DS noted that the highest dose of the EOGRTS (i.e. 180 mg/kg bw/day) was relatively low compared to the highest doses of the two reproductive/developmental screening studies (i.e. 300 mg/kg bw/day), and that hardly any general toxicity was seen in the animals of the EOGRTS including the top dose group. However, at the high dose some slight effects on developmental parameters were observed, which, if the top dose selected would have been higher would have been more pronounced, according to the DS.

<u>Conclusion by DS</u>

Based on treatment-related adverse effects on development (i.e. post-implantation loss) in three different studies in animals, seen together with only minimal general toxicity, the DS concluded that bisphenol S meets the criteria for classification for adverse effects on development in the category Repr. 1B; H360D.

Consequently, the DS concluded that a combined entry as Repr. 1B; H360FD is warranted.

Adverse effects on or via lactation

No information available. Consequently, no proposal for classification due to lack of data.

Comments received during consultation

Ten comments from seven commenting parties were received during the consultation: three Member State Competent Authorities (MSCAs), one Company-Manufacturer, two NGOs and one Academic Institution. The comments received from the MSCAs were all in agreement with the proposal for harmonised classification as Repr. 1B; H360FD. One MSCA noted that there were adverse effects on the weight of reproductive organs in males in several studies and atrophy of the mammary gland, which have no implications for reproductive performance in itself, but may provide an indication of hormonal disturbance, together with the increased pituitary weight observed in one of the reproductive/developmental screening studies. Furthermore, this MSCA provided several additional studies obtained from the public literature.

One NGO agreed with the proposal for harmonised classification as Repr. 1B; H360FD and noted that besides harmonised classification for reproduction, also harmonised classification for acute toxicity (adverse effects on the heart) should be scrutinised and also identification as an endocrine disruptor may be warranted. Another NGO also agreed with the proposal for Repr. 1B; H360FD and provided two additional studies obtained from the public literature.

Also a representative of an Academic Institution agreed with the proposal for harmonised classification as Repr. 1B; H360FD. This respondent also provided an additional study obtained from the public literature.

One Company-Manufacturer did not agree with the proposal for harmonised classification as Repr. 1B; H360FD. They provided comments in which specific elements were brought forward, noting incorrect information in the CLH dossier and placing certain adverse effects in the context of historical control data. They concluded that the EOGRTS should be seen as the most relevant and conclusive study to assess reproductive and developmental effects of bisphenol S, and that some of the information in this study, in their view, could be useful for classification and labelling of the substance. According to the Company-Manufacturer, safe use of the substance is however safeguarded as the EOGRTS provides a NOAEL for DNEL derivation, which will be included after an update of the REACH registration dossier.

All comments as well as the responses by the DS and RAC are compiled in the RCOM in Annex 2 to the RAC Opinion.

Additional key elements

In two epidemiological cross-sectional studies by Ghayda *et al*. (2019) and Wan *et al*. (2018), the effects of bisphenol S exposure on fertility and development were studied.

Ghayda *et al*. (2019) report that detectable urine concentrations were statistically significantly negatively associated with a decrease in sperm concentration, total sperm count, and total sperm motility among men (of which a large part was overweight or obese) in a model adjusting for abstinence time, specific gravity, age, BMI, year of

sample collection, and log-bisphenol A concentrations. However, among leaner men specifically, no statistically significant differences in semen parameters were found between detectable versus non-detectable urinary bisphenol S concentrations. Therefore, it is unsure whether the effects observed are the result of bisphenol S exposure specifically.

In Wan *et al*. (2018), urinary bisphenol S concentrations were evaluated in pregnant women. Bisphenol S concentrations were statistically significantly positively associated with an increase in pregnancy duration and gestational age at birth (estimated by first-trimester ultrasound) for both genders combined and for girls in particular, after adjustment for covariates. Among boys, effects on birth weight and pregnancy duration among pregnant women did not reach statistical significance after adjustment for covariates.

In non-guideline animal studies by Shi *et al*. (2017), Ashan *et al*. (2018), Ullah *et al*. (2018), Ullah *et al*. (2019) and Ijaz *et al*. (2019), (juvenile) mice and rats or dams were exposed via different routes (subcutaneous injection, oral via drinking water, intraperitoneally) to bisphenol S in non-conventional study designs to observe effects on reproductive function and/or development. Although several of these studies do use uncommon routes of exposure, such as subcutaneous or intraperitoneal, the effects observed are in line with findings observed in the oral reproductive screening studies and EOGRTS. Effects reported were, amongst others, a moderate increase in female body weights and an increase in abdominal fat pad, a decrease in fertility index, a decrease in the number of live pups, a decrease in weight of female reproductive organs (paired ovaries, uterus), an irregular oestrus cycling pattern, an increased incidence of cystic follicles, alterations in the number of specific follicles (corpus luteum, antral, atretic), a decrease in weight of male reproductive organs (epididymides, seminal vesicles), moderate changes in sperm parameters, and alterations in hormone levels (LH, FSH, progesterone, oestradiol, testosterone).

IND provided, amongst others, historical control data (HCD) for post-implantation loss, mean number of implantation sites per dam, mean number of pups delivered and the oestrus cycle duration.

The animals in the studies included in the CLH dossier were provided by the following laboratories:

- For the OECD TG 421 study (2000), the SD rats were provided by Tsukuba Breeding Center, Charles River Laboratories Japan.
- For the OECD TG 422 study (2017), the SD rats were provided by Charles River Laboratories, Research Models and Services, Germany GmbH/ Charles River Laboratories, UK.
- For the OECD TG 443 study (2019), the SD rats were provided by Charles River Laboratories, Research Models and Services, Germany GmbH / Charles River Laboratories, Italy.
- For the OECD TG 414 study (2014), the Wistar rats were provided by BASF Laboratories

The HCD provided were from the US (Charles River Ashland, Crl:CD(SD)), from France (RjHan:SD; rats CD®) (from Janvier or Charles River), or from BASF test lab (Wistar).

The HCD from the US summarise data from 89/91 OECD 412/422/443 studies in a time period from 12/2000 to 08/2018, and those from France summarise data from the F0 and F1 of an unknown number of two-generation studies from 02/2016 to 04/2020.

The Guidance on the application of the CLP criteria mentions that *"In a general sense, the historical control data set should be matched as closely as possible to the study being evaluated. The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc.".* Furthermore, the Guidance explains that *"the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study)".*

RAC observes the following:

- The strains of rats for which IND provided HCD are Crl:CD(SD), RjHan:SD, and Wistar which are the same type of strains as used in the studies included in the CLH dossier;
- The laboratories of which IND provided HCD are located in the US and France, which are not the same locations as the laboratories where the rats in the studies stem from (Japan, Germany, Italy);
- The period of the HCD from Charles River Ashland covers a period of over more than 10 years;
- The study year of one study (2000) is on the skewed end of the HCD range provided (2000-2018).

Hence, the HCD may provide an indication of the normal ranges, but its use may be limited due to the uncertainties mentioned above. The within-study controls are therefore used as most important reference to compare with treatment.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

In line with the DS, RAC places most weight on the guideline studies in the assessment of the reproductive effects of bisphenol S.

Mean number of implantation sites

The mean number of implantation sites was affected in three different guideline studies (OECD TG 421, OECD TG 443, OECD TG 422) from 180 mg/kg bw/day onwards, with increasing severity at 300 mg/kg bw/day (see table below). Considering the size of the effect and the consistency of the effect among studies, RAC considers this effect as relevant for classification for sexual function and fertility.

Table: *Mean number of implantation sites per dam*

Dose level (mg/kg bw/day)	0	10	20	30	60	100	180	300
OECD TG 421	15.9	13.3	-	-	14.8	-	-	10.7
OECD TG 443 F0	15.3 15.2	- -	14.8 14.6	- -	14.9 15.4	- -	14.3 13.7	- -

F1B								
OECD TG 422	15.8	-	-	15.0	-	15.5	-	10.4**
HCD (mean; range): 15.2 (12.3-17.8)[a] HCD (mean; range): 15.0 (13.8-16.0)[b] HCD (mean; range): 14.1 (12.1-15.3)[c]								

[a] Historical control data (Charles River Ashland, Crl:CD(SD), OECD 412/422/443, 89/91 studies in a time period from 12/2000 to 08/2018)

[b] Historical control data (Janvier or Charles River France, RjHan:SD (Rats CD®), two-generation studies F0, time period 02/2016 to 04/2020)

[c] Historical control data (Janvier or Charles River France, RjHan:SD (Rats CD®), two-generation studies F1, time period 02/2016 to 04/2020)

Fertility index

Both the OECD TG 421 and 422 studies show a dose-dependent decrease in fertility index at 300 mg/kg bw/day (see table below). In the OECD TG 421 study, most of the females at 300 mg/kg bw/day, which had a continued dioestrus phase, were not fertilised. In the OECD TG 422 study, there were two females without implantation sites at 300 mg/kg bw/day. Considering the severity, RAC considers these effects relevant for classification.

Table: *Fertility index*

Dose level (mg/kg bw/day)	0	10	20	30	60	100	180	300
OECD TG 421	91.7%	91.7%	-	-	100%	-	-	58%
OECD TG 443 F0 F1B	 100% 100%	 - -	 100% 100%	 - -	 100% 100%	 - -	 100% 100%	 - -
OECD TG 422	100%	-	-	90%	-	100%	-	80%[3]

Disturbed and prolonged oestrus cycle

The oestrus cycle was prolonged in female rats in several studies (OECD TG 421; OECD TG 443; OECD TG 422) (see table below). Furthermore, results from the OECD TG 443 illustrated that females in the F0, F1A and F1B cohorts tended to be in the diestrus stage for a longer period and in the proestrus stage for a shorter period in all treated dose-groups (>20 mg/kg bw/day) compared to controls (see table below).

In the OECD TG 421 study, the incidence of females with an irregular oestrus cycle was 0/12, 0/12, 1/12, and 5/12 at 0, 10, 60 and 300 mg/kg bw/day, respectively. Four out of five females in the highest dose group, which had a continued dioestrus phase, did not conceive at all and consequently this led to a steep decrease of the fertilisation index of 58%.

The biological relevance and adversity of the effects on the oestrus cycle, in the presence of indications of decreased fertility parameters (i.e. decreased mean number of implantation sites, decreased fertility index) is apparent. Taking this and the consistency of the effect among studies into account, RAC considers the effects on the oestrus cycle as relevant for classification.

[3] Note that this specific effect was initially mentioned to be 60% at the highest dose tested in the CLH dossier and on IUCLID, but during the consultation it was noted that this effect size was incorrect and should be changed into 80% fertility at the highest dose tested. The information in IUCLID was updated to this regard as well (ECHA dissemination website consulted on 18-08-2020). The RAC rapporteur was unable to verify the exact number as it had no access to the underlying study report. However, RAC wants to highlight that, either way, a dose-dependent decrease in fertility index is observed in this study.

Table: *Mean duration of the oestrus cycle in days*

Dose level (mg/kg bw/day)	0	10	20	30	60	100	180	300
OECD TG 421	4.08	4.01	-	-	4.14	-	-	5.57**
OECD TG 443 F0 F1A F1B	 3.9 4.1 3.9	 - - -	 3.9 4.1 4.0	 - - -	 3.9 4.1 4.0	 - - -	 4.1* 4.1 4.1[4]	 - - -
OECD TG 422	4.02	-	-	3.97	-	4.01	-	5.16**
HCD (mean; range): 4.2 (3.9-5.2)[a] HCD (mean; range): 4.9 (4.0-5.8)[b] HCD (mean; range): 4.5 (4.4-4.9)[c]								

[a] Historical control data (Charles River Ashland, Crl:CD(SD), OECD 412/422/443, 89/91 studies in a time period from 12/2000 to 08/2018)

[b] Historical control data (Janvier or Charles River France, RjHan:SD (Rats CD®), two-generation studies F0, time period 02/2016 to 04/2020)

[c] Historical control data (Janvier or Charles River France, RjHan:SD (Rats CD®), two-generation studies F1, time period 02/2016 to 04/2020)

Table*: Mean number of days in the proestrus, oestrus, metoestrus and dioestrus stage of the oestrus cycle for females in the OECD TG 443, measured during the last 3 weeks prior to mating.*

Dose level (mg/kg bw/day)		0	20	60	180
F0	prooestrus	4.7	3.5	3.8	2.2
	oestrus	5.1	5.1	5.0	5.2
	metoestrus	5.8	6.0	5.8	5.9
	dioestrus	6.3	7.4	7.7	9.0
F1A	prooestrus	2.2	2.0	2.2	1.3
	oestrus	3.5	3.6	3.5	3.2
	metoestrus	3.8	3.9	3.6	4.1
	dioestrus	4.5	4.6	4.8	5.4
F1B	prooestrus	4.7	2.8	2.2	1.3
	oestrus	5.4	5.2	5.4	4.6
	metoestrus	6.0	6.0	6.3	5.9
	dioestrus	6.8	8.4	9.2	11.2

General toxicity

The results from the repeated dose studies indicate that dosing with 600 to 1000 mg/kg bw/day for 28-90 days results in body weight changes in males (but not in females), as well as liver toxicity, kidney toxicity, and other systemic effects in both sexes. In the OECD TG 421/422 studies, adverse effects on sexual function and fertility were observed at the top dose of 300 mg/kg bw/day, and at this dose level only very slight to no general toxicity was observed. The results from the 90-day repeated dose toxicity study indicate that females tolerate doses up to 1000 mg/kg bw/day without marked general toxicity. In view of that, RAC is of the opinion that the effects observed on sexual function and fertility at 180 and 300 mg/kg bw/day are not due to general toxicity.

Comparison with the criteria

Bisphenol S was shown to consistently and severely disturb reproductive parameters. Overall, RAC observes the following:

[4] Note that this specific effect was initially mentioned to be 4.5 days at the highest dose tested in the CLH dossier and on IUCLID, but during the PC was noted that this effect size was incorrect and should be changed into 4.1 days. The information in IUCLID was updated to this regard as well (ECHA dissemination website consulted on 09-09-2020).

a) Exposure to bisphenol S consistently resulted in a decrease in mean number of implantation sites at 300 mg/kg bw/day;

b) Exposure to bisphenol S consistently resulted in a prolongation of the oestrus cycle at 300 mg/kg bw/day, as well as in an irregular oestrus cycle with a decreased pro-oestrus stage and an increased dioestrus phase from 20 mg/kg bw/day onwards;

c) Exposure to bisphenol S resulted in a decrease in the fertility index of 58% and 80% at 300 mg/kg bw/day.

RAC concludes that the adverse effects of bisphenol S on the mean number of implantation sites, the decrease in fertility index, and the effect on the oestrus cycle warrant classification as Repr. 1B; H360F.

RAC would like to emphasis that the dosing regimen in the guideline studies is also taken into account in the overall weight of evidence assessment for classification.

The top dose of 180 mg/kg bw/day, used in the EOGRTS, is not supported by adequate argumentation, and its correctness is questionable, especially in light of the effects seen in repeated dose studies where doses up to 1000 mg/kg bw did not exert severe toxicity in females, while 600 mg/kg bw/day did result in clear but not in severe toxicity in males.

Adverse effects on development

In the OECD TG 414, hardly any statistically significant effects were observed, either in foetuses or maternal animals. RAC notes that the dose levels were comparable to the reproductive screening studies, where slight effects were seen in the top dose. In the OECD TG 414, the following is stated:

"Unless limited by the physical/chemical nature or biological properties of the test substance, the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering. At least one intermediate dose level should produce minimal observable toxic effects."

The absence of effects in the TG 414 study is likely due to the shorter exposure duration and/or due to the different strain of rats used. RAC notes that considering the limited toxicity in the developmental study, this study is not fully in line with the prevailing guideline and is as such of limited relevance for the assessment of adverse effects on development.

<u>Increased post-implantation loss</u>

Three studies evaluated the post-implantation loss of offspring upon exposure to bisphenol S (see table below). In the OECD TG 422 study and the OECD TG 443 study (both cohorts), the mean number of post-implantation loss was statistically significantly increased from controls. This effect was apparent from 180 mg/kg bw/day onwards.

The DS noted that the post-implantation loss was increased by treatment and cannot be explained by maternal toxicity as the general condition of the animals was unaffected by treatment. Based on this, the DS concluded that the post-implantation loss was a clear adverse effect on development, that cannot be related to general toxicity, and observed in two studies.

Nitzsche *et al*. (2017) shows that post-implantation loss is, in general, not considered to be a secondary developmental effect resulting from non-specific maternal toxicity. Furthermore, there was no marked systemic toxicity observed at the doses at which these developmental effects were observed. Therefore, RAC agrees with the DS in this respect, and concludes that the consistently observed, severe effect on post-implantation loss justifies classification.

Table: *Mean percentage of post-implantation loss*

Dose level (mg/kg bw/day)	0	10	20	30	60	100	180	300
OECD TG 421	NA	NA	-	-	NA	-	-	NA
OECD TG 443 F0 F1B	 3.1 6.4	 - -	 5.9 5.3	 - -	 9.4* 11.1	 - -	 10.5** 24.6**	 - -
OECD TG 422	3.6	-	-	5.2	-	6.5	-	34.6*
OECD TG 414	4.7	-	-	3.9	-	3.9	-	6.3
HCD (mean; range): 7.1 (4.7-12.0)[b]								

[a] Charles River Ashland, Crl:CD(SD), OECD 412/422/443, 89/91 studies in a time period from 12/2000 to 08/2018

[b] BASF, Wistar, OECD TG 414

Number of pups delivered

The mean number of pups delivered/mean number of live foetuses was evaluated in four studies. In three of those (the OECD TG 421, OECD TG 443, and the OECD TG 422), there was a dose-dependent decrease in the mean number of pups delivered (see table below). RAC considers this effect to be a direct consequence of the exposure to the substance but notes that it is difficult to discriminate whether this is due to fertility or developmental effects.

Table*: Mean number of pups delivered*

Dose level (mg/kg bw/day)	0	10	20	30	60	100	180	300
OECD TG 421	14.3	12.5		-	13.5	-	-	9.1
OECD TG 443 F1 F2	 14.9 14.3	 - -	 14.0 13.8	 - -	 13.5 14.9	 - -	 12.7 11.4**	 - -
OECD TG 422	15.2	-	-	14.1	-	14.5	-	10.8**
OECD TG 414[a]	10.6	-	-	10.6	-	10.6	-	10.1
HCD (mean; range): 14.3 (12.1-15.9)[b]								

[a] Mean number of live foetuses

[b] Charles River Ashland, Crl:CD(SD), OECD 412/422/443, 89/91 studies in a time period from 12/2000 to 08/2018

Increased body weight in pups

Male and female pups consistently showed an increase in body weights from PND0 onwards (see table below) in the OECD TG 421 and OECD TG 443 studies; up to +14%

and +18% at 300 mg/kg bw/day on PND4 in females and males respectively.

The DS did not consider these effects for classification. RAC considers this consistent increase in body weights of the pups insufficient for classification on its own, but it contributes to the overall concern for effects on the developing organism and might be indicative of an endocrine mode of action.

***Table**: Change in body weight of pups (in grams (%))*

Dose level (mg/kg bw/day)	0	10	20	60	180	300
Females						
	PND0					
OECD 421	6.9	7.0 (+1%)	-	6.9 (+0%)	-	7.3 (+6%)
	PND1					
OECD 443 F1 F2	 6.7 7.2	 - -	 7.0 (+4%) 7.0 (-3%	 7.2* (+7%) 7.0 (-3%)	 7.3* (+9%) 7.7* (+7%)	 - -
	PND4					
OECD 421	11.7	11.7 (+0%)	-	11.5 (-2%)	-	13.3 (+14%)
OECD 443 F1 F2	 9.9 10.9	 - -	 10.3 (+4%) 10.5 (-4%)	 10.9* (+10%) 10.5 (-4%)	 10.9* (+10%) 11.8 (+8%)	 - -
	PND21					
OECD 443 F1 F2	 52.0 56.9	 - -	 54.3 (+4%) 56.2 (-1%)	 54.8* (+5%) 56.5 (-1%)	 53.7 (+3%) 58.3 (+2%)	 - -
Males						
	PND0					
OECD 421	7.4	7.5 (+1%)	-	7.3 (-1%)	-	7.8 (+5%)
	PND1					
OECD 443 F1 F2	 7.1 7.5	 - -	 7.4 (+4%) 7.5 (+0%)	 7.7* (+8%) 7.4 (-1%)	 7.7 (+8%) 8.0 (+7%)	 - -
	PND4					
OECD 421	12.0	12.4 (+3%)	-	12.1 (+1%)	-	14.1 (+18%)
OECD 443 F1 F2	 10.5 ND	 - -	 10.9 (+4%) ND	 11.5* (+10%) ND	 11.4* (+9%) ND	 - -
	PND21					
OECD 443 F1 F2	 54.0 59.1	 - -	 56.8 (+5%) 58.9 (+0%)	 57.8* (+7%) 58.6 (-1%)	 55.7 (+3%) 61.1 (+3%)	 - -

<u>Specific neuro- and immuno-developmental effects</u>

In cohort 2A and 3 of the OECD TG 443, there were some effects observed regarding brain morphometry and immune effects:

- statistically significant alteration in left nucleus caudatus width in males (10% reduced) and females (9% increased) at 180 mg/kg bw/day;
- reduction in the corpus callosum width in males (17% reduced) at 180 mg/kg bw/day;

- decrease in relative thymus weight in males (-19%) at 180 mg/kg bw/day;
- decrease T-cell dependent antibody response to sheep red blood cells (SRBC) in females at 20 and 60 mg/kg bw/day

Although marginal, some effects on specific neuro- and immune developmental effects were noted in the OECD TG 443 study. The DS did not consider these effects for classification. As with the increase in body weights of the pups, RAC considers these effects insufficient for classification on their own, but they contribute to the overall concern for effects on the developing organism.

Increased incomplete ossification

In the OECD TG 414 study, there were some effects on skeletal development observed at the highest dose tested (see table below). Although several of these effects were not outside the historical control range, it is noted that for some showed a dose-related trend. Especially the unossified sternebra was statistically significantly increased compared to controls in the high dose group.

According to the DevTox database[5], dumbbell ossification of the thoracic centrum and unossified sternebra are considered grey zone skeletal anomalies, and incomplete ossification of the pubis, incomplete ossification of supraoccipital, and incomplete ossification of the ischium are considered skeletal variations.

The DS did not consider these effects for classification. RAC notes that usually skeletal ossification is an effect indicative of decreased growth, not sufficiently severe for classification in itself. Although, considering the observation that this study is not in line with the prevailing guideline (no toxicity observed in any of the groups) and as such of minor relevance for the assessment of the developmental toxicity, the statistically significant effects are noteworthy. Especially since none of the effects observed in the pups could be attributed to reduced growth.

***Table**: Incidence of significant increased foetal skeletal variations (mean percentage of affected foetus/litter)*

Dose level (mg/kg bw/day)	0	30	100	300	HCD mean % (range)
Incomplete ossification of supraoccipital (unchanged cartilage)	34.1	35.2	37.6	45.2*	43.5 (10.3 – 64.3)
Dumbbell ossification of thoracic centrum (unchanged cartilage)	0.7	3.0	0.0	5.6**	6.9 (0.0 – 14.5)
Unossified sternebra (unchanged cartilage)	1.5	5.0	4.6	11.0**	8.2 (2.6 – 20.7)
Incomplete ossification of pubis (cartilage present)	0.0	0.8	2.0*	1.7	0.3 (0.0 – 2.4)
Incomplete ossification of ischium (cartilage present)	0.0	0.0	2.0*	1.7	0.2 (0.0 – 0.8)

Comparison with the criteria

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to

[5] https://www.devtox.org/nomenclature/ml_organ.php?lan=en

be a secondary non-specific consequence of other toxic effects.

Overall, RAC observes the following:

a. **Post-implantation loss** was increased in two studies, from 60 mg/kg bw/day onwards. Furthermore, a **decrease in the mean number of pups** delivered per dam was consistently observed in three studies from 180 mg/kg bw/day onwards. This effect is a result of both increased implantation loss and post-implantation loss.

b. A consistent pattern of **increased pup weight** was observed in both sexes (up to 14/18% in f/m at 300 mg/kg bw/day), that is attributed to gestational bisphenol S exposure.

c. And although marginal, some **specific neuro- and immunodevelopmental effects** were noted in the OECD TG 443 study. RAC considers these effects insufficient for classification as Repr. 1B; H360D on their own, but they contribute to the overall concern for effects on the developmental organism and therefore also contribute in the weight-of-evidence in support of this classification. For considerations regarding the dose selection, see Adverse effects on sexual function and fertility

RAC concludes that the adverse effect of bisphenol S on the post-implantation loss and the mean number of pups delivered per dam warrant **classification as Repr. 1B; H360D.** The effects observed are severe and are not resulting from maternal toxicity.

RAC notes the difficulty in determining whether the effect on the mean number of pups delivered per dam is related to an effect on fertility or development. However, the significant increase in post-implantation loss in two studies is a consistent and severe finding, which increased with treatment. The CLP Regulation states that classification in Category 2 is appropriate when "*there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1*". RAC considers that the evidence for bisphenol S on post-implantation loss is a clear effect development, and therefore does not consider classification in Category 2 appropriate.

Adverse effects on or via lactation

No information available. As a consequence, RAC proposes **no classification for lactation due to lack of data**.

Overall, RAC concluded that **bisphenol S warrants classification as Repr. 1B; H360FD**.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this dossier

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this dossier

10.13 Aspiration hazard

Not evaluated in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier

13 ADDITIONAL LABELLING

NA

14 REFERENCES

See confidential annex

Annaheim, 2007 : 4,4'-sulphonyldiphenol, Handbook, Chem. Ber.; 9; 1876; 1149, cited in Beilstein Institut zur Förderung der Chemischen Wissenschaften, Reg.-Nr.2052954

BASF AG, 2007 : 4,4'-sulphonyldiphenol, BASF AG, Department of Product Safety, unpublished calculation, EPISuite V3.20, 19.03.2007

BASF SE, 2008 : 4,4'-sulphonyldiphenol, BASF SE, Department of Product Safety, SPARC online calculator, v4.2, 12. Sep. 2008

Beilstein, 2007 : cited in Beilstein Institut zur Förderung der Chemischen Wissenschaften, Reg.-Nr.2052954

BGIA Gestis Stoffdatenbank,2008 : GESTIS - Substance database of 'Berufsgenossenschaftlichen Instituts für Arbeitsschutz' (BGIA)

Clairant, 2004 : 4,4'-sulphonyldiphenol, Material Safety Data Sheet for CAS 80-09-1, 16.01.2004

GESTIS Substance database, 2008 : 4,4'-sulphonyldiphenol, GESTIS - Substance database of 'Berufsgenossenschaftlichen Instituts für Arbeitsschutz' (BGIA)

Meylan WM, & Howard PH., 1995, 4,4'-sulphonyldiphenol, cited in SRC PhysProp database12. Sep. 2008

Meylan WM et al., 1996 : 4,4'-sulphonyldiphenol, cited in SRC PhysProp database12. Sep. 2008

Molina-Molina JM, Amaya E, Grimaldi M, Sáenz JM, Real M, Fernández MF, Balaguer P, Olea N. In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptor. Toxicology and applied pharmacology (2013); 272: 127-136

Neely WB & Howard PH, 1995: BASF AG, Department of Product Safety, unpublished calculation, EPISuite V3.20, 19.03.2007

Yaws, Carl L., 2009 : Yaws' Handbook of Physical Properties for Hydrocarbons and Chemicals, cited in © 2009 Knovel Corporation

Additional references

Ahsan, N., Ullah, H., Ullah, W., & Jahan, S. (2018). Comparative effects of Bisphenol S and Bisphenol A on the development of female reproductive system in rats; a neonatal exposure study. *Chemosphere, 197*, 336-343.

ECHA (2014). Opinion of harmonised classification and labelling at EU level of Bisphenol A; 4,4'-isopropylidenediphenol. Document no. CLH-O-0000004110-93-03/F. https://echa.europa.eu/documents/10162/51436fe4-c531-e3aa-dbe8-42f9c75c83db

Ghayda, R., Williams, P. L., Chavarro, J. E., Ford, J. B., Souter, I., Calafat, A. M., ... & Mínguez-Alarcón, L. (2019). Urinary bisphenol S concentrations: Potential predictors of and associations with semen quality parameters among men attending a fertility center. *Environment international, 131*, 105050.

Ijaz, S., Ullah, A., Shaheen, G., & Jahan, S. (2020). Exposure of BPA and its alternatives like BPB, BPF, and BPS impair subsequent reproductive potentials in adult female Sprague Dawley rats. *Toxicology Mechanisms and Methods, 30*(1), 60-72.

Nitzsche, D. (2017). Effect of maternal feed restriction on prenatal development in rats and rabbits–A review of published data. *Regulatory Toxicology and Pharmacology, 90*, 95-103.

Shi M, Sekulovski N, MacLean JA 2nd, Hayashi K. Effects of bisphenol A analogues on reproductive functions in mice. Reprod Toxicol. 2017 Oct; 73:280-291.
doi: 10.1016/j.reprotox.2017.06.134. Epub 2017 Jul 1. PubMed PMID: 28676390.

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Turi, N., Ullah, W., ... & Khan, M. M. (2018). Impact of low-dose chronic exposure to bisphenol A and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: A focus on the possible hormonal mode of action. *Food and chemical toxicology*, 121, 24-36.

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Razak, S., Rauf, N., ... & Mahboob, S. Z. (2019). Prenatal BPA and its analogs BPB, BPF, and BPS exposure and reproductive axis function in the male offspring of Sprague Dawley rats. *Human & experimental toxicology, 38*(12), 1344-1365.

Wan, Y., Huo, W., Xu, S., Zheng, T., Zhang, B., Li, Y., ... & Chen, Z. (2018). Relationship between maternal exposure to bisphenol S and pregnancy duration. *Environmental Pollution,* 238, 717-724.

15 ANNEXES

See confidential annex

16 ABBREVIATIONS

* : $p<0.05$

** : $p<0.01$

♂ or M : male

♀ or F: female

Abs. : absolute

AGD : anogenital distance

ALP : alkaline phosphatase

ALT : alanine aminotransferase

AST : aspartate aminotransferase

BW : body weight

BWG : body weight gain

Ca : calcium

Cat. : category

Chol : cholesterol

CMC : carboxymethylcellulose

Conc. : concentration

CSA : chemical safety assessment

DIT : developmental immunotoxicity

DNT : developmental neurotoxicity

DS : dossier submitter

EOGRTS : extended one generation reproductive toxicity study

Epith. : epithelium

FBW : final body weight

GD : gestational day

GGT_C = serum-γ-glutamyltransferase

GLP : good laboratory practice

GOT : glutamic oxaloacetic transaminase

Hb : haemoglobin

HCD : historical control data

Ht : haematocrit

LD : lactation day

LDH : lactate dehydrogenase

MCHC : mean corpuscular haemoglobin concentration

MCV : mean corpuscular volume

Min. : minimum

NA : not applicable

Nb or no : number

PND : post-natal day

Pt : prothrombin time

RBC : red blood cell

Rel : relative

RET : reticulocyte

S.d : standard deviation

SD : Sprague-Dawley

Sem. ves. : seminal vesicle

Sign : significant

STOT RE : specific target organ toxicity repeated exposure

TBD : to be determined

TG : test guideline

Tot : total

Tot. chol. : total cholesterol

Tot. prot. : total protein

Trig : triglyceride

WBC : white blood cell