CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate]

EC Number: **251-073-2**

CAS Number: **32509-66-3**

Index Number: **-**

Contact details for dossier submitter:

Belgian Federal Public Service Health, Food Chain Safety and Environment
Risk Management service
Avenue Galilée 5/2
1210 Brussels
Belgium

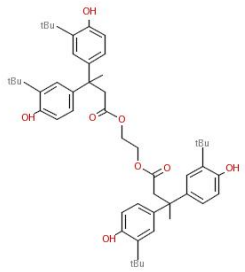
Version number: 3 **Date: October 2023**

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)	2-{[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butanoyl]oxy}ethyl 3,3-bis(3-tert-butyl-4-hydroxyphenyl)butanoate
Other names (usual name, trade name, abbreviation)	Ethane-1,2-diyl bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butanoate] Bis[3,3-bis-[4`-hydroxy-3`-tert-butylphenyl)butanoicacid] glycolester Benzenepropanoic acid, 3-(1,1-dimethylethyl)-beta-(3-(1,1-dimethylethyl)-4-hydroxyphenyl)-4-hydroxy-beta-methyl-, 1,1'-(1,2-ethanediyl) ester Hostanox O 3 P
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	251-073-2
EC name (if available and appropriate)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate]
CAS number (if available)	32509-66-3
Other identity code (if available)	-
Molecular formula	$C_{50}H_{66}O_8$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	795.07 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	>80 %

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 (CLP)	Current self-classification and labelling (CLP)
Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate]	> 80 wt % (mono-constituent)	-	Not classified (59 notifiers)

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
/				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not applicable					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	TBD	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate]	251-073-2	32409-66-3	Repr. 1B Lact.	H360D H362	GHS08	H360D H362	/	/	/

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

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	**Repr. 1B, H360D + Lact. H362**	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

The substance was not considered for harmonised classification so far.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

Ethylene bis[3,3-bis(3-ert-butyl-4-hydroxyphenyl)butyrate] has CMR properties (reproductive toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under article 36 of the CLP regulation.

5 IDENTIFIED USES

According to the registrants, the substance is used in coating products and polymers for machinery, plastic products, and vehicles.

Release to the environment is described for indoor use in long-life materials with low release rates (e.g. flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper/cardboard products, and electronic equipment) and outdoor use (e.g. metal, wooden- and plastic construction and building materials). The substance can be found in vehicles and machinery, mechanical appliances and electrical/electronic products (e.g. computers, cameras, lamps, refrigerators, washing machines), plastic (e.g. toys, mobile phones), fabrics, textiles and apparel used for articles with intense direct dermal (skin) contact during normal use (e.g. clothing, shirts, pants, shorts) and plastic used for articles intended for food contact (e.g. plastic dinner ware, food storage) (ECHA registration dossier: https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/28053)

6 DATA SOURCES

The sole data source is the registration dossier (ECHA registration dossier: https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/28053).

Full study report

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	Solid	ECHA, Registration dossier	Visual observation
Melting/freezing point	169 °C	ECHA, Registration dossier	Measured at 101.3 kPa
Boiling point	347 °C	ECHA, Registration dossier	Measured at 101.3 kPa
Relative density	1.11	ECHA, Registration dossier	Measured at 23 °C
Vapour pressure	$<1.0 \times 10^{-7}$ hPa	ECHA, Registration dossier	Measured at 20 °C
Surface tension	Not surface active	ECHA, Registration dossier	/
Water solubility	0.5 mg/L	ECHA, Registration dossier	Measured at 20 °C
Partition coefficient n-octanol/water	13.7	ECHA, Registration dossier	Measured at 25 °C
Flash point	No information available	ECHA, Registration dossier	/

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	Non-flammable	ECHA, Registration dossier	Measured
Explosive properties	No information available	ECHA, Registration dossier	/
Self-ignition temperature	No information available	ECHA, Registration dossier	/
Oxidising properties	No information available	ECHA, Registration dossier	/
Granulometry	MMAD: 30.6 µm	ECHA, Registration dossier	Measured
Stability in organic solvents and identity of relevant degradation products	No information available	ECHA, Registration dossier	/
Dissociation constant	No information available	ECHA, Registration dossier	/
Viscosity	Not determinable	ECHA, Registration dossier	/

8 EVALUATION OF PHYSICAL HAZARDS

Hazard class not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

According to the registration dossier, no studies are available in which the toxicokinetic properties of ethylene-bis[3,3-bis(3-tert.-butyl-4-hydroxyphenyl)butyrate] were investigated (ECHA, Registration dossier).

The registrant provided the following comments on toxicokinetic properties (ECHA, Registration dossier):

- Absorption

With reference to the Log P_{ow} of 13.66, the very limited water solubility and a molecular weight of 795 g/mol the registrants assumed poor absorption of ethylene-bis[3,3-bis(3-tert.-butyl-4-hydroxyphenyl)butyrate], an assumption supported by low acute toxicity. However, due to systemic effects observed in repeated dose studies some bioavailability needs to be assumed, which might occur by micellar solubilisation by bile salts and subsequent entering the circulation via the lymphatic system.

- Distribution

According to the registrants ethylene-bis[3,3-bis(3-tert.-butyl-4-hydroxyphenyl)butyrate] as a very lipophilic substance with a high molecular weight is expected to migrate into cells and concentrate in adipose tissues.

- Metabolism

According to the registrants the substance has a low tendency to hydrolyse. However, a cleavage of the ester functions in the molecular centre cannot be excluded.

- Excretion

According to the registrants, considering the high Log P_{ow} and molecular weight biliary excretion might be relevant and enterohepatic recycling resulting in a prolonged biological half-life cannot be excluded.

The Reproductive/developmental toxicity screening test, performed according to OECD TG 421 (Anonymous, 2019) indicates excretion of the substance via milk.

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

Based on the information given by the registrant it can be concluded that despite the Log P_{ow} of 13.66, the very limited water solubility and a molecular weight of 795 g/mol systemic absorption of ethylene-bis[3,3-bis(3-tert.-butyl-4-hydroxyphenyl)butyrate] is possible.

Transfer via breast milk has been demonstrated.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

10.7 Skin sensitisation

Hazard class not assessed in this dossier.

10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

10.9 Carcinogenicity

Hazard class not assessed 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, nb/group	Test substance, dose levels, duration of exposure	Results	Reference
Reproduction/developmental Toxicity Screening Test Oral (diet) OECD TG 421 GLP Rat (Wistar) Main group: 10/sex/group Additional groups: 5 females/group (control and high dose) Reliability 1 (according to registration dossier)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: 97.4 % Doses: 0, 3750, 7500 and 11250 ppm (corresp. approx. to 0, 271, 513 and 826 mg/kg bw/d in males and to 0, 501, 1013 and 1320 mg/kg bw/d in females) Duration of exposure: In males 29 or 34 D (min. 14 D prior mating and during mating period) In females: 57 to 71 D (14 D prior mating, variable time to conception, during gestation, and at least 21 D after delivery) 43 for females which failed to failed to deliver	F0 parental generation: No treatment-related mortality no clinical signs observed Bw: in males: trend towards lower BWG In females: BW comparable during pre-mating and gestation period, but sign. reduced at the highest dose on PND 13 and 21 (similar trend at the mid dose). Male fertility parameters: sperm not examined Female fertility parameters: Mating index 93 % at 11250 ppm vs 100 % in all other groups (within HCD range: 90 to 100 %) Fertility index: 80, 70, 80 and 93 %, resp. at 0, 3750, 7500 and 11250 ppm (HCD range: 80 to 100 %) Furthermore, precoital interval and mean nb of implantation sites not treatment-related modified Duration of gestation similar in all groups (between 21.1 and 21.6 d) No signs of difficult or prolonged parturition Necropsy: no treatment-related macroscopic findings in males (only 1 male with testes and epididymides reduced in size at 7500 ppm) while in females adrenal glands and thymus exhibited changes. Organ weight: Relative testes, prostate and epididymides weights were similar in all groups Microscopic examination: no treatment-related change observed	Anonymous, 2019

Method, guideline, species, strain, sex, nb/group	Test substance, dose levels, duration of exposure	Results	Reference
		F1 generation:	
		Tot. nb of pups comparable in all groups Mean nb of live born pups slightly, but not significantly, reduced in treated groups (11.6, 10.6, 10.3 and 10.3 pups, resp. at 0, 3750, 7500 and 11250 ppm). Modification not observed in cross-fostered group (10.0 and 11.6 pups, resp. in control and high cross-fostered groups) More information regarding F1 pups available in section 10.10.4	
One-generation reproductive toxicity study Oral (diet) No OECD guideline nor GLP (performed before GLP) Rat (Wistar) 15 males and 30 females/groups Reliability 2 (according the registration dossier, however only summary information available. Full tudy report was available to the DS but bad quality of the PDF file and tables mostly not readable)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 0.16, 0.4 and 1.0 % (corresp. approx. to 0, 70, 200 and 500 mg/kg bw/d) Duration of exposure: 7 weeks for males and 13 weeks for females (4 W of pre-mating and 3 W of mating, followed for females by 3 W of gestation and 3 W of lactation)	F0 parental generation: No treatment-related mortality nor clinical signs observed Bw: relatively low in males exposed to 0.4 and 1.0 %, similar in all groups in females (no more information available) Male fertility parameters: sperm not examined Female fertility parameters: Fertility index: 100, 100, 100 and 93 %, resp. at 0, 0.16, 0.4 and 1.0 % (corresp. approx. to 0, 70, 200 and 500 mg/kg bw/d) (only 1 female failed to be pregnant) No information available regarding nb of implantation sites, duration of gestation, nb of pre- and post-implantation loss Necropsy: not performed F1 generation Mean litter size: 10.8, 11.2, 10.6 and 10.7 pups, resp. at 0, 0.16, 0.4 and 1.0 % (corresp. approx. to 0, 70, 200 and 500 mg/kg bw/d) More information regarding F1 pups available in section 10.10.4	Anonymous, 1979
Reproductive toxicity study Oral (diet) No OECD guideline nor GLP Rat (Wistar) 11 males and 20 females/group Reliability 4 (according to the registration dossier (very restricted reporting, summary only, no individual data))	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 0.1, 0.4 and 1.6 % (corresp. approx. to 0, 50, 200 and 800 mg/kg bw/d) Duration of exposure: 7 weeks for males and 13 weeks for females (4 W of pre-mating and 3 W of mating, followed for females by 3 W of gestation and 3 W of	F0 parental generation: No treatment-related mortality no clinical signs observed Bw: relatively low in males exposed to 0.4 and 1.6 % (at the end of week 4: 284 g at 1.6 % vs 303 in control group) (no information about statistic) Male fertility parameters: sperm not examined Female fertility parameters: Fertility index: 100, 100, 100 and 90 %, resp. at 0, 0.1, 0.4 and 1.6 % (corresp. approx. to 0, 50, 200 and 800 mg/kg bw/d) (no information about statistic)	Anonymous, 1976

Method, guideline, species, strain, sex, nb/group	Test substance, dose levels, duration of exposure	Results	Reference
	lactation)	Necropsy: no information available F1 generation Mean litter size: 10.1, 11.1, 9.7 and 10.2 pups, resp. at 0, 0.1, 0.4 and 1.6 % More information regarding F1 pups available in section 10.10.4	
Sub-chronic (16-week) oral toxicity study Oral (diet) Rat (Wistar) (animals derived from parents which had received the test substance by diet during pre-mating, mating, gestation and lactation) 50/sex/dose No guideline followed Reliability 2 (according to the registration dossier)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 0.1, 0.4 and 1.6 % (corresp. approx. to 0, 70, 200 and 800 mg/kg bw/d) Duration of exposure: 16 weeks (and animals exposed *in utero*)	No treatment-related mortality nor clinical signs (one death occurs in control group (cause unknown)) Bw: Transiend significant increase of weight at 0.4% followed by a significant decrease at 1.6 % Necropsy: no treatment-related macroscopic or microscopic findings. Relative testes and ovaries weights significantly increased at the highest dose (ovary weight DR and significantly higher ≥0.4 %: 0.0321, 0.0325, 0.0375* and 0.0403**, resp. at 0, 0.1, 0.4 and 1.6 %; Testes weight significantly higher at the highest dose: 0.95, 0.90, 0.91 and 1.02*, resp. at 0, 0.1, 0.4 and 1.6 %)	Anonymous, 1977
Sub-chronic (90 days) oral toxicity study Oral (diet) Rat (Wistar) 15/sex/dose No guideline followed Reliability 2 (according to the registration dossier)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 0.1, 1.0 and 5.0 % (no corresp. approx. available) Duration of exposure: 90 days	Mortality: At high dose, all animals died between D 11 and 39 Clinical signs: At high dose, poor general condition Bw: severe decrease at 5 % Organ weight: (only performed in control, low and mid dose) no treatment-related change Histology: no spermatogenesis in 3 males	Anonymous, 1968
Combined chronic oral toxicity study and carcinogenicity study Oral (diet) Rat (Wistar) (animals derived from parents which had received the test substance by diet during pre-mating, mating, gestation and lactation) 60/sex/dose No guideline followed Reliability 1 (according to the registration dossie)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 0.16, 0.4 and 1.0 % (corresp. approx. to 0, 70, 200 and 500 mg/kg bw/d) Duration of exposure: 130 weeks (and animals exposed *in utero*)	Mortality and clinical signs: no treatment-effects observed Bw: not significantly modified Necropsy: no treatment-related effects	Anonymous, 1982
Sub-chronic (90 days) oral	Ethylene bis[3,3-bis(3-tert-butyl-4-	No mortality or treatment-related clinical signs	Anonymous,

Method, guideline, species, strain, sex, nb/group	Test substance, dose levels, duration of exposure	Results	Reference
toxicity study Oral (diet) Dog (Beagle) 4/sex/group No guideline followed Reliability 2 (according to the registration dossier)	hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 100, 500 and 600/750 ppm (highest dose corresp. approx. to 47.6 mg/kg bw/d in males and to 39.6 mg/kg bw/d in females) The highest dose (600 ppm) was increased to 750 ppm after a 30 of exposure period) Duration of exposure: 90 days	Bw unaffected Absolute testes weight slightly reduced at the highest dose (not dose-related and not significant) Absolute ovaries weight increased at the low dose and decrased at the mid and high dose groups, without statistical significance No macroscopic or microscopic findings observed in the reproductive organs	1977
Chronic (2 year) oral toxicity study Oral (diet) Dog (Beagle) 6/sex/dose No guideline followed Reliability 2 (according to the registration dossier)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 100, 500 and 600/1100 ppm (highest dose corresp. approx. to 84.6 mg/kg bw/d in males and to 75.2 mg/kg bw/d in females) The highest dose (600 ppm) was increased to 1100 ppm after a 93 of exposure period) Duration of exposure: 2 years	No mortality or clinical signs observed Bwg slightly reduced in males exposed to the highest dose (significance unknown) Testes weight slightly reduced at the highest dose (not dose-related, significance unknown) No macroscopic or microscopic findings observed in the reproductive organs	Anonymous, 1980

No human data or other data available

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

In a Reproduction/developmental Toxicity Screening Test (Anonymous, 2019), following OECD TG 421, groups of 10 male and 10 female Wistar rats were given, by diet, test substance at a concentration of 0, 3750, 7500 and 11250 ppm (corresponding approximately to 0, 271, 513 and 826 mg/kg bw/d in males and to 0, 501, 1013 and 1320 mg/kg bw/d in females). Males were exposed during 29 or 34 days (minimum 14 days prior mating and during mating period), while females were exposed during 57 to 71 days (14 days prior mating, during mating and gestation periods, and at least 21 days after delivery). Females which failed to deliver were treated for a period of 43 days. Furthermore, two satellite groups of 5 females (one satellite control group and one satellite high group) were treated in the same manner as main group. However, after littering (PND 1) litters of the satellite high group were cross-fostered with litters of the satellite control group, and *vice versa*.

F0 parental generation

No treatment-related mortality was observed during the study period, as one female of the mid dose group died on LD 23 (at blood sampling before scheduled necropsy, the necropsy revealed dark-red discolouration

of the glandular mucosa of the stomach. the death was considered incidental and not related to the treatment)) and one female of the control group was sacrificed in extremis on DPC 18 (severe scabs on a large area of the tail was observed). Furthermore, no treatment-related clinical signs was noted. As observed in Table 9, body weight examination revealed a trend towards lower body weight gain in males exposed to 11250 ppm. At the end of the study period, mean body weight in males of the highest dose was slightly lower (± -4 %, not significant). In females (see Table 10), body weight and body weight gain of the treated groups remained comparable to the control during pre-mating and post-coitum period. While during lactation period, body weight was significantly reduced at the highest dose (on PND 13 and 21) and a similar trend was observed for females of the mid dose group (although not significant).

Table 9: body weight (in g) and body weight gain (in %) in males

Dose level (in ppm)		0	3750	7500	11250
Nb animals examined		10	10	10	10
Pre-mating period	D 1	288	289	292	290
	D 8	311 (8 %)	315 (9 %)	316 (8 %)	305 (5 %)
Mating period	D 1	324 (12 %)	329 (14 %)	328 (12 %)	321 (11 %)
	D 8	336 (16 %)	342 (18 %)	336 (15 %)	324 (12* %)
	D 15	354 (23 %)	360 (25 %)	354 (22 %)	341 (18 %)

Table 10: body weight (in g) in females

Dose level (in ppm)		0	3750	7500	11250
Pre-mating period	D 1	225 (n= 15)	226 (n= 10)	230 (n= 10)	230 (n= 15)
	D 8	229 (n= 15)	230 (n= 10)	232 (n= 10)	230 (n= 15)
Mating period	D 1	231 (n=15)	232 (n= 10)	232 (n= 10)	234 (n= 15)
	D 8	238 (n= 2)	-	259 (n= 1)	273* (n= 2)
	D 15	267 (n= 1)	-	273 (n= 1)	287 (n= 2)
	D 22	309 (n= 1)	-	311 (n= 1)	303 (n= 2)
	D 29	-	-	-	261 (n= 1)
Post-coitum period	D 0	234 (n= 11)	233 (n= 7)	235 (n= 7)	232 (n= 11)
	D 4	246 (n= 11)	248 (n= 7)	248 (n= 7)	242 (n= 12)
	D 14	277 (n= 11)	279 (n= 7)	278 (n= 7)	272 (n= 12)
	D 20	339 (n= 11)	333 (n= 7)	333 (n= 7)	322 (n= 12)
Lactation period	D 1	261 (n= 12)	263 (n= 7)	257 (n= 8)	247 (n= 13)
	D 4	274 (n= 12)	276 (n= 7)	267 (n= 8)	261 (n= 13)
	D 7	279 (n= 12)	290 (n= 7)	279 (n= 8)	265 (n= 13)
	D 13	289 (n= 12)	285 (n= 7)	274 (n= 8)	255** (n= 13)
	D 21	280 (n= 12)	282 (n= 7)	265 (n= 8)	248** (n= 13)

Regarding male fertility, sperm was not examined in this study.

In females, one female exposed to 11250 ppm failed to be mated, which result in a mating index of 93 % compared to 100 % in the other groups (however, this result was not significant and comprised in the range

of HCD 90-100 % (Wistar rats, period 2015-2018)). As observed in Table 11, fertility index was low in all treated groups as well as in control group, and no correlating microspic findings was observed in the non-pregnant females. Mean precoital interval as well as mean number of implantation sites did not exhibited treatment-related changes. Furthermore, duration of gestation was similar in all groups (21.3, 21.6, 21.5 and 21.6 days, resp. at 0, 3750, 7500 and 11250 ppm in the main groups, and 21.1 and 21.4 days, resp. at 0 and 11250 ppm in the cross-fostered groups). And no signs of difficult or prolonged parturition were noted among the pregnant females.

Table 11: Reproduction data

Dose level (in ppm)	0	3750	7500	11250
Nb of females paired	15	10	10	15
Nb of females mated	15	10	10	14
Nb of pregnant females	12	7	8	13
Fertility index (in %)	80	70	80	93
Nb of females with living pups on D 1	12	7	8	13
Mean precoital interval (in d)	3.1	2.0	3.4	2.8
Mean nb of implantation sites	11.4	11.6	11.3	11.5

At necropsy, macroscopic examination revealed no treatment-related findings in males. Only 1 male exposed to 7500 ppm had testes and epididymides reduced in size. While in females, macroscopic abnormalities were observed in 2 organs (adrenal glands and thymus). Enlarged adrenal glands was noted in 3 females exposed to 11250 ppm and discoloration red-brown/black-brown was recorded in 4 females of this group. 1 female of the mid dose and 2 females of the highest dose exhibited a reduced size of the thymus. No significant difference in organ weight examination was observed. Relative testes, prostate and epididymides weights were similar in all groups. Histology did not revealed change in the reproductive organ.

Table 12: Organ weight (in g or %)

Dose level (in ppm)		0	3750	7500	11250
In males					
FBW (g)		335	340	333	320
Thyroids (g or %)	Abs	0.015	0.014	0.016	0.014
	Rela	0.004	0.004	0.005	0.005
Testes (g or %)	Abs	3.58	3.48	3.31	3.49
	Rela	1.07	1.03	1.00	1.09
Prostate (g or %)	Abs	0.887	0.797	0.870	0.851
	Rela	0.265	0.235	0.260	0.267
Epididymides (g or %)	Abs	1.099	1.064	1.034	1.060
	Rela	0.328	0.314	0.313	0.331
Seminal vesicles (g or %)	Abs	1.388	1.263	1.225	1.387
	Rela	0.416	0.373	0.368	0.434
In females					
FBW (g)		268	265	256	240**

Thyroids (g or %)	Abs	0.016	0.016	0.016	0.015
	Rela	0.006	0.006	0.006	0.006

F1 generation

At delivery, total number of pups was comparable in all groups. The mean number of live born pups was slightly reduced in treated groups (excluding the cross-fostered groups) (see Table 13).

Table 13: Litter data

		Main groups				Cross-fostered groups	
Dose level (in ppm)		0	3750	7500	11250	0	11250
Nb of litter		7	7	8	8	5	5
Nb of living pups at first litter check	Mean	11.6	10.6	10.3	10.3	10.0	11.6
	Tot.	81	74	82	82	50	58
Nb of dead pups at first litter check	Tot.	0	0	0	1	0	0

More information regarding F1 generation was available in section 10.10.4 Adverse effects on development.

In a one-generation reproductive toxicity study (Anonymous, 1979), groups of 15 male and 30 female Wistar rats were exposed via feed to the test substance at a concentration of 0, 0.16, 0.4 and 1.0 %, corresponding approximately to 0, 70, 200 and 500 mg/kg bw/d. Animals received the test substance daily during a study period of 7 weeks for males (4 weeks of pre-mating period and 3 weeks of mating period) and 13 weeks for females (4 weeks and 3 weeks, resp. for pre-mating and mating period, and during gestation period (3 weeks) and lactation until weaning of pups (PND 21). The pups from this study were used for a chronic toxicity study reported later in this CLH report.

During the study period, no mortality occurred and no treatment-related clinical signs was noted. Body weight was relatively low (no more information available) in males of the 2 highest doses, while it was similar in females. Food intake tended to be decreased in all tested groups (no more information available). No more information was available regarding general toxicity.

Sperm parameters were not examined in this study.

Regarding female fertility, all females of the control and of the 2 lowest doses casted a litter. At the highest dose, only 1 female out of 30 was not fertile, which result in a fertility index of 100 % in control, in 0.16 % and in 0.4 % groups, while it was of 93 % at 1.0 % (not significant). No information was available concerning the number of implantation, the duration of gestation, the number of pre- and post-implantation loss. At the end of the gestation period, the mean litter size was similar in all groups, as it was of 10.8, 11.2, 10.6 and 10.7 pups, respectively at 0, 0.16, 0.4 and 1.0 %.

Necropsy of the parental generation was not performed.

More information regarding the F1 generation was available in section 10.10.4 Adverse effects o development.

In another reproductive toxicity study (Anonymous, 1976), groups of 10 male and 20 female Wistar rats were exposed by diet at the test substance at a concentration of 0, 0.1, 0.4 and 1.6 %, corresponding approximetaly to 0, 50, 200 and 800 mg/kg bw/d. Animals were exposed during a study period of 7 weeks for males (4 weeks of pre-mating and 3 weeks of mating) and 13 weeks for females (4 weeks of pre-mating, 3 weeks of mating, 3 weeks of gestation and 3 weeks of lactataion periods). The pups from this study were used for a sub-chronic toxicity study which is reported later in this CLH report.

During the study period, no treatment-related mortality nor clinical signs were observed. As observed in Table 14, body weight was relatively low in males exposed to 0.4 and 1.6 %. The total food intake and food efficiency over 4 weeks were reduced in both sexes at 1.6 % and only in males at 0.4 % (no information regarding statistical analysis). Information regarding necropsy was not available.

Table 14: body weight data (in g)

		Males				Females			
Dose level (in %)		0	0.1	0.4	1.6	0	0.1	0.4	1.6
At the end of	Week 0	254	254	254	254	165	165	165	165
	Week 1	269	273	267	259	173	172	172	171
	Week 2	278	282	273	265	172	171	174	171
	Week 3	295	288	286	278	180	180	179	178
	Week 4	303	306	294	284	181	182	183	178

During the mating period, all females of the control, the low and the mid dose groups casted a litter, while 2 females exposed to 1.6 % failed to be pregnant, which results in a fertility index of 100, 100, 100 and 90 %, resp. at 0, 0.1, 0.4 and 1.6 % (no information regarding statistical analysis). At the end of the gestation, the mean litter size showed some variation amongst the groups, but the change was not dose-related (10.1, 11.1, 9.7 and 10.2 pups, resp. at 0, 0.1, 0.4 and 1.6 %) (no information about statistical analysis).

Regarding male fertility, sperm was not examined in this study.

More information regarding the F1 generation was available in section 10.10.4 Adverse effects o development.

In a sub-chronic (16 week) oral toxicity study (Anonymous, 1977), performed with the pups from the one-generation reproductive toxicity study, groups of 50 male and 50 female Wistar rats were received by diet the test substance at a concentration of 0, 0.1, 0.4 and 1.6 % (corresponding approximately to 0, 70, 200 and 800 mg/kg bw/d). Animals derived from parents which had also received the test substance by diet during a pre-mating period (31 days), mating, gestation and lactation (Anonymous, 1976). The test substance fed to rats at the same dietary levels as their parent.

During the study period, no treatment-related mortality nor clinical signs were observed. As observed in Table 15, bw in both sexes were significantly decreased in the highest dose from the start of the study (caused by growth depression during the lactation period as animals derived from the one-generation reproductive toxicity study). At the mid dose, significant increase was observed in both sexes but not during the all study period.

Table 15: body weight data (in g)

	Males				Females			
Dose level (in %)	0	0.1	0.4	1.6	0	0.1	0.4	1.6
W 0	46.5	46.5	45.9	37.1*** (-20.22 %)	43.9	45.0	46.2*	37.7*** (-14.12 %)
W 1	79	79	80	64*** (-18.99 %)	72	73	75*	59*** (-18.06 %)
W 2	116	116	119	97*** (-16.38 %)	98	97	101	84*** (-14.29 %)
W 4	184	186	193*	161*** (-12.5 %)	130	130	136*	118*** (-9.23 %)
W 8	255	258	264	233*** (-8.63 %)	159	169	164*	150*** (-5.66 %)
W 12	297	301	312**	273*** (-10.99 %)	177	176	184*	167** (-5.65 %)

W 16	328	337	346**	301*** (-8.23 %)	195	192	200	181*** (-7.18 %)

*: p<0.05; **: p<0.01; ***: p<0.001

At necropsy, the relative weights of ovaries showed a dose-related increase with significant effects in the mid and high dose groups. The relative testes weight was also significantly higher in the highest dose, however change was not dose-related. No changes were seen in absolute organ weight. Furthermore, macroscopic and microscopic examination did not reveal any abnormalities in these reproductive organs.

Table 16: FBW (in g) and relative organ weight (in g/100 g bw)

	Males				Females			
Dose level (in %)	0	0.1	0.4	1.6	0	0.1	0.4	1.6
FBW	333	340	355	304*	194	189	203	175**
Adrenals	0.0134	0.0134	0.0138	0.0138	0.0288	0.0268	0.0274	0.0292
Brain	0.54	0.54	0.51	0.59	0.86	0.88	0.85	0.95**
Heart	0.335	0.325	0.320	0.340	0.369	0.369	0.371	0.393
Kidneys	0.58	0.55	0.57	0.63*	0.66	0.66	0.66	0.68
Liver	3.16	3.13	3.22	3.31	2.96	3.13	3.08	3.23**
Spleen	0.161	0.152	0.148	0.166	0.175	0.199**	0.190*	0.206***
Thymus	0.112	0.103	0.100	0.118	0.139	0.130	0.129	0.150
Thyroid	0.0084	0.0079	0.0070	0.0093	0.0109	0.0114	0.0091*	0.0101
Testicule	0.95	0.90	0.91	1.02*	-	-	-	-
Ovary	-	-	-	-	0.0321	0.0325	0.0375*	0.0403**

*: p<0.05; **: p<0.01; ***: p<0.001

In another sub-chronic oral toxicity study (Anonymous, 1968), groups of 15 male and 15 female Wistar rats were exposed daily by diet to the test substance at a concentration of 0, 0.1, 1.0 and 5.0 % during 90 days.

During the study period, all animals exposed to the highest dose exhibited a poor general condition and a severe body weight decrease. At this dose, practically no food had been consumed. Between day 11 and 39 of the study period, all animals of the highest dose died.

At necropsy, testes and ovaries weight's examination did not revealed treatment-related change. Microscopic examination was only performed in 9 males and 8 females of the highest dose (other animals not present due to cannibalism). In 3 males exposed to 5 %, no spermatogenesis was noted. In the 5 remaining males of the highest dose and in the other groups, spermatogenesis was present.

In a combined chronic oral toxicity study and carcinogenicity study (Anonymous, 1982), groups of 60 male and 60 female Wistar rats were orally exposed during 130 weeks to the test substance at a concentration of 0, 0.16, 0.4 and 1.0 %, corresponding approximately to 0, 70, 200 and 500 mg/kg bw/d. Animals derived from parents which had also received the test substance by diet during a pre-mating period (31 days), mating, gestation and lactation (Anonymous, 1979). The test substance fed to rats at the same dietary levels as their parent.

During the study period, no treatment-related mortality nor clinical signs. After 18 months, ageing symptoms was observed in all groups (control and tested groups) such as dyspnoea, emaciation, blood discharge around

nostrils and eyes, cyanosis, weakness of muscles. At the end of the study, body weight was not significantly modified. Furthermore, necropsy did not revealed treatment-related effects.

Two repeated dose toxicity study performed in dogs were also available, a sub-chronic and a chronic studies.

In the sub-chronic oral toxicity (Anonymous, 1977), groups of 4 male and 4 female Beagle dogs were exposed orally during 90 days to the test substance at a concentration of 0, 100, 500 and 600/750 ppm (the highest dose corresponds approximately to 47.6 kg in males and to 39.6 kg in females). At the beginning of the study, the highest dose was set to 600 ppm, and after 30 days, the dose was increased to 750 ppm for the rest of the study.

All animals survived during the study period. Furthermore, no treatment-related clinical signs or body weight change were observed. As observed in Table 17, absolute testes weight was slightly reduced at the highest dose. This modification was due to 2 animals which had testes weight reduced (15.0 g). No correlated macroscopic or microscopic findings were noted.

Table 17: FBW (in kg) and absolute organ weight (in g)

	Males				Females			
Dose level (in ppm)	0	100	500	600/750	0	100	500	600/750
FBW	13.845	15.505	15.375	14.495	12.992	14.352	13.437	12.652
Adrenals	1.080	1.059	1.036	0.84	1.228	1.198	1.180	1.114
Brain	88.0	85.0	88.75	85.75	85.0	92.0	83.25	80.25
Heart	120.75	130.25	114.0	118.5	104.0	113.25	109.0	109.75
Kidneys	53.5	54.75	54.0	55.5	48.5	55.5	48.75	50.25
Liver	439.0	410.5	437.25	429.5	377.0	446.75	375.5	365.0
Lungs	127.75	122.5	140.5	116.5	122.75	121.5	121.0	107.5
Pancreas	27.75	30.0	31.75	27.0	29.5	32.0	31.5	28.75
Pituitary	0.098	0.077	0.116	0.081	0.092	0.083	0.086	0.094
Spleen	49.75	40.5	52.75	59.25	51.25	65.75	48.25	68.75
Testes	19.75	22.0	19.75	17.75	-	-	-	-
Ovaries	-	-	-	-	1.332	1.475	1.004	0.929

In a chronic oral toxicity study (Anonymous, 1980), groups of 6 male and 6 female Beagle dogs were given by diet the test substance at a concentration of 0, 100, 500 and 600/1100 ppm (highest dose corresponding approximately to 84.6 mg/kg bw/d in males and to 75.2 mg/kg bw/d in females). At the beginning of the study, the highest dose was of 600 ppm, and after 93 days, it was increased to 1100 ppm.

All animals survived during the study period, and no clinical signs was observed. In males, body weight gain was slightly reduced at the highest dose (3.47, 2.85, 3.37 and 2.18 g in M and 3.63, 2.67, 3.9 and 3.4 g in F; no information about statistical analysis). As observed in Table 18, testes weight was slightly reduced at the highest dose. However, modification was not significant and dose-related. In females, ovaries weight was increased at the low and the mid doses (not significant).

Table 18: FBW (in kg) and organ weight data (in g or %)

	Males				Females			
Dose level (in ppm)	0	100	500	600/110	0	100	500	600/110

FBW		16.786	15.886	16.045	14.921	15.740	15.155	14.956	14.401
Adrenals	Abs	1.116	1.270	1.309	1.335	1.262	1.378	1.712	1.520
	Rela	0.007	0.008	0.008	0.009	0.008	0.009	0.012	0.011
Brain	Abs	92.167	89.333	91.333	86.333	86.667	87.833	82.833	83.167
	Rela	0.550	0.563	0.572	0.579	0.554	0.585	0.560	0.581
Heart	Abs	147.33	152.33	144.83	146.00	133.50	127.83	128.67	126.33
	Rela	0.88	0.96	0.91	0.98	0.85	0.85	0.87	0.88
Kidneys	Abs	65.333	62.500	68.333	60.667	63.000	63.667	59.833	59.000
	Rela	0.391	0.394	0.427	0.407	0.403	0.423	0.402	0.411
Liver	Abs	484.50	461.17	494.17	511.33	525.67	497.17	472.50	468.33
	Rela	2.90	2.91	3.10	3.44	3.37	3.30	3.17	3.26
Lungs	Abs	138.83	144.67	134.67	135.33	134.67	130.33	120.67	125.50
	Rela	0.83	0.91	0.84	0.91	0.86	0.86	0.81	0.87
Pancreas	Abs	29.667	34.333	34.833	36.500	35.833	34.833	40.833	39.000
	Rela	0.177	0.216	0.218	0.244	0.230	0.232	0.267	0.271
Pituitary	Abs	0.087	0.081	0.081	0.092	0.065	0.096	0.088	0.100
	Rela	0.0005	0.0005	0.0005	0.0006	0.0004	0.0006	0.0006	0.0007
Slpeen	Abs	88.667	89.833	94.667	61.000	59.833	77.167	75.000	86.333
	Rela	0.532	0.566	0.588	0.409	0.372	0.508	0.504	0.603
Thyroid	Abs	0.819	0.980	0.939	0.874	0.767	0.846	0.975	0.974
	Rela	0.0049	0.0062	0.0059	0.0059	0.0049	0.0056	0.0064	0.0068
Testes/Ovaries	Abs	20.167	20.000	20.500	17.500	1.008	1.257	1.476	1.099
	Rela	0.121	0.127	0.128	0.118	0.006	0.008	0.010	0.008

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	"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

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.”	consequence of the other toxic effects."

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

In the available reproductive studies performed in rats as well as the available repeated dose toxicity studies in rats and dogs, no evidence of significant adverse effects on sexual function and fertility was demonstrated. However, limited information regarding male reproductive system is available, as sperm was not examined in the available studies. Based on the available results, a classification as Repr. 1B or 2 is then not warranted.

10.10.4 Adverse effects on development

Table 19: Summary table of animal studies on adverse effects on development

Method, guideline, species, strain, sex, nb/group	Test substance, dose levels duration of exposure	Results	Reference
Embryo-foetal developmental toxicity study Oral (gavage) Rat (Wistar) 24 pregnant females/group OECD TG 414 GLP Reliability 1 (according to the registration dossier)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: 97.4 % Doses: 0, 100, 300 and 1000 mg/kg bw/d Duration of exposure: GD 5 to 19 (ceasarean section and sacrificed on GD 20)	F0 parental generation No mortality occurred Clinical signs: 1000 mg/kg bw/d: reddish discharge from vagina (16 females out of 24) between GD 13 to 20 (associated to dams with resorption) Bw: sign. reduced at 1000 mg/kg bw/d (from GD 11 to GD 20) At GD 11: 244.28, 246.44, 243.78 and 231.52* g, resp. at 0, 100, 300 and 1000 mg/kg bw/d At GD 20: 302.53, 305.70, 286.72 and 224.38* g, resp. at 0, 100, 300 and 1000 mg/kg bw/d Mean nb of post-implantation loss: 0.52, 0.50, 3.08* and 10.25* (% per litter: 4.02, 5.39, 26.67 and 91.81 %), resp. at 0, 100, 300 and 1000 mg/kg bw/d Nb of dams with all resorption: 0, 0, 2 and 17*, resp. at 0, 100, 300 and 1000 mg/kg bw/d Necropsy: gross pathological examination: changes observed in thymus and adrenals at the highest dose Corrected bwg: stat. sign. reduced at 1000 mg/kg bw/d (15.68, 16.94, 12.71 and -11.26* g, resp. at 0,	Anonymous, 2017a

Method, guideline, species, strain, sex, nb/group	Test substance, dose levels duration of exposure	Results	Reference
		100, 300 and 1000 mg/kg bw/d) F1 pups Tot. nb of foetuses: 252, 267, 203 and 24*, resp. at 0, 100, 300 and 1000 mg/kg bw/d Mean litter size: 11.0, 11.1, 9.2 and 3.4, resp. at 0, 100, 300 and 1000 mg/kg bw/d Pups bw (M+F): 3.54, 3.59, 3.38 and 2.60* g, resp. at 0, 100, 300 and 1000 mg/kg bw/d	
14-day repeated dose and embryo-foetal developmental (EFD) toxicity study Oral (gavage) Rat (Wistar) 6/sex/group for main and satellite groups, 6 pregnant females/group for EFD Similar to OECD TG 414 and 407 Reliability 2 (according to the registration dossier)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: 97.4 % Doses: 0, 100, 300 and 600 mg/kg bw/d Duration of exposure: 14 days for main groups, 2 and 9 days for satellite groups and between GD 5 to 19 for EFD	EFD part: F0 generation: No mortality occurred Clinical signs: reddish discharge observed in 3 females of the highest dose Bw: sign. reduced at the highest dose at GD 20 (320.68, 313.82, 313.63 and 275.07* g, resp. at 0, 100, 300 and 600 mg/kg bw/d) Nb of post-implantation loss: 0.20, 1.17, 2.0 and 7.4*, resp. at 0, 100, 300 and 600 mg/kg bw/d Resorption: 2 dams out of 6 with complete resorption Corrected bwg: 11.63, 15.23, 8.25 and -7.55, resp. at 0, 100, 300 and 600 mg/kg bw/d (not mentioned as significant in the study report) F1 pups: Mean litter size: 13.8, 10.5, 11.6 and 11.0 pups, resp. at 0, 100, 300 and 600 mg/kg bw/d Tot. nb of foetuses: 69, 63, 58 and 33 foetuses, resp. at 0, 100, 300 and 600 mg/kg bw/d (statistical analysis not performed) Foetus weight: slightly reduced at the highest dose No foetal external or visceral abnormalities	Anonymous, 2017b
Reproduction/developmental Toxicity Screening Test Oral (diet) OECD TG 421 GLP Rat (Wistar) Main group: 10/sex/group Additional group: 5 females/group Reliability 1	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: 97.4 % Doses: 0, 3750, 7500 and 11250 ppm (corresp. approx. to 0, 271, 513 and 826 mg/kg bw/d in males and to 0, 501, 1013 and 1320 mg/kg bw/d in females) Duration of exposure: In males 29 or 34 D (min. 14 D prior mating and	F0 parental generation: See results in Table 8 F1 generation: Tot. nb of pups comparable in all groups Mean nb of live born pups slightly reduced in treated groups (11.6, 10.6, 10.3 and 10.3 pups, resp. at 0, 3750, 7500 and 11250 ppm). Modification not observed in cross-fostered group (10.0 and 11.6 pups, resp. in control and high cross-fostered groups) Pups bw: similar at birth. But trends towards lower bw from D 4, reaching to significant modification at the highest dose (from PND 13) and at the mid dose	Anonymous, 2019

Method, guideline, species, strain, sex, nb/group	Test substance, dose levels duration of exposure	Results	Reference
(according to registration dossier)	during mating period) In females: 57 to 71 D (14 D prior mating, during mating, gestation, and at least 21 D after delivery)	from (PND 21) Viability index: 99, 100, 99 and 98 %, resp. at 0, 3750, 7500 and 11250 ppm Lactation index: 100, 98, 100 and 100 %, resp. at 0, 3750, 7500 and 11250 ppm AGD and nipple retention: similar in all groups Post-implantation loss and resorption not examined	
One-generation reproductive toxicity study Oral (diet) No OECD guideline nor GLP (performed before GLP) Rat (Wistar) 15 males and 30 females/groups Reliability 2 (according the registration dossier, however only summary information available. Full tudy report was available to the DS but bad quality of the PDF file and tables mostly not readable)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 0.16, 0.4 and 1.0 % (corresp. approx. to 0, 70, 200 and 500 mg/kg bw/d) Duration of exposure: 7 weeks for males and 13 weeks for females (4 W of pre-mating and 3 W of mating, followed for females by 3 W of gestation and 3 W of lactation)	F0 parental generation: Information available in Table 8 F1 generation: Mean litter size: 10.8, 11.2, 10.6 and 10.7, resp. at 0, 0.16, 0.4 and 1.0 % Viability index at D 1: between 97 to 100 % Lactation index: unaffected by treatment Pups bw: significantly reduced at the highest dose at PND 21 No external malformation (visceral and skeletal examination not performed)	Anonymous, 1979
Reproductive toxicity study Oral (diet) No OECD guideline nor GLP Rat (Wistar) 11 males and 20 females/group Reliability 4 (according to the registration dossier (very restricted reporting, summary only, no individual data))	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: not specified Doses: 0, 0.1, 0.4 and 1.6 % (corresp. approx. to 0, 50, 200 and 800 mg/kg bw/d) Duration of gestation: 7 weeks for males and 13 weeks for females (4 W of pre-mating and 3 W of mating, followed for females by 3 W of gestation and 3 W of lactation)	F0 parental generation: Information available in Table 8 F1 generation: Litter size unaffected Mortality of pups at birth and at LD 20 sign. increased at the highest dose Bw at bith similar in all groups, while it was reduced at the highest during the rest of the lactation period No external malformation (visceral and skeletal examination not performed)	Anonymous, 1976

No human or other data available

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

In an Embryo-foetal developmental toxicity study (Anonymous, 2017a), performed according to OECD TG 414, groups of 24 pregnant female Wistar rats were exposed by gavage to the test substance at a concentration of 0, 100, 300 and 1000 mg/kg bw/d from gestational day 5 to 19. On gestational day 20, a caesarean section was performed and animal were sacrificed.

F0 parental generation

No mortality occurred during the study period. At the highest dose, reddish discharge from vagina was observed in most of the rats (16 out of 24) between GD 13 to 20 (associated to dams with resorption at necropsy). As observed in Table 20, body weight was significantly reduced from GD 11. At the mid dose, a trend towards a lower body weight was observed from GD 17. Furthermore, food consumption was significantly decreased during the treatment period.

Table 20: Body weight and body weight gain (in g)

Dose level (in mg/kg bw/d)	0	100	300	1000
Nb of animals examined	23	24	24	24
GD 0	212.58	212.41	212.42	212.38
GD 5	226.12	226.59	226.92	225.97
GD 11	244.28	246.44	243.78	231.52*
GD 17	277.73	280.57	268.41	228.81* (-17.61 %)
GD 20	302.53	305.70	286.72	224.38* (-25.83 %)
BWG				
Pre-treatment period (GD 0-5)	13.55	14.18	14.50	13.58
Treatment period (GD 5-20)	76.40	79.11	59.80*	-1.59*
Entire gestation period (GD 0-20)	89.95	93.29	74.30*	12.00*

Regarding reproductive data, as observed in Table 21, the mean number of pre-implantation loss was similar in all groups, while the mean number of post-implantation loss was significantly at the 2 highest doses. Two out of the 24 dams exposed to 300 mg/kg bw/d showed total resorption and at the highest dose, there was a significant increase of dams with complete resorption. The number of litter at the end of the gestation period was then severely reduced at the highest dose as well as the number of pups.

Table 21: Resorption and implantation loss data

Dose level (in mg/kg bw/d)	0	100	300	1000
Nb of pregnant rat	23	24	24	24
Mean nb of pre-implantation loss	1.70	2.08	2.00	2.04
Mean nb of post-implantation loss	0.52	0.50	3.08*	10.25*
Mean nb of early resorption (% per litter)	0.39 (2.94%)	0.42 (4.78%)	1.25 (10.79%)	0.88 (7.89%)
Mean nb of late resorption (% per litter)	0.13 (1.09%)	0.08 (0.62%)	1.83* (15.89%)	9.38* (83.92%)
Nb of dams with any resorption	7	9	19*	7
Nb of dams with all resorption	0	0	2	17*
Nb of litter	23	24	22	7
Tot. nb of fetuses	252	267	203	24*

At necropsy, at 300 and 1000 mg/kg bw/d, the gravid uterine weights were significantly reduced while the mean carcass weight of the dams is only statistically significantly lower in the highest dose group (see Table 22). In the mid dose group, mean carcass weight is comparable to the control group. Furthermore, corrected body weight gain is only statistically significantly reduced in the highest dose whereas at 300 mg/kg bw/d this number does not reach statistical significance. Macroscopic examination revealed findings at the highest dose, such as small thymus (in 10 females) or non-prominent thymus (9 females) and bilateral enlarged adrenals (12 females).

Table 22: Corrected bw and bwg (in g)

Dose level (in mg/kg bw/d)	0	100	300	1000
Nb of dams examined	23	24	24	24
BW at GD 5	226.12	226.59	226.92	225.97
Terminal BW on GD 20	302.53	305.70	286.72	224.38*
Uterine weight	60.73	62.17	47.09*	9.67*
Carcass weight (terminal bw on GD 20 – uterine weight)	241.80	243.53	239.63	214.70*
Corrected BWG (carcass weight – bw on GD 5)	15.68	16.94	12.71	-11.26*

F1 generation

At birth, the number of foetuses was severely reduced at the highest dose and at the mid dose, the number of foetuses was also decrease but not significantly (mean litter size: 11.0, 11.1, 9.2 and 3.4 pups, resp. at 0, 100, 300 and 1000 mg/kg bw/d). Moreover, weight of male and female foetuses was significantly lower at 1000 mg/kg bw/d (see Table 23). Foetal visceral and skeletal examination did not revealed no signs of major malformations. An increased incidence of minor anomalies and changes in normal variants (delayed skeletal ossification and incomplete/poor ossification) were noted at 300 and 1000 mg/kg bw/d (mentioned in the registration as “mainly due to poor development of the fetuses at theses doses).

Table 23: Live foetuses weight (in g)

Dose level (in mg/kg bw/d)	0	100	300	1000
M+F	3.54	3.59	3.38	2.60*
M fetuses	3.65	3.67	3.50	2.59*
F fetuses	3.40	3.49	3.29	2.78*

A 14-day repeated dose and embryo-foetal developmental toxicity study (Anonymous, 2017b) was performed in Wistar rats and exposed animals at the test substance at a concentration of 0, 100, 300 and 600 mg/kg bw/d. This study was divided in 3 parts:

- The main part examined groups of 6 male and 6 female Wistar rats per dose which were exposed during 14 days.
- The satellite groups were also composed of 6 animals per sex per dose which were exposed during an exposure period of 2 or 9 days.
- And an EFD part which exposed 6 presumed pregnant female per dose from the gestation day 5 to 19.

The main part as well as the satellite part did not examined developmental parameters and then is not described in this section (Methods and results are described in detail in the Annex I of the CLH report).

EFD part: F0 generation

During the study period of the EFD, no mortality occurred. Reddish discharge from vagina was noted in 3 females exposed to the highest dose (between GD 16-17 and 20). Moreover, mean body weight was significantly reduced at the end of the gestation period and at the highest dose (see Table 24).

Table 24: Body weight data (in g)

Dose level (in mg/kg bw/d)	0	100	300	600
Nb of animals examined	5	6	5	5
D 0	224.17	225.20	224.70	227.68
D 5	236.91	239.71	241.66	245.63
D 11	256.88	260.84	261.38	260.66
D 17	293.70	290.54	291.86	270.38
D 20	320.68	313.82	313.63	275.07*
Pre-treatment period (GD 0 – 5)	12.75	14.50	16.96	17.95
Treatment period (GD 5 – 20)	83.77	74.11	71.97	29.44*
Entire gestation period (GD 0 – 20)	96.51	88.61	88.93	47.39*

The number of post-implantatin loss was significantly increased at 600 mg/kg bw/d (0.20, 1.17, 2.0 and 7.4*, resp. at 0, 100, 300 and 600 mg/kg bw/d) with 2 dams of 6 showing complete resorption of litters. Dams with any resorption were seen in all groups (1, 3, 3 and 2 dams, resp. at 0, 100, 300 and 600 mg/kg bw/d).

Table 25: Resorption and post-implantation data

Dose level (in mg/kg bw/d)	0	100	300	600
Nb of females	6	6	6	6
Nb of pregnant females	5	6	5	5
Nb of dams with any resorption	1	3	3	2
Nb of dams with all resorption	0	0	0	2
Nb of dams with none resorption	4	3	2	1
Mean nb of post-implantation loss	0.20	1.17	2.0	7.4*

At necropsy, as observed in Table 26, the corrected bw was severely reduced at the highest dose and tend to decrease at the mid dose.

Table 26: Corrected bw (in g)

Dose level (in mg/kg bw/d)	0	100	300	600
Nb animals examined	5	6	5	5
Bw on GD 5	236.91	239.71	241.66	245.63
Terminal bw on GD 20	320.68	313.82	313.63	275.07*
Uterine weight	72.14	58.88	63.72	36.99
Carcass weight (corrected bw on GD 20)	248.55	254.94	149.91	238.08
Corrected BWG	11.63	15.23	8.25	-7.55

EFD part: F1 generation

At caesarean section, the mean litter size was of 13.8, 10.5, 11.6 and 11.0 foetuses, resp. at 0, 100, 300 and 600 mg/kg bw/d while the total number of pups was dose-dependendly decreased (69, 63, 58 and 33 pups, resp. at 0, 100, 300 and 600 mg/kg bw/d; statistical analysis not performed). The mean foetuse weight was marginally lower (5 to 9 %) and one foetus was found dead in the highest dose. Foetal external and visceral observations did not reveal any abnormalities (skeletal examination was not performed).

In a Reproduction/developmental Toxicity Screening Test (Anonymous, 2019), following OECD TG 421, groups of 10 male and 10 female Wistar rats were given, by diet, test substance at a concentration of 0, 3750, 7500 and 11250 ppm (corresponding approximately to 0, 271, 513 and 826 mg/kg bw/d in males and to 0, 501, 1013 and 1320 mg/kg bw/d in females). Males were exposed during 29 or 34 days (minimum 14 days prior mating and during mating period), while females were exposed during 57 to 71 days (14 days prior mating, during mating and gestation periods, and at least 21 days after delivery). Females which failed to deliver were treated for a period of 43 days. Furthermore, two satellite groups of 5 females (one satellite control group and one satellite high group) were treated in the same manner as main group. However, after littering (PND 1) litters of the satellite high group were cross-fostered with litters of the satellite control group, and *vice versa*.

F0 parental generation

No treatment-related mortality was observed during the study period as well as no treatment-related clinical signs. As observed and described in Table 10, body weight and body weight gain were unaffected by treatment during the pre-mating and post-coitum period. While during lactation, body weight was significantly lower at the highest dose (on PND 13 and 21) and a similar trends was noted in females of the mid dose group (but not significant). More details and necropsy data were available in section 10.10.2.

In this study, post-implantation loss and resorption were not examined.

F1 generation

At delivery, total number of pups was comparable in all groups. The mean number of live born pups was slightly reduced in treated groups (excluding the cross-fostered groups) (see Table 13).

As observed in Table 27, at birth, pups body weight was similar in all groups (male, female and combined male and female). However, a trend towards lower body weight was noted in male and female pups of the mid and high doses from PND 13 and PND 4, respectively, onwards, reaching statistical significance on PND 13 and 21 for pups at high dose (relative difference to concurrent control: -19 % and -34 % for M+F) and PND 21 for pups at the mid dose (relative difference to concurrent contro: -16 % for M+F). At the end of the observation period (PND 21), mean body weights of pups in both mid and high dose groups were below the historical control data range.

Table 27: Pups body weight data (in g) (excluding cross-fostered animals)

Dose level (in ppm)		0	3750	7500	11250	HCD (Wistar rats, period 2017-2018)
Nb animals examined		7	7	8	8	/
PND 1	M	6.3	6.7	6.6	6.3	NI
	F	6.0	6.3	6.4	6.0	NI
	M+F	6.2	6.5	6.5	6.1	NI
PND 4	M	9.6	10.1	10.0	9.2	NI
	F	9.3	9.6	9.7	8.9	NI
	M+F	9.5	9.8	9.9	8.9	NI
PND 13	M	30.4	33.0	28.6	24.9**	Mean= 31 (P5-P95= 27.0-34.7)
	F	29.5	31.4	28.1	24.0**	Mean= 30 (P5-P95= 26.7-34.4)

	M+F	30.0	32.1	28.4	24.3** (-19 %)	NI
PND 21	M	50.3	54.5	41.2**	33.7**	Mean= 51 (P5-P95= 43.0-59.0)
	F	48.5	52.1	41.4**	32.4**	Mean= 50 (P5-P95= 43.0-58.0)
	M+F	49.4	53.2	41.3** (-16 %)	32.8** (-34 %)	NI

The viability index was unaffected by treatment (99, 100, 99 and 98 %, resp. at 0, 3750, 7500 and 11250 ppm) as well as the lactation index which was of 100 % in all group (except in low group: 98 % due to one pups which found dead on LD 16). Furthermore, ano-genital distance and nipple retention were similar in all groups. On PND 13 and 21, mean body weight for male and female pups were reduced.

In a one-generation reproductive toxicity study (Anonymous, 1979), groups of 15 male and 30 female Wistar rats were exposed via feed to the test substance at a concentration of 0, 0.16, 0.4 and 1.0 %, corresponding approximately to 0, 70, 200 and 500 mg/kg bw/d. Animals received the test substance daily during a study period of 7 weeks for males (4 weeks of pre-mating period and 3 weeks of mating period) and 13 weeks for females (4 weeks and 3 weeks, resp. for pre-mating and mating period, and during gestation period (3 weeks) and lactation until weaning of pups (PND 21). The pups from this study were used for a chronic toxicity study reported later in this CLH report.

F0 parental generation

During the study period, no mortality occurred and no treatment-related clinical signs was noted. Body weight was relatively low in males of the 2 highest doses, while it was similar in females. Food intake tended to be decrased in all tested groups. No more information was available regarding general toxicity as well as resorption or post-implantation loss examination.

F1 pups

At birth, the mean litter size was unaffected by treatment, as it was of 10.8, 11.2, 10.6 and 10.7 pups, resp. at 0, 0.16, 0.4 and 1.0 %. The total number of pups born dead in each group was small (2, 0, 8 and 1 pups, resp. at 0, 0.16, 0.4 and 1.0 %), resulting in a high viability index at day 1 (between 97 to 100 % in all groups). Furthermore, mortality of offspring during lactation, as expressed by the viability index at day 4 and by the lactation index, was low and not adversely affected by the test substance. As observed Table 28, the mean pup body weight at day 1, 4 and 14 was similar in all groups, while at day 21 body weight in the highest dose was significantly lower in the highest dose. As animals were used for a repeated dose toxicity study, no skeletal and visceral examination were performed.

Table 28: Mean pups bdy weight data (in g)

Dose level (in %)	0	0.16	0.4	1.00
D 1	5.6	5.6	5.6	5.6
D 4	8.6	9.0	8.6	8.3
D 14	26.7	27.8	26.2	26.0
D 21	41.9	42.8	39.7	38.7*

In another reproductive toxicity study (Anonymous, 1976), groups of 10 male and 20 female Wistar rats were exposed by diet at the test substance at a concentration of 0, 0.1, 0.4 and 1.6 %, corresponding approximetaly to 0, 50, 200 and 800 mg/kg bw/d. Animals were exposed during a study period of 7 weeks for males (4 weeks of pre-mating and 3 weeks of mating) and 13 weeks for females (4 weeks of pre-mating,

3 weeks of mating, 3 weeks of gestation and 3 weeks of lactataion periods). The pups from this study were used for a sub-chronic toxicity study which is reported later in this CLH report.

F0 parental generation

During the study period, no treatment-related mortality nor clinical signs were observed. As observed in Table 14, body weight was relatively low in males exposed to 0.4 and 1.6 %. The total food intake and food efficiency over 4 weeks were reduced in both sexes at 1.6 % and only in males at 0.4 %. Information regarding necropsy was not available

F1 pups:

No dose-dependent effect was observed on litter size at birth. In addition, the resorption quotient was comparable in all groups. No information was available regarding post-implantation loss. At birth, mortality of the pups was significantly increased in the highest dose as well as at the lactation day 20 (see Table 29), while on PND 10 mortality was comparable in all groups. As observed in Table 30, the birth weight of the pups were similar in all groups but the body weight during lactation was significantly reduced at the highest dose. No external malformation observed (animals were used for a repeated dose toxicity then visceral and skeletal examination were not perfomed).

Table 29: Percentage of pups mortality

Dose level (in %)	0	0.1	0.4	1.6
At birth	0.5	0.5	0.5	4.4**
At LD 10	1.7	0.5	3.3	2.4
At LD 20	5.6	2.5	5.6	18.1***

Table 30: Pups body weight data (in g)

Dose level (in %)	0	0.1	0.4	1.6
At LD 1	5.3	5.4	5.4	5.3
At LD 10	17.5	17.7	17.6	14.5***
At LD 20	33.6	32.8	32.3	26.5***

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	"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

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.”	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 Repr. 1A for development is not appropriate.

As observed in Table 31, clear adverse effects on development were observed in Wistar rats. In the Embryo-foetal developmental toxicity study (Anonymous, 2017a), performed according to OECD TG 414, post-implantation losses (late resorption) were significantly increased in the mid and high dose groups with the result that the number of total live pups was reduced (significantly only at the highest dose). These results were confirmed in the other 14-day repeated dose and embryo-foetal developmental toxicity study (Anonymous, 2017b). The number of post-implantation loss was significantly increased at the highest dose as well as the number of dams with total resorption. Results of this study (Anonymous, 2017b) are based on statistical evaluation of 6 dams and their pups. Therefore, observations are used in support of the findings reported in the embryo-foetal developmental toxicity study (Anonymous, 2017a). Post-implantation loss was not examined in the 3 other available studies (Anonymous, 2019, 1979 and 1976), while in the Anonymous 1979 and 1976, resorption quotient was unaffected.

Table 31: Post-implantation loss, resorption and number of pups

Dose level (in mg/kg bw/d)	0	100	300	600	1000	1300
Mean nb of post-implantation loss (in % per litter)						
Embryo-foetal developmental toxicity study (Anonymous, 2017a)	0.52 (4.02 %)	0.50 (5.39 %)	3.08* (26.67)	/	10.25* (91.81)	/
14-day repeated dose and embryo-foetal developmental toxicity study (Anonymous, 2017b)	0.20	1.17	2.0	7.4*	/	/
Nb of dams with all resorption						
Embryo-foetal developmental toxicity study (Anonymous, 2017a)	0/23 (0 dams with any resorption)	0/24 (0 dams with any resorption)	2/24 (19 dams with any resorption)	/	17*/24 (7 dams with any resorption)	/
14-day repeated dose and embryo-foetal developmental toxicity study	0/5 (1 with any resorption)	0/6 (3 with any resorption)	0/5 (3 with any resorption)	2/5 (+ 2 with any resorption)	/	/

(Anonymous, 2017b)						
One-generation reproductive toxicity study (Anonymous 1979) Nb of resorption not available but resorption quotient	1.14	1.09 (at 70 mg/kg bw/d)	1.12 (at 200 mg/kg bw/d)	1.07 (at 500 mg/kg bw/d)	/	/
Reproductive toxicity study (Anonymous, 1976) Nb of resorption not available but resorption quotient	1.12	1.10 (at 50 mg/kg bw/d)	1.11 (at 200 mg/kg bw/d)	1.08 (at 800 mg/kg bw/d)	/	/
Tot nb of pups (mean litter size)						
Embryo-foetal developmental toxicity study (Anonymous, 2017a)	252 (11.0)	267 (11.1)	203 (9.2)	/	24* (3.4)	/
14-day repeated dose and embryo-foetal developmental toxicity study (Anonymous, 2017b)	69 (13.8)	63 (10.5)	58 (11.6)	33 (11.0)	/	/
Reproduction/developmental Toxicity Screeninf Test (Anonymous, 2019)	81 (11.6)	/	/	74 (10.6) (at ± 500 mg/kg bw/d)	82 (10.3)	82 (10.3)
One-generation reproductive toxicity study (Anonymous 1979)	(10.8)	(11.2) (at 70 mg/kg bw/d)	(10.6) (at 200 mg/kg bw/d)	(10.7) (at 500 mg/kg bw/d)	/	/
Reproductive toxicity study (Anonymous, 1976)	(10.1)	(11.1) (at 50 mg/kg bw/d)	(9.7) (at 200 mg/kg bw/d)	(10.2) (at 800 mg/kg bw/d)	/	/

The Guidance on the Application of the CLP Criteria (ECHA, July 2017) mentions that *"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."* and that *"Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a caseby-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant postnatal functional deficiencies."*.

Maternal toxicity was observed in the 2 embryo-foetal developmental toxicity studies. For both, maternal body weight was significantly reduced at the highest dose (600 and 1000 mg/kg bw/d, respectively in the embryo-foetal developmental toxicity study (Anonymous, 2017a) and in the 14-day repeated dose and embryo-foetal developmental toxicity study (Anonymous, 2017b)). Body weight gain was also significantly modified at the mid dose in the first embryo-foetal developmental toxicity study and at the highest dose in both studies.

In the embryo-foetal developmental toxicity study (Anonymous, 2017a), mean carcass weight of the dams

(terminal bw on GD 20 minus uterine weight) is only statistically significantly reduced at the highest dose (1000 mg/kg bw/d) (-11 % compared to control), while at 300 mg/kg bw/d, mean carcass weight is comparable to the control group. Uterine weight is statistically significantly lower at 300 and 1000 mg/kg bw/d. The corrected body weight gain (carcass weight minus bw on GD 5) is only statistically significantly decreased at 1000 mg/kg bw/d (-171 % compared to control) whereas the decrease does not reach statistical significance at 300 mg/kg bw/d (-19 % compared to control). These observations indicate that at the mid dose (300 mg/kg bw/d), maternal toxicity was not pronounced. Effects observed on the maternal body weight gain almost completely refer to uterine weight and therefore to the pups. At the mid dose, the effects occur in the absence of severe maternal toxicity and have to be considered as substance-related.

Furthermore, foetus weight are reduced in the embryo-foetal developmental toxicity study (Anonymous, 2017a). And the number of normal variants (delayed skeletal ossification and incomplete/poor ossification) and minor anomalies are elevated at both doses. The variants and anomalies observed are a sign of the poor and retarded development of the foetus. As explained above, the available data do not indicate a severe maternal toxicity at the mid dose which can explained these effects.

The effect on post-implantation loss and late resorptions in the mid dose and high doses, observed in the 2 more recent studies, cannot solely be attritubed to maternal toxicity. In conclusion, a classification as **Repr. 1B H360D** is warranted based on the severe effects.

10.10.7 Adverse effects on or via lactation

Table 32: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reproduction/developmental Toxicity Screening Test Oral (diet) OECD TG 421 GLP Rat (Wistar) Main group: 10/sex/group Additional group: 5 females/group Reliability 1 (according to registration dossier)	Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] Purity: 97.4 % Doses: 0, 3750, 7500 and 11250 ppm (corresp. approx. to 0, 271, 513 and 826 mg/kg bw/d in males and to 0, 501, 1013 and 1320 mg/kg bw/d in females) Duration of exposure: In males 29 or 34 D (min. 14 D prior mating and during mating period) In females: 57 to 71 D (14 D prior mating, during mating, gestation, and at least 21 D after delivery)	Pups body weight: Trend towards lower pups bw from PND 4 onwards in M and F pups from control females cross-fostered by exposed females and decrease was sign. and below HCD from PND 13 at the highest dose (already at the mid dose at PND 21) At PND 13: In M: 30.9 and 22.9** g, resp. at 0 and 11250 ppm (cross-fostered groups) and 30.4, 33.0, 28.6 and 24.9** g, resp. at 0, 3750, 7500 and 11250 ppm (excluding cross-fostered groups) HCD 27.0 – 34.7 g In F: 30.3 and 22.3** g, resp. at 0 and 11250 ppm (cross-fostered groups) and 29.5, 31.4, 28.1 and 24.0** g, resp. at 0, 3750, 7500 and 11250 ppm (excluding cross-fostered groups) HCD 26.7 – 34.3 g At PND 21: In M: 50.4 and 27.1** g, resp. at 0 and 11250 ppm (cross-fostered groups) and 50.3, 54.5, 41.2** and 33.7** g, resp. at 0, 3750, 7500 and 11250 ppm (excluding cross-fostered groups) HCD 43.0 – 59.0	Anonymous, 2019

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		In F: 48.1 and 27.2** g, resp. at 0 and 11250 ppm (cross-fostered groups) and 48.5, 52.1, 41.4** and 32.4** g, resp. at 0, 3750, 7500 and 11250 ppm (excluding cross-fostered groups) HCD 43.0 – 58.0 g Concentration in plasma: 1650 to 5660 ng/mL Concentration in milk: 1280 to 5990 ng/mL	

No human or other data available

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

In a reproduction/developmental toxicity screening test (Anonymous, 2019), performed according to OECD TG 421, groups of 10 male and 10 female Wistar rats were given, by diet, test substance at a concentration of 0, 3750, 7500 and 11250 ppm (corresponding approximately to 0, 271, 513 and 826 mg/kg bw/d in males and to 0, 501, 1013 and 1320 mg/kg bw/d in females). Furthermore, two satellite groups of 5 females (one satellite control group and one satellite high group) were treated in the same manner as main group. However, after littering (PND 1) litters of the satellite high group were cross-fostered with litters of the satellite control group, and *vice versa*.

As observed in Table 33, at birth, mean pup body weight for male pups, female pups and combined both sexes were similar between control and exposed group. From lactation day 4 onwards, a trend towards lower body weight was noted in males and females pups from control females, cross-fostered by exposed females, reaching statistical significance from PND 7 onwards. On PND 13 and 21, mean body weight for male and female pups were clearly below the available historical control data. At the end of the observation period, mean body weight for sexes combined was approximately 45 % lower compared to the mean body weight of pups from exposed females, cross-fostered by control females. Mean body weight of pups from exposed females cross-fostered by control females was similar to that of non-cross-fostered control pups or slightly higher throughout the observation period. Litters used for cross-fostering had a live litter size of 10.0 and 11.6 pups, resp. in control and 11250 ppm groups (after cross-fostering). No cross-fostered pups were found dead or missing during the study period, the viability index as well as the lactation index were 100 % for both control and exposure groups.

Table 33: Pups body weight data (in g) and viability index

		Cross-fostered groups		Excluding cross-fostered groups				
Dose level (in ppm)		0	11250	0	3750	7500	11250	HCD (Wistar rats, period 2017-2018)
Nb animals		5	5	7	7	8	8	/
PND 1	M	6.5	6.4	6.3	6.7	6.6	6.3	NI
	F	6.1	6.1	6.0	6.3	6.4	6.0	NI
	M+F	6.3	6.3	6.2	6.5	6.5	6.1	NI
PND 4	M	10.1	8.8	9.6	10.1	10.0	9.2	NI
	F	9.7	8.6	9.3	9.6	9.7	8.9	NI
	M+F	9.8	8.7	9.5	9.8	9.9	8.9	NI

PND 13	M	30.9	22.9** (-25.89 %)	30.4	33.0	28.6	24.9**	Mean= 31 (P5-P95= 27.0-34.7)
	F	30.3	22.3** (-26.4 %)	29.5	31.4	28.1	24.0**	Mean= 30 (P5-P95= 26.7-34.4)
	M+F	30.6	22.6** (-26.14 %)	30.0	32.1	28.4	24.3**	NI
PND 21	M	50.4	27.1** (-46.23 %)	50.3	54.5	41.2**	33.7**	Mean= 51 (P5-P95= 43.0-59.0)
	F	48.1	27.2** (-43.8 %)	48.5	52.1	41.4**	32.4**	Mean= 50 (P5-P95= 43.0-58.0)
	M+F	49.1	27.2** (-44.6 %)	49.4	53.2	41.3**	32.8**	NI
Mean living pups at first litter check		10.0	11.6	11.6	10.6	10.3	10.3	NI
Viability index (in %)		100.0	100.0	99.0	100.0	99.0	98.0	NI
Survival index (in %)		100.0	100.0	100.0	98.0	100.0	100.0	NI

Between LD 11 and 12, blood and milk samples from lactating dams used for cross-fostering were taken and analysed for the presence of the test substance. In exposed animals, the concentration in plasma ranged from 1650 to 5660 ng/mL, in milk the concentration ranged from 1280 to 5990 ng/mL.

10.10.9 Comparison with the CLP criteria

"However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the:

(a) Human evidence indicating a hazard to babies during the lactation period; and/or

(b) Results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."

For the test substance:

(a) No human data are available.

(b) Based on the observations from the reproductive/developmental toxicity screening test (Anonymous, 2019) with cross-fostering design reported above, adverse effects in the offspring caused by the transfer of the substance via milk of exposed mother have to be considered. Pups from non-exposed dams cross-fostered for three weeks by exposed dams showed severe effects on pup body weight (-45 %) compared to the control group.

Based on the available information, **classification for effects on or via lactation with the hazard phrase H362** is warranted for the test substance.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

In conclusion, a classification as **Repr. 1B H360D associated with Lact. H362** is warranted.

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

10.13 Aspiration hazard

Hazard class not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Hazard class not assessed in this dossier.

12 ADDITIONAL LABELLING

Not applicable

13 REFERENCES

ECHA, Registration dossier: https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/28053

ECHA, July 2017, Guidance on the Application of the CLP Criteria

14 ABBREVIATIONS

*: $p<0.05$

**: $p<0.01$

***: $p<0.001$

Abs: absolute

AGD: ano-genital distance

Approx.: approximately

Bw: body weight

Bwg: body weight gain

CMR: carcinogen, mutagen, reprotoxic

Corresp.: corresponding

DPC: day post-coitum

DS: dossier submitter

EFD : embryo-foetal developmental

F : female

FBW : final body weight

GD : gestationnal day

GLP: good laboratory practice

HCD: historical control data

Lact.: lactation

LD: lactation day

M: male

Min.: minimum

MMAD: mean mass aerodynamic diameter

Nb: nb

NI: no information available

PND: post-natal day

Rela: relative

Repr.: reproductive toxicity

Resp.: respectively

Sign: significant(-ly)

Stat.: statistical(-ly)

TBD: to be determined

TG: test guideline

Tot.: total

15 ANNEXES

Annex I to the CLH report