CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

tert-butyl 2-ethylhexaneperoxoate (TBPEH)

EC Number: **221-110-7**

CAS Number: **3006-82-4**

Index Number: **/**

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1 BACKGROUND INFORMATION

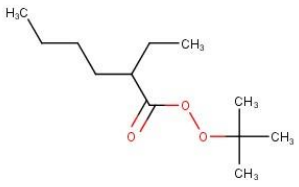
The opportunity to submit a classification proposal for different peroxyesters have been raised by France. Indeed, 5 substances was identified in the peroxyester family: *tert*-butyl-2-ethylperoxylhexanoate (TBPEH), (CAS3006-82-4), *tert*-butyl ethaneperoxoate (TBPA) (CAS 107-71-1), *tert*-butyl peroxypivalate (TBPP) (CAS 927-07-1), *tert*-amyl peroxypivalate (TAPP) (CAS 29240-17-3), *tert*-butyl peroxyneodecanoate (TBPN) (CAS 26748-41-4) and *tert*-butyl 3,5,5-tris(methylperoxy)hexanoate (TBPIN) (CAS 13122-18-4). However, regulatory actions are ongoing at European level, including EOGRTS requested under CCH process for some of these substances and an ECHA's group management work on a wider group "*tert*-alkyl/aryl peroxyesters".

Therefore, the present CLH proposal is only submitted for TBPEH (skin sensitisation and reprotoxicity) awaiting the expected EOGRTS and the GMT conclusion by ECHA before reopening the dossier in order to assess the relevance to propose a CLH report for the peroxyesters category.

2 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)	*tert*-butyl 2-ethylhexaneperoxoate
Other names (usual name, trade name, abbreviation)	*tert*-butyl 2-ethylperoxyhexanoate hexaneperoxoic acid, 2-ethyl-, 1,1-dimethylethyl ester T-BUTYLPEROXY-2-ETHYLHEXANOATE *tert*-butyl 2-(ethylperoxy)hexanoate *tert*-butyl 2-Ethyl-Perhexanoate *tert*-butyl 2-ethylhexaneperoxoate *tert*-butyl peroxy(2-ethyl)-hexanoate *tert*-butylperoxy-2-ethylhexanoat *Trade name:* Kayaester O, Trigonox 21 LUPEROX® 26 TBPEH Trigonox 21S
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	221-110-7
EC name (if available and appropriate)	*tert*-butyl 2-ethylperoxyhexanoate
CAS number (if available)	3006-82-4
Other identity code (if available)	*InChI=1/C12H24O3/c1-6-8-9-10(7-2)11(13)14-15-12(3,4)5/h10H,6-9H2,1-5H3*
Molecular formula	C12H24O3
Structural formula	
SMILES notation (if available)	CCCCC(CC)C(=O)OOC(C)(C)C
Molecular weight or molecular weight range	216.3172

Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	no information available
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
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 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
tert-butyl 2-ethylperoxyhexanoate EC no 221-110-7 CAS no 3006-82-4	>= 80 - <= 90 % (w/w)	None	Org. Perox. C – H242 Press. Gas (comp) – H280 Skin Irrit. 2 – H315 Eye Irrit. 2 – H319 Skin Sens 1 – H317 Repr. 1B – H360 Repr. 2 – H361 Aquatic Acute 1 – H400 Aquatic Chronic 1 – H410 Aquatic Chronic 2 – H411

Table 3: 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**
Hydrocarbons, C4, 1,3-butadiene-free, polymd., triisobutylene fraction, hydrogenated EC no 297-629-8 CAS no 93685-81-5		confidential	None	Flam. Liq. 3 – H226 Asp. Tox. 1 – H304 Skin Irrit. 2 – H315 Aquatic Chronic 4 – H413	No

3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

3.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4:

	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	None										
Dossier submitters proposal	tbd	*tert*-butyl 2-ethylperoxyhexanoate	221-110-7	3006-82-4	Repr. 1B Skin Sens.1B	H360FD H317	GHS08 Dgr	H360FD H317			
Resulting Annex VI entry if agreed by RAC and COM	tbd	*tert*-butyl 2-ethylperoxyhexanoate	221-110-7	3006-82-4	Repr. 1B Skin Sens.1B	H360FD H317	GHS08 Dgr	H360FD H317			

Table 5: 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	Harmonized classification proposed: Skin. Sens. 1B – H317	Yes
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	Harmonized classification proposed: Repr. 1B – H360 FD	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

4 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

TBPEH has no current harmonised classification according to CLP Regulation.

5 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Regarding proposal related to reproductive toxicity:

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

Regarding proposal related to skin sensitisation: Justification that action is needed at Community level is required:

Differences in self-classification for the following substances:

Among the 797 notifiers, 707 self-classified the substance as Skin. Sens. 1. The others do not classify the substance for skin sensitisation.

6 IDENTIFIED USES

This substance is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 1 000 to < 10 000 tonnes per annum.

This substance is used in polymers and plastic products by consumers, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing (ECHA, 2021).

The industrial uses reported are the following:

- Industrial use of organic peroxides as polymerisation initiators, cross linking agents or curing agents
- Other industrial uses of organic peroxides
- Use of reactive processing aid at industrial site (no inclusion into or onto article)
- Industrial use of chemicals for polymer processing
- Industrial use of coatings and paints
- Industrial use as polymerisation initiator and cross-linking agent
- Use of reactive process regulators in polymerisation processes at industrial site (inclusion or not into /onto article)

No public registered data are noted in ECHA website for widespread uses by professional but various PROC are indicated in the registered dossier, with PROC 5, 8a, 8b, 9, 10, 11, 13, 15, 19 and 28 (ECHA, 2021).

Regarding consumer uses, the substance is used in adhesives and sealants (PC1), coating and paints, thinners, paint removers (PC9a) and fillers, putties, plasters, modelling clay (PC9b) (ECHA, 2021).

7 DATA SOURCES

Data from the Reach registration dossier have been taken into account for elaboration of this CLH report. In addition, a bibliographic search was performed in 2021.

8 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid (100%)	ECHA disseminated website	TBPEH is a colourless organic liquid with perester-odour. Its aggregate state at 20 °C and 1013 hPa is liquid.
Melting/freezing point	-67.3°C	ECHA disseminated website	measured OECD Guideline 102 (Melting

Property	Value	Reference	Comment (e.g. measured or estimated)
			point / Melting Range)
Boiling point	-	ECHA disseminated website	technically not feasible The substance decomposes before boiling.
Relative density	0.9 (20°C)	ECHA disseminated website	DIN ISO 3507
Vapour pressure	2 Pa at 20°C 3 Pa at 25 °C 36 Pa at 50 °C	ECHA disseminated website	Extrapolated value from the Antoine equation
Surface tension	-	ECHA disseminated website	the study does not need to be conducted because based on structure, surface activity is not expected or cannot be predicted
Water solubility	46.3 mg/L (20°C)	ECHA disseminated website	Measured OECD Guideline 105 (Water Solubility)
Partition coefficient n-octanol/water	Log Pow: 4.79 (20°C)	ECHA disseminated website	Determinated by HPLC OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method)
Flash point	78.15°C (101325 Pa)	ECHA disseminated website ISO 3679 (Determination of flash point - Rapid equilibrium closed cup method)	Measured
Flammability	-	ECHA disseminated website	Based on the study results, the molecular structure and experience in handling and use, the substance should not be classified as flammable in contact with water, pyrophoric, self-reactive substance and self-heating substance according to Regulation (EC) No 1272/2008 (CLP). Based on the results of the UN-MTC tests and the decision logic, TBPEH should be classified as Organic Peroxide Type C with H242 (heating may cause a fire) according to Regulation (EC) No 1272/2008 (CLP).
Explosive properties	Non explosive	ECHA disseminated website	TBPEH has no explosive properties because it is not classified as Organic Peroxide Type B (reference CPL regulations 2.15.2.2)

Property	Value	Reference	Comment (e.g. measured or estimated)
Self-ignition temperature	-	ECHA disseminated website	the study does not need to be conducted for liquid organic peroxides, because the vapours decompose during the conduction of the test
Oxidising properties	-	ECHA disseminated website	the study does not need to be conducted for organic peroxides
Granulometry	-	ECHA disseminated website	the study does not need to be conducted because the substance is marketed or used in a non solid or granular form
Stability in organic solvents and identity of relevant degradation products	-	ECHA disseminated website	the study does not need to be conducted because the stability of the substance is not considered to be critical.
Dissociation constant	pKa -4.8	ECHA disseminated website	the study does not need to be conducted because the substance has no ionic structure. By using SPARC Performance Automated Reasoning in Chemistry (v.4.5) the dissociation constant of *tert*.-butylperoxy- 2-ethylhexanoate was calculated revealing a pKa of - 4.85
Viscosity	3.7 mPa.s	ECHA disseminated website	Measured OECD Test Guideline 114 (Viscosity of Liquids)

9 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

10 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

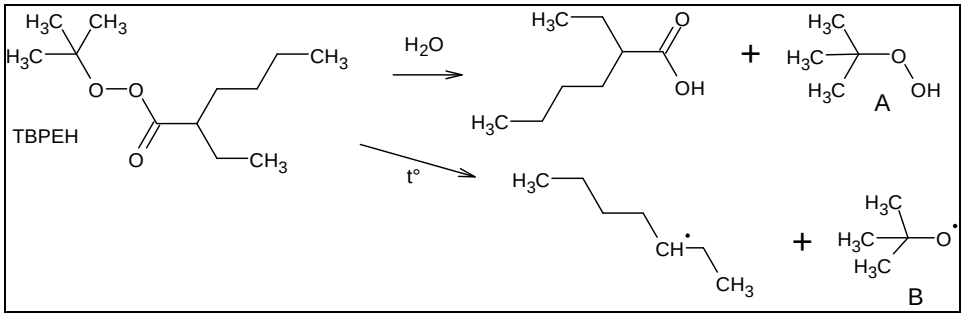
There is no experimental toxicokinetic data available on TBPEH.

TBPEH belongs to the peroxyester family, which is a class of organic peroxides that are relatively unstable under basic or acidic conditions in the presence of water, which catalyses the cleavage of the peroxyester molecule to form an organic acid and conjugate hydroperoxide. Peroxyesters are also expected to be oxidized by intrinsic peroxidases, resulting in the cleavage of the O-O bond (OECD SIDS, 2004).

Therefore, based on the structure of the molecule, the metabolism of TBPEH could possibly include the following pathways:

- enzymatic and non-enzymatic hydrolysis,
- direct reaction with biomolecules due to the reactivity of the peroxyester group of the parent or the hydroperoxide formed after hydrolysis.

The figure below represents the expected degradation products or metabolites of TBPEH:



Estimation of toxicokinetic parameters based on physico-chemical properties:

The molecular weight (< 500) is in favour of absorption. In contrast, water solubility (46.3 mg/L) and log Kow (4.79) are not favourable for significant absorption. Finally, absorption (oral and dermal) is suggested by the presence of systemic effects in toxicological studies performed by oral route but also by the skin sensitising properties of the substance. There is no experimental data by inhalation; the high vapour pressure indicates volatility of the substance and potential respiratory exposure.

When absorbed, TBPEH may be distributed throughout the organism (when considering the low molecular weight, the log Kow and the systemic effects reported). Expected hydrolysis products present a lower molecular weight, a relatively higher water solubility and a lower log Pow value than the parent itself, suggesting the lack of bioaccumulation. Based on these data, excretion is likely to occur mainly via the urine. This is supported by the effects on kidney reported in toxicological studies.

According to the Danish QSAR database, the absorptions from gastrointestinal tract are estimated to be 100% for 1 mg dose and 90% for 1000 mg dose. Dermal absorption is estimated at 0.00146 mg/cm^2/event. The log brain/blood partition coefficient was estimated at 0.5707. TBPEH was not expected to be CYP2C9 and CYP2D6 substrate.

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

There is no toxicokinetic data available for TBPEH. Physicochemical properties and data from toxicological studies generally favour systemic absorption. There is no bioaccumulation expected. The metabolism of TBPEH is expected to be mainly via hydrolysis. Excretion of parents and hydrolysis products is expected mainly via the urine.

11 EVALUATION OF HEALTH HAZARDS

11.1 Acute toxicity

Not evaluated in this dossier.

11.2 Skin corrosion/irritation

Irritation properties are summarized in the context of the classification proposal for skin sensitisation. This endpoint has not been assessed in regards to CLP criteria and thus is not open for public consultation.

According to the OECD SIDS related to t-butyl and t-amyl derived alkyl peroxyesters (2004), TBPEH was not irritating to the skin. No additional data is available in the registration dossiers.

11.3 Serious eye damage/eye irritation

Not evaluated in this dossier.

11.4 Respiratory sensitisation

Not evaluated in this dossier.

11.5 Skin sensitisation

Table 7: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Buehler test OECD Guideline 406 Low sensitivity to detect weak sensitizers for Buehler 3 inductions	Hartley-derived albino guinea pigs 10 males, 10 females 10 controls	**TBPEH** Purity: not stated. vehicle: mineral oil Positive control: Hexylcinnamaldehyde	Induction exposure 25 % w/v TBPEH once per week, for 3 consecutive weeks. Challenge exposure: Following a two week rest period, 5 % w/v TBPEH in mineral oil. Rechallenge exposure: Following a one week rest period, 2% w/v TBPEH in mineral oil. Challenge and rechallenge responses in the test animals were compared to those of the challenge control animals. epicutaneous, occlusive	**Skin sensitiser.** Following induction: mild irritation in the test animals. The dermal irritation increased slightly at induction 2 and 3. Following challenge: Dermal scores = 1 in 9/19 (47%) test animals and 3/10 control animals at the 24 hour scoring interval Dermal score = 1 in 3/19 (15%) test animals and 2/10 control animals at the 48 hour scoring interval. Dermal scores = 0 in the remaining test and challenge control animals. Group mean dermal scores similar in the test animals as compared to the challenge control animals. Following rechallenge: Dermal scores = 1 in 5/19 (26%) test animals at the 24 hour scoring interval which do not persist to the 48 hour scoring interval. Dermal scores = 0 in the remaining test and all challenge control animals. Group mean dermal scores slightly higher in the test animals as compared to the challenge control animals. **26% of positive cases following rechallenge** **➔ classification as skin sensitiser 1B**	Unnamed 1996 key study Klimisch score : 2

11.5.1 Short summary and overall relevance of the provided information on skin sensitisation

The dermal skin sensitisation was investigated using TBPEH in a Buehler test using 3 inductions.

The dermal sensitisation potential of TBPEH (purity not stated) was evaluated in Hartley-derived albino guinea pigs. Ten male and ten female guinea pigs were topically treated with 25 % w/v TBPEH in mineral

oil, once per week, for 3 consecutive weeks. One test female animal was found dead on study day 27. Gross necropsy observations included dark red mandibular and axillary lymph nodes, an adhesion in the thoracic cavity, mottled lungs, mottled liver, enlarged spleen and congested meningeal vessels in the brain. The majority of sensitisation study animals gained weight during the test period and generally appeared in good health. Following a two week rest period, a challenge was performed whereby the 19 tested and 10 previously untreated (naive) challenge control guinea pigs were topically treated with 5 % TBPEH in mineral oil. Challenge responses in the test animals were compared to those of the challenge control animals. Following a one-week rest period, a rechallenge was performed whereby the 19 tested and the 10 challenge control guinea pigs were topically treated with 2% w/v TBPEH in mineral oil. Rechallenge responses in the test animals were compared to those of the challenge control animals.

Following induction 1 with 25 % w/v TBPEH in mineral oil, mild irritation was noted in the test animals. The dermal irritation increased slightly at induction 2 and 3. Following challenge with 5 % w/v TBPEH in mineral oil, dermal scores of 1 were noted in 9/19 test animals and 3/10 challenge control animals at the 24 hour scoring interval and in 3/19 test animals and 2/10 challenge control animals at the 48 hour scoring interval. Dermal scores of 0 were noted in the remaining tested and challenge control animals. Group mean dermal scores were noted to be similar in the test animals as compared to the challenge control animals. Following rechallenge with 2 % w/v TBPEH in mineral oil, dermal scores of 1 were noted in 5/19 (26%) test animals at the 24-hour scoring interval; however, the dermal responses did not persist to the 48-hour scoring interval. Dermal scores of 0 were noted in the remaining tested and all challenge control animals. Group mean dermal scores were noted to be slightly higher in the test animals as compared to the challenge control animals.

11.5.2 Comparison with the CLP criteria

According to CLP criteria, classification as Skin Sens. 1B is required in the following cases:

Animal test results for sub-category 1B	
Assay	Criteria
Local lymph node assay	EC3 value > 2 %
Guinea pig maximisation test	≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
Buehler assay	≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

The dermal sensitisation potential of TBPEH was evaluated in a Buehler assay. Ten male and ten female guinea pigs were topically treated with 25 % w/v TBPEH in mineral oil, once per week, for 3 consecutive weeks. The study was concluded of reliability of 2 according to Klimish score. 26% of cases of sensitisation are reported after a topical induction of 25% (> 0.2 % to ≤ 20 %). Therefore a classification Category 1B is warranted for TBPEH.

11.5.3 Conclusion on classification and labelling for skin sensitisation

TBPEH (in mineral oil) was positive in a Buehler test (≥ 15 % to < 60 % animals responding at > 0.2 % to ≤ 20 % topical induction dose).

According to CLP criteria, a classification **Skin Sens. 1B – H317 (May cause an allergic skin reaction)** is warranted for TBPEH.

11.6 Germ cell mutagenicity

Not evaluated in this dossier.

11.7 Carcinogenicity

Not evaluated in this dossier.

11.8 Reproductive toxicity

11.8.1 Adverse effects on sexual function and fertility

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

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reproduction/developmental toxicity screening test (OECD TG 421) Wistar rat Males and females, n=10 animals/sex/dose	**TBPEH** purity not specified 0, 100, 300 and 1000 mg TBPEH/kg bw/day, Duration of Exposure : Treatment beginning (females/males): day-1 of pre-pairing -Pre-pairing (females/males): 14 days -Pairing (females/males): until mating (maximum 14 days) -Gestation (females): about 21 days -Parturition (females): expected : on day 21 or 22 post coitum -Lactation (females): until day 4 post partum -Treatment ending: females: on day 3 post partum	P0: At 1000 mg/kg bw/day: decreased food consumption in male and female animals during the first week of the pre-pairing period and lactation. Slight decreased bw in males until necropsy; in females: slight increase of bw during gestation but significant decreased bw during lactation. Some dams were noted with ruffled fur and/or bad condition. No test item-related effects were noted during necropsy. Reproduction parameters: All mated females were pregnant. At 1000 mg/kg bw/d: Increase in post-implantation loss and reduction in the number of live pups. F1 offsprings: Reduction in mean body weights of pups and increase in post-natal loss at 1000 mg/kg/day	Anonymous 2008 from ECHA website Klimisch score: 1 Key study
OECD 443, EOGRTS including cohorts 1A and 1B Han:WIST rats P0: n= 24/sex/group, 4 groups	**TBPEH** Purity : not specified 0 (vehicle), 100, 300 and 1000 mg/kg bw/day Gavage (oral): once daily Vehicle: sunflower oil	**Parental generations (P0 and P1):** No mortality related to treatment. No adverse clinical signs of systemic toxicity related to the test item at any dose level except salivation and nuzzling up the bedding material for P0 male and female animals at 300 and 1000 mg/kg bw/day shortly after the administration with variable incidence and duration in a dose related manner.	Anonymous 2020 Klimisch score: 1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Control animals received the vehicle, only. F1 for Cohort 1A n= 20 animals/sex/group F1 for Cohort 1B n= 20 animals/sex/group	Exposure : All animals of the parent (P) generation dosed prior to mating (10 weeks) and throughout mating. In addition, males received the test item or vehicle after mating up to the day before the necropsy (altogether for 153-156 days). Dams additionally exposed through the mating and gestation periods and up to lactation days 21-23 (altogether for 100-129 days).	From 300 mg/kg bw/day: elevated weights of kidneys and liver in male and female animals of Cohort 1A and Cohort 1B. <u>at 1000 mg/kg bw/day</u> Reduced body weight in P0 males : -13% of the control on day 152; cohort 1B males: -11% on day 154; cohort 1A males : -12% on day 90 and cohort 1B females : -4% of the control on day 36; cohort 1A females: -6% day on 42. Decreased bw gain in the most cases during the observation period only in P0 and P1 males. No effect on food consumption. Slightly elevated urine volume and lowered pH levels in both sexes in P0 and P1A generations. Slightly lower free triiodothyronine (FT3) and free tyroxine (FT4) levels in P0 and P1 animals as compared to the control. No alterations in the TSH levels, organ weight or histology of thyroid gland. Changes in various organ weights (including elevated liver weight) but not associated with histological alterations. Changes in kidneys: elevated weight (P0 and P1; both sexes) with chronic progressive nephropathy (only P0 males) **<u>Reproduction parameters in P0 and P1 generations:</u>** <u>at 1000 mg/kg bw/day</u> Irregular estrous cycle (P0), number and percentage of female animals in prolonged estrous slightly higher (P1) Reduced reproduction index in P0 and P1 animals. A second mating showed that the male animals of P0 generation – which did not fertilize their partners of main group – mated successfully with non-treated females, suggesting a female effect. Slightly longer mean duration of pregnancy of P0 dams. Decreased number of developing follicles and increased number of follicular atresia in P0 females (pregnant or non-pregnant) compared with their control. Not reported in P1. **<u>F1 offspring</u>** <u>at 1000 mg/kg bw/day:</u> Cohort 1B: Percentage of offspring showing signs (no milk in the stomach, cold, found dead, missing, alopecia) higher. Higher extra uterine mortality on PND0 (2% vs 0%) and PND0-21 (9% vs 1%), reduced pup body weight on PND0 (-6.5 %) and bw gain between PN0-21 (- 8.6 %). Similar effect on litter weight (-14% on PND21).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Lower percentage of pups with positive response and higher percentage of pups with negative response at surface righting reflex, pinna detachment - but not statistically significant - and eye opening (statistically significant; positive results: 31% versus 58%). Shorter absolute anogenital distance but not when normalized. Cohort 1A: Longer period of vaginal patency (ex. 65% versus 100% at PND34) and appearance of the first cornified vaginal smear (34.3 versus 34.2 day) **F2 offspring** at 1000 mg/kg bw/day: Percentage of found dead F2 offspring higher (+12%). Mortality higher from PND0. Lower BW (-8% on PND4) and BW gain of pups between PND0 and 4 (-13%). Also see for litter based. Shorter absolute anogenital distance but not when normalized.	

Table 9: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD Guideline 408 (90-Day Oral Toxicity Study) Hsd.Brl.Han: Wistar rats Oral:gavage Exposure time : 90 or 91 days GLP	TBPEH Doses tested : 0, 70, 150 and 450 mg/kg bw/d Vehicle : sunflower oil	10 animals per sex per dose in the main study. Additionally, 5 animals per sex in the control and high dose group (recovery group).	No mortality, no toxic signs in clinical observations and in the course of the functional observation battery. Salivation with variable frequency within a group but in a dose related manner regarding the incidence and onset. No difference in body weight and body weight gain. Daily mean food consumption similar in all groups. No changes in hematology and biochemistry. No macroscopic or histopathological alterations at necropsy. No change in mean weights of examined organs at any dose level. No effect on the estrous cycle. No effect on the sperm cells (count, motility and morphology).	Anonymous 2013 from ECHA website

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD guideline 407 (28-Day Oral Toxicity Study) Fischer 344/DuCrl male and female Oral : gavage Exposure time : 28 days GLP	TBPEH Doses tested : 0, 100, 316 and 1000 mg/kg bw/day Vehicle : corn oil	Dose levels selected based on data obtained during the 7-day dose-range-finding toxicity study. Two controls (with and without recovery) and four dose groups (n=5 animals per group and sex).	No mortality. No effect in clinical observations. No change in body weight and weight gain. No difference in food consumption in both sexes. Decrease in number of platelets in the blood of the mid and the high dose females groups and some minor liver changes (higher organ weights) in the high dose groups of both sexes, elevated plasma alkaline phosphatase level in the high dose females group. Indication for renal tubular alterations in the high dose females group.	Anonymous 2009 from ECHA website

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

TBPEH (purity not stated) was tested in a reproduction/developmental toxicity screening test according to OECD TG 421. TBPEH was administered orally (by gavage) to Wistar rats at repeated doses of 0, 100, 300 and 1000 mg/kg body weight/day, for 14 days during the pre-pairing period, and the pairing, the gestation and the lactation periods until 3 post partum.

All animals survived until scheduled necropsy. Treatment at 1000 mg/kg was associated with decreased food consumption in male and female animals during the first week of the pre-pairing period and during lactation. During pre-pairing period, mean body weight of the males exposed to 1000 mg/kg bw/d was slightly decreased starting on day 3 and continuing until necropsy. In females exposed to 1000 mg/kg bw/day, the mean body weight gain was slightly increased during gestation but statistically decreased during lactation. During the last two days of gestation or the first two days of lactation, seven dams (gestation period) and four dams (lactation period), respectively, were noted periodically to have ruffled fur and / or a generally bad condition. No test item-related effects were noted during necropsy.

For reproduction parameters, all mated females were pregnant leading to a fertility index of 100%. However, treatment at 1000 mg/kg was associated with an increase of post-implantation loss (no numerical value available). Developmental toxicity results obtained are evaluated under section “Adverse effects on development”.

An Extended One-Generation Reproduction Toxicity Study (EOGRTS) (Unnamed, 2020) in the rat according to OECD TG 443 (including production of a second generation) is also available using TBPEH, including the production of a second generation (cohort 1A and 1B; but without cohorts for developmental neurotoxicity and immunotoxicity).

The effect of the test item was examined on the male and female reproductive performance, such as gonadal function, estrous cycle, mating behavior, conception, parturition, gestation, lactation and weaning (P0 and Cohort 1B generation) and on the offspring viability, neonatal health and mortality, growth and development of the offspring (Cohort 1A and Cohort 1B later producing F2 generation) to adulthood following oral (by gavage) administration.

Four groups of Han:WIST rats (n= 24/sex/group) were administered with the test item orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day of TBPEH. Control animals received the vehicle, sunflower oil, only. The suitability of the vehicle at the intended concentrations of the test item was analytically verified up front (concentration and homogeneity). Concentration of the test item in the dosing

formulations varied in the acceptable range between 92 % and 107 % of the nominal values and formulations were homogenous thereby confirming the proper dosing.

All animals of the parent (P0) generation were dosed prior to mating (10 weeks) and throughout mating. In addition, males received the test item or vehicle after mating up to the day before the necropsy (altogether for 153-156 days). Dams were additionally exposed through the mating and gestation periods and up to lactation days 21-23 (altogether for 100-129 days).

Clinical observations (clinical signs, body weight, food consumption, estrous cycle) and pathology (clinical and organ pathology) examinations were performed on parental (P) animals for signs of toxicity, with special emphasis on the integrity and performance of the male and female reproductive systems. Estrous cycle was monitored by examining vaginal smears before the mating for two weeks and during the mating period until evidence of mating and on the day of the necropsy. The dams were allowed to litter and rear their offspring up to day 21 post-partum. As the number of pregnancies was low (16/21) in the high dose group, a second mating of the P0 males with untreated females (untreated group; n= 8 naïve females) was performed to clarify if the male fertility of the high group was impaired. Dams and offspring in the untreated group were terminated on post-partum/ PND (post-natal days) 5-8.

All F1 offspring were observed individually for the health, growth, development and function up to and including post-natal day 21 (clinical signs, body weight, surface righting reflex, pinna detachment, eye opening, anogenital distance). Twenty animals/sex/group for Cohort 1A and 20 animals/sex/group for Cohort 1B were randomly selected on post-natal day 21 for follow-up examinations. Dosing of offspring selected for follow-up examinations (Cohort 1A and Cohort 1B) begun on post-natal day 22 and treatment was continued up to the day before the necropsy. The foetal/offspring (from implantation) parameters are further evaluated under section ‘Adverse effects on development’.

F1 offspring (Cohort 1A and Cohort 1B) were observed during adulthood (P1) identically to parental animals – clinical signs, body weight, food consumption, estrous cycle, clinical pathology and organ pathology. Sexual maturity of offspring (Cohort 1A and Cohort 1B) was investigated by observation of balano-preputional separation, vaginal patency and appearance of first cornified vaginal smear. Cohort 1A animals were subjected to necropsy, organ weighing and sperm analysis – one day after the termination of the exposure – on PND 91-97. Cohort 1B animals were mated to produce a second (F2) generation after at least 90-day pre-mating period and were observed identically to parental (P0) animals. F2 offspring were observed and subjected to necropsy up to PND 5-8.

Blood samples were collected for determination of serum levels of thyroid hormones (FT3, FT4 and TSH) from 3-5 F1 pups per litter (where it was feasible) on PND 4, from 1-2 pups/10 litters on PND 22, from 10 dams (P) /group and from 10 parental (P) male animals/group at termination, from 10 male animals/ group and from 10 female animals in Cohort 1A, from all male and female animals in Cohort 1B at termination and from F2 pups on post-natal day 5 or shortly thereafter.

All adult animals (P0, Cohort 1A and Cohort 1B) were subjected to gross pathology with complete tissue preservation one day after the last treatment. Brain, spleen, thymus and mammary tissues were preserved for 10 male and 10 female pups per group – where feasible – in F1 offspring not selected for cohorts on PND22 or shortly thereafter and in F2 offspring on PND5 or shortly thereafter. Special attention was paid to the organs and tissues of the reproductive system for P, F1 or F2 animals.

Selected organ weights were determined in adult animals (P0, P1) (cohort 1B for males: kidneys, brain, testis, epididymides, seminal vesicle, prostate, pituitary gland; for females: brain, kidneys, uterus, ovaries, pituitary gland); (cohort 1A for males : brain liver, heart, thymus, spleen, testes, epididymides, seminal vesicle, prostate, adrenal glands, thyroids, pituitary gland; for females : brain, liver, kidneys, heart, thymus, spleen, uterus, ovaries, adrenal glands, thyroid, pituitary gland) and in offspring (PND22 or shortly thereafter and in F2 offspring on PND5) (brain, spleen, thymus)

Sperm parameters were determined in all control and high dose male animals in P0 generation and in P1 generation (Cohort 1A and Cohort 1B).

Full histopathology examinations were performed on the organs and tissues of adult animals (P0, Cohort 1A and Cohort 1B) in control and high dose groups with special emphasis on sexual organs and tissues.

Reproductive organs were also processed and examined histologically in non-mated and non-pregnant female animals and their mating partners (P0 and Cohort 1B) in the low and mid dose groups. In addition, organs showing macroscopic changes were also processed and examined histologically in adult animals in low or mid dose groups (P0, Cohort 1A and Cohort 1B) and in F1 offspring. The kidneys of male animals at 100 and 300 mg/kg bw/day were processed and evaluated histologically based on the organ weight data and histological observations at 1000 mg/kg bw/day.

Based on the low reproduction index and histopathological findings in parental (P0) female animals at 1000 mg/kg bw/day, a quantitative evaluation of primordial, small growing (secondary and tertiary) follicles, as well as corpora lutea was performed in all adult female animals (P0, Cohort 1A and Cohort 1B) in the control, 100, 300 and 1000 mg/kg bw/day.

The results were interpreted comparing treatment groups with respect to controls, which were treated concurrently with vehicle (sunflower oil) only.

There was no test item related mortality in 100, 300 or 1000 mg/kg bw/day groups in adult animals (P0, Cohort 1A and Cohort 1B) during the course of study. Salivation and nuzzling up the bedding material were noted for P0 male and female animals (but not in Cohort 1A or 1B) at 300 and 1000 mg/kg bw/day shortly after the administration with variable incidence and duration in a dose related manner.

The mean body weight gain and mean body weight were slightly reduced in male animals at 1000 mg/kg bw/day (P0 males : -13% of the control, day 152, Cohort 1B males: -11%, day 154; Cohort 1A males : -12%, day 90). The body weight of female animals in Cohort 1A and Cohort 1B at 1000 mg/kg bw/day was slightly lower during the first weeks of the treatment period (cohort 1B females : -4% of the control, day 36; Cohort 1A females: -6%, day 42) and the difference with respect to the control recovered during the following weeks. The food consumption was not adversely affected in male or female animals (P0, Cohort 1A and Cohort 1B) at 100, 300 and 1000 mg/kg bw/day.

There was no treatment-related toxicological relevant effect on hematological and clinical biochemistry parameters in any of the groups, sex and cohorts. At urinalysis, slightly elevated urine volume and lowered pH levels were apparent in both sexes in P0 generation and in both sexes in Cohort 1A at 1000 mg/kg bw/day.

There was a slightly lower FT3 and FT4 levels in males of P0 generation and of Cohort 1A and 1B at 1000 mg/kg bw/day as compared to the control. There were no accompanying alterations in the TSH levels, organ weight or histology of thyroid glands.

Specific macroscopic alterations related to the effect of the test item were not detected in male or female animals at 100, 300 or 1000 mg/kg bw/day at the terminal necropsy (P0, Cohort 1A and Cohort 1B).

Various organ weights were modified. Elevated weights of kidneys were indicative of the test item effect in P0 male animals at 1000 mg/kg bw/day and in male and female animals at 300 and 1000 mg/kg bw/day of P1 generation (cohort 1A and cohort 1B). Elevated liver weights were reported in male and female parental P0 animals at 1000 mg/kg bw/day and in male and female animals of Cohort 1A and Cohort 1B at 300 and 1000 mg/kg bw/day.

Histopathological investigations revealed chronic progressive nephropathy (CPN) in a higher incidence of P0 male animals at 1000 mg/kg bw/day but not in animals in Cohort 1A or Cohort 1B.

Effects on reproduction:

The estrous cycle was irregular in several P0 female animals at 1000 mg/kg bw/day during the two last weeks of the ten weeks pre-mating period. Statistical significance was noted for the lower percentage of female animals with regular cycle (54% versus 83%) and for the lower mean number of days in pro-estrous (1.5 versus 3) at 1000 mg/kg bw/day. The number of female animals in prolonged estrous was also higher than in the control group at 1000 mg/kg bw/day (29% versus 0%). In cohort 1A, the estrous cycle was irregular in several female animals in the control, 100, 300 and 1000 mg/kg bw/day groups during the two weeks observation period. The number and percentage of female animals in prolonged estrous was slightly higher than in the control group at 1000 mg/kg bw/day (15 % versus 0/20).

In the female animals of the control and 1000 mg/kg bw/day groups, the ovaries, uterus, cervix, vagina had a normal structure characteristic of the species, age and phase of the active sexual cycle. However, decreased number of developing follicles and increased number of follicular atresia (mean number follicular atresia: 13.2 versus 5.3 in control at quantitative evaluation of ovaries) were detected by qualitative histological as well as quantitative evaluation in P0 female animals at 1000 mg/kg bw/day compared with their control. The same effects were seen in the females not achieving pregnancy. Similar findings were not detected in female animals in Cohort 1A and Cohort 1B.

The investigated organs of male reproductive system (testes, epididymides, prostate, seminal vesicles, coagulating glands) were histologically normal and characteristic of the sexually mature organism in all P0, Cohort 1A and Cohort 1B male animals at 1000 mg/kg bw/day. Sperm examinations did not reveal any test item related influence on the sperm cell morphology and motility, and total sperm count at 1000 mg/kg bw/day (P0, Cohort 1A and Cohort 1B).

The reproductive performance, represented by the reproduction index (= percentage of pregnant females), was reduced in P0 animals at 1000 mg/kg bw/day (67% versus 91% in controls) and in Cohort 1B animals at 300 mg/kg bw/day (80% versus 95% in controls) and 1000 mg/kg bw/day (56% versus 95% in controls). A second mating showed that the male parental animals (P0) exposed at 1000 mg/kg bw/day group – which did not fertilize their partners of main group – mated successfully with non-treated females (n= 8 naïve females). This information suggests that the decrease in fertility of treated rats originate from alteration of reproductive function of the females.

The slightly longer mean duration of pregnancy of P0 dams at 1000 mg/kg bw/day was statistically significant (22.37 versus 21.97 days).

The mean number of implantation sites were comparable in all groups.

The foetal/offspring (from implantation) parameters are further evaluated below under section ‘Adverse effects on development’.

In summary, under the conditions of the EOGRTS, TBPEH caused:

- effects on reproductive function: irregular estrus cycle (P0 and P1 female), decreased number of developing follicles and increased number of follicular atresia (P0 females), decreased reproduction index (P0, Cohort 1B). These effects were mainly reported at 1000 mg/kg bw/day. At 300 mg/kg bw/day, reproduction index was also decreased in cohort 1B animals;
- general toxicity, including body weight reduction (mainly in males of P0, Cohort 1A, Cohort 1B generations), effects in kidneys (elevated weight, chronic progressive nephropathy (only in P0 males)). These effects were mainly reported at 1000 mg/kg bw/day. At 300 mg/kg bw/day, some changes in organ weights, without histopathological findings, were reported.

Based on data, the NOAEL for parental systemic toxicity and reproductive performance was determined at 100 mg/kg bw/day by the registrants based on slight changes in liver and kidneys weights and disturbed reproductive ability at 300 mg/kg bw/d, respectively. Dossier submitter notes that the NOAEL for parental systemic toxicity set by the registrants is highly conservative in the light of the effects observed at 300 mg/kg bw/day (only changes in organ weights).

Other data: 28-day and 90-day oral (gavage) studies (OECD 407 and OECD 408) have investigated systemic effects of TBPEH in rats. Data below are issued from disseminated ECHA website.

TBPEH was tested in a 28-day repeated dose toxicity study by oral route (gavage) in male and female Fischer, F344/DuCrl rats (n=5) according to OECD guideline 407, including recovery groups for control and high dose animals. The test item was administered at 100, 316 and 1000 mg/kg bw/day. Dose levels administered in the study were selected based on data obtained during a 7-day dose-range-finding toxicity study. The substance caused a decrease in the number of platelets in the blood of the mid and the high dose females groups and some minor liver changes (higher organ weights in the high dose groups of both sexes, elevated plasma alkaline phosphatase level in the high dose females group). There was also a borderline indication for renal tubular alterations in the high dose females group. There was a sex difference in the

response to the test substance with different effects in both sexes and a somewhat higher susceptibility of the females. The NOAEL set by the registrants was 316 mg/kg bw/day in males and 100 mg/kg bw/day in females.

TBPEH was tested over a prolonged period of time (90 days) followed by a 28-day recovery period in order to assess reversibility, persistence or delayed occurrence of potential toxicological effects. The substance was administered orally (by gavage) to Hsd.Brl.Han: Wistar rats (n=15 animals/sex in the control and high dose groups, n= 10 animals/sex in the low and middle dose groups) once a day at 0, 70, 150 or 450 mg/kg bw/day. 5 animals per sex in the control and high dose groups were observed without administration for four weeks (recovery observations). There was no substance related mortality, no toxic signs at any dose level in the daily and detailed weekly clinical observations and in the course of the functional observation battery. Salivation was observed in the male and female treated animals with variable frequency within a group but in a dose related manner regarding the incidence and onset. No substance-related effects on body weight, or body weight gain were observed with respect to controls at any dose level during the course of the study. The daily mean food consumption was similar in animals of the control and test item treated groups. There were no abnormalities in the eyes of animals in the high dose group at termination of the treatment. No substance-related changes were observed in investigated hematology, blood coagulation parameters and clinical chemistry examinations. Specific macroscopic and histopathological alterations related to the substance were not detected from necropsy observations. The mean weights (absolute and relative to the body and brain weights) of examined organs were not affected by the test item at any dose level. A substance influence on the estrous cycle was not detected. Sperm analysis did not reveal substance influence on the sperm cells (count, motility and morphology) at 450 mg/kg bw/day dose. The NOAEL set by the registrants was determined at 450 mg/kg bw/day for male and female animals.

11.8.3 Comparison with the CLP criteria

For potential classification on sexual function and fertility, criteria from CLP guidance (ECHA, 2017c) are applied.

- Adverse effects on sexual function and fertility are described as "*Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*." *(ECHA, 2017c).*
- *Known human reproductive toxicant. "The classification of a substance in this Category 1A is largely based on evidence from humans."*

There are no human data. Thus Cat. 1A is not fulfilled for TBPEH.

- *Presumed human reproductive toxicant. "The classification of a substance in this 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 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*" (ECHA, 2017c).

Two experimental studies have specifically investigated effects of TBPEH in rodent on reproductive function.

One reproduction/developmental toxicity screening study performed in rats according to TG 421 did not show clear effects on sexual function and fertility. Regarding the increase of post-implantation loss, this is rather considered as a developmental effect but it cannot be excluded that it is secondary to fertility alteration. In addition, it can be noted that the OECD 421 guideline study is a screening assay. According to the OECD guideline, this protocol is only "*designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour,*

conception, development of the conceptus and parturition". Statistical analysis is also rather limited since only 10 animals of both sexes were included.

One Extended One-Generation Reproduction Toxicity Study including cohort 1A and 1B according to TG 443 in Wistar is also available.

Regarding parental toxicity, at 1000 mg/kg bw/day, a slight reduction in mean body weight gain and mean body weight was noted in P0 and P1 generations. This effect is particularly observed in males, with decreases reaching a max. of -13% in P0 on day 152. Some changes were observed in various organ weights (including kidney and liver) at 1000 mg/kg bw/d in both parental generations with no supporting pathological alteration at histology, except for P0 males that presented chronic progressive nephropathy. At this dose, there was also a reduction of thyroid hormones (T3 and T4) in males of both generations and effects in urinalysis (higher volume, lower pH). At 300 mg/kg bw/day, the reported findings mainly consisted on some changes in organ weights.

Regarding fertility, the estrous cycle parameters were changed at the high dose in P0 and 1A cohorts. In P0 females, there was a statistically significant lower percentage of female animals with regular cycle (54% versus 83%) and lower mean number of days in pre-estrous (1.5 days versus 3 days). The number of female animals in prolonged estrous was higher (29% versus 0%). In P1 generation, the number and percentage of female animals at high dose in prolonged estrous was slightly higher (15 % versus 0%). This finding is consistent with observations in the P0 generation and considered substance related.

The reproductive performance was reduced in parental (P0) animals at 1000 mg/kg bw/day and in Cohort 1B animals at 300 and 1000 mg/kg bw/day. In P0 generation, the number of pregnant females (reproduction index = 67%) was statistically lower and the number of non-pregnant females animals was statistically higher (91%) with respect to their control group at 1000 mg/kg bw/day. In P1 generation, a lower reproduction index was also observed at 1000 mg/kg bw/day (56% versus 95%) and 300 mg/kg bw/day (80%). A second mating showed that the male parental animals (P0) exposed at 1000 mg/kg bw/day group – which did not fertilize their partners of main group – mated successfully with non-treated females. This suggests that the decrease in fertility of treated rats originate from alteration of female reproductive functions. A decreased number of developing follicles and increased number of follicular atresia (mean number follicular atresia: 13.2 versus 5.3 in control at quantitative evaluation of ovaries) were observed in pregnant or non-pregnant animals, but only in P0 females at 1000 mg/kg bw/d. Copulatory index was also lower in P1 exposed to 1000 mg/kg bw/day (90% versus 100%).

Finally, a slightly longer mean duration of pregnancy of dams at 1000 mg/kg bw/day was statistically significant (22.37 days versus 21.97 days) in P1 females. The value was at the upper of the historical control range (21.8-22.3 days; 13 studies).

There was no effect on reproductive organs reported in 28-day and/or 90-day studies in rats at tested doses up to 1000 or 450 mg/kg bw/day, respectively. No change in oestral cycles or sperm parameters was noted in the 90-day study with doses up to 450 mg/kg bw/day.

In conclusion:

Clear fertility effects, mainly characterised by a decrease of reproduction index, were consistently found in both generations. In particular, this effect occurred at lower doses in P1 (from 300 mg/kg bw/day) than in P0 (1000 mg/kg bw/day) suggesting that P1 animals are more sensitive to TBPEH toxicity than P0. Other findings that can be related to effects on sexual function and fertility included changes in oestral cycles in both generations, histopathological effects in ovaries in P0 females, lower copulatory index in P1 and slightly longer mean duration of pregnancy were reported at 1000 mg/kg bw/d.

In comparison, general toxicity is slight and particularly restricted to males exposed to TBPEH at 1000 mg/kg bw/day (decreased body weight < -13% mainly in males; histopathological effects in kidney only in P0 males). At 300 mg/kg bw/day, there are only changes in organ weights, without associated histopathological findings.

In summary, fertility effects are consistent between generations and cannot be considered secondary to a general toxicity. So, criteria for classification as Repr. 1B for fertility are fulfilled.

11.8.4 Adverse effects on development

Table 10: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal Developmental Toxicity Study Pregnant female Hsd. Brl. Han: WISTAR rats 24/dose group According to OECD TG 414 Deviations: no GLP: yes	**TBPEH** Purity: confidential 200, 400 and 1000 mg/kg bw/day Exposure: GD 5 to GD 19 Exposure via gavage	Dams: No death, no effect on corrected BW and no necropsy findings in treated groups. Only salivation and decreased food consumption during first 6 days of treatment reported. Offspring: No dose-related significant difference in the intrauterine mortality of the conceptuses, the number of implantations, the number of viable fetuses and their sex distribution. The incidence of visceral abnormalities was statistically significantly ($p<0.05$) higher in the 1000 mg/kg bw/day dose group (3 hydroureter + 1 umbilical hernia). No increase in the incidence of external and visceral variations in treated groups. No increase in skeletal malformations. Slightly but statistically significant ($p<0.01$) increase in the incidence of fetuses with incomplete ossification of the skull-bones and metacarpal/metatarsal at 1000 mg/kg bw/day.	Anonymous 2013 Klimisch score : 1 Key study
Prenatal Developmental Toxicity Study inseminated New Zealand White rabbits (26-27 animals/dose group) According to OECD TG 414 Deviations: no GLP: yes	**TBPEH** Purity: confidential 30, 100 and 300 mg/kg bw/day. Exposure: From GD 6 to GD27	**Dams**: At 30 mg/kg bw/day: 1 abortion At 100 mg/kg bw/day: 2 abortions. Lower bw gain (GD15-18) with reduced food consumption. At 300 mg/kg bw/day: 8 abortions. 4 moribund dams. Bleeding of the vagina in 6 animals. Lower mean body weight until GD24 (-6% on GD24) and bw gain between GD6-12 and GD15-18; reduced food consumption up to GD21. No stat. significant effect when corrected bw and bw gain considered. On GD28: 22, 24, 20 and 17 females with implantation site(s). **Offspring**: 20, 23, 16 and 10 litters evaluated in the control, 30, 100 and 300 mg/kg bw/day group respectively At 100 mg/kg bw/d: No effect on parameters assessed. At 300 mg/kg bw/day: Increase of early embryonic death (15% versus 6%) and post-implantation loss (17% versus 8%). Decrease of mean number of viable fetuses (not stat. signif). Significantly lower fetal weight (-15.6%) and crownrump length (-7.3%). No significant difference in incidence of overall fetal malformations among the experimental groups. Increasing skeletal variations: proximal and middle phalanges less than 7/7 ossified (18% versus 7%), misaligned and fused sternebra (2% versus 0%), multiple malformed ribs and vertebrae (3% versus 0%).	Anonymous 2018 Klimisch score :1 Key study
See also section 11.8.1: developmental effects are reported in the OECD TG 421 study and in an EOGRTS performed			

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
with TBPEH.			

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

Two prenatal developmental toxicity studies, in rats and rabbits, are available with TBPEH.

A first prenatal developmental toxicity study (Anonymous, 2013) was performed in rats according OECD TG 414. Groups of 24 sperm-positive female Hsd. Brl. Han: Wistar rats were treated with TBPEH by oral administration daily at three dose levels of 200, 400 and 1000 mg/kg bw/day from day 5 up to and including day 19 post coitum. A control group of 24 sperm positive females was given the vehicle sunflower oil. During the study, mortality was checked for and clinical observations were performed. Body weight and food consumption of the dams were also recorded. The day when sperm was detected in the vaginal smear was regarded as day 0 of gestation. A Caesarean section and gross pathology were performed on gestational day 20. The number of implantations, early and late resorptions, live and dead fetuses in each uterine horn and the number of corpora lutea were recorded. Each fetus was weighed and examined for sex and gross external abnormalities. The placentas were weighed and examined externally. About half of each litter was preserved for visceral examination and the other half of the litters were preserved for skeletal evaluation. At visceral examination the bodies were micro dissected by means of a dissecting microscope. The heads were examined by Wilson's free-hand razor blade method. After cartilage-bone staining the skeletons were examined by means of a dissecting microscope. All abnormalities found during the fetal examinations were recorded. In total, there were 21, 19, 23 and 19 evaluated litters in the control, 200, 400 and 1000 mg/kg groups, respectively.

One pregnant female in the control group was found dead on gestational day 20. No other clinical signs than alopecia in a few females unrelated to the treatment and salivation in the 400 and 1000 mg/kg bw/day groups immediately after treatment were observed. This was attributed to be an effect of the treatment, however as non-adverse. There were no findings observed at necropsy. There was no indication of an effect of the substance on body weight development and food consumption of the dams in the 200 and 400 mg/kg bw/day dose groups. The statistically significantly ($p<0.01$) reduced body weight gain on the first three days of treatment and the statistically significantly ($p<0.01$) reduced food consumption in the first week of treatment in the 1000 mg/kg bw/day dose were not considered adverse, in particular, considering that the corrected body weight and corrected body weight gain were not affected. There was no dose related significant difference in the intrauterine mortality of the conceptuses, the number of implantations, viable fetuses and their sex distribution.

The number of late embryonic death increased slightly in a statistically significant manner ($p<0.05$) in the 400 mg/kg bw/day dose group (sum = 7 versus 1 in the control group; but not significant if the mean percentage value was considered (3%)) and without a statistical significance in the 1000 mg/kg bw/day group (sum = 5; percentage = 2%). Registrants considered this finding as non adverse, since there was in the range of the historical control data of the laboratory (5 studies in Wistar rats using vehicle control groups and treated groups with no adverse effects: “inactive treatment”; 2009-2011): 0.0-1.4% in control group (n = 80 animals) and 0.9-5.0% in “inactive treatment” groups. Dossier submitter considers the use of this latter group as highly questionable. Thus, reference to this group has not been made thereafter.

There was a statistically significant ($p<0.01$) reduction in the body weight of the male and female fetuses in the 1000 mg/kg bw/day group (combined weight = 3.3 g). Registrants considered this finding as non adverse, since this was in the range of the historical control data of the laboratory (3.0-3.4 g in control group).

Absolute placental weight was similar in all experimental groups. There was a statistically significant

increase in the relative placental weight in the 1000 mg/kg bw/day dose group (204.3 mg/g). It should be noted that relative placental weights reported in this study (for control and treated groups) were below the historical control level of the laboratory (210.0 – 234.4 mg/g).

Regarding external examination, umbilical hernia occurred in one fetus in the 1000 mg/kg bw/day dose group.

The incidence of visceral abnormalities was statistically significantly ($p<0.05$) higher in the 1000 mg/kg bw/day dose group (4%), mainly due to variations (which are not significantly increased when considered individually: bilateral hydroureter (0, 1, 0, 1 in each group, respectively) and hydroureter with dilated renal pelvis (1, 0, 0, 2, in each group, respectively)).

There was no test item related effect at skeletal examination of the fetuses in the 200 and 400 mg/kg bw/day dose group. The incidence of the fetuses with skeletal variations increased significantly ($p<0.01$) in the 1000 mg/kg bw/day dose group due to the higher incidence of the delayed ossification of skull and metacarpal/metatarsal. Skeletal malformations were found only in the control and 1000 mg/kg bw/day dose group with an incidence of 2 and 1 respectively, thus the test item did not induce skeletal malformations.

The NOAEL were determined by the registrants as follows:

- NOAEL maternal toxicity: 1000 mg/kg bw/day

- NOAEL developmental toxicity: 1000 mg/kg bw/day

A second prenatal developmental toxicity study (Anonymous, 2018) was performed in rabbits according to OECD TG 414. It consisted of oral treatment of inseminated New Zealand White rabbits with TBPEH at dose levels of 30, 100 and 300 mg/kg bw/day respectively from day 6 up to and including day 27 post insemination. Four rabbits went into moribund state at 300 mg/kg bw/day. Eight aborted in the 300 mg/kg bw/day group, two in the 100 mg/kg bw/day group and one in the 30 mg/kg bw/day group. 1, 0, 2, 2 animals in each group died due to technical reason. Overall, there were 22, 24, 20 and 17 females with implantation site on GD28 and 20, 23, 16 and 10 litters evaluated in the control, 30, 100 and 300 mg/kg bw/day group respectively. Thus, it should be noted that the number of litters evaluated at the highest tested dose was rather low compared to control.

At the dose level of 100 mg/kg bw/d, TBPEH caused slightly reduced food consumption and lower body weight gain (only statisticaly significant on GD15-18). Two females aborted at this dose. At 300 mg/kg bw/day, there was a lower mean body weight in dams until GD24 (-6% on GD24; no statistical difference thereafter) and body weight gain between GD6-12 and GD15-18. This was associated with reduced food consumption up to GD21 (ex. -40.4% between GD 18-21; not statistical difference between GD21-GD28). There was also a lower body weight gain at 100 mg/kg bw/d (on GD6-9; GD9-12 and GD15-18; no statistical difference thereafter) with reduced food consumption. However, corrected body weight and body weight gain was not statistically significantly affected. Eight females aborted at this dose.

Early embryonic death (15% versus 6% in control) and post-implantation loss (17% versus 8% in control) (data compared to number of implantations) were increased at 300 mg/kg bw/day. These effects are statistically significant if the number and percent of resorptions are evaluated and not statistically significant if the mean number and SD are calculated, probably due to the high standard deviation) and mean number of viable fetuses were decreased (sum: 91 viable fetuses in treated group versus 191 in controls; not statistically significant) in the 300 mg/kg bw/day dose group.

There was no significant difference in incidence of overall fetal malformations among the experimental groups. However, significantly lower fetal weight (-15.6%) and crownrump length (-7.3%) were observed in the 300 mg/kg bw/day dose group. At this dose, were also reported increasing skeletal variations (proximal and middle phalanges less than 7/7 ossified (18% versus 7%), misaligned and fused sternebra (2% versus 0%) and multiple malformed ribs and vertebrae (3% versus 0%)).

The NOAELs were determined by the registrants as follows:

- NOAEL maternal toxicity: 30 mg/kg bw/day

- NOAEL developmental toxicity: 100 mg/kg bw/day based on statistical significant changes in body weight

and body weight gain, increased early embryonic death, increased post-implantation loss, slight decrease in mean number of viable fetuses, significant lower fetal weight and crown-rump length, increased incidence in number of fetuses with skeletal variations at 300 mg/kg bw/d.

In addition, developmental effects are reported in the OECD TG 421 study and EOGRTS.

In the OECD TG 421 study, treatment at 1000 mg/kg was associated with an increase of post-implantation loss. Post-natal loss was statistically increased (5/10 dams affected), with a reduction of live pups (43 dead and 20 pups missing until day 4 post-partum). The mean body weight of pups was also reduced at this dose, up to day 4 post-partum. The NOAEL set by the registrants was 300 mg/kg bw/day for parental and developmental toxicity. It should be noted that the level of information available for this study is very limited (no numerical value available).

In the EOGRTS, effects on development, characterised by a higher extra-uterine mortality (F1 and F2), a lower survival index (F2), clinical signs (F1 and F2), reduced body weight (F1 and F2) and slower development of reflexes (F1), were reported in offsprings. These effects were mainly reported at 1000 mg/kg bw/day. At 300 mg/kg bw/day, a lower body weight was also reported in pups. Details are summarized below:

The mean number of post-implantation loss, the mean number of total births, mean number of viable pups and live born and the live birth index (live pups/total birth) were comparable in all groups. Delivery data of dams was not adversely affected at 100, 300 or 1000 mg/kg bw/day dose level (P0 and Cohort 1B).

A higher mortality (F1 and F2) was reported on PND0 (2% versus 0% for F1; 12% versus 0% for F2), between PND0-4 (15% versus 3% for F2) and between PND0-21 (9% versus 1% for F1). A reduced body weight on PND0 (-6.5% in F1 and -5% in F2) and body weight gain (-8.6% between PND0-21 for F2; -13% between PND0-4 for F2) were observed in offspring at 1000 mg/kg bw/day. Lower pup body weight was also reported at 300 mg/kg bw/day (-3.2% for F1 and -3.4% for F2 on PND0). Clinical signs, such as no milk in the stomach, cold pups, were reported in the treated groups.

There was some statistical significance in the percentage of pups with positive response or lower percentage of pups with negative response at 100 mg/kg bw/day (pinna detachment, eye opening) or at 300 mg/kg bw/day (eye opening). At 1000 mg/kg bw/day, the percentage of pups with positive response was lower and the percentage of pups with negative response was higher than in the control group at surface righting reflex, pinna detachment and eye opening. Statistical significance was only reached for eye opening on PND14 (44% with positive response compared to 58% in the control group).

Regarding sexual maturity, the balano-preputional separation was completed in all F1 Cohort 1A male animal groups – control, 100, 300 and 1000 mg/kg bw/day – on post-natal day 35, although, the mean body weight was slightly lower with respect to the control group in male animals at 1000 mg/kg bw/day on PND35. In the F1 Cohort 1A female animals, statistical significance was noted for the longer period of vaginal patency at 1000 mg/kg bw/day (ex. 65% versus 100% at PND34) and at the longer period of appearance of the first cornified vaginal smear at 100 and 1000 mg/kg bw/day (34.2 day and 34.3 day versus 32.3 in the control group). The interval between days of vaginal patency and first cornified smear were similar in all groups (control, 100, 300 and 1000 mg/kg bw/day). Statistical significance was detected at the shorter absolute anogenital distance of male and female F1 pups and male F2 pups at 1000 mg/kg bw/day. However, the normalized anogenital distances were comparable. In F1 pups, nipples/areoles were not visible in any of the examined male offspring in the control or 100, 300 or 1000 mg/kg bw/day groups on post-natal day 13.

Histological investigations did not reveal test item related pathologic changes in the examined organs in F1 offspring.

The NOAEL for offsprings was set by the registrants at 300 mg/kg bw/day based on higher mortality (F1 and F2), lower survival index (F2), clinical signs (F1), reduced body weight (F1 and F2) and slower development of reflexes (F1) for F1, F2 at 1000 mg/kg bw/d. However, the dossier submitter notes that a lower pup body weight was also observed at 300 mg/kg bw/d.

In both studies (OECD 421 and EOGRTS), parental toxicity consisted mainly in slightly decreased body weight, mostly in males (see section 11.8.2).

11.8.6 Comparison with the CLP criteria

For potential classification on development, criteria from CLP guidance (ECHA, 2017c) were applied.

"Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency."

- *Known human reproductive toxicant "The classification of a substance in **Category 1A** is largely based on evidence from humans" (ECHA, 2017).*

There is no existing epidemiological studies. Therefore, classification as Repr. 1A is not fulfilled.

- *The classification of a substance in this **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 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.*

Data on animals report consistent effects on development.

Two experimental prenatal developmental toxicity studies were performed in rats and in rabbits according to OECD TG 414.

The first prenatal developmental toxicity study (OECD TG 414) in rats tested 0, 200, 400, 1000 mg/kg bw/day of TBPEH. At all doses tested, no effect on corrected body weight and body weight gain was observed in rats. Thus, no maternotoxicity was evidenced up to 1000 mg/kg bw/day. Regarding developmental toxicity, at 1000 mg/kg bw/day, there was a slight but statistically significant ($p<0.01$) reduction in mean body weight of male and female fetuses (within historical control data according to registrants). Incidence of skeletal variations (incomplete ossification of the skull-bones and metacarpal/metatarsal) was also increased at this dose.

The second prenatal developmental toxicity study (OECD TG 414) in rabbits tested 0, 30, 100 and 300 mg/kg bw/d of TBPEH. Abortion and moribund animals were found at the highest tested dose. In dams, body weight (until GD24), body weight gain (GD6-12 and 15-18) and food consumption (until GD21) were lower than control group at 300 mg/kg bw/day. However, there was no statistically significant effect when corrected body weight and body weight gain were considered. Regarding developmental toxicity, at 300 mg/kg bw/d, increase of early embryonic death and post-implantation loss (statistically significant if number and percent of resorptions evaluated) was noted. A slight decrease (without a statistical significance) in mean number of viable fetuses was observed. Fetal weight and crown-rump length were significantly lower. Incidence in number of fetuses with skeletal variations (proximal and middle phalanges less than 7/7 ossified, misaligned and fused sternebra, multiple malformed ribs and vertebrae) was observed.

Developmental toxicity of TBPEH was also assessed in a reproduction/developmental toxicity screening test (OECD TG 421) and in an extended one generation study, including the production of a second generation (cohort 1A and 1B) (OECD TG 443).

In the OECD TG 421 study, TBPEH was administered orally (by gavage) to Wistar rats at repeated doses of 0, 100, 300 and 1000 mg/kg body weight/day, for 14 days during the pre-pairing period, and the pairing, the gestation and the lactation periods until 3 post partum. At 1000 mg/kg bw/day, reduction of body weight

and/or body weight gain was reported in males during the entire study and in females during lactation. This was associated with a decreased food consumption. Treatment at 1000 mg/kg was associated with an increase of post-implantation. Post-natal loss was also statistically increased, with a reduction of live pups (until day 4 post-partum). The mean body weight of pups also was reduced at this dose, up to day 4 post-partum.

In the OECD TG 443 study, rats were administered with TBPEH orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day. TBPEH did not cause overt general toxicity in the mother up to the highest tested dose but interfered with development of the offsprings. Indeed, at 1000 mg/kg bw/day, higher post-natal mortality (observed from PND0) and reduction of body weight in both F1 and F2 offsprings were reported. There were also effects on post-natal development regarding absolute anogenital distance and eye opening, at this dose. At the mid dose of 300 mg/kg bw/day, lower body weight of F1 and F2 pups was also noted. In comparison, parental general toxicity is slight and particularly restricted to males exposed to TBPEH at 1000 mg/kg bw/day (decreased body weight < -13% mainly in males; histopathological effects in kidney only in P0 males). At 300 mg/kg bw/day, there are only changes in organ weights (in particular increase of kidney and liver weights) in both sexes, without associated histopathological findings.

The developmental adverse effect findings are consistent among studies and generations and occurred in the absence of overmaternotoxicity. They are visible in two species and cannot be considered as secondary non-specific consequence of other toxic effects. Therefore, the criteria for classification as Repr. 1B for development are fulfilled for TBPEH.

11.8.7 Adverse effects on or via lactation

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

There are no experimental data specifically related to adverse effects on or via lactation. However, some information can be derived from the OECD TG 421 study and the EOGRTS (see sections above). Post-natal losses and decreased body weight of pups were found in both studies.

In the OECD TG 421 study in rats, treatment at 1000 mg/kg was associated with an increase of post-natal loss and a reduction of live pups (until day 4 post-partum). The mean body weight of pups was also reduced at this dose, up to day 4 post-partum.

In the extended one generation according to OECD TG 443 in rats, at 1000 mg/kg bw/day, a higher percentage of offspring showed signs (no milk in the stomach, cold, found dead, missing, alopecia). There was also a higher extra-uterine mortality on PND 0 (2% versus 0% in F1 pups; 12% versus 0% in F2 pups), between PND0-4 (15% versus 3% in F2 pups) and between PND 0-21 (9% versus 1% in F1 pups). Reduction of body weight on PND 0 (-6.5 % for F1 pups; -5% for F2 pups) and of body weight gain between PND0-4 for F2 pups (-12.8%) and between PND0 - PND21 for F1 pups (- 8.6 %) were observed. At 300 mg/kg bw/day, F1 pups showed lower body weight (-6.5%) at PND0, due to male body weight and F2 pups showed a lower body weight (-3% on PND4).

11.8.9 Comparison with the CLP criteria

The two criteria suggested by ECHA (2017c) were checked for classification for adverse effects on or via lactation:

1. *"Substances which are absorbed by women and have been shown to interfere with lactation. This relates to effects in the mother that impact adversely on the breast milk, either in terms of the quantity produced or the quality of the milk produced (i.e. the composition). Any effect on the quantity or quality of the breast milk is likely to be due to systemic effects in the mother. However, overt maternal toxicity may not be seen (e.g. the substance may just affect the transfer of a nutrient into the milk with no consequence for the mother)."*

There is no study investigating the quantity and quality of the milk produces, or any suggestion in studies available that TBPEH can have an impact on breast milk production.

2. *"Substances which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child. This relates to the ability of the substance (including metabolites), to enter the breast milk in amounts sufficient to cause a concern. When the effect on the offspring is caused by the substance (or metabolite) after transport through the milk then the maternal toxicity has no relevance for classification."*

- *"Ideally, studies will be available which inform directly on whether the substance causes adverse effects in the offspring due to an adverse effect on lactation. One generation or multi-generation reproductive toxicity studies, which involve direct exposure or exposure via the milk of the offspring postnatally, usually provide information on this." (ECHA, 2017c).*

There is no study investigating the analysis and presence of metabolites of TBPEH in milk.

From the EOGRTS, an inadequate nursing behaviour of dams can be suggested considering the lower pup body weight during lactation and the higher extra-uterine mortality with the fact that some pups did not have milk in the stomach. In contrast, the fact that the observed adverse effects occurred from PND0 supports that they are consecutive to exposure during gestation. Overall, it cannot be clearly distinguished if these post-natal effects were caused by gestational exposure and/or lactation exposure.

A classification on or via lactation is not warranted for TBPEH.

11.8.10 Conclusion on classification and labelling for reproductive toxicity

Classification for reproductive toxicity addresses adverse effects on sexual function and fertility, developmental effects and adverse effects on or via lactation.

Adverse effects on sexual function and fertility

Impairment of fertility is reported in the EOGRTS 443 with decreased number of developing follicles and increased number of follicular atresia observed in pregnant or non-pregnant animals, irregular estrous cycle and lower reproduction index. These effects occurred in the absence of overtoxicity in parental animal. So a classification as Repr. 1B – H360F is warranted.

Adverse effects on development

In the OECD TG 421 study, rats were administered with TBPEH orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day. Treatment at 1000 mg/kg was associated with an increase of post-implantation. Post-natal loss was also statistically increased, with a reduction of live pups (until day 4 post-partum). The mean body weight of pups also was reduced at this dose, up to day 4 post-partum.

In the OECD TG 443 study, rats were administered with TBPEH orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day. TBPEH did not cause overt toxicity in the mother up to the highest tested dose but interferes with development of the offsprings. This include, at 1000 mg/kg bw/day, higher post-natal mortality (observed from PND0) and reduction of body weight in both F1 and F2 offsprings. There were also effects on post-natal development regarding absolute anogenital distance and eye opening, at this dose. At the mid dose of 300 mg/kg bw/day, lower body weight of F1 and F2 pups was also noted.

Therefore, these studies can be used by themself for classification purpose.

Two experimental Prenatal Developmental Toxicity Studied were performed in rat and in rabbit according guideline 414. The first OECD TG 414 study was performed in rat exposed to 0, 200, 400, 1000 mg/kg bw/day of TBPEH. Even of at all doses tested, no effect on corrected body weight and body weight gain was observed in rats. A slight but statistically significant reduction in mean body weight of male and female fetuses, and a higher incidence of skeletal variations was noted. The second OECD TG 414 study was performed in rabbits exposed to 0, 30, 100, 300 mg/kg bw/day of TBPEH. At high dose, abortions occurred. Moribund animals and clinical signs were also reported. Mean body weight of the animals was lower until

GD24 and body weight gain decreased at GD6-12 and GD15-18. But there was no statistically significant effect when corrected body weight and body weight gain were considered. Food consumption was reduced up to GD21. Increase of early embryonic death and post-implantation loss, and a slight decrease in mean number of viable fetuses were observed. Fetal weight and crown-rump length were significantly lower. Incidence in external variations increased significantly.

The available data in the four *in vivo* studies showed clear evidence of an effect of TBPEH on pup development. TBPEH should therefore be classified as Repr. 1B – H360D.

Adverse effects on or via lactation

There was no specific study assessing effects via or on lactation. From the EOGRTS, an inadequate nursing behaviour of dams can be suggested considering the lower pup body weight during lactation and the higher extra-uterine mortality with the fact that some pups did not have milk in the stomach. In contrast, the fact that the observed adverse effects occurred from PND0 support that they are consecutive to exposure during gestation. Overall, post-natal effects could not clearly be distinguished from effects caused by gestational exposure.

A classification on or via lactation is not warranted for TBPEH.

Table 57: Summary table of comparison between main adverse effects reported on general toxicity, fertility and development

Protocol	Parental toxicity	Fertility effect	Developmental toxicity
OECD 421 study in rats Dosage: 0, 100, 300, 1000 mg/kg bw/day Anonymous, 2008	**At 1000 mg/kg bw/day** Clinical signs in females at the end of gestation and beginning of lactation. Decreased BW in males during the whole study and in females during lactation. Decreased food consumption.	/	**At 1000 mg/kg bw/day** Increase of post-implantation loss, postnatal loss. Mean BW up to day 4 post-partum reduced.
OECD 443 in rats Dosage: 0, 100, 300, 1000 mg/kg bw/day Anonymous, 2020	**At 1000 mg/kg bw/day** **P0 generation** No mortality. Clinical signs as salivation and nuzzling up the bedding material. Lower BW in males (Day 152; -13% of the control) with decreased BW gain in the most cases during the observation period. No significant effect on BW and BW gain in females. Changes in various organ weights. Chronic progressive nephropathy in males, with increase kidney weight and changes of some urinalysis parameters (higher volume of urine and lower pH). Lower mean FT3 and FT4 in males. **P1 (second parental generation)** F1 cohort 1B: No test-item related mortality. BW reduced in males (-11%) and females from PND22-36 (max. -8% on PDN29) and higher on lactation day 4 (L4) (2%) (no effect on BW gain). Changes in various organ weights. No pathological alteration at histology. Reduction in T3 and T4 in males (and T4 in females). F1 cohort 1A: No test-item related mortality.	**At 1000 mg/kg bw/day** **P0 generation** Decreased number of developing follicles and increased number of follicular atresia (13.2 versus 5.3) Lower number animals with regular cycle (54% versus 83%), lower number of days in proestrous (1.5 versus 3). Reproduction index: 67% versus 91% Slightly longer mean duration of pregnancy of dams (22.37 versus 21.97 days). **P1 generation** Number and percentage of female animals in prolonged estrous slightly higher (15 %). Lower reproduction index (56% versus 95%). Copulatory index lower as 2 males failed to mate (95% versus 100%). **At 300 mg/kg bw/day:** Reproduction index reduced	**At 1000 mg/kg bw/day** **Cohort F1B generation pups** Higher percentage of offspring with clinical signs. Higher mortality on PND 0 (2% versus 0%) and between PND 0-21 (9% versus 1%). Reduced BW on PND 0 (-6.5 %). BW gain between PND0 - PND21 depressed (- 8.6 %). Similar effect on litter weight (-14% on PND21). Lower percentage of pups with positive response and higher percentage of pups with negative response at surface righting reflex, pinna detachment - but not statistically significant - and eye opening (statistically significant; positive results: 31% versus 58%). Shorter absolute anogenital distance but not when normalized. **Cohort 1A:** Longer period of vaginal patency and appearance of the first cornified vaginal smear (34.3 versus 34.2 day) **F2 generation** Clinical signs. Percentage of found dead F2 offspring higher (+12%) from PND0. Lower BW (-8% on PND4) and BW gain of pups between PND0 and 4 (-13%). Shorter absolute anogenital distance but not when normalized. **At 300 mg/kg bw/day:** Lower BW in F1 pups (-6.5% at PND0; due to male) and in F2 pups

	BW reduced in males (-12% on day 90) (with some decreases in BW gain) and females from PND22-42 (max. -10% on PDN25). No pathological alteration at histology. Higher volume of urine in males, lower pH of urine in females. Elevated kidney weight. Reduction of T3 and T4 in males. **At 300 mg/kg bw/day:** Some changes in organ weights in P0 and P1. Lower pH of urine in cohort 1A females. Reduction of T3 and T4 in cohort 1B males.	(80% versus 95%)	(-3% on PND4).
OECD 414 study in rats Dosage: 0, 200, 400, 1000 mg/kg bw/day Anonymous, 2013	**At all doses:** No effect on corrected BW and BW gain	**Not applicable**	**At 1000 mg/kg bw/day** Incidence of skeletal abnormalities increased with a statistical significance due to the increase in the variations (incomplete ossification of the skull-bones and metacarpal/metatarsal).
OECD 414 study in rabbits Dosage: 0, 30, 100, 300 mg/kg bw/day Anonymous, 2018	**At 300 mg/kg bw/day:** Abortions increased significantly (8 versus 0 in control). Moribund animals (16% versus 0%). Clinical signs reported. Mean body weight of the animals lower until GD24 (-6% on GD24). Decreased BW gain GD6-12 and GD15-18. No significant effect when corrected BW and BW gain considered. Reduced food consumption up to GD21.	**Not applicable**	**At 300 mg/kg bw/day:** Increase of early embryonic death and post-implantation loss (statistically significant if number and percent of resorptions evaluated): 41 versus 20 and 36 versus 13, respectively. 4 dams with total post-implantation loss Lower fetal weight (-15.6%) and crown-rump length (-7.3%) with one litter completely affected (i.e. 10/10 fetuses). Incidence of external variations increased due to growth retardation (15% versus 3%). Increase incidence of fetuses with skeletal abnormalities (42 versus 27%): proximal and middle phalanges less than 7/7 ossified, misaligned and fused sternebra, multiple malformed ribs and vertebrae

In summary, a classification as Repr. 1B – H360 FD is warranted for TBPEH.

11.9 Specific target organ toxicity-single exposure

Not assessed in this dossier.

11.10 Specific target organ toxicity-repeated exposure

Not assessed in this dossier.

11.11 Aspiration hazard

Not assessed in this dossier.

12 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

13 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

14 ADDITIONAL LABELLING

Not assessed in this dossier.

15 REFERENCES

OECD. SIDS INITIAL ASSESSMENT PROFILE (SIAP), CoCAM 6, September 30 - October 3, 2014, by NL ICCA.

See confidential Annex II.

16 ANNEXES

Annex I for study summaries.

Confidential Annex II.