CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: Acetophenone

EC Number: 202-708-7

CAS Number: 98-86-2

Index Number: 606-042-00-1

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TABLES

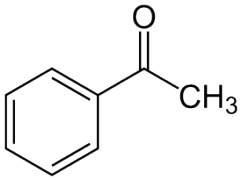
ABBREVIATIONS

abs	absolute
ASR	auditory startle response
BPS	balanopreputial separation
bw	body weight
ctrl	control
CAS	Chemical Abstract Service Registry number
DIT	developmental immunotoxicity
DNT	developmental neurotoxicity
DS	dossier submitter
EC	European Community number
EOGRTS	extended-one generation reproductive toxicity study
FOB	functional observational battery
GC-MS	gas chromatography-mass spectrometry
GD	gestational day
GLP	Good Laboratory Practice
HD	high-dose
LD	low-dose or lactation day
LOAEL	lowest observed adverse effect level
LD_{50}	lethal dose, 50%
m/f	male/female
MD	mid-dose
NADPH	nicotinamide adenine dinucleotide phosphate
Nb	number
NK	natural killer
NMR	Nuclear Magnetic Resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PND	post-natal day
rel	relative
stat sign	statistically significant
UV	ultraviolet-visible
VO	vaginal opening

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)	1-phenylethanone
Other names (usual name, trade name, abbreviation)	1-Phenylethanone Acetophenone Acetophenone, technical grade Acetylbenzene Hypnone Methyl phenyl cetone Methylphenylketone Phenylethanone Phenylmethylketone acetylbenzene;methyl phenyl ketone;1-phenylethanone;benzoyl methide
ISO common name (if available and appropriate)	n.a.
EC number (if available and appropriate)	202-708-7
EC name (if available and appropriate)	Acetophenone
CAS number (if available)	98-86-2
Other identity code (if available)	n.a.
Molecular formula	C8H8O
Structural formula	
SMILES notation (if available)	CC(=O)c1ccccc1
Molecular weight or molecular weight range	120.1485 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	n.a.
Description of the manufacturing process and identity of the source (for UVCB substances only)	n.a.
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

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)
1-phenylethanone EC number: 202-708-7 CAS number: 98-86-2	Mono-constituent ≥ 80 - ≤ 100 % (w/w)	Acute Tox. 4*, H302 Eye Irrit. 2, H319	Acute Tox. 4, H302 STOT SE 3, H336 Skin Irrit. 2, H315 Not classified

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Impurities not relevant for the classification of the substance.				

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
-					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP criteria

	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	606-042-00-1	acetophenone	202-708-7	98-86-2	Acute Tox. 4* Eye Irrit. 2	H302 H319	GHS07 Wng	H302 H319			
Dossier submitters proposal	606-042-00-1	acetophenone	202-708-7	98-86-2	**Add** Repr. 1B STOT SE 3 **Remove** Acute tox. 4*	**Add** H360FD H336 **Remove** H302	**Add** GHS08 **Modify** Dgr	**Add** H360FD H336 **Remove** H302			
Resulting Annex VI entry if agreed by RAC and COM	606-042-00-1	acetophenone	202-708-7	98-86-2	Repr. 1B STOT SE 3 Eye Irrit. 2	H360FD H336 H319	GHS08 GHS07 Dgr	H360FD H336 H319			

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	**data conclusive but not sufficient for classification**	Yes
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	**harmonised classification proposed (Repr. 1B, H360FD)**	Yes
Specific target organ toxicity-single exposure	**harmonised classification proposed (STOT SE 3, H336)**	Yes
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 acetophenone was introduced in the Annex I to Directive 67/548/EEC by Commission Directive 93/72/EEC of 1 September 1993 adapting to technical progress for the nineteenth time Council Directive 67/548/EEC with the following classification: Xn ; R 22 and Xi ; R 36.

This classification was then translated to the Annex VI to Regulation (EC) No 1272/2008 (CLP00) as Acute Tox. 4* (H302) and Eye Irrit. 2 (H319).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

There is no requirement for justification that action is needed at Community level since acetophenone is considered to fulfil the criteria for classification as toxic to reproduction (Repr. 1B, H360FD), and a harmonised classification is justified according to Article 36(1)(d) of the CLP Regulation.

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

Change in existing entry due to new interpretation/evaluation of existing data:

The minimum classification for Acute Tox. 4* (H302) has been revised in this proposal.

Disagreement by the DS with current self-classification:

The REACH registrant has not self-classified the substance for STOT SE 3. The DS considers that the data presented in this dossier supports classification as STOT category 3 following single exposure. Therefore, justification that action is needed at Community level for harmonised classification and labelling of acetophenone for STOT SE 3 is based on disagreement by the DS with the current self-classification.

5 IDENTIFIED USES

According to ECHA dissemination website, acetophenone is manufactured in the EU and it is used for the formulation of mixtures. At industrial sites, acetophenone is used as a cleaning agent, intermediate, solvent and in polymer manufacturing and processing.

Acetophenone is widespread used by professional workers and consumers in air care products, fillers, putties, plasters, modelling clay, cleaning and care products, lubricants, greases, release products, coatings and paints, thinners, paint removers and finger paints.

6 DATA SOURCES

The REACH registration dossier for acetophenone publicly available from ECHA dissemination site is the main source of information.

Additionally, the confidential registration dossier was available for evaluation as well as the original extended one-generation reproductive toxicity study report provided by registrants.

Scientific literature also served as information sources. Please see section 14. References for details.

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	Liquid	ECHA dissemination site (2022)	Reliable information from peer-reviewed handbook

Property	Value	Reference	Comment (e.g. measured or estimated)
Melting/freezing point	20 ºC	ECHA dissemination site (2022)	Reliable information from peer-reviewed handbooks and evaluated sources
Boiling point	202.11 ºC	ECHA dissemination site (2022)	Measured at 1013.25 hPa
Relative density	1.03 at 20 ºC	ECHA dissemination site (2022)	Reliable data from peer-reviewed and evaluated sources
Vapour pressure	45 Pa at 25 ºC	ECHA dissemination site (2022)	Experimental and calculated data from evaluated sources
Surface tension	39.04 nM/m at 25 ºC	ECHA dissemination site (2022)	Data from peer-reviewed handbook
Water solubility	6.2 g/L at 25 ºC	ECHA dissemination site (2022)	Reliable information from experimental studies and peer-reviewed handbooks
Partition coefficient n-octanol/water	Log Kow = 1.65 at 20 ºC	ECHA dissemination site (2022)	Experimentally derived
Flash point	105 ºC	ECHA dissemination site (2022)	Reliable data from peer-reviewed source
Flammability	Study technically not feasible	ECHA dissemination site (2022)	The study does not need to be conducted because the substance is a liquid
Explosive properties	Non explosive	ECHA dissemination site (2022)	The study does not need to be done since the substance does not contain any functional groups indicating explosive properties
Self-ignition temperature	535 ºC at 1013 hPa	ECHA dissemination site (2022)	Data from peer-reviewed source
Oxidising properties	Non oxidising	ECHA dissemination site (2022)	The study does not need to be done since the substance does not contain any chemical groups indicating oxidising properties
Granulometry	Study technically not feasible	ECHA dissemination site (2022)	The substance is a liquid
Stability in organic solvents and identity of relevant degradation products	Data waived	ECHA dissemination site (2022)	
Dissociation constant	21.55 at 25 ºC		Data from peer-reviewed handbook. The result is assignable as no acid group is present in the molecule (expert judgement).
Viscosity	1.681 mPa × s at 25 ºC		Reliable information from peer-reviewed handbook

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
8 male albino rats Single intraperitoneal administration Test material: acetophenone Doses: 100 mg/kg Investigation of urinary excretion of mandelic acid after injection of ^{14}C-methyl labelled acetophenone; exhalation of $^{14}CO_2$ was also determined. Non-guideline; non-GLP	Acetophenone was metabolised to mandelic acid. The formation of benzoic acid as a further metabolite was indicated by exhalation of CO_2, accounting for 10% during the first 4 h and 30% during 13 h post-administration.	2 (reliable with restrictions) Weight of evidence Experimental study	Sullivan *et al*., 1976
In vitro method Test material: acetophenone Assessment of metabolic conversion of acetophenone to 1-phenylethanol by ketone reductase in cytosols or microsomes from various tissues of untreated or phenobarbital pretreated rabbits and rats. Non-guideline; non-GLP	The NADPH-dependent formation of 1-phenylethanol was demonstrated in rabbit cytosols of liver, kidney, heart and lung after phenobarbital pre-treatment (initial rate of NADPH oxidation: liver > kidney > heart, lung). No activity was demonstrable in rabbit brain cytosol. Very little reduction of acetophenone took place in rabbit liver microsomes (10% of the rate of liver cytosol). The ketone reductase activity of rat liver cytosol was not inducible by phenobarbital pretreatment.	2 (reliable with restrictions) Weight of evidence Experimental study	Leibman, 1971
3 experiments with groups of 5 male rabbits each Single intraperitoneal administration Test material: acetophenone Doses: total dose per experiment was 5360 mg corresponding to ca. 250 mg/kg Investigation of urinary excretion of hydroxylated metabolites of acetophenone after 48 h post-administration. Non-guideline; non-GLP	The hydroxylated metabolites 1-phenylethanol, ω-, p- and m-hydroxyacetophenone were excreted via urine. Total excretion was approximately 4.5-5% of the dose with 1-phenylethanol as major metabolite (3.5% of the dose, up to half of this amount unconjugated) and ω-, p- and m-hydroxyacetophenone as minor metabolites (0.5-0.95%, 0.4 and 0.1%, respectively). Unchanged acetophenone comprised ca. 0.01% in urine.	2 (reliable with restrictions) Weight of evidence Experimental study	Kiese and Lenk, 1974
6 male Wistar rats per dose Inhalation exposure: 6 h, whole body Test material: ethylbenzene Doses: 0, 300, 600 ppm Urinary metabolites of ethylbenzene were investigated after inhalation exposure. As acetophenone is the main intermediate of ethylbenzene, a metabolism study with exposure to	About 93% of the total metabolites found in urine were related to acetophenone either as precursor, metabolic intermediate or final product, whereas only about 7% of the metabolites of ethylbenzene accounted for an alternate pathway independent of acetophenone with formation of phenylacetic acid. Acetophenone was found at 0.1-0.2% in the urine of ethylbenzene-exposed	2 (reliable with restrictions) Weight of evidence Experimental study	Engström, 1984

Method	Results	Remarks	Reference
ethylbenzene is evaluated. Non-guideline; non-GLP	rats and its precursor 1-phenylethanol at about 24%. Around 68-70% of the total urinary metabolites resulted from the biotransformation of acetophenone: mandelic acid and benzoic acid as main metabolites accounted for ca. 25% each, followed by about 10% phenylglyoxalic acid and 4.7% ω-hydroxyacetophenone. Phenylglyoxal, 1-phenyl-1,2-ethanediol and p-hydroxyacetophenone were identified as minor metabolites accounting for 0.5-1.5% in rat urine each.		

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

There are no studies on the absorption or distribution of acetophenone. Information on toxicokinetics is limited to an *in vitro* metabolic study and three *in vivo* studies investigating the metabolites excreted in the urine. However, there is no comprehensive quantitative investigation of excretion. Moreover, bioaccumulation potential could not be judged based on the results of the studies.

In the *in vitro* study (Leibman, 1971), a reduction of acetophenone to 1-phenylethanol took place in the presence of rabbit cytosols of liver, kidney, heart and lung after phenobarbital pretreatment. However, no activity was found in rabbit brain cytosol, and very little in the presence of rabbit liver microsomes (10% of the rate of liver cytosol).

The three non-guideline and non-GLP *in vivo* studies (Sullivan *et al*., 1976; Kiese and Lenk, 1974; Engström, 1984) investigated the urinary excretion of acetophenone or ethylbenzene, which is metabolised to acetophenone, after intraperitoneal administration or inhalation exposure of rats and rabbits.

In Sullivan *et al*. (1976), acetophenone was metabolised to mandelic acid in male rats after intraperitoneal injection of ^{14}C- acetophenone (labelled at methyl carbon). In the course of the formation of benzoic acid, the C-labelled methyl group is oxidized and eliminated via exhalation of CO_2, accounting for 30% within 13 h post-administration.

In rabbits exposed to a single intraperitoneal administration of acetophenone, total excretion of the hydroxylated metabolites 1-phenylethanol, ω-, p- and m-hydroxyacetophenone accounted for around 5% of the dose, while unchanged acetophenone comprised ca. 0.01% only. The major metabolite was 1-phenylethanol (3.5% of the dose) and ω-, p- and m-hydroxyacetophenone were minor metabolites which together amounted to less than 1.5% of the dose (Kiese and Lenk, 1974).

Acetophenone (0.1-0.2%) and its precursor 1-phenylethanol (ca. 24%) were found in the urine of male rats exposed via inhalation to ethylbenzene. About 93% of the total urinary metabolites were related to acetophenone either as precursor, metabolic intermediate or final product. The main metabolites were mandelic acid and benzoic acid, accounting for ca. 25% each, followed by phenylglyoxalic acid and ω-hydroxyacetophenone which together amounted to approximately 15% of the urinary metabolites. Minor metabolites identified in this study included phenylglyoxal, 1-phenyl-1,2-ethanediol and p-hydroxyacetophenone, representing less than 3% of the metabolites in urine (Engström, 1984).

The registrant proposes the following metabolism scheme of acetophenone based on the outcome of several experimental studies:

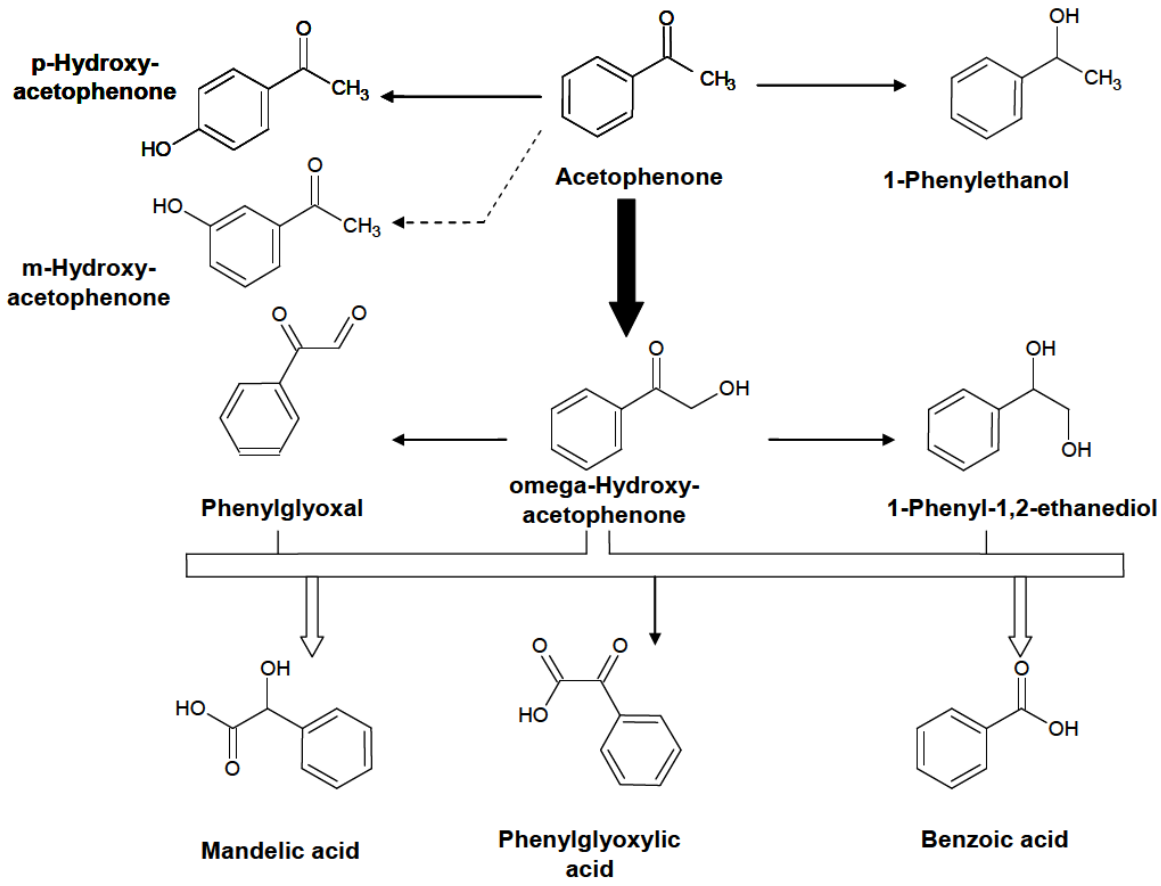


Figure 1: Metabolism of acetophenone reconstructed from metabolites found in urine (adopted from Engström, 1984). The thickness of the arrows represents the extent of the respective route. Broken arrows mean that only trace amounts were found. Unclear pathways are depicted by open arrows.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity Similar to OECD TG 401 Reliability 2 (reliable with restrictions) Non-GLP No information on the purity of the test substance.	Rat, Sprague-Dawley 5/sex/group	Acetophenone Purity: not specified No vehicle	0, 1030, 1648, 2575, 4120 mg/kg bw Single oral (gavage) dose 14 days post-treatment observation period	2081 mg/kg bw	Anonymous, 1981
Acute oral toxicity Similar to OECD TG	Rat, Sprague-Dawley	Acetophenone Purity: 99.7%	0, 710, 1400, 2000, 2800, 3900 mg/kg bw Single oral (gavage)	2200 mg/kg bw	Anonymous, 1978

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
401 Reliability 2 (reliable with restrictions) Non-GLP Less detailed documentation of testing procedure, no microscopic examination, confidence interval not provided and no response data specified by sex.	5/sex/group	No vehicle	dose 14 days post-treatment observation period		
Non-guideline acute oral toxicity Reliability 2 (reliable with restrictions; according to registration dossier) Reliability 4 (not reliable; according to the DS) Non-GLP No information on the purity of the test substance, dose levels, no results for dose groups or individual animals presented, no pathological findings reported.	Rat, Osborne-Mendel 5/sex/group	Acetophenone Purity: not specified No vehicle	Dose levels: not specified Single oral (gavage) dose 14 days post-treatment observation period	3200 mg/kg bw	Jenner *et al.*, 1964
Non-guideline acute oral toxicity Reliability 4 (not assignable) Non-GLP No information on the purity of the test substance, dose levels, no results for dose groups or individual animals presented, no clinical or pathological findings reported.	Rat, Sherman Number and sex of animals: not specified	Acetophenone Purity: not specified Vehicle: not specified	Dose levels: not specified Single oral (not specified) dose	900 mg/kg bw	Smyth and Carpenter, 1948
Non-guideline acute oral toxicity Reliability 4 (not assignable) Non-GLP No information on the	Mouse, strain not specified Number and sex of animals: not specified	Acetophenone Purity: not specified Vehicle: not specified	Dose levels: not specified Single oral (not specified) dose	740 mg/kg bw	Tiunov *et al.*, 1986

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
purity of the test substance, dose levels, study design and test results.					
Non-guideline acute oral toxicity Reliability 4 (not assignable) Non-GLP No information on the purity of the test substance, dose levels, no results for dose groups or individual animals presented, no clinical or pathological findings reported; authors designated the obtained LD_{50} as a rough approximation.	Rat, Wistar, male Number of animals: not specified	Acetophenone Purity: not specified Vehicle: cellosolve acetate	Dose levels: not specified Single oral (gavage) dose	3000 mg/kg bw (male)	Smyth and Carpenter, 1944

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

There are six non-GLP acute oral toxicity studies available in the REACH registration dossier.

Two studies similar to OECD TG 401 reported comparable LD_{50} values for rats (Anonymous, 1981; Anonymous, 1978).

In the key study (Anonymous, 1981), all rats showed piloerection and bending immediately after exposure. Decreased motility and staggering gait were also reported at 1030 and 1648 mg/kg bw. Mortality was observed from the lowest dose onwards (1m/1f at 1030 mg/kg bw, 2m/1f at 1648 mg/kg bw, 2m/4f at 2575 mg/kg bw, and 5m/5f at 4120 mg/kg bw), with unspecific clinical signs. These deaths occurred within 24 h post-administration, except for one female at 4120 mg/kg bw. The LD_{50} was determined at 2081 mg/kg bw. Deceased animals showed liver hyperemia, but no pathological findings were detected in animals sacrificed at the end of the observation period.

A supporting study (Anonymous, 1978) reported a LD_{50} of 2200 mg/kg bw. All rats died within 24 h at 2800 and 3900 mg/kg bw, and 3/10 animals at 2000 mg/kg bw within 48 h. Clinical signs such as staggering gait followed by inhibition of turn-around reflex, watery eyes, palpebral ptosis and slowed down breath were observed at all doses, with first signs appearing within 15 min after administration. Flabby appearance and cyanotic extremities from 2000 mg/kg bw, and prostration and flat breath from 2800 mg/kg bw were also reported.

In a non-guideline supporting study (Jenner *et al.*, 1964), some rats showed coma within 5 min after administration, persisting for up to 24 h. Death occurred between 1 h and 4 days. The LD_{50} was reported as 3200 mg/kg bw. However, the DS considers that these results are of limited reliability, since the study is deficient in reporting important aspects such as purity, dose levels, pathological examination, or results by sex and groups.

There are three other non-guideline studies included in the dossier with several deficiencies related to the purity of test substance, dose levels, number and sex of animals, clinical or pathological findings, or test results. In these studies (reliability 4), the LD_{50} reported was 900 mg/kg bw (Smyth and Carpenter, 1948), 740 mg/kg bw (Anonymous, 1986), and 3000 mg/kg bw (Anonymous, 1944). Since the reliability of these

studies is not assignable, these data are neither considered further in the assessment of acute oral toxicity nor used for classification purposes of acetophenone.

10.1.2 Comparison with the CLP criteria

According to the CLP Regulation, classification for acute oral toxicity is required for substances with a LD_{50} ≤ 2000 mg/kg bw.

Considering the most reliable studies (Anonymous, 1981; Anonymous, 1978), the lowest LD_{50} is 2081 mg/kg bw, obtained in a study similar to OECD TG 401 and flagged as key study in the dataset (Anonymous, 1981). This value does not meet the criteria for classification in Acute Tox. 4 for oral administration.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Acetophenone currently has a harmonised classification as Acute Tox. 4* (H302) due to the translation of a former classification as Xn; R 22 to the CLP Regulation.

Based on the most reliable studies, the existing classification as Acute Tox. 4* (H302) should be removed since the classification criteria for acute oral toxicity are not fulfilled.

10.2 Acute toxicity - dermal route

Not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Not assessed in this dossier.

10.4 Skin corrosion/irritation

Not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Not assessed in this dossier.

10.6 Respiratory sensitisation

Not assessed in this dossier.

10.7 Skin sensitisation

Not assessed in this dossier.

10.8 Germ cell mutagenicity

Not assessed in this dossier.

10.9 Carcinogenicity

Not assessed in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 10: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
EOGRTS with DNT OECD TG 443 Reliability 1 (reliable without restrictions) GLP Rat, Sprague-Dawley P0: 24/sex/group C1A: 20-21/sex/group in ctrl, LD and MD, and 10m/20f at HD C1B: 20/sex/group in ctrl, LD and MD. Not enough pups to constitute the HD C2A: 10/sex/group in ctrl, LD and MD, and 5/sex at HD C2B: 10/sex/group in ctrl, LD and MD. Not enough pups to constitute the HD	Acetophenone Purity: 99.79% Vehicle: 0.5% (w/v) carboxymethylcellulose 400-800cPs / 0.5% (w/v) Tween 80 in drinking water treated by reverse osmosis Oral (gavage) 0, 75, 225, 500 mg/kg bw/d P0 generation: 10 weeks before mating, during mating, until F1 weaning F1 generation: direct dosing from weaning (PND 22) to terminal sacrifice of respective cohorts C1A: until PND 90-93 C1B: until PND 98-100 C2A: until PND 76-78 C2B: sacrificed on PND 22 (no direct dosing)	See section 10.10.4 for a description of the effects on development. **P0 generation:** ***Sexual function and fertility*** Mating data (Table 11) 1 female at MD and 3 females at HD were sacrificed with no evidence of mating. As a consequence, there was a decrease in the mating index (100.0%, 100.0%, 95.7% and 87.5% in ctrl, LD, MD and HD groups, respectively). Delivery data (Table 11) 3 females at LD, 1 female at MD and 3 females at HD were sacrificed between GD 23-26 with clear evidence of dystocia. ***General toxicity*** Mortality (Table 11) 29 females were sacrificed due to the following reasons: - humane grounds: 1 in ctrl and 1 at MD (in premating) - no mating: 1 at MD and 3 at HD - non-pregnancy: 1 at LD and 1 at MD - dystocia: 3 at LD, 1 at MD and 3 at HD - dead litter: 3 at MD and 11 at HD There were no unscheduled deaths in P0 males. Clinical signs (Table 12) In both sexes, burrowing activity and ptyalism at all doses, hypoactivity and half-closed eyes from MD, and continuous chewing movement, staggering gait and recumbency at HD. In HD males, low incidence of loud/abdominal breathing and reflux at dosing. In HD females, abdomen increased in size. Body weight, body weight gain and food consumption (Tables 15-17) In males, ↓ bw at HD in several pre- and post-mating weeks (up to -6%), and also at LD and MD in some weeks (up to -5%); ↓ bw gain over the whole treatment period at HD (-9%), mainly due to ↓ bw change during the first week (-31%); ↓ food consumption at all doses during the first week. In females, ↑ bw at HD between premating days 8-50, and GD	Anonymous, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		0-14 (up to +8%); ↑ bw gain at MD and HD the first week of premating (+35% and +39%, respectively); ↓ food consumption at HD the first week of premating (-14%) and between LD 4-21 (up to -32%). Haematology In males, ↓ mean cell haemoglobin concentration at HD (-2%), ↑ % reticulocytes at MD (+26%) and HD (+40%). In females, ↑ fibrinogen at HD (+11%). Biochemistry In males, at HD, ↓ glucose (-12%) and chloride (-2%), and ↑ potassium (+10%), calcium (+5%), total protein (+10%), albumin (+11%), total cholesterol (+27%), alanine aminotransferase (+44%) and bile acids (+96%); at MD, ↓ chloride (-1%), and ↑ total protein (+5%) and total cholesterol (+24%). In females, at HD, ↓ chloride (-3%) and creatinine (-15%), and ↑ calcium (+8%), total protein (+5%), total cholesterol (+23%), alanine aminotransferase (+61%) and bile acids (+156%); at MD, ↓ chloride (-3%) and ↑ calcium (+5%), alanine aminotransferase (+48%) and bile acids (+140%); at LD, ↓ chloride (-2%). Organ weights (Table 22) *Liver*: ↑ abs/rel weight in both sexes, at MD and HD. *Kidneys*: in males, ↑ abs weight at MD and HD and ↑ rel weight at all doses. In females, ↑ abs/rel weight at LD and MD (non-stat sign ↑ at HD). Histopathology (Table 23) *Thyroid gland*: follicular cell hypertrophy in males at HD. *Liver*: hepatocellular hypertrophy in both sexes at all doses. *Kidneys*: increased severity and/or incidence of hyaline droplets, tubular basophilia, hyaline cast and pelvis dilation in males at all doses. *Spleen*: brown pigment in both sexes at all doses. **F1 generations:** ***Sexual function and fertility*** Sexual maturation (Tables 20-21) *Analysed separately*: Stat sign delays in BPS in C1A and C2A males at HD (2.6 and 6 days, respectively). In females, non-stat sign delays in VO in all cohorts at the highest doses tested (3.2, 2.9 and 2.4 days in C1A, C1B and	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		C2A, respectively). *Combining results of all F1 pups*: Stat sign delays in BPS and VO at HD (3 and 3.5 days, respectively). **C1A:** ***General toxicity*** <u>Mortality</u> 1 male found dead at LD on PND 22. 2 females sacrificed for humane grounds, 1 at LD on PND 55 and 1 at HD on PND 22. <u>Clinical signs (Table 13)</u> In both sexes, ptyalism at all doses, hypoactivity, half-closed eyes and loud breathing from MD, and staggering gait and piloerection at HD. With a lower incidence, loss of balance at HD. <u>Body weight, body weight gain (Table 18) and food consumption</u> In males, ↓ bw at HD during the treatment period, higher in the first three post-weaning weeks (up to -18%), partially recovering thereafter (-8% at the end of the exposure period); ↓ bw gain at HD in the first two post-weaning weeks (up to -20%). At MD, ↓ bw and bw gain in some weeks of the treatment period. In females, ↑ bw from PND 57 until the end of the exposure period at MD and HD (up to +10%); ↑ bw gain during several weeks from PND 22-64 (up to +36%) and over the whole post-weaning period at MD and HD (+10% and +14%, respectively). In both sexes, no changes in food consumption. <u>Haematology</u> In males, ↑ packed cell volume (+4%) and large unstained cells (+50%) at HD, and ↓ mean cell haemoglobin concentration at all doses (-3%, -3% and -4% at LD, MD and HD, respectively). In females, at HD, ↑ large unstained cells (+100%); at all doses ↓ mean cell haemoglobin concentration (-2%, -2% and -3% at LD, MD and HD, respectively), and ↑ % reticulocytes (+24%, +21% and +32% at LD, MD and HD, respectively). <u>Biochemistry</u> In males, at HD, ↑ alanine aminotransferase (+24%); at MD and HD, ↓ chloride (-2% and -2%, respectively), ↑ calcium (+4% and +3%, respectively), urea (+23% and +19%, respectively), total protein (+7% and +5%, respectively) and	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		albumin (+8% and +6%, respectively). In females, at HD, ↑ calcium (+4%), total protein (+5%) and albumin (+6%); at MD and HD, ↑ total cholesterol (+28% and +59%, respectively), alanine aminotransferase (+26% and +54%, respectively), and bile acids (+101% and +116%, respectively); at all doses, ↑ triglycerides (+44%, +51% and +32% at LD, MD and HD, respectively). Organ weights (Table 22) *Liver*: ↑ abs/rel weight at MD and HD in males and at all doses in females. *Kidneys*: in males, ↑ abs weight at LD and MD and ↑ rel weight at all doses. In females, ↑ abs weight at all doses and ↑ rel weight at LD and MD (non-stat sign ↑ at HD). *Spleen*: ↑ abs/rel weight in both sexes at MD and HD. Histopathology (Table 23) *Thyroid gland*: follicular cell hypertrophy in males at HD. *Liver*: hepatocellular hypertrophy in both sexes at all doses. *Kidneys*: increased severity and/or incidence of hyaline droplets, tubular basophilia and pelvis dilation in males at all doses. *Spleen*: brown pigment in both sexes at HD. **C1B:** ***General toxicity*** Clinical signs (Table 13) In both sexes, ptyalism from LD, and hypoactivity, half-closed eyes and burrowing activity at MD. Body weight, body weight gain and food consumption No effects on bw, bw gain and food consumption in males or females. Organ weights (Table 22) *Liver*: ↑ abs/rel weight at MD in both sexes and ↑ rel weight at LD in females. *Kidneys*: ↑ abs/rel weight from LD in males, and at MD in females. **C2A:** ***General toxicity*** Mortality 2 animals found dead, 1 male at MD on PND 41 and 1 female	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		at HD on PND 45. Clinical signs (Table 14) In both sexes, ptyalism at all doses, hypoactivity and half-closed eyes from MD, and piloerection and stagerring gait at HD. In MD and/or HD females, low incidence of burrowing activity, loud breathing and reflux at dosing. Body weight, body weight gain (Table 19) and food consumption In males, ↓ bw at MD and HD (up to -9% and -18%, respectively); ↓ bw gain in several weeks of the treatment period and over the whole treatment period at MD and HD (-10% and -15%, respectively). In females, ↑ bw at the end of the treatment period, stat sign only at LD and HD (+6% and +9%, respectively); ↑ bw gain over the whole treatment period at MD and HD (+11% and +13%, respectively). No effects on food consumption in both sexes. Organ weights (Table 22) *Liver*: ↑ rel weight at MD and HD in males, and ↑ abs/rel weight at all doses in females. *Kidneys*: in males, ↑ rel weight at HD. In females, ↑ abs weight at all doses and ↑ rel weight at LD and MD (non-stat sign ↑ at HD). **According to the registration dossier**: NOAEL P0 males (general toxicity) = 75 mg/kg bw/d, based on clinical signs. NOAEL P0 females (general toxicity) < 75 mg/kg bw/d, based on difficulties to deliver. NOAEL P0, C1A and C1B (reproductive/developmental toxicity) = 500 mg/kg bw/d, no adverse effects on reproductive parameters. **According to the DS**: NOAEL P0 males and females (general toxicity) = 75 mg/kg bw/d, based on clinical signs and liver effects. NOAEL F1 males and females (general toxicity) = 75 mg/kg bw/d, based on clinical signs, bw changes and liver effects. NOAEL P0 males (sexual function and fertility) = 500 mg/kg bw/d, no adverse effects on reproductive parameters. LOAEL P0 females (sexual function and fertility) = 75 mg/kg bw/d, due to the clear evidence of dystocia.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		NOAEL F1 (sexual function and fertility) = 225 mg/kg bw/d, based on the delay in sexual maturation.	
Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test OECD TG 422 Reliability 1 (reliable without restrictions) GLP Rat, Sprague-Dawley Short-term repeated dose part: 10 males and 5 females per group Screening for reproductive part: 10 additional females	Acetophenone Purity: 98.80% Vehicle: corn oil Oral (gavage) 0, 75, 225, 750 mg/kg bw/d Daily from 14 days before mating to lactation day 3	See section 10.10.4 for a description of the effects on development. **P0 generation:** ***Sexual function and fertility*** Delivery data 2 females in the ctrl group, 1 female at LD and 1 female at HD were sacrificed on post-breeding day 25 with evidence of mating but failing to deliver. At HD, 1 female showed prolonged parturition. ***General toxicity*** Mortality 11 females were sacrificed due to the following reasons: - no mating: 1 in ctrl (on post-breeding day 25) - failing to deliver: 2 in ctrl, 1 at LD and 1 at HD (on post-breeding day 25) - total litter loss: 6 at HD (on LD 1-4) All males survived to scheduled euthanasia after 28 days of exposure. Clinical signs (Table 24) In both sexes, salivation from MD, and wobbly gait at HD. Urine stain in females at all doses and in HD males. In HD females, low incidence of unkempt appearance, skin pale in color, rough coat, decreased activity, dark material around nose or few faeces. Body weight gain and food consumption (Tables 25-26) In males, stat sign bw loss and ↓ food consumption (-41%) at HD during days 0-3 of the premating period. In females, during days 0-3 of the premating period, non-stat sign bw loss from MD and stat sign ↓ food consumption at MD and HD (-13% and -44%, respectively). During GD 0-7, stat sign ↓ bw gain at HD (-39%). Haematology In males, ↑ mean corpuscular volume at HD (+7%). In females, ↓ erythrocytes (-8%) and haematocrit (-8%) at HD. Biochemistry In males, at HD, ↓ glucose (-16%) and ↑ total protein (+20%),	Kapp *et al*., 2003

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		albumin (+19%), globulin (+21%), sodium (+1%), potassium (+15%), calcium (+15%), cholesterol (+76%) and phosphorous (+25%); at MD, ↑ total protein (+10%), albumin (+11%), calcium (+10%), and phosphorous (+16%). In females, at HD, ↑ total protein (+10%), albumin (+13%), potassium (+12%), cholesterol (+51%) and alanine aminotransferase (+87%); at MD, ↑ total protein (+11%) and albumin (+12%). <u>Organ weights (Table 27)</u> *Heart*: ↓ abs weight in males at HD. *Liver*: ↑ abs/rel weight in unmated females from MD and ↑ rel weight in males at HD. *Kidneys*: in unmated females, ↑ rel weight at MD and HD. *Epididymides*: ↓ abs weight at HD. <u>Histopathology</u> *Liver*: vacuolar changes in hepatocytes in males and unmated females at HD. *Kidneys*: minimal to mild hyaline droplet formation sometimes accompanied by minimal tubular epithelial degeneration and regeneration in males at all doses. <u>Functional observational battery (Table 28)</u> In males, at HD, ↓ forelimb grip strength (-16%) and mean motor activity (-42%). **According to the registration dossier**: NOAEL P0 (maternal toxicity) = 225 mg/kg bw/d, based on clinical signs and reduced bw gain during GD 0-7. NOAEL P0 males = 225 mg/kg bw/d, based on clinical signs and neurobehavioural effects. NOAEL P0 (reproductive toxicity) = 750 mg/kg bw/d, no adverse effects on reproductive performance. **According to the DS**: NOAEL P0 females (sexual function and fertility) = 225 mg/kg bw/d, based on prolonged parturition in one female. NOAEL P0 males (sexual function and fertility) = 750 mg/kg bw/d, no adverse reproductive effects.	

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

Two experimental animal studies were considered to evaluate the reproductive toxicity of acetophenone. Both were performed with the substance in Sprague-Dawley rats, via gavage, according to OECD TG 443 and OECD TG 422, respectively, under GLP conditions.

Extended-one generation reproductive toxicity study (Anonymous, 2021)

In the OECD TG 443 study, acetophenone was administered once daily at dose levels of 0, 75, 225 and 500 mg/kg bw/d.

Sexual function and fertility

In P0, two females were sacrificed due to humane grounds in the premating period (on study day 32), one in the control group and one at mid-dose. Staggering gait and tremors were recorded, but no macroscopic findings were observed at necropsy in both cases.

No evidence of mating was reported in one and three females at mid- and high-dose, respectively. According to the registration dossier, the mating index was calculated including the two females prematurely sacrificed for humane grounds during the premating period in the control and mid-dose groups, resulting in a no dose-dependent decrease in the mating index (95.8%, 100%, 91.7%, and 87.5% in the control, low-, mid- and high-dose groups, respectively). This difference was considered to be of no biological significance because comparable fertility indexes were also observed in previous studies (90% - 100% in OECD 421/422 studies, September 2009 to January 2012, Charles River Laboratories Evreux).

However, in this sense, the DS considers that the two females sacrificed for humane grounds in the premating period should be excluded from the calculation of this index since these females had previously been sacrificed well before mating started. Thus, further analysis showed a dose-dependent decrease in the mating index (100%, 100%, 95.7%, 87.5% in the control, low-, mid- and high-dose groups, respectively), as shown in the Table 11.

Additionally, two more females, one at low- and one at mid-dose, did not become pregnant and were sacrificed.

On the other hand, according to the registration dossier, there was a high number of sacrificed females as a consequence of both, difficulties to deliver and/or dead litter (total litter loss), suggesting reproduction trouble and considered as a substance-related effect (Table 11). In this regard, the registration dossier reported three cases of females sacrificed on GD 23 and 24 due to difficulties to deliver, two at low-dose and one at mid-dose. In addition, one dam was sacrificed at 500 mg/kg bw/d for humane grounds on GD 25, and one and two dams at 75 mg/kg bw/d and 500 mg/kg bw/d, respectively, were sacrificed during GD 25-26 and identified as 'dams pregnant/no delivery'. It should be noted that all these females did not deliver and were sacrificed between GD 23-26 with dead foetuses, placentas and/or scars in the uterus at necropsy. Therefore, the DS considers that there was a clear evidence of dystocia (difficulties or no delivery) in three, one and three dams at low-, mid- and high-dose, respectively.

The DS concludes that both effects mentioned above, i.e the decreased mating index and dystocia, are adverse effects on sexual function and fertility.

There were also three and 11 dams sacrificed due to total litter loss between PND 1-4 at 225 and 500 mg/kg bw/d, respectively. However, the DS does not consider this a fertility effect, but a consequence of an adverse effect on development (refer to section 10.10.4. for more details).

Concerning the number of animals allocated to cohorts, it should be noted that, due to the fertility effects aforementioned along with the high rate of stillborns, resulting in a low birth index (refer to section 10.10.4. for more details), there were insufficient numbers of surviving pups at 500 mg/kg bw/d. Consequently, the test laboratory decided, in agreement with the Sponsor, to give precedent to C1A and C2A groups. Despite this, the high-dose group in C1A contained only ten males, and in C2A there were only five animals per sex

and group. In the case of C1B and C2B, there were not enough pups to constitute the high-dose group.

Table 11. Mating, fertility and delivery data of P0 females (Anonymous, 2021).

	Ctrl	LD	MD	HD
Nb of females	24	24	24	24
Sacrificed for humane grounds (FSR / **DS evaluation**)	1 (in premating)	0	1 (in premating)	1 / **0** (on GD 25)
No mating	0	0	1	3
Mating index (%) (FSR / **DS evaluation**)	95.8 / **100.0**	100.0	91.7 / **95.7**	87.5
Non-pregnant	0	1	1	0
Pregnant dams	23	23	21	21
Fertility index (%)	100.0	95.8	95.5	100.0
Dystocia (difficulties or no delivery) (FSR / **DS evaluation**)	0	2 / **3**	1	0 / **3**
Litters	23	20	20	18
Gestation index (%)	100.0	87.0	95.2	85.7
Females sacrificed (FSR / **DS evaluation**)	1	2 / **4**	5 / **7**	12 / **17**
Due to fertility effects (no mating and dystocia)	**0**	**4**	**3**	**6**
Due to dead litter	0	0	3	11

No statistically significant differences.

No effects were observed on the oestrous cycle, follicle and corpora lutea counts, the number of days taken to mate, implantation sites, gestation length and gestation index. Neither were affected sperm parameters nor the reproductive organs in both sexes.

General toxicity

In P0, 29 females were sacrificed due to the following reasons: two for humane grounds (one in the control group and one at mid-dose, both in the premating period), four with no evidence of mating (one at mid-dose and three at high-dose), two females were not pregnant (one at low-dose and one at mid-dose), seven with clear evidence of dystocia (three at low-dose, one at mid-dose and three at high-dose), and 14 dams were sacrificed due to total litter loss (three at mid-dose and 11 at high-dose).

In F1 generations, three animals were found dead: one male at 75 mg/kg bw/d in C1A on PND 22 with cranium fracture and red discolouration in the brain; and in C2A, one male at 225 mg/kg bw/d and one female at 500 mg/kg bw/d on PND 41 and 45, respectively, with no correlating findings. In addition, two C1A females were sacrificed for humane grounds, one at 75 mg/kg bw/d on PND 55, with scabs in the neck, chromodacryorrhea and exophthalmos in the right eye, and one at 500 mg/kg bw/d, with clinical signs such as hypoactivity, lateral recumbency, staggering gait and half-closed eyes. Due to the low incidence and the lack of dose-response, the DS considers that mortality in F1 generations was not related to the treatment.

In P0, clinical signs such as burrowing activity and ptyalism (at all doses), hypoactivity and half-closed eyes (at 225 and 500 mg/kg bw/d), and continuous chewing movement, staggering gait and recumbency (at 500 mg/kg bw/d) were observed in both males and females. At 500 mg/kg bw/d, abdomen increased in size was also recorded in females and loud/abdominal breathing and reflux occasionally in males (Table 12).

Table 12. Clinical signs of P0 animals (Anonymous, 2021).

	Males				Females			
	Ctrl	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
Nb of animals	24	24	24	24	23	22	19	12
Half-closed eyes			14	24			18	12
Hypoactivity			21	24			14	12
Loud/abdominal breathing				2				
Burrowing activity		10	24	24	1	13	19	12
Continous chewing movement				6			1	9
Ptyalism	1	24	24	24	1	20	19	12
Reflux at dosing				1				
Staggering gait				2			1	4
Recumbency				1				2
Increase in size abdomen								12

Nb of animals affected per group.

It should be noted that the clinical signs of dams did not worse during late gestation and/or after delivery. Therefore, it cannot be assumed that the reproductive effects (on sexual function and fertility and development) were due to a lack of maternal care.

Similar clinical signs were observed in C1A, C1B and C2A animals (Tables 13 and 14). In these cohorts, the most relevant findings were also hypoactivity and half-closed eyes. Staggering gait, burrowing activity, ptyalism, piloerection and loud breathing were also recorded in males and/or females of these cohorts.

Table 13. Clinical signs of C1A and C1B animals (Anonymous, 2021).

	C1A								C1B†					
	Males				Females				Males			Females		
	Ctrl	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**Ctrl**	**LD**	**MD**
Nb of animals	20	20	20	10	20	19	20	19	20	20	20	20	20	20
Half-closed eyes	1		4	10			4	19			4			2
Hypoactivity			2	10			1	19			2			3
Burrowing activity			3								2			2
Loss of balance				1				1						
Loud breathing			3	1			3	5			2			
Piloerection			1	3				9						
Ptyalism		15	20	10		13	20	19	1	16	20		15	20
Staggering gait				8				12						

Nb of animals affected per group. † In C1B, not enough pups to constitute the HD.

Table 14. Clinical signs of C2A animals (Anonymous, 2021).

	Males	Females

	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
Nb of animals	10	10	9	5	10	10	10	4
Half-closed eyes			2	5			2	4
Hypoactivity			2	5			2	4
Burrowing activity			1				1	1
Loud breathing							2	2
Piloerection				5				4
Ptyalism		6	9	5		10	10	4
Staggering gait				5				3
Reflux at dosing								1

Nb of animals affected per group.

All clinical signs observed in P0/F1 generations were considered adverse, except ptyalism, since it is commonly noted after a gavage procedure.

In P0 males, there were statistically significant decreases in body weight at 500 mg/kg bw/d during several pre- and post-mating weeks (up to -6%), and also at 225 and 75 mg/kg bw/d in some weeks (up to -5%). Body weight gain was reduced over the whole treatment period at the high-dose (-9%), mainly due to a reduction in body weight gain during the first week of treatment (-31%). Decreased food consumption was also recorded in the first week at all doses in P0 males, but afterwards, food consumption returned towards control values (Tables 15 and 16).

By contrast, P0 females showed an increase in body weight at 500 mg/kg bw/d between premating days 8-50 and GD 0-14 (up to +8% in both periods). Increased body weight gain was observed in the mid- and high-dose groups only the first week of premating (+35% and +39%, respectively), but not during the gestation or lactation periods. In addition, corrected maternal body weight gain showed no statistically significant differences between control and treated groups. Food consumption was reduced at 500 mg/kg bw/d in the first week of premating (-14%) and between LD 4-21 (up to -32%) (Tables 15 and 17).

According to the registration dossier, variations in body weight, body weight gain and food consumption were considered non-adverse due to the low magnitude or the reversibility of the changes. However, the DS considers the decrease in body weight and body weight gain in males at the highest dose as an adverse effect since the changes were not reversible during the treatment period.

Table 15. Body weight, body weight gain and food consumption of P0 animals in premating period (Anonymous, 2021).

		Males				Females			
	Days	**Ctrl**	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
Body weight (g)	**1**	266	265	262	266	173	173	172	175
	8	331	**321* (-3%)**	**315** (-5%)**	**312** (-6%)**	196	201	203	**207* (+6%)**
	15	388	380	**372** (-4%)**	**369** (-5%)**	217	220	223	**232** (+7%)**
	22	435	426	**416* (-4%)**	**416* (-4%)**	236	242	243	**254** (+8%)**
	29	472	464	**454* (-4%)**	**452* (-4%)**	250	255	254	**268* (+7%)**
	36	502	495	483	486	265	268	269	281
	43	531	521	510	510	276	277	279	292

		Males				Females			
	Days	**Ctrl**	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
	50	553	540	533	531	283	286	291	**301* (+6%)**
	57	571	558	550	547	291	293	295	305
	64	593	578	571	566	300	300	307	314
	71	602	590	584	576	304	303	312	318
Body weight gain (g)	**1 - 8**	+65	**+56# (-14%)**	**+53# (-19%)**	**+45# (-31%)**	+23	+27	**+31** (+35%)**	**+32# (+39%)**
	1 - 71	+336	+325	+322	+310	+130	+130	+140	+143
Food consumption (g/animal/d)	**1 - 8**	30	**28* (-7%)**	**27# (-10%)**	**25# (-17%)**	21	20	20	**18# (-14%)**

*/**/# Differences with control at p≤ 0.05/0.01/0.001. % in comparison to control group.

Table 16. Body weight and body weight gain of P0 males in post-mating period (Anonymous, 2021).

	Days	**Ctrl**	**LD**	**MD**	**HD**
Body weight (g)	**78**	606	590	585	578
	85	622	602	595	**588* (-5%)**
	92	633	616	605	**597* (-6%)**
	99	644	627	615	**603* (-6%)**
	106	656	633	**623* (-5%)**	**615** (-6%)**
	113	666	642	**632* (-5%)**	**623** (-6%)**
	120	672	646	**638* (-5%)**	**630** (-6%)**
	126	674	650	642	**636** (-6%)**
Body weight gain (g)	**1 - 126**	+408	+385	+380	**+370** (-9%)**

*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

Table 17. Body weight, body weight gain and food consumption of P0 females during gestation and lactation periods (Anonymous, 2021).

	Days	**Ctrl**	**LD**	**MD**	**HD**
Body weight (g)	**GD 0**	305	308	310	**326* (+7%)**
	GD 4	326	331	333	**350** (+7%)**
	GD 7	335	342	343	**361** (+8%)**
	GD 10	348	356	358	**373****

	Days	Ctrl	LD	MD	HD
					(+7%)
	GD 14	368	376	376	**390* (+6%)**
	GD 17	398	411	404	420
	GD 20	447	462	452	470
	LD 1	345	350	350	361
	LD 4	353	355	353	353
	LD 7	367	370	367	365
	LD 14	387	395	391	379
	LD 21	376	381	381	377
Body weight gain (g)	**GD 0-20**	+142	+154	+143	+143
	LD 1-21	+31	+32	+34	+34
Corrected maternal body weight gain (g)	**GD 0 - LD 1**	+40	+44	+42	+38
Food consumption (g/animal/d)	**GD 0-4**	29	33	26	27
	GD 17-20	32	34	33	33
	LD 1-4	38	40	35	28
	LD 4-7	56	57	51	**+41# (-27%)**
	LD 7-14	72	75	66	**+50# (-31%)**
	LD 14-21	90	96	83	**+61# (-32%)**

*/**/# Differences with control at p≤ 0.05/0.01/0.001. % in comparison to control group.

In turn, C1A males showed a statistically significant decrease in body weight at 500 mg/kg bw/d. This decrease was higher in the first three post-weaning weeks (up to -18%), with a tendency towards a return to control values thereafter. However, at the end of the exposure period, an 8% of decrease was still apparent. Lower body weight gain was observed in the first two post-weaning weeks at 500 mg/kg bw/d (up to -20%), but no body weight changes were recorded afterwards or over the whole treatment period. These findings were considered test item treatment-related and adverse due to the magnitude of the differences. At 225 mg/kg bw/d, there were a few statistically significant decreases in body weight and body weight gain in some weeks of the treatment period (Table 18).

Instead, a statistically significant body weight increase was observed in C1A females from PND 57 until the end of the exposure period at the two highest doses (up to +10%) (Table 18). Body weight gain also increased during several weeks from PND 22 to 64 and over the whole post-weaning period in the mid- and high-dose groups (+10% and +14%, respectively). The study authors concluded that this finding was non-adverse taking into account the reversibility of the change. However, since the effect on body weight was not reversible at the end of the treatment period, the DS considers this increase as an adverse effect.

No relevant changes in food consumption were observed in C1A males or females.

Table 18. Body weight and body weight gain of C1A animals (Anonymous, 2021).

		Males				Females			
	PND	**Ctrl**	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**

		Males				Females			
	PND	**Ctrl**	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
Body weight (g)	**22**	64	63	61	**54** (-16%)**	60	62	58	56
	29	114	112	109	**94** (-18%)**	100	103	100	91
	36	185	179	**173* (-6%)**	**154** (-17%)**	154	157	157	144
	43	252	246	239	**223** (-12%)**	190	198	199	193
	50	317	309	**299* (-6%)**	**281** (-11%)**	215	224	230	224
	57	376	369	**357* (-5%)**	**338** (-10%)**	234	244	**254** (+9%)**	**248* (+6%)**
	64	421	414	400	**385** (-9%)**	247	260	**267* (+8%)**	**268** (+9%)**
	71	458	448	436	**416** (-9%)**	260	275	**284** (+9%)**	**287** (+10%)**
	78	487	477	465	**443** (-9%)**	272	285	**295* (+8%)**	**297** (+9%)**
	85	503	501	487	**463* (-8%)**	282	295	**303* (+7%)**	**308** (+9%)**
Body weight gain (g)	**22-29**	+50	+49	+48	**+40** (-20%)**	+41	+41	+42	**+35** (-15%)**
	29-36	+71	+67	**+64** (-10%)**	**+60** (-15%)**	+53	+54	+56	+53
	36-43	+67	+67	+66	+69	+37	**+41* (+11%)**	**+42* (+14%)**	**+49** (+32%)**
	43-50	+65	+63	**+60* (-8%)**	**+58* (-11%)**	+24	+26	**+31** (+29%)**	**+31** (+29%)**
	50-57	+60	+60	+58	+57	+19	+20	**+25** (+32%)**	**+25** (+32%)**
	57-64	+45	+45	+43	+48	+14	+16	+13	**+19* (+36%)**
	64-71	+37	+35	+37	+31	+13	+15	+17	+19
	71-78	+29	+28	+29	+27	+12	+11	+11	+11
	78-85	+16	+24	+22	+21	+10	+10	+9	+11
	22-85	+439	+437	+426	+410	+222	+234	**+245** (+10%)**	**+252** (+14%)**

*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

In C1B, there were no effects on body weight, body weight gain and food consumption in males or females.

Finally, C2A males showed a decrease in body weight and body weight gain at the two highest doses, considered adverse (Table 19).

Whereas in C2A females, body weight tended to increase at all doses from PND 50, only achieving statistical significance at the end of treatment at 75 and 500 mg/kg bw/d. Body weight gain increased over the whole post-weaning period at 225 and 500 mg/kg bw/d (+11% and +13%, respectively), mainly due to a higher

change during PND 22-50, with a tendency towards a return to control values thereafter (Table 19). Due to the reversibility of the change, this finding was considered to be non-adverse.

Neither males nor females showed changes in food consumption.

Table 19. Body weight and body weight gain of C2A animals (Anonymous, 2021).

		Males				Females			
	PND	**Ctrl**	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
Body weight (g)	**22**	64	67	59	**56* (-13%)**	60	63	57	59
	29	114	117	**104* (-9%)**	**93** (-18%)**	104	104	**97* (-7%)**	**93** (-11%)**
	36	183	187	**167** (-9%)**	**153** (-16%)**	160	157	150	145
	43	250	256	**231* (-8%)**	**217** (-13%)**	195	197	193	193
	50	315	319	**288** (-9%)**	**271** (-14%)**	220	226	225	227
	57	373	374	**342** (-8%)**	**325** (-13%)**	239	245	245	253
	64	416	424	**382** (-8%)**	**362** (-13%)**	250	259	261	270
	71	455	459	**412** (-9%)**	**388** (-15%)**	262	**277* (+6%)**	280	**286** (+9%)**
Body weight gain (g)	**22-29**	+51	+49	**+45* (-12%)**	**+38** (-25%)**	+44	+41	+40	**+34** (-23%)**
	29-36	+70	+70	**+63* (-10%)**	**+60** (-14%)**	+55	+53	+53	+52
	36-43	+67	+70	+64	+64	+36	+41	**+43* (+19%)**	**+48** (+33%)**
	43-50	+66	+63	**+57* (-14%)**	**+53** (-20%)**	+25	+28	+32	**+35* (+40%)**
	50-57	+58	+55	+55	+54	+18	+20	+20	+27
	57-64	+43	+51	+40	+37	+12	+13	+16	+17
	64-71	+38	+35	**+30* (-21%)**	**+26** (-32%)**	+11	+19	+19	+17
	22-71	+391	+392	**+353** (-10%)**	**+332** (-15%)**	+201	+214	**+223* (+11%)**	**+228** (+13%)**

*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

Concerning haematological parameters, a statistically significant increase in % reticulocytes was recorded in P0 males at mid- and high-dose (+26% and +40%, respectively), and in C1A females at all doses (+24%, +21% and +32% at low-, mid- and high-dose, respectively), within the range of historical control data (HCD), considered non-adverse. Other statistically significant differences were noted with no dose level relationship, minimal magnitude, in one sex only and/or within the range of HCD.

As regards clinical chemistry, there were statistically significant increases in bile acids at 225 mg/kg bw/d and 500 mg/kg bw/d in P0 and C1A females (in P0, +140% and +156%, respectively; in C1A, +101% and +116%, respectively) and at high-dose also in P0 males (+96%). According to the registration dossier, the

amplitude of the changes was moderate and the increases were not considered adverse in the absence of associated pathology findings. Other statistically significant differences were noted with no dose level relationship, minimal magnitude, in one sex only and/or within the range of HCD.

Sexual maturation

There were statistically significant delays in the mean age at BPS in C1A and C2A males at 500 mg/kg bw/d (2.6-day and 6-day delay, respectively) (Table 20). In females, non-statistically significant delays in the VO were recorded in all cohorts at the highest doses tested (3.2-day, 2.9-day and 2.4-day delays in C1A, C1B and C2A, respectively). These delays were considered non-adverse due to the minimal magnitude of the changes. However, the DS notes that the delays in the mean age at BPS and VO were consistently observed in C1A and C2A at 500 mg/kg bw/d. No significant changes in body weight at sexual maturation were reported in males or females.

Table 20. Sexual maturation in C1A, C1B and C2A animals (Anonymous, 2021).

	C1A				**C1B†**			**C2A**			
	Males										
	Ctrl	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**Ctrl**	**LD**	**MD**	**HD**
Bw (g)	301	285	283	289	295	285	292	296	301	277	295
BPS (PND)	48.4	47.0	48.3	**51.0****	49.9	48.4	49.3	47.4	47.8	48.7	**53.4***
	Females										
Bw (g)	148	154	150	155	138	140	152	151	152	147	156
VO (PND)	35.4	35.9	35.6	38.6	34.5	34.8	37.4	34.9	35.7	35.7	37.3

† In C1B, not enough pups to constitute the HD. */** Differences with control at p≤ 0.05/0.01.

It should be highlighted that both landmarks were not statistically analysed by combining the results of all F1 pups. Combining the results of all F1 pups (Table 21), statistically significant delays were noted at 500 mg/kg bw/d on the day of achievement of both BPS and VO (3-day and 3.5-day delays, respectively). The DS concludes that these findings are adverse effects.

Table 21. Sexual maturation of all F1 animals combined (Anonymous, 2021).

	F1			
	Males			
	Ctrl	**LD**	**MD**	**HD**
Bw (g)	297	287	286	291
BPS (PND)	48.8	47.7	48.8	**51.8****
	Females			
Bw (g)	144	148	151	155
VO (PND)	34.9	35.4	36.3	**38.4***

*/** Differences with control at p≤ 0.05/0.01.

Organ weights and histopathology

Statistically significant increases in the absolute and relative liver and kidneys weights were consistently reported in all cohorts and sexes (Table 22).

In the case of liver, this was correlated with an increase in the incidence of hepatocellular hypertrophy in P0 and C1A males and females at all doses (Table 23). According to the registration dossier, the hepatocellular hypertrophy was considered to be secondary to the induction of hepatic microsomal enzymes and, given the low magnitude of the effect and in the absence of a degenerative associated process, it was not considered as adverse but rather an adaptive change. However, since liver changes were consistently observed in both sexes and generations together with histopathological correlation, the DS considers these findings as treatment-related and adverse.

Microscopic changes were also reported in kidneys (tubular basophilia, accumulation of hyaline droplets, hyaline casts, and pelvic dilatation) in P0 and C1A males at all doses (Table 23). Even though, the changes in kidneys were consistently observed in males, α2μ-globulin in male rats was confirmed by immunohistochemistry in an OECD TG 408 (Anonymous, 2016b). Therefore, the DS considers these findings adverse but not toxicologically relevant.

Increased absolute and relative spleen weights were recorded in C1A animals from 225 mg/kg bw/d (Table 22). Brown pigment was seen in P0 animals at all doses and in C1A animals at 500 mg/kg bw/d (Table 23). According to the registration dossier, this pigment was suggestive of hemosiderin, and due to the low severity and nature of this change, it was considered to be non-adverse. However, the DS considers the spleen alterations consistently observed in P0 and C1A as adverse effects. Furthermore, it should be noted that there were also adverse changes in the absolute splenic lymphocyte population in C1A males (see section 10.10.4).

There was also a higher incidence of thyroid follicular cell hypertrophy in P0 and C1A males at the highest dose (5/24 and 5/10, respectively) (Table 23). According to the registration dossier, this microscopic finding was a known secondary effect of the hepatocellular hypertrophy observed, and both findings were considered as non-adverse effects. Instead, the DS considers the follicular cell hypertrophy observed in both cohorts, with a higher incidence in C1A, as an adverse effect.

Table 22. Organ weights data of P0, C1A, C1B and C2A animals (Anonymous, 2021).

			Terminal bw (g)	Liver		Kidneys		Spleen	
				abs wt	rel wt	abs wt	rel wt	abs wt	rel wt
P0	males	Ctrl	662	16.6	2.5	3.3	0.49	1.44	0.22
		LD	638	17.2	2.7	3.5	**0.54** (+10%)**	1.41	0.22
		MD	631	**19.5** (+17%)*	**3.1** (+24%)**	**3.7** (+13%)**	**0.58** (+18%)**	1.45	0.23
		HD	623	**21.7** (+31%)**	**3.5** (+40%)**	**4.0** (+23%)**	**0.64** (+31%)**	1.44	0.23
	females	Ctrl	334	12.0	3.6	2.2	0.66	1.05	0.31
		LD	340	12.5	3.7	**2.4** (+7%)*	**0.69** (+5%)*	1.13	0.33
		MD	341	**13.7** (+15%)*	**4.0** (+12%)**	**2.4** (+7%)*	**0.69** (+5%)*	1.15	0.34
		HD	337	**14.0** (+17%)**	**4.2** (+16%)**	2.3 (+5%)	0.69 (+4%)	1.11	0.33
C1A	males	Ctrl	485	11.5	2.4	2.9	0.60	1.33	0.27
		LD	486	12.5	2.6	**3.1** (+8%)*	**0.64** (+8%)**	1.46	0.30
		MD	470	**14.0** (+21%)**	**3.0** (+26%)**	**3.1** (+8%)*	**0.66** (+11%)**	**1.54** (+16%)*	**0.33** (+20%)**

			Terminal bw (g)	Liver		Kidneys		Spleen	
				abs wt	rel wt	abs wt	rel wt	abs wt	rel wt
		HD	**441* (-9%)**	**13.3** (+15%)**	**3.0** (+28%)**	2.9 (+1%)	**0.66** (+11%)**	**1.63* (+23%)**	**0.37** (+35%)**
	females	**Ctrl**	266	6.4	2.4	1.7	0.62	0.93	0.35
		LD	280	**7.2* (+13%)**	**2.6** (+7%)**	**1.9** (+13%)**	**0.67** (+7%)**	1.05	0.37
		MD	**286* (+8%)**	**8.1** (+27%)**	**2.8** (+18%)**	**1.9** (+15%)**	**0.66* (+6%)**	**1.15** (+24%)**	**0.40** (+15%)**
		HD	**287* (+8%)**	**8.8** (+39%)**	**3.1** (+28%)**	**1.9** (+12%)**	0.65 (+4%)	**1.14** (+23%)**	**0.40* (+15%)**
C1B†	**males**	**Ctrl**	503	12.7	2.5	2.9	0.57	-	-
		LD	507	13.2	2.6	**3.1** (+8%)**	**0.61** (+8%)**	-	-
		MD	510	**15.5** (+22%)**	**3.0** (+20%)**	**3.3** (+14%)**	**0.64** (+13%)**	-	-
	females	**Ctrl**	278	6.7	2.4	1.7	0.62	-	-
		LD	281	7.4	**2.6* (+10%)**	1.8	0.65	-	-
		MD	286	**8.1** (+21%)**	**2.8** (+18%)**	**1.9** (+8%)**	**0.65* (+5%)**	-	-
C2A	**males**	**Ctrl**	443	14.4	3.3	3.4	0.77	1.28	0.29
		LD	456	15.8	3.5	3.8	0.84	1.29	0.28
		MD	**407** (-8%)**	15.4	**3.8** (+16%)**	3.4	0.83	1.21	0.30
		HD	**383** (-13%)**	16.3	**4.3** (+31%)**	3.4	**0.89* (+15%)**	1.21	0.31
	females	**Ctrl**	256	8.3	3.2	2.0	0.78	1.00	0.38
		LD	271	**10.3** (+23%)**	**3.8** (+17%)**	**2.4* (+20%)**	**0.89* (+14%)**	1.07	0.40
		MD	271	**11.1** (+33%)**	**4.1** (+27%)**	**2.5** (+23%)**	**0.91** (+16%)**	1.12	0.41
		HD	279	**10.8** (+30%)**	**3.9* (+19%)**	**2.4* (+18%)**	0.85 (+9%)	1.02	0.37
C2B†	**males**	**Ctrl**	63.36	2.53	3.99	0.63	1.00	0.40	0.63
		LD	63.32	2.57	4.05	0.65	1.03	0.40	0.63
		MD	60.77	2.44	4.01	0.62	1.02	0.43	0.70
	females	**Ctrl**	58.30	2.24	3.84	0.61	1.04	0.39	0.67
		LD	61.26	2.42	3.95	0.67	1.09	0.42	0.68
		MD	59.07	2.40	4.05	0.62	1.05	0.41	0.69

Absolute weight expressed in grams. Relative weight: g/100 g final bw. † In C1B and C2B, not enough pups to constitute the HD.
*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

Table 23. Microscopic findings in P0 and C1A animals (Anonymous, 2021).

				Thyroid	Liver	Kidneys				Spleen
			Nb animals	FCH	Heph	Hyad	Tubb	Hyac	Peld	Pigm
P0	males	Ctrl	24	0	0	0	4	2	0	0
		LD	24	0	4	2	8	2	3	3
		MD	24	0	14	7	10	7	1	5
		HD	24	5	24	23	20	13	8	15
	females	Ctrl	23	0	0	n.a.	1	0	0	0
		LD	22	0	2	n.a.	0	0	0	2
		MD	19	0	6	n.a.	n.a.	n.a.	n.a.	2
		HD	12	1	7	n.a.	0	1	1	9
C1A	males	Ctrl	20	0	0	0	2	0	6	0
		LD	20	0	2	14	3	0	3	0
		MD	20	0	13	14	10	1	3	0
		HD	10	5	8	10	8	0	6	4
	females	Ctrl	20	n.a.	0	n.a.	n.a.	2	4	0
		LD	19	n.a.	1	n.a.	n.a.	n.a.	n.a.	0
		MD	20	n.a.	2	n.a.	n.a.	0	1	0
		HD	19	n.a.	14	n.a.	n.a.	0	0	9

FCH: follicular cell hypertrophy. Heph: hepatocellular hypertrophy. Hyad: hyaline droplet. Tubb: tubular basophilia. Hyac: hyaline casts. Peld: pelvis dilation. Pigm: pigment. n.a.: not applicable.

According to the registration dossier, the NOAEL for systemic toxicity (excluding reproductive and developmental toxicity) was considered to be 75 mg/kg bw/d in P0 males, based on the adverse clinical signs observed from 225 mg/kg bw/d, and lower than 75 mg/kg bw/d in P0 females, based on difficulties to deliver from this dose level. Given the absence of adverse effects on reproductive parameters, the NOAEL for reproductive/developmental toxicity was set at 500 mg/kg bw/d in P0, C1A, and C1B animals.

Otherwise, the DS concludes that the NOAEL for parental general toxicity in both sexes should be established at 75 mg/kg bw/d, based on the clinical signs and liver effects observed. For F1 males and females, the NOAEL should be also set at 75 mg/kg bw/d, based on clinical signs, body weight changes and liver effects. On the other hand, due to the lack of adverse effects, the NOAEL for sexual function and fertility in P0 males was 500 mg/kg bw/d. However, since clear evidence of dystocia was observed in all treated groups, the DS established a LOAEL for sexual function and fertility in P0 females at 75 mg/kg bw/d. Moreover, the NOAEL for sexual function and fertility in F1 should be set at 225 mg/kg bw/d, based on the delay in sexual maturation.

Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Kapp *et al*., 2003)

In a GLP compliant OECD TG 422 study, Sprague-Dawley rats were given 0, 75, 225 and 750 mg acetophenone/kg bw/d by gavage administration. The short-term repeated dose study was performed with ten males and five females per group, and for the reproductive toxicity part, ten additional females were used.

Sexual function and fertility

In the reproductive part, one female in the control group was sacrificed with no evidence of mating. Some parental females were sacrificed on post-breeding day 25 with evidence of mating but failing to deliver (two, one and one females, in the control, low- and high-dose groups, respectively). In addition, six dams were euthanised between LD 1-4 due to total litter loss at the highest dose (refer to section 10.10.4. for more details).

At 750 mg/kg bw/d, one female showed prolonged parturition.

No other effects on reproductive parameters such as corpora lutea counts, implantation sites, and mating, gestation and fertility indices were described. Sperm measures and oestrus cyclicity were not examined.

General toxicity

As mentioned before, in the reproductive part, 11 females were sacrificed. In the short-term repeated dose part, all males and females survived after treatment.

Parental animals showed salivation from 225 mg/kg bw/d, and wobbly gait at 750 mg/kg bw/d. Urine stain was recorded in males at the highest dose and in females at all doses. Other clinical signs were observed in individual females at 750 mg/kg bw/d, such as unkempt appearance, skin pale in color, rough coat, decreased activity, dark material around nose or few faeces (Table 24). Effects observed at the high dose were considered to be of toxicological significance due to incidence or type of effect as e.g. wobbly gait indicating central nevous system depression (see also section 10.11).

Table 24. Clinical signs of parental animals (Kapp *et al.*, 2003).

	Males				Females			
	Ctrl	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
Nb of animals	10	10	10	10	10	10	10	10
Urine stain				2		2	3	6
Unkempt appearance								1
Skin pale in color								1
Rough coat								2
Decreased activity								1
Dark material around nose					1		1	3
Wobbly gait				10				10
Salivation			9	10			7	10
Few faeces								4

Nb of animals affected per group.

Few statistically significant changes in body weight gain and food consumption were observed. In males, there was statistically significant body weight loss accompanied by significant decreases of food consumption during days 0-3 of the premating period at the highest dose (Table 25). In females, non-significant decreases of body weight gain accompanied by significant decreases of food consumption during days 0-3 of the premating period were observed from 225 mg/kg bw/d. In addition, a significant decrease in body weight gain was observed at 750 mg/kg bw/d during GD 0-7 (-39%) and returned to control values thereafter (Table 26). The effects on body weight gain were considered to be of no toxicological significance.

Table 25. Body weight gain and food consumption of P0 males (Kapp *et al.*, 2003).

	Days	Ctrl	LD	MD	HD
Body weight gain (g)	**0-3**	2	-1	-4	**-21****
	3-7	7	9	11	10
	12-16	-22	-26	-23	-19
Food consumption (g/animal/d)	**0-3**	22	22	22	**13**** **(-41%)**
	3-7	23	24	25	23
	12-16	27	27	25	22

** Differences with control at p≤ 0.01. % in comparison to control group.

Table 26. Body weight gain and food consumption of P0 females (Kapp *et al.*, 2003).

		Days	Ctrl	LD	MD	HD
Body weight gain (g)	**Prior mating**	**0-3**	-3	-1	-6	-10
		3-7	4	3	1	6
		7-12	13	7	10	9
	Gestation	**0-7**	36	36	33	**22* (-39%)**
		7-14	23	30	31	27
		14-20	55	67	62	54
	Lactation	**1-4**	12	17	14	8
Food consumption (g/animal/d)	**Prior mating**	**0-3**	16	15	**14* (-13%)**	**9**** **(-44%)**
		3-7	18	17	17	17
		7-12	18	16	16	17
	Gestation	**0-7**	22	23	22	19
		7-14	20	24	24	22
		14-20	22	23	22	22
	Lactation	**1-4**	33	35	37	25

*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

Regarding haematological parameters, males showed statistically significant increases in mean corpuscular volume at 750 mg/kg bw/d. In turn, females showed decreases in erythrocytes and haematocrit at 750 mg/kg bw/d. According to the registration dossier, these findings did not follow a consistent pattern, and the mean values remained within the range of HCD.

Concerning the clinical chemistry, there were statistically significant changes in several parameters, such as the levels of total protein and albumin from 225 mg/kg bw/d in males and females or the levels of sodium, potassium, or globulin at 750 mg/kg bw/d. Changes in total protein, albumin and globulin were related to the increased liver weight. Most of the values were within the range of HCD, whereby the toxicological significance of the changes was considered questionable.

Organ weights were recorded in the short-term repeated dose part in both sexes but not in the females of the reproductive part. In males, there were statistically significant decreases in the absolute weight of heart and epididymides and a statistically significant increase in the relative liver weight at 750 mg/kg bw/d. In unmated females, there were increases in the liver and kidney relative weights and in the absolute liver

weight from 225 mg/kg bw/d (Table 27). The changes in heart and epididymides weights were not considered relevant due to the lack of histopathological correlation.

Microscopic alterations were noted in liver (vacuolar changes in hepatocytes in males and unmated females at 750 mg/kg bw/d) and kidneys (minimal to mild hyaline droplet formation sometimes accompanied by minimal tubular epithelial degeneration and regeneration in males at all doses). According to the registration dossier, liver changes were interpreted as a background lesion based on the nature and distribution of the changes. However, since similar effects were reported in the EOGRTS and the sub-chronic study, the DS considers liver changes as adverse effects. Kidneys findings were not considered toxicologically significant for humans.

Table 27. Organ weights data of P0 animals (Kapp *et al.*, 2003).

		Males				Females			
		Ctrl	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
Heart	**abs wt**	1.49	1.44	1.42	**1.31** (-12%)**				
	rel wt	0.306	0.294	0.299	0.296				
Liver	**abs wt**	17.20	17.00	18.09	19.84	7.40	7.69	**9.12* (+23%)**	**10.63** (+44%)**
	rel wt	3.470	3.487	3.779	**4.457** (+28%)**	2.784	2.884	**3.345** (+20%)**	**4.006** (+44%)**
Kidneys	**abs wt**					1.82	2.08	2.12	2.08
	rel wt					0.687	0.779	**0.779* (+13%)**	**0.784* (+14%)**
Epididymides	**abs wt**	1.36	1.31	1.29	**1.24* (-9%)**				
	rel wt	0.280	0.268	0.272	0.283				

Absolute weight expressed in grams. Relative weight: g/100 g final bw.
*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

Functional observational battery

In the FOB, males showed statistically significant decreases in the forelimb grip strength and mean motor activity at the highest dose (Table 28). Both decreases were considered toxicologically meaningful findings. No relevant effects were reported on unmated females.

Table 28. Functional observational battery data of P0 males (Kapp *et al.*, 2003).

	Ctrl	**LD**	**MD**	**HD**
Forelimb grip strength	1.880	1.817	1.684	**1.572* (-16%)**
Motor activity	2796.4	3222.4	2675.6	**1632.8* (-42%)**

No unit of measurement was indicated for the forelimb grip strength. Motor activity was indicated by the total number of squares entered during the 1-h test interval.
* Differences with control at p≤ 0.05. % in comparison to control group.

According to the registration dossier, in the reproductive part, the NOAEL for maternal toxicity was established at 225 mg/kg bw/d, based on clinical signs and reduced body weight gain during GD 0-7

recorded at 750 mg/kg bw/d. The NOAEL for reproductive toxicity was set at 750 mg/kg bw/d, since reproductive performance was not affected. In the short-term repeated dose part, the NOAEL was 225 mg/kg bw/day, based on clinical signs and neurobehavioural effects.

However, the DS considers that the NOAEL for sexual function and fertility in females should be set at 225 mg/kg bw/d, based on the prolonged parturition registered in one female at the highest dose. Since males did not show adverse reproductive effects, the NOAEL for sexual function and fertility in males is considered to be 750 mg/kg bw/d.

10.10.3 Comparison with the CLP criteria

According to the CLP, the criteria for classifying substances with regard to adverse effects on sexual function and fertility can be allocated in one of two categories.

Substances are classified in Category 1 for reproductive toxicity (known or presumed human reproductive toxicant) when they are known to have produced an adverse effect on sexual function and fertility 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 data (Category 1A) or from animal data (Category 1B).

Substances are classified in Category 2 for reproductive toxicity (suspected human reproductive toxicant) when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

There are no human data to support the classification of acetophenone in Category 1A.

Instead, sexual function and fertility was investigated in two animal studies, included in the registration dossier, an extended-one reproductive toxicity study (Anonymous, 2021) and a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Kapp *et al*., 2003).

The EOGRTS reported two dams at 75 mg/kg bw/d and one dam at 225 mg/kg bw/d sacrificed on GD 23-24 due to difficulties to deliver, and three dams at 500 mg/kg bw/d and one at 75 mg/kg bw/d on GD 25-26 for humane grounds or due to no delivery. However, it should be noted that all the abovementioned dams did not deliver and were sacrificed between GD 23-26 with dead foetuses, placentas and/or scars in the uterus at the moment of necropsy. Therefore, the DS considers that all these dams suffered dystocia. In fact, general condition of these females did not worse over the course of pregnancy (i.e. body weight, food consumption or clinical signs). Moreover, adverse effects on weight and histopathology of liver, kidneys and spleen were observed in both sexes in the EOGRTS and in a subchronic study. Thus, the DS concludes that organ toxicity did not trigger the poor condition of dams leading to their sacrifice. Additionally, in the OECD TG 422, one dam was also described with prolonged parturition at 750 mg/kg bw/d. In this screening study, females did not show apparent systemic toxicity either. This finding supports the evidence of dystocia observed in the EOGRT study. Although in this latter study, dystocia did not follow a dose-dependent pattern in the treated groups (three, one and three dams in the low-, mid-, and high-dose groups, respectively), due to the severity of the effect, leading to the sacrifice of seven dams, it should be considered clear evidence of an adverse effect on sexual function and fertility and therefore, relevant for the classification.

It should be noted that the substance does not cause marked systemic toxicity, since no increase in the incidence of mortality, and in the incidence and/or severity of clinical signs or post-mortem findings is observed, neither in males nor in mated and unmated females. In fact, only the dystocia is the trigger of the sacrifices of the females at delivery.

Despite there was a higher number of females sacrificed due to total litter loss between PND 1-4 at 225 and 500 mg/kg bw/d in the OECD TG 443 (three and 11 dams, respectively) and at 750 mg/kg bw/d in the OECD TG 422 (six dams), the DS does not consider this a fertility effect, but a consequence of an adverse

effect on development (refer to section 10.10.4. for more details).

In addition, in the EOGRTS, there was a dose-dependent decrease in the mating index (100%, 100%, 91.7%, and 87.5% in the control, low-, mid- and high-dose groups, respectively) due to the fact that one and three females did not mate at 225 and 500 mg/kg bw/d, respectively. Even though there were no effects on the mating index in the OECD TG 422, it should be noted that the premating period in the screening study was only 14 days, compared to the premating exposure duration of ten weeks in the EOGRTS. Consequently, the DS considers that the decreased mating index in the OECD TG 443 contributes to the classification.

Furthermore, significant delays in sexual maturation in the F1 generation were described in the EOGRTS, in the absence of significant change in mean body weight on the day of occurrence. At 500 mg/kg bw/d, there were statistically significant 2.6-day and 6-day delays in BPS in C1A and C2A males, respectively. Moreover, combining the results of all F1 pups, as recommended to achieve higher statistical power, a significant delay was noted in the mean age at BPS at 500 mg/kg bw/d (3-day delay).

In females, delays in the mean age at VO did not reach statistical significance at the highest doses tested but achieved 3.2 days in C1A at 500 mg/kg bw/d, 2.9 days in C1B at 225 mg/kg bw/d, and 2.4 days in C2A at 500 mg/kg bw/d. Moreover, further analysis combining the results of all F1 pups showed a statistically significant delay in the attainment of VO at 500 mg/kg bw/d (3.5-day delay).

In conclusion, the severity of the dystocia along with the decreased mating index and the delayed sexual maturity, are considered clear evidence of adverse effects on sexual function and fertility and classification as Repr. 1B (H360F) is therefore proposed.

10.10.4 Adverse effects on development

Table 29: 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
EOGRTS with DNT OECD TG 443 Reliability 1 (reliable without restrictions) GLP Rat, Sprague-Dawley P0: 24/sex/group C1A: 20-21/sex/group in ctrl, LD and MD, and 10m/20f at HD C1B: 20/sex/group in ctrl, LD and MD. Not enough pups to constitute the HD C2A: 10/sex/group in ctrl, LD and MD, and 5/sex at HD C2B: 10/sex/group in ctrl, LD and MD. Not enough pups to constitute the HD	Acetophenone Purity: 99.79% Vehicle: 0.5% (w/v) carboxymethylcellulose 400-800cPs / 0.5% (w/v) Tween 80 in drinking water treated by reverse osmosis Oral (gavage) 0, 75, 225, 500 mg/kg bw/d P0 generation: 10 weeks before mating, during mating, until F1 weaning F1 generation: direct dosing from weaning (PND 22) to terminal sacrifice of respective cohorts C1A: until PND 90-93 C1B: until PND 98-100 C2A: until PND 76-78	See section 10.10.1 for a description of the effects on general toxicity and sexual function and fertility. **Developmental toxicity** <u>Offspring mortality / viability (Tables 30-31)</u> ↑ post-implantation losses in treated groups, stat sign at HD (16.1%, 20.3%, 24.1%, 29.8% in ctrl, LD, MD and HD, respectively), associated with ↓ total nb of pups delivered (284, 253, 212, 196 in ctrl, LD, MD and HD, respectively). ↓ nb of liveborn pups, stat sign from MD (total: 274, 237, 167, 75 in ctrl, LD, MD and HD, respectively; mean: 11.9, 11.9, 8.8, 7.5 in ctrl, LD, MD and HD, respectively). ↑ total nb of stillbirths (10, 16, 45, 121 in ctrl, LD, MD and HD, respectively). ↓ live birth index (96.8%, 94.4%, 79.6%, 34.1% in ctrl, LD, MD and HD, respectively). Absence of milk in the stomach, autolysis and/or cannibalism in pups found dead. ↓ mean nb of live pups on PND 4 (11.9, 11.6, 9.2, 5.9 in ctrl, LD, MD and HD, respectively), and ↑	Anonymous, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	C2B: sacrificed on PND 22 (no direct dosing)	nb of dead pups on PND 0-4 (11, 20, 55, 155 in ctrl, LD, MD and HD, respectively), both stat sign from MD. ↓ viability index (96.3%, 92.9%, 77.7%, 38.1% in ctrl, LD, MD and HD, respectively). ↓ mean nb of live pups on PND 21, stat sign at HD (9.4, 9.3, 8.7, 5.9 in ctrl, LD, MD and HD, respectively). <u>Clinical signs (Table 32)</u> On PND 1 and during lactation, F1 pups had more findings at macroscopic examination (qualitative external examination and assessment of body temperature) at MD and HD. <u>Body weight and body weight changes (Table 33)</u> In males, ↓ bw from PND 1 to 21 at HD (from -20% to -23%) and ↓ bw change during PND 1-21 (-24%). On PND 1, ↓ bw at MD in males (-11%). In females, ↓ bw on PND 1 at MD and HD (-9% and -20%, respectively). <u>C2A: Auditory startle response (Table 34)</u> In males, stat sign ↓ magnitude of response in almost all trials at HD. This decrease was also apparent at MD, but not stat sign. <u>C2A: Morphometrics (Table 35)</u> In males, ↓ entire hippocampus thickness at HD; ↓ dentate gyrus thickness and cornu ammonis thickness from LD. <u>C1A: Immunophenotyping (Table 36)</u> In males, ↓ in abs splenocytes, B cells, T cells, helper T cells and cytotoxic T cells in all treated groups and ↓ in abs NKT cells at MD and HD. **According to the registration dossier**: NOAEL developmental toxicity = 500 mg/kg bw/d, based on absence of adverse effects. NOAEL developmental neurotoxicity < 75 mg/kg bw/d, based on lower hippocampus measurements. **According to the DS**: NOAEL developmental toxicity = 75 mg/kg bw/d, based on post-implantation losses and low nb of pups delivered, high nb of stillbirths and dead pups on PND 0-4. LOAEL developmental neurotoxicity = 75 mg/kg	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		bw/d, based on lower hippocampus measurements. LOAEL developmental immunotoxicity = 75 mg/kg bw/d, based on immunophenotyping effects.	
Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test OECD TG 422 Reliability 1 (reliable without restrictions) GLP Rat, Sprague-Dawley 10/sex/group	Acetophenone Purity: 98.80% Vehicle: corn oil Oral (gavage) 0, 75, 225, 750 mg/kg bw/d Daily from 14 days before mating to lactation day 3	See section 10.10.1 for a description of the effects on general toxicity and sexual function and fertility. **Developmental toxicity** <u>Offspring mortality / viability (Table 37)</u> ↑ nb stillborn (2, 6, 4, 30 in ctrl, LD, MD and HD, respectively), and ↓ total nb liveborn pups (105, 136, 145, 109 in ctrl, LD, MD and HD, respectively), both stat sign at HD. ↓ live birth index at HD (non-stat sign, but out of HCD). ↑ pups dying, missing and/or cannibalized during PND 1-4 (6, 5, 8, 79 in ctrl, LD, MD and HD, respectively), ↑ nb litters with total litter loss up to PND 4 (0, 0, 0, 6 in ctrl, LD, MD and HD, respectively), and ↓ nb of live pups/litter on PND 0, 1, 2, 3 and 4, all these effects stat sign at HD. ↓ viability index on PND 4, stat sign at HD (94.3%, 96.3%, 94.5%, 22.9% in ctrl, LD, MD and HD, respectively). <u>Clinical signs (Table 38)</u> ↑ incidence of desquamation, cool to touch, changes in skin appearance, and gasping and skin pale in color at HD. Desquamation also at MD. <u>Body weight (Table 37)</u> ↓ pup bw/litter on PND 1 and 4, stat sign at HD, both days out of HCD. <u>Gross pathology (Table 39)</u> In pups with scheduled euthanasia on PND 4: ↑ incidence of scabbing and desquamation at MD and HD. In stillborn pups: single incidence each of cleft palate and edema, ↑ incidence of atelectasis, milk not present in the stomach and dermal hypoplasia at HD. Atelectasis and milk not present in the stomach also at LD and MD. In died pups: ↑ incidence of milk not present in the stomach, dermal hypoplasia, scabbing and desquamation, and 22 pups with autolysis at HD.	Kapp *et al.*, 2003

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		NOAEL developmental toxicity = 225 mg/kg bw/d, based on pup viability, pup weight, external observations, and gross necropsy.	
Prenatal developmental toxicity study OECD TG 414 Reliability 1 (reliable without restrictions) GLP Rat, Wistar 35 females in ctrl and HD 25 females in LD and MD	Acetophenone Purity: 99.79% Vehicle: corn oil Oral (gavage) 0, 125, 300, 750 mg/kg bw/d Daily from GD 5 to GD 19	**Maternal toxicity** <u>Clinical signs</u> Salivation, transiently reduced spontaneous activity associated with half eyelid closure and prone position, ataxia and piloerection on several occasions, and fully closed eyes and apathy on few days of treatment, at HD and/or MD. <u>Body weight, body weight gain (Table 40) and food consumption</u> At MD and HD, ↓ bw from GD 8 to 20 (up to -8% and -12%, respectively). At MD and HD, ↓ bw gain (-23% and -37%, respectively) and food consumption (-13% and -23%, respectively) between GD 0-20. <u>Organ weight (Table 41)</u> At MD and HD, ↓ uterus weight (-14% and -25%, respectively), and ↓ mean adjusted maternal weight (-7% and -9%, respectively). NOAEL maternal toxicity = 125 mg/kg bw/d, based on clinical signs, bw and bw gain, food consumption and organ weights. **Developmental toxicity** <u>Prenatal data (Table 42)</u> ↓ mean nb of live foetuses at HD (no stat sign). ↓ mean nb of implantation sites, ↑ % early resorptions and % post-implantation loss at HD (no stat sign). <u>Foetal and litter weight (Table 43)</u> ↓ foetal weight at MD and HD (-6% and -15%, respectively), and ↓ total litter weight at HD (-23%). <u>External malformations</u> Agnathia was observed in one single foetus in ctrl and HD. No skeletal correspondence in the ctrl foetus, whereas the mandible was short in the HD foetus (micrognathia). <u>Skeletal malformations (Table 44)</u>	Anonymous, 2016a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↑ incidence per litter of bilateral pelvic girdle caudal shift from MD, stat sign and above HCD at HD. ↑ litter and foetal incidence of 14th full bilateral rib at HD, no stat sign but above HCD. NOAEL developmental toxicity = 125 mg/kg bw/d, based on foetal bw changes and skeletal malformations.	
Prenatal developmental toxicity study OECD TG 414 Reliability 1 (reliable without restrictions) GLP Rabbit, New Zealand White 22 females/group	Acetophenone Purity: 99.79% Vehicle: 0.5% (w/v) carboxymethylcellulose 400-800cPs/0.5% (w/v) Tween® 80 in purified water Oral (gavage) 0, 60, 170, 500 mg/kg bw/d Daily from GD 6 to GD 28	**Maternal toxicity** <u>Mortality</u> At HD, two females aborted (9%, above 2% in the HCD), and a third female was found dead, with red discharge in the cage. <u>Clinical signs</u> Transient clinical signs such as decreased activity, abnormal gait, laboured and shallow breathing, lying on the side and subdued behaviour at HD. Higher frequency of few faeces from MD. <u>Body weight, body weitght gain and food consumption</u> At HD, bw loss between GD 6-9, but non-stat sign differences in bw and bw gain between GD 6-29. At LD and MD, transient low bw gain or bw loss between GD 18-21 and GD 15-21, respectively. ↓ food consumption between GD 6-21 at MD and between GD 6-24 at HD. NOAEL maternal toxicity = 170 mg/kg bw/d, based on clinical signs, bw and bw gain, food consumption, mortality and nb of abortions. **Developmental toxicity** <u>Prenatal data (Table 45)</u> ↑ nb of late resorption (out of HCD) and total nb of resorption at HD (both non-stat sign). Consequently, non-stat sign ↑ % post-implantation loss at HD (11.13% vs 9.20% in ctrl). <u>Foetal weight (Table 46)</u> ↓ foetal weight at MD and HD (-7% and -9%, respectively), stat sign and below HCD at HD.	Anonymous, 2020

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		NOAEL developmental toxicity = 170 mg/kg bw/d, based on foetal bw changes.	

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

The developmental toxicity of acetophenone was investigated in four experimental animal studies, an extended one-generation reproductive toxicity study, a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, and two prenatal developmental toxicity studies.

Extended-one generation reproductive toxicity study (Anonymous, 2021)

In the EOGRTS, conducted in accordance with OECD TG 443, acetophenone (purity of 99.79%) was administered by gavage to Sprague-Dawley rats at concentrations of 0, 75, 225 and 500 mg/kg bw/d (see section 10.10.1 for a description of the effects on sexual function and fertility and general toxicity).

Developmental toxicity

There was a dose-dependent increase in the post-implantation losses in the treated groups, statistically significant at 500 mg/kg bw/d (16.1%, 20.3%, 24.1%, 29.8% in the control, low-, mid- and high-dose groups, respectively). This effect was associated with a dose-dependent decrease in the total number of pups delivered (284, 253, 212, 196 in the control, low-, mid- and high-dose groups, respectively). Moreover, from 225 mg/kg bw/d, there was also a statistically significant decrease in the number of liveborn pups (total: 274, 237, 167, 75; mean: 11.9, 11.9, 8.8, 7.5 in the control, low-, mid- and high-dose groups, respectively) and a high total number of stillbirths (10, 16, 45, 121 in the control, low-, mid- and high-dose groups, respectively). Consequently, there was also a decrease in the live birth index (96.8%, 94.4%, 79.6%, 34.1% in the control, low-, mid- and high-dose groups, respectively) (Table 30). At necropsy of pups found dead, absence of milk in the stomach, autolysis and/or cannibalism were noted (Table 31).

In addition, there was a statistically significant increase in the number of dead pups on PND 0-4 (11, 20, 55, 155 in the control, low-, mid- and high-dose groups, respectively), and a statistically significant reduction in the mean number of live pups on PND 4 (11.9, 11.6, 9.2, 5.9 in the control, low-, mid- and high-dose groups, respectively). As a result, there was a low viability index on PND 4 (before culling) at 225 and 500 mg/kg bw/d (96.3%, 92.9%, 77.7%, 38.1% in the control, low-, mid- and high-dose groups, respectively) (Table 30).

It should be noted that these findings were observed in the absence of maternal systemic toxicity since no increase in the incidence of mortality and in the incidence and/or severity of clinical signs or post-mortem findings were observed in dams. In addition, there were no statistically significant differences in corrected maternal body weight gain between the control and treated groups. In fact, mortality was triggered during the gestation period due to a fertility effect, i.e. dystocia (please refer to section 10.10.2 for more details). Moreover, dams were sacrificed in the lactation period due to total litter loss between PND 1-4, as a consequence of an adverse effect on development at the two highest doses.

Likewise, there was also a statistically significant decrease in the mean number of live pups on PND 21 at 500 mg/kg bw/d (9.4, 9.3, 8.7, 5.9 in the control, low-, mid- and high-dose groups, respectively). However, after culling on PND 4, the lactation index was not affected. According to the registration dossier, the

decreased number of live pups on PND 1 and live birth indices were consequences of the increases in the post-implantation losses and were considered treatment-related and adverse.

Table 30. Offspring survival indices (Anonymous, 2021).

	Ctrl	LD	MD	HD
Implantations (total/mean)	338 / 14.7	318 / 15.9	283 / 14.1	282 / 15.7
Post-implantation loss %	16.1	20.3	24.1	**29.8* (+85%)**
Number of litters	23	20	20	18
Pups delivered (total)	284	253	212	196
Liveborn pups (total)	274	237	167	75
(mean)	11.9	11.9	**8.8** (-26%)**	**7.5** (-37%)**
Stillbirths (total)	10	16	45	121
Live birth index %	96.8	94.4 (-2%)	79.6 (-18%)	34.1 (-65%)
Live pups on PND 4 / litter (mean)	11.9	11.6	**9.2** (-23%)**	**5.9** (-50%)**
Died and/or cannibalized PND 0-4 (total)	11	20	**55****	**155****
Dead pups on PND 1-2 (total)	1	3	9	30
Dead pups on PND 2-3 (total)	0	0	1	3
Dead pups on PND 3-4 (total)	0	1	0	1
Viability index %	96.3	92.9 (-4%)	77.7 (-19%)	38.1 (-60%)
Live pups on PND 21 / litter (mean)	9.4	9.3	8.7	**5.9** (-37%)**

*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

Table 31. Macroscopic post-mortem observations in found dead F1 pups (Anonymous, 2021).

	Ctrl	LD	MD	HD
Nb of litters examined	5	8	8	17
Nb of found dead pups	8	16	42	132
Absence of milk in stomach (P/L)	3/2	6/5	12/5	34/7
Autolysis (P/L)	4/2	9/5	23/6	79/15
Partial cannibalism	0/0	0/0	1/1	3/2
Litters affected, Nb (%)	4 (80.0)	7 (87.5)	8 (100)	17 (100)
Pups affected, Nb (%)	7 (87.5)	15 (93.8)	36 (85.7)	115 (87.1)

P/L: Number of pups/Number of litters affected.

As already mentioned, due to the low birth index given by the high rate of stillborns along with the fertility

effects (refer to section 10.10.2 for more details), there were insufficient numbers of surviving pups at 500 mg/kg bw/d. Consequently, the test laboratory decided, in agreement with the Sponsor, to give precedent to C1A and C2A groups. Despite this, the high-dose group in C1A contained only ten males, and in C2A there were only five animals per sex and group. In the case of C1B and C2B, there were not enough pups to constitute the high-dose group.

On the other hand, on PND 1, F1 pups had more findings at macroscopic examination (qualitative external examination and assessment of body temperature) from 225 mg/kg bw/d. In addition, during lactation, there was a high percentage of pups with external findings such as malformed, short tail and/or hematoma, desquamation, scab, and some pups were observed cold to touch, dehydrated, or with hypoactivity and/or thin appearance (Table 32). According to the registration dossier, these findings were likely to represent nursing difficulties or lack of maternal care, and were considered treatment-related and adverse. However, the DS notes that most of the reported deaths and clinical signs in pups occurred at birth and between PND 1-2. Moreover, clinical signs of dams did not worse during gestation and/or after delivery. Therefore, the DS does not consider that the high number of dead pups was due to lack of maternal care.

Table 32. Findings and clinical signs in F1 pups on PND 1 and during lactation (Anonymous, 2021).

	Findings in F1 pups on PND 1			
	Ctrl	**LD**	**MD**	**HD**
Nb of litters examined	23	20	19	10
Nb of pups examined	274	237	167	75
External examination (P/L)	8/7	23/7	61/10	49/7
Assessment of body temperature (P/L)	3/3		3/1	16/1
	Clinical signs in F1 pups during lactation			
Malformed / shortened tail (P/L)			1/1	2/1
Cold to touch (P/L)	3/3		7/3	24/2
Dehydration (P/L)	1/1			1/1
Thin appearance (P/L)				1/1
Hematoma/desquamation/scab (back, abdomen, head, hindlimb, tail)	3/3	50/9	158/8	45/9
Hypoactivity				1/1

P/L: Number of pups/Number of litters affected.

At 500 mg/kg bw/d, there was a decreased body weight in male F1 pups from PND 1 to 21 (from -20% to -23%) and a decreased body weight gain during PND 1-21 (-24%). On PND 1, a decrease in body weight was also observed at 225 mg/kg bw/d in males (-11%) and from 225 mg/kg bw/d in females (-9% and -20% at 225 mg/kg bw/d and 500 mg/kg bw/d, respectively), with a return to control values afterwards (Table 33).

Table 33. F1 pup body weights and body weight changes (Anonymous, 2021).

			Ctrl	**LD**	**MD**	**HD**
Males	**Body weight** (g)	**PND 1**	8.0	7.8	**7.1** ** **(-11%)**	**6.4** ** **(-20%)**
		PND 4	11.5	11.3	11.3	**9.2** ** **(-20%)**

			Ctrl	LD	MD	HD
		PND 7	17.7	17.7	17.2	**13.9** (-21%)**
		PND 14	35.3	36.1	33.9	**27.3** (-23%)**
		PND 21	58.2	60.0	56.0	**44.7** (-23%)**
	Body weight change (g)	**PND 1-21**	+50.2	+52.2	+48.7	**+38.2# (-24%)**
Females		**PND 1**	7.5	7.3	**6.8** (-9%)**	**6.0** (-20%)**
		PND 4	11.0	10.9	10.8	10.1 (-8%)
	Body weight (g)	**PND 7**	16.8	17.2	16.6	15.9 (-5%)
		PND 14	33.9	35.4	32.8	31.8 (-6%)
		PND 21	56.0	58.2	54.5	52.0 (-7%)
	Body weight change (g)	**PND 1-21**	+48.5	+50.9	+47.6	+45.7

**/# Differences with control at p≤ 0.01/0.001. % in comparison to control group.

Developmental neurotoxicity

Regarding the developmental neurotoxicity, statistically significant decreases in the magnitude of response in the auditory startle test were recorded in C2A males in trial 1 to 10 (-32%), 11 to 20 (-35%), 31 to 40 (-42%) and 41 to 50 (-45%) at 500 mg/kg bw/d (Table 34). No effects were observed in females as well as no variations in the latency of response were reported. These results were considered adverse, taking into account the magnitude of the difference.

Table 34. Auditory startle response (ASR) data in C2A animals (Anonymous, 2021).

	Males				Females			
	Ctrl	**LD**	**MD**	**HD**	**Ctrl**	**LD**	**MD**	**HD**
Trial 1 to 10 Amp. (N)	1.85	1.86	1.64 (-12%)	**1.26* (-32%)**	1.71	1.73	1.45 (-15%)	1.45 (-16%)
Trial 11 to 20 Amp. (N)	1.72	1.43	1.44 (-17%)	**1.11* (-35%)**	1.31	1.49	1.21 (-7%)	1.36 (+4%)
Trial 21 to 30 Amp. (N)	1.65	1.34	1.36 (-18%)	1.11 (-33%)	1.22	1.40	1.14 (-7%)	1.37 (+13%)
Trial 31 to 40 Amp. (N)	1.63	1.33	1.32 (-19%)	**0.94* (-42%)**	1.27	1.32	1.14 (-11%)	1.25 (-2%)
Trial 41 to 50 Amp. (N)	1.54	1.31	1.27 (-18%)	**0.85** (-45%)**	1.23	1.32	1.09 (-11%)	1.28 (+5%)

N: Newton. Amp.: Magnitude of response. */** Differences with control at p≤ 0.05/0.01. % in comparison to control group.

On the other hand, in C2A males, the morphometric analysis showed a statistically significant decrease in the entire hippocampus thickness at the highest dose (-12%). Moreover, at all doses, decreases were also observed in the dentate gyrus thickness (-8%, -11%, -19% in the low-, mid- and high-dose groups,

respectively) and cornu ammonis thickness (-18%, -20%, -15% in the low-, mid- and high-dose groups, respectively) (Table 35). No effects on C2A females and C2B animals were reported. In addition, there was no treatment-related gross abnormalities or microscopic alteration in the central nervous system in P0, C1A, C2A or C2B. Moreover, the study authors questioned the relationship between the hippocampal findings and the ASR and the clinical signs described before, referring to the main roles of the hippocampus, i.e. learning, memory and spatial coordination. Overall, according to the registration dossier, the decreases in morphometric measurements were related to a more general effect on growth resulting from maternally toxic doses rather than a specific neurotoxic effect in males. Thus, the study authors considered unlikely the relationship with the test item, although they do not rule out a possible relation with the test substance.

However, the DS considers the hippocampus morphometric alterations in males as an adverse effect since the magnitude of the effect is pronounced. Moreover, regarding the ASR, the role of the hippocampus cannot be disregarded (Zhang *et al.*, 2022). In addition, alterations in the auditory startle test, as well as morphometric findings were only observed in C2A males, so both findings could be specific developmental neurotoxic effects in males rather than a general effect on animals growth.

Table 35. Hippocampus thickness measurements in C2A males (Anonymous, 2021).

	Ctrl	LD	MD	HD
Nb of examined animals	10	10	7	5
Dentate gyrus thickness (µm)	678	**626** (-8%)**	**605** (-11%)**	**549** (-19%)**
Cornu ammonis thickness (µm)	689.5	**565.8** (-18%)**	**554.4** (-20%)**	**587.3** (-15%)**
Entire hippocampus thickness (µm)	1623	1538 (-5%)	1551 (-4%)	**1427** (-12%)**

** Differences with control at p≤ 0.01. % in comparison to control group.

Developmental immunotoxicity

Regarding immunotoxicity, although the EOGRTS did not include the developmental immunotoxicity cohort, there were statistically significant decreases in the absolute counts of splenocytes, B cells, NKT cells, T cells, helper T cells and cytotoxic T cells, in C1A males (Table 36). Moreover, in terms of relative counts, there was a decrease in B cells and an increase in NK cells at 225 mg/kg bw/d. No effects were observed in C1A females. Due to the clear shift down observed in the absolute splenic lymphocyte subpopulations in males, the DS considers these variations as an adverse effect.

Table 36. Splenic lymphocyte immunophenotyping in C1A males (Anonymous, 2021).

		Ctrl	LD	MD	HD
Splenocytes	abs	460602	**287506** (-38%)**	**240755# (-48%)**	**274871** (-40%)**
B cells	abs	180516	**102732** (-43%)**	**82127# (-55%)**	**111328* (-38%)**
	rel	39.0	35.2	**34.2* (-12%)**	39.2
NK cells	rel	3.1	4.1	**4.5* (+45%)**	4.1
NKT cells	abs	13354	8743	**7874* (-41%)**	**6262** (-53%)**
T cells	abs	170115	**100819** (-41%)**	**93513# (-45%)**	**95467# (-44%)**

		Ctrl	LD	MD	HD
Helper T cells	abs	117433	**66287# (-44%)**	**60762# (-48%)**	**64343# (-45%)**
Cytotoxic T cells	abs	48590	**31801* (-35%)**	**30091** (-38%)**	**28835** (-41%)**

Abs values: mean, cells/mg spleen. Rel values: mean percent vs splenocytes.
*/**/# Differences with control at p≤ 0.05/0.01/0.001. % in comparison to control group.

According to the registration dossier, the NOAEL for developmental toxicity was established at 500 mg/kg bw/d, based on the absence of adverse effects. On the other hand, since the lower hippocampus measurements could possibly be related to the test item, the NOAEL for developmental neurotoxicity was considered lower than 75 mg/kg bw/d.

Nevertheless, the DS considers that the NOAEL for developmental toxicity should be set at 75 mg/kg bw/d due to the increase in the post-implantation losses, the low number of pups delivered and the high number of stillbirths and dead pups on PND 0-4, along with the clinical signs and the body weight effects observed in F1 pups. Regarding developmental neurotoxicity, a LOAEL of 75 mg/kg bw/d was established based on the hippocampus morphometric alterations. Even though a DIT cohort was not included, based on immunophenotyping effects in C1A, a LOAEL for developmental immunotoxicity should be also established at 75 mg/kg bw/d.

Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Kapp *et al*., 2003)

In the OECD TG 422 study, Sprague-Dawley rats received oral gavage administrations of 0, 75, 225 and 750 mg/kg bw/d acetophenone (see section 10.10.1 for a description of the effects on sexual function and fertility and general toxicity).

Developmental toxicity

There was a statistically significant increase of number of stillborn (2, 6, 4, 30 in the control, low-, mid- and high-dose groups, respectively; out of HCD) and a statistically significant decrease in the number of liveborn (105, 136, 145, 109 in the control, low-, mid- and high-dose groups, respectively) at 750 mg/kg bw/d. As a result, the live birth index was also lower at the highest dose (98.1, 95.8, 97.3, 78.4 in the control, low-, mid- and high-dose groups, respectively), and out of HCD. There was also a statistically significant increase of F1 pups dying, missing and/or cannibalized during PND 1-4 (6, 5, 8, 79 in the control, low-, mid- and high-dose groups, respectively), and litters with total litter loss up to PND 4 (0, 0, 0, 6 in the control, low-, mid- and high-dose groups, respectively) at 750 mg/kg bw/d. Consequently, the viability index on PND 4 was significantly lower in the high-dose group (94.3, 96.3, 94.5, 22.9 in the control, low-, mid- and high-dose groups, respectively) (Table 37).

Table 37. Summary of F1 pup viability and body weights (Kapp *et al.*, 2003).

		Ctrl	LD	MD	HD	HCD
Females with stillborn pups (%)		2 (28.6%)	4 (44.4%)	3 (30.0%)	7 (77.8%)	
Litters with liveborn pups		7	9	10	9	
Dams with total litter loss between PND 0-4 (%)		0	0	0	**6* (66.7%)**	
Pups delivered	total	107	142	149	139	
	mean	15.3	15.8	14.9	15.4	

	Ctrl	LD	MD	HD	HCD
Nb of liveborn	105	136	145	**109****	
Live birth index	98.1	95.8	97.3	**78.4**	**95.5-99.1%**
Nb of stillborn (%)	2 (1.9%)	6 (4.2%)	4 (2.7%)	**30**** **(21.6%)**	**0.9-4.5%**
Nb of pups missing	2	2	4	22	
Pups died or missing and/or cannibalized on PND 0 (%)	0	0	0	5 (4.6%)	
Pups died or missing and/or cannibalized during PND 1 -4 (%)	6 (5.7%)	5 (3.7%)	8 (5.5%)	**79**** **(72.5%)**	
Nb of pups surviving to PND 4	99	131	137	**25****	
Viability index on PND 4 (%)	94.3	96.3	94.5	**22.9****	
Mean nb of live pups/litter on					
PND 0	15.0	15.1	14.5	12.1	
PND 1	14.6	14.8	13.8	**4.1****	
PND 2	14.4	14.7	13.8	**3.0****	
PND 3	14.1	14.7	13.7	**2.9****	
PND 4	14.1	14.6	13.7	**2.8****	
Pup bw/litter on					
PND 1	6.3	6.5	6.3	**5.0****	**6.5-7.5**
PND 4	8.7	9.2	8.4	**5.4****	**8.5-11.1**

*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group. HCD: data provided in the registration dossier.

External observations included a high incidence of desquamation observed in F1 pups from 225 mg/kg bw/d, and gasping, cool to touch and changes in skin appearance at 750 mg/kg bw/d (Table 38).

Table 38. Summary of observations from external examination of F1 pups on PND 0 and 4 (Kapp *et al.*, 2003).

	Ctrl	LD	MD	HD
Nb of dams with litters examined	7	9	10	9
Within normal limits	191/7	254/9	216/10	53/4
Respiration: gasping	0/0	0/0	0/0	4/2
Skin pale in color	0/0	1/1	1/1	4/3
Cool to touch	0/0	9/3	0/0	31/5
Desquamation	0/0	0/0	42/5	15/3
Skin: shiny appearance	0/0	0/0	0/0	43/4
Skin: tight – restricts movement	0/0	0/0	0/0	27/3
Overall evaluation †		**No remarkable effects**	**Desquamation as single remarkable effect**	**All compiled effects considered as remarkable**

Frequency of observation/nb of dams with the specific observation in their litter. † reported in the registration dossier.

There was a statistically significant decrease in pup body weight per litter on PND 1 and 4 at 750 mg/kg bw/d (PND 1: 6.3, 6.5, 6.3, 5.0; PND 4: 8.7, 9.2, 8.4, 5.4, in the control, low-, mid- and high-dose groups, respectively), both below the range of HCD (Table 37).

On the other hand, at gross necropsy, stillborn pups showed a single incidence each of cleft palate and edema, increased incidences of atelectasis, milk not present in the stomach and dermal hypoplasia at the

highest dose. Pups found dead during lactation showed scabbing, desquamation, milk not present in the stomach, dermal hypoplasia, and in addition, 22 pups showed autolysis at 750 mg/kg bw/d. Findings in pups with scheduled euthanasia on PND 4 were scabbing and desquamation from 225 mg/kg bw/d (Table 39).

Table 39. Summary of gross necropsy observations in stillborn, died (on PND 0 to 4) and euthanised F1 pups (Kapp *et al.*, 2003).

			Ctrl	LD	MD	HD
	Location	**Observation**				
Stillborn		Litters evaluated	1	4	3	7
		Pups evaluated	1	6	4	28
	Whole body	Edema	0	0	0	1/1
	Oral cavity	Cleft palate	0	0	0	1/1
	Lung	Atelectasis	1/1	6/4	3/2	15/4
	Skin	Dermal hypoplasia	0	0	0	12/2
	Stomach	Milk not present	1/1	6/4	3/2	23/5
	Overall evaluation †			**No remarkable findings**	**No remarkable findings**	**Compiled effects considered as remarkable**
Died pups		Litters evaluated	3	3	2	8
		Pups evaluated	4	3	4	62
	Skin	Scabbing	1/1	0	0	8/2
		Desquamation	0	0	0	3/1
		Dermal hypoplasia	0	0	0	6/2
	Stomach	Milk not present	2/2	1/1	1/1	43/7
	Gross examination	Autolysis	0	2/2	1/1	22/7
	Overall evaluation †			**No remarkable findings**	**No remarkable findings**	**Compiled effects considered as remarkable**
Scheduled euthanasia		Litters evaluated	7	9	10	3
		Pups evaluated	99	131	137	25
	Skin	Scabbing	2/2	2/1	8/4	12/2
		Desquamation	0	0	43/5	14/2
	Overall evaluation †			**No remarkable findings**	**No remarkable findings**	**Compiled effects considered as remarkable**

Pup incidence/Litter incidence. † reported in the registration dossier.

Due to developmental toxicity up to PND 4 based on findings in pup viability, pup body weight, external observations, and gross pathology, the NOAEL was established at 225 mg/kg bw/d.

According to the registration dossier, as all the effects observed in F1 pups occurred only at the highest dose being associated with maternal toxicity, including reduced weight gain during GD 0-7, there is a high probability that the reproductive effects were a non-specific secondary consequence of a general toxic effect of acetophenone.

The DS acknowledges the decrease in body weight change of P0 female rats recorded during GD 0-7 (-39%).

However, during the rest of the gestation period as well as during the lactation period, body weight gain was comparable to the control group. In addition, body weight was only slightly, non-statistically significant, lower on GD 7 (ca. -6%), and there were no statistically significant effects on body weight during the gestation or the lactation period. In this screening study, the main clinical signs observed in parental females included wobbly gait, salivation, urine stain and few faeces. Increased liver and kidney weights were reported in females without histopathological correlation in the short-term repeated dose toxicity phase of the screening test. Therefore, the DS considers that the maternal toxicity was not severe enough to account for the severe developmental effects observed. Consequently, the developmental effects referred to in this section should be considered treatment-related and not as a secondary consequence of maternal systemic toxicity.

Prenatal developmental toxicity study in rats (Anonymous, 2016a)

In the first prenatal study, conducted in accordance with OECD TG 414, acetophenone was administered to female Wistar rats via oral gavage from GD 5 to 19 at 0, 125, 300 and 750 mg/kg bw/d.

Maternal toxicity

Salivation was recorded transiently in a few females at mid-dose and all females at high-dose. Transiently reduced spontaneous activity short post-administration was observed in three single females at mid-dose on 1-2 days of treatment and in most females at high-dose on several treatment days short and late post-administration, associated with half eyelid closure and prone position in several cases. At high-dose, short and late post-administration ataxia and piloerection were recorded on several occasions and fully closed eyes and apathy in single females on a few days of treatment. These signs were considered treatment-related due to the known hypnotic effect of acetophenone, used in the past as “hypnone” (see section 10.11 for more information regarding specific target organ toxicity – single exposure).

In pregnant females, a significant decrease in body weight from GD 8 to 20 was recorded at 300 mg/kg bw/d and 750 mg/kg bw/d (up to -8% and -12%, respectively). In the mid- and high-dose groups, there were also reductions in body weight gain (-23% and -37%, respectively) and food consumption (-13% and -23%, respectively) between GD 0-20 (Table 40).

Table 40. Mean body weight and body weight gain of parental females (Anonymous, 2016a).

	GD	Ctrl	LD	MD	HD
Body weight (g)	**0**	226	225	223	224
	5	240	240	239	239
	8	245	244	**237**** **(-3%)**	**230***** **(-6%)**
	11	255	254	**246**** **(-3%)**	**240***** **(-6%)**
	14	265	264	**255**** **(-4%)**	**248***** **(-6%)**
	17	290	287	**276**** **(-5%)**	**265***** **(-9%)**
	20	329	321	**303***** **(-8%)**	**290***** **(-12%)**
Body weight gain (g)	**0-20**	104	97	**79***** **(-23%)**	**66***** **(-37%)**

/* Differences with control at p≤ 0.01/0.001. % in comparison to control group.

The uterus weight was lower at 300 mg/kg bw/d and 750 mg/kg bw/d (-14% and -25%, respectively). Likewise, lower mean adjusted maternal weight (maternal weight minus gravid uterus weight) was also reported in the mid- and high-dose groups (-7% and -9%, respectively) (Table 41).

Table 41. Mean uterus weight and mean adjusted maternal weight of parental females (Anonymous, 2016a).

	Ctrl	LD	MD	HD
Terminal bw	329.44	321.46	**302.67*** (-8%)**	**289.94*** (-12%)**
Uterus weight	63.16	58.89	**54.59* (-14%)**	**47.67*** (-25%)**
Adjusted maternal weight	266.28	262.57	**248.08*** (-7%)**	**242.27*** (-9%)**

*/*** Differences with control at p≤ 0.05/0.001. % in comparison to control group.

The NOAEL for maternal toxicity was considered to be 125 mg/kg bw/d, based on the effects on body weight, body weight gain, food consumption, clinical signs and organ weights (uterus weight).

Developmental toxicity

There were non-statistically significant decreases in the mean number of implantation sites and the mean number of foetuses. According to the registration dossier, as treatment was not initiated before actual nidation and values were within the normal range of variation, these findings were considered incidental. Non-statistically significant increases in the mean number of early resorptions and the percentage of post-implantation loss were also observed at 750 mg/kg bw/d, related to 100% post-implantation loss in one single female. Due to these non-statistically significant values were within the range of normal variation, these effects were also considered incidental (Table 42). However, the DS notes the increasing trend in both early resorptions and the percentage of post-implantation loss, as well as, the decreasing trend in the number of live fetuses at all doses.

Table 42. Prenatal data (Anonymous, 2016a).

	Ctrl	LD	MD	HD
Implantation sites (mean)	11.70	11.13 (-5%)	11.13 (-5%)	10.88 (-7%)
Live foetuses (mean)	11.22	10.50 (-6%)	10.38 (-7%)	9.81 (-13%)
Dead foetuses (mean)	0	0	0	0
Early resorptions (mean)	0.41	0.54	0.71	1.06
Post-implantation loss (%)	3.74	5.26	7.30	10.70

No statistically significant differences. % in comparison to control group.

Mean foetus weight, calculated both on individual and litter basis, was statistically significantly lower in the mid- and high-dose groups (-6% and -15%, respectively). This resulted in a lower total litter weight at 750 mg/kg bw/d (-23%) (Table 43).

Table 43. Mean foetal weight and total litter weight (Anonymous, 2016a).

	Ctrl	LD	MD	HD
Mean foetus weight (g) (individual basis)	3.67	3.68	**3.45*** (-6%)**	**3.13*** (-15%)**
Mean foetus weight (g) (litter basis)	3.67	3.69	**3.44** (-6%)**	**3.11*** (-15%)**
Total litter weight (g)	41.17	38.64	35.60 (-14%)	**31.68*** (-23%)**

/* Differences with control at p≤ 0.01/0.001. % in comparison to control group.

External examination of foetuses revealed agnathia in one single foetus in the control and high-dose group, considered incidental due to the low incidence. While no correspondence for this finding was observed at skeletal examination of the control foetus, a short mandible was noted in the foetus of the high-dose group (micrognathia). Bilateral pelvic girdle caudal shift was recorded in more litters at 750 mg/kg bw/d (2, 0, 3, 7 in the control, low-, mid- and high-dose groups, respectively). Both foetal and litter incidences of this skeletal malformation were above HCD. Moreover, the observed malposition of the pelvic girdle relative to the number of pre-pelvic vertebrae was associated with a moderately higher foetal and litter incidences of supernumerary bilateral full 14th thoracolumbar rib, non-statistically significant, but above HCD (Table 44).

Table 44. Foetal skeletal findings (Anonymous, 2016a).

		Ctrl	LD	MD	HD	HCD (maximum values)
Pelvic girdle (B) caudal shift	Nb incidences	2	0	4	13	
	Total nb observed foetuses	177	121	115	119	
	% foetal incidence	1.13	0.00	3.48	**10.92**	6.20
	Nb litters with at least 1 incidence	2	0	3	**7***	
	Total nb observed litters	30	22	21	23	
	% litter incidence	6.67	0.00	14.29	**30.43**	30.00
Rib (14th) (B) full	Nb incidences	7	2	5	17	
	Total nb observed foetuses	177	121	115	119	
	% foetal incidence	3.95	1.65	4.35	**14.29**	11.65
	Nb litters with at least 1 incidence	7	2	3	10	
	Total nb observed litters	30	22	21	23	
	% litter incidence	23.33	9.09	14.29	**43.48**	31.58

B: bilateral. * Differences with control at p≤ 0.05. HCD: data provided in the registration dossier. In bold, values above HCD.

The NOAEL for developmental toxicity was established at 125 mg/kg bw/d, based on the foetal body weight reduction and the skeletal malformations observed.

Prenatal developmental toxicity study in rabbits (Anonymous, 2020)

The second OECD TG 414 study was conducted in New Zealand White rabbits. Acetophenone was administered from GD 6 to 28 by daily oral gavage at dose levels of 0, 60, 170 and 500 mg/kg bw/d.

Maternal toxicity

At 500 mg/kg bw/d, two females aborted, representing 9% of the dams, above 2% of the HCD. A third female was found dead, with red discharge in the cage. Although the cause of death was not clearly determined at the necropsy, the possibility of a treatment association was not excluded.

All females showed decreased activity and/or abnormal gait at 500 mg/kg bw/d, 1 h after dosing, from GD 6 to GD 27, recovering within the day. In addition, two females had laboured and shallow breathing, lying on the side and subdued behaviour on GD 19-20 and GD 9, respectively, 1-2 h after dosing, also recovering within the day. Moreover, a higher frequency of few faeces was reported from mid-dose.

Transient reductions in body weight gain or body weight loss was observed at all doses in different gestational periods, with no impact on the terminal body weight on GD 29. There were also no statistically significant differences in body weight gain over the entire gestation period (GD 6-29). A decreased in food consumption was recorded up to GD 21 and GD 24 at 170 mg/kg bw/d and 500 mg/kg bw/d, respectively.

The NOAEL for maternal toxicity was established at 170 mg/kg bw/d, based on clinical signs, body weight and body weight gain, food consumption, mortality and number of abortions.

Developmental toxicity

A non-statistically significant higher number of late resorptions was observed at 500 mg/kg bw/d (0.7 vs 0.5 in the control group), above HCD. The total number of resorptions was also slightly higher at the highest dose (1.1 vs 0.9 in the control group). This was mainly due to three females, two with three late resorptions and one with no viable foetuses (all early resorptions). Consequently, the percentage of post-implantation loss was slightly not-statistically significant higher at 500 mg/kg bw/d (11.13% vs 9.20% in the control group), close to the higher values of HCD. It should be mentioned that the female with no viable foetuses, i.e. 100% post-implantation losses, was excluded from that calculation. Despite 18 pregnant females were reported at terminal caesarean, only 17 were included in the assessment of the uterine content examination. Therefore the prenatal data values would be higher than the registration dossier provided (Table 45).

Table 45. Prenatal data (Anonymous, 2020).

	Ctrl	LD	MD	HD	HCD
Pregnant females at terminal caesarean	17	20	17	18	
Pregnant females with ovarian and uterine content examination	17	20	17	17†	
Total nb of resorptions (mean)	0.9	**0.3***	0.4	1.1 (+22%)	
Nb of early resorptions (mean)	0.4	0.2	0.3	0.4	
Nb of late resorptions	0.5	0.2	0.1	0.7 (+40%)	0.1-0.6
Post-implantation loss (%)	9.20	3.02	5.47	11.13 (+21%)	2.4-11.4

* Differences with control at p≤ 0.05. % in comparison to control group. HCD: data provided in the registration dossier.
† Female with no viable foetuses (all early resorptions) excluded from the data analysis provided in the Table.

There was a decrease in the foetal weight at 170 mg/kg bw/d and 500 mg/kg bw/d (-7% and -9%, respectively), statistically significant and below HCD at the highest dose. The difference was more pronounced for female foetuses than for the males (Table 46).

Table 46. Mean foetal weight (Anonymous, 2020).

	Ctrl	LD	MD	HD	HCD

	Ctrl	LD	MD	HD	HCD
Mean foetal weight (both) (g)	41.35	39.96 (-3%)	38.45 (-7%)	**37.44* (-9%)**	**38.4-43.2**
Mean foetal weight (M) (g)	41.19	40.42 (-2%)	39.97 (-3%)	37.44 (-9%)	
Mean foetal weight (F) (g)	41.41	39.66 (-4%)	**37.55* (-9%)**	**36.07** (-13%)**	

*/** Differences with control at p≤ 0.05/0.01. % in comparison to control group. HCD: data provided in the registration dossier.

The NOAEL for developmental toxicity was considered to be 170 mg/kg bw/d, based on foetal body weight changes.

10.10.6 Comparison with the CLP criteria

According to the CLP, substances are classified in Category 1 (known or presumed human reproductive toxicant) for reproductive toxicity when they are known to have produced an adverse effect 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. While the classification in Category 1A is largely based on evidence from humans, the classification in Category 1B is largely based on data from animal studies.

Since there are no human data available on developmental toxicity, classification for Category 1A is not proposed.

Developmental toxicity was observed in several animal studies with acetophenone. Consistent findings regarding pup viability were recorded in the extended one-generation reproductive toxicity study (Anonymous, 2021) and the reproduction/developmental toxicity screening test (Kapp *et al*., 2003), both performed in Sprague-Dawley rats. These included a decreased number of liveborn pups and an increased number of stillbirths, a decreased number of live pups on PND 4 and an increased number of dead pups between PND 0-4. Consequently, in both studies, there was a decrease in the live birth index and the viability index. In the EOGRTS, all these findings were reported at the two highest doses, i.e. 225 and 500 mg/kg bw/d, whereas in the screening test, pup viability was affected at the highest dose (750 mg/kg bw/d), but not at the mid-dose (225 mg/kg bw/d). The higher duration of premating exposure could explain the differences in the dose levels at which the developmental effects occurred in these studies since the premating exposure duration in the EOGRTS was ten weeks, while in the screening study was two weeks.

Marked systemic toxicity was not reported in either study, since there was no increase in the incidence of mortality and in the incidence and/or severity of clinical signs or post-mortem findings in dams.

Moreover, a statistically significant increase in the percentage of post-implantation loss was observed in the OECD TG 443 at 500 mg/kg bw/d, associated with a decrease in the number of pups delivered. Two prenatal developmental toxicity studies in rats (Anonymous, 2016) and rabbits (Anonymous, 2020) also reported non-statistically significant higher post-implantation losses at the highest doses tested (750 and 500 mg/kg bw/d, respectively). In the prenatal study in Wistar rats, there was a non-statistically significant increase in the mean number of early resorptions and a non-statistically significant decrease in the mean number of foetuses, at 750 mg/kg bw/d. However, it has to be noted that an increasing trend in both early resorptions and the percentage of post-implantation loss, as well as, a decreasing trend in the number of implantation sites and the number of live foetuses were observed at all doses. In the OECD TG 414 in New Zealand White rabbits, a non-statistically significant increase in the number of late resorptions and the total number of resorptions was reported at 500 mg/kg bw/d. However, it has to be mentioned that, in the evaluation of the uterine content, a female with 100% post-implantation losses (all early resorptions) was not included.

The differences in exposure duration, the exposure window in the life cycle and/or the species and strains used may explain the slight differences observed between studies.

Similar clinical signs were observed in pups during lactation at the two highest doses tested in the OECD TG 443 and the OECD TG 422, including desquamation, scabbing and cold to touch.

In the EOGRTS, a decrease in pups body weight was recorded on PND 1 at 225 and 500 mg/kg bw/d in both sexes. The decrease in male pups was maintained until PND 21 (between -20% to -23%), while in female pups, body weight recovered from PND 4 onwards. In the screening study, there was also a statistically significant reduction in pup body weight per litter on PND 1 and 4 at 750 mg/kg bw/d. A decreased foetal weight was also described at the two highest doses in both prenatal developmental toxicity studies.

On the other hand, developmental neurotoxicity effects were observed in the EOGRTS. Specifically, in C2A, statistically significant decreases in the magnitude of response in the auditory startle test were recorded in almost all the trials in males at 500 mg/kg bw/d. In addition, decreases in three hippocampus measurements were also detected in males from the low-dose. According to the study authors, these decreases in hippocampus measurements may be due to decreased body weight and brain weight observed in males. They questioned the relationship between the hippocampal findings with the ASR and the clinical signs described in C2A males, which included hypoactivity, half-closed eyes and staggering gait. Therefore, they concluded that the brain alterations could be related to a more general effect on growth, resulting from maternally toxic doses, rather than a specific neurotoxic effect. However, the DS considers that the magnitudes of both decreases, in the ASR and the hippocampus measurements, are statistically and biologically significant. Furthermore, there is increasing evidence of the relationship between the hippocampus with the auditory system. Therefore, the role of the hippocampus in the ASR or vice versa cannot be disregarded. In addition, the findings in both the auditory startle test and the morphometrics were noted only in C2A males, which the DS considers a specific developmental neurotoxic effect in males rather than a general effect on the animals growth.

Regarding developmental immunotoxicity, effects on immunophenotyping were observed in the OECD TG 443. C1A males showed statistically significant decreases in the absolute counts of splenocytes, B cells, T cells, helper T cells and cytotoxic T cells in all treated groups as well as decreases in the absolute count of NKT cells at mid- and high-dose levels. The DS considers this to be a downward shift in the absolute splenic lymphocyte subpopulations in males.

Finally, the prenatal developmental toxicity study in rats reported an increase in the incidence of skeletal malformations related to the pelvic girdle. Bilateral pelvic girdle caudal shift was statistically significantly recorded in more litters at 750 mg/kg bw/d. Although foetal and litter incidences of this skeletal malformation were not statistically significant, both incidences were outside HCD. Moreover, this malformation was associated with non-statistically significant higher foetal and litter incidences of supernumerary bilateral full 14th thoracolumbar rib, also above HCD. These skeletal findings were only observed in the prenatal study in Wistar rats, but not in the prenatal study in rabbits, the EOGRT study or the screening study in Sprague-Dawley rats. The differences in exposure duration, the exposure window in the life cycle and/or the species and strains used may account for this different finding relative to skeletal malformations.

Overall, there is consistency between the studies investigating developmental toxicity, mainly regarding the decreased offspring viability and the reduction in pup/foetus body weight. Moreover, the developmental neurotoxicity findings and the effects in the immunophenotyping observed in the EOGRTS, along with the skeletal malformations observed in the prenatal study in rats, support the classification of acetophenone as Repr. 1B, H360D for developmental toxicity.

10.10.7 Adverse effects on or via lactation

No effects of acetophenone on or via lactation have been reported in the extended one-generation reproductive toxicity study (Anonymous, 2021) or in the reproduction/developmental toxicity screening test (Kapp *et al*., 2003). Therefore, no classification is proposed.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Based on the available animal data, classification as Repr. 1B, H360FD is considered warranted, based on the adverse effects observed on sexual function and fertility and development.

10.11 Specific target organ toxicity-single exposure

Table 47: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Acute oral toxicity Similar to OECD TG 401 Reliability 2 (reliable with restrictions) Non-GLP No information on the purity of the test substance, and duration of clinical signs. Rat, Sprague-Dawley 5/sex/group	Acetophenone Purity: not specified No vehicle 0, 1030, 1648, 2575, 4120 mg/kg bw Single oral (gavage) dose 14 days post-treatment observation period	At 1030 mg/kg bw, decreased motility and staggering gait in all rats. At 1648 mg/kg bw, decreased motility in 1m/1f, and staggering gait in 1m/3f. (See section 10.1 for information on acute oral toxicity).	Anonymous, 1981
Acute oral toxicity Similar to OECD TG 401 Reliability 2 (reliable with restrictions) Non-GLP Less detailed documentation of testing procedure, no microscopic examination, confidence interval not provided and no response data specified by sex Rat, Sprague-Dawley 5/sex/group	Acetophenone Purity: 99.7% No vehicle 0, 710, 1400, 2000, 2800, 3900 mg/kg bw Single oral (gavage) dose 14 days post-treatment observation period	At all doses, transient clinical signs such as staggering gait followed by inhibition of turn-around reflex and slowed down breath, observed within 15 min after exposure up to 48 h. Flabby appearance was also recorded from 2000 mg/kg bw and reduced spontaneous activity and prostration from 2800 mg/kg bw, up to 6 h. LOAEL = 710 mg/kg bw, indicating the nervous system to be target of an adverse action. (See section 10.1 for information on acute oral toxicity).	Anonymous, 1978
Acute dermal toxicity Similar to OECD TG 402 Reliability 2 (reliable with restrictions) Non-GLP Less detailed documentation of testing procedure, no	Acetophenone Purity: 99.7% No vehicle 0, 1820, 2360, 3000, 4000, 5200 mg/kg bw Single dermal (occlusive) dose, 24 h	Reduced spontaneous activity was observed from 1820 mg/kg bw, and staggering gait from 2360 mg/kg bw. Prostration was also reported from 3000 mg/kg bw, and inhibition of turn-around reflex from 4000 mg/kg bw. Clinical symptoms were transient, mainly observed up to 48 h. LOAEL = 1820 mg/kg bw, typical of effects on the nervous system. LD_{50} = 3300 mg/kg bw.	Anonymous, 1978

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
microscopic examination, confidence interval not provided and no response data specified by sex Rat, Sprague-Dawley 5/sex/group	14 days post-treatment observation period		
Subchronic toxicity OECD TG 408 Reliability 1 (reliable without restrictions) GLP Rat, Wistar 10/sex/group	Acetophenone Purity: 99.67% Vehicle: corn oil 0, 125, 250, 500 mg/kg bw/d Daily oral (gavage) doses for 90 days	At HD, slightly to moderately reduced spontaneous activity in all males and most females on almost all days of treatment, and transient ataxia in some males and one female on a few days in the first week of treatment. Clinical signs were related to the known hypnotic effect of acetophenone. NOAEL = 250 mg/kg bw/d	Anonymous, 2016b
Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test OECD TG 422 Reliability 1 (reliable without restrictions) GLP Rat, Sprague-Dawley Short-term repeated dose part: 10 males and 5 females per group Screening for reproductive part: 10 additional females	Acetophenone Purity: 98.80% Vehicle: corn oil Oral (gavage) 0, 75, 225, 750 mg/kg bw/d Daily from 14 days before mating to lactation day 3	At HD, wobbly gait in all males and females on a few days. NOAEL = 225 mg/kg bw/d	Kapp *et al*., 2003
EOGRTS with DNT OECD TG 443 Reliability 1 (reliable without restrictions) GLP Rat, Sprague-Dawley P0: 24/sex/group	Acetophenone Purity: 99.79% Vehicle: 0.5% (w/v) carboxymethylcellulose 400-800cPs / 0.5% (w/v) Tween 80 in drinking water treated by reverse osmosis	Transiently, in P0 generation and all cohorts, males and females showed hypoactivity and half-closed eyes at MD and HD. In addition, staggering gait was recorded at HD in C1A and C2A males and females and P0 males. **According to the registration dossier**: NOAEL P0 males = 75 mg/kg bw/d	Anonymous, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
C1A: 20-21/sex/group in ctrol, LD and MD, and 10m/20f at HD C1B: 20/sex/group in ctrl, LD and MD. Not enough pups to constitute the HD C2A: 10/sex/group in ctrl, LD and MD, and 5/sex at HD C2B: 10/sex/group in ctrl, LD and MD. Not enough pups to constitute the HD	Oral (gavage) 0, 75, 225, 500 mg/kg bw/d P0 generation: 10 weeks before mating, during mating, until F1 weaning F1 generation: direct dosing from weaning (PND 22) to terminal sacrifice of respective cohorts C1A: until PND 90-93 C1B: until PND 98-100 C2A: until PND 76-78 C2B: sacrificed on PND 22 (no direct dosing)	**According to the DS**: NOAEL P0 males and females = 75 mg/kg bw/d NOAEL F1 males and females = 75 mg/kg bw/d	
Prenatal developmental toxicity OECD TG 414 Reliability 1 (reliable without restrictions) GLP Rat, Wistar 35 females in ctrl and HD 25 females in LD and MD	Acetophenone Purity: 99.79% Vehicle: corn oil Oral (gavage) 0, 125, 300, 750 mg/kg bw/d Daily from GD 5 to GD 19	At MD, transiently reduced spontaneous activity short post-administration was observed in three single females on 1-2 days of treatment. At HD, slightly to severely reduced spontaneous activity in most females on several treatment days short (mid-morning) and late post-administration (afternoon), associated with half eyelid closure and prone position in several cases, and short and late post-administration ataxia. Fully closed eyes and apathy were also recorded in single females on a few days of treatment. All these signs were considered treatment-related due to the known hypnotic effect of acetophenone, used in the past as “hypnone”. NOAEL = 125 mg/kg bw/d	Anonymous, 2016a
Prenatal developmental toxicity OECD TG 414 Reliability 1 (reliable without restrictions) GLP Rabbit, New Zealand White 22 females/group	Acetophenone Purity: 99.79% Vehicle: 0.5% (w/v) carboxymethylcellulose 400-800cPs/0.5% (w/v) Tween® 80 in purified water Oral (gavage) 0, 60, 170, 500 mg/kg bw/d Daily from GD 6 to	At HD, all females showed decreased activity and/or abnormal gait 1 h after dosing, from GD 6 to GD 27, recovering within the day. In addition, two females had laboured and shallow breathing, lying on the side and subdued behaviour on GD 19-20 and GD 9, respectively, 1-2 h after dosing, also recovering within the day. NOAEL = 170 mg/kg bw/d	Anonymous, 2020

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	GD 28		

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

For the assessment of specific target organ toxicity, single exposure (STOT SE), seven experimental animal studies via oral and one via dermal exposure were considered. Two of these studies, the subchronic and prenatal toxicity studies in rats, specifically refer to the known hypnotic characteristics of the substance.

The two acute oral toxicity studies included (Anonymous, 1981; Anonymous, 1978) are also referred to in section 10.1. In Anonymous (1981), decreased motility and staggering gait was recorded in all rats at 1030 mg/kg bw, and in individual animals at 1648 mg/kg bw. In Anonymous (1978), staggering gait, inhibition of turn-around reflex and slowed down breath was also detected at all doses, up to 48 h. This study also described flabby appearance from 2000 mg/kg bw, and prostration from 2800 mg/kg bw, up to 6 h.

In turn, the acute dermal toxicity study (Anonymous, 1978) also reported transient clinical symptoms, mainly observed up to 48 h, such as reduced spontaneous activity at all doses, staggering gait from 2360 mg/kg bw, and prostration at 3000 mg/kg bw. At 4000 and 5200 mg/kg bw, similar clinical signs were also observed.

On the other hand, in the subchronic toxicity study (Anonymous, 2016b), all male and most female rats showed slightly to moderately reduced spontaneous activity on almost all days of treatment at 500 mg/kg bw/d. In addition, transient ataxia was also reported at this dose level in some males and one female for a few days in the first week of treatment.

Furthermore, in the short-term repeated dose toxicity part of the OECD TG 422 (Kapp *et al*., 2003), wobbly gait was noted in all males and females at 750 mg/kg bw/d for a few days. Likewise, in the reproductive/developmental screening part, parental females also exhibited wobbly gait at the same dose level.

Similarly, transient clinical signs after exposure were also described in the EOGRT study (Anonymous, 2021). These effects included hypoactivity and half-closed eyes from 225 mg/kg bw/d in P0 generation and C1A, C1B, and C2A adult animals. Moreover, staggering gait was also observed at 500 mg/kg bw/d in P0 males and C1A and C2A males and females.

Finally, two prenatal developmental toxicity studies reported similar clinical signs in pregnant females. In the first one (Anonymous, 2016a), reduced spontaneous activity was observed transiently in three rats at 300 mg/kg bw/d on 1-2 days of treatment and in most females at 750 mg/kg bw/d on several treatment days. In addition, half eyelid closure and prone position, along with ataxia were also detected in several cases. Single females also exhibited fully closed eyes and apathy for a few days of treatment. In turn, in the second prenatal developmental study (Anonymous, 2020), all female rabbits showed decreased activity and/or abnormal gait at 500 mg/kg bw/d, recovering within the day. Moreover, three females exhibited subdued behaviour, lying on the side, on individual days, also recovering during the day.

10.11.2 Comparison with the CLP criteria

According to the CLP, the criteria for classifying substances as Category 3 for narcotic effects are:

“(a) central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgment, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness.

(b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure."

On the one hand, acetophenone was used in medicine in the late 19th and early 20th centuries as an anesthetic agent, and also as a hypnotic agent under the brand name Hypnone (Dujardin-Beaumetz, 1885; Limousin, 1886; Norman, 1887). Indeed, this is one of the trade names currently referred to in the ECHA dissemination web.

On the other hand, three acute and five repeated dose toxicity studies showed similar clinical signs, consistent with the narcotic characteristics of acetophenone such as decreased activity, staggering/wobbly/abnormal gait, flabby appearance, half-closed eyes, prostration or ataxia. As mentioned before, the subchronic and prenatal toxicity studies in rats (Anonymous, 2016b; Anonymous, 2016a) specifically refer to the known hypnotic characteristics of the substance. In addition, ataxia is reported as a transient effect in both studies. Similarly, in the prenatal study, the reduction in spontaneous activity is also described as transient. Moreover, the narcotic effects (decreased activity and abnormal gait) observed in the prenatal study in rabbits (Anonymous, 2020) were recovered within the day. Consistently, the OECD TG 422 (Kapp *et al*., 2003) reported wobbly gait only on a few days. In the same way, according to the results of both oral and dermal acute toxicity studies (Anonymous, 1978), staggering gait, reduced spontaneous activity, prostration and flabby appearance were reversible effects.

Therefore, since the substance was used in the past as a hypnotic agent and the narcotic effects were transiently observed in experimental animals, a proposal for the classification of acetophenone as STOT SE 3; H336 is considered appropriate.

10.11.3 Conclusion on classification and labelling for STOT SE

According to the CLP Regulation, based on the available data, classification for acetophenone as STOT SE 3 (H336: may cause drowsiness or dizziness) is considered warranted.

10.12 Specific target organ toxicity-repeated exposure

Not assessed in this dossier.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

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15 ANNEXES

Confidential Annex.