CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

4-hydroxy-4-methylpentan-2-one; diacetone alcohol

EC Number: 204-626-7

CAS Number: 123-42-2

Index Number: 603-016-00-1

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)
14 rue Pierre Marie Curie
F-94701 Maisons-Alfort Cedex
classification.clp@anses.fr

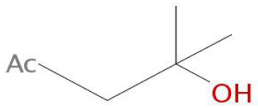
Version number: V2 **Date: June 2024**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	4-hydroxy-4-methylpentan-2-one
Other names (usual name, trade name, abbreviation)	2-Hydroxy-2-methyl-4-pentanone 2-Pentanone, 4-hydroxy-4-methyl- Acetonyldimethylcarbinol diacetone alcohol dimethylacetonylcarbinol
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	204-626-7
EC name (if available and appropriate)	4-hydroxy-4-methylpentan-2-one; diacetone alcohol
CAS number (if available)	123-42-2
Other identity code (if available)	/
Molecular formula	$C_6H_{12}O_2$
Structural formula	
SMILES notation (if available)	CC(=O)CC(C)(C)O
Molecular weight or molecular weight range	116.16
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 99% < 100% w/w

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
4-hydroxy-4-methylpentan-2-one (CAS 123-42-2)	≥ 99% < 100% w/w	Eye Irrit. 2 – H319	Flam. Liq 2 – H225 Flam. Liq. 3 – H226 Skin Irrit. 2 – H315 Eye Irrit. 2 – H319 STOT SE 1 – H370 STOT SE 3 – H335 STOT RE 1 – H372 STOT RE 2 – H373 Repr. 2 – H361

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

See confidential annex

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-016-00-1	4-hydroxy-4-methylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	Eye Irrit. 2	H319	GHS07 Wng	H319		Eye Irrit. 2; H319: C ≥ 10 %	
Dossier submitters proposal	603-016-00-1	4-hydroxy-4-methylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	**Add** Repr. 1B	**Add** H360D	**Add** GHS08 Dgr	**Add** H360D			
Resulting Annex VI entry if agreed by RAC and COM	603-016-00-1	4-hydroxy-4-methylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	Eye Irrit. 2 Repr. 1B	H319 H360D	GHS07 GHS08 Dgr	H360D		Eye Irrit. 2; H319: C ≥ 10 %	

Table 5: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	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 – H360D**	**Yes**
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has an harmonised classification as Eye Irrit.2 – H319 (CLP00).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

5 IDENTIFIED USES

According to ECHA website (consulted in June 2023), this substance is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 10 000 to < 100 000 tonnes per annum.

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

Consumer Uses

This substance is used in the following products: coating products, anti-freeze products, biocides (e.g. disinfectants, pest control products), lubricants and greases, fillers, putties, plasters, modelling clay and finger paints.

Widespread uses by professional workers

This substance is used in the following products: coating products, fillers, putties, plasters, modelling clay, polymers, adhesives and sealants, air care products, anti-freeze products, biocides (e.g. disinfectants, pest control products), finger paints, fertilisers, plant protection products, perfumes and fragrances and washing & cleaning products.

Uses at industrial sites

This substance is used in the following products: coating products, inks and toners, textile treatment products and dyes and adhesives and sealants.

This substance is used in the following areas: printing and recorded media reproduction and building & construction work.

This substance is used for the manufacture of chemicals, food products, textile, leather or fur, wood and wood products, pulp, paper and paper products, fabricated metal products, electrical, electronic and optical equipment and machinery and vehicles.

6 DATA SOURCES

Data are issued from the registration dossier. Lead registrant was contacted in February 2023 in order to obtain the study reports for the endpoints concerned. Study reports for the OECD TG 421 (Unnamed, 2020) and 443 (Unnamed, 2020) studies were obtained in February 2023. Study report for the OECD TG 422 study (Unnamed, 1997) was not available since it was written in Japanese but a summary and the figures in English were obtained. Study reports for the OECD TG 414 studies (Unnamed, 2016b; Unnamed, 2019) were obtained in February 2023.

For the OECD TG 408 (Unnamed, 2017) and 412 (Unnamed, 1979, 1980) studies, data are issued from the registration dossier (public ECHA website).

The litterature was reviewed on Scopus and Pubmed, in August 2023, but no other data were available.

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid (100%)	ECHA website	Observed

Property	Value	Reference	Comment (e.g. measured or estimated)
Melting/freezing point	-44°C at 1013 hPa	ECHA website	Information was sourced from two separate handbooks. No information on atmospheric pressure provided.
Boiling point	167.9°C at 1013 hPa	ECHA website	Information was sourced from two separate handbooks.
Relative density	0.9 at 20°C	ECHA website	Information was sourced from two separate handbooks.
Vapour pressure	1.29 hPa at 20 °C	ECHA website	Information was sourced from three separate handbooks.
Surface tension	/	ECHA website	Not justified
Water solubility	400 g/L at 20 °C	ECHA website	Information was sourced from two separate handbooks.
Partition coefficient n-octanol/water	-0.09 at 20 °C	ECHA website	Calculated
Flash point	42°C at 1013 hPa	ECHA website	Measured
Flammability	/	ECHA website	Acetone is identified as impurity (0-1%) and is classified as Flam. Liq 2. Classification of DAA as Flam. Liq 3 depends on the impurity content.
Explosive properties	Non-explosive (100%)	ECHA website	In accordance with column 2 of REACH Annex VII, the explosiveness study (required in section 7.11) does not need to be conducted as there are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature	643°C	ECHA website	Information was sourced from two separate handbooks. No information on atmospheric pressure reported.
Oxidising properties	No (100%)	ECHA website	In accordance with column 2 of REACH Annex VII, the oxidising properties study (required in section 7.13) does not need to be conducted as the substance does not possess chemical groups associated with oxidising properties.
Granulometry	/	ECHA website	In accordance with section 2 of REACH Annex XI, the particle size distribution (required in section 7.14) does not need to be conducted as the substance is a liquid.
Stability in organic solvents and identity of relevant degradation products	/	ECHA website	The study does not need to be conducted because the stability of the substance is not considered

Property	Value	Reference	Comment (e.g. measured or estimated)
			to be critical
Dissociation constant	/	ECHA website	In accordance with section 1 of REACH Annex XI, the dissociation constant study (required in section 7.16) does not need to be conducted as inspection of the chemical structure shows no functional groups which are associated with dissociation behaviour at relevant environmental conditions.
Viscosity	2.798 mPa.s at 20°C	ECHA website	Information was sourced from two separate handbooks.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this report.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

Oral route

The plasma pharmacokinetic profile of 4-hydroxy-4-methylpentan-2-one or diacetone alcohol (DAA), and its potential metabolites, methyl isobutyl carbinol (MIBC) and methyl isobutyl ketone (MIBK) were evaluated following a single oral administration (gavage) at the dose level of 580 mg/kg (5 mmol/kg) to male Sprague-Dawley rats (Unnamed, 2015). DAA was quantifiable in plasma from 0.25h to 24h. An initial plasma concentration peak at 4.40 mmol/L was reached after 1 hour post-dosing but C_{max} was observed (at 4.82 mmol/L) after 6 hours post-dosing, indicating a prolonged absorption phase. The terminal half-life was of 2.3 hours for DAA. DAA was not significantly metabolized in MIBC and MIBK since plasma levels of metabolites were below the lower Limit Of Quantification at all time-points.

Inhalation route

After a 6-hour inhalation exposure (nose only) to DAA at doses of 500 ppm (3.43 mg/L) and 1000 ppm (7.42 mg/L) of male Sprague-Dawley rats (Unnamed, 2016a):

- Mean plasma concentrations of DAA was quantifiable fsourcerom 0.5 h to 24 h after the end of exposure,
- plasma concentrations of DAA were maximum at 0.5 h after the end of exposure (500 ppm, 389 µg/ml; 1000 ppm, 848 µg/ml),
- the elimination rate of DAA was lower and the half-life higher at 1000 ppm than 500 ppm, indicating a saturation of the excretion pathway,
- MIBC and MIBK were not quantifiable.

DAA was quickly and extensively absorbed by inhalation exposure.

Dermal absorption

In vitro dermal penetration rate of DAA was determined using human cadaver skin mounted in a diffusion cell model (Fasano and McDougal, 2008). Skin penetration was 0.04, 0.15 and 5.71 % of the dose of 25 mg/cm^2 after 10 min, 60 min and 24 h, respectively.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Not assessed in this report.

10.2 Skin corrosion/irritation

Not assessed in this report.

10.3 Serious eye damage/eye irritation

Not assessed in this report.

10.4 Respiratory sensitisation

Not assessed in this report.

10.5 Skin sensitisation

Not assessed in this report.

10.6 Germ cell mutagenicity

Not assessed in this report.

10.7 Carcinogenicity

Not assessed in this report.

10.8 Reproductive toxicity

10.8.1 Adverse effects on sexual function and fertility

Table 7: 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**
OECD Guideline 422 (Combined Repeated Dose	Diacetone alcohol (purity: confidential) Route of	<u>General toxicity (P0)</u> - *Mortality:*	Unnamed, 1997. Study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) GLP compliance is not specified. Reliability 2 (DS assigned reliability) Sprague-Dawley rat Male/Female 10/sex/dose	administration: oral (gavage) Vehicle: Japanese Pharmacopeia purified water. Doses levels: 0, 30, 100, 300, or 1000 mg/kg bw/day Duration and frequency of test/exposure period: daily exposure, 44 days (males: from 14 days before stating to mate) and 41-45 days (females: until lactation day 3)	one **female** in the 1000 mg/kg bw/day group was weakened during delivery and was euthanized once reaching a near death state. - *Clinical signs:* decreased spontaneous locomotion (300 mg/kg bw/day: **males** (7/10) and **females** (6/10) ; 1000 mg/kg bw/day: **both sexes** (10/10)) and less response to stimulation (300 mg/kg bw/day, **both sexes** (5/10); 1000 mg/kg bw/day, **both sexes** (10/10)). - *Body weight*: **Females:** significant reduction in the amount of weight gain (<u>no numerical data</u>) during the premating period in the 1000 mg/kg bw/day group. - *Hematological and blood chemical examinations*: **Males:** significant increases in platelet count (+16%), glutamic oxalacetic transaminase (+49%), choline esterase (+178%), total protein (+10%), total cholesterol (+76%), total bilirubin (+33%), blood urea nitrogen (+19%), creatinine (+17%), and calcium (+6%), as well as a significant decrease in glucose (-23%), at 1000 mg/kg bw/day. **Females:** not examined. - *Organ weight and pathological examinations:* **Males:** statistically significant increase in the weights (absolute (+15%) and relative (+16%)) of the kidneys in the 300 mg/kg bw/day groups, and increases in the weights of the liver (relative (+18%)) and adrenals (absolute (+21%) and relative (+25%)) in the 1000 mg/kg bw/day group). Histologically in the kidneys: increased severity of deposition of hyaline droplets in the proximal tubular epithelium in the groups of 100 mg/kg bw/day or more, increased basophilic tubules in the 300 and 1000 mg/kg bw/day groups and dilation of the distal tubules in the 1000 mg/kg bw/day group. Hepatocellular hypertrophy (5/10) was noted in the liver in the 1000 mg/kg bw/day group and vacuolization of the cells of the zona fasciculata (5/10) in the adrenals of the 1000 mg/kg bw/day group was noted. **Females:** statistically significant increase in the weights (absolute (+26%) and relative (+25%) of the liver in the 1000 mg/kg bw/day group. Histopathologically: hepatocellular hypertrophy (6/10) was noted in the liver in the 1000 mg/kg bw/day group. <u>Reproductive function / performance (P0) (not statistically significant effects)</u> - number of pregnant females: 9, 10, 8, 9, 6 in each group, respectively - fertility index: 90%, 100%, 80%, 90%, 60% - implantation index: 97.3%, 95.4%, 96.3%, 98.2%, 79% - in the 1000 mg/kg bw/day group, one female was euthanized with vaginal hemorrhagia on GD22 (complete stillborn) and another case where none of the pups survived after delivery due to death or	report

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		cannibalism. - No changes were noted for the *copulation rate, number of corpus luteums, gestation period.* See Table 14 for developmental toxicity	
OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test) No deviations. GLP compliant. Reliability 1 (DS assigned reliability) Sprague Dawley Rats Male/Female 10 /sex/dose	Diacetone alcohol (purity confidential) Route of administration – oral (gavage) Doses levels: 0, 50, 250, 750 mg/kg bw/day Vehicle: corn oil Duration and frequency of test/exposure period: daily exposure, from 2 weeks prior pairing until day 30-31 of the study (males) and until Day 13 *post partum* or the day before sacrifice (females)	<u>General toxicity (P0)</u> - *Mortality:* one **female** from the 250 mg/kg bw/day group was sacrificed for humane reasons on Day 23 of gestation, since difficulty in parturition was noted. - *Body weight gain:* **females**: mean body weight gain was decreased in all treated groups on Day 7 *post partum* (-56%, -66%, -25% at 50, 250 and 750 mg/kg bw/day, respectively, not statistically significant). No effect on mean body weight. - *Food consumption:* **females**: on Days 7 and 13 *post partum*, a statistically significantly decrease of food consumption was recorded (down to -18%) at 750 mg/kg bw/day. - *Thyroid hormone:* **males:** significant increase of TSH (1.8 fold higher) at 750 mg/kg bw/day. - *Microscopic observations:* <u>Liver:</u> statistically significant changes as hepatocytic hypertrophy in **males** (10/10) and **females** (9/10) compared to control in both sexes (0/10). In **males**, hepatocytic vacuolization (6/10) at 750 mg/kg bw/day was observed compared to control (0/10). No effects on liver weight. <u>Kidneys:</u> **males:** increased severity of hyaline droplets accumulation at 250 and 750 mg/kg bw/day. Increased incidence of inflammatory cell foci (7/10) at 750 mg/kg bw/day compared to control (0/10). Even if not statistically significant, nephropathy was more frequently observed at 750 mg/kg bw/day (8/10) compared to control group (3/10). No effects on kidney weight. <u>Reproductive function / performance (P0)</u> In both sexes, no significant effects observed. See Table 14 for developmental toxicity	Unnamed, 2020. Study report
OECD Guideline 443 (Extended One-Generation Reproductive Toxicity Study) including a 2nd generation	Diacetone alcohol (purity confidential) Route of administration – oral (gavage) Dose levels: 0, 50, 200 and 600 mg/kg	<u>General toxicity (P0)</u> - *Mortality:* unscheduled deaths occurred in one **male** and one **female** receiving 50 mg/kg bw/day. - *Body weight gain:* **Males:** at 600 mg/kg bw/day, a statistically significant decrease in mean body weight gain was noted on Day 20 of mating phase (-19%) when compared with control animals. **Females:** at 600 mg/kg bw/day, a slightly lower mean body weight	Unnamed, 2020. Study report

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Deviations: at arrival the body weight range of males was 224 to 263 g instead of 225-250 g. Mesenteric lymph nodes weights were measured in all females and not in 10 females per group. Cervical lymph nodes were measured in 10 males per group and not in all males. GLP compliant Reliability 1 (DS assigned reliability) Parental: 25/sex/group Sprague-Dawley Rats Male/Female <u>Parental (P0) :</u> 25/sex/group <u>Cohort 1A and 1B:</u> 20/sex/group	bw/day Vehicle: corn oil Duration and frequency of test/exposure period: <u>Parental males</u> were treated once a day, 7 days/week, for at least 2 weeks prior to pairing and during mating up to the day before sacrifice, for at least 10 weeks (68-72 days). <u>Parental females</u> were treated once a day, 7 days/week at least 2 weeks prior to pairing, during mating, gestation and *post partum* periods until the day before sacrifice (Day 21 *post partum*). <u>Cohort 1A:</u> Males and females were treated starting from Day 21 of age up to the day before necropsy (approximately 13/14 weeks of nominal age). <u>Cohort 1B:</u> Males were treated starting from Day 21 of age for at least 10 nominal consecutive weeks prior to pairing (on nominal Day 91) and during pairing up to the day before sacrifice (after the weaning of the majority F2 litters). Females	gain was recorded on Day 7 *post coitum* (-10%). On Day 14 *post partum*, all dose levels showed a dose-related decrease in mean body weight gain when compared to the control group (-40%, -43% and - 45%), statistically significant only at 600 mg/kg bw/day. No effect on mean body weight. - *Food consumption:* **females**: on Day 14 *post partum*, decreases in food consumption were observed with a statistically significant difference only at 200 mg/kg bw/day (around -6%). - *Coagulation and haematology:* significant decrease in partial thromboplastin time in **males** (-16.3%) and in **females** (-8.0%) at 600 mg/kg bw/day. Significant decrease of hemoglobin in **males** (-5%) at 600 mg/kg bw/day. - *Clinical biochemistry:* significant increases of cholesterol in **males** (+52%) and in **females** (+31%) at 600 mg/kg bw/day. Significant increases of total protein (+5%) and in globulin (+9%) in **males** at 600 mg/kg bw/day. Significant decrease of glucose (-11%) in **females** at 600 mg/kg bw/day. - *Urinalysis*: increased ketonuria was observed in **males** dosed at 200 and 600 mg/kg bw/day and in **females** receiving 600 mg/kg bw/day. - *Thyroid hormones:* **males**: statistically significant increase of T3 (+37%) at 600 mg/kg bw/day. - *Organs weights and histopathological findings:* <u>Liver:</u> **males:** significant increases in absolute and relative mean liver weight (+16.2%) and significant hepatocellular hypertrophy, from minimal to moderate degree at 600 mg/kg bw/day (14/25) compared to control (0/25). <u>Kidneys:</u> significant increases in absolute and relative kidney weights in **males** (+22-23%) and in **females** (+7-8%) at 600 mg/kg bw/day. Statistically significant increase in severity of hyaline droplets accumulation, from mild to moderate, in all **males** dosed at 600 mg/kg bw/day was observed. This was associated with significant increase of nephropathy at 600 mg/kg bw/day (13/25) compared to control (0/25). <u>Adrenals:</u> **males:** significant increases in absolute adrenals weight at 200 mg/kg bw/day (+11.0%), in absolute and relative adrenals weight at 600 mg/kg bw/day (+15.5%). No histopathology findings associated. <u>Reproductive function / performance (P0)</u> - *Sperm analysis:* morphology: non significant decrease of normal % spermatozoa (-7%) and increases of abnormal head (1.6-fold) and headless (2.5-fold) in 600 mg/kg bw/day group. - *Organ weights:* <u>Epididymides:</u> significant decreases in absolute (-7.6%) and relative (-8.5%) epididymides weights at 600 mg/kg bw/day. At 200 and	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	were treated starting from Day 21 of age for at least 10 nominal consecutive weeks prior to pairing (on nominal Day 90), during mating, gestation and *post partum* periods until Days 21 *post partum* or the day before sacrifice.	600 mg/kg bw/d, absolute (-5.6% and -8.3%) and relative (-6.6% and -9.1%) epididymide left weights were significantly decreased. No effects on epididymide right weight. Seminal vesicles: significant decreases in seminal vesicles absolute (-11%) and relative (-13%) weights at 600 mg/kg bw/day. No histopathology findings associated with these weight modifications. - *Copulatory index:* 100%, 100%, 100%, 100%, in each group respectively (0, 50, 200 and 600 mg/kg bw/day). - *Fertility index:* 100%, 100%, 96%, 92%. A total of 3 females were found not pregnant at necropsy: one female receiving 200 mg/kg bw/day and two receiving 600 mg/kg bw/day. No statistically significant effect on oestrous cycle, gestation length or number of implantation. General toxicity (Cohort 1A) No mortality or clinical signs occurred. - *Body weight gain:* **males** showed statistically significant decreases in mean body weight gain on Days 56 (-11%) and 70 (-19%) at 600 mg/kg bw/day. No effect on mean body weight. - *Food consumption:* **Males:** significant reductions in food consumption were observed on Day 56 (-9%) in males receiving 200 mg/kg bw/day and in all treated groups between Days 77 and 91 (-5% to -12%). - *Clinical chemistry:* **Males:** as for P0 males at 600 mg/kg bw/day group, significant increase of cholesterol (+32%) was observed, compared with controls. Significant increases of total protein (+6%), urea (+23%) and decrease of glucose (-17%) at 600 mg/kg bw/day. - *Haematology*: monocytes were statistically significantly lower than controls in males dosed at 200 and 600 mg/kg bw/day (-38% and -31%, respectively). Monocytes were lower than controls in females dosed at 600 mg/kg bw/day (-32%), even if not statistically significant. - *Coagulation:* as for P0 animals, significant decrease in partial thromboplastin time in **males** (-11%) at 600 mg/kg bw/day. - *Urinalysis:* **Males:** as for P0 animals, an increase of urinary ketones was recorded in almost all treated males. In **females:** minimal increases of urinary ketones at 200 and 600 mg/kg bw/day. - *Organs weights and histopathological findings:* Liver: **males:** similarly to P0 generation, a statistically significant	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		increase in relative mean liver weight (+19%) was recorded with centrilobular hepatocellular hypertrophy of liver in 14/20 males treated at the 600 mg/kg bw/day compared to control (0/20). Kidneys: similarly to P0 generation, statistically significant increases in absolute (+17%) and relative (+22%) kidneys weights for **males** receiving the test item at 600 mg/kg bw/day and in relative (+8%) weight at 200 mg/kg bw/day. Increased severity (mild to moderate) of hyaline droplets accumulation in kidneys in 8/20 and in all males dosed at 200 and 600 mg/kg bw/day, respectively. Nephropathy in males dosed at 600 mg/kg bw/day (9/20) compared to control (0/9). **Females:** Significant increases of kidneys relative weights at 600 mg/kg bw/day (+10%) and at 200 mg/kg bw/day (+6%) compared to control. No histopathology findings associated. Adrenals: **males:** significant increases of absolute (+14%) and relative (+20%) weights in 600 mg/kg bw/day group compared to control. No histopathology findings associated. Other organs (mesenteric nodes, heart): significant decreases of mesenteric nodes weight in **males** and in **females,** and heart weight in **females,** at 600 mg/kg bw/day, compared to control, without any histopathology findings associated. Reproductive function / performance (Cohort 1A) No statistically significant effect on reproductive organs, ovarian follicle number, sperm analysis and oestrous cycle. Immunotoxicity (Cohort 1A) - *Lymph nodes weight and splenic lymphocyte subpopulation:* A statistically significant difference ($p<0.05$) in CD8 marker expression was observed in **male** animals dosed at 600 mg/kg bw/day (-11.4%). General toxicity (Cohort 1B) - *Mortality:* one male was found dead in the control group, and one **female** was killed due to human reason at 200 and at 600 mg/kg bw/day. The female in this latter group was killed on GD22 and presented clinical signs coherent with the dystocia. - *Body weight:* **males**: significant decrease on PND35 (-7.7%) and PND42 (-7%). No effect in females. - *Body weight gain:* **males:** significant decreases at 600 mg/kg bw/day on nominal Days 35 (-12%) and 91 (-22%) before pairing. Significant increase on nominal Day 77 (+22%) at 200 mg/kg bw/day. From pairing, significant increase on nominal Days 43 (+63%) at 600 mg/kg bw/day. **Females:** significant increases at 600 mg/kg bw/day on LD7 (+103%) and at 200 mg/kg bw/day on GD	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		14 (+18%). - *Food consumption:* **females:** On Day 14 *post partum*, females dosed at 600 mg/kg bw/day showed a statistically significant decrease in food consumption (-7%). - *Hematology*: **males:** significant decreases of haemoglobin (-7%), mean corpuscular Hb (-5%) and mean corpuscular Hb concentration (-3%); significant increase of neutrophils (+6%) at 600 mg/kg bw/day. Significant decrease of platelets (-24%) at 200 mg/kg bw/day. - *Coagulation:* significant decreases of prothrombin time (-4%) at 600 mg/kg bw/day and significant decrease in partial thromboplastin time in **males** at 200 mg/kg bw/day (-10%) and at 600 mg/kg bw/day (-14%). - *Clinical chemistry:* **Males** dosed at 600 mg/kg bw/day showed statistically significant increases of cholesterol (+49%), triglycerides (+143%), albumin (+5%), creatinine (+29%) and calcium (+6%), and decreases of aspartate aminotranferase (-27%) and chloride (-3%). Males dosed at 200 mg/kg bw/day showed statistically significant increases of triglycerides (+92%) and glucose (+23%), and decreases of chloride (-1%) and sodium (-2%). **Females** dosed at 600 mg/kg bw/day showed statistically significant increase of alanine aminotransferase (+20%), bilirubin (+72%) and cholesterol (+27%). - *Urinalysis:* increases of urine volume in **males** at 200 mg/kg bw/day (+142%) and at 600 mg/kg bw/day (+222%). As for the P0 and Cohort 1A animals, **males** dosed at 200 and 600 mg/kg bw/day showed an increase of urinary ketones, with a dose-related trend while **females** of the same groups only showed a minimal increase. - *Thyroid hormones:* **males:** significant increase of T3 level (+48%) at 600 mg/kg bw/day and a non significant increase of T4 level related to the dose (9%, 16%, 24% respectively for 50, 200 and 600 mg/kg bw/day). Decreased trend of TSH related to the dose (-48%, -50%, -59% respectively for 50, 200 and 600 mg/kg bw/day). **Females:** significant increase of T3 level (+23%) at 200 mg/kg bw/day. Decreased trend of TSH related to the dose (-14%, -50%, -59% respectively for 50, 200 and 600 mg/kg bw/day). - *Organs weights and histopathological findings:* Liver: **males:** non significant increase of liver absolute weight (+14.8%) but significant increase of liver relative weight (+17%) at 600 mg/kg bw/day. **Females:** non significant increases of absolute (+5.6%) and relative (+7.5%) weights at 600 mg/kg bw/day. Kidneys: **males:** significant increases of kidneys absolute (+12.7%) and relative (+14.2%) weights at 600 mg/kg bw/day. **Females:** significant increases of absolute (+6.5%) and relative (+7.5%) weights at 600 mg/kg bw/day. Adrenals: **males:** trend to a dose-dependent increase (+4.7%,	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		+11.6%, +13.8%). Thyroid: **males:** significant increases of absolute (+12.1%) and relative (+10.6%) weights at 600 mg/kg bw/day. No histopathology findings associated with any organ. Reproductive function / performance (Cohort 1B) - *Organ weights and histopathology findings:* Prostate: significant decrease of absolute (-18.3%) and relative (-17.2%) weights at 600 mg/kg bw/day. No histopathology findings associated. The number of pregnant females was: 18, 19, 19 and 20 in control, low (50 mg/kg bw/day), mid-dose (200 mg/kg bw/day) and high dose (600 mg/kg bw/day) groups, respectively. No statistically significant effect on oestrous cycle, reproductive index, spermatogenic cycle, ovarian follicle number, gestation length or number of implantation. See table 14 for developmental toxicity	

Table 8: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents) No deviations GLP Reliability 1 (DS assigned reliability) 15 animals per sex at dose-levels of 0 and 600 mg/kg bw/day and 10 animals per sex at dose-levels at 25 and 150 mg/kg bw /day.	DAA (purity confidential)	Sprague-Dawley rats Male/Female Oral gavage, daily 0, 25, 150 and 600 mg/kg bw/day in corn oil 13 weeks followed by a 6-week recovery period (for the control and the high dose group)	General toxicity - *Mortality:* no effects - *Clinical signs:* no treatment-related effects. - *Body weight and body weight gain:* At 600 mg/kg bw/d: statistically significantly lower mean body weight in males from week 10 (-9%) and lower body weight gain (-13%) all over the treatment period (partial recovery after treatment free period). No effect in females. -*Hematology:* significantly higher neutrophil count at 600 mg/kg bw/d in males; significantly lower red blood cell count from 150 mg/kg bw/d in females associated with lower haemoglobin concentration and packed cell volume at highest dose. Significantly lower white blood cell with lower lymphocytes count in females at 600 mg/kg bw/d. These effects were reversible after recovery	Unnamed, 2017. Study report

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			period. - *Biochemistry:* higher cholesterol concentration in both sexes at 600 mg/kg bw/d (reversible after recovery period). - *Organ weights and histology*: **Liver:** significant increase of absolute and relative liver weight in males (+20% and +31%, respectively) and in females (+18% and +12%) associated with minimal to sligh centrilobular hypertrophy and vacuolation in males and females at 600 mg/kg bw/day. (completely reversible after recovery period). **Kidney:** in males, there was a higher incidence of grade 3 or 4 tubular hyaline droplets from 25 mg/kg bw/day compared to controls. At 600 mg/kg bw/day, statistically significant increase of absolute and relative kidney weights were recorded for males receiving the test item at the highest dose (+16% and +26%, respectively). These changes correlated with microscopic tubular hyaline droplets (consistent with a2u-globulin) and tubular basophilia and granular casts. (almost completely reversible after recovery period). In females, a statistically significant increase in absolute but not in relative kidney weight was detected in animals given 150 (+13%) or 600 mg/kg bw/day (+14%) with no microscopic correlates. **Adrenals**: significant increase of absolute and relative weights (+26% and +38%, respectively) associated with minimal to moderate vacuolation of cortical cells in males at 25 and 600 mg/kg bw/d (not reversible during recovery period). <u>Oestrous Cycles</u> 600 mg/kg bw/day: non significant decreased number of cycles (2.4 vs 3.5 in the control group) with a longer cycle length (7.8 days vs 4.7 in the control group) in the treatment period. A the end of the treatment-free period, the cycle length was 5.2 at 600 mg/kg bw/day vs 4.0 in the control group.	

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			Seminology Mean epididymal sperm motility and morphology, mean testicular sperm head and the daily sperm production rate: no effects at 25 and 150 mg/kg bw/day. 600 mg/kg bw/day: non significant decrease of mean epididymal sperm counts (-10% as number/cauda and -11% as number/g cauda) compared to control at the end of treatment. Similar effects were reported at the end of treatment-free period. No effect reported in weight and histopathology of the reproductive organs.	
OECD Guideline 412 (Subacute Inhalation Toxicity: 28-Day Study). No deviations. No GLP compliant Reliability 2 (Registrant assigned reliability) 12 animals/sex/dose	DAA (purity confidential)	Wistar rats Male/Female Inhalation: vapour (whole body) 50, 225 and 1000 ppm = 233, 1041 and 4685 mg/m^3 6 hours, 5 days/ week 6 weeks	Histopathology examined: ovaries or testes, uterus or prostate and seminal vesicles. No results described	Unnamed, 1979, 1980. Study report

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

1- An OECD TG 422 combined reprotoxicity study (Unnamed 1997)

The reproductive toxicity of diacetone alcohol (DAA) was evaluated in a combined oral repeated-dose toxicity and reproductive/developmental toxicity screening test (OECD TG 422) in Sprague Dawley rats. Dossier Submitter (DS) only has access to the English summary of the study report, in addition to the disseminated registration dossier.

DAA was administered at dose levels of 0 (water vehicle), 30, 100, 300, or 1000 mg/kg bw/day. Males were exposed for 44 days, from 14 days before starting to mate, and females until day 3 of lactation after delivery (41~45 days).

One female of the highest tested dose (1000 mg/kg bw/day) was euthanized around delivery. Vaginal hemorrhaging was noted during the evening of the expected delivery date in this animal but at necroscopy, nothing abnormal was noted. Clinical findings in parental animals included decreased locomotion and

decreased response to stimulation at 300 mg/kg bw/day (13/20 animals and 10/20 animals respectively) and at 1000 mg/kg bw/day in all animals.

Reduced premating body weight gain was noted in the high-dose females. No change was reported in males and in females in other phases. No effect was observed on mean body weight. Numerical values is not available to DS in order to check these conclusions.

Results of hematology and blood chemistry in males revealed statistically increases in platelet count (+16%), glutamic oxalacetic transaminase (+49%), choline esterase (+178%), total protein (+10%), total cholesterol (+76%), total bilirubin (+33%), blood urea nitrogen (+19%), creatinine (+17%), and calcium (+6%), as well as a statistically decrease in glucose (-23%), at 1000 mg/kg bw/day.

In males, statistically significant increases in absolute (+15%) and relative (+16%) kidneys weights were noted at 300 mg/kg bw/day. There was also an increase at 1000 mg/kg bw/day but not statistically significant. Statistically significant increased relative liver weight (+18%) and absolute and relative adrenal weights (+21% and +25%) were noted in the 1000 mg/kg bw/day in males.

Histological evaluation of kidney tissues in males identified an increased severity of hyaline droplets accumulation in the proximal tubular epithelium at 100 mg/kg bw/day or more, and basophilic tubules at 300 and 1000 mg/kg bw/day. Centrilobular hepatocellular hypertrophy was noted in the liver of males at 1000 mg/kg bw/day (5/10). Vacuolization of the cells of the zona fasciculata were noted in the adrenals of males at 1000 mg/kg bw/day (5/10).

In females, there was a statistically significant increase in absolute (+26%) and relative (+25%) liver weights at 1000 mg/kg bw/day. This was associated with significant centrilobular hepatocellular hypertrophy in 6/10 females.

No statistically significant effect was reported in the reproductive parameters. However, a non statistical reduction was found in fertility index, number of implantations, and implantation index at the 1000 mg/kg bw/day dose level. Statistically significance may not have been reached due to the high standard deviation at this dose. DS does not have access to individual data.

Table 9. Fertility data in females of the OECD TG 422 study

Dose (mg/kg bw/day)	0	30	100	300	1000
Number of pregnant females	9	10	8	9	6
Fertility index (%)	90	100	80	90	60
Number of implantation sites (mean ±SD)	16.6 ± 1.3	17.4 ± 1.3	17.8 ± 1.5	18.1 ± 1.5	14.2 ± 6.1
Implantation index (% ±SD)	97.3 ± 6.1	95.4 ±5 .8	96.3 ± 8.4	98.2 ± 3.8	79.0 ± 32.4
Number of pregnant females with parturition	9	10	8	9	5

Data are expressed as % or mean ± SD

Fertility index =[(number of pregnant animals/number of pairs with successful copulation) x 100

Implantation index = (number of implantations/number of corpus luteums) x 100

Developmental effects are reported and described in section 10.8.4.

2- An OECD TG 421 fertility study preliminary to OECD TG 443 (Unnamed , 2020)

This study was performed according to OECD Guideline no. 421 and in compliance with GLP. All doses (0, 50, 250 and 750 mg/kg/ bw/day) were administered orally, by gavage. The control group received corn oil.

Males were treated daily for 2 weeks prior to pairing and during pairing with females until the day before necropsy, for a total of 29 or 30 days. Females were treated for 2 weeks prior to pairing, during pairing up to

Day 13 *post coitum*. The non pregnant females and the females sacrificed for humane reasons were dosed up to the day before necropsy.

One female receiving 250 mg/kg bw/day was sacrificed for humane reasons on Day 23 of gestation, since difficulty in parturition was noted. No significant clinical signs were observed throughout the study in all treated animals of both sexes.

No relevant difference in body weights and body weight gain were recorded in treated males compared to the control group. Transient decrease in body weight gain on Day 7 *post partum* was noted in all treated females with no clear dose-response relationship (-56%, -66%, -25% at 50, 250 and 750 mg/kg bw/day, respectively – not statistically significant; details available in Annex I). Looking at the individual data, a transient decrease in the body weight was evident in some females in all treated groups. After this, a recovery was noted and on Day 13 *post partum* there was an increase in body weight gain in treated groups, that was significant (+250%) only at 250 mg/kg bw/day, when compared to control (see details in Annex I). Overall, no significant adverse effect on mean body weight was reported in females.

On Days 7 and 13 *post partum*, food consumption was statistically significantly decreased in females receiving 750 mg/kg bw/day (down to -18%). At all dose levels, no effects on food consumption were observed in treated males during the study.

No changes were observed on absolute and relative organ weights of treated animals that completed the treatment. Liver and kidney weights were not examined but histological changes were observed at microscopic level.

Hepatocytic hypertrophy in males (10/10) and females (9/10) (compared to 0/10 in both sexes of the control group) and hepatocytic vacuolization (6/10) in males (compared to 1/10 in control group) at 750 mg/kg bw/day were noted.

Concerning the kidneys, there was an increased severity of hyaline droplets accumulation in males at 250 and 750 mg/kg bw/day, from mild to moderate degree compared to controls (hyaline droplet also reported for all control males but with minimal severity). Increased incidence of inflammatory cell foci (7/10) at 750 mg/kg bw/day was observed (compared to 0/10 in the control group). Even if not statistically significant, nephropathy was more frequently observed at 750 mg/kg bw/day (8/10 versus 3/10 in control group).

No effects were noted in the spermatogenic cycle. The total number of oestrous cycles observed in females before pairing, the pre-coital intervals, the copulatory index and fertility index did not show any differences between control and treated groups. Gestation length was similar between treated and control groups.

Corpora lutea and number of implantation sites of treated females were comparable to the control values.

Developmental effects are reported and described in section 10.8.4.

3- An extended one generation study with F2 extension (Unnamed, 2020 - OECD TG 443)

The effects of DAA on the reproductive and developmental toxicity were investigated in an OECD Guideline no. 443 study in compliance with GLP.

Males and females Sprague-Dawley rats (25/sex/group) were given DAA at doses levels of 0, 50, 200 or 600 mg/kg bw/day, by gavage, 7 days per week, for 2 weeks prior to mating. The control group received corn oil. For male animals, the administration continued for at least 10 weeks (68-72 days). Treatment of females continued throughout the mating, gestation and lactation periods up to Day 21 *post partum*. At weaning (post natal Day 21), F1 pups/sex/group were randomly selected to serve Cohort 1A (20/sex/group) and Cohort 1B (20/sex/group). For each group, one or two male and female pups were selected from each litter. The treatment of selected pups started on the day of selection, Day 21 of age. Pups of Cohort 1A were given the test item for at least 10 weeks and then sacrificed at 13/14 weeks of nominal age. Pups of Cohort 1B were given the test item for at least 10 nominal weeks before pairing, and then animals were mated. Treatment of Cohort 1B males continued during the mating period up to 17 nominal weeks. Treatment of Cohort 1B females continued during the mating period and up to Days 21/22 *post partum*.

General toxicity (Parental generation, Cohorts 1A and 1B)

In parental generation (P0), unscheduled deaths occurred in one male and in one female receiving 50 mg/kg bw/day. In cohort 1B, one male was found dead in the control group, and one female was killed due to human reason at 200 and at 600 mg/kg bw/day.

In P0 and Cohort 1A animals, no effect on body weight was recorded but changes in mean body weight gain occurred.

In P0 animals, a statistically significant decrease in mean body weight gain was noted in males on Day 20 of mating phase (-19%) and in females (-45%) on Day 14 of lactating phase, at 600 mg/kg bw/day group (details on body weight gain available in Annex I). Decreases in food consumption were only observed in females on PND (post-natal day) 14 in the dose levels ≥ 200 mg/kg bw/day, with a statistically significant difference only at 200 mg/kg bw/day (around -6%).

In Cohort 1A animals, statistically significant decreases in mean body weight gain occurred in males on PND 56 (-11%) and on PND 70 (-19%) at 600 mg/kg bw/day. For females receiving 50 mg/kg bw/day a significant increase of body weight gain on PND 63 (+29.9%) was noted. Significant reductions in food consumption were observed in males receiving 200 mg/kg bw/day on PND 56 (-9%) and in all treated males on PND 77, 84, 91 (-5% to -12%).

In Cohort 1B animals, statistically significant decreases of body weight on PND 35 (-7.7%) and PND 42 (-7%) before pairing were observed in males as well as significant decreases of body weight gain on PND 35 (-12%) and PND 91 (-22.3%) followed by an increase on PND 134 (+62.5%), at 600 mg/kg bw/day. A statistically significant increase of body weight gain on PND 77 (+22.3%) was observed at 200 mg/kg bw/day. In females, no effects on body weight were observed but there were statistically significant increases of mean body weight gain on GD (gestation day) 14 (+18.2%) at 200 mg/kg bw/day and on LD (lactation day) 7 (+103.1%) at 600 mg/kg bw/day (table available in Annex I). Food consumption was modified only in females, with a statistically significant decrease (-7%) on PND 14, at 600 mg/kg bw/day.

Changes in haematology parameters were observed. There was a significant decrease of haemoglobin (-5% in P0 and -7% in 1B cohort) at 600 mg/kg bw/day in males. This was associated in cohort 1B with significant decreases of mean corpuscular Hb (-5%) and mean corpuscular Hb concentration (-3%) at the same dose level in males. There was a significant increase of neutrophils (+6%) at 600 mg/kg bw/day in the cohort 1B males. In Cohort 1A animals, only effects on monocytes were observed. A statistically significant decrease in males dosed at 200 and 600 mg/kg bw/day (-38% and -31%, respectively) was observed. There was no statistically significant change when considering monocytes %. In Cohort 1A females, monocytes were lower than controls at 600 mg/kg bw/day (-32%), even if not statistically significant.

Changes in coagulation parameters were consistently observed among generations. Partial thromboplastin time was statistically significantly reduced in P0 males (-16%) and females (-8%), in Cohort 1A males (-11%) and Cohort 1B males (-14%) at 600 mg/kg bw/day group and in Cohort 1B males at 200 mg/kg bw/day (-10%). Additionally, prothrombine time was reduced (-4%) in Cohort 1B males at 600 mg/kg bw/day group.

Results of clinical chemistry revealed statistically changes in all generations consistent with liver alterations at 600 mg/kg bw/day. Significant increases of cholesterol were found in P0 males (+52%) and females (+31%), in Cohort 1A males (+32%), in Cohort 1B males (+49%) and females (+27%). In addition, there was a significant increase of total protein (+5% in P0 males, +6% in F1B males) and globulin (+9% in P0 males). Triglycerides were also increased in P0 and Cohort 1A animals, reaching statistically significance in Cohort 1B males at 200 and 600 mg/kg bw/day (+ 92% and + 143%). Triglycerides were higher than controls in some Cohort 1B females treated at 200 and 600 mg/kg/day (+47% and +55%, respectively), even if changes were not statistically significant. Glucose was significantly decreased in P0 females (-11%) and Cohort 1A males (-17%). In cohort 1B males, there were also a significant increase of albumin (+5%), creatinine (+29%) and calcium (+6%), and a significant decrease of aspartate aminotranferase (-27%) and chloride (-3%). In Cohort 1B females, there was a significant increase of alanine aminotransferase (+20%) and bilirubin (+72%). These modifications were associated with increased liver weight as well as hypertrophy at 600 mg/kg bw/day.

Urinalysis findings showed increased ketonuria in P0, Cohorts 1A and 1B males with a dose-related trend at 200 and 600 mg/kg bw/day while females of the same groups showed no effect or only a minimal increase. No detailed data are available in the study report. In Cohort 1B males, increases of urine volume at 200 mg/kg bw/day (+142%) and at 600 mg/kg bw/day (+222%) were observed.

Thyroid hormones measurement showed statistically significant increases of T3 level in P0 males (+36.7%), in Cohort 1B males (+47.7%) at 600 mg/kg bw/day and in Cohort 1B females (+22.9%) at 200 mg/kg bw/day. In Cohort 1B animals, there were also a dose-related increased trend of T4 in males (9%, 16%, 24%) and dose-related decreased trend of TSH in males (-48%, -50%, -59%) and in females (-14%, -50, -59%). Additionally, in Cohort 1B males significant increases of absolute (12.1%) and relative (+10.6%) thyroid weight were observed. These changes were not associated with histopathological findings in the thyroid.

Statistically significant increases in absolute (+16.2%) and relative (+16.2%) mean liver weights in P0 males, in relative mean liver weight of Cohort 1A males (+19.4%) and Cohort 1B males (+17.0%), were recorded at 600 mg/kg bw/day. These changes were associated with hepatocellular hypertrophy in 14/25 of P0 males and in 14/20 of Cohort 1A males, at 600 mg/kg bw/day. No histological analyses were performed for Cohort 1B despite the changes in liver weight. No histopathological liver alterations were noticed in any of the generation for females.

There were statistically significant increases in absolute and relative kidneys weights in P0 males (+22.6%/+21.5%) and in P0 females (+7.3%/8.5%) dosed at 600 mg/kg bw/day. Statistically significant increases of relative kidneys weight were observed in Cohort 1A males (+8.3%) and females (+6.5%) dosed at 200 mg/kg bw/day and in absolute and relative kidneys weight of Cohort 1A males (16.5%/+22.2%) and in relative kidneys weight of Cohort 1A females (+10%) dosed at 600 mg/kg bw/day. Similarly to P0 and Cohort 1A generations, statistically significant increases in absolute and relative kidneys weights for Cohort 1B males (+12.7%/+14.2%) and females (+6.5%/+7.5%) receiving the test item 600 mg/kg bw/day were noted. These modifications of weights were associated with an increased severity of hyaline droplets accumulation, from mild to moderate in kidneys in all P0 males at 600 mg/kg bw/day and in Cohort 1A males dosed at 200 mg/kg bw/day (8/20) and 600 mg/kg bw/day (all males). Nephropathy was also reported in P0 males (13/25) and Cohort 1A males (9/20) at 600 mg/kg bw/day. Kidney histology was not examined in Cohort 1B generation. No histological damages in kidneys of females were found.

Table 10. Liver and kidney toxicities in males of the OECD TG 443 study

Parameters	P0				C1A				C1B			
	Dose (mg/kg bw/day)				Dose (mg/kg bw/day)				Dose (mg/kg bw/day)			
	0	**50**	**200**	**600**	**0**	**50**	**200**	**600**	**0**	**50**	**200**	**600**
Cholesterol (% compared to control)	0	-6.3%	+5.2%	**+52.3%****	0	-16.4%	+6.0%	**+31.6%****	0	+3.3%	+13.5%	**+49.2%****
Triglycerides (% compared to control)	0	+55.1%	+27.5%	+62.8%	0	+16.7%	-4.4%	-18.2%	0	+42.3%	**+92.0%****	**+142.9%****
Ketonuria (no group mean data and/or raw data)	0	No effects	↑	↑	No effects	No effects	↑	↑	No effects	No effects	↑	↑
Liver absolute weight (% compared to control)	0	-6.3%	+1.1%	**+16.2%****	0	-3.2%	-3.7%	+14.0%	0	-5.7%	+8.6%	**+20.1%**
Liver relative weight (% compared to control)	0	-4.0%	-0.2%	**+16.2%****	0	-2.8%	-0.8%	**+19.4%****	0	-5.7%	+8.1%	**+17.0%***
Hepatocyte hypertrophy (number of affected animals/examined animals)	0/25	0/24	2/25	**14/25***	0/20	0/20	1/20	**14/20***	-	-	-	-
Kidneys absolute weight (% compared to control)	0	-0.7%	+6.5%	**+22.6%****	0	+2.1%	+4.7%	**+16.5%****	0	-1.7%	+2.5%	**+12.7%***
Kidneys relative weight (% compared to control)	0	+1.1%	+4.7%	**+21.5%****	0	+3.2%	**+8.3%***	**+22.2%****	0	-5.8%	+4.0	**+14.2%****
Hyaline droplet accumulation (number of affected animals/examined animals)	25/25	24/24	25/25	**25/25***	20/20	20/20	**20/20***	**20/20***	-	-	-	-
Nephropathy (number of affected animals/examined animals)	0/25	1/24	3/25	**13/25***	0/20	0/20	2/20	**9/20***	-	-	-	-

*/**: Differences with control at p≤ 0.05/0.01 are in red and **bold**. -: not examined. P0: parental generation; C1A: cohort 1A; C1B: cohort 1B

Increases in adrenals weight appeared in males from all generations: increases in absolute adrenals weight in P0 males at 200 mg/kg bw/day (+11%), in absolute (+15.6%) and relative (15.4%) adrenals weight in P0 males at 600 mg/kg bw/day and in absolute (+14.0%) and relative (20.0%) adrenals weight in Cohort 1A males at 600 mg/kg bw/day. There were no microscopic organs damages.

Other organs weights were significantly modified at 600 mg/kg bw/day such as, a decrease in absolute weight of mesenteric nodes in Cohort 1A males (-29%) and females (-31%); decreases of absolute (-14%) and relative (-10%) heart weights in Cohort 1A females; increases of absolute (+12.1%) and relative (+10.6%) thyroid weights of Cohort 1B males. These modifications were not associated with histopathological findings.

Reproductive toxicity (Parental Generation, Cohort 1A and 1B)

In P0 males, epididymides and seminal vesicles weights were statistically significantly decreased at 200 and 600 mg/kg bw/day. Similar effects were found in Cohort 1A, but without statistically significance. Statistically significant decrease in prostate weight was found in Cohort 1B at 600 mg/kg bw/day. There were no microscopic damages in these organs.

Additionally, no statistical significant effects occurred on sperm parameters. Nevertheless, non significant increases of abnormal head spermatozoa were reported in P0 and Cohort 1A males. Headless spermatozoa was decreased at 50 mg/kg bw/day in both generation in P0 and Cohort 1A and at 200 mg/kg bw/day in P0 males. At higher doses, increase of headless spermatozoa was observed in both generation. The increase in P0 males at 600 mg/kg bw/day is mainly caused by one animal with extreme values. These effects are not dose-related. No anomaly in the different stages of spermatogenic cycle was observed.

The effects are described in the table below.

Table 11. Male reproductive organ weights and sperm parameters of the OECD TG 443 study

	P0				C1A				C1B		
	Dose (mg/kg bw/day)				Dose (mg/kg bw/day)				Dose (mg/kg bw/day)		
	0	50	200	600	0	50	200	600	50	200	600
Epididymides (left) abs weight	-	-5.0%	**-5.6%***	**-8.3%***	-	-0.6%	+1.6%	-8.1%	-0.5%	-3.0%	+1.4%
Epididymides (left) rel weight	-	-3.0%	**-6.7%***	**-9.1%****	-	+0.9%	+5.8%	-3.7%	+0.3%	-3.8%	+2.9%
Epididymides (total) abs weight	-	-4.3%	-4.5%	**-7.6%***	-	+0.01%	+1.1%	-8.3%	+0.4%	-2.2%	+0.9%
Epididymides rel weight	-	-2.5%	-5.7%	**-8.5%***	-	+1.5%	+5.2	-3.9%	+0.1%	-3.2%	+2.3%
Seminal vesicles abs weight	-	-2.5%	-10.6%	**-12.6%***	-	-3.7%	-0.7%	-10.7%	+2.7%	-7.5%	-1.4%
Seminal vesicles rel weight	-	-0.6%	-11.1%	**-13.1%***	-	-3.0%	+2.7%	-6.5%	+1.2%	-8.5%	-1.5%
Prostate abs weight	-	**-14.6%***	+1.5%	-5.3%	-	-	-	-	-9.3%	-10.4%	**-18.3%****
Prostate rel weight	-	-12.2%	-3.3%	-5.7%	-	-	-	-	-9.8%	-11.4%	**-17.3%***
Sperm parameters											
Motility	-	No effects	No effects	No effects	-	No effects	No effects	No effects	-	-	-
Concentration	-	No effects	No effects	No effects	-	No effects	No effects	No effects	-	-	-
Morphology: *Abnormal head*	17.48 ± 9.41	22.79 ± 12.69	18.56 ± 10.06	28.08 ± 24.08	27.35 ± 14.35	27.30 ± 13.94	33.55 ± 14.65	34.20 ± 19.70	-	-	-
Morphology: *Headless*	1.04 ± 1.81	0.67 ± 1.27	0.60 ± 1.08	2.56 ± 5.16	0.45 ± 1.00	0.10 ± 0.45	0.70 ± 1.08	0.55 ± 1.05	-	-	-
Differents stages of spermatogenic cycle	-	No effects	No effects	No effects	-	No effects	No effects	No effects	-	-	-

*/**: Differences with control at p≤ 0.05/0.01 are in red and **bold**. -: not assessed.

In F0 generation, a total of 3 females were found not pregnant at necropsy: one female receiving 200 mg/kg bw/day and two receiving 600 mg/kg bw/day.

Regarding reproductive index, the copulatory index was unchanged in any generation by treatment of the test item. The fertility index was slightly, but non statistically significantly, reduced in P0 animals to 96% and 92%, at 200 and 600 mg/kg bw/day respectively, compared to 100% in control animals. However, the fertility index was not affected in Cohort 1B treated animals. After request for historical control data (HCD), data from 4 OECD TG 443 studies were provided by the registrant but none of the studies were conducted with the same breeder and/or vehicle than those in the OECD TG 443 with DAA. Thus, DS considers HCD not relevant for analysis. Overall, DS considers the decrease of fertility index in P0 animals cannot be clearly attributed to the treatment since the decrease was only observed in the P0 generation and was not statistically significant.

Table 12. Fertility data of the OECD TG 443 study

	P0				C1B			
Males / Females	**0**	**50**	**200**	**600**	**0**	**50**	**200**	**600**
Fertility index (%)	100	100	96	92	90	95	100	100

There was no treatment-related significant effect on oestrous cycle, ovarian follicle count, gestation length or number of implantation.

Developmental effects are reported and described in section 10.8.4.

4- Information from other studies

Other studies that can bring additional information regarding reproductive toxicity potential of DAA are available. Information below was issued from the disseminated registration dossier:

OECD TG 408 study (Repeated Dose 90-Day Oral Toxicity Study in Rodents)

DAA was administered daily for 13 weeks by gavage to male and female Sprague-Dawley rats at the dose-levels of 0, 25, 150 and 600 mg/kg bw/day in corn oil (Unnamed, 2017). On completion of the treatment period, designated animals of the control- and high-dose groups were held for a 6-week treatment-free period in order to evaluate the reversibility of any findings. The study was performed based on the OECD guideline No. 408 and in compliance with the Good Laboratory Practices. Regarding reproductive parameters, weight and histology of reproductive organs and estrous cycle stage and seminology investigations were performed at the end of the treatment or treatment-free period for recovery animals.

At the end of the treatment period, the number of cycles measured during a period of 21 days in the high dose group was slightly lower (not statistically significant) than in control group, due to a higher number of females which remained in diestrous during several consecutive days (6/10 vs. 2/10 in the control group), leading to longer cycles. This was not associated with effects on weight and/or microscopic effects on female reproductive organs. In the high-dose group, 1/5 females remained in estrous for 13 consecutive days before returning to normal. A normal cycle started from Day 18 for this female.

At 600 mg/kg bw/day and when compared with controls, there was a non significant decrease of mean epididymal sperm counts (-10 and -11%, as number/cauda and number/g cauda, respectively) at the end of the treatment period. At the end of the treatment-free period, there were still lower mean epididymal sperm counts (-14 and -5%, as number/cauda and number/g cauda, respectively). This was not associated with effects of weight and/or microscopic finding in the testis and epididymidis.

Regarding general toxicity, no mortality or clinical signs related to the treatment occurred. Lower body weight from week 10 (-9%) and lower body weight gain (-13%) all over the treatment period in males at 600

mg/kg bw/day were recorded. Body weight gain returned to normal at the end of the treatment-free period, suggesting reversibility. No effects were observed in females.

At hematology, a slightly higher neutrophil count was noted in males treated at 600 mg/kg bw/day (+53%). In females, mean red blood cell count was statistically significantly decreased at 150 and 600 mg/kg bw/day when compared with controls (up to -6%) and was associated with lower hemoglobin (-5%) and packed cell volume (-7%) at 600 mg/kg bw/day. Lower total white blood cell (-24%) and lymphocyte counts were also noted (-26%) at the highest dose. These effects were reversible after recovery period.

At blood biochemistry, in both sexes, moderately higher cholesterol concentration was noted at 600 mg/kg bw/day (+40 % for males and +43% for females). It was reversible after the recovery period.

Test item administration at 600 mg/kg bw/day increased absolute and relative liver weight in both males (+20% and +31%, respectively) and females (+18% and +12%) associated with minimal to sligh centrilobular hypertrophy and vacuolation in males and females at 600 mg/kg bw/day. These effects were completely reversible after the recovery period.

In the kidneys, in males only, there were increased incidence and severity of tubular hyaline droplets (consistent with a2u-globulin), tubular basophilia and granular casts, from 25 mg/kg bw/day, which correlated with increased kidney absolute (+16%) and relative (+26%) weights at 600 mg/kg bw/day. In females, a statistically significant increase in absolute but not in relative kidney weight was detected in animals given 150 (+13%) or 600 mg/kg bw/day (+14%) with no microscopic correlates.

The mean absolute and relative weights of the adrenal glands were statistically significantly increased (+26% and +38%, respectively) in males at 600 mg/kg bw/day. These changes were mainly due to higher weights in two individuals. Furthermore, in females, mean absolute and relative values were also above those of controls (+17% and +11%, respectively) at the highest dose, but these differences were not statistically significant. There was no microscopic correlates.

Equivalent OECD TG 412 study (Subacute inhalation study)

A 6-week whole body inhalation study was conducted with DAA in male and female Wistar rats. Animals were exposed to analytical concentrations of 0, 233, 1041 and 4685 mg/m^3 of DAA for 6 hours per day for 5 days per week for 6 weeks (Unnamed, 1979, 1980). This non-GLP study was equivalent to an OECD Test Guideline 412. Histopathology examinations were performed on ovaries or testes, uterus or prostate and seminal vesicles. No results were described for these organs.

10.8.3 Comparison with the CLP criteria

For classification on sexual fertility and reproduction the CLP criteria have been followed. Adverse effects on sexual function and fertility are defined as "*any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*".

According to the CLP criteria for Category 1A for known human reproductive toxicant, Annex I: 3.7.2.1.1 of CLP Regulation 1272/2008: "*The classification of a substance in this Category 1A is largely based on evidence from humans*."

Classification of diacetone alcohol in Repr. 1A is not fulfilled as no human data are available.

Regarding the CLP criteria for Category 1B for presumed human reproductive toxicant, Annex I: 3.7.2.1.1 stipulates: "*The classification of a substance in this Category 1B is largely based on data from animal*

studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects".

Considering the CLP critera for Category 2 for suspected human reproductive toxicant, Annex I: 3.7.2.1.1 stipulates: "*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects*."

Three experimental studies have investigated the effects of DAA in rats on reproductive function. The screening studies were performed at doses up to 750 mg/kg bw/day for the OECD 421 study and 1000 mg/kg bw/day for the OECD TG 422 study. The OECD TG 443 study, including production of a second generation, is available with DAA using doses levels up to 600 mg/kg bw/day. **No systemic overt toxicity has been observed at the highest dose in the OECD TG 443, presuming that the dose level setting was not optimal, and higher doses could have been tested without compromising the study.**

Fertility index and number of implantations sites

In the OECD TG 422 study, non statistically significant decreases in fertility index and number of implantation sites at 1000 mg/kg bw/day were recorded. However, the decrease of implantation sites number is weak and comparable to the control in C1B generation in the OECD TG 443 study. In the OECD TG 421 and OECD 443 studies, no such effects were observed.

Table 13. Comparative data of diacetone alcohol toxicity on fertility parameters

OECD TG study	443								422					421			
Generation	P0				C1B				P0					P0			
Doses (mg/kg bw/day)	**0**	**50**	**200**	**600**	**0**	**50**	**200**	**600**	0	**30**	**100**	**300**	**1000**	0	**50**	**250**	**750**
Fertility index (%)	100	100	96	92	90	95	100	100	90	100	80	90	60	90	100	100	100
Number of implantation sites (mean ±SD)	17.0 ± 1.35	16.83 ± 2.67	16.17 ± 4.26	17.44 ± 3.69	14.8 ± 2.53	16.6± 1.46	16.6 ± 1.72	14.9 ± 3.63	16.6 ± 1.3	17.4 ± 1.3	17.8 ± 1.5	18.1 ± 1.5	14.2 ± 6.1	15.8	15.4	15.6	16.6

Regarding toxicity in parental animals at 1000 mg/kg bw/day in the OECD TG 422 study:

One female was euthanized around delivery due to humane reason.

All males and females presented a significant reduction of locomotor activity and of response to stimuli. A significant decrease of body weight gain (without impact on mean body weight) in females on pre-mating day 15 was recorded (numerical data not available). Furthermore, liver alterations were observed as indicated by significant changes in biochemistry, increases of liver weight and hepatocellular hypertrophy in males and females. Kidneys alterations were seen in males with increase of absolute and relative kidneys weight and increased severity of hyaline droplet accumulation.

In summary, non statistically significant effects were reported on fertility index and implantation parameters in the OECD TG 422 study but only at high dose (1000 mg/kg bw/day) which is higher than the doses used in other studies. These effects occurred in males and in females with reduced locomotor activity and response to stimuli at high dose. These effects were not reported in other studies and did not reach statistically significance in the OECD TG 422 study. Thus, it does not justify a classification for fertility.

Oestrous cycle

Effects on estrous cycles were reported from the OECD TG 408 study. Indeed, decreased number of cycles with a longer cycle length was observed in females dosed at 600 mg/kg bw/day. No microscopic lesions were reported in female reproductive organs No measurement of hormones levels was performed. These effects did not reach statistically significance, and were not reported in other studies (OECD TG 421 and 443). Thus, they do not justify a classification for fertility.

Male reproduction

Effects were reported on **reproductive organ weights** in males in the OECD TG 443 study. In particular, decreases in relative and absolute weights of epididymides (driven by left epididymides; about -9%) and seminal vesicles (about -13%) were seen in P0 animals at 600 mg/kg bw/day but without histopathological findings or with no influence on reproductive performance. The effects observed in P0 animals were not consistently observed between generations. Isolated decreased effects on prostate weight were observed on absolute weight at 50 mg/kg bw/day in P0 animals (about -15%) and on absolute and relative weight in Cohort 1B animals at 600 mg/kg bw/day (about -18%). No significant effects on reproductive organ weights occurred in the screening studies but these studies have a lower statistical power than an EOGRTS to identify reproductive effects.

About **sperm parameters**, non statistically significant modifications of morphology (abnormal head or headless spermatozoa) in P0 and Cohort 1A males of the OECD TG 443 study were observed. At the high dose, the effects seems to bo more severe in P0 animals but it was mainly due to one animal with extreme values. Overall, these effects were not dose related. They were not associated with anomaly in the different stages of spermatogenic cycle.

In the OECD TG 408 study, there was a non significant decrease of mean epididymal sperm counts at 600 mg/kg bw/day at the end of the treatment period (-10 and -11%, as number/**cauda** and number/g cauda, respectively) and the treatment-free period (-14 and -5%, as number/cauda and number/g cauda, respectively). This was not associated with effects of weight and/or microscopic finding in the testis and epididymidis. At this dose, general toxicity in males mainly consisted on lower body weight from week 10 (-9%) and lower body weight gain (-13%) all over the treatment period, reversible modifications in biochemistry as well as centrilobular hypertrophy and vacuolation.

In summary, effects on sperm parameters were observed in 2 different studies (OECD TG 443 and 408 studies). These effects did not reach statistically significance and were not associated with other altered parameters (such as histopathological lesion or effect on reproduction). These effects are thus not sufficient to warrant a classification for fertility.

Overall, these data are not sufficient to provide evidence of adverse effects on sexual function and fertility. In conclusion, classification for DAA is not warranted for fertility.

10.8.4 Adverse effects on development

Table 14: 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
OECD Guideline 422. (Combined	Diacetone alcohol (purity confidential)	Toxicity in dams (F0) - *Mortality:* one female in the 1000 mg/kg bw/day group was weakened during delivery and was euthanized once reaching a near	Unnamed, 1997. Study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) GLP compliance is not specified. Reliability 2 (DS assigned reliability) Sprague-Dawley rat Male/Female 10/sex/dose	Route of administration: oral (gavage) Vehicle: Japanese Pharmacopeia purified water. Doses levels: 0, 30, 100, 300, or 1000 mg/kg bw/day. Duration and frequency of test/exposure period: daily exposure, 44 days (males: from 14 days before stating to mate) and 41-45 days (females: until lactation day 3)	death state. - *Clinical signs:* decreased spontaneous locomotion (4/10 and 10/10) and less response to stimulation (5/10 and 10/10) in the 300 and 1000 mg/kg bw/day groups. - *Body weight*: significant reduction in the amount of weight gain during the premating period in females in the 1000 mg/kg bw/day group (no numeric value). No impact on mean body weight. - Statistically increase in the *weights (absolute and relative)* of the liver (+26% / 25%) in the 1000 mg/kg bw/day group. - *Histopathologically:* hepatocellular hypertrophy was noted in the liver in the 1000 mg/kg bw/day group (6/10 animals). <u>Offspring toxicity (F1)</u> - *Mortality/Viability:* Non statistically significant reduction in the 1000 mg/kg bw/day group for the overall *birth rate, delivery rate, number of live pups, live birth rate, number of live pups* at day 4 of lactation and *survival rate* at day 4 of lactation. - *Sex ratio:* Non statistically significant reduction in the 1000 mg/kg bw/day group (0.78) compared to control (1.07).	report
OECD Guideline 421. (Reproduction / Developmental Toxicity Screening Test) No deviations. GLP compliant Reliability 1 (DS assigned reliability) Sprague Dawley rats Male/Female 10/sex/dose	Diacetone alcohol (purity confidential) Route of administration – oral (gavage) Doses/concen-tration levels: 0, 50, 250, 750 mg/kg bw/day Duration and frequency of test/exposure period: daily exposure, 30-31 days (males) and until Day 13 *post partum* or the day before sacrifice (females)	<u>Toxicity in dams (F0)</u> - *Mortality:* one female from the 250 mg/kg bw/day group was sacrificed for humane reasons on Day 23 of gestation, since difficulty in parturition was noted. - *Body weight gain:* mean body weight gain was decreased in all treated groups on Day 7 *post partum* (-56%, -66%, -25% at all doses, respectively, not statistically significant). Increased in all groups with significant increase by 2.5-fold of body weight gain per day on Day 13 *post partum* at 250 mg/kg bw/day. No effect on mean body weight. *Corrected body weight gain during pregnancy:* no statistical significant decreases were recorded at 50 and 750 mg/kg bw/day, -14% and -15%, respectively. - *Food consumption:* on Days 7 and 13 *post partum*, a statistically significantly decrease of food consumption was recorded (down to -18%) at 750 mg/kg bw/day. - *Microscopic observations:* hepatocytic hypertrophy in 9/10 females at 750 mg/kg bw/day. <u>Offspring toxicity (F1)</u> **750 mg/kg bw/day** - *Pre-natal loss:* 20% versus 8% in the control group, without statistical significance.	Unnamed, 2020. Study report

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		- *Litter data:* Live litter size was gradually reduced (mean value), from birth with statistical significance on Day 13 *post partum* (6.30 versus 8 in the control group). In the same period, an non statistically significant increase in post-natal loss (7.73%) was also recorded at 750 mg/kg bw/day compared to the control group (0%). Starting from Day 1 *post partum* until termination, litter weight and mean pup weight were decreased when compared to the control group and they were statistically significant on Days 4 (litter weight, -31%) and 13 *post partum* (litter weight, -32% ; mean pup weights, -16%). - *Sex ratios of pups:* Starting from birth until Day 14 *post partum*, a non statistically significant decrease in the number of male pups (as absolute value or expressed as percentage) was noted at the dose level of 750 mg/kg bw/day (40.5% males) compared to the control group (51.0% males).	
OECD Guideline 443 (Extended One-Generation Reproductive Toxicity Study) including a 2nd generation Deviations: at arrival the body weight range of males was 224 to 263 g instead of 225-250 g. Mesenteric lymph nodes weights were performed in all females and not in 10 females per group. Cervical lymph nodes were performed in 10 males per group and not in all males. Reliability 1 (DS assigned	Diacetone alcohol (purity confidential) Route of administration – oral (gavage) Dose levels: 0, 50, 200 and 600 mg/kg bw/day for parental animals Parental males were treated once a day, 7 days a week for at least 2 weeks prior to pairing and during mating up to the day before sacrifice, for at least 10 weeks (68-72 days). Parental females were treated once a day, 7 days a week at least 2 weeks prior to pairing, during mating, gestation and *post partum* periods	Toxicity in dams (P0) - *Mortality:* unscheduled death of one female receiving 50 mg/kg bw/day. - *Body weight gain:* at 600 mg/kg bw/day, a slightly lower mean body weight gain was recorded on Day 7 *post coitum* (-10%). On Day 14 *post partum*, all dose levels showed a dose related decrease in mean body weight gain when compared to the control group (-40%, -43% and - 45%), significant only at 600 mg/kg bw/day. No effect on mean body weight. *Corrected maternal body weight gain:* non statistical significant decrease at 600 mg/kg bw/day (-13%) in P0 females and increase at 200 (+13%) and 600 (+11%) mg/kg bw/day in C1B females. - *Food consumption:* on Day 14 *post partum*, decreases in food consumption were observed in the dose levels ≥ 200 mg/kg bw/day, with a statistically significant difference only at 200 mg/kg bw/day (around -6%). - *Coagulation:* significant decrease in partial thromboplastin time (-8.0%) at 600 mg/kg bw/day. - *Clinical biochemistry:* significant increase of cholesterol (+31%) and decrease of glucose (-11%) at 600 mg/kg bw/day. - *Urinalysis:* increased ketonuria at 600 mg/kg bw/day. - *Organs weights:* *Kidneys:* significant increases in absolute and relative kidney weights (+7-8%) at 600 mg/kg bw/day. No histopathology findings associated. Offspring toxicity (F1)	Unnamed, 2020. Study report

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
reliability) GLP compliant Parental: 25/sex/group Sprague-Dawley Rats Male/Female Parental (P0) : 25/sex/group Cohort 1A and 1B: 20/sex/group	until the day before sacrifice (Day 21 *post partum*). <u>Cohort 1A:</u> Males and females were treated starting from Day 21 of age up to the day before necropsy (approximately 13/14 weeks of nominal age). <u>Cohort 1B:</u> Males were treated starting from Day 21 of age for at least 10 nominal consecutive weeks prior to pairing (on nominal Day 91) and during pairing up to the day before sacrifice (after the weaning of the majority F2 litters). Females were treated starting from Day 21 of age (Day 21 *post partum*) for at least 10 nominal consecutive weeks prior to pairing (on nominal Day 90), during mating, gestation and *post partum* periods until Days 21 *post partum* or the day before sacrifice.	- *Pre-natal loss:* non statistically significant increase at 200 (9.85%) and statistically significant increase at 600 mg/kg bw/day (12.06%) compared to control (4.30%). - *Post-natal loss at birth (based on live litter size):* non statistically increased at 600 mg/kg bw/day group (2.4% versus 0.2% in the control). - *Post-natal loss on Day 4 post partum (based on live litter size):* non statistically increased at 600 mg/kg bw/day group (6.8% versus 2.9% in the control). - *Mortality:* non statistically significant increase in the total number of lost pups at 600 mg/kg bw/day (mean: 1.4), when compared with control (mean: 0.6) between PND0-21. - *Anogenital distance (AGD) (*$mm/g^{1/3}$*):* Slight significant decreases in mean AGD (normalized) were noted in treated male and female pups (200 and 600 mg/kg bw/day) when compared to the control value: **Males:** Control (2.38), 50 mg/kg bw/day (2.37), 200 mg/kg bw/day (2.05), 600 mg/kg bw/day (2.00). **Females**: Control (1.34), 50 mg/kg bw/day (1.35), 200 mg/kg bw/day (1.11), 600 mg/kg bw/day (1.05). <u>Toxicity in dams (Cohort 1B)</u> - *Mortality*: one female was killed due to human reason at 200 and at 600 mg/kg bw/day. The female in this latter group was killed on GD22 and presented clinical signs coherent with the dystocia. - *Body weight gain:* significant increases at 600 mg/kg bw/day on LD7 (+103%) and at 200 mg/kg bw/day on GD 14 (+18%). - *Food consumption:* on Day 14 *post partum*, females dosed at 600 mg/kg bw/day showed a statistically significant decrease in food consumption (-7%), but as for P0 females the change was followed by an increment comparable to the control group. - *Clinical chemistry:* statistically significant increase of alanine aminotransferase (+20%), bilirubin (+72%) and cholesterol (+27%). Triglycerides were higher than controls in some females treated at 200 and 600 mg/kg/day (+47% and +55%, respectively), even if changes were not statistically significant. - *Urinalysis*: minimal increase of urinary ketones at 200 and 600 mg/kg bw/day. - *Thyroid hormones:* significant increase of T3 level (+23%) at 200 mg/kg bw/day. Decrease trend of TSH related to the dose (-14%, -50%, -59% respectively for 50, 200 and 600 mg/kg bw/day). - *Organs weights and histopathology findings:* <u>Liver:</u> non significant increases of absolute (+5.6%) and relative (+7.5%) weights at 600 mg/kg bw/day.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Kidneys: significant increases of absolute (+6.5%) and relative (+7.5%) weights at 600 mg/kg bw/day No histopathology findings associated with any organ. Offspring toxicity (F2) - *Pre-natal loss:* non significant increase at 200 mg/kg bw/day (8.79%), statistically significant increase at 600 mg/kg bw/day (12.98%) compared to control (6.80%). -*Post-natal loss at birth (based on live litter size in the study report):* non significant increase at birth (2.40% versus 0% in the control) at dose level of 600 mg/kg bw/day. - *Post-natal loss on day 4 post partum* (*based on total litter size in the study report, recalculated by DS with live litter size*): significant increase at dose level of 600 mg/kg bw/day (12.14%) versus control (0.68%). - *Total dead pups:* significant increases at 200 mg/kg bw/day (0.61) and 600 mg/kg bw/day (1.47) were recorded compared to control (0.17) between PND0-21. - *Anogenital distance (AGD*): contrary to F1 pups, no effects observed.	
OECD Guideline 414 (Prenatal Developmental Toxicity Study). No deviations. GLP compliant. Sprague Dawley Rats 24 females/dose Reliability 1 (DS assigned reliability)	Diacetone alcohol (purity confidential) Route of administration – oral (gavage) Doses levels: 0, 100, 300 and 1000 mg/kg bw/day Vehicle: Corn oil Duration and frequency of test /exposure period: daily exposure, Gestation Day 6 to Day 20	Toxicity in dams No mortality, no effect on body weight and on net body weight change - *Body weight gain*: at 1000 mg/kg bw/day, significantly higher mean body weight gain (+25 g vs. +20 g in controls, $p<0.05$) between GD 9-12, associated with increased food consumption, mainly due to 1 animal. - *Clinical signs:* excessive salivation (recorded as ptyalism) at 1000 mg/kg bw/day (6/24) compared to control (0/24). - *Organ weight:* Liver: a statistically significant increase was noted in mean absolute and relative liver weight value (+15.5%; +14.7%) at 1000 mg/kg bw/day Kidneys: a statistically significant increase was noted in mean absolute and relative kidneys weight value (+9.0%; +6.3%) at 1000 mg/kg bw/day. Offspring toxicity - *Fetal skeletal variations:* At 1000 mg/kg bw/day, 23/23 dams had fetuses with unossified or incomplete ossification of various part of the skeleton: hyoid (fetal	Unnamed, 2016b. Study report.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		incidence: 1.3% *vs.* 0%), lumbar vertebra: bipartite ossification of centrum (0.7% *vs.* 0%), caudal vertebra: unossified centrum (3.3% *vs.* 0%), extra stenebral ossification site (0.7% versus 0%), unossified 1st metacarpals (0.7% *vs.* 0%), incomplete ossification of metacarpals (11.1% *vs.* 10.5%), forepaw: unossified proximal phalanx (68.0% *vs.* 49.7%). These findings were associated with presence of cartilage - *Fetal skeletal malformations:* At 1000 mg/kg/ bw/day, one litter had a fetus with knobby ribs. This malformation was associated with other thoracic skeletal variations (thickened and wavy ribs).	
OECD Guideline 414 (Prenatal Developmental Toxicity Study). Deviations: some animals had 4 days of acclimation only instead of 5 days as recommended per guideline. Photoperiod (hrs dark / hrs light): 8h dark / 16h light. GLP compliant. Reliability 2 (DS assigned reliability) New Zealand White Rabbits: 24 females/dose	Diacetone alcohol (purity confidential) Route of administration – oral (gavage) Doses levels: 0, 100, 300 and 800 mg/kg bw/day Vehicle: water Duration and frequency of test /exposure period: daily exposure, Gestation Days 6 to 28 inclusive	<u>Toxicity in dams</u> - *Mortality:* At 800 mg/kg bw/day, one female was prematurely euthanized on GD 20. - *Body weight, weight changes and food consumption:* At 800 mg/kg bw/day, statistically significant mean body weight loss was noted at the beginning of the treatment period (-73 g on GD 6-9, $p<0.001$). Mean food consumption was lower, especially at the beginning of the treatment period (down to -40% on GD 6-9, $p<0.001$). This can be due to the female that was euthanized (body weight loss of 18% between GD6-9). Thereafter, mean body weight gains and mean food consumption returned towards control values in surviving pregnant females. - *Net body weight :* no significant effect <u>Offspring toxicity</u> - *Malformations*: 1 foetus with multiple malformation and 1 foetus with face malformation at 100 mg/kg bw/day. At 300 mg/kg bw/day: 2 foetus with dilated aorta. At 800 mg/kg bw/day: 5 foetus from 5 different litters with malformations: 1 foetus with dilated and overriding aorta, 1 foetus with omphalocele and dilated aorta, 2 foetus with meningoencephalocele, 1 foetus with split frontal. In addition, 2 foetus with tail shape abnormalities. - *Soft tissue examination:* At 800 mg/kg bw/day and when compared with controls, there were statistically significant increases in the numbers of fetuses with a small (5.4% versus 0% in the control) and/or pale spleen (4% versus 0% in the control). - *Skeletal examination*: Cartilage: From 300 mg/kg bw/day, there were increased incidences of non-ossified metacarpal bones and proximal phalanxes (only the respective cartilages were present) reaching statistical significance at 800 mg/kg bw/day. Variation: From 300 mg/kg bw/day onwards, there was a tendency towards a dose-dependent increase in the incidences of skeletal variations (interparietal, ribs and metacarpal bones) reaching	Unnamed, 2019. Study report.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		statistical significance at 800 mg/kg bw/day. The incidence of unossification or incomplete ossification of the metacarpal bones correlates with the cartilage findings.	

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

1- An OECD TG 422 combined reprotoxicity study (Unnamed 1997)

The reproductive toxicity of DAA was evaluated in a combined oral repeated-dose toxicity and reproductive/developmental toxicity screening test (OECD TG 422) in Sprague Dawley rats. DS only has access to the English summary of the study report, in addition to the disseminated dossier.

DAA was administered at dose levels of 0 (water vehicle), 30, 100, 300, or 1000 mg/kg bw/day. Males were exposed for 44 days, from 14 days before starting to mate, and females until day 3 of lactation after delivery (41~45 days).

A non significant decrease was observed in the 1000 mg/kg bw/day group for the overall number of pups born, delivery index, live birth index, viability index. In one 1000 mg/kg bw/day litter, no pups survived due to death or cannibalism. Statistically significance may be not reached due to the high standard deviation at this dose. DS does not have access to individual data. It can be questioned if the decrease in pups born is due to the decrease of implantation sites also reported in this study. However, decreases in delivery, live birth and viability index are also reported and these parameters are not dependent of the number of implantation sites. DS considers these effects are rather an effect on a development.

Additionally, a non significant decrease in the sex ratio (day not mentioned) of the 1000 mg/kg bw/day group compared to control was observed.

Table 15. Litter data of the OECD TG 422 study

Dose (mg/kg bw/day)	0	30	100	300	1000
Number of pups born	15.9 ± 1.1	16.4 ± 1.9	16.5 ± 1.6	16.9 ± 1.6	11.6 ± 6.3
Delivery index (%)	96.1 ± 4.0	94.1 ± 5.7	93.0 ± 4.9	93.2 ± 3.9	78.7 ± 26.7
Live birth index (%)	98.7 ± 2.6	98.8 ± 2.6	98.6 ± 2.7	97.4 ± 4.3	88.7 ± 21.8
Viability index (%)	99.2 ± 2.4	97.5 ± 4.3	98.6 ±2 .6	94.5 ± 10.6	69.4 ± 45.1
Sex ratio (day not mentioned) (Male/Female)	1.07	1.13	0.89	1.03	0.78

Data are expressed as mean ± SD except sex ratio.

Delivery index = (number of pups born/number of implantation sites) x 100

Live birth index = (number of live pups on day 0/number of pups born) x 100

Viability index = (number of live pups on day 4/ number of live pups on day 0) x 100

Sex ratio = total number of male pups/ total number of female pups

Regarding general toxicity in dams:

One female of the highest tested dose (1000 mg/kg bw/day) was euthanized around delivery. Vaginal hemorrhaging was noted during the evening of the expected delivery date in this animal. During necropsy, in addition to the one stillborn in the vagina, confirmation was made of 17 stillborns still in the uterus.

Clinical findings included decreased locomotion and decreased response to stimulation at 300 mg/kg bw/day (6/10 animals and 5/10 animals respectively) and at 1000 mg/kg bw/day in all animals.

There was no effect on body weight and body weight gain, except a reduced premating body weight gain in the high-dose females (no numerical value available). The corrected maternal body weight and body weight gain are not available and cannot be calculated (no numerical value available).

There was a statistically significant increase in absolute (+26%) and relative (+25%) liver weights at 1000 mg/kg bw/day. This was associated with significant centrilobular hepatocellular hypertrophy in 6/10 females.

2- An OECD TG 421 fertility study preliminary to OECD TG 443 (Unnamed, 2020)

This study design was performed according to OECD Guideline no. 421 and in compliance with GLP. All doses (0, 50, 250 and 750 mg/kg bw/day) were administered orally, by gavage. The control group received corn oil.

Males were treated daily for 2 weeks prior to pairing and during pairing with females until the day before necropsy, for a total of 29 or 30 days. Females were treated for 2 weeks prior to pairing, during pairing up to Day 13 *post coitum*.

A non significant increase of pre-natal loss was noted in females receiving 750 mg/kg bw/day when compared to the control value. Pre-natal loss data was particularly evident in two females. The effect was considered to be treatment-related in the study report and DS agrees with it, despite the lack of statistical significance.

Post-natally, there were a non significant decrease in live litter size associated with a non significant increase in post-natal loss at birth which continued during the lactation period at the dose level of 750 mg/kg bw/day. The decreased live litter size became statistically significant on Day *13 post partum*.

The total number of lost pups (found dead plus missing pups) was higher in females at 750 mg/kg bw/day group when compared to the control group. This is particularly evidenced in 3 females of this group.

Decreases in mean litter weight and mean pup weight were evident at 750 mg/kg bw/day compared to the control group, starting from Day 1 *post partum* until termination. They were statistically significant on Days 4 (litter weight, -31%) and 13 *post partum* (litter weight, -32% and mean pup weight, -16%).

Table 16. Litter data of the OECD TG 421 study

Dose (mg/kg bw/day)	**0**	**50**	**250**	**750**
Pre-natal loss %	8.08 ± 4.41	4.18 ± 3.85	4.79 ± 7.44	20.08 ± 27.19
	At birth			
Live litter size (nb)	14.56 ± 3.40	14.70 ± 4.97	14.78 ± 0.97	13.00 ± 5.54
Post-natal loss (%)	0.00 ± 0.00	0.63 ± 1.99	0.00 ± 0.00	7.73 ± 22.45
	Day 1 *post partum*			
Live litter size (nb)	14.33 ± 3.04	14.00 ± 5.14	14.33 ± 1.12	12.10 ± 5.49
Post-natal loss (%)	1.11 ± 3.33	5.50 ± 7.50	3.03 ± 3.61	7.48 ± 8.96
Litter weight (g)	105.63 ± 19.14	98.28 ± 35.88	99.82 ± 13.17	81.29 ± 32.92
Mean pup weight (g)	7.46 ± 0.88	7.15 ± 0.93	6.97 ± 0.61	6.93 ± 1.32
	Day 4 *post partum*			
Live litter size	14.33 ± 3.04	13.60 ± 5.13	14.00 ± 1.22	11.80 ± 5.25

Post-natal loss (%)	1.11 ± 3.33	8.12 ± 8.08	5.26 ± 5.47	9.50 ± 9.43
Litter weight (g)	152.67 ± 24.52	137.51 ± 46.23	136.51 ± 15.51	**106.10*** ± 42.70
Mean pup weight (g)	10.84 ± 1.58	10.49 ± 1.79	9.76 ± 0.96	9.53 ± 2.53
	Day 13 *post partum*			
Live litter size (nb)	8.00 ± 0.00	7.40 ± 1.26	8.00 ± 0.00	**6.30*** ± 2.41
Post-natal loss (%)	41.16 ± 16.72	39.25 ± 20.14	42.46 ± 5.10	38.90 ± 22.76
Post-natal loss (%) PND 0-13	41.65 ± 17.26	45.04 ± 15.74	45.65 ± 3.70	44.40 ± 21.85
Litter weight (g)	280.19 ± 29.55	247.24 ± 37.58	262.92 ± 19.71	**190.27*** ± 88.76
Mean pup weight (g)	35.00 ± 3.71	33.74 ± 3.28	32.88 ± 2.47	**29.47*** ± 4.71

Data are expressed as mean ± SD.

*: Differences with control at $p \leq 0.05$ are in red and **bold**. nd: not defined. After checking individual data, DS notes that values of post natal loss on day 13 *post partum* were not correct and were recalculated.

Post natal loss at Day 0 *post partum* was calculated as a percentage from the formula: (Total litter size – Live litter size) x 100 / Total Litter size.

Post natal loss at Day 1 *post partum* (before culling) was calculated as a percentage from the formula: (Live litter size at birth - live litter size at Day 1 (before culling) x 100 / Live litter size at birth.

Post natal loss at Day 4 *post partum* (before culling) was calculated as a percentage from the formula: (Live litter size at birth - live litter size at Day 4 (before culling) x 100 / Live litter size at birth.

Post natal loss at Day 13 *post partum* (after culling) was calculated as a percentage from the formula: (Live litter size on Day 4 (after culling) – Live litter size on Day 13) x 100 / Live litter size on Day 4 (after culling).

Post natal loss PND0-13 was calculated as a percentage from the formula: (Live litter size at birth – Live litter size on Day 13) x 100 / Live litter size at birth.

Starting from birth until Day 14 *post partum*, non statistically significant decreases in the number of male pups, % of males and ratio of male/female were noted at 750 mg/kg bw/day, compared to control. After DS request, registrants provided HCD from 13 OECD 421 studies. Among them, only four studies were conducted with the similar experimental conditions than the OECD TG 421 study with DAA, in particular with similar breeder and vehicle. Thus, DS only considers HCD from these studies in order to interpret the biological relevance of the effects on sex ratio. In all occasions the values for the number of male pups, recorded at 750 mg/kg/day, were below the incidences of the HCD, and a possible test item-related effect is suggested in the study report.

Table 17. Sex ratio data of the OECD TG 421 study

Dose (mg/kg bw/day)	**0**	**50**	**250**	**750**	HCD mean [min; max]
	At birth				
Male pups	7.33 ± 2.18	7.50 ± 3.21	7.67 ± 2.24	5.90 ± 3.41	7.87 [6.20; 9.78]
% Males	50.96 ± 12.63	49.78 ± 13.02	51.71 ± 14.08	40.47 ± 17.84	49.08 [39.95; 56.49]
M/F	1.25 ± 0.96	1.19 ± 0.68	1.26 ± 0.76	0.83 ± 0.61	-
	Day 4 *post partum*				
Male pups	7.22 ± 2.17	7.00 ± 3.06	7.33 ± 2.18	6.00 ± 2.83	7.71 [6.00; 9.67]
% Males	50.83 ±12.76	51.51 ± 14.89	52.04 ± 13.05	40.89 ± 21.32	48.97 [39.51; 57.44]
M/F	1.25 ± 0.96	1.33 ± 1.08	1.25 ± 0.70	0.91 ± 0.74	-
	Day 14 *post partum*				
Male pups	4.11 ± 0.33	3.80 ± 1.03	4.00 ± 0.00	3.22 ± 1.39	-
% Males	51.39 ± 4.17	51.08 ± 8.91	50.00 ± 0.00	40.83 ± 17.88	-

M/F	1.07 ± 0.22	1.15 ± 0.70	1.00 ± 0.00	0.81 ± 0.49	-

M/F was calculated except for HCD. -: not assessed. HCD from 4 OECD 421 studies were expressed as mean ± SD. Further details are reported in the Annex 1.

No effects in anogenital distance were seen in treated male or female pups, when compared to the control group. No nipples were observed in male pups on Day 13 *post partum*, the day before necropsy. No relevant necropsy findings were noted in control and treated pups sacrificed on Days 4 and 14 *post partum*.

Regarding general toxicity in dams:

One female receiving 250 mg/kg bw/day was sacrificed for humane reasons on Day 23 of gestation, since difficulty in parturition was noted. No significant clinical signs were observed throughout the study in treated animals.

No effect on mean body weight was reported in females. Transient decrease in body weight gain on Day 7 *post partum* was noted in all treated females with no clear dose-relationship (-56%, -66%, -25% at 50, 250 and 750 mg/kg bw/day, respectively – not statistically significant). Recovery was noted thereafter (see annex I for details).

The corrected maternal body weight gain during pregnancy from generational studies was calculated by DS as maternal body weight at LD1 minus maternal body weight at GD0. Statistits were performed with Dunnett`s test if group variances are homogeneous and modified t test if group variances are inhomogeneous. Decreases of the corrected maternal body weight gain were observed in females dosed at 50 and 750 mg/kg bw/day, -14% and -15% respectively,but with no statistical significance and without clear dose response relationship. After delivery (at LD1), dams body weight was not significantly different in treated groups (-7% at 750 mg/kg bw/d).

Table 18. Body weight and corrected body weight gain during pregnancy in females

Dose level (mg/kg bw/day)	0	50	250	750
Body weight (g)				
DD1 (pretest)	251.66 ± 13.65	253.02 ± 14.53	252.79 ± 14.92	252.63 ± 14.46
preMD1 (start of premating period)	267.56 ± 17.52	267.47 ± 15.31	261.13 ± 12.58	263.01 ± 19.60
MD1 (start of mating period)	282.94 ± 22.25	279.35 ± 15.95	274.57 ± 15.76	276.26 ± 19.99
GD0 (start of pregnancy)	292.25 ± 25.84	285.19 ± 15.32	277.70 ± 13.04	280.64 ± 17.05
GD20 (end of pregnancy)	453.37 ± 41.06	442.32 ± 36.88	442.54 ± 25.72	437.51 ± 27.93
LD1 (start of *lactation*)	357.72 ± 27.87	341.59 ± 18.94	343.86 ± 27.99	335.90 ± 23.79
Termination (lactation day 14)	377.35 ± 40.18	373.58 ± 22.85	376.31 ± 25.45	359.37 ± 23.21
Corrected body weight gain (g) during pregnancy LD1 – GD0	65.5 ± 8.2	56.3 ± 11.1 (-14%)	69.4 ± 20.4 (+6%)	55.6 ± 14.9 (-15%)

DD: dosing day. preMD: pre mating phase day. MD: mating phase D. GD: gestation day. LD: lactation day. Body weight gain per day = mean daily body weight gain over the previous period starting from the day of allocation

On Days 7 and 13 *post partum*, food consumption was statistically significantly but transiently decreased in females receiving 750 mg/kg bw/day (down to -18%). At all dose levels, no effects on food consumption were observed in treated males during the study.

Hepatocytic hypertrophy in 9/10 females (compared to 0/10 of the control group) was noted at 750 mg/kg bw/day.

3- An extended one generation study with F2 extension (Unnamed, 2020 –OECD TG 443)

The effects of DAA on the reproductive and developmental toxicity were investigated in an OECD Guideline no. 443 study in compliance with GLP.

Male and female Sprague-Dawley rats (25/sex/group) were given DAA at doses levels of 0, 50, 200 or 600 mg/kg bw/day, by gavage, 7 days per week, for 2 weeks prior to mating. For male animals, the administration continued for at least 10 weeks (68-72 days). Treatment of females continued throughout the mating, gestation and lactation periods up to Day 21 *post partum*. At weaning (post natal Day 21), F1 pups/sex/group were randomly selected to serve as Cohort 1A (20/sex/group) and Cohort 1B (20/sex/group). For each group, one or two male and female pups were selected from each litter. The treatment of selected pups started on the day of selection, Day 21 of age. Pups of Cohort 1A were given the test item for at least 10 weeks and then sacrificed at 13/14 weeks of nominal age. Pups of Cohort 1B were given the test item for at least 10 nominal weeks before pairing, and then animals were mated. Treatment of Cohort 1B males continued during the mating period up to 17 nominal weeks. Treatment of Cohort 1B females continued during the mating period and up to Days 21/22 *post partum*.

DS requested HCD (data from 4 OECD TG 443 study provided) but none of the studies were conducted with the same breeder and/or vehicle than those used in the OECD TG 443 study with DAA. Thus, in this context, DS considers that these HCD cannot be considered to interpret the results of this study.

F1/F2 pups

The litter observations revealed consistent effects on viability of the offspring before and after birth.

There were increases in pre-natal loss in F1 and F2 pups for doses ≥ 200 mg/kg bw/day reaching statistical significance at 600 mg/kg bw/day in F1 pups. The magnitude of pre-natal loss is similar between both generations.

Non statistically significant increases in post-natal loss were reported at birth in F1 and F2 pups at 600 mg/kg bw/day. The percentage of post-natal loss on PND 4 was increased without significance in F1 pups at 600 mg/kg bw/day but was statistically significantly increased in F2 pups at 600 mg/kg bw/day. Additionally, the total number of lost pups between PND 0-21 was non significantly increased in F1 pups at 600 mg/kg bw/day and became statistically significant in F2 pups for doses ≥ 200 mg/kg bw/day. These results may suggest that the severity of these effects increased with duration of exposure.

Table 19. Litter data of the OECD TG 443 study

	F1				F2			
Doses (mg/kg bw/day)	**0**	**50**	**200**	**600**	**0**	**50**	**200**	**600**
Prenatal loss %	4.28 ± 5.16	5.56 ± 4.57	9.85 ± 10.64	**12.06*** ± 14.01	6.79 ± 8.67	8.63 ± 7.61	8.78 ± 8.79	12.98 ± 13.37
Postnatal loss % birth	0.21 ± 1.05	0.00 ± 0.00	0.79 ± 2.90	2.36 ± 4.82	0.00 ± 0.00	0.70 ± 2.03	1.48 ± 3.66	2.40 ± 6.38
Postnatal loss % PND 4 based on live litter size in study report (recalculated by DS for F2 pups)	2.85 ± 5.15	1.80 ± 3.76	3.32 ± 4.18	6.77 ± 11.63	0.68 ± 1.99	2.20 ± 5.57	2.12 ± 3.12	**12.14*** ± 26.05
Nb of pups dead (mean) (PND 0 to 21)/litter	0.56 ± 0.87	0.84 ± 2.27	0.71 ± 0.75	1.39 ± 1.83	0.17 ± 0.51	0.53 ± 0.84	**0.61*** ± 0.70	**1.47*** ± 2.48

*: Differences with control at p≤ 0.05 are in red and **bold**.

Prenatal loss %: in the study report, analysis with the nonparametric version of the Williams test was only performed when significance has been previously found with a Kruskal-Wallis test. Since Kruskal-Wallis test was almost significant (p: 0.051) for F1 pups at 600 mg/kg bw/day, DS performed statistical analysis with the nonparametric version of the Williams test. This test showed statistical significance in F1 pups at 600 mg/kg bw/day.

Postnatal loss % PND 4: in the study report, calculations made for F1 pups were based on live litter size whereas for F2 pups it was based on total litter size. DS recalculated postnatal loss % at PND 4 for F2 pups based on live litter size and performed statistical analysis with new data.

The anogenital distance (AGD) was statistically significantly and dose-dependently decreased in F1 males and females but was unchanged in F2 pups.

Table 20. Anogenital distance data in the OECD TG 443 study

Pup generation	F1				F2			
Doses (mg/kg bw/day)	0	50	200	600	0	50	200	600
AGD (mm/g$^{1/3}$) Males	2.38 ± 0.33	2.37 ± 0.37	**2.05*** ± 0.39	**2.00*** ± 0.39	1.98 ± 0.30	2.05 ± 0.31	2.07 ± 0.39	1.99 ± 0.45
AGD (mm/g$^{1/3}$) Females	1.34 ± 0.27	1.35 ± 0.27	**1.11*** ± 0.27	**1.05*** ± 0.28	1.14 ± 0.21	1.13 ± 0.21	1.21 ± 0.25	1.20 ± 0.21

*: Differences with control at p≤ 0.05 are in red and **bold**.

The nipple count was performed in all male pups on PND 13 and re-counted on PND 22/23 at necropsy. Nipple count on Day 22 *post partum* showed that no nipples were found in F1 and F2 pups. However, no information regarding nipple retention on PND 13 and no individual nipple retention data on any PND were provided.

Immunotoxicity was explored in adult F1 pups (cohort 1A animals). Results showed a statistically significant decrease of CD8 marker expression in male animals dosed at 600 mg/kg bw/day (-11.4%). No statistically significant differences were observed in the frequencies of all the other cell populations. Expression of surface markers was similar for all groups for both genders. This result may be associated with the significant decrease in absolute weight of mesenteric nodes in C1A males (-29%) and females (-31%). The relevance of these results is questionable but immune effects cannot be excluded.

Regarding general toxicity in dams (P0 and cohort 1B)

There was no treatment-related effect on viability of dams.

In P0 animals, no effect on body weight was recorded but body weight gain was significantly, but only punctually, decreased at 600 mg/kg bw/day (-45%) on day 14 *post-partum*, associated with a decreased food consumption (-6%) on the same date (see Annex I).

In cohort 1B, there was no effect on body weight but body weight gain was significantly increased at 200 mg/kg bw/day (on GD14; +18%) and at 600 mg/kg bw/day (on LD7; +103%) (see Annex I). Food consumption was modified in cohort 1B with a statistically significant decrease (-7%) on PND 14, at 600 mg/kg bw/day.

Table 21. Body weight (g) of P0 and 1B females during pre-mating until termination

Dose (mg/kg bw/d)	Start of premating	Start of mating	Start of gestation (GD0)	Start of *post-partum* (LD1)	Termination (Day 21 pp)
P females					
0	256.14 ± 13.58	277.34 ± 17.83	286.55 ± 15.22	348.10±21.22	365.72 ± 20.51
50	256.90 ± 13.82	284.09 ± 24.16	290.32 ± 20.24	356.10±22.43	368.87 ± 24.10
200	255.87 ± 11.73	279.07 ± 15.55	286.50 ± 15.97	345.80±19.14	363.88 ± 17.30

600	257.52 ± 10.56	285.27 ± 23.35	292.37 ± 15.12	346.11±23.41	372.79 ± 19.42
Cohort 1B					
0	281.46 ± 22.28	277.34 ± 17.83	286.55 ± 15.22	348.10 ± 21.22	365.72 ± 20.51
50	277.88 ± 15.86	284.09 ± 24.16	290.32 ± 20.24	356.10 ± 22.43	368.87 ± 24.10
200	272.03 ± 20.68	279.07 ± 15.55	286.50 ± 15.97	345.80 ± 19.14	363.88 ± 17.30
600	267.14 ± 21.38	285.27 ± 23.35	292.37 ± 15.12	346.11 ± 23.41	372.79 ± 19.42

The estimated corrected maternal body weight gain during gestation in P0 and cohort 1B was calculated by DS and are described in the table below. Statistics were performed with Dunnett`s test if group variances are homogeneous and modified t test if group variances are inhomogeneous. The modifications observed were not statistically significant and were not consistent between generations.

Table 22. Estimated corrected maternal body weight (g) during gestation of P0 and 1B females

Dose level (mg/kg bw/day)	0	50	200	600
corrected maternal body weight gain (g) in P0	61.54 ± 15.4	65.78 ± 14.13 (+7%)	59.30 ± 13.49 (-4%)	53.74 ± 15.78 (-13%)
corrected maternal body weight gain (g) in cohort 1B	48.52 ± 11.07	52.00 ± 11.59 (+7%)	54.84 ± 13.24 (+13%)	53.63 ± 17.87 (+11%)

Corrected maternal body weight gain: body weight at LD 1 – body weight at GD 0

At 600 mg/kg bw/day, there were some altered parameters, in relation to minimal liver toxicity, reported on clinical chemistry (mainly characterized by an increase of cholesterol), urinalysis (increased ketonuria) in P0 and Cohort 1B and organ weight (non significant increase of absolute and relative liver weight) only in Cohort 1B. However, there were no histopathological associated findings in the liver. In addition, P0 females presented significant decrease in partial thromboplastin time at 600 mg/kg bw/day (-6%). In Cohort 1B, some changes were reported regarding thyroid hormones, with increase of T3 level without dose-response relationship and non-statistically significant decrease of TSH. Concerning kidney toxicity, increased absolute and relative organ weight (< 10%) was reported in both generations at 600 mg/kg bw/day. No histopathological findings were observed.

4- An *OECD Guideline 414 (Prenatal Developmental toxicity study)*

DAA was daily administered as a solution in corn oil at dose-levels of 0, 100, 300 or 1000 mg/kg bw/day, from gestation Day 6 to Day 20, by gavage, to pregnant Sprague-Dawley female rats. This GLP study was carried out according to OECD test guideline No. 414.

The pregnancy status were 21/24, 21/24, 22/24 and 23/24 pregnant females in controls, 100, 300 and 1000 mg/kg/ bw/day groups, respectively. All pregnant females had viable fetuses.

There was no mortality observed in dams. Excessive salivation as recorded as ptyalism (6/24) at 1000 mg/kg bw/day was reported.

There were no effects on body weight and net body weight. At 1000 mg/kg bw/day, there was a significantly higher mean body weight gain (+25 g vs. +20 g in controls, $p<0.05$) between GD9-12, associated with increased food consumption, mainly due to 1 animal. Net body weight change was increased at 100 and 300 mg/kg bw/day compared to control, but withoutstatistical significance and no clear dose-response relationship.

Table 23. Body weight, body weight change and net body weight change in dams

Dose level (mg/kg bw/day)	0	100	300	1000
Body weight (g)				
GD 6	300 ± 30	298 ± 23 (-1%)	298 ± 21 (-1%)	300 ± 23 (0%)
GD 21	448 ± 43	443 ± 40 (-1%)	446 ± 35 (0%)	445 ± 32 (-1%)
Net body weight (g) **(terminal body weight minus uterus weight)**	340.9 ± 33.0	347.0 ± 25.8 (+1.8%)	345.9 ± 23.2 (+1.5%)	341.3 ± 23.5
Body weight change (g)				
GD 9 - 12	+20 ± 7	+19 ± 7 (-5%)	+24 ± 6 (+20%)	+25* ± 5 (+25%)
GD 6 - 21	+147 ± 16	+145 ± 23 (-1%)	+149 ± 29 (+1%)	+145 ± 18 (-1%)
Net body weight change from GD 6 (g)	40.6 ± 9.5	49.1 ± 11.1 (+21%)	48.3 ± 12.6 (+19%)	41.2 ± 14.3 (+1%)

GD : gestation day. *p<0.05. Net body weight change was calculated as: body weight on GD 21 - body weight on GD 6 - gravid uterine weight.

A statistically significant increase was noted in mean absolute and relative liver (+15.5%; +14.7%) and kidney (+9%; +6.3%) weight values at 1000 mg/kg bw/day when compared with controls.

There were no test item treatment-related effects on gravid uterus and carcass weights and hysterectomy data. There were no effects on mean fetal body weight and sex ratio. There were no test item treatment-related findings at external examination, and soft tissue examination of the fetuses.

Unossified or incomplete ossification of various part of the skeleton, although not statistically significant, was noted in all litters at 1000 mg/kg bw/day.

It should be noted that incidence of skeletal variations at the highest tested dose is higher than HCD presented in the study report. However, very little information is provided on HCD for proper interpretation by DS but this points to a treatment-related effect, despite the lack of statistical significance.

Table 24. Fetal (Litter) incidences % of dose-related skeletal variations in the OECD 414 study (rat)

Dose level (mg/kg bw/day)	0	100	300	1000	HCD
Number of litters	21	21	22	23	182
Number of fetuses	143	131	146	153	1249
Hyoid: unossified	0 (0)	0 (0)	0 (0)	1.3 (8.7)	0 (0)
Lumbar vertebra: bipartite ossification of centrum	0 (0)	0 (0)	0 (0)	0.7 (4.3)	0 (0)
Caudal vertebra: unossified centrum	0 (0)	0 (0)	0.7 (4.5)	3.3 (8.7)	0.4 (2.7)
Extra stenebral ossification site	0 (0)	0 (0)	0 (0)	0.7 (4.3)	0.2 (1.6)
Unossified 1st metacarpals	0 (0)	0 (0)	0 (0)	0.7 (4.3)	0.2 (1.1)
Incomplete ossification of metacarpals	10.5 (28.6)	7.6 (28.6)	8.9 (31.8)	11.1 (30.4)	0.2 (1.6)
Forepaw: unossified proximal phalanx	49.7 (95.2)	41.2 (95.2)	56.2 (86.4)	68.0 (100)	5.3 (21.4)

F: fetal incidence, L: litter incidence. Noticeable adverse changes (higher fetal and/or litter incidences vs. upper limits of the HCD and contemporaneous controls) are in red (not statistically significant). HCD: Historical Control Data (Sprague-Dawley rat, at the test facility, March 2013 to June 2014).

4- An *OECD Guideline 414 (Prenatal Developmental)*

DAA was daily administered in water by gavage from Gestation Days 6 to 28 at 0, 100, 300 or 800 mg/kg bw/day in New Zealand White rabbits.

The pregnancy status were 17/24, 18/24, 20/24 and 21/23 pregnant dams in the groups treated at 0, 100, 300 or 800 mg/kg bw/day, respectively.

At 800 mg/kg bw/day, one female was prematurely euthanized on GD 20 for ethical grounds (emaciated appearance and absence of feces were observed from GD 15). The female lost 18% of body weight between GD 6 and 19 associated with a progressive decrease in food consumption from the start of the dosing period and this female did not eat from GD 12. Consistent with this finding, there was a statistically significant mean body weight loss (-73 g on GD 6-9, $p<0.001$) associated with a lower mean food consumption (down to -40% on GD 6-9, $p<0.001$) (more details are available in Annex I). The decreases observed in net body weight change were not statistically significant probably due to high variability in the results and not dose-dependent. In rabbits, the body weight gain may not be a useful indicator of maternal toxicity because of normal fluctuations in body weight during pregnancy. (CLP guidance, Annex I: 3.7.2.4.4, p402).

Table 25. Body weight, body weight change and net body weight change

Dose level (mg/kg bw/day)	0	100	300	800
Mean body weight (g)				
GD 6	3561 ± 293	3658 ± 283 (+3%)	3597 ± 274 (+1%)	3579 ± 216 (+1%)
GD 29	3991 ± 339	4083 ± 278 (+2%)	4030 ± 308 (+1%)	3940 ± 211 (-1%)
Net body weight (g) **(terminal body weight minus uterus weight)**	3481.0 ± 103.4	3530 ± 288.1 (+1%)	3508.2 ± 245.3 (+1%)	3424.3 ± 220.1 (-2%)
Mean body weight change (g)				
GD 6 – 9	+57 ± 54	+37 ± 61	+33 ± 31	-73** ± 53
GD 6 – 29	+430 ± 114	+425 ± 141	+434 ± 93	+348 ± 95
Net body weight change (g)	-79.6 ± 175.9	-127.5 ± 196.5	-88.5 ± 137.9	-168.0 ± 124.3

GD: gestation day. **p<0.001. Net body weight change was recalculated by DS as: body weight on Day 29 p.c. - body weight on Day 6 p.c. - gravid uterine weight.

There were no clinical signs or necropsy findings in surviving females. There were no effects on mean gravid uterus, carcass weights and on hysterectomy parameters.

No effects were noted on mean fetal weight and mean sex ratio.

Concerning the fetal malformations, external, visceral and skeletal malformations were noted: closure defects at 800 mg/kg bw/day such as omphalocele (two fetuses from two different litters), meningoencephalocele (one fetus from another litter) and split frontal bone (one fetus from another litter); malformed aortas at 300 and 800 mg/kg bw/day (dilatation in two fetuses from two different litters at both dose levels, associated with overriding aorta in one fetus at 800 mg/kg bw/day). With five affected fetuses from five different litters and these malformations being not seen in HCD or with a lower incidence in HCD, the study report concludes that an effect of the test item was considered likely at 800 mg/kg bw/day and was suspected at 300 mg/kg bw/day. However, very little information is provided on HCD for proper interpretation by DS but this points to a treatment-related effect, despite the lack of statistical significance.

Table 26. Relevant malformed foetuses of the OECD TG 414 study (rabbit)

Doses (mg/kg bw/day)	0	100	300	800

Number of litters/total	0/17	0/18	2/20	5/21
Number of fetuses/total	0/159	0/171	2/186	5/202
Malformations			Dilated aorta Dilated aorta	Dilated and overriding aorta Omphalocele, dilated aorta Meningoencephalocele Omphalocele Split frontal

HCD: Historical Control Data (NZW Rabbits, at the test facility, June 2015 to September 2016 and October 2016 to December 2017, n = 8 + 2 studies).

- dilated aorta, L(F) %: 0.0 (0.0)
- overriding aorta, L(F) %: 0.0 (0.0)
- omphalocele, L(F) %: 4.5 (0.5) (a)
- meningoencephalocele, L(F) %: 0.0 (0.0)
- split frontal, L(F) %: 0.0 (0.0)

(L): litter incidence; (F): fetal incidence; (a): maximum value.

At 800 mg/kg bw/day and when compared with controls, there was a statistically significant increase in the number of fetuses with a small and/or pale spleen.

From 300 mg/kg bw/day, there are increased incidences of non-ossified metacarpal bones and proximal phalanxes (only the respective cartilages were present) reaching statistical significance at 800 mg/kg bw/day. From 300 mg/kg bw/day onwards, there is a tendency towards a dose-dependent increase in the incidences of skeletal variations (interparietal, ribs and metacarpal bones). The incidence of unossification or incomplete ossification of the metacarpal bones correlates with the cartilage findings.

Table 27: Soft tissue examination, cartilage examinations and skeletal variations data of the OECD TG 414 study (rabbit)

Dose level (mg/kg bw/day)	**0**	**100**	**300**	**800**	**HCD**
Dams with live fetuses, n	17	18	20	21	159 + 42
Live fetuses evaluated, n	159	171	186	202	1558 + 391
Spleen: paleness, L (F) %	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	14.3 **(4.0*)**	11.1 (1.2) (a)
Spleen: small, L (F) %	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	9.5 **(5.4**)**	8.3 (0.9) (a)
Metacarpal bone: cartilage present, L (F) %	17.6 (4.4)	16.7 (1.8)	45.0 (5.9)	**52.4* (14.4**)**	36.4 (5.7) (a)
Forepaw phalanx: proximal cartilage present, L (F) %	0.0 (0.0)	0.0 (0.0)	15.0 (2.2)	19.0 **(3.0*)**	9.1 (1.0) (a)
Interparietal: split, L (F) %	0.0 (0.0)	0.0 (0.0)	5.0 (1.1)	23.8 **(3.0*)**	20.0 (2.5) (a)
Ribs: supernumary 13th, L (F) %	94.1 (56.6)	88.9 (59.1)	100 **(68.8*)**	100 **(73.3**)**	100.0 (81.2) (a)
Metacarpal: unossified 1st, L (F) %	5.9 (1.9)	5.6 (0.6)	25.0 (3.2)	**47.6** (7.9*)**	33.3 (7.4) (a)
Metacarpal: incomplete ossification 1st, L (F) %	11.8 (2.5)	11.1 (1.2)	20.0 (2.7)	33.3 **(8.4*)**	61.1 (8.4) (a)

*/**: Differences with control at p ≤ 0.05/0.01 are in red and **bold**. L: Litter incidence. F: Fetal incidence. (a): maximum value. HCD: Historical Control Data (NZW Rabbits, at the test facility, June 2015 to September 2016 and October 2016 to December 2017, n = 8 + 2 studies).

10.8.6 Comparison with the CLP criteria

According to the CLP criteria for Category 1A for known human reproductive toxicants, Annex I: 3.7.2.1.1 of CLP Regulation 1272/2008: " *The classification of a substance in this Category 1A is largely based on evidence from humans.*"

Classification of DAA in Repr. 1A is not fulfilled as no human data are available.

Regarding the CLP criteria for Category 1B for presumed human reproductive toxicants, Annex I: 3.7.2.1.1 stipulates: "*The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects*".

Considering the CLP critera for Category 2 for suspected human reproductive toxicant, Annex I: 3.7.2.1.1 stipulates: "*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.*"

Developmental toxicity of DAA was assessed in a reproduction/developmental toxicity screening test (OECD TG 421), a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422), an extended one generation study, including the production of a second generation (cohort 1A and 1B) (OECD TG 443) and in two prenatal developmental toxicity studies (OECD TG 414).

Lethality

- **Pre-natal mortality**

Non significant increases of prenatal loss or post implantation loss were recorded at 750 mg/kg bw/day in the OECD TG 421 study, at 300 and 1000 mg/kg bw/day in the OECD 422 study. In the OECD TG 443 study, there were increases in pre-natal loss in F1 and F2 pups for doses ≥ 200 mg/kg bw/day, reaching statistical significance at 600 mg/kg bw/day in F1 pups **Although most of these findings were not statistically significant, they are consistently found in 3 different well-conducted studies and are consistent with subsequent post-natal mortality observed in these same studies (see below).**

In constrast, no increase of post-implantation loss was reported in the two available OECD TG 414 studies. **This may suggest that a sufficient duration of maternal exposure may be needed to induce lethal effects in fetuses.**

In this context, this effect can not be considered, as such, as clear evidence of adverse effect on development but as supportive evidence for classification.

- **Post-natal mortality**

At birth, post-natal loss was non significantly increased at the highest dose tested in all reproductive toxicity studies (600 mg/kg bw/d in the OECD TG 443 study, 750 mg/kg bw/day in the OECD TG 421 study and 1000 mg/kg bw/day in the OECD TG 422 study).

On day 4 *post partum,* the post-natal loss was non significantly increased at 750 mg/kg bw/day in the OECD TG 421 study and in F1 pups dosed at 600 mg/kg bw/day in the OECD TG 443 study. Statistically significance was reached in the 2nd generation of pups dosed at 600 mg/kg bw/day in the OECD TG 443 study.

On day 14 *post partum*, at the highest dose tested (1000 mg/kg bw/day) in the OECD TG 421 study, live litter size was significantly reduced.

Between birth and PND 21, the number of pups dead was increased at 600 mg/kg bw/day in the F1 pups (not statistically significant) and from 200 mg/kg bw/day in F2 pups (statistically significant) in the OECD TG 443 study.

Overall, there were consistent effects on post-natal mortality reported in 3 different well-conducted studies. In particular, statistically significances were reached in 2 studies, with severity increasing in F2 pups in the OECD TG 443 study. This effect is considered as clear evidence of developmental toxicity.

Table 28. Comparative litter data (pups mortality) of DAA toxicity

OECD TG study	443								422					421			
Generation	F1				F2				F1					F1			
Dose level (mg/kg bw/day)	**0**	**50**	**200**	**600**	**0**	**50**	**200**	**600**	**0**	**30**	**100**	**300**	**1000**	**0**	**50**	**250**	**750**
Prenatal loss or post implantation loss (%)	4.30 ± 2.18	5.58 ± 4.57	9.85 ± 10.64	**12.06 *** ± 14.01	6.80 ± 8.68	8.63 ± 7.61	8.79 ± 8.78	12.98 ± 17.09	5.42	6.90	8.43	9.39	29.58	8.08 ± 4.41	4.18 ± 3.85	4.79 ± 7.44	20.08 ± 27.19
PN loss % birth Based on live litter size	0.21 ± 1.06	0.00 ± 0.00	0.79 ± 2.90	2.36 ± 4.82	0.00 ± 0.00	0.66 ± 1.99	1.48 ± 3.66	2.40 ± 6.38	1.26	1.22	1.21	2.96	13.79	0.00 ± 0.00	0.63 ± 1.99	0.00 ± 0.00	7.73 ± 22.45
PN loss % On Day 4 *post partum*	2.85 ± 5.15	1.80 ± 3.76	3.32 ± 4.18	6.77 ± 11.63	0.68 ± 1.99	2.20 ± 5.57	2.12 ± 3.12	**12.14 *** ± 26.05	-	-	-	-	-	1.11 ± 3.33	8.13 ± 8.07	5.26 ± 5.47	9.22 ± 8.92
PN loss % On Day 13 or 14 *post partum*	0.40 ± 2.00	0.00 ± 0.00	0.44 ± 2.09	0.00 ± 0.00	0.56 ± 2.36	1.05 ± 3.15	0.56 ± 2.35	0.00 ± 0.00	-	-	-	-	-	0.00 ± 0.00	1.25 ± 3.95	0.00 ± 0.00	7.50 ± 16.87
Nb of pups dead/litter (days 0 to 21 PND)	0.56 ± 0.87	0.84 ± 2.27	0.71 ± 0.75	1.39 ± 1.82	0.17 ± 0.51	0.53 ± 0.84	**0.61*** ± 0.70	**1.47*** ± 2.48	-	-	-	-	-	-	-	-	-
Live litter size on Day 13 or 14 *post partum*	9.96 ± 0.20	9.83 ± 0.64	9.78 ± 0.85	9.52 ± 1.65	9.67 ± 0.84	9.90 ± 0.32	9.94 ± 0.24	9.72 ± 0.75	-	-	-	-	-	8.00 ± 0.00	7.40 ± 1.26	8.00 ± 0.00	**6.30*** ± 2.41

PN: post-natal; *: Differences with control at p≤ 0.05 are in red and **bold**. -: not examined.

Prenatal loss %: in the study report, analysis with the nonparametric version of the Williams test was only performed when significance has been previously found with a Kruskal-Wallis test. Since Kruskal-Wallis test was almost significant (p: 0.051) for F1 pups at 600 mg/kg bw/day DS performed statistical analysis with the nonparametric version of the Williams test. This test showed statistical significance in F1 pups at 600 mg/kg bw/day.

Postnatal loss % PND 4: in the study report, calculations made for F1 pups were based on live litter size whereas for F2 pups it was based on total litter size. DS recalculated postnatal loss % at PND 4 for F2 pups based on live litter size and performed statistical analysis with new data.

Regarding general toxicity in dams:

In the OECD TG 421 study, developmental effects occurred at the dose of 750 mg/kg bw/day. At this dose, there were some punctual variations of body weight gain, without significant change of body weight, and liver hypertrophy in 9/10 females. The estimated corrected body weight gain, expressed as body weight on LD1 minus body weight on GD0 was non statistically significantly decreased at this dose and without a clear dose response relationship and is not considered to reflect significant maternal toxicity.

About the OECD TG 422 study, developmental effects occurred at the dose of 1000 mg/kg bw/day. At this dose, there was a significant decrease of locomotor activity and of response to stimuli and liver alterations (increased weight at about +25%, hypertrophy in 6/10 females) in dams.

In the OECD TG 443 study, developmental effects occurred from the dose of 200 mg/kg bw/day. In dams, there was a punctual variations in body weight gain (i.e. significant decrease at 600 mg/kg bw/day in P0 dams on *post partum* Day 14 and significant increases at 200 mg/kg bw/day on gestational day 14 and at 600 mg/kg bw/day on *post partum* day 7 in Cohort 1B dams) without impact on mean body weight. The estimated corrected body weight gain was only decreased at 600 mg/kg bw/d in P0 females (and increased in cohort 1B females) but these modifications were not statistically significant. There were also changes in coagulation (decrease in partial thromboplastine time), biochemistry (including significant increases of cholesterol), urinalysis (ketonuria) and in organ weight (increase in absolute and relative kidney and/or liver weights < 10%) without associated histopathological findings, in dams, at 600 mg/kg bw/day.

Thus, changes in prenatal loss and postnatal loss occurred in the absence of significant maternal systemic toxicity.

Table 29. Comparative general toxicity data in dams

OECD TG study	443									422				421		
Generation	P0			C1A			C1B			P0				P0		
Doses (mg/kg bw/day)	**50**	**200**	**600**	**50**	**200**	**600**	**50**	**200**	**600**	**30**	**100**	**300**	**1000**	**50**	**250**	**750**
Mortality	1/25	0/25	0/25	0/20	0/20	0/20	0/20	1/20	1/20	0/10	0/10	0/10	1/10	0/10	1//10	0/10
Clinical signs ↘ *in locomotor activity* ↘ *of response to stimuli*	No effects	No effects	No effects	No effects	No effects	No effects	No effects	No effects	No effects	No effects	No effects	**6 /10**** **5/10****	**10/10**** **10/10****	No effects	No effects	No ffects
Body weight	No effects	No effects	No effects	No effects	No effects	No effects	No effects	effects	No effects	No effects	No effects	No effects	No effects	No effects	No effects	No effects
Body weight gain	PPD 14 (-40%)	PPD 14 (-43%)	**PPD 14 (-45%*)**	**PND 63 (+30%*)**	No effects	No effects	No effects	**GD 14 (+18%*)**	**PPD 7 (+103%*)**	No effects	No effects	No effects	**↘* on PMD 15**	PPD 7 (-56%)	PPD 7 (-66%) **PPD 13 (+250%**)**	PPD 7 (-25%)
Corrected maternal body weigt gain	+7%	-4%	-13%	-	-	-	+7%	+13%	+11%	-	-	-	-	-14%	+6%	-15%
Clinical Chemistry Cholesterol Triglycerides Total protein	↗ ↘ ↗	↗ ↗ ↗	**↗**** ↗ 0	↘ ↘ ↘	↘ ↘ ↘	**↗**** ↗ ↘	0 ↘ ↘	↗ ↗ ↘	**↗**** ↗ 0	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Haematology and coagulation Haemoglobin Partial thromboplastin time	↘ ↘	↘ ↘	**↘*** **↘***	↘ ↗	↗ ↗	↗ ↘	↘ ↗	↗ ↗	↘ ↘	- -	- -	- -	- -	- -	- -	- -
Urinalysis Ketonuria Urinary volume	No effects ↘	↗ ↗	↗ ↗	No effects ↘	↗ ↗	↗ ↗	No effects ↘	↗ ↘	↗ 0	- -	- -	- -	- -	- -	- -	- -
Liver abs / rel wt	+0.5% / -0.4%	-1.4% / -0.5%	+2.2% / +3.3%	+0.05% / -3.0%	+4.1% / +2.7%	+2.7% / +5.1%	-0.9% / - 1.4%	-1.0% / +0.1%	+5.6% / +6.6%	+4.4% / 0.0	+6.0% / +4.8%	+10.8% / +9.7%	**+26.2%* / +25.3%****	- / -	- / -	- / -
Liver Hepatocyte hypertrophy Vacuolization	0/25 0/25	0/25 0/25	0/25 0/25	0/20 0/20	0/20 0/20	0/20 0/20	- -	- -	- -	0/10 -	0/10 -	0/10 -	6/10** -	0/10 0/10	0/10 0/10	**9/10*** 0/10
Kidneys abs / rel wt	+0.8% / -0.3%	+1.5% / +2.4%	**+7.3%* / +8.5%***	+4.2% / +0.6%	+8.2% / **+6.5%***	+7.5% / **+9.9%****	-3.9% / +3.4%	+2.5% / +4.0%	**+6.5%* / +7.5%****	+3.2% / - 1.8%	+8.1% / +7.0%	+9.2% / +8.7%	+15.1% / +14.0%	- / -	- / -	- / -
Nephropathy	0/25	0/25	0/25	0/20	0/20	0/20	-	-	-	-	-	-	-	0/10	0/10	1/10
Hyaline droplet accumulation	0/25	0/25	0/25	0/20	0/20	1/20	-	-	-	-	-	-	-	0/10	0/10	0/10

Results are expressed as % compared to control or as number of affected animals/examined animals. */**: Differences with control at p≤ 0.05/0.01 are in red and **bold.** P0: parental generation; C1A: cohort 1A; C1B: cohort 1B; -: not examined; PPD: *post-partum* day; PND: post-natal day, GD: gestation day; PMD: pre-mating day.

Variations and malformations

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

Skeletal variations are reported in both studies.

In rats, skeletal variations are reported with unossified or incomplete ossification of various parts of the skeleton in all litters at 1000 mg/kg bw/day higher fetal and/or litter incidences vs. upper limits of the HCD and contemporaneous controls). These findings were associated with presence of cartilage.

Similar findings were reported in rabbits. From 300 mg/kg bw/day, there are statistically significant increases of the incidence of supernumerary 13th ribs and at 800 mg/kg bw/day, significant increases of non-ossified/ incomplete ossified metacarpal bones and proximal phalanxes. At 800 mg/kg bw/day and when compared with controls, there was statistically significant increases in the numbers of fetuses with a small and/or pale spleen.

In addition, **at fetal examination in rabbits**, at 800 mg/kg bw/day, **five fetus from 5 different litters presented malformations (omphalocele, meningoencephalocele, dilated and/or overriding aorta, and/or split frontal bone).** These malformations were not seen in the control group nor in the HCD provided or to a lower incidence.

Regarding maternal toxicity:

Regarding the rat study: there was no mortality observed in dams. Some clinical signs were reported as excessive salivation (recorded as ptyalism) at 1000 mg/kg bw/day. There were no significant effects on body weight and net body weightAt 1000 mg/kg/bw/day, there was a significantly higher mean body weight gain between GD9-12, associated with decreased food consumption, mainly due to 1 animal. Net body weight change was increased at 100 and 300 mg/kg bw/day compared to control, but without statistical significance and no clear dose-response relationship. A statistically significant increase was noted in mean absolute and relative liver and kidney weight values at 1000 mg/kg bw/day when compared with controls.

Regarding the rabbit study: at 800 mg/kg bw/day, one female was prematurely euthanized on Day 20 post coitum for ethical grounds (no food consumption associated with body weight loss). There were no clinical signs or necropsy findings in surviving females. Mean body weight and food consumption was negatively affected at this dose on GD6-9, due to the female that was euthanized. Net body weight change was negative is all groups, without statistically significant differences between groups. The net body weight was not affected. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy (CLP guidance, Annex I: 3.7.2.4.4, p402)

Thus, skeletal variations in both species and malformations in rabbits occurred in the absence of over-maternal toxicity. Variations are generally not used as basis of classification since they are not considered as a major manifestation of developmental toxicity, as defined in CLP guidance. However, malformations are observed in rabbits and are considered as clear evidence of developmental toxicity.

In addition, effects described below are not sufficient to warrant a classification but are considered as supportive evidence of developmental toxicity. Indeed, these effects were either not found consistently between studies and/or did not reach statistical significance and/or not considered as major manifestations of developmental toxicity.

Sex ratio, litter/mean pup weight

The sex ratio was non significantly reduced at the highest dose tested in the OECD TG 422 study (1000 mg/kg bw/day) and in the OECD TG 421 study (750 mg/kg bw/day). The values recorded at 750 mg/kg bw/day, were below the incidences of the physiological range, and a possible test item-related effect is suggested in the OECD TG 421 study report. In the OECD TG 443 where the doses were lower than OECD TG 422 and 421 studies, and in OECD TG 414 studies, this was not observed.

On day 13 *post partum*, the litter weight and the mean pup weight were significantly reduced at the highest dose tested (750 mg/kg bw/day) in the OECD TG 421 study. Similar effects were not found in other studies.

Table 30. Comparative litter data (sex ratio and litter/mean pup weights) of DAA toxicity.

OECD TG study	443								422					421			
Generation	F1				F2				F1					F1			
Dose level (mg/kg bw/day)	**0**	**50**	**200**	**600**	**0**	**50**	**200**	**600**	**0**	**30**	**100**	**300**	**1000**	**0**	**50**	**250**	**750**
Sex ratio (M/F) *Day not specified*	-	-	-	-	-	-	-	-									
Day 4 *post partum*	1.19 ± 0.98	1.19 ± 0.62	1.34 ± 0.90	1.24 ± 0.68	1.04 ± 0.49	0.94 ± 0.33	1.01 ± 0.44	1.17 ± 0.82	1.07	1.13	0.89	1.03	0.78	1.24 ± 0.96	1.33 ± 1.08	1.25 ± 0.70	0.91 ± 0.74
Day 14 *post partum*	-	-	-	-	-	-	-	-	-	-	-	-	-	1.07 ± 0.22	1.15 ± 0.70	1.00 ± 0.00	0.81 ± 0.49
Sex ratio % males on day 4 *post partum*	49.74 ± 12.60	51.27 ± 11.72	52.73 ± 12.76	54.05 ± 15.83	47.29 ± 10.32	46.84 ± 9.80	48.11 ± 10.20	47.54 ± 16.20	-	-	-	-	-	50.96 ± 12.63	49.78 ± 13.02	51.71 ± 14.08	40.47 ± 17.84
Litter weight (g) on Day 13 or 14 *post partum*	348.10 ± 26.86	349.98 ± 35.63	344.84 ± 31.32	325.81 ± 59.58	340.77 ± 35.30	344.81 ± 24.56	347.87 ± 21.19	325.50 ± 32.02	-	-	-	-	-	280.19 ± 29.55	247.24 ± 37.58	262.92 ± 19.71	**190.27*** ± 88.76
Mean pup weight (g) on Day 13 or 14 *post partum*	34.97 ± 2.81	35.58 ± 2.57	35.35 ± 2.30	34.41 ± 3.12	35.36 ± 3.27	34.87 ± 2.35	34.97 ± 2.40	33.48 ± 2.05	-	-	-	-	-	35.00 ± 3.71	33.74 ± 3.28	32.88 ± 2.47	**29.47*** ± 4.71

PN: post-natal; AGD: anogenital distance

*: Differences with control at p≤ 0.05 are in red and **bold.** -: not examined

Sex ratio (M/F) in Prenatal Developmental Toxicity (OECD TG 414) studies was calculated:

- Rat: 0 (1.24 ± 1.02) ; 100 (1.01 ± 0.74) ; 300 (1.10 ± 0.90) ; 1000 mg/kg bw/day (0.96 ± 0.50)
- Rabbit: 0 (1.62 ± 1.09) ; 100 (1.54 ± 1.51) ; 300 (1.28 ± 0.75) ; 800 mg/kg bw/day (1.41 ± 0.93)

AGD

The anogenital distance was significantly reduced in males and in females from 200 mg/kg bw/day in the F1 pups but not in F2 pups in the OECD TG 443 study. This effect was not reported in other studies.

Table 31. Comparative anogenital distance (AGD) of DAA toxicity

OECD TG study	443								422					421			
Generation	F1				F2				F1					F1			
Dose level (mg/kg bw/day)	**0**	**50**	**200**	**600**	**0**	**50**	**200**	**600**	**0**	**30**	**100**	**300**	**1000**	**0**	**50**	**250**	**750**
AGD (mm/g$^{1/3}$) **Males**	2.38 ± 0.33	2.37 ± 0.37	**2.05*** ± 0.39	**2.00*** ± 0.39	1.98 ± 0.30	2.05 ± 0.31	2.07 ± 0.39	1.99 ± 0.45	-	-	-	-	-	2.02 ± 0.23	2.03 ± 0.20	2.11 ± 0.26	2.00 ± 0.25
AGD (mm/g$^{1/3}$) **Females**	1.34 ± 0.27	1.35 ± 0.27	**1.11*** ± 0.27	**1.05*** ± 0.28	1.14 ± 0.21	1.13 ± 0.21	1.21 ± 0.25	1.20 ± 0.21	-	-	-	-	-	1.25 ± 0.17	1.23 ± 0.17	1.30 ± 0.16	1.27 ± 0.20

AGD: anogenital distance; *: Differences with control at p≤ 0.05 are in red and **bold**. -: not examined

In conclusion, the available data provide clear evidence of developmental adverse effects (principally characterised by post-natal lethality and malformations) among studies performed in either rats or rabbits. They cannot be considered as secondary non-specific consequence of other toxic effects. Additionally, other effects as increased prenatal loss, decreased litter and mean pups weights, decreases of

sex ratio and anogenital distance, as well as skeletal variations provide supportive evidence of developmental adverse effects. There is no mechanistic evidence to indicate that the observed effects are not relevant for humans.

Classification of DAA in Repr. 2 is not considered appropriate as the evidence for adverse effects on development from the experimental data is considered as clear evidence.

Therefore, criteria for classification Repr. 1B for development are fulfilled for DAA.

10.8.7 Adverse effects on or via lactation

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

There is no specific data with DAA that provide information on effects on or via lactation.

10.8.9 Comparison with the CLP criteria

There is no specific data with DAA that provide information on effects on or via lactation.

Thus, no classification can be proposed for this endpoint.

10.8.10 Conclusion on classification and labelling for reproductive toxicity

Based on the available information, the dossier submitter considers that classification in category 1B is warranted for effects on development.

10.9 Specific target organ toxicity-single exposure

Not assessed in this report.

10.10 Specific target organ toxicity-repeated exposure

Not assessed in this report.

10.11 Aspiration hazard

Not assessed in this report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this report.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this report.

13 ADDITIONAL LABELLING

Not assessed in this report.

14 REFERENCES

Unnamed, 1979, 1980. Study reports. Guideline 412 (Subacute Inhalation Toxicity: 28-Day Study).

Unnamed, 1997. Study report. OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)

Unnamed, 2015. Study report. OECD Guideline 417 (Toxicokinetics)

Unnamed, 2016a. Study report. No guideline followed.

Unnamed, 2016b. Study report. OECD Guideline 414 (Prenatal Developmental Toxicity Study)

Unnamed, 2017. Study report. OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents).

Unnamed, 2019. Study report. OECD Guideline 414 (Prenatal Developmental Toxicity Study)

Unnamed, 2020a. Study report. OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)

Unnamed, 2020b. Study report. OECD Guideline 443 (Extended One-Generation Reproductive Toxicity Study)

15 ANNEXES

Annex 1 for study summaries.

Confidential annex.