Agency technical report on the classification and labelling of:

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

EC Number: 204-626-7
CAS Number: 123-42-2

June 2026

Contents

Brief summary

The conclusion of the Agency technical report is that 4-hydroxy-4-methylpentan-2-one (diacetone alcohol; DAA) meets the classification criteria for:

Repr. 1B; H360D (May damage the unborn child)

Is this in agreement with the RAC opinion? YES

At the time of publication, this mandatory classification and labelling (MCL) has not been agreed and/or adopted in Great Britain.

This is a targeted technical report which only considers reproductive toxicity. This was the only hazard class considered in the EU Committee for Risk Assessment (RAC) Opinion.

This substance has an existing MCL which includes Eye Irrit. 2; H319. Eye irritation is not assessed in this technical report, therefore Eye Irrit. 2; H319 should be retained in the GB MCL.

Introduction

Under Article 37 of the GB CLP Regulation[1], the Agency[2] is required to produce a technical report for each substance on which the Committee for Risk Assessment (RAC) of the European Chemicals Agency produces an opinion[3].

This technical report documents an independent scientific assessment, conducted by HSE technical specialists of the classification and labelling of diacetone alcohol.

Table 1. Information considered in the scientific assessment

Document	Included in assessment
EU CLH report	Yes
Annexes to the EU CLH report	Yes
RAC opinion	Yes
Background document	Yes
Information submitted during the EU public consultation process (RCOM table, including attachments)	Yes
RAC minority opinion(s)	Not applicable
Other information:	No

This information has been evaluated against the classification and labelling criteria set out in the GB CLP Regulation.

[1]The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

[2] HSE acting in its capacity as the GB CLP Agency

[3] Under Article 37(4) of Regulation (EU) No 1272/2008 on classification, labelling and packaging of substances and mixtures

Overview of current and proposed classification and labelling

Table 2. Current and proposed classification and labelling

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)		
GB MCL List entry	603-016-00-1	4-hydroxy-4-menthylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	Eye Irrit. 2	H319	GHS07 Wng	H319		Eye Irrit.2; H319: C ≥ 10 %	
EU dossier submitter's proposal	603-016-00-1	4-hydroxy-4-menthylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	**Add** Repr. 1B	**Add** H360D	**Add** GHS08 Dgr	**Add** H360D			
EU RAC opinion	603-016-00-1	4-hydroxy-4-menthylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	**Add** Repr. 1B	**Add** H360D	**Add** GHS08 Dgr	**Add** H360D			
Agency technical report conclusion	603-016-00-1	4-hydroxy-4-menthylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	**Add** Repr. 1B	**Add** H360D	**Add** GHS08 Dgr	**Add** H360D			
Resulting MCL entry on GB MCL list	603-016-00-1	4-hydroxy-4-menthylpentan-2-one; diacetone alcohol	204-626-7	123-42-2	Repr. 1B Eye Irrit. 2	H360D H319	GHS08 GHS07 Dgr	H360D H319		Eye Irrit.2; H319: C ≥ 10 %	

Background

Active substance in Plant Protection Products: ☐

Active substance in Biocidal Products: ☐

Chemical registered under REACH: ☒

Diacetone alcohol is a water-soluble liquid solvent used in printing, recorded media reproduction, building and construction work in industrial sites. In this context, the substance is used in the manufacture of chemicals, food products, textile, leather and fur, wood and wood products, pulp, paper and paper products, fabricated metal products, electrical, electronic, optical equipment, machinery and vehicles.

In terms of professional use, diacetone alcohol is used in coating products, fillers, putties, plasters, modelling clay, polymers, adhesives and sealants, air care products, anti-freeze products, biocides (disinfectant, pest control products), finger paints, fertilisers, plant protection products, perfumes and washing and cleaning products.

In terms of consumer uses, this substance is used in coating products, anti-freeze products, biocides, lubricants and greases, fillers, putties, plasters, modelling clay and finger paints (CLH, 2024).

The chemical structure of diacetone alcohol is shown below:

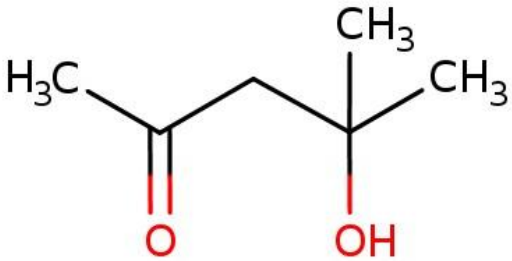

Figure 1: structural formula of diacetone alcohol (4-hydroxy-4-methylpentan-2-one 100.004.207 | Identity - ECHA CHEM, accessed date: 03/26)

Scientific assessment of the physical, human health and environmental hazard classes

Physical Hazards

Not assessed in the CLH report or RAC opinion.

Health Hazards

Acute Toxicity

Not assessed in the CLH report or RAC opinion.

Specific target organ toxicity – single exposure (STOT SE)

Not assessed in the CLH report or RAC opinion.

Skin corrosion/irritation

Not assessed in the CLH report or RAC opinion.

Serious eye damage/irritation

Not assessed in the CLH report or RAC opinion.

Respiratory sensitisation

Not assessed in the CLH report or RAC opinion.

Skin sensitisation

Not assessed in the CLH report or RAC opinion.

Specific target organ toxicity – repeated exposure (STOT RE)

Not assessed in the CLH report or RAC opinion.

Germ cell mutagenicity

Not assessed in the CLH report or RAC opinion.

Carcinogenicity

Not assessed in the CLH report or RAC opinion.

Reproductive toxicity

Classification agreed by RAC:

Sexual function and fertility

RAC considered three key studies for the assessment of sexual function and fertility, and two further supporting studies. All key studies were performed in Sprague-Dawley rats. Only findings relating to sexual function and fertility are discussed in this section; findings relating to development can be found in the section on “developmental toxicity” in this report.

Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422, GLP status not specified (Anonymous (1997))

In this key study, Sprague-Dawley rats (10/sex/dose) were administered DAA (vehicle: purified water) via oral gavage at doses of 0, 30, 100, 300 or 1000 mg/kg bw/d. The exposure period lasted 44 days in males (from 14 days prior to mating), and 41-45 days in females (from 14 days prior to mating until Day 3 of lactation). RAC noted that this study was given a reliability rating of 2 by the DS because GLP-compliance was not specified, and the exposure duration was shorter than recommended in the test guideline.

Parental toxicity

One female was euthanised at the top dose after reaching a near death state post-delivery. No other deaths occurred.

A ‘significant reduction in body weight’ was noted during the pre-mating period in top-dose females at the top dose (no numerical data provided).

Signs of neurotoxicity were observed from 300 mg/kg bw/d. Decreased spontaneous locomotion was observed in animals at 300 mg/kg bw/d (7/10 males; 6/10 females) and 1000 mg/kg bw/d (10/10, both sexes). At the same doses, there was also a reduced response to stimulation (both sexes: 5/10 at 300 mg/kg bw/d; 10/10 at 1000 mg/kg bw/d).

Haematology and clinical chemistry were assessed in males only. At the top dose, the following statistically significant changes were observed: increased platelet count (+16%), glutamic oxalacetic transaminase (+49%), choline esterase (+179%), total protein (+10%), total cholesterol (+76%), total bilirubin (+33%), blood urea nitrogen (+19%), creatinine (+17%), calcium (+6%), and decreased glucose (-23%).

At the top dose, increased liver weight was observed in males (+18% relative weight) and in females (+26% absolute / +25% relative). This was accompanied by hepatocellular hypertrophy (5/10 males, 6/10 females).

Kidney effects were reported in males only. Increased kidney weight was observed at 300 mg/kg bw/d (+15% absolute / +16% relative). There was an increased severity of deposition of hyaline droplets in the proximal tubular epithelium from 100 mg/kg bw/d, increased basophilic tubules from 300 mg/kg bw/d and dilation of the distal tubules at 1000 mg/kg bw/d.

Effects on the adrenals were also reported in males only. At 1000 mg/kg bw/d there was a statistically significant increase in adrenal weight (relative: +25%, absolute: +21%) accompanied by vacuolization of the cells of the zona fasciculata (5/10 animals).

Reproductive findings

No statistically significant effects were reported in the reproductive parameters, and no changes were noted in the copulation rate, number of corpora lutea or the length of the gestation period. However, a non-statistically significant reduction was found in the fertility index, number of implantations and implantation index at 1000 mg/kg bw/day (see Table 3). The DS noted that statistical significance may not have been reached due to the high standard deviation at this dose (individual animal data were not available to the DS, preventing further analysis).

Table 3. Fertility data in female rats of the OECD TG 422 study

Dose (mg/kg bw/d)	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

At the top dose there was also one female euthanised due to vaginal haemorrhage on GD22 with a completely stillborn litter, and one other case where no pups survived past delivery due to death or cannibalism.

Reproduction/developmental screening test (OECD TG 421, GLP (Anonymous, 2020))

In this key study, Sprague-Dawley rats (10/sex/dose) were administered DAA (purity - confidential; vehicle – corn oil) via oral gavage at doses of 0, 50, 250 and 750 mg/kg bw/d. The exposure period began 2-weeks prior to pairing, until day 30-31 of the study for males, and until day 13 post-partum or the day before sacrifice for females.

Parental toxicity

Mortality was observed at 250 mg/kg bw/d in one female who was sacrificed for humane reasons on GD 23 due to difficulty in parturition. No effects on body weight were noted, however a non-statistically significant decrease in body weight gain was noted in females at all doses at day 7 post-partum (-56%, -66%, -25% at 50, 250 and 750 mg/kg bw/day, respectively. At 750 mg/kg bw/d, there was a statistically significant decrease in food consumption on GD 7 and GD 13 post-partum (down to -18%).

At 750 mg/kg bw/d, there was a significant increase in TSH (1.8 folder higher) in males.

Changes in the liver were noted in both sexes at 750 mg/kg bw/d. There was a statistically significant increase in hepatocytic hypertrophy (males: 10/10, females: 9/10 controls: 0/10). In males there was a significant increase in hepatocytic vacuolization (6/10 vs control 0/10). There were no effects on liver weight in either sex.

Changes in the kidneys were reported in males only. An increase in the severity of hyaline droplet accumulation was noted from 250 mg/kg bw/d. At 750 mg/kg bw/d, an increase in inflammatory cell foci (7/10 vs control 0/10) was noted. Also at the top dose, a non-statistically significant increase in nephropathy was observed (8/10 vs 3/10 in controls). There were no effects on kidney weight in either sex.

Reproductive function / performance (P0)

In both sexes, no significant effects were observed relating to sexual function and fertility.

Extended one-generation reproductive toxicity study (OECD TG 443, GLP (Anonymous, 2020))

In this key study, Sprague-Dawley rats were administered DAA (purity: confidential) via oral gavage at doses of 0, 50, 200, and 600 mg/kg bw/d. For the P0 generation, 25 rats/sex/dose were used. For Cohort 1A and 1B, 20 rats per sex/dose were used. The exposure period in P0 males was 68-72 days. They were treated once a day, 7 days a week, from 2 weeks pre-mating to up to 10 weeks post mating. The exposure period in P0 females was similar - they were treated once a day, 7 days a week from 2 weeks pre-mating, through mating, gestation, and post-partum period until the day before sacrifice, day 21 post-partum. In cohort 1A the exposure period started from day 21 until the day before necropsy, typically 13/14 weeks. In cohort 1B, males were treated from day 21 for 10 weeks, prior to mating on day 91, and then until the majority of F2 litters were weaned. Females were treated the same, with treatment occurring through gestation and post-partum periods.

P0 Generation

General toxicity

Mortality was observed at 50 mg/kg bw/d in one male and one female. No clinical signs were observed. No effect on mean body weight was observed, however, a statistically significant decrease in body weight gain was reported at 600 mg/kg bw/d in both males (-19%) and females at day 7 post coitum (-10%). At all dose levels, a dose-dependent decrease in body weight gain when compared to the control group was observed, but was only statistically significant at 600 mg/kg bw/d. Food consumption was decreased in

females at doses ≥ 200 mg/kg bw/d on day 14 post-partum, however the finding was only statistically significant at 200 mg/kg bw/d (around -6%).

In the liver, there was a statistically significant increase for males in absolute and relative mean liver weight (+16.2%). The CLH report also noted 'significant' hepatocellular hypertrophy, from minimal to moderate degree (600 mg/kg bw/d: 14/25, control: 0/25). No histopathological changes were reported in P0 females.

In the kidneys, there was a statistically significant increase in absolute and relative kidney weight at 600 mg/kg w/d (males: +22.4%, +22.23%, females: +7.3%, 8.5%). There was also a statistically significant increase in hyaline drop accumulation in all males dosed at 600 mg/kg bw/d. The DS noted that this was associated with nephropathy at this dose (13/25).

In the adrenals, males had a significant increase in absolute adrenal weight at 200 mg/kg bw/d (+11%), as well as absolute and relative adrenal weight at 600 mg/kg bw/d (+15.5%). However, the DS noted that no histopathology findings were associated with this.

At 600 mg/kg bw/d, there was statistically significant decrease of haemoglobin (-5%) in males. Partial thromboplastin time was statistically significantly reduced in males (-16%) and females (-8%),

Results of clinical chemistry analysis revealed statistically significant changes consistent with liver alterations at 600 mg/kg bw/day. Statistically significant increases in cholesterol levels were found in P0 males (+52%) and females (+31%). In addition, there was a statistically significant increase in total protein (+5%) and globulin (+9%) in males. Glucose was statistically significantly decreased in females (-11%). Triglycerides were increased in P0 animals, but the change was not statistically significant.

Urinalysis findings showed increased ketonuria in males, with a dose-related trend at 200 and 600 mg/kg bw/day while females showed no effect or had only a minimal increase. According to the DS, no detailed data on this finding are available in the study report.

Thyroid hormone measurements showed statistically significant increase of T3 in P0 males (+36.7%) at 600 mg/kg bw/day (not associated with any histopathological changes in the thyroid).

Sexual function and fertility

There was a non-statistically significant decrease of normal spermatozoa (-7%), as well as an increase in the number of headless sperm or sperm with an abnormal head at the 600 mg/kg bw/d dose. RAC noted that this was not considered dose-related or associated with altered parameters such as a histopathological lesion or an effect on reproduction.

A statistically significant decrease in absolute (-11%) and relative (-13%) seminal vesicle weight was noted at 600 mg/kg bw/d. Similarly, a statistically significant decrease in absolute (-7.6%) and relative (-8.5%) weight of the epididymides was reported at this dose. However, the DS noted that there were no histopathology findings associated with the weight changes.

Presented in the table below are the results of the copulatory index and the fertility index.

Table 4. Copulatory and fertility indices for the P0 generation

	0 mg/kg bw/d	**50 mg/kg bw/d**	**200 mg/kg bw/d**	**600 mg/kg bw/d**
Copulatory index	100%	100%	100%	100%
Fertility index	100%	100%	96%	92%

Three females were found to be not pregnant at necropsy; one at 200 mg/kg bw/d and two at the top dose. No effects were seen on the oestrous cycle, gestation length or number of implantations.

1A generation cohort

General toxicity

No mortality or clinical signs were observed in this generation. A statistically significant reduction in mean body weight gain was observed on day 56 (-11%) and 70 (-19%) at the top dose in males. However, there was no effect on bodyweight noted.

The main target organs were the liver and kidneys.

In top dose males, there was a statistically significant increase in relative (+19%) mean liver weight, with centrilobular hepatocellular hypertrophy of the liver in 14/20 animals (vs o 0/20 males in controls).

Statistically significant increases in relative kidney 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 (17%/+22%) and in relative kidneys weight of Cohort 1A females (+10%) dosed at 600 mg/kg bw/d. In males, the weight changes were associated with an increased severity of hyaline droplet accumulation, from mild to moderate in Cohort 1A males dosed at 200 mg/kg bw/d (8/20) and 600 mg/kg bw/d (all

males). Nephropathy was also reported in Cohort 1A males (9/20) at 600 mg/kg bw/d. No histological changes were reported in the kidneys of females.

In males there was a significant increase in absolute (+14%) and relative (+20%) adrenals weight at the top dose compared to the control. No histopathological findings were associated with this change in adrenal weight. No change was noted in females.

Reproductive function/performance

No statistically significant effects were observed on the reproductive organs, ovarian follicle number, sperm analysis or the oestrous cycle.

Generation 1B cohort

General toxicity

One male was found dead in the control group. One female was sacrificed at 200 and 600 mg/kg bw/d for humane reasons. The female sacrificed at 600 mg/kg bw/d was killed on GD22 and presented clinical signs which were associated with dystocia.

In males, there was a statistically significant decrease in bodyweight on PND35 (-7.7%) and PND42 (-7%). With bodyweight gain, males showed a statistically significant decrease at the top dose on day 35 (-12%) and day 91 (-22%) pre-pairing. After pairing there was a statistically significant increase at the top dose on day 43 (+63%). In females, there was a statistically significant increase in body weight gain at the top dose on LD7 (+103%) and at 200 mg/kg bw/d on GD14 (+18%). Food consumption was affected in females only, with a statistically significant decrease (-7%) on PND 14 at 600 mg/kg bw/day.

Results of the clinical chemistry analysis revealed a statistically significant increase of cholesterol in Cohort 1B males (+49%) and females (+27%), and a statistically significant increase in total protein in 1B males. Triglycerides were also increased in Cohort 1B males at 200 and 600 mg/kg bw/day (+ 92% and + 143%, statistically significant). Triglycerides were higher than controls in some Cohort 1B females treated at 200 and 600 mg/kg/day (+47% and +55%, respectively), although the changes were not statistically significant. In cohort 1B males, there were statistically significant increases in albumin (+5%), creatinine (+29%) and calcium (+6%), and a statistically significant decrease of aspartate aminotranferase (-27%) and chloride (-3%). In Cohort 1B females, there was a statistically significant increase in alanine aminotransferase (+20%) and bilirubin (+72%).

In males, statistically significant decreases in haemoglobin (-7%), mean corpuscular Hb (-5%) and mean corpuscular Hb concentration (-3%) were observed at 600 mg/kg bw/d. A statistically significant increase in neutrophils (+6%) was also observed at the top dose. At 200 mg/kg bw/d, there was a significant decrease in platelets (-24%). Additionally, at the

top dose there was a significant decrease of prothrombin time (-4%) and thromboplastin time (14%).

In the liver, males at the top dose showed a non-statistically significant increase in absolute weight (+14.8%) and a statistically significant increase in relative weight (+17%). In females there was a non-statistically significant increase in absolute (+5.6%) and relative (+7.5%) liver weight at the top dose. No histopathology findings were reported in the liver.

In the kidneys, males at the top dose showed a statistically significant increase in absolute (+12.7%) and relative (14.2%) kidney weight. In females there was also a statistically significant increase in absolute (+6.5%) and relative (+7.5%) kidney weight at the top dose. There were no histopathological findings in the kidneys. Urinalysis findings showed increased ketonuria in 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. In 1B males, increases of urine volume at 200 mg/kg bw/day (+142%) and at 600 mg/kg bw/day (+222%) were observed.

In males there was a dose-dependent increase in adrenal weight (50 mg/kg bw/d: +4.7%, 200 mg/kg bw/d: +11.6%, 600 mg/kg bw/d: 13.8%), without any associated histopathological findings.

In males at the top dose there was a significant increase in absolute (+12.1%) and relative (10.6%) thyroid weight. The changes in thyroid hormones are summarised in Table 5, below.

Table 5. Statistically significant changes in thyroid hormones

	50 mg/kg bw/d	**200 mg/kg bw/d**	**600 mg/kg bw/d**
T3	Males: non-sig Females: non-sig	Males: non-sig Females: 23%	Males: +48% Females: non-sig
TSH	Males: -48% Females: -14%	Males: -50% Females: -50%	Males: -59% Females: -59%

Reproductive function and performance

At 600 mg/kg bw/d, there was a statistically significant decrease in absolute (-18.3%) and relative (-17.2%) prostate weight. However, there were no associated histopathology findings.

The number of pregnant females per dose is shown in the table below.

Table 6. Number of pregnant females per dose in the OECD 443 study

	50 mg/kg bw/d	**200 mg/kg bw/d**	**600 mg/kg bw/d**	**control**
Number of pregnant females	18/20	19/20	19/20	20/20

There were no statistically significant effects on the oestrous cycle, reproductive index, spermatogenic cycle, ovarian follicle number, gestation length or number of implantation.

In addition to the key studies summarised above, RAC considered some additional supporting studies.

Repeated dose 90-day oral toxicity study (OECD TG 408, GLP (Anonymous, 2017))

In a repeated dose 90-day toxicity study, Sprague Dawley rats (10/sex/dose: 25, 150 mg/kg bw/d; 15/sex/dose: 0, 600 mg/kg bw/d) were administered DAA (purity: confidential) via oral gavage at doses of 0, 25, 150 and 600 mg/kg bw/d. The exposure period lasted 13 weeks, followed by a 6-week recovery period.

No mortality or clinical signs were observed. In males at 600 mg/kg bw/d there was a statistically significant decrease in mean body weight from week 10 (-9%), and a reduction in body weight gain (-12%) across the treatment period, with a partial recovery post treatment.

The target organs were the liver, kidneys and adrenals.

Liver: at the top dose, there was a statistically significant increase in absolute (male: +20%, female: +18%) and relative (male: +31%, female: +12%) liver weight, which was associated with minimal to slight centrilobular hypertrophy. This was completely reversible within the recovery period.

Kidneys: at the top dose in males, there was a statistically significant increase in absolute (+16%) and relative (+26%) kidney weight. These changes correlated with microscopic tubular hyaline droplets, tubular basophilia and granular cats. All findings were almost completely reversed by the end of the recovery period. In females, there was a statistically significant increase in absolute kidney weight at 150 mg/kg bw/d (+13%) and 600 mg/kg bw/d (+14%), with no associated histopathological findings.

Adrenals: at the top dose in males there was a statistically significant increase in absolute (+26%) and relative (+38%) adrenal weight, associated with moderate vacuolation of cortical cells at 25 and 600 mg/kg bw/d. This was not reversible after the recovery period.

Reproductive function and performance

There were no statistically significant effects on the oestrous cycle or spermatogenic cycle.

Subacute inhalation toxicity 28-day study (OECD TG 412, non-GLP (Anonymous (1980))

In this study, Sprague-Dawley rats (12/sex/dose) were exposed to DAA vapour at concentrations of 50, 225, and 1000ppm (233, 1041, and 4685 mg/m^3), and the animals were subject to whole-body exposure. The exposure period lasted 6 hours, 5 days per week for a total of 6 weeks. The ovaries, testes, uterus, prostate and seminal vesicles histopathology was examined, but no results were described.

RAC's assessment of the data on sexual function and fertility and conclusion on classification

Across all of the available studies, RAC considered that there was some evidence for effects on the fertility index and number of implantations, effects on the oestrous cycle, and effects on male reproduction.

Fertility index and number of implantations

In the OECD TG 422 study, there was a decrease in both the fertility index and number of implantation sites at the top dose (1000 mg/kg bw/day). RAC noted that no effects were observed on these parameters in the OECD TG 421 and 443 studies (although the Agency notes that the top dose in these studies was 750 mg/kg bw/d and 600 mg/kg bw/d, respectively). Regarding the effects seen in the OECD TG 422 study, RAC considered the findings to be weak, not statistically significant and noted that they occurred in the presence of overt maternal toxicity (liver and kidney effects, neurotoxicity). Therefore, RAC concluded that the findings on fertility index/number of implantations were not sufficient for classification.

Oestrous cycle

Effects on the oestrous cycle (decreased number of cycles, longer cycle length) were reported in the supportive OECD TG 408 study in females dosed at 600 mg/kg bw/day. The effects were not statistically significant, and no histopathological findings were reported in the female reproductive organs. Hormone levels were not measured. As similar effects were not reported in the other studies (OECD TG 421 and 443), RAC concluded that the effects on the oestrous cycle were not sufficient for classification.

Male reproduction

RAC noted that effects on sperm parameters were observed in two studies. The OECD TG 443 study reported modifications of morphology (abnormal heads or headless spermatozoa) in P0 and Cohort 1A males, although the changes were not statistically significant, not dose-related and were not associated with other altered parameters (such as histopathological lesions or effects on reproduction) nor with anomalies in the different stages of the spermatogenic cycle. In the OECD TG 408 study, a non-statistically significant decrease in mean epididymal sperm counts was reported at the top dose (600 mg/kg bw/d) at the end of the treatment and recovery periods. These findings were not associated with effects on the weight and/or microscopic findings in the testis and epididymides, and were concomitant with lower body weight from week 10 (-9 %), lower body weight gain (-13 %), reversible changes in biochemistry and centrilobular hypertrophy/vacuolation. The OECD TG 443 study also reported decreases in relative and absolute weights of epididymides in P0 animals at 600 mg/kg bw/day. No histopathological findings were observed, and the effects were not consistently observed across the other generations in the study. In addition, decreased absolute prostatic weights were reported at 50 mg/kg bw/day in P0 animals (about -15 %), and decreased absolute and relative prostatic weights in Cohort 1B animals at 600 mg/kg bw/day (about -18 %). However, RAC noted that none of the observed male organ alterations resulted in statistically significant changes in reproductive performance.

The only study assessing fertility at 1000 mg/kg bw/day (OECD TG 422 (Anonymous (1997) showed some indications for effects on fertility parameters (fertilisation and implantation). However, the differences between controls and high dose groups were not statistically significant, and they occurred in the presence of overt maternal toxicity. As such, RAC considered that the findings were not sufficient for classification. This study reported high standard deviations on parameters at the top dose, which adds to the uncertainty relating to the observed effects. RAC noted that CLP Annex I 3.7.2.3 allows biologically significant positive results to be considered in a weight of evidence evaluation, even in the absence of statistical significance. However, RAC acknowledged the contradiction between the results of the three key studies and considered that the findings in the OECD TG 422 study (Anonymous,1997) alone were not robust enough to justify classification.

Overall, RAC noted that the three key studies that investigated the effects of DAA in rats on reproductive function found no statistically significant changes in the female (oestrous cycles) and male (epididymides, seminal vesicles, prostate histopathological parameters and sperm parameters) reproductive organs and functions. In addition, fertility index and number of implantation sites were not statistically significantly affected. RAC also noted the hysterectomy investigations relevant to sexual function and fertility from the two Prenatal Developmental Toxicity Studies (see “Developmental Toxicity” section). These studies showed no statistically significant differences in any parameter for both species.

RAC concluded that the only study assessing fertility at 1000 mg/kg bw/day (OECD TG 422, Anonymous (1997)) showed some indications for effects in fertility parameters. However, the differences between control and high dose groups were not statistically significant for any given parameter, and they occurred in the presence of overt maternal toxicity. Taking into account all available data, RAC considered that the observed effects were not sufficient to justify classification for effects on sexual function and fertility.

Developmental toxicity

Five key studies were presented in the CLH report for the assessment of developmental toxicity. Four studies were conducted on Sprague Dawley rats, with a fifth study conducted on New Zealand White rabbits.

<u>Prenatal developmental toxicity study (OECD TG 414, GLP (Anonymous, 2016b))</u>

In this prenatal developmental toxicity study, Sprague Dawley rats (24/female/dose) were administered DAA (purity: confidential) in corn oil via oral gavage at doses of 0, 100, 300 and 1000 mg/kg bw/d. The exposure period lasted from GD 6 to GD 20.

Parental toxicity

No mortalities or significant body weight changes were noted in the study. At 1000 mg/kg bw/d, there was a significantly increase body weight gain between GD 9-12 (+25g vs 20g in controls). This was associated primarily with increased food consumption in 1 animal. Excessive salivation was also noted at the top dose (6/24, compared to control 0/24)

Organ weight changes were noted at the top dose. There was a statistically significant increase in absolute (+15.5%) and relative (+14.7%) liver weight, and a statistically significant increase in absolute (+9%) and relative (+6.3%) kidney weight.

Developmental toxicity

At the top dose, 23/23 dams had foetuses with unossified or incomplete ossification of various parts of the skeleton: hyoid (fetal incidence: 1.3% vs. 0%), lumbar vertebra: bipartite ossification of centrum (0.7% vs. 0%), caudal vertebra: unossified centrum (3.3% vs. 0%), extra sternebral 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 the presence of cartilage. A skeletal malformation 'knobby ribs' was reported in one fetus in one litter at the top dose; this was associated with other thoracic skeletal variations such as thick and wavy ribs.

Reproduction/ developmental toxicity screening study (OECD TG 421, GLP (Anonymous, 2020b))

In this reproduction and developmental toxicity screening test, Sprague Dawley rats (10/sex/dose) were administered DAA (purity: confidential) via oral gavage at doses of 0, 50, 250 and 750 mg/kg bw/d. The exposure period was over 30-31 days for males, and until day 13 post-partum or the day before sacrifice for females. The effects relevant to sexual function and fertility in this study are discussed in the previous section of this report.

Toxicity in dams (F0)

At 250 mg/kg bw/d one female was sacrificed on GD 23 for humane reasons, as difficulty in parturition was observed. Body weight gain was decreased in all dose groups on day 7 post-partum (-56%, -66%, -25% at 50, 250 and 750 mg/kg bw/d, respectively), although the finding was not statistically significant. Food consumption was statistically significantly decreased on day 7 and day 13 post-partum (up to 18%) at the top dose. Hepatocytic hypertrophy (9/10 females) was also observed at the top dose.

Toxicity in offspring (F1)

Several effects were observed at the top dose. There was a non-statistically significant increase in pre-natal loss (20% versus control 8%). Live litter size gradually decreased from birth, reaching statistical significance on day 13 post-partum (6.30 versus 8 control). In the same period, an increase in post-natal loss was noted (7.73% vs 0% control). Litter weight and mean pup weight also decreased from day 1 post-partum when compared to the control group, and the difference was statistically significant on Days 4 (litter weight, -31%) and 13 post-partum (litter weight, -32%; mean pup weights, -16%). In terms of the sex ratio of the pups, from day 1 to day 14 post-partum, there was a non-statistically significant decrease in the proportion of male pups (40.5% vs 51% control).

Repeated dose toxicity study with reproduction/developmental toxicity screen test (OECD TG 422, GLP status not specified (Anonymous, 1997))

In this repeated dose toxicity study with a reproduction and developmental toxicity screening test, Sprague Dawley rats (10/sex/dose) were administered DAA (purity: confidential) in purified water via oral gavage at doses 0, 30, 100, 300 and 1000 mg/kg bw/d. The exposure period was over 44 days for males, and 41-45 days for females. Males were dosed from 14 days before mating, and females until lactation day 3. GLP compliance was not specified, and therefore the DS assigned a reliability rating of 2. The results relating to sexual function and fertility are discussed in the previous section of this report.

Toxicity in dams (F0)

At 1000 mg/kg bw/d there was one female that was weakened during delivery and was therefore euthanised. No other deaths were observed. At 1000 mg/kg bw/d there was a "significant reduction in the amount of weight gain during the premating period in females", although the DS notes there are no numerical data available for this observation. At this dose level a statistically significant increase in absolute (+26%) and relative (25%) liver weight was observed; this was accompanied by hepatocellular hypertrophy (6/10 animals).

Offspring toxicity (F1)

At 1000 mg/kg bw/d, there were non-statistically significant reductions in the following: 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.

Extended one-generation reproductive toxicity study (OECD TG 443, GLP (Anonymous, 2020))

This method and general/parental toxicity observed in this study are described in the previous section, "Sexual function and fertility".

Offspring toxicity (F1)

At 200 mg/kg bw/d, there was a non-statistically significant increase (9.85%) in pre-natal loss, and a statistically significant increase (12.06%) at 600 mg/kg bw/d compared to the control (4.30%).

At 600 mg/kg bw/d, the following were observed: increase in post-natal loss at birth (based on live litter size) (2.4% vs control 0.2%, not statistically significant); increase in post-natal loss on day 4 (6.8% vs control 2.9%, not statistically significant); and an increase in the

total number of lost pups (mortality) between PND0-21 (mean:1.4 vs 0.6 in controls; not statistically significant).

From 200 mg/kg bw/d, ”slight significant” (description in the CLH report, CLH 2024) decreases in mean anogenital distance AGD (normalised) were noted in male and female pups compared to controls; distances were 2.38, 2.37, 2.05 and 2.00 mm/g in males and 1.34, 1.35, 1.11 and 1.05 mm/g in females at 0, 50, 200 and 600 mg/kg bw/d, respectively.

Offspring toxicity (F2)

A statistically significant increase in total dead pups between PND 0-21 was observed at 200 mg/kg bw/ (0.61) and 600 mg/kg bw/d (1.47) compared to the control (0.17).

At 200 mg/kg bw/d, there was a non-statistically significant increase in pre-natal loss (8.79%), and a statistically significant increase (12.98%) at 600 mg/kg bw/d compared to the control (6.80%).

Several effects were noted at 600 mg/kg bw/d. Post-natal loss at birth (based on live litter size) was increased (2.4% vs control 0%, not statistically significant). Post-natal loss on day 4 post-partum ad a significant increase (12.14%) compared to the control (0.68%). Post-natal loss on day 4 post-partum (based on total litter size in the study report, recalculated by DS with live litter size) was statistically significantly increased (12.14% vs. 0.68% in controls).

The DS notes that contrary to the findings in the F1 generation, no effect on anogenital distance was observed in the F2 pups.

<u>Prenatal developmental toxicity study (OECD TG 414, GLP (Anonymous, 2019))</u>

In this key study, New Zealand White rabbits (24/female/dose) were administered DAA (purity: confidential) in water via oral gavage at doses of 0, 100, 300 and 800 mg/kg bw/d. The exposure period lasted from GD 6 to GD 28. A deviation from the test guideline was noted, in which some animals had 4 days acclimation instead of 5 days. The DS assigned a reliability score of 2.

Toxicity in dams

At the top dose, one female was prematurely euthanised on GD 20. There was a statistically significant reduction in mean body weight at the beginning of the treatment period (-73g on GD 6-9). Additionally, mean food consumption was reduced at the beginning of the treatment period (-40% on day 6-9). This in part could be due to the female that was euthanised, as values returned to control levels in surviving pregnant females.

Offspring toxicity

A number of malformations were noted. At the low dose (100 mg/kg bwd), 1 foetus with multiple malformations and 1 foetus with a face malformation was observed. At 300 mg/kg bw/d, 2 foetuses were noted to have a dilated aorta. At the top dose, five foetuses from five different litters presented malformations (omphalocele, meningoencephalocele, dilated and/or overriding aorta, and/or split frontal bone). RAC noted that these malformations were not seen in the control group nor in the provided historical control data (HCD), or they had a low background incidence. Additionally, 2 foetuses were noted to have tail shape abnormalities.

At 800 mg/kg bw/d, there was a statistically significant increase in the number of foetuses with a small (5.4% vs 0% controls) or pale (4% vs 0% controls) spleen.

From 300 mg/kg bw/d, there were dose-dependent increased incidences of non-ossified bones, proximal phalanxes, and skeletal variations. All observations reached statistical significance at 800 mg/kg bw/d.

RAC's assessment of the data and conclusion on the classification

The CLP criteria for Category 1A (known human reproductive toxicants) state: "The classification of a substance in this Category 1A is largely based on evidence from humans." (Annex I: 3.7.2.1.1 of CLP). Classification of DAA in Repr. 1A is not appropriate as no human data are available.

The CLP criteria for Category 1B (presumed human reproductive toxicants) state: "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 … 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" (Annex I: 3.7.2.1.1 of CLP).

The CLP criteria for Category 2 state: "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 … 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."

In the OECD TG 414 study in rabbits, a significant increase in foetuses with skeletal variations and pale spleen was reported at the top dose (800 mg/kg bw/d), as well as malformations in five foetuses from five different litters (omphalocele, meningoencephalocele, dilated and/or overriding aorta, and/or split frontal bone). RAC noted that these malformations occurred in the absence of maternal toxicity and were absent or very rare in the control group/provided HCD.

Furthermore, two rat studies reported statistically significant increases in post-natal mortality – the OECD TG 443 study (F2 pups only, PND4, 600 mg/kg bw/day) and the OECD TG 421 study (PND14, 750 mg/kg bw/day). RAC noted that the other findings indicative of developmental toxicity reported in the rat studies (increased prenatal loss, decreased litter and mean pup weights, reduced anogenital distance, and skeletal variations) were inconsistent across studies, and occurred at the same doses as maternal toxicity (mainly liver and kidney enlargement and neurotoxic symptoms). Therefore, RAC considered the data from the rat studies as only indicative for developmental toxicity.

Overall, RAC concluded that the rabbit data provided **clear evidence** of developmental toxicity that cannot be considered a secondary non-specific consequence of maternal toxic effects. The observed developmental effects in the rat studies provide supporting evidence for developmental toxicity. In the absence of any mechanistic evidence to indicate that the observed effects are not relevant for humans, RAC proposed classification as Repr. 1B; H360D.

Effects on or via lactation

RAC noted that there were no specific data with DAA that provided information on effects on or via lactation, and concluded that classification was not warranted.

Classification proposed by the Agency:

Sexual function and fertility

The Agency agrees with RAC's conclusion – classification for adverse effects on sexual function and fertility is not warranted.

Developmental toxicity

The Agency agrees with RAC's conclusion on the classification. DAA meets the classification criteria for Repr. 1B; H360D (May damage the unborn child).

Adverse effects on or via lactation

The Agency agrees with RAC’s conclusion - classification for effects on or via lactation is not warranted.

Aspiration hazard

Not assessed in the CLH report or RAC opinion.

Environmental hazards

Hazardous to the aquatic environment

Not assessed in the CLH report or RAC opinion.

Other hazards

Hazardous to the ozone layer

Not assessed in the CLH report or RAC opinion.

Overall conclusion

The Agency has evaluated the RAC Opinion, its rationale and any additional scientific evidence that may have been made available to HSE against the criteria for classification and labelling in the GB CLP Regulation and technical guidance.

The Agency technical report **agrees** with the classification proposed by RAC for the following hazards:

Repr. 1B; H360D (May damage the unborn child)

Overall, the conclusion is to **agree** with the RAC opinion.

References

ECHA (2024) Guidance on the Application of the CLP Criteria, Part 3: Health Hazards. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 5.0, ref: ECHA-24-G-06-EN. Available at https://www.echa.europa.eu/

For all other references, please see the EU CLH report and the EU RAC opinion (available at: https://echa.europa.eu/registry-of-clh-intentions-until-outcome)

CLH (2024) CLH report (including Annexes): Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: 4-hydroxy-4-methylpentan-2-one;diacetone alcohol; Date: 2024; Written by: ANSES (on behalf of the French MSCA) Accessed date: 03/2026

ECHA (2025) Committee for Risk Assessment (RAC) Opinion (including Annexes) proposing harmonised classification and labelling at EU level of 4-hydroxy-4-methylpentan-2-one;diacetone alcohol; Reference CLH-O-0000007588-59-01/F; Date: 06/06/2025, Accessed date: 03/26

Documents published as part of the EU CLH process: Source: European Chemicals Agency, http://echa.europa.eu/

Glossary of terms used in Agency technical reports

Agency, the	HSE, acting in its capacity as the GB CLP Agency
AR	Applied radioactivity
ATE	Acute toxicity estimate
BCF	Bioconcentration factor
BOD	Biological Oxygen Demand
bw	Body weight
CAR	Competent Authority Report
CAS	Chemical Abstracts Service
CI	Confidence interval
CL	Confidence limits
CLH	Harmonised Classification and Labelling
CLP	Classification, labelling and packaging (of substances and mixtures)
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CV	Coefficient of Variation
d	Day
DAR	Draft Assessment Report
DAA	Diacetone alcohol
DOC	Dissolved Organic Carbon
DS	Dossier Submitter
DT	Dissipation time OR degradation time (also DissT or DegT where apparent)
DT_{50}	Dissipation half-life OR degradation half-life (hours or days), see also above
dw	Dry weight
ECHA	European Chemicals Agency
ECx	x% effect concentration
EFSA	European Food Safety Authority
E_rCx	x% effect concentration based on growth rate
EU	European Union
GLP	Good Laboratory Practice
GD	Gestation day
h	Hours
HCD	Historical control data
K_{OC}	Organic carbon-water partition coefficient
K_{OW}	Octanol-water partition coefficient
LC_x	x% lethal effect concentration

LD	Lactation day
MCL	Mandatory Classification and Labelling
M-factor	Multiplying factor
MW	Molecular weight
NOEC	No-observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PND	Post-natal day
QSAR	Quantitative structure-activity relationship
RAC	Risk Assessment Committee
RAR	Renewal Assessment Report
RCOM	Response to comments document
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals regulation
STOT-RE	Specific target organ toxicity – repeated exposure
STOT-SE	Specific target organ toxicity – single exposure
TG	Test Guideline
TSH	Thyroid-stimulating hormone
US EPA	United States Environmental Protection Agency
wt	Weight
wwt	Wet weight
DDA	Diacetone alcohol

Further information

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