COMPILED COMMENTS ON CLH CONSULTATION

Comments provided during consultation are made available in the table below as submitted through the web form. Please note that the comments displayed below may have been accompanied by attachments which are listed in this table and included in a zip file if non-confidential. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Last data extracted on 05.12.2023

Substance name: acetophenone
CAS number: 98-86-2
EC number: 202-708-7
Dossier submitter: Spain

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2023	Belgium	BASF SE, dsm-firmenich, International Flavors & Fragrances, MANE, Robertet, Sotraflor, Takasago International Corp.	Company-Downstream user	1
Comment received				
BASF, dsm-firmenich, IFF, MANE, Robertet, Sotraflor, Takasago took note of the CLH dossier proposing to update the classification of 1-phenylethanone (Acetophenone, EC 202-708-7) to Repr. 1B; H360(FD), STOT SE 3; H336 and Eye Irritant 2; H319 and welcome the opportunity to comment, specifically on the proposed classification for Repr. 1B; (H360 FD). ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to CLH report Proposal for Harmonized Classification Acetophenone 20231129.pdf				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	France	Novapex	Company-Manufacturer	2
Comment received				
Novapex, as Lead registrant of the REACh registration dossier, as well as the P&D consortium (REACh consortium), REACh co-registrants i.e., Shell, Domo and Versalis welcome this opportunity to comment this proposal during the period of the public consultation. Please find below our comments prepared jointly. General comments: In Table 23 (page 31), the abbreviation 'n.a.' is used inappropriately. For example, the C1A females are listed as 'n.a.' for thyroid follicular cell hypertrophy. Rather, the thyroids of 20 and 19 C1A females were evaluated in the control and high dose groups, respectively. No thyroid follicular cell hypertrophy was noted in these animals. Thus, a '0' rather than 'n.a.' should be shown for C1A females in the control and high dose groups. Other uses of 'n.a.' in this table should be similarly corrected.				

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 231201_ACP_CLH_comments_Novapex.zip

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	Germany	BASF SE	Company-Downstream user	3

Comment received

Acetophenone is used as a fragrance ingredient of diverse consumer products and has been approved as flavouring food additive by the US FDA. Acetophenone has a strong and persistent odor (resembling bitter almonds, cherry-/orange blossom and convallaria = lily-of-the valley). In the late 19th century acetophenone has been used as pharmaceutical for treatment of insomnia under the name Hypnone.

It is surprising that the narcotic effects of acetophenone resulting in clear clinical signs of toxicity in the reproduction toxicity studies are largely discounted by the Dossier submitter as evidence for maternal toxicity, although these findings in the reproduction toxicity studies are highlighted in CLH Report Section 10.11 to justify the STOT SE 3 classification proposal.

In addition to maternal toxicity resulting from narcotic effects of acetophenone, it is possible that the strong fragrant properties of acetophenone caused further maternal stress postnatally affecting pup recognition and nursing of the offspring. This point is discussed in more detail in the comments on reproduction toxicity. It is mentioned here only to indicate that the strong and persistent odor of substances like acetophenone should be considered when evaluating toxicological studies with rodents and other species that are highly smell-sensitive.

HEALTH HAZARDS – Acute toxicity

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2023	France		MemberState	4

Comment received

FR agrees to remove the current harmonised classification of acetophenone as Acute Tox.4* (H302) based on the lowest LD50 value (2081 mg/kg bw) that does not meet the classification criteria (≤ 2000 mg/kg bw).

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2023	Belgium	BASF SE, dsm-firmenich, International Flavors & Fragrances, MANE, Robertet, Sotraflor, Takasago International Corp.	Company-Downstream user	5

Comment received

We agree with the CLH proposal

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to CLH report Proposal for Harmonized Classification Acetophenone

20231129.pdf

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	France	Novapex	Company-Manufacturer	6
Comment received				
We agree with the evaluation of the oral acute toxicity done by the Dossier Submitter (DS) in the CLH report. It is aligned with the assessment and the conclusion reported in the REACh dossier for this endpoint by the registrants. We support the proposal to remove the existing classification as Acute Tox. 4 (H302), as the classification criteria are not fulfilled. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 231201_ACP_CLH_comments_Novapex.zip				

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2023	Germany		MemberState	7
Comment received				
The DE CA agrees that in the available OECD TG 401 of 1981 the LD50 value was exceeding 2000 mg/kg bw, which precludes classification into the Acute Tox. 4 category.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	Netherlands	<confidential>	Company-Downstream user	8
Comment received				
No comments				

HEALTH HAZARDS – Reproductive toxicity

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2023	Germany		MemberState	9
Comment received				
Fertility and sexual function: The DE CA supports the proposed classification of acetophenone as Repr. 1B (H360F) based on the clear evidence of effects on sexual function and fertility observed in the GLP-compliant, reliable OECD TG 443 study. The adverse effects which warrant the proposed classification include observation of dystocia in treated pregnant females (0/23, 3/23, 1/21 and 3/21 at 0, 75, 225 and 500 mg/kg bw/day, respectively), in which parturition either did not start or females had difficulties to deliver, leading to their premature death starting from the low dose group. The effects on parturition are independent of severe overt toxicity or other toxicities, as the hypoactivity in P0 females was observed transiently after dosing and in the absence of other clinical signs. Albeit dystocia is a rare finding, it represents clear evidence of the disturbed parturition. To the best of our knowledge, there are two previous RAC decisions on substances that induced dystocia, thiacloprid (List no. 601-147-9) and BENPAT (EC no. 273-227-8), both fulfilling the classification criteria as Repr. 1B (H360F). Furthermore, reduced mating index in the 500 mg/kg bw/day-dosed females (87.5 % vs 95.8 % in the controls) supports the concern that acetophenone affects sexual function and				

fertility. Provided historical control data, comprising the data from two studies of 2017 and 2018, report mating index at the level of 100 %.

The observed delayed sexual maturation in males of F1 generation (2.6 days in Cohort 1A and 6 days in Cohort 2A) can be considered as an additional adversity supporting the classification proposal of the DS.

Development:

The DE CA supports the proposed classification of acetophenone as Repr. 1B (H360D) based on the clear evidence of developmental toxicity observed in the GLP-compliant, reliable OECD TG 443 study. The adverse effects which warrant the proposed classification include increased post-implantation loss in the high dose group (+85 % vs. controls), reduced number of live-born pups (-18 % in mid and -65 % in high dose vs. controls) and reduced pups' viability between PND 0 and PND 4 (-19 % in mid and 60 % in high dose vs. controls).

Date	Country	Organisation	Type of Organisation	Comment number
24.11.2023	United Kingdom	Health and Safety Executive	National Authority	10

Comment received

'The DS has proposed a classification of Repr. 1B(F) primarily based on the findings from the OECD TG 443 study (EOGRTS). There are uncertainties with interpretation of the some of the findings from the EOGRTS and therefore we would welcome a discussion regarding the adverse effects on sexual function and fertility based on the following:

• Classification as STOT SE 3 has also been proposed, based on the narcotic effects of acetophenone in oral and dermal studies, including the EOGRTS. We support the proposal to classify acetophenone with STOT SE for narcotic effects. Taking into consideration the clear narcotic properties of acetophenone it is possible the apparent adverse effects on sexual function and fertility in particular, dystocia observed in the extended one generation reproduction toxicity study (OECD TG 443) are a secondary consequence of narcosis.'

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	United Kingdom	IFRA UK	Industry or trade association	11

Comment received

IFRA UK does not support the Reproductive toxicity classification that has been proposed. IFRA UK supports the work and comments that have been provided by BASF, dsm-firmenich, IFF, MANE, Sotraflor, Takasago in their report submitted to the consultation (also attached to this response). IFRA UK agrees with their report and supports justifying a Repr. 2 classification, rather than the proposed Repr. 1B classification.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment IFRA UK CLP Consultation Response Acetophenone CAS 98-86-2– November 2023.pdf

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	France	Novapex	Company-Manufacturer	12

Comment received

ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

a) Mating index

With regard to the mating index discussed on page 20 of the CLH report, we acknowledge that including females who died before the mating period in the mating index is incorrect (EOGRT study).

Nonetheless, regardless of whether these females are included or not, it should be noted that the high dose incidence (87.5%) is very similar to the historical control range of 90%. Further, the lack of reproducibility in these data, as evidenced by the absence of a similar finding in the combined repeat dose toxicity and DART screening study (in which doses higher than the EOGRT study were administered), further substantiates that there is not a significantly clear effect of treatment on mating. Therefore, these data should not be used to support categorization of acetophenone for effects on fertility and sexual function.

Furthermore, the reduced mating index at the high dose in the EOGRT study is highly suspect as an effect of treatment because it was not replicated in the combined repeat dose toxicity and DART screening study of acetophenone conducted at a considerably higher top dose (750 mg/kg/day versus 500 mg/kg/day in the EOGRT study). Although the EOGRT study was designed with a longer premating period (10 weeks versus 4 weeks in the combined repeat dose toxicity and DART screening study), extension of the premating period is done only to unmask potential effects on male sperm development (which in the rat, is approximately 65 days for a full spermatogenic cycle); this difference should have no impact on the mating index. As such, the reduced mating index at the high dose in the EOGRT study should be given reduced (or no) weight in the overall assessment of acetophenone for classification for effects on sexual function and fertility.

Finally, the impact of the neurological effects observed in the male and female parental generation during the mating period of the EOGRT study should not be underestimated. A narcotic state, such as hypoactivity and half-closed eye, impacts the mating behaviour (duration of pairing before mating, absence of mating). In the high dose group, where the parental males and females shows a mean of 41 days of hypoactivity and 33 days with half-closed eyes (with a maximum of 72 and 68 days, respectively), the females with the most severe symptoms (i.e. high dose females R21093, R21081, R21090, R21079, and R21082) during the mating period are also the ones who did not mate or who had the longest pairing duration before mating.

b) Dystocia

On page 35 of the CLH report, the Dossier Submitter performs a summary comparison of the sexual function and fertility data for acetophenone with the CLP criteria for reproductive toxicity classification. We agree that dystocia, as observed in the EOGRT study and the combined repeat dose toxicity and DART screening study, should be considered for the classification of acetophenone for effects on reproduction. It is noted, however, that dystocia (difficulty with parturition) is not an effect on fertility (the ability to conceive), nor an effect on sexual function (the ability to be aroused or be sexually active) ; it is, however, and indication of reproductive toxicity. It must be kept in mind that, in contrast to the conclusion reached by the Dossier Submitter, this reproductive outcome was observed in the presence of significant maternal clinical signs of toxicity, consistent with the narcotic effects of acetophenone (and for which the compound has been proposed to be classified as STOT SE-3). Narcotics have been shown in the literature to cause muscle relaxation in general, but also of the uterus (some of them are used as drugs for stopping of premature labour and weakening of labour activity).

It should be further noted that the trigger for parturition in rodents differs dramatically from that in humans. In rodents, surfactant production in late gestation (around GD 19) plays an

important role in triggering the start of parturition. Perturbation in the timing of lung surfactant synthesis or in the quality of surfactant contributes to prolonged gestation and peripartum death in fetal rats (Mysore et al, 1998; Gao et al, 2015). By way of contrast, the appearance of pulmonary surfactant in humans (which occurs around gestational week 25) plays no role in triggering parturition. Rather, the timing of human parturition (typically in gestational week 38) coincides with deterioration of the term placenta and appears to be triggered by the release of exosomes from senescent tissues on the fetal side of the placenta (Menon, 2019). Thus, the finding of dystocia in rats is likely of no relevance for prolonged gestation in humans. As such, dystocia in rats may warrant more appropriate classification of acetophenone as Cat 2 reproductive toxicant.

c) NOAEL for sexual function and fertility
The Dossier Submitter concludes on page 31 of the CLH report that the NOAEL for sexual function and fertility in the F1 animals in the EOGRT study should be 225 mg/kg/day based on delays in sexual maturation. As noted in the comments on the adverse effect on the development (delays in male pubertal development), the effect in males is primarily a reflection of delayed growth of the male offspring rather than a direct effect on sexual development. As such, this finding should not be interpreted as an effect on fertility, but rather, as a finding secondary to an effect on offspring development, and thus, should be considered for classification of acetophenone for developmental toxicity. Further, as noted in the comments on the adverse effect on the development (sexual maturation in females), the delay in females was not significant, was within the laboratory's HCD range, and was solely due to the influence of two outliers at the high dose that should be excluded from the statistical analysis. When this is done, no effect on female pubertal development can be concluded. Thus, effects on pubertal development should not be used to support classification of acetophenone for sexual function and fertility effects. Moreover, because cohort C1B was not bred to derive an F2 generation, no NOAEL for sexual function and fertility should be established for the F1 generation.

d) General toxicity (liver changes)
On page 34 of the CLH report, the Dossier Submitter interprets the liver changes observed in the combined repeat dose toxicity and DART screening study at 750 mg/kg/day (vacuolar changes) as being adverse "since similar effects were reported in the EOGRTS and the subchronic study." The liver changes that were observed in the sub-chronic study are not chronicled in the CLH report. However, based on information provided in the ECHA database (ECHA, 2023), "…a minor, dose dependent hepatocellular hypertrophy was noted in the liver of several animals from all dose groups with males being more affected." This finding from the sub-chronic study is similar to that reported in the EOGRTS (i.e., liver hypertrophy), with the exception that it was not associated with changes in serum liver enzymes or other serum biomarkers indicative of liver toxicity. The liver vacuolar change reported in the combined repeat dose toxicity and DART screening study (i.e., the presence of cytoplasmic vacuoles in the cells) is NOT the same as or similar to hepatic hypertrophy (which is characterized by enlargement of the liver cells). In fact, in the US National Toxicology Program (NTP) Atlas of Nonneoplastic Lesions (US NTP, 2023), it is specifically stated that "If the hepatocytes are enlarged due to the presence of cytoplasmic vacuoles or inclusions, hypertrophy should not be diagnosed." Thus, the vacuolar changes observed in the combined repeat dose toxicity and DART screening study are not similar to the findings from the EOGRT study and sub-chronic study. Moreover, in the absence of changes in serum liver enzymes or other biomarkers indicative of toxicity, they should not be interpreted as adverse or toxicologically significant.

e) Functional observational battery
With regard to the neurobehavioral assay results discussed on page 34 of the CLH report, the lack of consistency in findings across sexes for forelimb strength and motor activity in

the FOB should be taken into account. Specifically, only males at the high dose showed an effect of treatment on these two endpoints of the functional observation battery; females were unaffected. Additionally, the motor activity data are not very robust, as they are based on evaluation of only 5 animals per sex per group. Moreover, these evaluations were conducted on animals exposed in adulthood only, and thus, are not relevant to classification of acetophenone for developmental and reproductive toxicity. Finally, the contribution to these findings of the pharmacologic effect of acetophenone as a narcotic (and for which classification as STOT SE-3 has been proposed) cannot be excluded, especially since the high dose animals all displayed a wobbly gait (as shown in Table 24 of the CLH report).

f) Summary comparison with the CLP criteria
With respect to the summary comparison of the CLP criteria for classification presented on page 35, we agree that the total litter losses are an effect on development rather than on fertility or sexual function.

Based on the observed effect in the studies, we consider that the reduced mating index at the high dose in the EOGRT study should be given reduced (or no) weight in the overall assessment of acetophenone for classification for effects on sexual function and fertility. We agree that dystocia should be considered relevant for the classification for effects on sexual function and fertility. However, this reproductive outcome was observed in the presence of significant maternal clinical signs of toxicity, consistent with the narcotic effects of acetophenone (and for which the compound has been proposed to be classified as STOT SE-3). It should be further noted that the trigger for parturition in rodents differs from that in humans such that the finding of dystocia in rats is likely of no relevance for prolonged gestation in humans. As such, dystocia in rats may warrant more appropriate classification of acetophenone as Repro. category 2.

ADVERSE EFFECTS ON DEVELOPMENT
a) Maternal toxicity
Page 22 of the CLH report makes a claim that, because clinical signs in the dams did not worsen in gestation and/or after delivery in the EOGRT study, it cannot be assumed that the reproductive effects were due to a lack of maternal care. This is incorrect. Even if the clinical signs did not worsen, they remained present during a sensitive life stage and are indicative of marked systemic toxicity that was present in the dams of the mid- and high dose groups during gestation and after delivery. Further, most of the observed clinical signs are indicative of the narcotic effects associated with acetophenone exposure (for which the compound has been proposed for classification as STOT SE-3), which could definitely affect maternal care. In the high dose group, the parental females show a mean of 41 days of hypoactivity and 33 days with half-closed eyes (with a maximum of 72 and 68 days, respectively); these figures show that the maternal systemic toxicity (narcotic effects and other clinical signs) is significant and observed during a large part of the study. Narcotic affects are also observed in the mid dose group, to a lesser extent.
With regard to the maternal clinical signs discussed on page 22 of the CLH report, Annex I: 3.7.2.4.3 of the CLP guidance (2017) states "…when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects." This statement clearly provides allowance within the CLP criteria for no classification as a reproductive toxicant. Narcotic effects (such as hypoactivity and half-closed eyes) were evident in the dams whose litters did not thrive or survive. The nature of these clinical signs could definitely have affected maternal care. It is further notable that the Dossier Submitter has proposed classification for STOT-SE 3; H336, reflecting the view that the substance is a hypnotic agent and causes narcotic effects in experimental animals. To argue these narcotic and hypnotic changes would not impact maternal care is contradictory.

It is concluded on Page 41 of the CLH report that the neonatal pup findings in the EOGRT study were "observed in the absence of maternal systemic toxicity." As stated in a previous comment, this is not correct. Further, page 21 of the CLH report states "In P0, clinical signs such as burrowing activity and ptyalism (at all doses), hypoactivity and half-closed eyes (at 225 and 500 mg/kg bw/d), and continuous chewing movement, staggering gait and recumbency (at 500 mg/kg bw/d) were observed in both males and females. At 500 mg/kg bw/d, abdomen increased in size was also recorded in females and loud/abdominal breathing and reflux occasionally in males." Thus, maternal systemic toxicity (consistent with the known pharmacologic [hypnotic and narcotic] effect of acetophenone for which this compound is also being proposed for classification as STOT SE-3) was seen at the same doses at which neonatal pup effects were observed in the EOGRT study (during a mean of 41 days of hypoactivity and 33 days with half-closed eyes in the high dose females all along the study period, with a maximum of 72 and 68 days respectively, and to a lesser extend in the mid dose group) and likely resulted in a lack of maternal care, which is especially critical in the early postnatal period. Additionally, the NOAEL for systemic toxicity was concluded to be 75 mg/kg/day. Moreover, as previously noted, Annex I: 3.7.2.4.3 of the CLP guidance (2017) clearly provides allowance within the CLP criteria for no classification as a reproductive toxicant when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups. pups. As such, these data do not warrant classification of acetophenone as a developmental toxicant.

On page 43 of the CLH report, the Dossier Submitter argues that the high number of dead pups in the EOGRT study was not due to a lack of maternal care, in part, because most of the reported deaths and clinical signs in pups occurred at birth or between PND 1-2. As already discussed, this is incorrect. Pups were not assessed at birth (PND 0), but rather beginning on PND 1. Thus, it cannot be concluded that these deaths occurred at birth. The Dossier Submitter further states that maternal clinical signs did not worsen in gestation or after delivery. Nonetheless, as mentioned above, significant maternal clinical signs of toxicity (narcotics effects among others) were present during both gestation and in the postnatal period at both 225 and 500 mg/kg/day, and thus, could have affected – and most likely did affect – maternal care in the early postnatal period, which is the time of greatest maternal interaction with her pups (Grota and Ader, 1969; Hughes et al., 1978). The lack of maternal care is further evidenced by the findings of no milk in the stomach of pups that died in this period.

b) delays in male pubertal development (EOGRT study)

With regard to the delays in male pubertal development noted on page 26 of the CLH report, such delays can be secondarily induced by reductions in male pup body weights. Melching-Kollmuß et al. (2014) found that the age at preputial separation (PPS) in control rats was inversely related to pup body weights, with body weight at the time of weaning (on postnatal day [PND] 21) showing the best correlation, "presumably because any potentially confounding variation due to lactation differences as a result of litter size at birth (lower pup weights in large litters and higher pup weights in smaller litters) was minimized by pup culling at day 4 p.p. and the subsequent comparable lactation conditions." Melching-Kollmuß et al. (2014) further noted that a reduction of approximately 10 g in the body weight of male pups on PND 21 may lead to a one-day delay in preputial separation in control Crl:WI rats and likely an even greater delay in Chbb = THOM rats; they did not evaluate Sprague-Dawley, RjHan:SD (CD®) rats, the strain used in the OECD 443 study of acetophenone. When the individual animal data for the C1A and C2A males in the acetophenone EOGRT study are combined, the mean male pup weight at the high dose on PND 22 was 9.5 g less than control or 85% of control; thus, the significance of the delay in general male pup growth to the delay in male pubertal development observed at the high dose cannot be discounted. Further, when the day of PPS was evaluated using PND 22 pup body weight as a covariate in the analysis (ANCOVA), the difference at the high dose was no

longer statistically significant unless two outliers (control animal #R20308 and high dose animals #R20457) were removed (see Appendix B in Attachement). Finally, the mean body weight at the time of preputial separation was unaffected by treatment, indicating that the males went through normal pubertal development once they reached the appropriate degree of postnatal growth. It should be additionally noted that no other male endocrine-sensitive endpoints were reported to be affected in the study, and ToxCast models show that acetophenone is not expected to affect androgen receptor function (US EPA, 2023; see Appendix A in Attachement), further indicating that this finding likely is not an indication of endocrine disruption.

c) Sexual maturation in females (age of vaginal opening, EOGRT study)
On page 28 of the CLH report, the Dossier Submitter argues that vaginal opening was affected by acetophenone treatment. The day of vaginal opening was not significantly altered in the F1 females (combined C1A and C2A) when properly evaluated using the PND 22 pup body weight as a covariate in the analysis (ANCOVA; see Appendix B in Attachement). Moreover, the mean day of vaginal opening at the high dose was affected by two outliers: animal #R21249, which reached vaginal opening at 48 days of age, and animal #R21264, which reached vaginal opening at 70 days of age. When these animals are removed from the analysis or when a robust regression ANCOVA is used to account for the effect of these outliers, statistical significance is not shown. Finally, despite these two outliers, the mean day of vaginal opening at the high dose was still within the laboratory's historical control data (HCD) range (<confidential>, 2023a; see Appendix C in Attachement). Additionally, it should be noted that, according to ToxCast Models (see Appendix A in Attachement), acetophenone is not expected to interact with the estrogen receptor (US EPA, 2023).
As previously discussed, proper statistical analysis shows that there was no effect of acetophenone treatment on vaginal opening. Further, it is our opinion that the effects on preputial separation are a developmental outcome (resulting primarily due to a general delay in development for the males) rather than an outcome that should be classified as an effect on sexual function/fertility. Thus, these findings should not be used as a basis for classification of acetophenone for developmental effects, nor for effects on fertility or sexual function.

d) post-implantation loss (EOGRT study)
On page 41 of the CLH report, a dose-dependent increase in post-implantation loss in the treated groups of the EOGRT study is discussed along with changes in associated parameters (i.e., numbers of total pups liveborn pups, and stillborn pups and live birth index). It should be noted that, as detailed on page 53 of the study report, both post-implantation loss and the live birth index were calculated based on the mean number of live pups per litter observed on PND 1; these parameters were not calculated based on the actual number of pups born (i.e., those observed on PND 0). Total litter size likewise was recorded on PND 1 rather than PND 0. Because of this, and because pup viability to PND 4 was affected by treatment, it cannot be concluded that these findings are indicative of an effect in gestation. Rather, they are blended values composed of stillbirths plus pups that died or were cannibalized during the first 24 hours after birth, and thus reflect effects in the early postnatal period. Moreover, significant increases in post-implantation loss were NOT seen in the rat and rabbit prenatal developmental toxicity studies (OECD TG 414) of acetophenone; the rates of post-implantation loss reported in these two studies were within the laboratories' reported HCD ranges (as provided in appendices to the original study reports; discussed further below). Thus, the data on post-implantation loss are consistent across the acetophenone database in that acetophenone does NOT affect this parameter. The conclusion that the post-implantation losses reported in the EOGRT study are a reflection of early postnatal pup deaths due to lack of maternal care in the presence of maternal toxicity is further supported by the fact that early neonatal pup survival to PND 4

was also low in the EOGRT study. This fact should also be taken into consideration in discussion of these data in the Comparison with CLP criteria, as found on page 53 of the CLH report. More specifically, the available data do not support a conclusion that acetophenone exposure is associated with an increase in post-implantation loss. Thus, post-implantation losses should not be considered as a basis for the classification of acetophenone as a developmental toxicant.

Pages 41-42 of the CLH report states that "According to the registration dossier, the decreased number of live pups on PND 1 and live birth indices were consequences of the increases in the post-implantation losses and were considered treatment-related and adverse." However, as previously noted, it is more likely that the reported increased post-implantation losses are due to the manner in which this parameter was calculated (based on PND 1 rather than PND 0 data) and thus, a reflection of the reduced early neonatal pup survival observed in the study in the presence of maternal toxicity.

Furthermore, the % post-implantation losses reported in both the rat and rabbit developmental toxicity studies were not statistically significant and were within the laboratory's expected HCD ranges (data provided in Table below, as reported in the original study reports). This does not constitute clear evidence of adverse developmental toxicity; therefore, the data do not warrant Repro. Cat 1B classification.
See in attachment table "Mean early resorptions per litter (± SD) and mean % post-implantation loss (± SD) in the rat and rabbit prenatal developmental toxicity studies of acetophenone"

Likewise, the mean numbers of early resorptions noted at the high dose in the rat and rabbit developmental toxicity studies were not significantly different from control. In the rat study, the rate was within the laboratory's HCD range. Further, the slightly higher early resorption rate and % post-implantation loss at 750 mg/kg/day in the rat study was due primarily to a single dam (#77) that experienced total litter resorption of 12 implantations. In the rabbit study, the rate was just inside the laboratory's HCD range, and the exceedance was based primarily on a single doe that lost all eight implantations. Thus, neither post-implantation loss nor early resorptions should be considered as a basis for the classification of acetophenone as a developmental toxicant.

e) reductions in the magnitude of the auditory startle response (ASR, EOGRT study)
On page 44 of the CLH report, it is noted that there were reductions in the magnitude of the auditory startle response (ASR) in the high dose C2A males. This test was conducted on PND 24, when the high dose males weighed significantly and substantially less than the control males. As discussed in the NAFTA Technical Working Group on Pesticides (TWG) Developmental Neurotoxicity Study (DNT) Guidance Document (NAFTA TWG, 2016), body weight has a significant impact on the magnitude of the startle response, wherein increased body weight results in an increased amplitude of the startle response. For this reason, the ASR data should be evaluated using body weight as a covariate in the analysis. When this is done, the ASR data at the high dose are no longer statistically significant (See Appendix D in Attachement). Thus, the reduced ASR in males at the high dose is a function of the reduced pup body weights observed at this dose. As such, the ASR findings should not be considered an adverse, treatment related effect on neurodevelopment.

f) brain morphometric measurements (EOGRT study)
With regard to the C2A brain morphometric measurements discussed on page 45 of the CLH report, the three affected measurements (dentate gyrus thickness [L4-1], cornu ammonis thickness [L4-2], and hippocampus thickness [L4-3]) are all measures within the same brain structure: the hippocampus (Garman et al., 2016). Thus, the laboratory's reporting of all three endpoints was redundant (although confirmatory). It must be also remembered

that, at the time these measurements were made, the mid- and high dose males weighed 9% and 15% less than controls, respectively. Further, mean absolute brain weights for males in the mid- and high dose groups were 3.4% and 6.4% less than that of controls, respectively. Thus, reductions in the brain morphometric measures at these doses is not unexpected in the C2A males. It should be additionally noted that the control value for the cornu ammonis (L4-2) is the highest control value reported in the laboratory's HCD (Appendix C in Attachement); all other control measures for this structure were considerably less and more in line with those reported for the treated groups.
This fact, in combination with the absence of a dose-response for the length of the cornu ammonis, suggests that the finding is not due to treatment.

On page 45 of the CLH report, the Dossier Submitter attempts to correlate the hippocampus morphometric measurements with the ASR findings in C2A males. However, the hippocampus is not involved in the auditory startle response. As addressed by Graham et al. (2012), "The ASR circuit comprises the auditory nerve, ventral cochlear nucleus, nuclei of the lateral lemniscus, and the pons (the nucleus reticularis pontis caudalis), before connecting with the spinal interneurons (which project to the periphery and the neuromuscular junction) to elicit a flinch response. The habituation component of the ASR is one of the simplest forms of learning and is mediated by the forebrain." Additionally, the ASR is a defensive mechanism in which memory is not involved. In contrast, the hippocampus is involved in long-term memory; the dentate gyrus is involved in the memory associated with exploratory actions and the cornu ammonis is involved in information processing, the reproductive cycle, and has been implicated in Alzheimer's disease (Eichenbaum, 2017; Jonas and Lisman, 2014; Gaillard et al., 2023). Further, as discussed in a previous comment, the ASR in the high dose C2A males is due to their delayed growth, not an effect on neurodevelopment. Thus, no correlation can be made between the morphometric findings and results of neurobehavioral testing.

g) splenocyte immunophenotyping data (EOGRT study)
With regard to the splenocyte immunophenotyping data discussed on pages 45-46 of the CLH report, each of the lymphocyte subpopulations represents a proportion of the total splenocytes present. As shown in Table 36 of the CLH report, the absolute number of each of the splenocyte subpopulations in the treated groups of C1A males was decreased from control by approximately the same relative percentage as was the number of total splenocytes. Further, the relative percentage of each of the different subpopulations (with two exceptions) were not changed with treatment. Moreover, the data for the treated groups are highly consistent with those provided in the laboratory's HCD (<confidential>, 2023a; see Appendix C in Attachement).
These data thus indicate that there was not a treatment-related shift in the types of lymphocytes present. The exceptions to the above relate to significant differences in the relative percentage of B cells and NK cells present at the mid-dose. However, since these differences were not seen at the high dose (i.e., they are not dose-related), it is unlikely that they are due to treatment. Moreover, as shown in Table 36 of the CLH report, the differences in absolute numbers of splenocytes and splenocyte subpopulations did not show a relation to dose, suggesting that these differences compared to control are instead related to the control value being unusually high, as shown in the laboratory's HCD data (Appendix C in Attachement). Because the findings do not show a dose-response relationship and are considered an artifact of unusually high control values, the NOAEL for immunotoxicity should be 500 mg/kg/day, not 75 mg/kg/day as suggested by the Dossier Submitter on page 46 of the CLH report. The likelihood that the non-dose-related reductions in splenocytes and splenocyte subpopulations is an artifact of the unusually high control values should also be considered in the Comparison with the CLP criteria found on page 54 of the CLH report. Finally, these data are considered not sufficiently convincing as clear evidence for developmental toxicity; therefore, they do not warrant classification for Cat 1B.

h) OECD 414 in rabbits
With regard to the two rabbits that aborted on GD 20 (discussed on page 52 of the CLH report), it should be noted that both animals experienced negligible food consumption prior to abortion and one of them (#76) developed body weight loss beginning at GD 12. In addition to the treatment-related clinical sign of abnormal gait observed in all high dose animals, the two does that aborted also showed labored and shallow breathing, lying on side, and subdued behavior. Thus, the two abortions seen at the high dose were only seen in maternal animals with severe toxicity and, therefore, the abortions are considered secondary to systemic toxicity. This does not constitute clear evidence of developmental toxicity and therefore does not warrant classification for Cat 1B.

i) Summary comparison with the CLP criteria
On pages 53-54 of the CLH report, the Dossier Submitter does a summary comparison of the developmental data for acetophenone with the CLP criteria for classification. As previously discussed, we disagree that reduced early postnatal pup viability should be considered for the classification of acetophenone for effects on development. It must be kept in mind that, in contrast to the conclusion reached by the Dossier Submitter, this reproductive outcome was observed in the presence of significant maternal clinical signs of toxicity, consistent with the narcotic effects of acetophenone (and for which the compound has been proposed to be classified as STOT SE-3). Although maternal systemic toxicity was not considered by the Dossier Submitter to be "marked" at the high dose in the EOGRT study or in the combined repeat dose toxicity and DART screening study, we disagree. The systemic toxicity was specifically characterized by the presence of clinical signs consistent with the narcotic effects of acetophenone that have been proposed for STOT-SE 3 classification. In the EOGRT study, clinical signs included hypoactivity, half-closed eyes, continuous chewing, and staggering gait after treatment at both the mid- and high doses and increased size of the abdomen at the high dose. In the combined repeat dose toxicity and DART screening study, treatment-related clinical signs were also observed at both the mid and high dose, including urine stains, salivation, feces few/small in size, decreased activity, pale skin, unkempt appearance, rough coat, dark material around the nose, and wobbly gait. This maternal toxicity contributed to a lack of maternal care as evidenced by the absence of milk in the stomach of pups that died in the early postnatal period. Because effects on pup viability occurred in the presence of substantial maternal toxicity that interfered with maternal care, no classification for developmental toxicity is warranted.

Also, with regard to the Comparison with the CLP criteria presented on pages 53-54, it must be remembered that, because live litter size and post-implantation loss in the EOGRT study were calculated based on the number of pups found on PND 1 instead of those born on PND 0, and because there was a significant effect of treatment on early neonatal pup viability in the presence of maternal toxicity, the % post-implantation loss seen in this study is most likely a reflection of the reduced neonatal pup viability and not an effect of treatment on gestational development (i.e., post-implantation loss).

We disagree that the reduced pup weights, as recorded in the EOGRT study and the combined repeat dose toxicity and DART screening study, also may serve as a basis for the classification of acetophenone as a developmental toxicant. toxicant. As previously discussed, the presence of significant maternal clinical toxicity (for which the compound has been proposed for classification as STOT SE-3) interfered with the ability to provide maternal care which includes the provision of nutrition through lactation. Thus, these data do not warrant the classification of acetophenone as a developmental toxicant.

With regard to the neurodevelopmental findings discussed on page 54 of the CLH report, Garman et al. (2016) provides a decision tree for evaluating neurodevelopmental data (see

Appendix E in Attachement). As shown in the decision tree, the evidence available for acetophenone from the EOGRT study should be judged as ambiguous, at best. There are no macroscopic or microscopic abnormalities associated with treatment, and dose related differences in linear measures were not seen at both ages of evaluation, nor across sexes. Further, as previously noted in other comments, the ASR in C2A males is a consequence of the reduced pup body weights at the high dose and, thus, not a specific neurobehavioral finding. Due to the ambiguous nature of these findings, they do not constitute sufficiently clear evidence to warrant classification as a Cat 1B.

As already discussed, the immunophenotyping data from the EOGRT study of acetophenone show only that the numbers of total splenocytes present in the treated groups were less than that in the control group. Moreover, the changes were not treatment related and all findings were within the HCD range. The pattern of changes observed suggest that, rather than an effect of treatment, the findings are an artifact of the unusually high control value for total splenocytes. Because it is unlikely that the immunophenotyping findings are related to treatment, these data should not be used to support classification of acetophenone for developmental toxicity.

With regard to bilateral pelvic girdle caudal shift reported at the high dose in the rat prenatal developmental toxicity study (discussed on page 54 of the CLH report), although rats typically have 26 vertebrae in their vertebral column above the sacrum (termed "presacral vertebrae"), the normal range among control rats is 25-27 presacral vertebrae (<confidential>, 2023b; see Appendix F in Attachement). The observation of 27 pre-sacral vertebrae is often called a caudal pelvic shift. When 27 presacral vertebrae occur in isolation (or in concert with a 14th rudimentary rib), there are no adverse sequelae, and the observation is considered a variation. Human thoracolumbar (TL) vertebral counts vary between 16-18 (typical number is 17 TL) with no gross pathology observed when 18 TL are present (Kawashima and Sato, 2023). While the pelvic caudal shift appears dose-related in the rat prenatal developmental toxicity study, it is important to note that two control fetuses also exhibited the finding. The relatively high incidence of 27 presacral vertebrae in this study as whole suggests that the rats used in this study were predisposed to the condition and the high dose exacerbated that predisposition. Further, this finding was not reported in the rabbit prenatal developmental toxicity study of acetophenone. Because this finding is a normal variation and its relation to treatment is questionable, the pelvic caudal shift should not be used as evidence to support the classification of acetophenone as a developmental toxicant.

In conclusion, we consider that the effects observed in the studies do not warrant classification of acetophenone for toxicity to the development.

REFERENCES

<confidential>. 2023a. Historical Control Data in Rats. 2017-2022_HCD_OECD443. <confidential>, Safety Assessment SAS. BP 563 – 27005 Evereux, France.
<confidential>. 2023b. Historical Control Data in Rats. Developmental Parameters for Crl:CD(SD). ASH_RS_EFD_GD21-TM-50-S_2019.01. <confidential>.
European Chemicals Agency (ECHA). 2023. Acetophenone. Repeated dose toxicity: oral. 001 Key | Experimental Result. Available at: Registration Dossier - ECHA (europa.eu).
Eichenbaum H. 2017. The role of the hippocampus in navigation is memory. J Neurophysiol 117: 1785-1796.
Gaillard F, Haouimi A, El-Feky M, et al. 2023. Hippocampus. Reference article, Radiopaedia.org (Accessed on 27 Oct 2023). doi: 10.53347/rID-1454.
Gao L, EH Rabbitt, JC Condon, NE Renthal, JM Johnston, MA Mitsche, P Chambon, J Xu, BW O'Malley, CR Mendelson. 2015. Steroid receptor coactivators 1 and 2 mediate fetal-to-maternal signaling that initiates parturition. J Clin Invest. 125:2808-2824. doi:

10.1172/JCI78544.
Garman RH, AA Li, W Kaufmann, RN Auer, B Bolon. 2016. Recommended Methods for Brain Processing and Quantitative Analysis in Rodent Developmental Neurotoxicity Studies. Toxicologic Pathology 44:14-42.
Graham DL, TL Schaefer, CV Vorhees. 2012. Neurobehavioral testing for developmental toxicity. Chapter 11. In: Developmental and Reproductive Toxicology. A Practical Approach, 3rd Ed. (RD Hood, Ed.), Informa healthcare, NY.
Grota LJ, R Ader. 1969. Continuous recording of maternal behavior in Rattus norvegicus. Animal Behavior 17:722-729.
Hughes CW, RS Harlan, SM Plaut. 1978. Maternal behavior of wild and domestic Rattus norvegicus recorded continuously in dual-chambered cages. Dev. Psychobiol. 11:329-334.
Jonas P, Lisman J. 2014. Structure, function, and plasticity of hippocampal dentate gyrus microcircuits. Front Neural Circuits 10:8 article 107. doi: 10.3389/fncir.2014.00107.
Kawashima T, F Sato. 2023. Prominent caudal shift of the lumbar plexus roots in spines with 18 thoracolumbar vertebrae. Surgical and Radiologic Anatomy 45:1245-1256.
Melching-Kollmuß S, KC Fussell, R Buesen, M Dammann, S Schneider, H Tennekes, B van Ravenzwaay. 2014. Anti-androgenicity can only be evaluated using a weight of evidence approach. Regulatory Toxicology and Pharmacology 68:175-192.
Menon R. 2019. Initiation of human parturition: signaling from senescent fetal tissues via extracellular vesicle mediated paracrine mechanism. Obstet Gynecol Sci 62:199-211. doi:10.5468/ogs.2019.62.4.199.
Mysore MR, LR Margraf, MA Jaramillo, DR Breed, VL Chau, M Arévalo, FR Moya. 1998. Surfactant protein A is decreased in a rat model of congenital diaphragmatic hernia. Am J Respir Crit Care Med 157:654-657.
NAFTA Technical Working Group on Pesticides (NAFTA TWG). 2016. Developmental Neurotoxicity Study (DNT) Guidance Document. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/developmental-neurotoxicity-study-guidance.
Spanish Ministry of Health. 2023. CLH report. Proposal for Harmonised Classification and Labelling. Acetophenone. Version 2.0. Dated 12/09/2023.
US Environmental Protection Agency (US EPA). 2023. Acetophenone. Bioactivity – ToxCast: Models. CompTox Chemicals Dashboard. V2.2.1. Available at: https://comptox.epa.gov/dashboard/chemical/bioactivity-toxcast-models/DTXSID6021828.
US National Toxicology Program (US NTP). 2023. US NTP Nonneoplastic Lesion Atlas. Hepatobiliary System. Liver, Hepatocyte – Hypertrophy. Available at: https://ntp.niehs.nih.gov/atlas/nnl/hepatobiliary-system/liver/Hepatocyte-Hypertrophy.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 231201_ACP_CLH_comments_Novapex.zip

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	Germany	BASF SE	Company-Downstream user	13

Comment received

(1) In reproduction toxicity studies (EOGRTS and OECD 422) acetophenone was administered daily to rats at very high dose levels by gavage over an extended period of time. Gavage treatment at high dose levels was associated with clinical signs of narcotic effects and with post-gavage salivation/ptyalism of treated dams (see Table 12 page 22). Evidently pups were dermally exposed to acetophenone during the lactation period via ptyalism/salivation of mothers (this would explain reported pup skin findings on PND 0 and 4 in the OECD 422 study at 225 and/or 750 mg/kg bw/d (desquamation, shiny appearance, pale in color; see Table 38, page 47). The additional whole-body dermal exposure of pups is

not representative of a relevant human exposure scenario and raises the question to which extent postnatal adverse findings from such studies should be considered relevant for human health classification purposes. These pups were heavily acetophenone-perfumed, which may have caused further stress of the dizzy/disoriented mothers shortly after birth, and which potentially impaired pup recognition (via smelling and licking) and nursing of the pups. The available evidence indicates that insufficient maternal care as a consequence of maternal toxicity plus potentially odor-mediated altered behaviour caused increased incidences of pre-weaning pup deaths, cannibalism and delays in pup development. All these developmental effects can be considered secondary to maternal toxicity and to rodent-specific responses to high-dose acetophenone exposure, which are not relevant for humans and should not trigger a classification for reproduction toxicity.
(2) From the offspring survival and macroscopic data of the EOGRT study presented in Tables 30 and 31 it is difficult to derive the correct percentage of dead pups with no milk present in the stomach (which indicates insufficient maternal care). At first glance, the data in Table 31 suggests that 34 of 132 dead pups of the high-dose HD group (25%) examined macroscopically had no milk in the stomach. However, this conclusion is probably not correct because the132 dead pups likely include stillborn pups which were not examined. Instead, it is plausible to assume that 100% pups of the HD group that died between PND 1-4 had no milk present in the stomach:
According to Table 30, 196 pups of the high-dose (HD) group were delivered, of which 75 pups were liveborn and 121 pups were stillborn. An overall total of 155 HD pups were reported to have died/cannibalized between PND 0-4 a subset of 34 pups died between PND 1-4. Thus, the remaining 121 pups (=155-34) pups, which were counted as dead or cannibalized on PND 0, were apparently all classified as stillborn. The reported mean percentage of live pups on PND 4 and PND 21 are identical (5.9%), indicating that no HD pups died after Day 4.
According to Table 31, a total of 132 dead HD pups were examined by post-mortem macroscopy. As indicated from information in Table 30, overall, 34 HD pup deaths occurred, all between PND 1-4, thus a subset of 87 stillborn pups of the HD group (= 121-34) seemed to have been macroscopically examined as well. By definition, stillborn pups should not contain any milk in the stomach, therefore it is unlikely stillborn pups were examined for stomach content. It is noted that according to Table 30, the number of HD pups died between PND 1-4 is identical to the number of HD pups found with absence of milk in the stomach as listed in Table 31, both 34. It is therefore highly probable that all deaths occurring in the HD group between PND 1-4 were a consequence of insufficient maternal care. To be 100% sure, individual animal data would need to be consulted.

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	Netherlands	<confidential>	Company-Downstream user	14

Comment received

Comparison with CLP criteria for sexual function and fertility (page 35):
For the EOGRTS the DS states (page 22) “that the clinical signs of dams did not worse during late gestation and/or after delivery. Therefore, it cannot be assumed that the reproductive effects (on sexual function and fertility and development) were due to a lack of maternal care.” This is reiterated on page 35 “general condition of these females did not worse over the course of pregnancy (i.e. body weight, food consumption or clinical signs)”

We respectfully disagree that clinical signs need to worsen during gestation or lactation in order to be considered as relevant for maternal toxicity. Acetophenone, has historically been used as a hypnotic and anticonvulsant agent. The clinical signs linked to the hypnotic effects (burrowing activity and ptyalism at all doses, hypoactivity and half-closed eyes from MD,

and continuous chewing movement, staggering gait and recumbency at HD) were observed in the MD and HD and could have affected the maternal care of the litters. The presence of these clinical signs is evidence that the dams were affected by the test item, although the clinical signs did not worsen during gestation or after delivery the presence of the clinical signs in itself are sufficient to potentially affect maternal care.
In several studies clinical signs consistent with the narcotic effects of acetophenone were reported for which the DS proposes a STOT-SE3 for narcotic effects. Thus, these effects are considered by the DS as adverse.

Furthermore, according to Annex I:3.7.2.4.3 from Guidance on the application of the CLP criteria:
"Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity."

Considering the presence of the clinical signs linked to the known narcotic effects of acetophenone, could have impacted the maternal care, it should be considered if a Cat 2 classification could be more appropriate.

Page 35: Furthermore, the DS considers dystocia as evidence of an adverse effect on sexual function and fertility. However, dystocia is not an effect on sexual function or fertility but is an indication of reproductive toxicology. According to Hood (2006, Second edition developmental and reproductive toxicology a practical approach.) "dystocia may be a manifestation of maternal toxicity resulting from exposures that cause prolonged labor, difficult labor, or termination of the contractions necessary to evacuate the uterus."
Several anesthesia (eg ether) have been reported to have an impact on labor by reducing or terminating the uterine contractions (Wulf, Anaesthesist 1998 Jun. 47(6)):496-500) and as such can impact via maternal effects on the uterus. Considering acetophenone also has narcotic effects it could have impacted on the labor of the rats via maternal mediated toxicity.

Page 35: "Despite there was a higher number of females sacrificed due to total litter loss between PND 1-4 at 225 and 500 mg/kg bw/d in the OECD TG 443 (three and 11 dams, respectively) and at 750 mg/kg bw/d in the OECD TG 422 (six dams), the DS does not consider this a fertility effect, but a consequence of an adverse effect on development (refer to section 10.10.4. for more details)."
The clinical signs linked to narcotic effects were observed in the MD and HD females. Since maternal care is important in the first couple of days it cannot be excluded that the lack of maternal care due to the narcotic effects of acetophenone has contributed to the pup mortality and as such that this is caused by secondary effect of maternal toxicity.

Comparison with CLP criteria for developmental toxicity (page 53)

The DS indicates that the increased pup mortality in the OECD 443 was observed in the absence of worsening clinical signs in the dams. As discussed previously, the dams showed clear clinical signs correlating to the narcotic effects of acetophenone, and the presence of these signs in itself can already affect the maternal care. Furthermore, in the EOGRTS pups

were not observed on PND0 but on PND1 instead, so it is unknown if pups were stillborn or died after birth. Considering that in the OECD 414 study in rat no dead foetuses were recorded (Table 42) it is possible that in the OECD 443 study the pups died in the first couple of days after birth, due to lack of maternal care when the maternal interaction with the pups is important.

Developmental neurotoxicity (page 54)
The DS considers the reduced auditory startle reflex adverse in the high dose males. It should be noted that for the HD males a clear reduction in body weight was observed. According to the Developmental Neurotoxicity Study Guidance Document from the NAFTA Technical Working Group on Pesticides (NAFTA TWG, 2016), body weight has a significant impact on the magnitude of the startle response. And the ASR data should have been evaluated correcting for body weight. Based on Table 34, body weight was not taken into consideration when evaluating this parameter. This should be evaluated, in order to understand if the effect can be explained by reduced body weight.

Furthermore, it is important to determine if the auditory startle reflex was determined when the C2A pups were direct dosed, which started on PND 22 (Table 10) the auditory startle reflex should be determined on PND 24 (±1 day) according to the OECD 443 guideline. Based on this it is likely that the animals were direct dosed at the time of the ASR measurement. With the known narcotic effects of acetophenone it is possible that the effects observed in the HD could have been caused by the direct dosing of the pups and as such are not a developmental effect. MD and HD animals showed clinical signs related to the narcotic effects of acetophenone (Table 14)

The DS considers the hippocampus morphometric alterations in males as adverse effects. However, these effects were observed in the presence of reduced body weight and brain weight. Furthermore, in C2A females these effects were not observed, neither were they reported in C2B animals. Considering the C2B animals were not directly dosed and no effect on hippocampus morphometric alterations were reported, it is uncertain if the effects observed in the C2A males (only) after direct dosing are relevant for the evaluation of developmental toxicity.

Developmental immunotoxicity (page 54)
The DS considers the reduction in absolute splenic lymphocyte subpopulation in males only, as an adverse effect. However, there is no clear dose response in the absolute splenic lymphocyte subpopulation and the effect is not present in the relative numbers. Considering there is no clear dose response and it was only observed in males it would be helpful to compare these values with historical control data to understand the biological relevance of these changes.
Furthermore, it is questionable if this is a developmental toxicity effect since the animals were directly dosed with the test item before determination of the splenic lymphocyte subpopulation.

According to the CLP criteria Category 1B "The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate".

Based on the previous considerations, a Category 2 classification could be more appropriate considering the narcotic effects of acetophenone. The pregnant dams which displayed clear

clinical signs linked to the narcotic effects during gestation and lactation, which could have resulted in secondary non-specific effect on reproduction and the potential lack of maternal care leading to high pup mortality.
For the developmental immunotoxicity, only effects in males were observed in the absence of a clear dose response and since the animals were directly dosed it is unclear of this is a developmental toxic response.
For the developmental neurotoxicity, only effects were observed in C2A males which were directly dosed, no effects were observed in the C2B animals (not dosed) or C2A females, as such it is unclear if this is a developmental toxicity response.

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2023	France		MemberState	15

Comment received

FR agrees with the classification of acetophenone as Repr. 1B, H360F based on clear evidence of adverse effects on sexual function and fertility : dystocia, decreased mating index and delayed sexual maturity (males and females) (OECD TG 443 study).

FR agrees with the classification of acetophenone as Repr. 1B, H360D based on clear evidence of developmental toxicity : decreased offspring viability (live birth and viability index, (OECD TG 422 and 443 studies ; post-implantation loss, OECD TG 443 and 414 studies), the reduction in pup/foetus body weight (OECD TG 443 and 414 studies), developmental neurotoxicity findings and immunophenotyping effects (OECD TG 443), skeletal malformations (pelvic girdle, OECD TG 414 rat study).

There is no data to adequately assess the effects of acetophenone on or via lactation. FR agrees that no classification is justified based on the lack of data.

FR suggests adding a paragraph on the experimental design of OECD 443 for a better overview and comprehension of the results in the section "10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility" (page 20) and in the section "10.10.5 Short summary and overall relevance of the provided information on adverse effects on development (page 41).

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2023	Belgium	BASF SE, dsm-firmenich, International Flavors & Fragrances, MANE, Robertet, Sotraflor, Takasago International Corp.	Company-Downstream user	16

Comment received

The extensive set of reproductive toxicity studies conducted with acetophenone demonstrates biological effects in animals. However, several elements in the CLH dossier proposing a classification for adverse effects on the development of the offspring and on sexual function and fertility deserve an in-depth review and reconsideration as they lack the necessary justification or scientific weight to support the classification conclusion. In particular, the acknowledgement of this material exhibiting well known narcotic effects in the classification proposal as STOT SE 3 and its relation as such to maternal care and the observed effects in these studies warrant further consideration as to their relevance related to the overall CLH proposal.

According to the CLP criteria, the classification of a substance in 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. Narcotic effects are to be considered as "toxic effects", and even if they are not considered as being "severe" or "marked"- the reproductive toxicity observed can be considered a secondary and non-specific consequence of these effects. The transient nature of the narcotic effect is a criterium for the Classification and Labelling as STOT SE 3. However, a transient narcosis does not mean a contradiction with a marked maternal toxicity and a secondary, non–specific parental mediated mechanism. Narcosis at the right point in time will, as discussed in the previous chapters, have an adverse impact on the dams and pups during the parturition process. In general, narcosis / anesthesia of pregnant animals will cause secondary effects on reproduction via effects on the dams as reported in the literature. Based on the above considerations, a Repr. 2 classification (H361) would be better in line with CLP criteria. This is also in line with the RAC opinion on Salicylic acid where (hypothetical) Human exposure considerations have been used to justify a category 2 for Repro.

The different elements which lead us this conclusion are elaborated in the provided attachment.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to CLH report Proposal for Harmonized Classification Acetophenone 20231129.pdf

HEALTH HAZARDS – Specific target organ toxicity - single exposure

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2023	France		MemberState	17
Comment received				
FR agrees with the STOT SE 3 classification (H336: may cause drowsiness or dizziness) based on narcotic characteristics of acetophenone (decreased activity, staggering / wobbly / abnormal gait, flabby appearance, half-closed eyes, prostration or ataxia).				

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2023	Belgium	BASF SE, dsm-firmenich, International Flavors & Fragrances, MANE, Robertet, Sotraflor, Takasago International Corp.	Company-Downstream user	18
Comment received				
We agree with the CLH proposal ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to CLH report Proposal for Harmonized Classification Acetophenone 20231129.pdf				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	France	Novapex	Company-Manufacturer	19
Comment received				
We agree with the evaluation of the specific target organ toxicity-single exposure done by the Dossier Submitter (DS) in the CLH report. We support the proposal to add classification for acetophenone as STOT SE 3 (H336: may cause drowsiness or dizziness). The lowest adverse effect level (LOAEL) is 225 mg/kg bw, observed in the the EOGRT study (Anonymous, 2021) in males and females of the P0 and F1 generation. The corresponding NOAEL is 75 mg/kg bw, as reported by the Dossier Submitter (DS). ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 231201_ACP_CLH_comments_Novapex.zip				

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2023	Germany		MemberState	20
Comment received				
The DE CA supports the proposal of the DS to classify acetophenone as STOT SE 3 based on the observed narcotic effects.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2023	Netherlands	<confidential>	Company-Downstream user	21
Comment received				
No comments				

PUBLIC ATTACHMENTS
1. IFRA UK CLP Consultation Response Acetophenone CAS 98-86-2– November 2023.pdf [Please refer to comment No. 11]
2. Response to CLH report Proposal for Harmonized Classification Acetophenone 20231129.pdf [Please refer to comment No. 1, 5, 16, 18]

CONFIDENTIAL ATTACHMENTS
1. 231201_ACP_CLH_comments_Novapex.zip [Please refer to comment No. 2, 6, 12, 19]