IFRA UK
Ivy Cottage
Cosgrove MK19 7JJ
secretariat@ifrauk.org

IFRA UK CLP Consultation Response – Acetophenone CAS 98-86-2– November 2023

Thank you for the opportunity to give feedback on the proposals to amend the classification of Acetophenone CAS – 98-86-2 under Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures. IFRA UK has some comments about this which we would like to set out.

About IFRA UK.

As a respected trade association, IFRA UK strives to support the development and advancement of the British fragrance industry and highlight the benefits of fragrance to health and well-being. IFRA UK actively works with legislators as an advisory body and influences legislation through advocacy and policy. The Association works to protect the industry's future by setting a strict requirement for its members to comply with current legislation and industry standards that ensure consumer safety.

Comments

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.

Thank you for taking note of our feedback, we hope it is helpful and will aid constructive dialogue on the classification of Acetophenone CAS – 98-86-2.

Response to CLH report Proposal for Harmonized Classification and Labelling of Acetophenone

Chemical names:
1-phenylethanone

EC Numbers:
202-708-7

CAS Numbers:
98-86-2

Comments on the CLH report Proposal for Harmonized Classification and Labelling of 1-phenylethanone (Acetophenone, EC 202-708-7)

Introduction

The undersigned companies (BASF, dsm-firmenich, IFF, MANE, 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).

Even though exposure is not considered for hazard conclusion, it should be noted that Acetophenone exposure in toxicology studies exceeded by several orders of magnitude the Acetophenone levels used in fragrances, flavors (including also naturally occurring Acetophenone) and (in the past) pharmaceuticals. Acetophenone is an important fragrance ingredient with an estimated 95th percentile consumer exposure of 0.53µg/kg BW/day.[1] It is also a flavor recognized as FEMA-GRAS [2] and it is found in a wide range of natural products such as fruits, dairy products and vegetables.[1] Human exposure in this case should be used to assess human relevancy of the findings, as considered in the case of Salicylic acid where postulated human plasma levels have been compared to rat plasma levels.[3] In the late 19th and early 20th centuries, acetophenone was also used in medicine as a hypnotic and anticonvulsant under the brand name Hypnone [4]. The typical dosage was 0.12 to 0.3 milliliters which corresponds roughly to 2-5 mg/kg BW. This former use demonstrates the intrinsic issue with the effects seen in some of the reported studies and why it is relevant to view effects seen in the studies in relation to acetophenone's inherent hypnotic and anticonvulsant properties. With the proposed classification as STOT SE 3; H336 for narcotic effects, the Dossier Submitter acknowledges the relevance of this hazard.

An extensive set of *in vivo* studies is available in the REACH dossier for acetophenone, i.e., a Repeated Dose 90-Day Oral Toxicity Study in Rats (OECD TG 408), a Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD TG 422), Prenatal Developmental Toxicity Studies in rats and rabbits (OECD TG 414) and an Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443). We agree that these studies show effects which warrant a classification for reproductive toxicity, but we would like to highlight several elements in the CLH dossier which in our view deserve reconsideration and which ultimately should lead to a different appreciation of the relevance of the findings justifying a Repr. 2 classification.

Maternal toxicity

Considering the specific pharmacologic effects of acetophenone (i.e., narcosis and anesthesia), hence also the (proposed) STOT SE 3 classification for which we agree with the DS, we believe that the influence of maternal toxicity was underestimated. Neurological findings were assessed using the standard battery of determinations routinely applied in these types of studies, and effects such as hypoactivity and half-closed eyes were typically observed at doses around 200 mg/kg/day and higher. However, these dose levels are still between 40 and 100 higher than the therapeutic doses when using acetophenone as a hypnotic and anticonvulsant. As such, it cannot be excluded that maternal care was affected in a way which would not be picked up with the standard batteries.

The CLH report states on page 27 "*It should be noted that the clinical signs of dams did not worse [sic] 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 point is reiterated on page 43: "*Moreover, clinical signs of dams did not worse [sic] during gestation and/or after delivery. Therefore, the DS does not consider that the high number of dead pups was due to lack of maternal care*."
We disagree with the importance that the DS assigns to the fact that clinical signs in dams did not worsen. Groups showed neurological effects indicative of a narcotic state throughout the different studies, and the dams referred to in the excerpts above were also consistently affected. Whether or not their status affected maternal care is not driven by the fact that this status further worsened, but by the status itself.
The critical effects on parturition (i.e., dystocia) and pup survival occurs together with other toxicity effects and the adverse effect on reproduction is considered to be a secondary non-specific consequence of the other toxic effects. The causal link between Acetophenone maternal toxicity and the observed reproductive effects are discussed in the following chapters.

Assessment of parturition and pups at/after birth in EOGRTS

On page 43, the DS notes that most of the reported deaths and clinical signs in pups occurred at birth and between PND 1-2, thus dismissing the explanation provided in the registration dossier that these findings were likely due to nursing difficulties or lack of maternal care. However, pups were not assessed at birth, but only from PND1. As such, nursing difficulties and lack of maternal care during the important first hours post-partum were not captured in the statistics or in observations, and therefore the CLH dossier likely overestimates the deaths/effects observed at birth.
According to the CLP Guidance, it is usually difficult to prove a causal relationship between a parentally mediated mechanism and adverse effects in the offspring. However, based on expert judgement and the historical use of Acetophenone, sufficient certainty for a conclusion can be drawn. It is known from historical use and in literature, that if a narcotic and anticonvulsant chemical is applied to pregnant animals around birth, it can be assumed that this has secondary consequences. Acetophenone, as well as various other narcotics have been shown in the literature to cause muscle relaxation in general, but also of the uterus.[5, 6] This can be expected to interfere with pregnant animals in parturition impacting labor and the overall birthing process, which can be interpreted as a maternally mediated mechanism resulting from an effect on the uterus. Moreover, pups will be exposed to the narcotic in utero as well. It is generally accepted that xenobiotic metabolism in pups is not fully developed at this time of parturition. Both scenarios lack human relevance as narcotic exposure around birth is usually avoided, except for Cesarean section and / or in utero surgery. These are historically well-known phenomena for anesthetics used in the 19th and 20th century, i.e., both diethyl ether and chloroform suppress uterus contractility and press reflexes of the abdominal muscles leading in extremum, to uterus atonia and increased offspring mortality. The more recent anesthetic propofol is also known to interfere with uterus contraction in the rat.[7-9]

Lack of dose response

The DS identified a number of effects in the low dose groups of the EOGRTS which was used as a justification to identify a LOAEL at the lowest dose (75 mg/kg/day) for sexual function

and fertility (based on dystocia), and for developmental neurotoxicity (based on brain morphology) and immunotoxicity (based on immunophenotyping), this further supported a classification of acetophenone, as these findings were apparently observed in the absence of systemic/maternal toxicity. However, these findings lack in all cases but one (dentate gyrus thickness) a dose-response relationship, despite more than 6-fold dose difference between the low and high dose, which suggests that causes other than acetophenone might be at play.

Effects driven/influenced by body weight differences.

On page 44, the DS identifies the observed decreased magnitude of responses (up to -45%) in the auditory startle test (ASR) of the C2A males as an indication of developmental neurotoxicity. The difference in the ASR between control and high dose females fluctuated between -16% and +13%. However, as indicated in the NAFTA Technical Working Group on Pesticides (TWG) Developmental Neurotoxicity Study (DNT) Guidance Document [10], body weight has a significant impact on the parameter, and the body weights of the high dose C2A males were significantly lower at PND24 (i.e. the day of the measurement). It does not appear that the body weight differences were taken into consideration when assessing the ASR data, which renders the pertinence of the developmental neurotoxicity claim questionable.

The DS explains as from page 44 of the CLH dossier that the hippocampus morphometric alteration in C2A males only should be considered as an adverse effect. However, according to Garman et al.[11] these findings should be viewed at best as ambiguous evidence of DNT because they were accompanied by differences in brain and body weight. Moreover, the morphometric findings in the C2A findings were not corroborated by findings in the C2A females and C2B males and females because none of the three parameters showing significant changes in the C2A group were reproduced in the other three cohorts.

Link ASR and hippocampal morphometric alterations

The DS puts forward a link between the morphometric changes in the hypothalamus and the decreased startle response in the cohort 2A (only) by referring to Zhang et al.[12]. Whereas Zhang et al point towards several interactions between the hypothalamus and auditory function, the authors do not link morphometric changes in the hypothalamus with delays in the ASR, which is a reflex reaction. According to Gomez-Nieto et al "*In rats, the primary ASR circuit encompasses three serially connected structures: cochlear root neurons (CRNs), neurons in the caudal pontine reticular nucleus (PnC), and motoneurons in the medulla and spinal cord.*"[13]. As such, linking both observations is, in our view, too speculative and does not meet the level of certainty required in a classification rationale.

Absolute vs relative splenocyte numbers

The DS considers the shift in the absolute splenic lymphocytes subpopulations in males (only) as an adverse effect and an indication of developmental immunotoxicity. It is noteworthy that contrary to the absolute numbers, the picture for the relative numbers of the different splenocytes sub-populations is different, less consistent and does not show a dose-response relationship. As such, the data provided in the CLH dossier is to be considered at least incomplete and not allowing to conclude on developmental immunotoxicity. To allow a founded conclusion, the nature of the lower absolute splenocytes counts needs in depth (re-)evaluation to exclude technical confounders and a comparison with historical control data. Weighing all evidence argues against a biologically

relevant immunotoxic effect because of the lack of consistent effects in the other sex, the lack of histologic alterations in the spleen, e.g. reduced number of primary follicles, reduced density of white pulp, reduced peri-arteriolar lymphocyte sheath, the increased rather than decreased spleen weight, the lack of effects on white blood cell counts in hematology, the lack of effects on other immune organs such as lymph nodes and bone marrow and the lack of any immune system-related parameters in both sexes of the parental generation.

Conclusions

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.[3]

Signatories

- **BASF SE**
- **dsm-firmenich**
- **International Flavors & Fragrances**
- **MANE**
- **Sotraflor**
- **Takasago International Corp.**

References

1. Api, A. M.; Belsito, D.; Botelho, D.; Bruze, M.; Burton, G. A., Jr.; Buschmann, J.; Dagli, M. L.; Date, M.; Dekant, W.; Deodhar, C.; Francis, M.; Fryer, A. D.; Jones, L.; Joshi, K.; La Cava, S.; Lapczynski, A.; Liebler, D. C.; O'Brien, D.; Patel, A.; Penning, T. M.; Ritacco, G.; Romine, J.; Sadekar, N.; Salvito, D.; Schultz, T. W.; Sipes, I. G.; Sullivan, G.; Thakkar, Y.; Tokura, Y.; Tsang, S., RIFM fragrance ingredient safety assessment, acetophenone, CAS Registry Number 98-86-2. *Food Chem Toxicol* **2018,** *118 Suppl 1*, S162-S169.
2. Adams, T. B.; McGowen, M. M.; Williams, M. C.; Cohen, S. M.; Feron, V. J.; Goodman, J. I.; Marnett, L. J.; Munro, I. C.; Portoghese, P. S.; Smith, R. L.; Waddell, W. J., The FEMA GRAS assessment of aromatic substituted secondary alcohols, ketones, and related esters used as flavor ingredients. *Food Chem Toxicol* **2007,** *45* (2), 171-201.
3. Assessment, R. C. f. R. *Opinion proposing harmonised classification and labelling at EU level of Salicylic acid EC Number: 200-712-3, CAS Number: 69-72-7*; CLH-O-0000001412-86-110/F; ECHA: Helsinki, Finland, 2016; p 22.
4. Bartholow, R.; Bartholow, P., *A practical treatise on materia medica and therapeutics*. 12th ed.; D. Appleton and company: New York and London,, 1906; p xxiv, 866 (i.e. 890) p.
5. Bremerich, D. H.; Kaufmann, M.; Dudziak, R., Geburtshilfliche Anästhesie. *Deutsches Ärzteblatt* **2001,** *98* (13), 834.
6. Childress, C. H.; Katz, V. L., Nifedipine and its indications in obstetrics and gynecology. *Obstet Gynecol* **1994,** *83* (4), 616-24.
7. Kimizuka, M.; Tokinaga, Y.; Azumaguchi, R.; Hamada, K.; Kazuma, S.; Yamakage, M., Effects of anesthetic agents on contractions of the pregnant rat myometrium in vivo and in vitro. *J Anesth* **2021,** *35* (1), 68-80.
8. Tsujiguchi, N.; Yamakage, M.; Namiki, A., Mechanisms of direct inhibitory action of propofol on uterine smooth muscle contraction in pregnant rats. *Anesthesiology* **2001,** *95* (5), 1245-55.
9. Eun, S. S.; An, T. H.; Jung, K. T.; Jung, J. D.; So, K. Y.; Yu, B. S.; Lim, K. J.; Kim, S. H.; Lee, H. Y.; Song, C. H.; Shi, H.; Lin, J., The comparison of the relaxant effects of propofol, thiopental, ketamine, and etomidate on isolated rat uterine smooth muscle. *Korean J Anesthesiol* **2008,** *55* (6), 723-730.
10. *Developmental Neurotoxicity Study (DNT) Guidance Document*; NAFTA: 2016.
11. Garman, R. H.; Li, A. A.; Kaufmann, W.; Auer, R. N.; Bolon, B., Recommended Methods for Brain Processing and Quantitative Analysis in Rodent Developmental Neurotoxicity Studies. *Toxicol Pathol* **2016,** *44* (1), 14-42.
12. Zhang, L.; Wang, J.; Sun, H.; Feng, G.; Gao, Z., Interactions between the hippocampus and the auditory pathway. *Neurobiology of Learning and Memory* **2022,** *189*, 107589.
13. Gomez-Nieto, R.; Horta-Junior Jde, A.; Castellano, O.; Millian-Morell, L.; Rubio, M. E.; Lopez, D. E., Origin and function of short-latency inputs to the neural substrates underlying the acoustic startle reflex. *Front Neurosci* **2014,** *8*, 216.