Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one

EC Number: 438-340-0
CAS Number: 119344-86-4

CLH-O-0000007134-80-01/F

Adopted

2 June 2022

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-(DIMETHYLAMINO)-2-[(4-METHYLPHENYL)METHYL]-1-[4-(MORPHOLIN-4-YL)PHENYL]BUTAN-1-ONE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one
EC number: 438-340-0
CAS number: 119344-86-4
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.11.2021	Netherlands		MemberState	1
Comment received				
NL-CA agrees with the dossier submitter (DS) that read-across for 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (EC 438-340-0) based on 2-benzyl-2 dimethylamino-4'-morpholinobutyrophenone (EC 404-360-3) to further support classification is justified. Both substances belong to the same chemical group and are structural similar. Read-across is further supported by an analogues toxicological profile; target organs (liver, kidney, adrenals and male reproductive system) and adverse reproductive effects (changed weight testes, reduced fetal body weight and increased pup mortality) of both substances are similar.				
Dossier Submitter's Response				
Thank you for your support of the applied read-across.				
RAC's response				
Thank you, your support for the proposed read across is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.11.2021	Germany		MemberState	2
Comment received				
Tables: EC-, CAS-number and purity of the test substance should be added in the 2nd column of Table 7 (pp 13-14). Read-across from EC no. 404-360-3 regarding reproductive toxicity is considered reliable. However, some uncertainties surround the read across from the similar substance (EC no.				

404-360-3), which was assessed by RAC in 2016. RAC concluded based on an OECD TG 415 study and three subacute oral toxicity studies in rat that no classification is warranted. „RAC agrees with the DS that classification for fertility is not warranted, given that the only relevant findings, i.e. changes in the weights of male reproductive organs in the one-generation study, were relatively small and not accompanied by histopathological or functional changes." Thus, it appears that there are toxicological differences (e. g. with regard to histopathology and spermatogenesis) in spite of the structural similarity of both substances. Sometimes, an effect is not detected because the doses were too low or relevant parameters were simply not measured. However, this does not seem to be the case at least for some of the studies performed with EC no. 404-360-3. Therefore, it appears that the read-across does not support the Cat. 1B-classification proposed by the DS for EC no. 438-340-0. An explanation for this discrepancy would be helpful.

Dossier Submitter's Response

Thank your for the clarifying question to Table 7. The purity of the test substance used for OECD 421 as well as the 14-day range finding study was 96.4%. For the 28-day study a purity of 99.1% is reported. The OECD 415 study with the read-across substance 2–benzyl-2-dimethylamino-4'- morpholinobutyrophenone reports a purity of 99.9%.

The classification proposal for Repr. 1B, H360F is solely based on the clear effects seen after exposure to the substance itself. Data from the similar substance is not used as supportive evidence for fertility. We agree that there is some variance concerning effects on fertility, however testes could be identified for both substances as target organ. In addition it has to be mentioned that first effects are documented in the OECD 421 study at 200 mg/kg bw but main effects on testes were seen in the 28-day study at a dose of 450 mg/kg bw (and a NOAEL of 150 mg/kg bw), while for EC 404-360-3 only doses up to 300 mg/kg bw have been tested (where some small effects on reproductive organs are documented).
For developmental toxicity both substances show similar effects and a classification is proposed based on data with the substances itself supported by the read-across substance.

RAC's response

Thank you for your comment. RAC agrees that despite many similarities in the toxicological profile of the two substances, there is a significant difference in testicular toxicity and this difference cannot be explained solely by dose levels used. The 28-d studies and the 1-generation study with the source substance did not reveal any effect on testicular histopathology up to 300 mg/kg bw/d nor any changes in testes weight up to 500 mg/kg bw/d.
RAC is of the view that all information from the source substance should be taken into account in a weight-of-evidence assessment, i.e.; both presence of effects (such as pup mortality) and absence of effects (testicular degeneration).

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
19.11.2021	France		MemberState	3

Comment received

FR agrees with the proposal to classify the substance as Repr. 1B for fertility. The effects on testes and epididymis (decreased weights, oligospermia, not reversible reduced spermatogenesis) observed in males rats provide clear evidence, even if without reducing mating or fertility indexes. Although a drastic reduction in spermatogenesis is needed to impact fertility in rodent, these effects on sperm are of particular relevance for (subfertile) humans.

Concerning developmental endpoint, FR would like to have some discussions on the cause of the dead pups at first litter check and the post-natal losses. Indeed, as observed, the 20 dead pups at the first litter check come from only 2 litters out of 10. The 12 post-natal losses were from 4 litters, out of which, 7 were from only one litter, in common with the 2 litters of the 20 dead pups mentioned above. This might suggest a bad state of dams rather than a direct effect of the substance itself. This deserves to be discussed at RAC meeting.

Also, it is interesting to note that no histopathological effect on reproductive organs was observed with the source substance (2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone), contrarily to the target substance. Clear effects on developmental outcomes are seen with the source substance, while with the target substance they are less convincing to our opinion. This deserves to be discussed at RAC meeting.

Dossier Submitter's Response

Thank you for your support for Repr. 1B for fertility.

The interpretation of the developmental effects seen with the target substance leave some room for discussion as described in your comment and in detail in the dossier. However, some dose-dependancy of effects could be established (effects at 60 and 200 mg/kg bw) and it cannot be concluded that the two dams were in a general bad state: at the highest concentration maternal toxicity is described as (1) body weight loss in females in week 1 of treatment (recovering during the remainder of the study),(2) decreased food consumption in females (on some points in time) and (3) some findings (moderate lymphoid atrophy, kidney in female #71) as described in Table 29. Maybe dam #71 showed a general bad state but not #79.
In addition, statistically significant reduced pup weights for both sexes at 200 mg/kg bw are reported (and also documented for the read-across substance) and considered relevant for classification.
Based on the effects seen with the substance supported by data with the read-across substance we think that a classification as Repr. 1B for developmental toxicity is warranted.

See also response to comment No 2 for effects on reproductive organs.

RAC's response

Thank you for your comments.
RAC agrees to apply the read across from the source substance consistently to both adverse effects on sexual function and fertility and development. Given the lack of effect on fertility-related findings in the 1-generation study with the source substance, RAC prefers Category 2 for adverse effects on sexual function and fertility.
As to the OECD TG 421 study with the target substance, it is possible that the total litter loss in dam no. 71 could be secondary to general toxicity in this animal (clinical signs: lethargic, pale; histopathology: marked glomerular and tubular necrosis of the kidneys). Some toxicity (hunched posture, lower food consumption, thymus atrophy) was also observed in the other dam (no. 79) who lost the whole litter. No marked toxicity was apparent in top dose females no. 73 (1 pup missing), no. 76 (1 pup dead, 1 missing) and no. 78 (1 pup dead, 1 missing).
During the RAC discussion, several members expressed a view that without read across, the evidence from the OECD TG 421 study alone would not be sufficiently strong to justify classification in Category 1B for development. Nevertheless, it was agreed that read across is justified. The RAC conclusion was thus Repr. 1B; H360Df.

Date	Country	Organisation	Type of Organisation	Comment number
11.11.2021	Germany		MemberState	4
Comment received				

Adverse effects on sexual function and fertility:
Various adverse effects on the male gonads (testes, epididymides) of rats were detected in a reproduction/developmental toxicity screening study (OECD 421; 0, 20, 60, 200 mg/kg bw/d) and an oral 28-d-study (OECD 407; 0, 15, 50, 150 and 450 mg/kg bw/d), which widely did not reverse after a recovery period at the highest dose of 450 mg/kg bw/d of the oral 28-d-study. However, the reproduction/developmental toxicity screening study (OECD 421) did not detect effects on the reproductive parameters (fertility index etc.) up to 200 mg/kg bw/d, which might be explained by the high sperm reserve of rats. The gonadal effects were observed in the presence of reduced bw gain, clinical signs and other systemic effects (kidney, liver, haematology, bone marrow, thymus) especially at the high dose of the oral 28-d-study (450 mg/kg bw/d). As to the 28-d-study a systemic NOAEL of 50 mg/kg bw/d and a gonadal NOAEL of 150 mg/kg bw/d were determined. With regard to the OECD 421-study a systemic NOAEL of 20 mg/kg bw/d and a gonadal NOAEL of 60 mg/kg bw/d were observed. Thus, in both studies the NOAEL for systemic effects is lower than the NOAEL for gonadal effects. The various effects on the male gonads cannot be attributed solely to general toxicity and a classification is clearly justified.
Some uncertainties surround the read across from a similar substance (EC 404-360-3), which was assessed by RAC in 2016. RAC concluded based on an OECD 415 and three subacute oral toxicity studies in rats, that no classification is warranted. „RAC agrees with the DS that classification for fertility is not warranted, given that the only relevant findings, i.e. changes in the weights of male reproductive organs in the one-generation study, were relatively small and not accompanied by histopathological or functional changes." Thus, it appears that there are toxicological differences (e. g. with regard to histopathology and spermatogenesis) in spite of the structural similarity of both substances. Sometimes, an effect is not detected because the doses were to low or relevant parameters were simply not measured. However, this does not seem to be the case at least for some of the studies performed with EC 404-360-3. Therefore, it appears that the read across does not support the Cat. 1B-classification proposed by the DS for EC 438-340-0. An explanation for this discrepancy would be helpful.

Adverse effects on development:
The reproduction/developmental toxicity screening test (OECD 421; 0, 20, 60, 200 mg/kg bw/d) in rats determined a NOAEL (development) of 20 mg/kg bw/d and a NOAEL (maternal toxicity) of 60 mg/kg bw/d. At the developmental LOAEL of 60 mg/kg bw/d 4 dead pups in one litter at the first check (control: 0) were observed. There is also 1 postnatal loss (but also 1 in the control). At the highest dose of 200 mg/kg bw/d 20 dead pups in two litters (total litter loss) at the first check were found and 12 pups in 4 litters as postnatal loss. Furthermore a significantly reduced pup weight at d 1 (-15%) and d 4 (-20%) and a significantly reduced viability index (87.9) was detected. The cause of the absence of milk in the stomach of dead pups is not fully clear, but maternal care is described as normal.
With regard to maternal toxicity at 200 mg/kg bw/d it is noted, that one of the two dams with total litter loss showed macroscopic findings in the kidney (general pale discolouration, many reddish foci (marked glomerular and tubular necrosis)) and thymus (moderate lymphoid atrophy), what indicates a significant maternal toxicity in this animal. The other dam with total litter loss showed a reduced thymus size, only. The other maternal effects at 200 mg/kg bw/d, as far as these are investigated in an OECD 421, are

clinical signs and a transient bw loss during the first week of treatment, which recovered during the remainder of the study and a corresponding decreased food consumption. Apart from one animal with total litter loss, which showed a pronounced toxicity in kidneys (marked glomerular and tubular necrosis) and thymus, maternal toxicity was only slight at 200 mg//kg bw/d. Thus, the developmental effects cannot be attributed solely to maternal toxicity and a classification is justified. Similar developmental effects were observed in the OECD 415 study with the similar substance EC 404-360-3, which was classified by RAC as Cat. 1B. With regard to developmental toxicity the read across approach supports the classification proposed by the DS. The Cat.1B-classification is justified.

Dossier Submitter's Response

See also response to comment No 2.

Thank you for your support of Cat 1B for developmental toxicity and also reflecting on the situation around maternal toxicity. We clearly follow your arguments that developmental effects cannot be attributed solely to maternal toxicity (see response to comment No 3).

RAC's response

Thank you for your comments. RAC agrees with your conclusions, including the statement that the read across does not support classification in Category 1B for adverse effects on sexual function and fertility.

Date	Country	Organisation	Type of Organisation	Comment number
11.11.2021	Netherlands		MemberState	5

Comment received

Fertility and sexual function
The DS proposes classification of Repr. 1B for fertility and sexual function based on adverse effects noted in the male reproductive system (changed weight testes and histopathological changes) in OECD 421 and OECD 407 studies with 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one. This is further supported by read-across from a one-generation reproduction toxicity study (changes in weight of the male reproductive organ) with 2-benzyl-2 dimethylamino-4'-morpholinobutyrophenone (EC 404-360-3).

Reduced weight of the epididymides and testes including histopathological changes (intraluminal cell debris, up to moderate; oligospermia, slight degree; germ cell exfoliating without degeneration, moderate degree; reduced spermatogenesis, slight to marked degree; tubular atrophy, marked degree) were noted at the highest dose level. No effects on male and female fertility were noted. In addition, clinical signs (piloerection, hunched posture, macroscopic findings kidneys and/or thymus) in females and reduced body weight together with microscopic findings in testes and epididymides in males were observed at the highest dose level.

The DS referred to an OECD 415 study for 2-benzyl-2 dimethylamino-4'-morpholinobutyrophenone (EC 404-360-3) for read-across. In a previous opinion on this substance, RAC agreed with the DS that classification of fertility was not warranted for EC 404-360-3 as changes in weight of the male reproductive organs were small and not accompanied with histopathological findings (RAC, 2016).

Since the effects on fertility were limited to the male reproductive organs and male fertility was not reduced in both the OECD 421 study with the target substance and the

OECD 415 study with the read-across substance, the NL-CA is of the opinion that a classification in category 2 is more appropriate than category 1B. Humans may be more sensitive, but it remains uncertain if fertility can be impaired nonetheless and therefore category 2 seems more appropriate.

Developmental toxicity
The NL-CA agrees with the DS that Repr 1B for adverse effects on development is warranted.

In the OECD 421 study with the target substance, a dose dependent increase (mid- and high-dose) in dead pups were found. At the high dose, adverse effects on viability index (88% vs 99% in control), postnatal loss (-12.1%) and decreased pup body weights (up to -20%) were noted as well. In the OECD 415 study with the source (read-across) substance, a statistically significant and dose dependent increase in dead pups was also observed. Other similar effects compared to the target substance were observed as well including increased postnatal mortality and a decrease in pup weight. The effects in this study have resulted in classification for effects on development (cat. 1B) before (RAC, 2016). The target substance should therefore be classified in the same way.

Overall, both studies indicate similar adverse effects on development in the absence of clear maternal toxicity. Therefore Repr. 1B for development is warranted.

Lactation
The NL-CA agrees with the DS there is insufficient evidence supporting classification based on adverse effects on or via lactation for 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (EC 438-340-0).

Dossier Submitter's Response

Fertility and sexual function:
The classification proposal for Repr. 1B, H360F is solely based on the clear effects in males seen after exposure to the substance itself. Effects on male fertility have not been seen maybe due to the high sperm reserve in rats (laboratory animals are still fertile even if the sperm counts drop by 90 to 99%; Mangelsdorf 2003). Data from the similar substance (where effects on testes, prostate and seminal vesicles weight were seen but no histopathological findings) is not used in a read-across approach for this endpoint. See also response to comment No 2.

Remark on general toxicity:
In our opinion the effects seen in male rats cannot be explained by general toxicity.
In the 28 day study the effects on male reproductive organs at 450mg/kg bw (testes weight (-49%, $p<0.01$) and epididymides weight (-34%, $p<0.01$), small testes not recovering, reduced spermatogenesis not recovering, etc.) were accompanied by a bodyweight reduction of 11.3% as well as changes in livers weights (+15% $p<0.01$) and kidneys (+13%, $p<0.01$). During the recovery period, the mean body weights of rats in this dose group remained lower than those of the controls, although the mean body weight gain improved. Females showed a bw reduction of 13.8% accompanied with increased liver weights (+42%, $p<0.05$), increased absolute ovary weights (+23%, not significant) and ovary-to-body weight ratios (+41%, $p<0.05$) which may be normal variations due to oestrus.
In the OECD 421 study with dosing up to 200 mg/kg bw male bodyweight was reduced by about 7% on day 28, weight of the epididymides was statistically significantly reduced with -17% and microscopic findings in tests and epididymides were described. Effects in

females mentioned in your comment were recorded on day 42 (or even up to 52) and were only reported for single animals.
According to the CLP guidance "*there is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity"* and "*parental toxicity that is less than marked should not influence the classification for reproductive toxicity independent of the specific parental effects observed"*. Marked systemic toxicity is described as e.g. lethality, dramatic reduction in absolute body weight, coma. In the 28 day study and the screening study no marked toxicity has been described.

Developmental toxicity and Lactation:
Thank you for your support.

RAC's response

Thank you for your comments. RAC agrees with Category 2 for adverse effects on sexual function and fertility, Category 1B for adverse effects on development and no classification for effects on or via lactation.

Date	Country	Organisation	Type of Organisation	Comment number
22.10.2021	Slovakia		MemberState	6

Comment received

In the classification system, reproductive toxicity is subdivided into two main headings: 1. adverse effect on sexual function and fertility, and 2. adverse effect on development of the offspring. CLH report - Proposal for Harmonised Classification and Labelling of the substance 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (Omnirad 379, EC Number: 438-340-0) includes an evaluation of both parameters of reproductive toxicity.

1. Adverse effects on sexual function and fertility
Results of the Reproduction/developmental toxicity screening test (OECD 421) showed reduction of epididymides weight and slight morphology changes of testes and epididymides (germ cells exfoliated into the lumen of the testicular tubules, but without degenerative changes; decreased epididymides weight correlated with microscopic findings of intraluminal cell debris and oligospermia) in male rats. On the other hand, important reproductive parameters (spermatogenic staging profiles, mating, fertility and conception indices, precoital time, number of corpora lutea and implantation sites) have not changed. Although the target substance indicates a slight adverse effect on male reproductive organs, ultimately the fertility of rats was
unaffected.
Results 28-day oral toxicity study (OECD 407) clearly demonstrated a dose-related adverse effect of the target substance on male reproductive organs. The changes were more extensive in the high dose group (450 mg/kg bw/day). Increased testicular and epididymal weights correlated with histopathological changes in most of males at the end of treatment and after the recovery period. Histopathology of gonads was the most sensitive parameters to detect adverse effects on male fertility and was used to derive the NOAEL in 28-day oral toxicity study. It is also necessary to mention systemic adverse effect of the target substance (reduction of hemoglobin and hematocrit values in both sexes, changes in the kidneys, fatty bone marrow atrophy, increased extramedullary hematopoietic activity of the spleen in males) as well as clinical signs of intoxication

(hunched posture, piloerection in some animals on some days, and dark feces in all high-dose animals from day 12) and changes in parameters of clinical biochemistry founded in 28-day oral toxicity study.
However, the clarity of the effects rather than the sensitivity of the effects observed, are important for classification and labelling and will affect the category into which the substance is classified. Thus, to address the fertility also for the classification and labelling purposes, including the categorisation, it is necessary to consider how well all the available parameters address the fertility endpoint. Due to its limitations, a screening study cannot be used to fulfil the information requirement of the Extended one-generation reproductive toxicity study (Chapter R.7a: Endpoint specific guidance).
Although the results of the Extended one-generation reproductive toxicity study are not available, CLH report contains results of One-generation reproduction toxicity study (OECD 415) on a structurally similar substance (EC 404-360-3). In this case, only weight of the male reproductive organs was affected. Source substance did not show adverse effects on reproduction in terms of fertility (no histopathological and functional changes). This was also the reason why the source substance was not classified as toxic for reproduction due to fertility.
Conclusion: While the results of the screening study suggested adverse effects on male reproductive organs, the observed changes in this study did not impair spermatogenesis and the overall fertility of both sexes. The changes in testes and epididymides contributed to the evidence for the reprotoxic effect of the substance on males, but the range of parameters observed does not cover all aspects of fertility assessment in 28-day oral toxicity study. Also there are no human data on the adverse reprotoxic effect of the target substance. Therefore we are of the opinion, that target substance does not meet the criteria for classification as Repr.1B, H360F.

2. Adverse effects on development of the offspring
The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency (Annex I, CLP). With regard to developmental toxicity, screening tests do not provide sufficient information on prenatal developmental toxicity because the pups are not examined for external, skeletal and visceral anomalies (Chapter R.7a: Endpoint specific guidance). The source of such important information is the Reproductive Developmental Toxicity Study (OECD 414), which play a key role in deciding on the classification of a substance for developmental toxicity.
The results of available studies (OECD 421, OECD 415) confirm only few manifestations of developmental toxicity: death of the developing organism - but only to a limited extent (reduced viability index and increased dead of pups) and partially altered growth (reduced pup weights). These adverse effects are limited to fetal development just before and 4 days after birth. We have no information about structural abnormalities and functional deficiencies of developing organism.
Moreover, we cannot fail to mention influence of maternal toxicity. The adverse effects of target substance on developing pups observed in the reproductive/developmental toxicity screening test were clearly associated with maternal stress and the disruption of homeostasis, as evidenced by the presence of weight loss, general clinical signs, decreased food consumption at higher doses. Hematological changes found in females (fatty bone marrow atrophy at 450 mg/kg bw/d, test substance-related effects at 150 and 450 mg/kg bw/d, decreased hemoglobin and hematocrit values at 450 mg/kg bw/d) during 28-daily oral toxicity study suggests that even at a dose of 200 mg/kg bw/d, adverse hematological changes may have contributed to maternal toxicity in the screening reproduction study (examination of hematological parameters is not part of the screening test methods). Increased absolute liver weights, reduced bw/bw gain and food consumption and increased adrenal weights (stress adaptive) were found in females at

100 mg/kg bw/d and 300 mg/kg bw/d in one-generation reproductive toxicity study with the source substance. The results of available studies indicate that the systemic effect begins at doses higher than 50 mg/kg bw/d. It follows that at doses higher than 100 mg/kg bw/d, the incidence of adverse effects in pregnant females should be considered. Therefore we assume, that the observed effects on fetuses are a secondary non-specific consequence of the observed maternal toxicity.
Conclusion: According to the available reprotoxic studies we can consider, that results of these studies do not provide sufficient evidence to classify substance Omnirad 379 as a developmental toxicant. Hence in our opinion, the classification criteria for the substance Omnirad 379 for developmental reproductive toxicity are not met.

Dossier Submitter's Response

An EOGRT study for this substance is not available but a well conducted screening study. A general retrospective evaluation of more than 100 screening studies (OECD 421/422) has shown that the required number of animals as stated in the guidelines, is appropriate for detecting developmental and reproductive toxicity (Beekhuijzen, 2014).

Adverse effects on sexual function and fertility
The classification proposal for Repr. 1B, H360F is based on the clear effects in males seen after exposure to the substance itself. Effects on male fertility have not been seen maybe due to the high sperm reserve in rats (laboratory animals are still fertile even if the sperm counts drop by 90 to 99%; Mangelsdorf 2003). In our opinion the missing of effects on female fertility can not be used as argument against a classification in Cat 1B. Human data is not needed for classification in Category 1B. Data from the similar substance is not used in a read-across approach for this endpoint.

Adverse effects on development of the offspring
For discussion of maternal toxicity please see response to comment No 3 and 5. In addition it has to be mentioned that the clear developmental toxicity seen with EC 438-340-0 in a screening study is supported by a read-across. The source substance has been classified by RAC (2016) based on the results of the EOGRTS, presented in the dossier, as Repr. 1B. In the RAC opinion also a closer look at maternal effects has been done and RAC considered it unlikely that the observed increase in stillborn pups and postnatal mortality was secondary to maternal effects for EC .

Reference:
Beekhuijzen et al (2014). The underestimated value of OECD 421 and 422 repro screening studies: putting it in the right perspective. Reproductive Toxicology, Volume 48, September 2014, Pages 81-87.
of the offspring

RAC's response

Thank you for your comments.
Adverse effects on sexual function and fertility: RAC agrees that Category 2 is more appropriate than Category 1B mainly because of lack of testicular degeneration in the 1-generation study with the source substance (EC 404-360-3).
Adverse effects on development: RAC has to conclude based on the information available. The top doses in the reproductive studies did cause some maternal toxicity but the degree of toxicity was not sufficient to explain the pup mortality except for two dams in the OECD TG 421 study with Omnirad 379; maternal toxicity in these animals is taken into account in the RAC opinion. When considering only the pup mortality in the OECD TG 421 study, without read across, the evidence may not be sufficiently strong to justify Category 1B. However, considering the clear effect in the 1-generation study with the structurally related substance Omnirad 369 Category 1B is considered justified.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
19.11.2021	France		MemberState	7
Comment received				
FR agrees with the conclusion that observed effects are not considered significant (as defined in the guidance) to propose a classification.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you, RAC agrees with no classification for STOT RE.				

Date	Country	Organisation	Type of Organisation	Comment number
11.11.2021	Germany		MemberState	8
Comment received				
The proposed non-classification is supported. The slight substance related anaemia and its compensatory effects are not sufficient for a STOT RE-classification.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you, RAC agrees with no classification for STOT RE.				

Date	Country	Organisation	Type of Organisation	Comment number
11.11.2021	Netherlands		MemberState	9
Comment received				
The NL-CA agrees with the DS that no classification for STOT RE is warranted for 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (EC 438-340-0). Slight hematological effects were observed in mid and high dose groups in rats (male: reduced mean corpuscular volume, and increased platelets and leukocyte count; female: reduced red blood cell count and hematocrit) in an OECD 407 study. However, no hematological changes were noted in a 14-day dose range finding study and was not investigated in the OECD 421 study. In addition, the adversity is questionable and the hematological effects were only slightly changed at relevant dose levels for STOT RE.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you, RAC agrees with no classification for STOT RE.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
11.11.2021	Netherlands		MemberState	10

Comment received

Chronic toxicity to aquatic invertebrates: Currently, it is not clear why the results obtained from Test 2 are regarded as relevant over the results obtained from Test 1. The first test shows significant signs of toxicity in the reproduction endpoint (reduction of cumulative offspring per introduced parent) at lower concentrations than the suggested NOEC of 0.064 mg/L from Test 2 (NOEC <0.039 mg/L).

On p. 63, it is described that a second test is performed as no NOEC could be established in Test 1. However, as the effect on this endpoint in Test 1 is a reduction of > 10 and < 20 % compared to the control (18.9%), the NOEC can be calculated as LOEC/2, described in the Chapter R.10: Characterisation of dose [concentration]-response for environment guidance document (Table R.10-1). This would give a NOEC of 0.0195 mg/L.

If the results of Test 1 are considered to be not reliable (perhaps due the observed deviations in the water quality parameters), this should clearly be explained.

p. 62/63: Chronic toxicity to aquatic invertebrates: results on group mean body length and reduction of length in final test 1 are tabulated. In this table, even very marginal levels of reduction are displayed as statistically significant. Is it correct that the three lowest test concentrations (mean measured concentrations of 39, 74 and 125 µg/L) also significantly reduce the growth parameters?

Based on the available data, we agree with the proposed classification Aquatic Acute 1 (M-factor=1) and Aquatic Chronic 1 (M-factor=1).

Dossier Submitter's Response

Ad usage of a NOEC from Test 1 of the chronic aquatic invertebrates test:
We consider Test 1 of the chronic toxicity to aquatic invertebrates test as valid and agree that for toxicity in the reproduction endpoint (reduction of cumulative offspring per introduced parent) a reduction of 18.3% is observed. It is agreed that, as this value is > 10% and < 20% compared to the control, the NOEC can be calculated as LOEC/2, resulting in a NOEC of 0.0195 mg/L usable as basis for classification.

Ad statistical significance of body length in Test 1 of the chronic toxicity to aquatic invertebrates test:
According to the original study report, the reductions in mean body length were statistically significant ($p \leq 0.05$) at all concentrations including the three lowest concentrations (Williams Multiple Sequential t-test Procedure was performed).

Ad Agreement with the proposed classification:
Thank you for your support.

RAC's response

RAC considers results from both test 1 and test 2 as valid although some deviations in oxygen content were observed. From such point of view, all obtained results have to be taken into account for final endpoint calculation, that is why EC_{10} (reproduction) value of 65 µg/L (39-108 µg/L, 95 % CI) is obtained and finally endpoint for mortality, reproduction and growth 0.064 mg/L was based on average exposure concentrations.

As far as only final results were presented for group mean body length and reduction of length calculated statistical differences has to accepted as reliable. Thank you for your support for classification.

Date	Country	Organisation	Type of Organisation	Comment number
19.11.2021	United Kingdom	Health and Safety Executive	National Authority	11

Comment received

Bioaccumulation:
Given fish lipid data appear to be available, we think it would be useful to present lipid normalised fish BCFs as per CLH guidance (ECHA, 2017).
Ecotoxicity:
Experimental GLP data from OECD guideline studies are available for acute toxicity to fish, invertebrates and algae.
The acute toxicity to fish study showed no effect up to the limit of solubility using a 100 mg/L loading equating to >0.13 mg/L geometric mean measured concentrations.
The 72-hour ErC50 for algae was considered above the limit of maximum achievable concentration in test media and the NOErC at or greater than this treatment. While analytical verification was limited, the data does support that the treatments were correctly dosed and given no acute effects were observed, the study supports no acute ecotoxicity < 1 mg/L.
The acute toxicity to invertebrates study also has limitations with regard to analytical verification as the test item was not detected above the LoQ (0.0643 mg/L). The GLP study appears to be part of a suite with the fish and algal studies with treatments similarly prepared and study validity criteria were met. Given no acute effects were observed up to the limit of maximum achievable solubility, we are unclear why the predicted QSAR endpoint would be used in preference as the key endpoint for the aquatic acute hazard classification.
It would be useful if further information was available to:
1) confirm if the test item was detected above the LoD in the acute toxicity to invertebrates study (especially at the start of the test), and
2) consider the increasing reduction in test item solubility in test media at pH >7 given the pH range of ecotoxicity studies and the pKa of the test substance of 6.22.

Dossier Submitter's Response

Ad Bioaccumulation:
In the study the lipid content was determined before and after the experiment, with a mean of 4.3% and 4.6%, respectively (for this, fish in the control group (n=2) was used). The lipid normalisation was determined according to OECD 305, Annex 5. The BCF_{SSL} is for the high exposure level 821 and for the low exposure level 743, respectively.

Ad Ecotoxicity:
1) In the experimental acute invertebrates study only LoQ is provided, while no information on LoD is given. The results of the analytical measurements at the beginning and end of the test in the original study report do only state that the substance was below the LOQ of 0.0643 mg/L (sampling day 0) or below the LOQ of 0.0477 mg/L (sampling day 2). As the substance could not be measured at the beginning or end of the study, the validity of this study is severly hampered and is not considered suitable for classification purposes. Therefore, the QSAR data were used.

2) In the experimental acute invertebrates study it is stated that acccording to a pre-test (not performed under GLP) the solubility limit of EC 438-340-0 in the test water was approximately 0.1 mg/L. The pH in the experimental acute invertebrates study was 7.9 at the start and at the end of the test.
While quantification of the substance was successful in the algae test (pH: 8.0 at the beginning and 9.3 to 9.5 at the end of the test; range due to different concentrations) and the acute fish test (pH: 7.8 -7.9, measured each day during four days), the limit of quantification was not reached in the acute invertebrates study. Considering the pKa of 6.22, the substance is in the neutral form in all the acute tests and the water solubility of this substance is considered to be in the same range.

RAC's response

Bioaccumulation
RAC agree with DS for the recalculated values for BCF taking into account lipid content.
Ecotoxicity.

1. RAC consider data from acute toxicity studies to fish and alga valid and reliable and agree that to the highest exposure concentration (measured) toxicity was not observed. RAC consider results from toxicity studies to invertabrates unsuitable for classification:

- Experimental study for invertebrates (GLP) showed no toxicity effect at highest test concentration however this concentration is below the LOQ of HPLC method used (0.0643 mg/L). It is worth mention that LOD (limit of detection is usually two/three times lower than LOQ (limit of quantification or limit of determination).
- RAC noted that in the test medium two test organisms were immobile at the observation after 24 hours. Registrant accepted this immobilization rate as unsignificant toxic effect, because according to the test guidelines an immobilization rate of 10 % is tolerated in the control. Data for 48 h are not presented.
- The test substance solution is prepared according to the well-defined procedure - filtration of 100 mg/L supersaturated solution (obtained after 15 min. ultrasound treatment and 3 h stirring), but in this experiment unexpectedly concentration after filtration is below 0.0643 mg/L (LOQ of HPLC method). First, the concentration is much lower than typically achieved concentrations using the same preparation procedure (0.28 mg/L fish acute study and 17 mg/L alga study) and second evidently quite unsuitable HPLC method is used as far as this concentration is in the possibilities of UPLC. From such point of view results from this study might not be used for classification.

RAC considers that results from acute toxicity studies should be supplemented by QSAR calculation for substance classification.

2. Usually in analytical chemistry Limit of detection is calculated as 3 × SD of blank sample and LOQ is calculated as 10 × SD of blank sample, LOD is about LOQ/3 e.g. in this case 0.0214.
Test for fish and alga are performed at pH-7-9, same for invertebrates. pK_a 6.22 means that as higher pH as higher substance solubility. Substance solubility should be same for all acute tests.