Committee for Risk Assessment

RAC

Annex 2
Response to comments document (RCOM) to the Opinion proposing harmonised classification and labelling at EU level of

Bis(2-(2-methoxyethoxy)ethyl)ether; tetraglyme

EC Number: 205-594-7

CAS Number: 143-24-8

CLH-O-0000001412-86-215/F

Adopted

8 June 2018

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public 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 public 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 public 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.

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Substance name: Bis(2-(2-methoxyethoxy)ethyl)ether; tetraglyme
EC number: 205-594-7
CAS number: 143-24-8
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2017	Germany		MemberState	1
Comment received				
The German CA supports the CLH proposal for tetraglyme (CAS No. 143-24-8) as Repr. 1B, H360. The classification proposal for tetraglyme as Repr. 1B is based on a read across approach to the glycol ethers mono-, di- and triglyme, and on adverse effects to fertility and foetal development found in two dose range finding studies in rats by oral treatment with tetraglyme.				
Dossier Submitter's Response				
Thank you for your review and support.				
RAC's response				
Your position is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
12.10.2017	United Kingdom	CS Regulatory Ltd 1L-9	Company-Importer	2
Comment received				
CS Regulatory Ltd 1L-9 has access to additional data for the substance under review from a GLP compliant OECD 422 study completed in November 2012, but as a late pre-registrant was not previously in a position to provide this to the lead registrant prior to registration. These data are submitted to the RAC to assist in the consideration of effects.				

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment OECD422_Report.pdf
Dossier Submitter's Response
Thank you for this additional information indicating a parental NOAEL below the reproduction and developmental NOAEL. We expect that RAC will take this additional information into consideration for their assessment.
RAC's response
Thank you for the information. The study has been included in the RAC evaluation.

Date	Country	Organisation	Type of Organisation	Comment number
26.10.2017	Netherlands		MemberState	3
Comment received				
'Pubs' should be replaced with 'pups' in the entire document. Further, it is suggested to differentiate between effect on sexual function and effects on fertility.				
Dossier Submitter's Response				
Thank you, the typo needs correction by RAC. The Dossier Submitter is of the view that the observed adverse effects are transparently described and considers that further differentiation would not be necessary to arrive at a conclusion on classification for reproductive toxicity.				
RAC's response				
Thank you for the comments. RAC has corrected the typo's in the opinion, however, the CLH report cannot be altered. RAC has noted your comment about differentiation between effects on sexual function and fertility.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	France		MemberState	4
Comment received				
Effects of tetraglyme on the reproductive system, in particular in males, have been observed in two different studies in absence of general toxicity. It mainly consists in decreased testis weight but it is accompanied in the 90-day study by serious histological effects (degradation of germinal epithelium, increased single cell necrosis, reduced number of matured sperm cells) that may result in male fertility impairment. Similarly, an effect of tetraglyme on post-implantation loss was observed from 500 mg/kg and in absence of maternal toxicity consistently in the OECD 421 study as well in the prenatal development study. In addition malformations were observed from the low dose of 250 mg/kg. It is further noted that these effects were identified despite the very low sensitivity of the available studies that were performed on a very limited number of animals. On this basis, the data of tetraglyme by themselves are relevant for a classification Repro 1B for fertility and development. This is further supported by the consistency of the findings with effects of other glymes that are expected to be metabolised in the same known reproductive toxicant 2-ME and 2-MAA. Testicular toxicity, induction of post-implantation loss and of malformations are common features of these compounds, although the profile of malformations may be difficult to compare across substances and species tested. A classification in category 1B for fertility and development is therefore supported. It is finally noted that the proposed labelling Repr. 1B – H360 'May damage fertility or the				

unborn child' is vague regarding the possible target of the substance and should apply when the effects cannot be specified with respect to fertility and/or developmental toxicity. In this specific case, tertraglyme induces effects both on the reproductive organs with a possible impact on fertility and on development and both target should be identified through the use of labeling H360 FD 'May damage fertility. May damage the unborn child', in line with Table 3.15 of the CLP guidance. NB: a description of reprotoxic effects induced by 2-ME and 2-MAA would have been relevant to include in this dossier as these are the common metabolites on which read-across is built. Data are presented in the respective registration dossiers of 2-ME (https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14919/7/9/1) and 2-MAA (https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/2127/7/9/2)
Dossier Submitter's Response
Thank you for your review. We acknowledge the view of FR with regard to specifying H360FD. The data are transparent and the arguments supporting not to specify for f/F or d/D are summarized in the last paragraph in 4.10.5. (page 40). It is upon RAC to decide this. The read across is built on the source substances Triglyme, Diglyme, Monoglyme and a hypothesis for common metabolites, 2-ME and 2-MAA. All of these substances have a harmonized classification for Reprotox 1B. All details available in the CSR, the IUCLID summary and the classification dossiers for the 3 source substances are presented in the CLP report. For the hypothesised metabolites 2-ME and MAA the reproductive toxicity data used for their classification are summarized in the Annex of the tetraglyme CLH dossier. The level of detail provided for these is judged sufficient to show toxicological similarity of the target substance primarily with the three source substances. We consider this to be sufficient evidence for classification.
RAC's response
Thank you for your comments. RAC has noted your position with regard to classification for effects on fertility and development.

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	Belgium		MemberState	5
Comment received				
BE CA would thank the Environment Agency Austria for this CLH proposal. We support the Repr. 1B (H360) classification for tegraglyme. In an OECD Guideline 421 study, tetraglyme demonstrated a specific toxicity on rat reproduction, with a prolonged gestation period at 500 mg/kg bw/day and reduced testes and epididymis weights at 1000 mg/kg bw/day in all males. The same study also showed a developmental toxicity based on no live pups at 1000 mg/kg bw/day and reduced number of live pups at 500 mg/kw bw/day. These findings have been confirmed by an OECD guideline 414 study. At 250 mg /kg bw/day pups showed overall external effects, soft tissue and skeletal abnormalities. The number of post-implantation losses at the same dose was also increased, with no maternal toxicity up to 1000 mg/kg bw/day. Conclusively, conditions for a Repr. 1B (H360) classification are fulfilled.				
Dossier Submitter's Response				
Thank you for your review and support.				
RAC's response				
Thank you for your comments. RAC has noted your position.				

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2017	Germany		MemberState	6
Comment received				
Tetraglyme has structural similarity to other glymes of this category (mono-, di- and triglyme). It is assumed, that glymes (including tetraglyme) are metabolized to 2-methoxyacetic acid and 2-methoxyethanol which are both reproductive toxicants and classified as Repr. 1B, H360FD. It seems to be conclusive that these metabolites are responsible for the adverse effects on fertility and foetal development. In addition to the supported read across approach two dose range finding studies on rats by oral treatment with tetraglyme are available (BSL Bioservice 2011 & 2012). In these studies clear adverse effects on fertility and foetal development become apparent, even if the number of tested animals is very low. A causal relationship has been established between the treatment with tetraglyme and a dramatic increase in post implantation loss, foetal mortality and malformation in addition with decreased testis weight in male rats. Similar effects could be found in Prenatal Development Toxicity Studies on mono-, di- and triglymes. An increased incidence of post implantation loss, foetal mortality and malformations were found in several studies with these glymes in three different species (rat, mouse, and rabbit) on both routes of administration, oral or inhalation. All of this taken together, the read across approach to mono-, di- and triglymes and the results of the dose range finding studies, we consider it as appropriate to classify tetraglyme as Repr. 1B, H360. However, regarding the limited data of the dose range finding studies further differentiation toward H360F or H360D might be difficult. For a more reliable risk assessment it would be helpful to get more information regarding the adverse effects observed in the studies (e.g. number and type of malformations). On the other hand, source substances have a very similar toxicity profile compared to tetraglyme regarding all endpoints investigated, including effects on (male) fertility. With the exception of triglyme, all source substances have a harmonised classification as Repr. 1B, H360FD. In the case of triglyme the reason for different classification (Repr. 1B, H360Df) is not clear. Combining the data available for tetraglyme and the source substances classification as Repr. 1B, H360FD might be also a possible option for consideration.				
Dossier Submitter's Response				
Thank you for your review and support. The arguments supporting not to specify for f/F or d/D are summarized in the last paragraph in 4.10.5. (page 40). It is upon RAC to decide whether a differentiation is possible.				
RAC's response				
Thank you for your comments. RAC has noted your position. RAC has access to (confidential) additional details from the dose-range finding studies (e.g. types and number of malformations) which will be used in the evaluation of effects on fertility and development.				

Date	Country	Organisation	Type of Organisation	Comment number
12.10.2017	United Kingdom	CS Regulatory Ltd 1L-9	Company-Importer	7
Comment received				
Treatment with tetraglyme by oral gavage in male and female Wistar Han rats was conducted according to the OECD 422 test guideline at dose levels of 100, 300 and 1000 mg/kg revealed parental toxicity at 300 and 1000 mg/kg. Reproduction				

and developmental toxicity was observed at 1000 mg/kg. Based on these results, the study established a parental No Observed Adverse Effect Level (NOAEL) of 100 mg/kg. The reproduction and developmental NOAEL was derived as 300 mg/kg. These data are largely consistent with the data presented by the lead registrant from the OECD 421 study which supported classification as reprotoxic category 2 under CLP (reprotoxic category 3, R62 under DSD) ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment OECD422_Report.pdf
Dossier Submitter's Response
Thank you for this additional information indicating a parental NOAEL below the reproduction and developmental NOAEL. Please note that the classification proposal is based on several studies for tetraglyme and also for the three source substances. Using multiple studies and read across enhances the reliability of the assessment since in this way reproducibility and other uncertainties from individual animal tests and assessments can be compensated. We anticipate that RAC will take the additional information referenced in your comment into consideration for their assessment.
RAC's response
Thank you for the additional information. The study has been included in the RAC evaluation and your position is noted.

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	Sweden		MemberState	8
Comment received				
All source substances (monoglyme, diglyme, triglyme, 2-ME and 2-MAA) used in the read-across to the target substance Tetraglyme contain "D" as part of their harmonized classifications for Repr. 1B, H360. Tetraglyme show clear developmental effects as presented in the classification proposal and further elaborated in the confidential Annex 2 (e.g. in the extracted study report Table B.4.10.2.1.1. "Remarkably increased skeletal malformation"). In the CLH proposal summary it is stated that clear effects on development were observed for Tetraglyme in the absence of maternal toxicity. A clarification from the dossier submitter about what uncertainties that lead to the conclusion not to specify the differentiation (d/D) in the classification proposal is lacking. We consider the current explanation too vague and unspecific. All source substances contain either "F" (Monoglyme, Diglyme, 2-ME and 2-MAA) or "f" (Triglyme) as part of their harmonized classifications for Repr. 1B, H360. It is noted that the harmonised classification of the source substances were concluded by Technical Committee for Classification and Labelling and hence included in Annex I of Directive 67/548/EEC and later translated and included in CLP Annex VI. It would be valuable to know what the underlying reasons are for the weaker differentiation "f" of Triglyme, the closest homologue to Tetraglyme, in its harmonized classification. Therefore, more detailed data on adverse effects on fertility of Triglyme than presented in the current CLH proposal is necessary to make a robust conclusion to be used in read-across and to conclude whether category 1B (H360F) or 2 (f) is warranted. In the CLH proposal summary it is stated that clear effects on fertility were observed for Tetraglyme. A clarification from the dossier submitter about what uncertainties that lead to the conclusion not to specify the differentiation (f/F) in the classification proposal is lacking. We consider the current explanation too vague and unspecific.				

Dossier Submitter's Response
Thank you for your review. All available information for the three source substances was extracted from the CSR, the IUCLID summary and the classification dossiers (which were the basis for the harmonised classification of the other glymes). The reason why triglyme was categorized differently compared to the other glymes and the potential metabolites is not clear: According to the classification dossiers (from 2001 and 1998) in all cases, classification for fertility effects is based on effects on testis, epididymis and spermatogenesis in repeated dose toxicity studies. The arguments for not differentiating for d/D or f/F are provided in chapter 4.10.5. It is upon RAC to decide this question.
RAC's response
Thank you for your comments. The underlying basis for the committee decision on the classification of triglyme is presented in the ODD.

Date	Country	Organisation	Type of Organisation	Comment number
26.10.2017	Netherlands		MemberState	9
Comment received				
Although it is expected that the reprotoxic metabolites will also be formed in the case of tetraglyme, it could be helpful if quantitative data or an estimate would be available. • Since there are reproductive toxicity studies available for tetraglyme itself, resulting in adverse effects on both sexual function and development, in principal, classification should be based primarily on these data. However, since only range finding studies, with low power are available, the read across to the other glymes and the data for the suggested metabolites may be equally important. • We support the proposed classification of tetraglyme as Repro 1B because: o The data with tetraglyme itself (28 day study rat, dose-range finding studies for reproductive/developmental toxicity in rat) result in clear effects on sexual function (reduced testes and epididymal weight accompanied by histopathological effects on testes and reduction in mature sperm (Is it possible to indicate the level of reduction?) and increased precoital interval) and development (prolonged gestation period, increased post implantation loss, decreased no of live pups at birth and increased soft tissue and skeletal abnormalities). However, it should be noted that the range finding studies can only be used as evidence that tetraglyme indeed induces effects on sexual function and development, but, due to the low power not to determine the potency; o Supported by the fact that the shorter chain glymes show similar effects on fertility and development. Would it be possible to provide a table comparing the type of malformations of the different glymes in more detail than in table B. 4.10.3.4 ? o Also supported by the fact that the assumed metabolites (2-MAA and 2-ME) show similar effects on fertility and development. Since 2-MAA is suggested by QSAR to be formed for all glymes and identified as metabolite in in vitro and in vivo studies for diglyme, we agree that it can be indeed expected to be one of the metabolites of tetraglyme. It is noted however, that no further information is shown for the formation of the other suggested metabolite: 2-ME. The in vivo results with diglyme show that the ether linkage can be cleaved although at low percentage. Therefore, it is likely that also the tetraglyme ether linkage can be cleaved resulting in the formation, amongst others, of 2-ME and its oxidation product 2-MAA. However, the percentage of formation of the active metabolite is considered relevant to assess whether the substance is capable of inducing severe effects at the limit dose. Is it possible to give a quantitative estimate? o Additional evidence for the in vivo formation of 2-MAA is available for DEGME, also showing the cleavage of the ether linkage although at a low percentage (registration dossier).				

o It is noted that the other glymes as well as the suggested metabolites are all classified as Repro 1B.
• However, we do not agree not to indicate f/F or d/D. The studies with tetraglyme itself (although mostly from range-finding studies) already show clear effects on sexual function and development that are consistent with the data from the other glymes and the assumed metabolites.
• Effects on sexual function (reduced testis and epididymis weight) are only observed at the limit dose of 1000 mg/kg bw, however, they are observed in the absence of other general toxicity and, as indicated, it is noted that despite the limited power of the studies they show clear effects on fertility parameters .
• Further, although there was no reduction in the number of implants in the OECD TG 421 range-finding study indicating that the observed effects on male sexual function in this study at the limit dose of 1000 mg/kg bw/day do not result in an effect on male fertility, the power of such a range-finding study (with n=3) is too low to conclude there is no reduction in implants.
• In addition, it cannot be excluded that longer exposure periods of male rats may result in much stronger effects on the male sexual organs and a stronger reduction of sperm concentration due to the 10 week period required for the development of sperms.
• Further, it is known that human male fertility is more sensitive to reduced sperm concentration than rats. Would this justify a Cat 1B also for effects on fertility? If so how can this be justified?
• In addition, it is questioned why only range finding studies are available and no follow up (full) studies for example from a statement by the registrant?.

Dossier Submitter's Response

Thank you for your review. All data and details available within the CSR, the IUCLID summary and the classification dossiers for the three source substances are summarized in the draft CLH report. The arguments for not differentiating for d/D or f/F are provided in chapter 4.10.5.
As mentioned in footnote 6 referring to ECHA guidance R7a we agree, that the subacute oral rat study and the range finding studies with tetraglyme could eventually also suffice for classification, but read across further supports the conclusion.
The level of reduction of mature sperm cannot be further specified on the basis of the available data.
We agree that potency differentiation should not be suggested.
No more details are available, therefore we cannot provide a table comparing the type of malformations of the different glymes in more details (reformatting the table is of course an option for RAC).
No quantitative metabolism data are available.
We are not sure that a low statistical power may be blamed for obscuring effects on implants/dam (in the dose-range finding study for repro/dev tox screening BSL Bioservice 2011) - since there is practically no difference at all: from control to high dose: 12.67, 12, 10.5, 11.
Also in the 28 day study effects on fertility were observed (i.e. relative testis weight minus 10%; germinal epithelium histopath. in 2/5 animals in 28d study; significantly reduced mature sperm in 28d study) – but only at 1000 mg/kg bw day. Therefore we are not sure what the impact of a longer exposure period in the repro/dev tox screening study could be with regard to more severe effects on sexual organs and sperm or the overall conclusion.
We agree that the knowledge about human male fertility beeing more sensitive to reduced sperm concentration compared to rats should be considered. Therefore the "significantly reduced mature sperm in 28d study" may be a concern, regardless of the magnitude of the effect.

We also agree that the fact that toxicological investigation stopped after the range finding studies indicates that the registrant agrees to the severe reproductive toxicity concern. However these fertily effects were observed only at 1000 mg/kg bw day and furthermore triglyme as the nearest neighbour in the read across approach was classified with H360Df and it is unclear why triglyme was categorized differently compared to the other glymes and the potential metabolites: According to the classification dossiers (from 2001 and 1998) in all cases, classification for fertility effects is based on effects on testis, epididymis and spermatogenesis in repeated dose toxicity studies. Only for diglyme in addition a rat dominant lethal test is available indicating reduced male fertility. However, indicating f instead of F because of the lack of data is not considered adequate, especially in the case where classification is supported by read across. In addition the classification dossier for monoglyme available to the dossier submitter (Repr. Cat. 3, R62) is inconsistent with the Annex VI to the CLP regulation (Repr. Cat 2, R60). Acknowledging the uncertainties related to testing, assessment, data documentation and read across, we supported not to indicate f/F or d/D. For us as dossier submitters it would be helpful to hear the view of RAC about the practical regulatory impact of classification with H360 D/f versus H360 D/F versus just H360. However in any case it is upon RAC to decide whether a specification as D/d and F/f is justified.
RAC's response
Thank you for your comments. RAC has noted your position with regard to classification for effects on fertility and development.

CONFIDENTIAL ATTACHMENTS
1. OECD422_Report.pdf [Please refer to comment No. 2, 7]