Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Reaction mass of *N*,*N*'-ethane-1,2-diylbis(decanamide) and 12-hydroxy-*N*-[2-[(1-oxodecyl)amino]ethyl]octadecanamide and *N*,*N*'-ethane-1,2-diylbis(12-hydroxyoctadecanamide); [1]

Reaction mass of *N*,*N*'-ethane-1,2-diylbis(decanamide) and 12-hydroxy-*N*-[2-[(1-oxodecyl)amino]ethyl]octadecanamide; [2]

EC Number: 430-050-2 [1] - [2]
CAS Number: - [1] - [2]

CLH-O-0000007073-80-01/F

Adopted

18 March 2022

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON REACTION MASS OF N,N'-ETHANE-1,2-DIYLBIS(DECANAMIDE) AND 12-HYDROXY-N-[2-[(1-OXODECYL)AMINO]ETHYL]OCTADECANAMIDE AND N,N'-ETHANE-1,2-DIYLBIS(12-HYDROXYOCTADECANAMIDE)

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: Reaction mass of N,N'-ethane-1,2-diylbis(decanamide) and 12-hydroxy-N-[2-[(1-oxodecyl)amino]ethyl]octadecanamide and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide); [1]
Reaction mass of N,N'-ethane-1,2-diylbis(decanamide) and 12-hydroxy-N-[2-[(1-oxodecyl)amino]ethyl]octadecanamide; [2]
EC number: 430-050-2 [1] - [2]
CAS number: - [1] - [2]
Dossier submitter: Spain

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
04.03.2021	Netherlands		MemberState	1
Comment received				
P11 Table 10; – Summary of relevant information on acute aquatic toxicity: The fish and daphnia studies were labelled as reliability 1, which is not consistent with the dossier submitter conclusions of not reliable in the text. Four studies on algae are available, two with marine algae Skeletonema costatum and two with freshwater algae Chlorella vulgaris. The description in the text was available only for two marine studies. One marine study has been described in detail and another without details. Are there additional detailed description of marine and freshwater algae tests? Similar to the fish and daphnia tests, the reliability of the freshwater algae studies were not correctly labelled or was missed.				
Dossier Submitter's Response				
The reliability scores indicated in the Table 10 as well as in the other overview tables on available studies refer to the scores given by the registrants, as instructed by ECHA. The DS's ratings for reliability are indicated in the text.				

Regarding the other marine and freshwater algae tests, as indicated in the Annex I of the CLH report, very limited information on study conditions and results is available. However, this is not considered to affect the classification proposal because these studies are not considered reliable as indicated in the CLH report. In the second marine alga test, the loading rates were above the water solubility limit of the substance and there is no information on whether the test concentrations were analytically verified. Therefore, the study is not considered reliable for classification purposes.

The two freshwater algae studies are not considered reliable as they used nominal concentrations/ loading rates well above the water solubility limit of the substance, the results are based on nominal concentrations/loading rates and no analytical measurement of the test concentrations were made.

RAC's response
Thank you for your comment. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
12.03.2021	France		MemberState	2
Comment received				
We have some reservations about the alga test with Skeletonema costatum as key study for the classification proposal. See comments for this specific endpoint.				
Dossier Submitter's Response				
See our responses below.				
RAC's response				
Thank you for your comment. Noted. See response under comment number 5.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
12.03.2021	Belgium		MemberState	3
Comment received				
BE CA thanks the dossier submitter for the well-substantiated CLH report. BE CA supports the proposal for the environmental classification of the substance Thixatrol plus: Aquatic Acute 1, H400, M=100 and Aquatic Chronic 1, H410, M=10. We also agree with the way the dossier submitter came to his conclusion, based on the most stringent outcome between NOEC for the most sensitive species and the surrogate approach (no chronic data available for the 3 trophic levels). Nevertheless, it is not clear why the aquatic acute and chronic studies are given a reliability 1 in the respective overview tables while in the descriptive text most of them are considered unreliable. In the confidential annex there are constituents reported that are in our view impurities.				

A multi-constituent substance is a substance consisting of several main constituents present at concentrations generally ≥ 10% and < 80% (w/w) (Guidance for identification and naming of substances under REACH and CLP, v.2.1 of May 2017).
Dossier Submitter's Response
The reliability scores indicated in the Table 10 as well as in the other overview tables on available studies refer to the scores given by the registrants, as instructed by ECHA. The DS's ratings for reliability are indicated in the text. Regarding the information on constituents, the information included in the confidential annex is reported as in the registration dossiers.
RAC's response
Thank you for your comment. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
04.03.2021	Netherlands		MemberState	4
Comment received				
Toxicity to Algae The main issue is over the key study on marine algae Skeletonema costatum. Available information suggested that there was a problem in chemical analysis. Additionally, the EC50 was expressed for 48 hours but not for 72 hours because the validity criterion of the mean coefficient of variation for section-by-section specific growth rates were not met for 72 hours. Overall, the chemical analysis was based on the measurement of 0 and 72 hours; whereas the toxicity values were expressed for 48 hours, which were different from the 72 or 96 hour ErC50 for algae required by the CLP guidance. Read-across to Thixatrol Max The results of the Daphnia reproduction test was based on the read across results of Thixatrol Max (EC No. 432-430-3). As the composition, structure etc of Thixatrol Plus was not provided in the dossier, it cannot be determined whether read across is valid. Furthermore, the TG211 results of Thixatrol Max and the details of the study method, e.g. nominal concentrations and sampling for chemical analysis, were not described. Acute aquatic hazard No reliable acute toxicity studies with Thixatrol plus are available for fish and aquatic invertebrates. The current classification was based on the study on marine alga Skeletonema costatum, with a 48-h ErC50 of 0.0012 mg/L (mean measured concentration). The key issue is whether this study is reliable and a 48-h ErC50 value, instead of 72-h EC50, can be used for classification. Based on the current description, it seems that the substance should not be classified in the absence of adequate data for classification purposes. It would be useful if the dossier submitter could expand on using the 48-hour ErC50 for classification purposes and provide the 72-hour ErC50 in order to compare it with the 48-hour endpoint. Long-term aquatic hazard (including bioaccumulation potential and degradation) Since fully adequate chronic data are not available for fish and aquatic invertebrates, the surrogate approach was considered for the chronic classification. However, valid acute				

data are not available for fish and aquatic invertebrates too. It is not possible to apply the surrogate approach for these trophic levels. We agree with this conclusion. The proposed classification was exclusively based on the marine algae study, with a 48h- ErC10 of 0.00087 mg/L. The NL CA concern regarding the key study is whether we can use the 48-hour exposure period to classify chronic toxicity and its impact on the classification proposal for the substance.

Based on the information provided regarding degradation and bioaccumulation we agree with the dossier submitter that Thixatrol plus is considered to be rapidly degradable and has bioaccumulation potential for classification purposes.

Dossier Submitter's Response

Toxicity to Algae

Regarding the test duration, in the ISO 10253 guideline it is indicated that if the control cultures show declining growth rate towards the end of the exposure period, inhibited cultures may tend to catch up with the controls, falsely indicating a decreased growth inhibiting effect. In such a case the guideline indicates to perform the calculations of results based on the last measurement within the exponential growth period in the control cultures.

Also according to paragraph 11 of the OECD TG 201, test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached. The cell density had multiplied by a factor well above 16 in the controls after 48 hours. Also other validity criteria of the OECD TG 201 were met after 48 hours exposure.

Furthermore, in the ECHA Guidance document R.7b, the following is stated with regard to algal toxicity tests: "*It is sometimes seen also when test was done according to standard test guidelines, that the exponential growth ceased in the control before the end of the test period. Likewise it may be seen that the validity criteria of the test were not fulfilled (pH increase etc.) or growth of the algae in the exposed concentrations was increased (due to e.g. loss of test substance from the test system) at the end of the test. In such cases only data from the part of the test where exponential growth occurs and the validity criteria for the controls are fulfilled, should be used. In many such cases this may be achieved by excluding data from the last test day from the calculation of ErC50 and NOEC or ErC10.*"

Hence, both in the ISO 10253 and OECD 201 guidelines as well as in the ECHA Guidance it is indicated that a shorter test duration than the typical 72h can be used to calculate the results (including EC10 and NOEC) in algal toxicity tests if all validity criteria are met at the shorter duration. Therefore, we consider that the results at 48 hours from the ISO 10253 study with *S.costatum* can be used for classification, also for the chronic classification.

Regarding the problems with the analytical methods, as indicated in the section 11.5.3, based on the initial method validation trials and procedural recovery trial, it seems that the analytical method can be considered applicable for most of the test substance concentrations used in the definitive test but less aplicable for the lowest test susbtance

concentration (0.00029 mg/L). Since the test substance has low water solubility and high adsorption potential, the real exposure concentrations were likely lower than the nominal concentrations used in the test, especially in the case of the higher test concentrations. Therefore, and considering a precautionary approach, it is considered justified to determine the results based on the geometric mean of the measured concentrations instead of the nominal concentrations.

As you mention in your comments, the test concentrations were measured only at 0 and 72 hours. However, since the substance has low water solubility and high adsorption potential, it can be assumed that any lost of the test substance due to adsorption occurred relatively fast, and hence, the real exposure concentrations at 48 hours is expected to be similar to the measured concentrations at 72 hours.

See also our response to the comment by FR CA below for further information on the applicability of the analytical methods.

Read-across to Thixatrol Max

The main constituents of Thixatrol Plus are indicated in the table 2 of the CLH report, and their concentration ranges as well as further minor constituents/impurities are reported in the confidential annex to the CLH report. In the section 11.6.2 of the CLH report, the structural similarities and differences of Thixatrol Plus and Thixatrol Max are discussed. As indicated in the Annex I of the CLH report, no detailed information e.g. on the purity or composition (e.g. concentrations of different constituents) of the test substance is available for the Long term Daphnia study with Thixatrol Max. Furthermore, as stated in section 11.7.2 of the CLH report, there can be some differences in the toxicities of the two substances and hence the Thixatrol Max study is not considered fully adequate for the classification of Thixatrol Plus and is only used as supporting information.
Further information on the results (including e.g. a table on observed mortality and number of young) and study design of the OECD 211 study with Thixatrol Max are provided in the Annex I of the CLH report.
The test solutions were prepared by using the saturated solution approach, i.e. introducing excess test substance in water followed by stirring and filtration, as explained in the CLH report. The nominal concentrations used in the test were reported to be 0.0070, 0.0022, 0.22 and 0.70 mg/L. The concentration of the test substance was verified by chemical analysis in all surviving test groups on days 0 (fresh media), 2, 5, 7, 9, 12, 14, 16, 19 (fresh and old media) and 21 (old media). The chemical analysis of the freshly prepared test solutions showed measured concentrations in the saturated solution, which was equivalent to the highest nominal test concentration of 0.70 mg/L, to be variable and in excess of the nominal concentration. This was considered to be possibly due to slight variation in the stirring speed and/or slight differences in the water quality. Therefore, the registrant considered appropriate to express the exposure concentrations in terms of time-weighted mean measured concentrations, which were 0.025, 0.071, 0.24, 0.90 and 2.5 mg/L, as also indicated in the CLH report.

Acute aquatic hazard
Regarding your comment whether the key study is reliable and a 48-h ErC50 value, instead of 72-h EC50, can be used for classification, we think that the study is reliable and, as we already indicate above, in our opinion the 48h ErC50 value can be used for

classification. We also note that in the CLH case of etridiazole (CAS: 2593-15-9), mentioned by the UK national authority in their comment, RAC agreed that a 48h ErC50 value for *P. subcapitata* from an OECD TG 201 study could be used to conclude on the acute classification as the exponential growth in the controls was not maintained at 72 h.

We calculated a 72h ErC50 of 0.00138 mg/L (95% CI 0.00121-0.00153 mg/L) based on the mean measured concentrations. However, as can be seen in the Figure 2 in the Annex I of the CLH report and in the below table, at the measured concentration of 0.00153 mg/L, the inhibition of growth at 72h was very high and at the measured concentration of 0.00107 mg/L no inhibition at 72h was observed. Hence, for the 72h test duration, the inhibition increased from zero to almost 100% between two test concentrations. Therefore, the fit of the data to estimate ECx values is quite poor. The 72h ErC50 reported in the study based on the nominal concentrations is 0.0054 mg/L. Hence, these values would also justify a classification as Aquatic Acute 1 with an M-factor of 100.

Long-term aquatic hazard

We have calculated a 72h-ErC10 of 0.00123 mg/L (0.00103-0.00139 mg/L) based on mean measured concentrations. As i dicated above, the inhibition increased from zero to almost 100% between two test concentrations and therefore, the fit of the data to estimate ECx values is quite poor. However, the calculated 72h-ECr10 for mean measured concentrations is in agreement with the graphical method that migth be applied according to ISO 10253 if data are few or uncertain for regression analysis. As can be seen in Figure 2 in the Annex I of the CLH the 72h-ECr10 is somewhere between 0.0029 and 0.0093 mg/L nominal concentrations, which correspond to 0.00107 and 0.00153 m,g/L mean measured concentrations. The registrant reported a 72h-NOErC of 0.0029 mg/L based on the nominal concentrations, which corresponds to a NOEC of 0.00107 based on the mean measured concentrations. Hence, also when using the nominal concentrations and the study duration of 72 hours, classification as Aquatic Chronic 1 is justified. However, the M factor based on the 72h-NOErC values (based on nominal or mean measured concentrations) or the 72h ErC10 of 0.00123 mg/L (based mean measured concentrations) would be 1 (instead of 10 as proposed by the DS based on the 48h ErC10 determined based on mean measured concentrations).

Table. Inhibition of growth rates at 48 and 72h test duration.

Test concentration (mg/L)		Inhibition of growth rate(%)	
Nominal	Mean measured	48h	72h
0.00029	0.00036	-2.27	-2.89
0.00093	0.00038	3.81	-2.43
0.0029	0.00107	11.96	-2.63
0.0093	0.00153	112.26	91.11
0.029	0.02355	112.26	111.25

However, as explained above, we think that the 48h ErC10 determined based on the mean measured concentrations can be used for chronic classification. See also our below response to the comment by the UK national authority.

RAC's response
Thank you for your comment. RAC agrees with the DS that exponential growth is important for the reliability of the results therefore the data up to 48 hours should be taken in to account. As indicated by the DS the validity criteria for the controls according to ISO 10253 and OECD TG 201 were met for exposure period of 48 hours. The use of shorter exposure period (48 h) is acceptable according to ISO 10253, OECD TG 201 and ECHA Guidance document R.7b. In the CLP guidance (version 5.0, July 2017) it is indicated "The algal growth inhibition test is a short-term test that provides both acute and chronic endpoints." Hence RAC supports the use of the acute algae study with *S. costatum* as a source for a chronic NOEC/EC_{10}. Overall, RAC agrees with the DS that growth rate reduction endpoints ErC_{50}, NOE_rC and E_rC_{10} after 48 hours of exposure should be considered for the classification of the substance. RAC agrees with the DS that since the substance has low water solubility and high adsorption potential, it can be assumed that any loss of the test substance due to the high adsorption potential occurred relatively fast, therefore the real exposure concentrations at 48 hours are expected to be similar to the measured concentrations at 72 hours. This assumption in also in line with the CLP guidance (version 5.0, July 2017, p. 561) where it is stated that in the case of adsorption: "this can occur for substances of high adsorption characteristics such as high log Kow substances. Where this occurs, the loss of concentration is usually rapid and exposure may best be characterised by the end of test concentrations;". The RAC opinion regarding the chemical analysis is provided under the comment number 5. RAC agrees with the DS to read-across aquatic chronic toxicity data from Thixatrol Max. The main assumption to justify the read-across approach is structural similarity of the constituents of the Thixatrol Plus and Thixatrol Max. Both substances have three main constituents, one common constituent and the other two constituents differ only in the length of the shorter alkyl sidechain. RAC supports the DS's view that two constituents of Thixatrol Plus with longer alkyl chains are expected to be less water soluble and to have higher log Kow values than the two constituents of Thixatrol Max with the shorter sidechains. Consequently, this could lead to some differences in the toxicity of the two substances to *D. magna*. RAC agree with the DS that chronic toxicity study with *D. magna* using Thixatrol Max should be considered as supporting information due to deficiencies pointed out by the DS (lack of information and difference in toxicity). RAC agrees that due to lack of acute data for fish and invertebrates the surrogate approach for these trophic levels could not be applied. The support for rapid degradability and bioaccumulation potential conclusions is noted by RAC.

Date	Country	Organisation	Type of Organisation	Comment number
12.03.2021	France		MemberState	5

Comment received

Before to conclude on a classification based on the key study with algae, we believe that some interrogations needs to be first clarified. Indeed, as it is explained in the CLH report the constant exponential growth occurred only up to 48 hours exposure in the controls and at 72h exposure the growth had slowed down. In an algae test, in order to arrive to an unrestricted exponential growth during exposition, sufficient nutrients conditions and continuous light need to be ensured. Since the control does not reach the exponential growth at 72h, we believe that the experimental conditions (test medium, initial cell concentration, light) needs to be verified. Another critical point is the stability of the substance, due to the low water solubility and high adsorption potential, the substance is not stable in the medium even at 0 hour, it is indicated that the measured test concentrations ranged from 15 to 124% of the nominal at 0 hour and from 18 to 227 % after 72 hours. The dossier submitter took into account the concentrations measured at 0h and 72h in order to estimate the concentrations tested at 48hours. The EC50 estimated is based on the effect measured at 48h and not at 72h as it is recommended in the CLP guidance for algae, because, as the control did not follow an exponential growth at 72h. Looking the figure 2 in the annex I document (page 11), it is possible to hypothesize that the EC50 at 72h will be < 1 mg/L. In order to strengthen the assumption, it would be useful to have information about historical controls for the species of algae used in this study. The information could allow to make estimations about the growth inhibition at 72h.

Classification proposal for Acute toxicity category 1 can be acceptable. However, regarding to the M factor proposed for acute hazards, due to the uncertainties explained above, FR thinks that it is not possible to select the appropriate M factor.

Regarding to the proposal of classification as Chronic 1, FR has some reservations. It may be expected an EC10 (72h) <0.01 mg/L, however FR considers, as for acute classification, that further information is necessary in order to strengthen the assumption.

Dossier Submitter's Response

Regarding whether the 48h ErC50 and ErC10 values can be used for concluding on acute and chronic classification and M factors, please see our responses to the above comment by NL CA and the below comment by UK national authority.

According to the information in the registration dossier, the experimental conditions used in the test were in line with the ISO 10253 guideline. The composition and preparation of test medium followed the indications of the guideline. Natural sea water with added nutrients was used as recommended for *Skeletonema costatum* by the guideline. The incubation was done at 20 ±1 °C under continuous illumination (intensity approx. 7000 lux) provided by warm white lighting (380-730 nm), and at constant shaking (ca. 150 rpm). The nominal initial cell density was 3000 cells/ml. The measured cell densities of the control replicates were in the range of 1650-5000 cells/ml at 0h (see the table included in our response to the UK national authority's comment). According to the ISO

10253 guideline, the initial cell density shall not exceed 10^4 cells/ml. However, the guideline recommends a lower cell density (three to fivefold lower) for *Skeletoma sp* due to its higher cell volume and growth rate. Hence, the initial cell density used in the study was also in line with the guideline.

Regarding the stability of the test substance, in the below tables the results of the measured concentrations at 0 and 72 are presented as well as the procedural recoveries and results of the pre-study media preparation trials. In the study report it is stated that the detection system had acceptable linearity, and that the procedural recoveries were acceptable except for the lowest test concentrations. As shown in the tables and indicated in the CLH report, all control samples, both of the definitive test as well as of the paralel procedural recovery trial, gave positive responses at the same retention times as the samples with the test substance. The study authors say that there was significant and variable interference seen around the test samples´ peaks in the chromatogram analyses. However, as seen in the below tables, no test item was detected in the control samples of the pre-study trials. In conclusion, there is some uncertainty in the analytical results. However, in all the procedural recoveries, most of the test substance concentrations had accpetable recoveries. Taking into account that the substance is poorly soluble and highly adsorptive and consequently the real exposure concentrations were likely lower than the nominal ones, especially in the case of the highest test concentrations, in our opinion the measured concentrations can be used to calculate the results. For the lowest test concentration only the measured concentration at 0h was used in the calculation of the results as the concentration at 72h was well above the nominal one. Hence, the mean measured concentrations used for the recalculation of the results were 0.000359, 0.000383, 0.00107, 0.00153 and 0.0235 mg/L.

Table. Measured concentrations of the test solution samples.

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
0 hours	Control	0.0000741	-
	0.00029	0.000359	124
	0.00093	0.000328	35
	0.0029	0.00157	54
	0.0093	0.00137	15
	0.029	0.0308	106
72 hours	Control	0.000635	-
	0.00029	0.000660	227
	0.00093	0.000447	48
	0.0029	0.000724	25
	0.0093	0.00170	18
	0.029	0.0180	62

Table. Measured concentrations of the procedural recovery samples

Sample	Fortification (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Fortification (%)
0 hours	Control	0.000908	-
	0.000302	0.000455	151
	0.00101	0.000958	95
	0.00302	0.00283	94
	0.0101	0.0103	103
	0.0302	0.0319	105
Sample	Fortification (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Fortification (%)
72 hours	Control	0.0110	-
	0.000283	0.00112	397
	0.000940	0.00186	197
	0.00283	0.00331	117
	0.00940	0.00907	96
	0.0283	0.0233	82

Table. Pre-study media preparation trials

Stirring Period and Treatment	Concentration Found (mg/l)
Control	<LOQ
24 Hours Centrifuged 10000 *g*	3.06
24 Hours Centrifuged 40000 *g*	2.75
24 Hours Filtered ~500 ml discarded	0.0785
24 Hours Filtered ~1 litre discarded	0.0290
Stirring Period and Treatment	Concentration Found (mg/l)
48 Hours Control	<LOQ
48 Hours Centrifuged 10000 *g*	3.24
48 Hours Centrifuged 40000 *g*	2.22
48 Hours Filtered ~500 ml discarded	0.0813
48 Hours Filtered ~1 litre discarded	0.0536

It is also indicated in the FR CA's comment that it would be useful to have information about the "historical controls" for *Skeletonema costatum* in order to make an estimation about the growth inhibition at 72h. It is not clear for us whether this request regards to

the culture line used in the laboratory or to a general information regarding the growth of *S. costatum*. However, we can not see clearly how this information would clarify the decreased growth observed in the controls at 72h in this specific test. As indicated in test guidelines with algae, i.e. ISO 10523 and OECD 201, exponential growth is expected during the whole test duration with a growth rate of 0.9 d^{-1}. It is also mentioned that growth rates above 1.0 d-1 are normally obtained in results from interlaboratory test. So, it is supposed that something happened during the test performance that resulted in a slower growth in the controls in the period from 48h to 72h. We note that the test was performed in accordance with the ISO guideline, and hence, we cannot think of any obvious reason that could explain the lack of exponential growth during the last day. In any case, in our opinion, the last day of the test did not fulfil the validity criteria, independently of the reason for growth inhibition, and therefore, only the first two days should be used for calculating the results.

RAC's response

Thank you for your comment.

RAC's opinion regarding the use of endpoints ErC_{50}, NOE_rC and E_rC_{10} after 48 hours of exposure for the classification of the substance is provided under the comment number 4.

Experimental conditions: Noted.

Historical controls: Noted.

RAC is of the opinion that there are some uncertainties with respect to analytics, i.e. Thixatrol Plus was detected in the control of the definitive test and procedural recovery test. However, no Thixatrol Plus was detected in control in pre-study media preparation trials, detection system had acceptable linearity and the procedural recoveries were acceptable except for lowest test concentration therefore RAC is of the opinion that the analytical method could be considered applicable. Taking in to account that the substance has low water solubility and high adsorption potential, the real exposure concentartions were likely lower that the nominal concentrations used in the test therefore the effect value should be related to geometric mean measured concentrations. This is also in line with ECHA guidance (Chapter R.7b, p.26) where it is indicated that if measured concentrations are < 80% of nominal concentrations, for static test the geometric mean measured concentrations should be calculated.

Date	Country	Organisation	Type of Organisation	Comment number
12.03.2021	United Kingdom	Health and Safety Executive	National Authority	6

Comment received

Reaction mass of N,N'-ethane-1,2-diylbis(decanamide) and 12-hydroxy-N-[2-[(1-oxodecyl)amino]ethyl]octadecanamide and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide) (EC: 430-050-2; CAS: -)
The DS proposes the key endpoints for Aquatic Acute and Aquatic Chronic hazard classification are a 48-h ErC50 of 0.0012 mg/L and a 48-h ErC10 of 0.00087 mg/L (mean

measured) for Skeletonema costatum. These endpoints were derived from a 72 hour study conducted according to the ISO 10253 test method (Harlan Laboratories Ltd, 2011) and the DS notes the ISO 10253 validity criteria were met for the study 72 hour test period. The DS considers that the CoV for section-by-section growth rates in the controls exceed the OEDC TG 201 validity criterion of 35% and that the controls are only reliable up to 48 hours. However, raw cell data are not presented in the CLH report and it is unclear how many replicates were employed and what the section–by-section CoV values were.

Please can the DS present the raw cell data for controls and calculated CoV section-by-section values for each time point? This is necessary to consider the OECD TG 201 validity criteria and its relevance to S. costatum. OECD TG 201 validity criteria assess the performance of the controls although it is unclear whether the criteria are applicable to a species not included as an OECD TG 201 test species. The pydiflumetofen (CAS 1228284-64-7) CLH public consultation response noted that Skeletonema costatum is composed of chains (unlike most other OECD TG 201 recommended test species that are single cells or rods) and as a result more clumping of Skeletonema costatum may be expected which could contribute to relatively high count variability.

In addition, if 48 hour endpoints are the considered the most reliable, we are unclear whether 48 hours is sufficient duration for chronic classification – it may be appropriate to consider the surrogate approach as was previously the case for etridiazole (CAS: 2593-15-9).

Dossier Submitter's Response

In the below table the raw data for cell densities and the section-by-section growth rates are shown for the control replicates. The growth rates for the section 0-24h are calculated using the nominal cell density as the initial cell density, as recommended by the ISO 10253 and OECD TG 201. The mean coefficient of variation for section-by-section specific growth rates was calculated to be 55.5% for the 0-72 h study period and 12.5% for the 0-48 study period. From this data it can be seen that the growth was very fast in the control cultures during the first two days after which it slowed down significantly.

Table. Cell densities and section-by-section growth rates for the control replicates.

Control replicate	Nominal cell density (cells/ml)	Measured cell densities (cells/ml)				Section-by-section Growth rates (day-1)		
		0h	24h	48h	72h	0-24h	24-48h	48-72h
1	3000	3350	23400	503000	533000	2.054	3.068	0.058
2	3000	1650	26700	393000	533000	2.186	2.689	0.305
3	3000	3350	23400	373000	692000	2.054	2.769	0.618
4	3000	1650	26700	360000	617000	2.186	2.601	0.539
5	3000	5000	28400	340000	650000	2.248	2.483	0.648
6	3000	1650	23400	413000	650000	2.054	2.871	0.454

In the information included in the registration dossier, it is indicated that given that the algae used as test species form long chains that are not always detected by multisizer particle counters, the number of algal cells was counted using haemocytometer and light

microscope in order to obtain accurate count. Hence, the study authors were aware of the challenges in counting the cells of the algae and therefore it can be assumed that all effort was made to obtain as accurate count as possible.

Regarding the two other CLH cases mentioned in the comment, it is noted that for pydiflumetofen (CAS 1228284-64-7) the study with Skeletoma costatum was not a key study for acute and long-term environmental hazards as algae were not the most sensitive
aquatic organisms. For etridiazole (CAS: 2593-15-9), in an OECD TG 201 study with P. subcapitata the 48-h ErC50 was chosen as the most relevant value as the CoV of the growth rate in the control cultures was more than 35% due to reduced growth rate at 72-h and 120-h. This value was used to conclude on the acute toxicity of the substance. However, for the chronic toxicity RAC considered that the 48h NOEC/EC10 of this study could not be directly compared with the CLP criteria and used the surrogate approach instead, i.e. the chronic classification was based on the 48h ErC50 for P. subcapitata.

As stated in our response to the above comment from NL CA, both in the ISO 10253 and OECD TG 201 as well as in the ECHA Guidance R.7b it is indicated that a shorter test duration than the typical 72h can be used to calculate the results (including EC10 and NOEC) if exponential growth in the controls is not maintained until 72h and all validity criteria are met at the shorter duration. Therefore, we consider that the results at 48 hours can be used for classification, also for the chronic classification.

It is also noted that using the surrogate approach for the algal chronic toxicity, as was done in the etridiazole case, the 48h- ErC50 of 0.0012 mg/L (based on mean measured concentrations) would also justify classification as Aquatic chronic 1 (ErC50 < 1 mg/L and the substance is considered bioaccumulative) but the M-factor would be 100 (0.001 < EC50 ≤ 0.01 mg/L) instead of 10 as proposed by the DS based on the 48h ErC10 of 0.00087 mg/L (based on mean measured concentrations) (0.0001 < EC10 ≤ 0.001 mg/L and the substance is rapidly degradable).

RAC's response

Thank you for your comment.

RAC's opinion regarding the use of endpoints ErC_{50}, NOE_rC and E_rC_{10} after 48 hours of exposure for the classification of the substance is provided under the comment number 4. As reliable chronic data are available for algae the surrogate approach was not considered by RAC.