Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

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**
CAS Number: **-**
Index Number: **616-127-00-5**

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1 PHYSICAL HAZARDS

Not assessed in this dossier. No public consultation proposed.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not relevant for the classification proposal in this dossier.

3 HEALTH HAZARDS

Not assessed in this dossier. No public consultation proposed.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Study 1: Determination of the ready biodegradability of Thixatrol Plus (EA2525)

Study reference:

Chemex International plc, 1998

Detailed study summary and results:

Test type:

Ready Biodegradability – CO2 Evolution Test, OECD TG 301B, EU Method C.5. GLP.

Test substance:

Thixatrol Plus (EA2525) was used as test substance, with a reported purity of 96.9 %. However, there is no information on the concentrations of different constituents or impurities in the test substance.

Materials and methods:

Activated sludge from Letchworth Sewage Treatment Works was used as inoculum. The inoculum was sieved, settled, decanted and resuspended using deionised water. These pre-treatment steps were done twice after which the inoculum was centrifuged. The concentration of suspended solids in the final inoculum used in the test was 30.0 mg/L.

The test substance and inorganic nutrient medium were inoculated with activated sewage sludge. 55 mg of substance was used as sole source of organic carbon. It is indicated a Total Organic Carbon (TOC) of 40 mg in 2 L of mineral medium, which results in 20 mg C/L. The test included two replicates with the test substance plus inoculum, two replicates for inoculum blank and one test vessel with the reference substance (anhydrous sodium acetate) plus inoculum.

The test vessels were incubated in dark at 23 °C for 28 days and aereated by CO_2-free air. The degradation was followed by determining the produced CO_2. The CO_2 was trapped in barium hydroxide and was

measured by titration of the residual hydroxide. The amount of CO_2 produced (corrected for that determined for the inoculum blanks) is expressed as a percentage of the theoretical CO_2. The measurement of CO_2 was performed on days 2, 4, 7, 10, 14, 17, 21, 25 and 28. On day 28, 1 ml of concentrated hydrocholoric acid is added to each vessel which are aereated overnight to drive off the remaining CO_2, and the last analysis of evolved CO_2 was made on day 29.

Results:

The degradation of the substance was determined to be 69.3 % after 28 days based on CO_2 evolution. The degradation did not meet the criteria for the 10-days window although it was close to meeting them. After 10 days the degradation was 9.62 % and after 21 days it had reached a level of 59.27 %. The validity criteria of the test were met. The difference of the replicate values of the removal of the test substance at the plateau, at the end of the test and at the end of the 10-d window was less than 20 %. The reference substance, sodium acetate, reached 66.9 % degradation after 14 days and the mean blank CO_2 evolution was 19.9 mg/L.

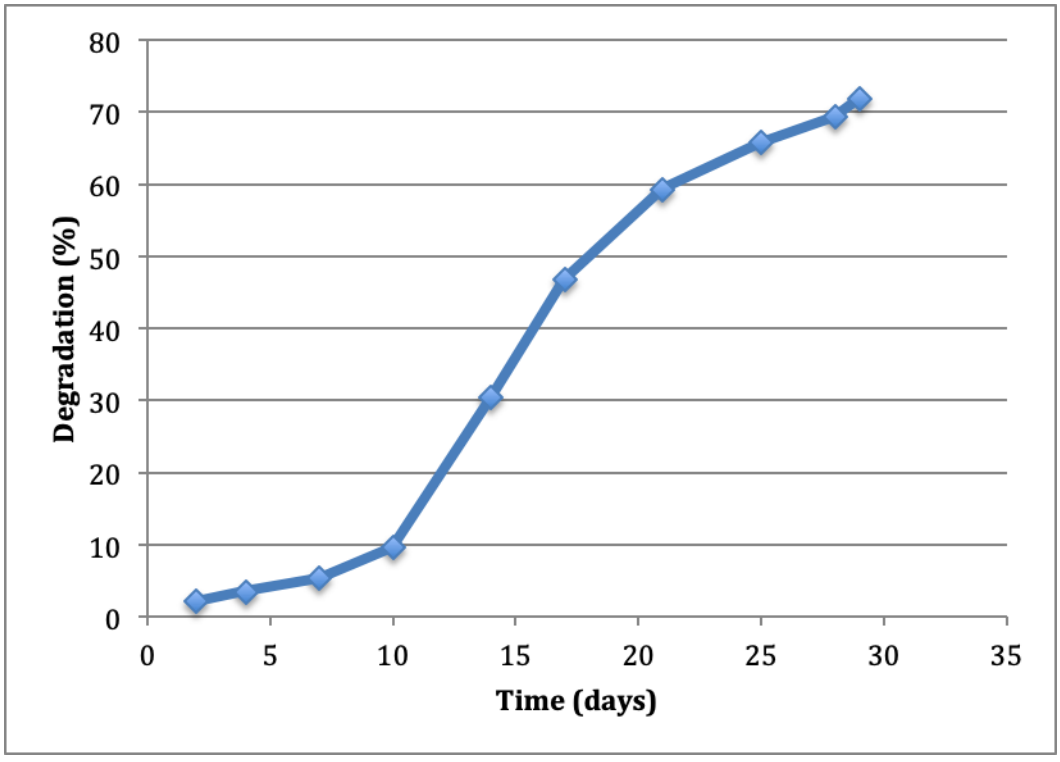

Figure 1. Degradation of Thixatrol Plus based on CO_2 evolution (mean of the two replicates) in the OECD TG 301B study.

Study 2: Estimation of ready biodegradability of the main constituents of Thixatrol Plus

EPISuite BIOWIN QSAR models were performed to predict the ready biodegradability of the main constituents of Thixatrol Plus. In the BIOWIN 1, 2, 5 and 6 models, a biodegradability probability

score above 0.5 predicts fast or ready biodegradability of the substance. In BIOWIN 3 model, a score in the range of ≥ 2.25 - <2.75 predicts ultimate biodegradation in "weeks to months" and a score of ≥ 2.75 ultimate biodegradation in "weeks" (or faster). According to the REACH Guidance R.11: PBT/vPvB Assessment (ECHA, 2017), the output of the models BIOWIN 2, BIOWIN 3 and BIOWIN 6 of the EPISuite BIOWIN QSAR models can be used to make a screening assessment of persistence. The following outcome indicate that a substance may potentially be persistent: BIOWIN 2 <0.5 and BIOWIN 3 <2.2 or BIOWIN 6 <0.5 and BIOWIN 3 <2.2. However, borderline cases should be carefully examined, e.g. when the estimate of the BIOWIN 3 gives a result in the range 2.25 to 2.75.

The results of the BIOWIN models for the main constituents are shown in the below table. The BIOWIN 1, 2, 5 and 6 models predict that all three constituents are readily biodegradable as the results are well above 0.5. For the constituent A and B, the results of the BIOWIN 3 model also indicate fast ultimate biodegradation as they are 2.75 or above. However, it is noted that the result of BIOWIN 3 model for the constituent C is a borderline case (in the range 2.25 to 2.75) as it is close to the screening criterion specified in the ECHA Guidance R.11 for potential persistence. The BIOWIN models include a coefficient for amide fragments, and hence, this type of structures are taken into account in the predictions. However, it is noted that there is some inconsistency between the models as BIOWIN models 1, 2, 4, 5 and 6 have a positive coefficient for the amide fragment whereas in BIOWIN 3 model the coefficient for amides is slightly negative. The training sets of the BIOWIN models 1-2 and 3-4 include 12 and 13 compounds, respectively, with a maximum instance of one amide group per compound. The training set of the models 5-6 contain 23 compounds with a maximum instance of 2 amide groups per compound. Hence, the BIOWIN models 5 and 6 may predict better the degradation of the constituents of Thixatrol Plus.

Table 1 Episuite Biowin V4.10 Models For The Main Constituents

Constituent	Smiles	BIOWIN model				
		1	2	3	5	6
1-[2-(decanoylamino)ethylamino]-1-decanone	C(C)CCCCCCCC(NCCNC(CCCCCCCCC)=O)=O	1.2092	0.9989	2.8729	0.7591	0.8063
1-[2-(decanoylamino)ethylamino]-12-hydroxy-1-octadecanone	CCCCCCCCCC(=O)NCCNC(=O)CCCCCCCCCCC(O)CCCCCC	1.3069	0.9979	2.7495	0.8340	0.8369
12-hydroxy-1-[2-(12-hydroxyoctadecanoylamino)ethylamino]1-octadecanone	CCCCCCC(O)CCCCCCCCCCC(=O)NCCNC(=O)CCCCCCCCCCC(O)CCCCCC	1.4046	0.9957	2.6261	0.9090	0.8635

4.1.2 BOD_5/COD

No data.

4.1.3 Aquatic simulation tests

No data.

4.1.4 Other degradability studies

No data.

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

No data.

4.2.2 Bioaccumulation test with other organisms

No data.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

[Study 1: Determination of the toxicity of Thixatrol Plus (EA2525) to Rainbow Trout (*Oncorhynchus mykiss)*

Study reference:

Chemex International Plc (1998b)

Detailed study summary and results:

Test type:

OECD Guideline 203 (Fish, Acute Toxicity Test) , EU Method C.1 (Acute Toxicity for Fish), GLP

Test substance:

Thixatrol Plus (EA2525) was used as test material. There is no further information on the purity, concentrations of the different constituents and/or impurities.

Materials and methods:

Rainbow trout *(Oncorhynchus mykiss)* were exposed to a water accommodated fraction (WAF) of the test substance at a loading rate of 1000 mg/L under static conditions during 96 hours. Tween 80 was used as solvent in the test solutions at a concentration of 100 mg/L. There is no further information on the test conditions and test design.

Results:

No mortalities or other adverse effects were observed during the study. There is no information on whether the test concentration was analytically measured.

4.3.2 Short-term toxicity to aquatic invertebrates

Study 1: The Acute Toxicity of Thixatrol Plus (EA2525) to Daphnia magna

Study reference:

Chemex International Plc (1998)

Detailed study summary and results:

Test type:

OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) , EU Method C.2 (Acute Toxicity for Daphnia) , GLP

Test substance:

Thixatrol Plus (EA2525) was used as test material. There is no further information on the purity, concentrations of the different constituents and/or impurities.

Materials and methods:

Daphnia magna were exposued to the test substance under static conditions during 48 hours. Tween 80 was used as solvent in the test solutions at a concentration of 100 mg/L. There is no further information on the test conditions and test design.

Results:

All concentrations tested caused immobilisation of D. magna, with 50% immobilisation being observed at a nominal concentration of 31.25 mg/l and 40% immobilisation at a nominal concentration of 62.5 mg/l. However, there is no further information on the test concentrations or on the results. Observation was not dose related and thought to be due to Daphnia becoming coated with particles of suspended material during the study rather than toxicity of test material. No visible sub-lethal effects such as floating were seen.

4.3.3 Algal growth inhibition tests

Study 1: Thixatrol Plus (EA2525): Marine algal inhibition test

Study reference:

Harlan Laboratories Ltd, 2011

Detailed study summary and results:

Test type:

The study followed ISO 10253 (Water quality - Marine Algal Growth Inhibition Test with Skeletonema costatum and Phaeodactylum tricornutum) and was GLP compliant.

Test substance:

Thixatrol Plus was used as test material (Batc number 1325G-12601). However, there is no further information on the concentrations of the different constituents and/or impurities.

Due to the low solubility of the substance, preliminary solubility trials were performed. These showed that it was not possible to obtain stable test substance solutions using the traditional test solution preparation methods (e.g. ultrasonification, high shear mixing). Furthermore, the test substance was not readily soluble in the recognised solubilising agents. The pre-trial indicated that a dissolved test substance concentration of approx. 0.029 mg/L could be obtained from a saturated solution method. The saturated solution was prepared by stirring 50 mg/L of the test material in culture medium during 24 hours after which any undissolved test substance was removed by filtration (0.2 µm Gelman Acrocap, discarding the first 1 litre in order to pre-condition the filter).

Materials and methods:

• *Test species: Skeletonema costatum* (Strain CCAP 1077/5)

• *Initial cell concentration:* 3 x 10^3 cells/ml

• *Test conditions:* Natural sea water (sterilised by membrabe filtration with mean pore size of 0.2 µm) with added nutrients was used as culture medium. The test vessels were 250 ml glass flasks each containing 100 ml of the culture medium. The test vessels were maintained under continuous illumination (approximately 7000 lux) provided by warm white lightning (380-730 nm) and constant agitation by orbital shaker. Temperature within the incubator was recorded daily and the pH of the test solutions was measured at 0 and 72 hours of exposure. The temperature was maintained at 20 ± 1 °C throughout the test. The pH of the control cultures varied from pH 8.0 at 0 h to pH 8.7-8.8 at 72 h, and the pH of the treatment cultures varied from pH 8.0-8.1 at 0 h to pH 8.2-8.8 at 72 h.

• *Test duration/total exposure duration:* 72 hours

• *Test desig:* The nominal test substance concentrations were 0.00029, 0.00093, 0.0029, 0.0093 and 0.29 mg/L. The test concentrations were measured at 0 and 72 h by high performance liquid chromatography –

mass spectrometry (HPLC-MS) using an external standard. Three peaks were observed in the analysis and the results were calculated using the total peak area associated with the test material.
The test included three replicate vessels for each treatment group and 6 vessels for control containing the mineral medium without the test substance. Furthermore, potassium dichromate was used as reference substance (positive control).
Samples of the algal population were taken at 0, 24, 48 and 72 hours from each treatment and control group and the cell densities determined using a haemocytometer and light microscope. Particle counter was not used since the algae forms long chains that are not always detected by particle counter.

Results:
The measured test concentrations ranged from 15 to 124 % of the nominals at 0 hours and from 18 to 227 % after 72 hours. However, there was significant and variable interference seen around the test sample peaks. Furthermore, all control samples, both of the definitive test as well as of the paralel procedural recovery trial, gave positive responses at the test sample retention times. These responses were lower or in the same order of magnitude as the ones measured for the samples from the lowest test substance treatment, except in the case of the procedural recovery trial control sample where the concentration of the substance measured at 72 hours was similar to the concentration measured in the second highest treatment concentation. The registrant considered that the analytical method used was not applicable for the test substance and the results were based on nominal concentrations.

However, based on the initial method validation trials and procedural recovery trial, it seems that the method can be considered applicable for most of the test substance concentrations used in the final test but less aplicable for the lowest test susbtance concentration (0.00029 mg/L). Furthermore, it is not clear why the controls gave a positive response in the final test and procedural recovery trial because in the initial trial comparing different test solution preparation methods, the measured concentrations in the controls were below the limit of quantification (LOQ 0.0068 μg/L). Therefore, it cannot be excluded that the samples of the controls that gave positive response were contaminated with the test substance.

In conclusion, 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, it is considered justified to determine the results based on the geometric mean of the measured concentrations at 0 and 72 hours in case of the nominal test concentrations of 0.00093, 0.0029, 0.0093 and 0.29 mg/L. In case of the lowest test concentration (0.00029 mg/L) only the measured concentration at 0 hours is used for calculating the results because the measured concentration at 72 hours was well above the nominal concentration (227 %), and thus, there could have been some error in the measurement. 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.

In the control cultures the number of cells increased by a factor in the range of 177-230 and the growth rates were in the range of 1.73-1.81 day^{-1}. The coefficient of variation of the growth rates was below 7 % in the controls. Hence, the validity criteria of the ISO 10253 guideline regarding the growth in the control cultures were met. However, it is noted that constant exponential growth occurred only up to 48 hours exposure and at 72h exposure the growth had slowed down (see Figure 2). The validity criterion of the OECD TG 201 regarding the mean coefficient of variation for section-by-section specific growth rates not exceeding 35% is fulfilled until 48 hours exposure but not for the 72 hours study duration. Although the ISO 10253 guideline does not include this validation criterion, constant exponential growth in the control cultures is considered important for the reliability of the results.

Therefore, the dossier submitter considered only the data up to 48 hours exposure and recalculated the results. EC50 and EC10 values were determined using the Probit Analysis. ANOVA with LSD post-hoc test was used for determination of NOEC. The results were calculated both based on the nominal concentrations and measured concentrations. As indicated above, 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 expected that any lost of the test substance due to adsorption in the test vessels occurred relatively fast, and hence, the actual exposure concentrations at 48 hours can be expected to have been similar to the measured concentration at 72 hours. Therefore, the mean measured concentrations as explained above were used by the dossier submitter to calculate the results at 48 hours of exposure.

A 48h ErC50 of 0.0012 mg/L (95% CI of 0.0011-0.0013 mg/L) and ErC10 of 0.00087 mg/L (95% CI of 0.00068-0.0010 mg/L) for inhibition of growth rate were determined based on the mean measured concentrations. The results based on nominal concentrations were 48h ErC50 of 0.0047 mg/L (95% CI of 0.0036-0.0071 mg/L) and ErC10 of 0.0025 mg/L (95% CI of 0.0014-0.0035 mg/L). The 48h-NOEC for growth rate was determined to be 0.000359 mg/L based on measured concentrations and 0.00029 mg/L based on nominal concentrations.

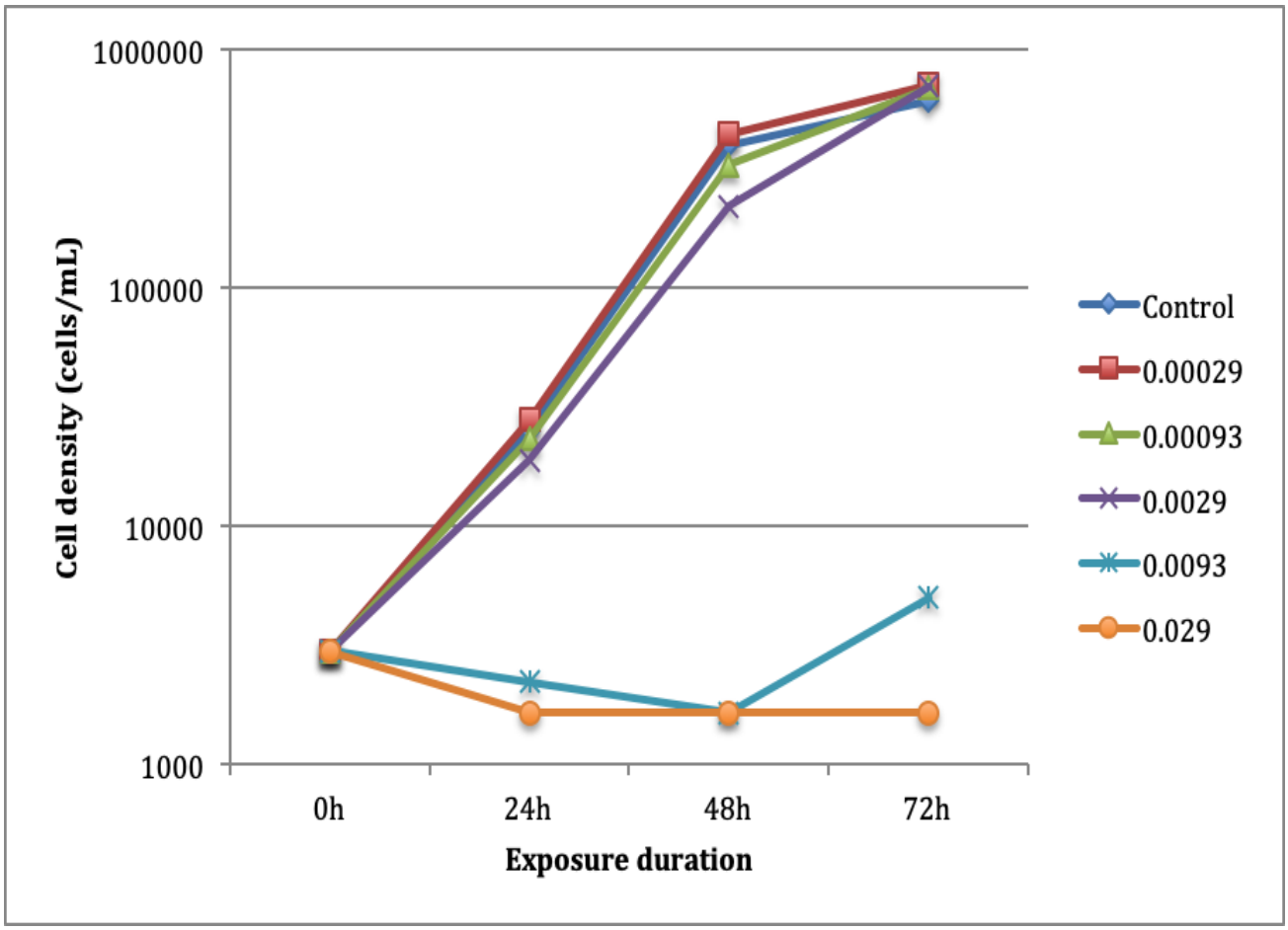

Figure 2 Mean cell densities in the control and treatment (nominal concentrations) groups during the 72h test period

No abnormalities in the algal cultures were detected in any of the treatment or control groups at 72 hours. The results with the reference substance (72h-ErC50 5.9 mg/L, 72h-NOEbC 0.625 mg/L) were within the normal ranges.

Study 2: The Acute Toxicity of EA2525 to the Marine Alga *Skeletonema costatum*

Study reference:

Chemex International Plc (1998)

Detailed study summary and results:

Test type:

ISO 10253 (Water quality - Marine Algal Growth Inhibition Test with Skeletonema costatum and Phaeodactylum tricornutum)

Test substance:

Thixatrol Plus was used as test material. There is no further information on the purity, concentrations of the different constituents and/or impurities.

Materials and methods:

water accomodated fractions over the range of 1 to 10 mg/l loading rate were used. The 72-h EC50 for growth rate was determined to be 4.08 mg/L loading rate. It was not possible to determine a NOEC value. The marine algae *Skeletonema costatum* were exposed to qater accomodated fractions of the test substance in the range of 1 to 10 mg/l loading rate under static conditions during 72 hours. Tween 80 was used as solvent in the test solutions at a concentration of 100 mg/L. There is no further information on the test conditions and test design.

Results:

The 72-h EC50 for growth rate was determined to be 4.08 mg/L loading rate. It was not possible to determine a NOEC value. There is no information on whether the test concentrations were analytically measured.

Study 3: Determination of the toxicity of EA2525 to the unicellular green alga Chlorella vulgaris

Study reference:

Chemex International Plc (1998)

Detailed study summary and results:

Test type:

EU Method C.3 (Algal Inhibition test), GLP

Test substance:

Thixatrol Plus was used as test material. There is no further information on the purity, concentrations of the different constituents and/or impurities.

Materials and methods:

Chlorella vulgaris were exposued to the test substance under static conditions during 72 hours. Tween 80 was used as solvent in the test solutions at a concentration of 100 mg/L. There is no further information on the test conditions and test design.

Results:

A 72h-NOErC of 25.6 mg/L is reported, and it is stated that no ErC50 could not be calculated as the dissolved concentration of test substance was not determined.

Study 4: The Growth Inhibition of the alga Chlorella vulgaris by EA2525, using a water accommodated fraction (WAF)

Study reference:

Chemex International Plc (1998)

Detailed study summary and results:

Test type:

OECD Guideline 201 (1984), GLP

Test substance:

Thixatrol Plus was used as test material. There is no further information on the purity, concentrations of the different constituents and/or impurities.

Materials and methods:

Chlorella vulgaris were exposued to water accommodated fractions (WAFs) of the test substance under static conditions during 72 hours. The WAFs were prepared by using loading rates of 1, 10, 100 and 1000 mg/L, stirring for 20-24 hours after which they were left to settle for 4 hours. The supernatant was decanted and used for testing. No analytical measurement of the test concentrations were performed. There is no further information on the test conditions and test design. There is no further information on the test conditions and test design.

Results:

No significant effects on the growth of the algae were observed in any of the test solutions.

4.3.4 *Lemna* sp. growth inhibition test

No relevant data available.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

No relevant data available.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No relevant data available.

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

No relevant data available.

4.4.4 Chronic toxicity to aquatic invertebrates

Study 1: Thixatrol Max (EA-2854): Daphnia Magna Reproduction Test

Study reference:

Harlan Laboratories Ltd, 2009

Detailed study summary and results:

Test type:

OECD Guideline 211 (Daphnia magna Reproduction Test) , EU Method C.20 (Daphnia magna Reproduction Test) , GLP

Test substance

The substance Reaction mass of N, N'-ethane1,2-diylbis(hexanamide) and 12-hydroxy-N-[2-[(1-oxyhexyl)amino]ethyl]octadecanamide and N, N'-ethane-1,2-diylbis(12-hydroxyoctadecan amide), referred to as Thixatrol Max in this document, (EC 432-430-3), was used as test material. No further information e.g. on the purity of the test substance or on the concentrations of different constituents available.

Due to the low solubility of the substance, preliminary solubility trials were performed. These showed that it was not possible to obtain stable test substance solutions using the traditional test solution preparation methods (e.g. ultrasonification, high shear mixing). Furthermore, the test substance was not readily soluble in the recognised solubilising agents. The pre-trial indicated that a saturated solution method followed by filtration was the most appropriate method for test solution preparation. The saturated solution was prepared by stirring (1500 rpm) 50 mg/L of the test material in dechlorinated tap water during 24 hours after which any undissolved test substance was removed by filtration (0.2 µm Gelman Acrocap, discarding the first 100 ml in order to pre-condition the filter).

Materials and methods:

• *Test species and origin:* Daphnia magna, from in-house laboratory cultures
• *Species life stage:* less than 24 hours old at the start of the test
• *Preliminary test*
• *Test duration:* 21 days
• *Test design (e.g. test concentrations, number of controls, number of replicates, number of animals, etc.)*

Daphnids were exposed during 21 days to five test concentrations of Thixatrol Max and to a dilution water control under semi-static conditions. The time-weighted (TW) mean measured test substance concentrations were 0.025, 0.071, 0.24, 0.90 and 2.5 mg/L. Ten daphnids per treatment were held individually in 150 ml glass vessels with 100 ml of the test solution. The test vessels were covered with plastic lid and maintained at 20 °C with a photoperiod of 16 hours light and 8 hours of darkness. Dechlorinated tap water (total hardness approx. 108-160 mg/L as CaCO3) was used as test medium. The test solutions were renewed three times per week (on days 0, 2, 5, 7, 9, 12, 14, 16 and 19). The number of live and dead adult Daphnia, young daphnids (live and dead) and unhatched eggs were determined daily. Also the number of Daphnia with eggs or young in the brood pouch was determined daily and observations were made on the general condition and size of the adults. At the end of the test the lengths of the surviving adults were measured. The Daphnia were fed daily with an algal suspension.

The results from control and each treatment group were compared using one way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control. Furthermore, the mortality data was statistically analysed by using the corrected chi-squared statistics (Breslow and Day, 1980).

Results:

In the robust study summary it is stated that the temperature was maintained at approximately 20 °C and that there were no treatment related differences in oxygen concentration and pH. However, the actual pH and oxygen concentrations are not reported.

Immobilisation of the parent Daphnia was statistically significantly different ($p < 0.05$) in the 2.5 mg/L test group compared to the control group. Immobilisation occurred also in the 0.90 (on days 6, 15 and 19) and 0.071 (on day 4) mg/L groups but they were not significantly different ($p > 0.05$) from the control group.

Also the number of live young produced per adult (which did not die accidentally or inadvertently during the test) after 21 days was significantly lower in the 2.5 mg/L group when compared to the control group. The other treatment groups did not differ statistically significantly from the control.

There was also a statistically significant different in the size of the surviving adults at the end of the test in the 2.5 mg/L compared to the surviving adults in the control group.

Hence, a 21d-NOEC of 0.90 mg/L (based on TW mean measured concentration) is reported for immobilisation, reproduction and growth.

In the control group, one offspring was observed on day 7 and on day 8 several more offspring were observed. The time to first brood was 8 days in the treatment groups 0.025, 0.071, 0.24 and 0.90 mg/L and 11 days in the highest test concentration (2.5 mg/L).

It is also indicated that the colouration of the surviving daphnids in the 2.5 mg/L treatment group was paler than that of the daphnids in the control group and in the other treatment groups.

TW mean measured concentrations [mg/L]	Mortality on day 21 [%]	Total number of live young	Number of live young produced per adult after 21 days
Control	10	646	72
0.025	0	674	67
0.071	10	589	65
0.24	0	680	68
0.90	30	431	52
2.5	70	67	9

4.4.5 Chronic toxicity to algae or aquatic plants

See short-term toxicity.

4.5 Acute and/or chronic toxicity to other aquatic organisms

Study 1: The Acute Toxicity of EA2525 to Sediment Re-Worker Corophium volutator

Study reference:

Hyder Environmental Laboratories (1998b)

Detailed study summary and results:

Thixatrol Plus was used as test material. There is no further information on the purity, concentrations of the different constituents and/or impurities.

A Corophium volutator sediment reworker test was performed on the test substance following the PARCOM Guidence 190.5 and GLP. Adult Corophium were exposed to natural sediment spiked with the test substance for 10 days. Test concentrations up to 10,000 mg/kg dry weight sediment were used. The 10-day LC50 value was determined to be >10000 mg/kg dry weight of sediment, with a slight indication of a concentration response at the tested range. The 10-d NOEC was determined to be 1000 mg/kg dry weight of sediment. None of the concentrations tested induced 100% mortality. There is no further information on the test conditions results.