CLH report

Proposal for Harmonised Classification and Labelling

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

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

Contact details for dossier submitter:
Spanish Ministry for the Ecological Transition
Plaza de San Juan de la Cruz, s/n
28071-Madrid. Spain

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CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	**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]**
Other names (usual name, trade name, abbreviation)	Thixatrol Plus
ISO common name (if available and appropriate)	*N/A*
EC number (if available and appropriate)	430-050-2
EC name (if available and appropriate)	**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]**
CAS number (if available)	-
Other identity code (if available)	-
Molecular formula	Not applicable as the substance is a multi-constituent substance
Structural formula	Not applicable as the substance is a multi-constituent substance
SMILES notation (if available)	Not applicable as the substance is a multi-constituent substance
Molecular weight or molecular weight range	368-625 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*N/A*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*N/A*
Degree of purity (%) (if relevant for the entry in Annex VI)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
1-[2-(decanoylamino)ethylamino]-1-decanone (EC: -, CAS 51139-08-3)	See confidential annex	-	-
1-[2-(decanoylamino)ethylamino]-12-hydroxy-1-octadecanone (EC: -, CAS 146781-64-8)	See confidential annex	-	-
12-hydroxy-1-[2-(12-hydroxyoctadecanoylamino)ethylamino]1-octadecanone (EC 204-613-6, CAS 123-26-2)	See confidential annex	-	Skin Sens. 1B Skin Irrit. 2 Eye Irrit. 2 STOT SE 3 Aq. Chronic 3 Aq. Chronic 4

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No relevant impurities				

Some of the registrants of the substance have reported impurities. These have been taken into consideration and are not considered to affect the classification proposed in this dossier. Further information on the impurities is considered to be confidential. See confidential annex.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: For substance with an existing entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-127-00-5	reaction mass of: *N,N'*-Ethane-1,2-diylbis(decanamide); 12-Hydroxy-*N*-[2-[1-oxydecyl)amino]ethyl]octadecanamide; *N,N'*-Ethane-1,2-diylbis(12-hydroxyoctadecanamide)	430-050-2	-	Skin Sens. 1 Aquatic Chronic 2	H317 H411	GHS09 GHS07 Wng	H317 H411			
Dossier submitters proposal	616-127-00-5	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]	430-050-2 [1] - [2]	- [1] - [2]	**Add** Aquatic Acute 1 **Modify** Aquatic Chronic 1	**Add** H400 **Modify** H410	**Retain** GHS09 Wng	**Modify** H410		**Add** M-factor acute=100 M-factor chronic=10	
Resulting Annex VI entry if agreed by RAC and COM	616-127-00-5	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]	430-050-2 [1]	- [1]	**Skin Sens. 1** **Aquatic Acute 1** **Aquatic Chronic 1**	**H317** **H400** **H410**	**GHS07** **GHS09** **Wng**	**H317** **H410**		**M=100** **M=10**	

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

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	*hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/irritation	*hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*hazard class not assessed in this dossier*	No
Respiratory sensitisation	*hazard class not assessed in this dossier*	No
Skin sensitisation	*hazard class not assessed in this dossier*	No
Germ cell mutagenicity	*hazard class not assessed in this dossier*	No
Carcinogenicity	*hazard class not assessed in this dossier*	No
Reproductive toxicity	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	*hazard class not assessed in this dossier*	No
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	Hazard classification proposed	Yes
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The harmonised classification of Thixatrol Plus as R43 and N;R51-53 was agreed under the Dangerous Substances Directive 67/548/EEC (DSD) and was included in the Annex I of DSD. The harmonised classification was translated to the CLP Classification as Skin Sensitisation 1: H317, and Aquatic Chronic 2: H411, and included in the Annex VI of CLP.

This proposal aims to update the current environmental classification by including Aquatic Acute 1 (M-factor 100) and changing Aquatic Chronic 2 to Aquatic Chronic 1 (M-factor 10).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Change in existing entry due to new data
Change in existing entry due to changes in the criteria

5 IDENTIFIED USES

Thixatrol Plus is used as a rheological additive in coating products, fillers, putties, plasters, modelling clay, finger paints and adhesives and sealants. Uses at industrial sites, by professional workers and by consumers as well as article service-life are registered under the REACH Regulation ((EC) No 1907/2006).

6 DATA SOURCES

Registration dossiers submitted for the substance under the REACH Regulation ((EC) No 1907/2006).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid (powder), off-white		
Melting/freezing point	>= 122.6 - <= 126.1 °C	Zeneca Specialties, 1997	EU Method A.1 (Capillary method)
Boiling point	ca. 352 °C at 101.3 kPa	Zeneca Specialties, 1997	EU Method A.2 (Differential Scanning Calorimetry.)
Relative density	1.04 at 20 °C	Zeneca Specialties, 1997	EU Method A.3 (Pycnometer method)
Vapour pressure	< 0 Pa at 25 °C < 3.5 x 10^{-9} Pa at 25 °C	Zeneca Specialties, 1997	EU Method A.4, effusion method by loss of weight,estimated value EPISuite MPBPVP (v1.43) QSAR model (using smiles of the three main constituents)
Surface tension	51.9 mN/m at 23 °C	Zeneca Specialties, 1997	EU Method A.5 (Plate method)
Water solubility	< 0.034 mg/L at 22 °C	Zeneca Specialties, 1997	EU Method A.6 (flask method)

Property	Value	Reference	Comment (e.g. measured or estimated)
Partition coefficient n-octanol/water	5.4 - 6.6 at 25 °C 6.12-11.31	Zeneca Specialties, 1997	EU Method A.8 (HPLC method) EPISuite KOWWIN (v1.68) QSAR model (using smiles of the three main constituents)
Flash point	Not applicable		
Flammability	Not flammable	Zeneca Specialties, 1997	EU Method A.10
Explosive properties	Based on the chemical structures of the components of the substance, the result for the explosive properties has been predicted negative.		
Self-ignition temperature	The substance did not ignite below its melting point range of 122.6 - 126.1°C.	Zeneca Specialties, 1997	EU Method A.16
Oxidising properties	Based on the chemical structures of the components of the substance, the result for the oxidising properties has been predicted negative.		
Granulometry	D50 4.13 - 596 µm	Zeneca, 1998	Air elutriation method/ Laser diffraction method
Stability in organic solvents and identity of relevant degradation products	Solubility in n-octanol 3290 mg/L at 25 °C		EU Method A.8 (estimation method)
Dissociation constant	No data		
Viscosity	Not applicable		

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not relevant for the classification proposal in this dossier.

10 EVALUATION OF HEALTH HAZARDS

Not assessed in this dossier. No public consultation proposed.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 8: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Test type: ready biodegradability OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test) GLP	Readily biodegradable (not meeting 10d window) % Degradation of test substance: 69.3 after 28 d (CO_2 evolution)	1 (reliable without restriction) experimental result **Test material (EC name): Thixatrol Plus (purity 96.9 %)**	Chemex International plc, 1998 (Study summary included in the REACH registration dossier)

11.1.1 Ready biodegradability

A ready biodegradation screening test according to OECD 301B is available for Thixatrol Plus. The test substance and inorganic nutrient medium were inoculated with activated sewage sludge (concentration of suspended solids 30 mg/L) and incubated for up to 28 days at 23 °C. 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, and hence, is within the range of 10-20 mg C/L indicated in the OECD guideline. 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 very 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 reference substance, sodium acetate, reached 66.9 % degradation after 14 days and the mean blank CO_2 evolution was 19.9 mg/L.

It is noted that Thixatrol Plus is a multiconstituent substance consisting of three main constituents and the degradation of different constituents may differ. Ready biodegradability tests are intended for pure substances and are generally not applicable for complex compositions containing different types of constituents. However, the OECD "Guidelines for the Testing of Chemicals, Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3 Part I: Principles and Strategies Related to the Testing of Degradation of Organic Chemicals" (OECD, 2006) indicates that "*it is sometimes relevant to examine the ready biodegradability of mixtures of structurally similar chemicals".* Still "*a case by case evaluation should however take place on whether a biodegradability test on such a complex mixture would give valuable information regarding the biodegradability of the mixture as such (i.e. regarding the degradability of all the constituents) or whether instead an investigation of the degradability of carefully selected individual components of the mixture is required*". The OECD document and the ECHA Guidance on the Application of CLP criteria (Annex II, Version 5.0, July 2017) also state that the 10-day window need not be applied if the test is carried out on a mixture of structurally similar constituents and if it is anticipated that a sequential biodegradation of the individual constituents is taking place. This applies to multi-constituent and certain UVCB substances (such as oils and surfactants) consisting of structural similar constituents with different chain-lengths, degree and/or site of branching or stereo-isomers, even in their most purified commercial forms.

The main constituents of Thixatrol Plus are structurally similar; they all have two amide groups connected by an ethyl group and two linear alkyl sidechains. The alkyl sidechains are either C10 or C18, the latter having an hydroxyl group. The constituent with two short sidechains (referred to as constituent A in the this dossier)

is more watersoluble and hence more bioavailable to the microorganisms than the other two constituents that have either one short and one long sidechain (constituent B in this dossier) or two long sidechains (constituent C in this dossier). Therefore, a sequential degradation of the constituents can be expected, and as a consequence, the 10 day window criteria do not need to be applied.

It is also noted that according to ECHA Guidance R.7b (ECHA, 2017b), the pass levels for ready biodegradability tests relate to measured sum parameters for DOC depletion, oxygen use or CO2 production and imply total degradation (assume that 30-40 % of the organic carbon of the test substance is either assimilated by the microbial biomass for growth or present as products of biosynthesis). Therefore, as the substance reached 69 % degradation, it can be assumed that not much of the substance remained after 28 days. There is no information on the proportions of the three constituents in the test material, but according to the registration information on typical concentrations, all the constituents are present at a significant concentration (above 10 %) and the most abundant constituent is the constituent B followed by the constituent C. Consequently, since almost complete degradation of the entire substance was observed in the ready biodegradation test, and considering that the constituents are structurally relatively similar (they differ in the length of the linear alkyl chains), it can be assumed that all three main constituents have degraded either almost completely or at least to a significant extent.

EPISuite BIOWIN v4.10 models were performed for the main constituents of the substance as supporting information (see Table 9). 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 $\geq$ 2.25 - <2.75 predicts ultimate biodegradation in “weeks to months” and a score $\geq$ 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.

Table 9 Episuite Biowin V4.10 Models For The Main Constituents

Constituent	BIOWIN model				
	1	2	3	5	6
1-[2-(decanoylamino)ethylamino]-1-decanone (constituent A)	1.2092	0.9989	2.8729	0.7591	0.8063
1-[2-(decanoylamino)ethylamino]-12-hydroxy-1-octadecanone (constituent B)	1.3069	0.9979	2.7495	0.8340	0.8369
12-hydroxy-1-[2-(12-hydroxyoctadecanoylamino)ethylamino]1-octadecanone (constituent C)	1.4046	0.9957	2.6261	0.9090	0.8635

11.1.2 BOD_5/COD

No relevant data available.

11.1.3 Hydrolysis

No relevant data available.

11.1.4 Other convincing scientific evidence

No relevant data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No relevant data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No relevant data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

No relevant data available.

11.1.4.4 Photochemical degradation

No relevant data available.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.3 Environmental fate and other relevant information

Not relevant.

11.4 Bioaccumulation

11.4.1 Estimated bioaccumulation

EPISuite KOWWIN (v1.68) QSAR model predicts log Kow values of 6.12, 8.51 and 11.31 for the three main constituents of Thixatrol Plus.

11.4.2 Measured partition coefficient and bioaccumulation test data

There is no experimental information on the bioaccumulation of the Thixatrol Plus or of the similar substances.

The log Kow values of the constituents measured using the HPLC method are in the range of 5.4-6.6. There is uncertainty in the measured values because the HPLC method is applicable only for log Kow values up to 6 and the log Kow values of the constituents predicted by the KOWWIN QSAR model are in the range of 6.12-11.31.

In conclusion, since there is no experimental data on bioaccumulation and the measured and predicted log Kow values of all main constituents are above 4, Thixatrol Plus is considered to have a high bioaccumulation potential for classification purposes.

11.5 Acute aquatic hazard

Table 10: Summary of relevant information on acute aquatic toxicity. As the name of the substance is quite long, 'Thixatrol Plus' has been used instead in the document. Information on substance purity was not available.

Method	Species	Test material	Results	Remarks	Reference
OECD Guideline 203 (Fish, Acute Toxicity Test) EU Method C.1 (Acute Toxicity for Fish) GLP freshwater static	*Rainbow trout (Oncorhynchus mykiss)*	**Thixatrol Plus** Exposure to a water accommodated fraction (WAF)	LL50 (96 h): > 1000 mg/l loading rate test mat. (nominal) based on: mortality NOELR (96 h): 1000 mg/l loading rate test mat. (nominal) based on: mortality	1 (reliable without restriction)	Chemex International Plc (1998b) (Study summary included in the REACH registration dossier)
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) EU Method C.2 (Acute Toxicity for Daphnia) GLP freshwater static	*Daphnia magna*	**Thixatrol Plus**	EL50 (48 h): 15.63 — 250 mg/L test mat. (nominal) based on: immobilisation	1 (reliable without restriction)	Chemex International Plc (1998c) (Study summary included in the REACH registration dossier)
OECD Guideline 201 (algal growth inhibition) EU Method C.3 (Algal Inhibition test) GLP freshwater static	*Chlorella vulgaris*	**Thixatrol Plus**	NOEC (72 h): 25.6 mg/L based on: growth rate (Freshwater study on Chlorella vulgaris. No ErC50 could not be calculated as the dissolved concentration of test substance was not determined.)	1 (reliable without restriction)	Chemex International Plc (1998d) (Study summary included in the REACH registration dossier)
OECD 201 (1984) (algal growth inhibition) EU Method C.3 (Algal Inhibition test)	*Chlorella vulgaris*	**Thixatrol Plus**	EL50 (72 h): > 1000 loading rate WAF test mat. (nominal) based on: growth rate and biomass	1 (reliable without restriction)	Chemex International Plc (1998e) (Study summary included in the REACH registration dossier)

GLP freshwater static					
ISO 10253 (Water quality - Marine Algal Growth Inhibition Test with Skeletonema costatum and Phaeodactylum tricornutum) GLP saltwater static	*Skeletonema costatum*	**Thixatrol Plus**	ErC50 (48 h): 0.0012 mg/L (95% CL of 0.0011-0.0013 mg/L) (meas.) based on: growth rate ErC10 (48 h): 0.00087 mg/L (95% CL of 0.00068-0.0010 mg/L) (meas.) based on: growth rate NOErC (48 h): 0.000359 mg/L (meas.) based on: growth rate	1 (reliable without restriction) **Key study**	Harlan Laboratories Ltd (2011) (Study summary and full study report included in the REACH registration dossier)
ISO 10253 (Water quality - Marine Algal Growth Inhibition Test with Skeletonema costatum and Phaeodactylum tricornutum) saltwater static	*Skeletonema costatum*	**Thixatrol Plus**	EC50 (72 h): 4.08 mg/L loading rate, water accomodated fraction (nominal) based on: growth rate		Hyder Environmental Laboratories (1998a) (Study summary included in the REACH registration dossier)
short-term toxicity PARCOM 190.5 GLP saltwater static	*Corophium volutator*	**Thixatrol Plus**	NOEC (10 d): 1000 mg/kg sediment dw test mat. (nominal) based on: mortality LC50 (10 d): > 10000 mg/kg sediment dw test mat. (nominal) based on: mortality	1 (reliable without restriction)	Hyder Environmental Laboratories (1998b) (Study summary included in the REACH registration dossier)

11.5.1 Acute (short-term) toxicity to fish

One acute study following OECD 203 is available for Thixatrol Plus. Rainbow trout were exposed to a water accommodated fraction (WAF) at a loading rate of 1000 mg/L during 96 hours. No mortality or other adverse effects were observed. Therefore the reported 96h LL50 is >1000 mg/L. It is noted that the loading rate is well above the water solubility limit of the constituents of the substance, there is no information on the measured concentrations or on the method used for the preparation of the WAFs. Therefore, it is not possible to confirm that the fish were actually exposed to the test substance, and hence, the study is considered not reliable.

Due to the low solubility of the substance, long-term testing is considered more relevant for the substance. However, no long-term tests with fish are available for the substance.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

In an acute study performed according to OECD 202, *Daphnia magna* were exposed to the registered substance for a period of 48 hours. In the study summary, immobilisation is reported for all the concentrations tested but it is not stated what the test concentrations were. It is stated that the immobilisation did not follow a clear concentration response and that it may have been caused by physical effects due to undissolved substance particles. 50% immobilisation was observed at 31.25 mg/l and 40% immobilisation at 62.5 mg/l. According to the registrants, the 48-hr EC50 value could not be calculated with any degree of confidence but is thought to lie between 15.63 and 250 mg/l based on nominal concentrations. As the nominal test concentrations were well above the water solubility of the substance, there is no further information on the measured test concentrations and test conditions, and some of the effects may have been caused by undissolved test material, the study is not considered reliable.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Four studies on algae are available for the substance; two marine algal growth inhibition tests with *Skeletonema costatum* performed according to ISO 10253 and two freshwater algae tests following OECD TG 201. In the key study, *Skeletonema costatum* was exposed to five concentrations of the test substance in aqueous solution and to a dilution water control at 20°C under static conditions for 72 hours. The saturated solution method was used to prepare the test solution 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). 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). The test included three replicate vessels for each treatment group and six vessels for the control group. 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.

The measured test concentrations ranged from 15 to 124 % of the nominals at 0 hours and from 18 to 227 % after 72 hours. There was significant and variable interference seen around the test samples´ peaks in the chromatogram analyses. 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. Therefore, the registrant considered that the analytical method used was not applicable for the test substance, and they reported the results based on nominal concentrations.

However, 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). Furthermore, it is not clear why the controls gave a positive response in the definitive 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 average growth rates were in the range of 1.73-1.81 day^{-1} during the 72 hour study period . 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 for the 72 hour study period. These criteria were also met after 48 hours of exposure. However, it is noted that constant exponential growth occurred only up to 48 hours exposure in the controls and at 72h exposure the growth had slowed down. Hence, 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 of exposure but not for the whole 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 valid and recalculated the results. 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 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. Therefore, the mean measured concentrations as explained above were used by the dossier submitter to calculate the results at 48 hours of exposure. This resulted in a 48h-ErC50 of 0.0012 mg/L (95% CI of 0.0011-0.0013 mg/L) for inhibition of growth rate (based on the mean measured concentrations).

In another marine algal study with *Skekeletonema costatum*, 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. There is very little information on the study available, 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.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

A *Corophium volutator* sediment reworker test was performed on the test substance following the PARCOM Guidence 190.5. Adult Corophium were exposed to 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.

11.6 Long-term aquatic hazard

Table 11: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD Guideline 211 (Daphnia magna	*Daphnia magna*	**Reaction mass of N, N'-ethane1,2-diylbis(hexanamide) and 12-hydroxy-N-[2-[(1-oxyhexyl)amino]ethyl]octadecanamide**	mat. (meas. (TWA)) based on: immobilisation	2 (reliable with restrictions)	Harlan Laboratories Ltd, 2009 (Study

Reproduction Test) EU Method C.20 (Daphnia magna Reproduction Test) GLP freshwater semi-static		**and N, N'-ethane-1,2-diylbis(12-hydroxyoctadecan amide) (EC 432-430-3)**	NOEC (21 d): 0.9 mg/L test mat. (meas. (TWA)) based on: reproduction LOEC (21 d): 2.5 mg/L test mat. (meas. (TWA)) based on: immobilisation LOEC (21 d): 2.5 mg/L test mat. (meas. (TWA)) based on: reproduction	Read-across from supporting substance (structural analogue or surrogate)	summary included in the REACH registration dossier)

11.6.1 Chronic toxicity to fish

No relevant data available.

11.6.2 Chronic toxicity to aquatic invertebrates

No long-term studies are available for Thixatrol Plus but a semi-static Daphnia Reproduction study according to OECD 211 is available for the structurally similar substance Thixatrol Max (EC No. 432-430-3). Daphnids (10 individuals per treatment, held individually) were exposed during 21 days to five test concentrations of Thixatrol Max and to a dilution water control under semi-static conditions. The saturated solution method was used to prepare the test solution 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 100 ml in order to pre-condition the filter). The time weighted mean measured test substance concentrations were 0.025, 0.071, 0.24, 0.90 and 2.5 mg/L. The number of live and dead adult Daphnia, young daphnids (live and dead) and unhatched eggs were determined daily. Also observations on the general condition and size of the adults were made daily. At the end of the test the lengths of the surviving adults were measured. A 21d-NOEC of 0.90 mg/L is reported for reproduction (mean number of live offspring produced per adult), immobilisation and length based on time-weighted mean measured concentration.

The validity criteria of the OECD TG 211 regarding parent mortality (≤ 20%) and the mean number of living offspring produced per parent animal surviving at the end of the test (> 60) were met. It is noted that according to the guideline, the same validity criterion for mortality (20%) can be used for accidental and inadvertent parental mortality for each of the test concentrations. In the second highest test concentration (0.90 mg/L) 30% mortality occurred but according to the study information, this was not statistically significantly different from the control. However, even if the mortalities in this groups are considered accidental and inadvertent, they are above the validity criterion of 20%.

On the other hand, as 30% mortality occurred in the test concentration 0.90 mg/L (which was the second highest concentration) and 70% mortality in the highest concentration, it could be considered that the mortality follows a dose-response at the two highest concentrations. In this case the NOEC for mortality could be the next lowest concentration (0.24 mg/L). If the mortalities in the 0.90 mg/L treatment are considered to be caused by the test substance, the NOEC for reproduction could also result in a lower value than the one reported in the study. This is because the NOEC for reproduction reported in the study is calculated by omitting from the analysis the adults that died during the study (and their offspring) in the 0.90 mg/L treatment. However, if the mortalities in this concentration are not considered accidental or inadvertent, the individuals that died and their offspring should be included in the analysis when calculating the mean number of offspring per adult. However, since raw data on the number of offspring per adult is not available, it is not possible to re-calculate the results.

Thixatrol Plus is similar with the substance Thixatrol Max. Both substances have three main constituents out of which one (EC 204-613-6) is common for both substances and the other main constituents differ only in the length of the shorter alkyl sidechain attached to the amide group(s). In Thixatrol Plus the shorter chain is C10 and in Thixatrol Max it is C6. The constituents of Thixatrol Plus with longer alkyl chains are expected to be less water soluble and to have higher log Kow values than the constituents of Thixatrol Max with shorter sidechains. This could lead to some differences in the toxicity of the two substances to daphnia. However, since the available chronic toxicity value of Thixatrol Plus for algae (ErC10 of 0.00087 mg/L, see next section) is three orders of magnitude lower than the reported NOEC of Thixatrol Max for daphnia (0.9 mg/L, or potentially 0.24 mg/L, see the paragraph above), it is expected that daphnia are not more sensitive to Thixatrol Plus than the algae.

11.6.3 Chronic toxicity to algae or other aquatic plants

In the key study following ISO 10253, a 48h- ErC10 of 0.00087 mg/L (95% CL of 0.00068-0.0010 mg/L) and 48h-NOErC of 0.000359 mg/L (based on mean measured concentrations) are determined for the marine alga *Skeletonema costatum*. See section 11.5.3 for more information on the study.

According to the current ECHA Guidance on the application of the CLP criteria (Version 5.0, July 2017), when EC10 values are available these are preferred over NOEC values in chronic toxicity studies. This applies in cases where EC10 and NOEC values are available for the same endpoint. EC10 values are considered more appropriate for aquatic chronic classification because NOEC values strongly depend on the experimental design (number of doses, width of the inter-dose interval, etc.), whereas EC10 values are derived from the whole concentration-response curve. Therefore, the 48h- ErC10 of 0.00087 mg/L is used for the classification.

11.6.4 Chronic toxicity to other aquatic organisms

No relevant data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

No reliable acute studies with Thixatrol plus are available for fish and aquatic invertebrates. One valid acute study with the marine alga *Skeletonema costatum* is available and it resulted in a 48-h ErC50 of 0.0012 mg/L (mean measured concentration). In an acute study with the sediment dwelling organism *Corophium volutator* a 10-day LC50 value of >10000 mg/kg dry weight of sediment was determined.

In conclusion, the lowest available acute value is the 48-h ErC50 of 0.0012 mg/L for *Skeletonema costatum* which is below the classification threshold of 1 mg/L for Aquatic Acute 1 and in the range of $0.001 < L(E)C50 \leq 0.01$ mg/L leading to an acute M-factor of 100.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The degradation of Thixatrol Plus was 69.3 % after 28 days (based on CO_2 evolution) in an OECD TG 301B study. The degradation did not meet the 10 day window criteria. However, since Thixatrol Plus is a multiconstituent substance consisting of structurally similar constituents and it can be anticipated that a sequential biodegradation of the individual constituents takes place, the 10 day windows criteria does not need to be applied. BIOWIN QSAR models performed for the main constituents support the conclusion of rapid degradability of the substance. In conclusion, Thixatrol Plus is considered to be rapidly degradable for classification purposes.

No experimental information on the bioaccumulation of Thixatrol Plus is available. The measured and estimated log Kow values of the three main constituents are above the cut-off value of 4 indicated in the CLP. Therefore, the substance is considered to have bioaccumulation potential for classification purposes.

No chronic toxicity data for Thixatrol plus is available for fish and aquatic invertebrates. For the marine alga *Skeletonema costatum* a 48h- ErC10 of 0.00087 mg/L (based on mean measured concentration) is available. This is below the classification threshold of 0.01 mg/L for Aquatic Chronic 1 for rapidly degradable substances and in the range of $0.0001 < NOEC \leq 0.001$ mg/L justifying a chronic M-factor of 10.

A *Daphnia magna* Reproduction study with the similar substance Thixatrol Max is available. Two of the main constituents of Thixatrol Plus are expected to be less water soluble and to have higher log Kow values than two of the main constituents of Thixatrol Max with shorter sidechains. This could lead to some differences in the toxicity of the two substances to daphnia, and therefore, the study is not fully adequate for classification of Thixatrol Plus. However, it can be used as supporting information. As the available chronic toxicity value of Thixatrol Plus for algae (ErC10 of 0.00087 mg/L) is three orders of magnitude lower than the reported NOEC of Thixatrol Max for daphnia (0.9 mg/L, or potentially 0.24 mg/L, see the section 11.6.2) it is expected that daphnia are not more sensitive to Thixatrol Plus than the algae.

Since (fully) adequate chronic data is not available for fish and aquatic invertebrates, the surrogate approach (Figure 4.1.1 and Table 4.1.0 in Annex I of CLP) should also be applied in the chronic classification, and the most stringent outcome should be selected. However, valid acute data is not available for fish and aquatic invertebrates either, and hence, it is not possible to apply the surrogate approach for these trophic levels.

As no reliable information on fish is available, some uncertainty remains. However, this only affects the M-factor as the substance already receives the most stringent category for aquatic chronic classification based on the available algae data.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1, H400, M-factor 100

Aquatic Chronic 1, H410, M-factor 10

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier. No public consultation proposed.

13 ADDITIONAL LABELLING

Not relevant for this dossier.

14 REFERENCES

15 ANNEXES

Confidential Annex