SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

A mixture 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)

EC No 430-050-2

CAS No -

Evaluating Member State(s): Spain

Dated: 31 May 2019

Template Version 2.1
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Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2017

The evaluating Member State concluded the evaluation without any further need to request further information from the registrants under Article 46(1).

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

A mixture 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) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Wide dispersive use
- Exposure of environment

During the evaluation no other concerns were identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

No other other process / EU Legislation are applicable to the substance or its constituents.

3. CONCLUSION OF SUBSTANCE EVALUATION

The above concerns identified for substance evaluation were analyzed. The evaluation of the available information on the substance has led the evaluating Member State to conclude that a) no PBT properties have been identified regarding the whole substance nor its constituents and b) no risk has been estimated for all environmental compartments based on the information provided by the registrants.

Conclusions are summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

The concern *Suspected PBT/vPvB* could be removed based on the performed PBT assessment of the constituents of the substance concluding "no-Persistency" based on: ready biodegradation screening test with the substance, QSAR predictions with the constituents and read-across approach from other substances with structural similarity (see chapter 8 – PBT assessment for additional information).

The concern *Wide dispersive use and environmental exposure* could be removed based on the performed exposure assessment concluding no risk for the environmental compartments (see Chapter 9 and 10 for additional information).

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Thixatrol Plus has a harmonised classification for aquatic hazards as Aquatic Chronic 2 in the Annex VI to CLP (Index number 616-127-00-5). However, the available aquatic toxicity data justifies a more stringent classification.

The lowest available LC/EC50 value is the 72h ErC50 of 0.0054 mg/L determined for the marine algae *Skeletonema costatum.* This results in an acute classification as Aquatic Acute 1 with M-factor of 100. It is noted that there is no reliable acute data on fish or aquatic invertebrates. The substance receives the most stringent classification category for acute aquatic hazards based on the available acute data for algae. The M-factor could potentially be affected if further acute data on fish or aquatic invertebrates was available. However, due to the low solubility of the substance, potential long-term effects are expected to be more relevant for these organisms.

Chronic data for Thixatrol Plus is only available on algae. In addition, chronic data on *Daphnia magna* is available for the similar substance Thixatrol Max. Since chronic data is not available for all three trophic levels, the substance should be classified for chronic hazards according to both Tables 4.1.0. (b) (i) or (ii) and 4.1.0. (b) (iii) of CLP and the most stringent outcome is selected.

The substance is considered rapidly degradable for classification purposes as the pass level was reached after 28 days in the OECD 301 B test with the substance. The 10 days window criteria was not met but according to CLP this is not required in case of complex multiconstituent substances consisting of structurally similar constituents, which is considered to be the case of Thixatrol Plus. The lowest available chronic value is the 72h NOErC of 0.0029 mg/L for *Skeletonema costatum.* This justifies classification as Aquatic Chronic 1 with M-factor of 1.

As mentioned above reliable acute data is only available for algae, and hence, this is used in the surrogate approach for chronic classification. Based on the lowest acute value, the 72h ErC50 of 0.0054 mg/L, and considering the substance as bioaccumulative for classification purposes (based on log Kow > 4), classification as Aquatic Chronic 1 with M-factor of 100 is considered justified. This is the most stringent outcome and is selected for classification.

It is noted that the available aquatic data leads to a more stringent classification than the current harmonised classification of the substance. In the classification inventory we note

that 41 out of 42 notifiers out apply the current less stringent harmosided classification for the aquatic chronic toxicity[2].

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable

5.2. Other actions

No other actions are proposed at national or EU level.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Proposal for Harmonised Classification and Labelling	*May/ 2020*	*ESCA-Env*

[2] https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/96552.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

A mixture 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) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Wide dispersive use
- Exposure of environment

During the evaluation no additional concern was identified.

7.2. Procedure

The substance evaluation of A mixture 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) was initiated on 21 March 2017.

A targeted assessment of endpoints related to PBT properties was performed. Also environmental exposure information was evaluated. The evaluation included relevant information from the aggregated registration dossier of the substance, from other similar substances and literature search. The environmental exposure assessment has been performed using the EUSES default releases factors unless stated otherwise.

During the 12 month-evaluation period, full study reports and additional information (e.g. QSAR predictions) were provided by the Registrant(s) regarding the biodegradability and bioaccumulation.

Based on the evaluation of the available information, the eMSCA concluded that some uncertainty remained on the degradation of one of the constituents and it was necessary to request new data. Therefore, a draft decision was submitted to ECHA on 21 March 2018.

The Registrant(s) were invited to provide comments on the draft decision. After receiving the comments from the Registrant(s), the eMSCA re-evaluated all the available information and concluded that no concern remains on potential PBT properties of the whole substance or its constituents, and hence, the Substance Evaluation of the substance was concluded without requesting further information. The eMSCA sent to ECHA the substance evaluation conclusion and report document on 20 January 2019.

7.3. Identity of the substance

Table 3

SUBSTANCE IDENTITY	
Public name:	A mixture 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)
EC number:	430-050-2
CAS number:	-
Index number in Annex VI of the CLP Regulation:	616-127-00-5
Molecular formula:	Not applicable as the substance is multi-constituent
Molecular weight range:	
Synonyms:	THIXATROL PLUS 12-hydroxy-N-[2-(12-hydroxyoctadecanamido)ethyl]octadecanamide; N-(2-decanamidoethyl)-12-hydroxyoctadecanamide; N-(2-decanamidoethyl)decanamide A mixture 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) A reaction product of decanoic acid, 12-hydroxystearic acid and 1,2-ethandiamine in the mole ratio of 1:1:1 Diamid wax mixture~ Reaction mass of N,N'-ethane-1,2-diylbis(alkanamide), 12-hydroxy-N-[2-[1-oxyalkyl)amino]ethyl]octadecanamide and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide) Reaction mass of N,N'-ethane-1,2-diylbis(decanamide), 12-hydroxy-N-[2-[1-oxydecyl)amino]ethyl]octadecanamide and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide)

Type of substance ☐ Mono-constituent ■ Multi-constituent ☐ UVCB

Structural formula: See the information on the constituents below.

The whole multi-constituent substance is referred as Thixatrol Plus in this document.

Multi-constituent/UVCB substance/others

Thixatrol Plus is a multi-constituent substance consisting of three main constituents (see the below Table 4, Table 5, and Table 6). Information on the impurities is included in the confidential Annex. In this document the constituents are referred as Constituent A, Constituent B and Constituent C (see the below tables).

Table 4. Constituent A

CONSTITUENT A	
Public name:	N,N'-1,2-ETHANEDIYLBIS-DECANAMIDE
EC number:	-
CAS number:	51139-08-3
Index number in Annex VI of the CLP Regulation:	-
Smiles:	C(C)CCCCCCCC(NCCNC(CCCCCCCCC)=O)=O
Molecular formula:	C22 H44 N2 O2
Molecular weight range:	368.61
Synonyms:	

Structural formula:

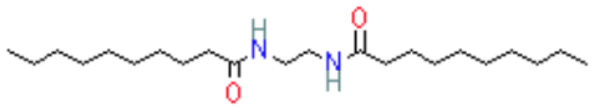


Table 5. Constituent B

CONSTITUENT B	
Public name:	12-HYDROXY-N-[2-[1-OXYDECYL)AMINO] ETHYL]OCTADECANAMIDE
EC number:	604-536-1
CAS number:	146781-64-8
Index number in Annex VI of the CLP Regulation:	-
Smiles:	CCCCCCCCCC(=O)NCCNC(=O)CCCCCCCCCCC(O)CCCCCC
Molecular formula:	C30 H60 N2 O3
Molecular weight range:	496.82
Synonyms:	

Structural formula:

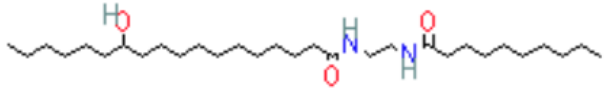
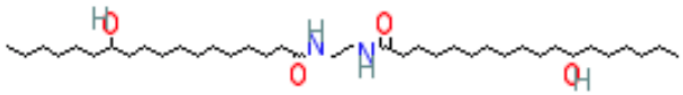


Table 6. Constituent C

CONSTITUENT C	
Public name:	N,N'-ETHANE-1,2-DIYLBIS(12-HYDROXYOCTADECANAMIDE)
EC number:	204-613-6
CAS number:	123-26-2
Index number in Annex VI of the CLP Regulation:	-
Smiles:	CCCCCCC(O)CCCCCCCCCCC(=O)NCCNC(=O)CCCCCCCCCCC(O)CCCCCC
Molecular formula:	C38 H76 N2 O4
Molecular weight range:	625.04
Synonyms:	

Structural formula:



Similar substances

There are several di-or polyamides with long linear alkylchains as sidechains. In this section only some of them are included, those which are most similar with Thixatrol Plus or its constituents. In the section on Degradation, information on further similar substances is included.

Reaction mass of Octadecanamide, 12-hydroxy-N-[2-[(1-oxodecyl)amino]ethyl]- and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecan-1-amide) and Decanamide, N,N'-1,2-ethanediylbis- (EC 907-495-0) has the same main constituents (A, B and C in the above tables) as Thixatrol Plus but the concentration ranges are slightly different.

Table 7

SIMILAR SUBSTANCE	
Public name:	Reaction mass of Octadecanamide, 12-hydroxy-N-[2-[(1-oxodecyl)amino]ethyl]- and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecan-1-amide) and Decanamide, N,N'-1,2-ethanediylbis-
EC number:	907-495-0
CAS number:	-
Index number in Annex VI of the CLP Regulation:	
Molecular formula:	Not applicable as the substance is multiconstituent
Molecular weight range:	
Synonyms:	

Structural formula: See the structural formulas of constituents A, B and C of Thixatrol Plus above.

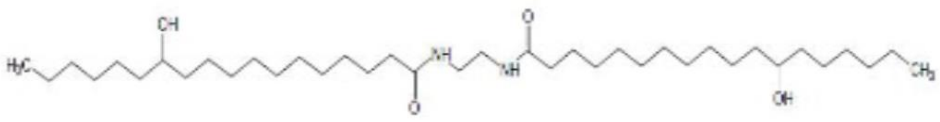
Thixatrol Plus is similar with the substance Thixatrol Max (see Table 8 below). Both substances have three main constituents out of which one (EC 204-613-6) is common for both substances and the other main constituents differ only in the length of the shorter carbon chain attached to the amide group(s). In Thixatrol Plus the shorter chain is C10 and in Thixatrol Max it is C6. According to the registration information, in the NONS procedure the UK MSCA accepted read across between the substances in some endpoints, e.g. in ready biodegradation and ecotoxicity.

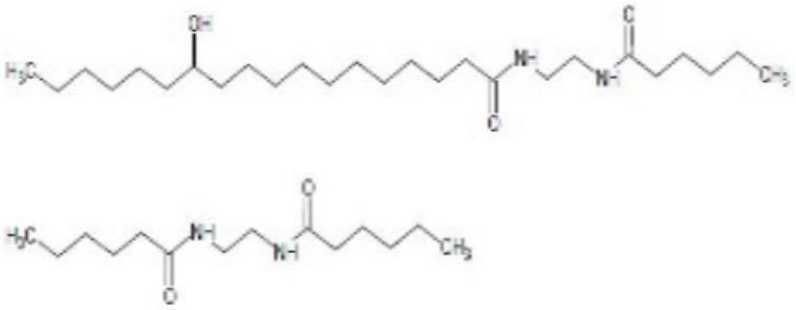
Table 8

SIMILAR SUBSTANCE	
Public name:	reaction mass of N,N'-ethane-1,2-diylbis(hexanamide) and 12-hydroxy-N-[2-[(1-oxyhexyl)amino]ethyl]octadecanamide and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecan amide)
EC number:	432-430-3
CAS number:	-
Index number in Annex VI of the CLP Regulation:	616-200-00-1
Molecular formula:	Not applicable as the substance is multiconstituent
Molecular weight range:	
Synonyms:	Thixatrol MAX 12-hydroxy-N-[2-(12-hydroxyoctadecanamido)ethyl]octadecanamide; N-(2-hexanamidoethyl)hexanamide; N-[(9R,10S)-10-acetamido-22-hydroxydocosan-9-yl]acetamide 12-hydroxy-N-[2-[(1-oxyhexyl)amino]ethyl]octadecanamide Complex mixture of diamide waxes N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide) Reaction mass of N,N'-ethane-1,2-diylbis(heanamide);12-hydroxy-N-[2-[(1-oxyhexyl)amino]ethyl]octadecanamide;N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide) reaction mass of: N,N'-ethane-1,2-diylbis(hexanamide)

Type of substance ☐ Mono-constituent ■ Multi-constituent ☐ UVCB

Structural formula:





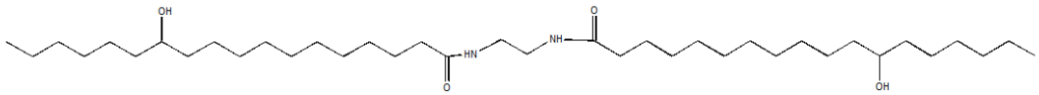
Thixatrol Plus and the UVCB substance octadecanoic acid, 12-hydroxy-, reaction products with ethylenediamine (see below Table 9) have one main constituent in common, EC 204-613-6 (the constituent C in Thixatrol Plus) and another constituent of the UVCB substance is very similar to constituent C (differing only in having two OH-groups less in the alkyl chains). The other seven constituents of the UVCB substance are bigger molecules formed through esterification reaction(s) between 12-hydroxystearic acid and the OH-group(s) in N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide) (EC 204-613-6) or in similar constituents. In the registration dossier of EC 204-613-6, read across from the UVCB substance has been used in some endpoints, e.g. in ready biodegradation.

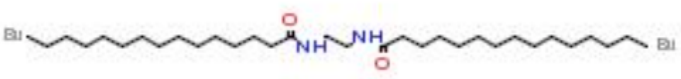
Table 9

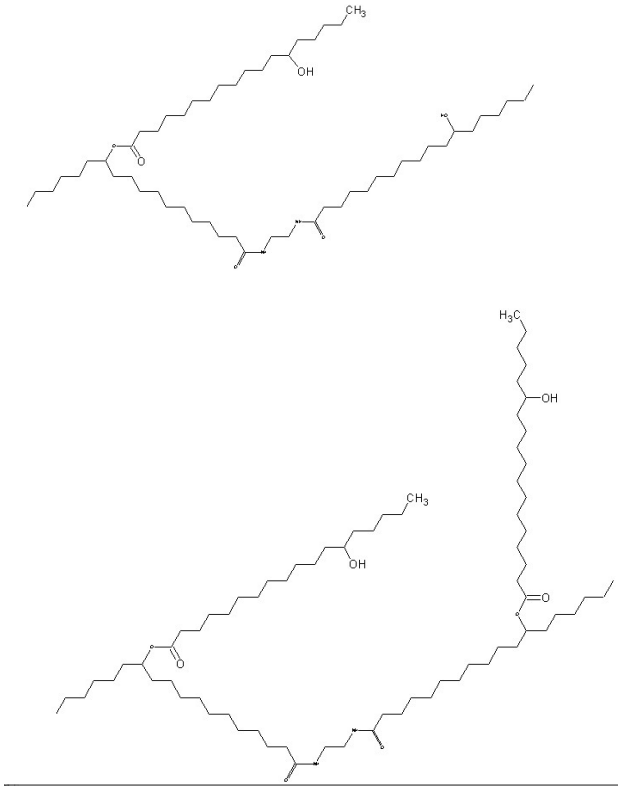
SIMILAR SUBSTANCE	
Public name:	Octadecanoic acid, 12-hydroxy-, reaction products with ethylenediamine
EC number:	309-629-8
CAS number:	100545-48-0
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	Not applicable as the substance is UVCB
Molecular weight range:	Not applicable as the substance is UVCB
Synonyms:	Reaction products of 12-hydroxyoctadecanoic acid with ethane-1,2-diamine 80005005

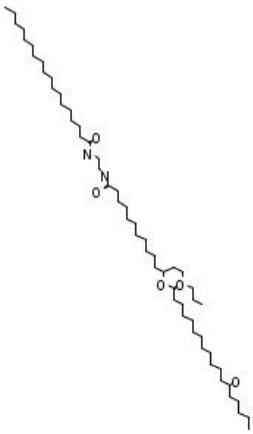
Type of substance ☐ Mono-constituent ☐ Multi-constituent ■ UVCB

Structural formula:









Additonal information provided in the confidential annex.

7.4. Physico-chemical properties

Table 10

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF THIXATROL PLUS	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Solid
Vapour pressure	< 0 Pa at 25 °C (EU Method A.4, effusion method by loss of weight,estimated value, at ECHA dissemination website) 0.000001 at 25 °C (calculated using the Clausis-Clapeyron equation)
Water solubility	< 0.034 mg/L at 22 °C (EU Method A.6, flask method)
Partition coefficient n-octanol/water (Log Kow)	5.4 - 6.6 at 25 °C (EU Method A.8, HPLC method) There is some uncertainty in these log Kow values since the HPLC method is applicable for substances with log Kow up to 6 and based on the KOWWIN QSAR model the constituents of the substance may have log Kow values above 6 (see Table 11 below).
Flammability	Non-flammable (EU Method A.10 (Flammability (Solids))
Explosive properties	nNon-explosive (based on chemical structure)
Oxidising properties	Non-oxidising (based on chemical structure)
Granulometry	The mass mean diameter is 596 µm according to the Air elutriation method Proportion of test material having a particle size less than 115 µm: 3.62 %. Proportion of test material having a particle size less than 75 µm: 1.93 %. Proportion of test material having a particle size less than 50 µm: 0.60 %. Proportion of test material having a particle size less than 35 µm: 0.13 %. Proportion of test material having a particle size less than 15 µm: 0.08 %.
Surface activity	51.9 mN/m at 23 °C (EU Method A.5) Based on the available information the substance shows some surface-active properties as the surface tension is below 60 mN/m which is the criterion for surface active substances according to this EU method. This may result in uncertainties in additional calculations, such as estimations on bioaccumulation. However, as the

	value is close to the threshold value, any effect is expected to be low.
Stability in organic solvents and identity of relevant degradation products	idem
Dissociation constant	idem

Table 11

MEASURED/PREDICTED PHYSICOCHEMICAL PROPERTIES OF THE MAIN CONSTITUENTS OF THIXATROL PLUS				
Constituent	**Water solubility (mg/L)**		**Log Kow**	
	Measured	**Predicted (WSKOW v1.42 / WATERNT v1.01)**	**Measured**	**Predicted (KOWWIN v1.68)**
A	-	0.02814 / 0.19636	5.4*	6.12
B	-	4.019e-005 / 0.00054486	6.0 *	8.51
C	0.104** <0.115***	2.409e-008 /1.4112e-006	6.6 *	11.31

* Measured in a study with Thixatrol Plus according to EU Method A.8 using HPLC method

** Measured in a study with Thixatrol Max according to EU method A.6. using shake flask method

*** Reported in the registration dossier of Component C (EC 204-613-6), according to OECD 105, using shake flask method

7.5. Manufacture and uses

7.5.1. Quantities

At the time of this assessment, there are two companies with active submissions for registration.

The substance is manufactured and/or imported in the European Economic Area, but the tonnage data is confidential.

Additional information is provided in the confidential Annex.

Table 12

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☒ Confidential

7.5.2. Overview of uses

There is no information on the uses of the substance on the ECHA dissemination site. Further information on the registered uses is included in the confidential annex of this document.

Based on information found on the internet[3] (http://www.elementis-specialties.com/esweb/webproducts.nsf/allbydocid/8885CC873FA666DE8525799C004AC223/$FILE/ELEMENTIS-THIXATROL%20PLUS.pdf), Thixatrol Plus can be used as a rheological additive in coatings, paints, adhesives, sealants and two component polyurethane systems. Typical concentrations of the substance in the above mentioned products range from 0.2% to 2.0% of the total system weight.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 13

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	International Chemical Identification	EC No	CAS No	Classification		Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)		
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	-	-

[3] Information accessed on 17 December 2018.

7.6.2. Self-classification

- In the registration(s):

Aquatic Acute 1, H400
Aquatic Chronic 1, H410, M-chronic=100

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

No additional classifications.

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

7.7.1.1.1. Hydrolysis

No relevant information available. The study has been waived as it is technically not feasible due to the low solubility of the substance.

7.7.1.1.2. Phototransformation in air

No relevant information available.

7.7.1.1.3. Phototransformation in water

No relevant information available.

7.7.1.1.4. Phototransformation in soil

No relevant information available.

7.7.1.2. Biodegradation

7.7.1.2.1. Biodegradation in water

Estimated data

According to the REACH Guidance R.11: PBT/vPvB (ECHA, 2017a) assessment, 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.

EPISuite BIOWIN v4.10 models were performed for the main constituents of the substance. The results of the QSAR models are shown in the below table.

Table 14

EPISUITE BIOWIN V4.10 MODELS FOR THE MAIN CONSTITUENT			
Constituent	**BIOWIN 2**	**BIOWIN 3**	**BIOWIN 6**
A	0.9989	2.8729	0.8063
B	0.9979	2.7495	0.8369
C	0.9957	2.6261	0.8635

The BIOWIN 2 and 6 models predict that all three constituents are readily biodegradable, and hence, the constituents do not fulfil the screening criteria for potentially P/vP based on BIOWIN models. For the constituent A, the result of the BIOWIN 3 model also indicates ready biodegradability. However, it is noted that the results of BIOWIN 3 model for the constituent B and especially for the constituent C, are borderline cases (in the range 2.25 to 2.75) as they are close to the screening criterion specified in the ECHA Guidance R.11 for this model.

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 do predict better the degradation of the constituents of Thixatrol Plus.

Degradation pathways of the constituents were predicted by using EAWAG-BBD model[4] (see the below figures). The model predicts plausible pathways for microbial degradation of chemical compounds. Predictions use biotransformation rules, based on reactions found in the EAWAG-BBD database or in the scientific literature. It is noted that according to the model none of the first transformation steps are likely. The model predicts that the degradation starts either at the end of the alkyl chains or that one of the amide groups hydrolyses to the parent carboxylic acid and amine.

[4] http://eawag-bbd.ethz.ch/predict/

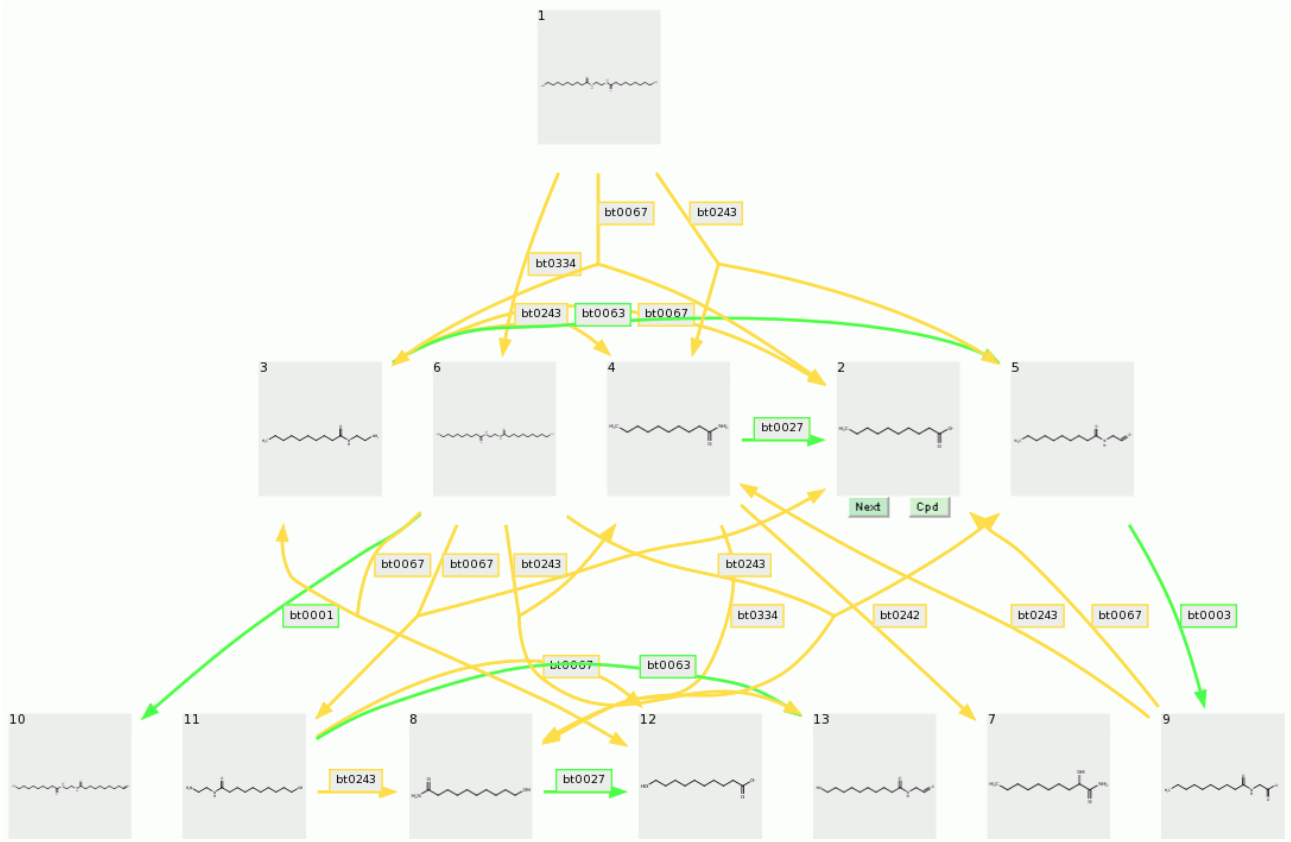


Figure 1 Predicted degradation pathway of the Constituent A using EAWAG-BBD model. Aerobic likelihood: very likely (not displayed in this case), likely and neutral. Predicted products shown in the grey squares.

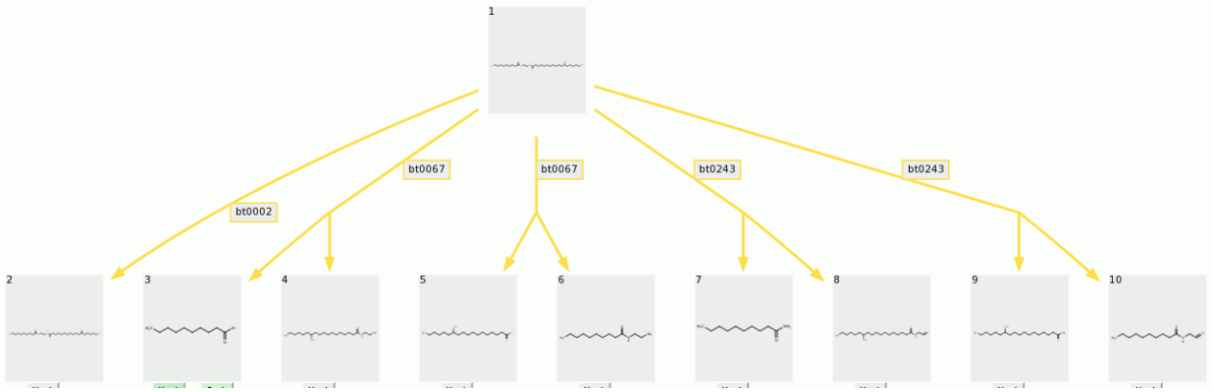


Figure 2 Predicted degradation pathway of the Constituent B using EAWAG-BBD model. Aerobic likelihood: very likely (not displayed in this case), likely (nos displayed in this case) and neutral. Predicted products shown in the grey squares.

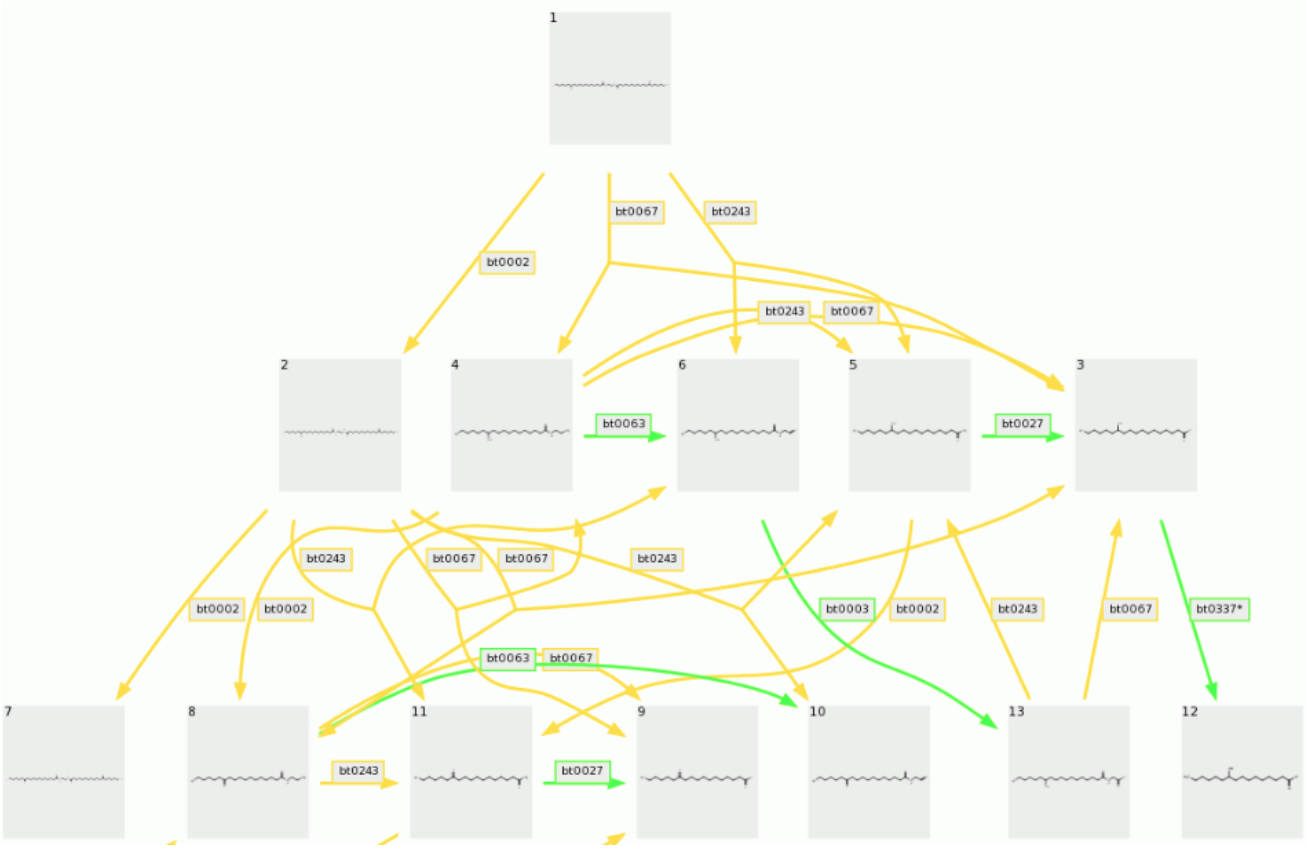


Figure 3 Predicted degradation pathway of the Constituent C using EAWAG-BBD model. Aerobic likelihood: very likely (not displayed in this case), likely and neutral. Predicted products shown in the grey squares.

Screening tests

Table 15

OVERVIEW OF AVAILABLE DEGRADATION STUDIES		
Method	**Results**	**Remarks**
Test type: ready biodegradability OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)	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): A mixture of: N,N'-ethane-1,2-diyl bis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]ethyl]octadecanamide; N,N'-ethane-1,2-diyl bis(12-hydroxyoctadecanamide)**

Test type: ready biodegradability (enhanced test) OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test)	% Degradation of test substance: 52 % after 28 days 61 % after 42 days 67% after 60 days (O_2 consumption)	2 (reliable with restrictions) read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): 907-495-0**
Test type: ready biodegradability OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test)	% Degradation of test substance: 63 % after 28 days	2 (reliable with restrictions) read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): 907-495-0**
Test type: ready biodegradability OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test) EU Method C.4-C (Determination of the "Ready" Biodegradability - Carbon Dioxide Evolution Test) EPA OPPTS 835.3110 (Ready Biodegradability)	Not readily biodegradable % Degradation of test substance: 20 after 28 d (CO2 evolution)	2 (reliable with restrictions) read-across from supporting substance (structural analogue or surrogate) **Test material (EC number): 432-430-3**
Test type: ready biodegradability OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test)	Not readily biodegradable % Degradation of test substance: 22 after 28 d 37 after 60 d	Supporting information **Test material (EC number): 309-629-8 Octadecanoic acid, 12-hydroxy-, reaction products with ethylenediamine**

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. Based on the molecular formula provided at the ECHA website ($C_{90}H_{180}N_6O_9$) equal contribution for the components A, B and C has been assumed in the composition of the substance. The degradation of the substance was determined to be 69.3 % after 28 days based on CO_2 evolution. The criteria for the 10-days window was not met. 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.

No information is available on the concentrations of the constituents in the test material. Thixatrol Plus consisting of three components, which contribute to the total carbon content with different percentages. Based on the registration information all three constituents are present at a significant concentration (above 10 %). However, the concentration ranges of the constituents are wide and therefore the composition of the substance varies. This raises uncertainities regarding the real concentration of the constituents, which have very different solubilities in the test material (component A is the most soluble, and components B and C are of very low solubility). Furthermore, information on the preparation of the test solution and on test conditions (i.e volume of bottles, pH, dispersion method used if any…) is lacking.

An OECD 301D and an enhanced OECD 301D tests are included in the registration dossier of the similar substance EC 907-495-0. This substance has the same main constituents as Thixatrol Plus but concentration ranges for the three constituents are slightly different. Also the purity of EC 907-495-0 is less than that of Thixatrol Plus, and it contains some impurities that are not reported for Thixatrol Plus. However, most of these impurities are relatively similar to the main constituents or monoesterification products of the main constituents, and hence, they are expected to have similar degradation characteristics as the main constituents. In both tests there were some deviations from the 301D guideline but these are not considered to affect the validity of the test; a) Activated sludge from a plant treating predominantly domestic wastewater was used instead of secondary effluent or surface water. The activated sludge was preconditioned to reduce the endogenous respiration rates. To precondition the sludge (approximately 400 mg/L), it was aerated for a period of approximately one week. b) Ammonium chloride was omitted from the medium to prevent nitrification, c) The contents of the bottles with silicone oil were mixed with a magnetic stirrer to improve the bioavailability. The studies were conducted using 10 bottles containing only inoculum, 10 bottles containing inoculum, silicone oil and polyalkoxylate alkylphenol, 10 bottles containing inoculum, test substance, silicone oil and polyalkoxylate alkylphenol, and 6 bottles containing sodium acetate and inoculum. The concentrations of the test substance, polyalkoxylate alkylphenol and sodium acetate in the bottles were 2.0, 2.0 and 6.7 mg/L, respectively. The silicone oil concentration was 6 mL/L. The inoculum was diluted to 2 mg DW/L in the closed bottles. Two duplicate bottles of all series were withdrawn for analyses of the dissolved oxygen concentration at day 7, 14, 21, and 28. The enhanced test was prolonged by measuring the course of the oxygen decrease in the bottles of day 28 using a special funnel at days 42 and 60. 63 % of the test substance was degraded after 28 days based on O_2 consumption in one of the test. In the enhanced test, the test substance reached 52 % degradation after 28 days, 61 % after 42 days and 67 % after 60 days based on O_2 consumption. The tests are reported to meet the validation criteria as shown by an endogenous respiration of 1.1-1.2 mg/L, 70-76% % degradation of the reference compound, sodium acetate, after 14 days, and the oxygen concentrations >0.5 mg/L in all bottles during the test period.

A screening test following OECD 301 B guideline is also available for the similar substance Thixatrol Max. Thixatrol Plus and Thixatrol Max have one main constituent in common (constituent C) and the other two main constituents only differ in the length of the shorter side chain (C6 in Thixtarol Max vs. C10 in Thixatrol Plus). After different methods tested, the test material was dispersed with the aid of a high shear mixing resulting in a cloudy dispersion with fine particles of test material visible dispersed troughtout. A concentration of 14.4 mg/L (equivalent to 10 mg C/L) was exposed to activated sewage sludge microbes (from a plant treating predominantly domestic

sewage) with culture medium for 28 days at 21°C. The concentration of suspended solids in the test solution was 30 mg/L. The test substance reached 20 % degradation (based on CO_2 evolution) after 28 days. Toxicity control was included in the study and it showed no toxic effects of the test substance to the inoculum.

Two of the three main constituents of Thixatrol Max have shorter carbon chains than the constituents of Thixatrol Plus. Hence, they are expected to have higher water solubility and thus be more available to the microorganisms (based on the registration information, the measured water solubilities of the different constituents of Thixatrol Max are 147 mg/L, <0.4 mg/L and 0.1 mg/L). Therefore, they would be expected to be more rapidly degradaded than the constituents of Thixatrol Plus. However, this was not the case in the available screening studies. This could have been related to limited bioavailability of Thixatrol Max to the microbes in the test. However, the concentration of Thixatrol Plus in the test was higher than that of Thixatrol Max and a similar problem could be expected for that, too. There is no information on the method of test solution preparation from the Thixatrol Plus test, therefore it is not possible to assess whether the bioavailability could explain the observed differences in degradation. Both tests used activated sewage sludge as inoculum. In the Thixatrol Max test this is stated to come from a plant treating predominantly domestic sewage. In the case of Thixatrol Plus only the name of the treatment plant is given, but it can also be assumed to treat predominantly domestic sewage.

A ready biodegradation screening test according to OECD 301D (with some deviations) is also available for the UVCB substance EC 309-629-8 which has equal or similar constituents as Constituent C (see table bellow). This study was used for read across in the registration dossier of the substance EC 204-613-6 (i.e. constituent C). Based on the information on the ECHA dissemination site[5], the concentration of the constituent EC 204-613-6 was 64 % in the UVCB substance used as test item in the OECD 301D study and 14.3 % of the UVCB substance consisted of a constituent very similar to the constituent EC 204-613-6 (differering only in having one OH-group less). The UVCB substance constains also other constituents, e.g. some bigger constituents which may have slower degradation and be less bioavailable to the micro-organisms (at least 12 % of the test substance used in the OECD 301D[5]). Therefore, a direct read across from this UVCB substance to the constituent C of Thixatrol Plus is not possible.

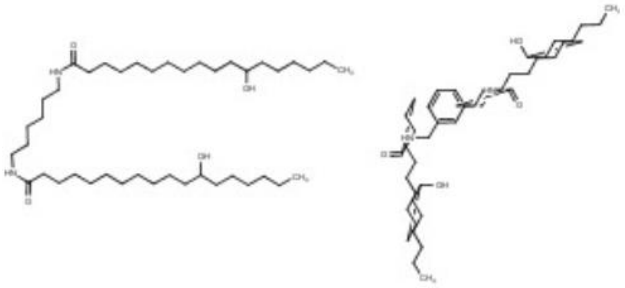
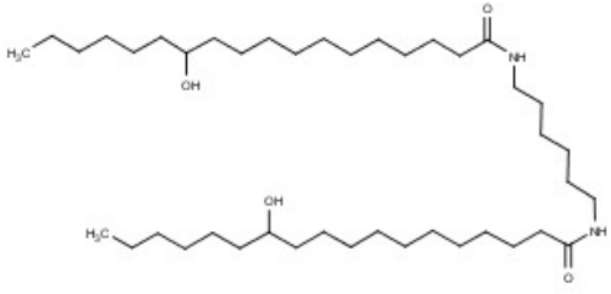
In the study, a solution of the test substance (EC 309-629-8) at 1 mg/L was inoculated with undiluted non-adapted river water activated sludge and placed in closed bottles in the dark for 28 d. Because of the low solubility of the test substance, the test solution was prepared using an emulsion of silicone oil and water (1:1) with 0.5 g/L of Tween 85. The degradation of the test substance was assessed by the determination of the dissolved oxygen concentration (DOC) on days 7, 14, 21 and 28. Control solutions containing the reference substance, sodium acetate (6.7 mg/L), together with abiotic control and inhibition control were used for validation purposes. The test substance was biodegraded 22% after 28 days and 37% after 60 days (during the prolonged closed bottle test).

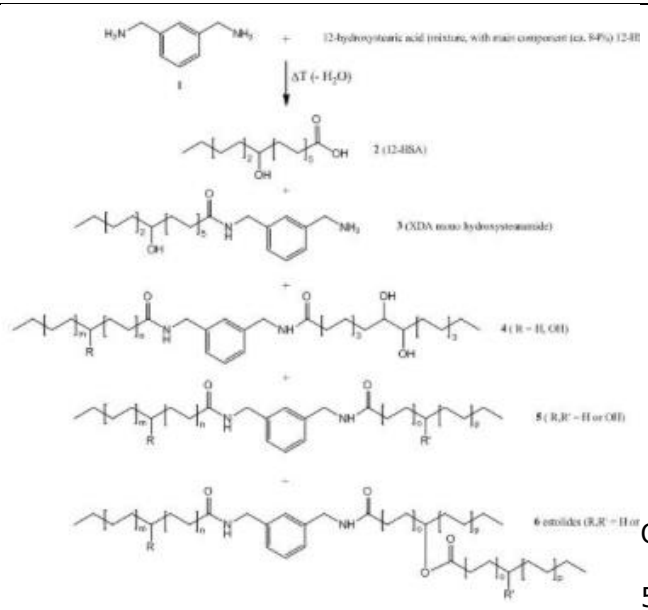
There are also other registered substances that have similar constituents as Thixatrol Plus. The results of the (extended) ready biodegradation tests with these substances are

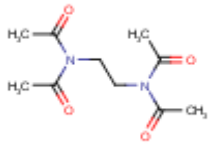
[5] Information disseminated from the registration dossier of constituent C (EC 204-613-6) where the OECD 301D study with EC 309-629-8 is used as read across.

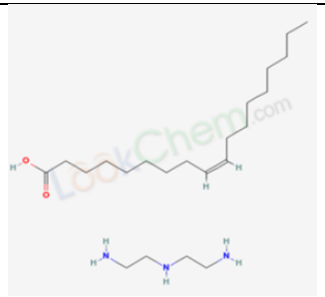
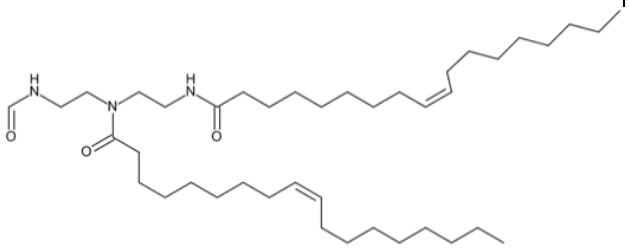
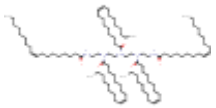
included in the below table. The reliability of the studies has not been evaluated by the eMSCA. However, they can be used as supporting information. The degradation of these substances did not reach the pass level after 28 days but in some of the extended tests over 60 % degradation was observed after 60 days. This could suggest that the low degradation observed in the ready biodegradation tests after 28 days might be due to the low solubility and bioavailability of the test substances.

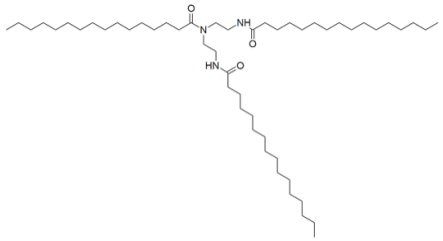
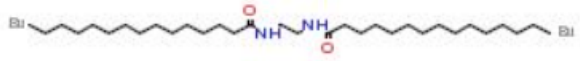
Table16. Summary on Ready biodegradation tests available on further similar substances identified by ECHA and the eMSCA.

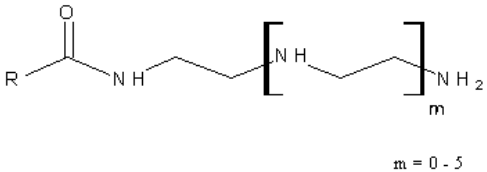
Substance name and EC number	Molecular structure	Study (year) and results	Remarks
12-hydroxyoctadecanoic acid, reaction products with 1,3-benzenedimethanamine and hexamethylenediamine EC 432-840-2 UVCB		OECD 301B (1999): 9% after 28 d (CO_2 evolution), Enhanced closed bottle test EPA OPPTS 835.3120 (2008): 4.2 % after 56 days (inorg. C analysis) 37% after 56 days, silicone oil used (inorg. C analysis)	In the EPA OPPTS 835.3120 test initial test concentration 6mg C/L See the confidential annex for further information.
12-hydroxy-N-[6-(12-hydroxyoctadecanamido)hexyl]octadecanamide EC 434-430-9 Multi-constituent		OECD 301B (2005): 7% in 28 days (CO_2 evolution)	Low solubility of the substance, substance was floating on the surface , initial concentration ca. 16 mg/L (WS <0.1 mg/L - < 0.01 mg/L) See the confidential annex for further information.

1,3-bis[12-hydroxy-octadecamide-N-methylene]-benzene 423-300-7 UVCB		OECD 301B: 5 % after 29 days (CO2 evolution)	
Reaction mass of N, N'-hexane-1,6-diylbis [12-hydroxyoctadecanamide] and 12-hydroxy-N-[6-[1-oxoalkyl)amino] hexyl] octadecanamide 469-110-8 UVCB	Not disseminated in ECHA website	OECD 301B (2006): 3 % after 28 days (CO2 evolution) OECD 310 (2009): 5 % after 28 days (inorg. C analysis)	In the OECD 301B, the tested concentration (16 mg/L) exceeded the maximum water solubility (<0.0007 mg/l) by a factor of about 23000. In the OECD 310, the test concentration was 2.8 mg/107 mL = c.a. 28 mg/L. See the confidential annex for further information.

N,N'-ethylenedi(stearamide) **EC 203-755-6** **CAS 110-30-5** **Mono constituent**		OECD 301 C (1988): 1.1 % degradation (O2 consumption) in 14 d (initial concentration 100 mg/l) EPA OTS 796.3260 (2000): 15% after 28 days (CO2 evolution) (initial conc. 10 mg/L) 6% after 28 days (initial conc. 20 mg/L)	Very similar to constituent C of Thixatrol Plus (the only difference is that there are no hydroxyl groups in the alkylchains) WS predicted 0 µg/L (WSKOW)
N,N'-ethylenebis[N-acetylacetamide] **EC 234-123-8** **CAS 10543-57-4** **Mono constituent**		OECD 301B (1995): 75.1 - 104.6% after 28 days	
Oleic acid, compound with N-(2-aminoethyl)ethane-1,2-diamine **EC 241-924-6**	UVCB consisting of reaction products of the following starting materials:	OECD 301F (1998): 0-10% after 28 d (O2 consumption) (initial concentration 50 mg/L) unadapted activated sludge	WS 3-34 mg/L

CAS 18016-43-8 **UVCB**	 Example structure: 		
Amides, Fatty acids C18 unsaturated, reaction products with tetraethylenepentamine **EC 630-459-8** **CAS 1225197-81-**	UVCB Example structure: 	Read across from tall oil diethylenetriamine imidazoline (CAS 68442-97-7) OECD 301D (2010): 24% in 28 days, 61% in 60 days (Initial concentration 2 mg/L, silica gel used)	

8 **UVCB**			
Amides, from diethylenetriamine and hydrogenated palm oil **EC 810-543-2** **CAS 1618093-67-6** **UVCB**	UVCB Example structure: 	OECD 301B: 34% in 28 d, 73% in 56 d	WS < 0.01 mg/L (OECD 105)
Amides, C16-C18 (even) , N,N'-ethylenebis **EC 931-299-4** **CAS –** **UVCB (one of the main constituents is EC 203-755-6)**	UVCB Example structure: 	OECD 301B (1991): The test substance was not degraded over 60% on a 10-day window (days 2-12) for any of the tested concentrations (10 and 20 mg/L) activated sludge, non-adapted	No measured or predicted WS reported in the registration dossier, but the substance is concluded to be not soluble in water by the registrant.

Fatty acids, C18 unsat, reaction products with triethylenetetramin, tetraethylenepentamine and pentaethylenehexamine **EC 945-133-3** **CAS –** **UVCB**	UVCB  R is fatty alkyl with chain lengths C 18 unsaturated chainlengths	Read across: Fatty acid C18 unsaturated diethylenetriamine imidazoline (CAS 68442-97-7) tested in the presence of silica gel was biodegraded 24% at day 28, 61% at day 60. Fatty acids C18 unsaturated reaction products with polyethylenepolyamines 35 and 38% biodegradation was achieved after 28 and 56 days.	

Simulation studies (water and sediment)

No relevant information available.

7.7.1.2.2. Biodegradation in soil

No relevant information available.

7.7.1.2.3. Summary and discussion on degradation

There is no information available on abiotic degradation. In a ready biodegradation screening test (OECD 301B) with Thixatrol Plus, 69 % degradation was observed after 28 days but the 10-d window was not met. 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 also states that the 10-day window need not be applied only 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.

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 implies total degradation (assumes 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 remains 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, and considering that the constituents are structurally relatively similar (they mainly differ in the length of the linear alkyl chains), it can be assumed that the three constituents have degraded either almost completely or at least to a significant extent.

The substance EC 907-495-0, which has the same main constituents A, B and C as Thixatrol Plus, reached a 63% degradation after 28 days in an OECD 301D test and 52 % degradation after 28 days, 61 % after 42 days and 67 % after 60 days in an enhanced OECD 301D test. This supports the result of the OECD 301B study with Thixatrol Plus.

Based on the BIOWIN QSAR models the constituent A is readily biodegradable and the constituents B and C do not fulfil the screening criteria for P/vP according to ECHA Guidance R.11 either because the BIOWIN 2 and 6 model results are well above 0.5. It is noted that the results of the BIOWIN 3 model for these two constituents are in the range of 2.25-2.75 and hence they are close to meeting the screening criterion defined for this QSAR model in the ECHA Guidance R.11. However, in the case of constituent B the value is just in the borderline (2.749), and hence, it can still be considered to screen ready

biodegradable based on the BIOWIN models. Therefore, the BIOWIN QSAR models support the results of the OECD 301B test with Thixatrol Plus although some uncertainty remains on the degradation of the constituent C.

Some uncertainty arises from the results of ready biodegradation tests with Thixatrol Max and other similar substances. In these tests low degradation was observed after 28 days. In addition, only 22 % degradation after 28 days and 37 % after 60 days was observed in an OECD 301D study with the UVCB substance EC 309-629-8 that consists mainly of constituents that are equal or very similar to the constituent C of Thixatrol Plus. However, none of these tests show a lack of degradation, and a continuous degradation over prolonged exposure times is observed. According to ECHA Guidance Document R.7b, given that ready biodegradability tests may sometime fail because of the stringent test conditions, positive test results should generally supersede negative test results. The low degradation observed in the tests with similar substances may be due to low bioavailablity of the substances to the microorganisms as high initial concentrations compared with the water solubilities of the substances were used, especially in the OECD 301B tests. Therefore, the lower degradation observed in the tests with some of the similar substances is not considered to override the results of the screening tests with Thixatrol Plus and the substance EC 907-495-0 (which has the same main constituents as Thixatrol Plus).

In conclusion, considering all the available test data and QSAR predictions in a weight-of-evidence analysis, it can be concluded that all constituents of Thixatrol Plus are non-persistent.

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

There is no experimental information on the adsorption/desorption coefficient of the substance. The log Koc values of the constituents were predicted using EPISuite KOCWIN QSAR model, which resulted in log Koc of 4.74, 5.71 and 6.69 based on MCI, and log Koc of 4.26, 5.17 and 6.31 based on log Kow method, for the constituents A, B and C, respectively.

The adsorption coefficients of the constituent of the similar substance Thixatrol Max have been determined to be in the range of 188 to greater than 4.27×10^5, (log Koc from 2.28 to >5.63), using a HPLC screening method, designed to be compatible with OECD 121.

For the chemical safety assessment a geometric mean of the log Koc values of the three constituents predicted by EPISuite KOCWIN model based on MCI method is calculated. This resulted in a log Koc of 5.66 (Koc 457088).

7.7.2.2. **Volatilisation**

Based on the low vapour pressure of the substance, volatisation is not expected to be a significant distribution pathway.

EPISuite HENRYWIN (v3.20) QSAR model predicts Henry's Law Constants (H) of $1.62 \times 10^{-4,}$ 5.73×10^{-8} and 2.02×10^{-8} Pa*m3/mol (bond estimation method) for the constituents A, B and C, respectively. For the chemical safety assessment a geometric mean of the H values is calculated. This resulted in a HLC of 5.7×10^{-7} Pa*m3/mol.

7.7.2.3. **Distribution modelling**

Level III fugacity model in EPI Suite (v4.11) was performed to predict the distribution of the constituents if equal emissions to water, soil and sediment are assumed. The results are shown in the below table.

The EPISuite STP Fugacity Model predicts a similar partitioning for all the constituents: 92-93 % will partition to sludge, 0 % to air, 6-7 % to effluent water. Total removal from STP is estimated >92% and < 1 % is biodegraded under anaerobic conditons. Biowin7 (Anaerobic Model Predictions) indicates not fast biodegradation.

Table 17

PARTITIONING OF THE MAIN CONSTITUENTS BASED ON THE LEVEL III FUGACITY MODEL IN EPI SUITE (V4.11)				
Constituent	**Air (%)**	**Water (%)**	**Soil (%)**	**Sediment (%)**
A	0.3	15.8	61.3	22.5
B	0.1	12	67.3	20.6
C	0.1	16.6	82.8	0.47

7.7.3. Bioaccumulation

7.7.3.1. Bioaccumulation in aquatic organisms (pelagic and sediment organisms)

There is no experimental information on the bioaccumulation of the substance 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. Since the measured and predicted log Kow values are above 4.5, all the constituents screen B/vB. However, it is noted that the predicted log Kow value of the constituent C is above 10, which may, together with its high molecular size, indicate hindered uptake and low potential for bioaccumulation.

The BCF values of the constituents were predicted using the BCFBAF QSAR model based on both the measured and predicted log Kow (see the below table). The predicted BCFs are low for all the constituents based on the regression method and Arnot-Gobas method with biotransformation. Only if zero biotransformation rate is assumed, the Arnot-Gobas method predicts high BCF values for the constituent A and also for the constituent B and C if the measured log Kow values are used as input.

Table 18

PREDICTED BCF VALUES (EPISUITE BCFBAF) FOR THE MAIN CONSTITUENTS BASED ON THE MEASURED AND PREDICTED (KOWWIN) LOG KOW VALUES				
Constituents	**Log Kow (meas./pred.)**	**BCF (L/kg) (regression method)**	**BCF (L/kg) (Arnot-Gobas, upper trophic with biotransformation estimate)**	**BCF (L/kg) (Arnot-Gobas, upper trophic assuming zero biotransformation)**
A	5.4 6.12	72 215	39.6 53.9	13,980 21,010
B	6.0 8.51	178 610	20.8 2.9	20,820 1,700
C	6.6 11.31	444 26	8.6 0.9	17,470 5.0

7.7.3.2. **Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates)**

No experimental information is available on the bioaccumulation of the substance in terrestrial organisms. The EPISuite KOAWIN model predicts a log Koa values of 13, 19 and 22 for the constituents A, B and C, respectively. According to the ECHA guidance R11, an efficiently absorbed, non-biotransformed neutral organic substance with a log Koa ≥ 5 in combination with a log Kow ≥ 2 has the potential to biomagnify in terrestrial food chains and air-breathing marine wildlife as well as in humans. Hence, based on the log Koa and log Kow values, all the constituents meet the screening criteria for bioaccumulation in terrestrial organisms.

The substance is classified as skin sensitiser. Recent chemical reactivity kinetic studies suggest that the rate of protein binding is a major determinant of allergenic potency. Low molecular weight chemical allergens must complex with proteins to be recognized by the immune system (Divkovic et al., 2005[6]; Chipinda et al, 2011[7]). Once absorbed, some partitioning of the absorbed dose into fat deposits would be expected, based on the high log Pow values. Therefore positive response of the substance in the skin sensitization test may suggest the substance may bind to carrier proteins in the circulatory system.

There is no specific information on the sensitising potential of main constituents. Applaying silico models, the QSAR-ToolBox indicates that the necessary conditions for eliciting direct or indirect protein interaction, described in a general mechanistic profile, are met in relation to amides (Protein binding OASIS v1.4). However, the specific structural boundaries providing sufficiency for interaction to proteins may not be identified. This indication referes to the main constituents A, B and C.

[6] Divkovic, M; Pease, CK; Gerberick, GF and Basketter, DA. 2005. Hapten–protein binding: from theory to practical application in the *in vitro* prediction of skin sensitization. Contact Dermatitis: Environmental and Occupational dermatitis, 189-246 53(4): http://onlinelibrary.wiley.com/doi/10.1111/j.0105-1873.2005.00683.x/full

[7] Chipinda I, Hettick JM, Siegel PD. 2011. Haptenation: chemical reactivity and protein binding. J Allergy (Cairo). doi: 10.1155/2011/839682. Epub 2011 Jun 30.

7.7.3.3. **Summary and discussion of bioaccumulation**

There is no experimental information on the bioaccumulation potential of the constituents of the substance or on similar substances. The predicted and measured log Kow values of the constituents meet the screening criterion for bioaccumulation in aquatic organisms. There is some uncertainty in the measured log Kow values (5.4-6.6) as the HPLC method is applicable only for log Kow values up to 6 and the predicted values are in the range of 6.12-11.31. It is also noted that the predicted log Kow of the constituent C is above 10 which may, together with its high molecular size, indicate hindered uptake and low potential for bioaccumulation.

The BCFBAF QSAR model predicts low bioaccumulation potential for all constituents based on the regression method and Arnot-Gobas method including biotransformation estimate. High BCF values are predicted only by the Arnot-Gobas method when assuming biotransformation rate of zero for the constituent A and also for the constituent B and C if the measured log Kow values are used as input.

The constituents meet the screening criteria for bioaccumulation in terrestrial organisms based on predicted log Koa and log Kow values. Furthermore, there are some indications of potential binding to proteins. Based on the positive response of the substance observed in a skin sensitisation study (see section 7.9.1), at least some of the constituents/impurities of the substance may bind to carrier proteins in the circulatory system. In addition, QSAR-ToolBox indicates that the necessary conditions for eliciting direct or indirect Protein interaction, described in a general mechanistic profile, are met by the main constituents (Protein binding OASIS v1.4).

Since there is no experimental information on the bioaccumulation potential and based on the log Kow values the constituents screen B/vB, a firm conclusion on the bioaccumulation of the constituents cannot be drawn.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

Table 19

OVERVIEW OF AVAILABLE AQUATIC TOXICITY STUDIES		
Method and test species	**Results**	**Remarks**
Fish		
Rainbow trout (Oncorhynchus mykiss) freshwater static OECD Guideline 203 (Fish, Acute Toxicity Test) EU Method C.1 (Acute Toxicity for Fish)	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	3 (not reliable) Experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diy lbis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]eth yl]octadecanamide; N,N'-ethane-1,2-diy lbis(12-hydroxyocta decanamide)** Form: powder

Aquatic invertebrate		
Daphnia magna freshwater static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) EU Method C.2 (Acute Toxicity for Daphnia)	EL50 (48 h): 15.63 — 250 mg/L test mat. (nominal) based on: immobilisation	3 (not reliable) Experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diyl bis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]ethyl]octadecanamide; N,N'-ethane-1,2-diyl bis(12-hydroxyocta decanamide)** Form: powder
Daphnia magna freshwater semi-static OECD Guideline 211 (Daphnia magna Reproduction Test) EU Method C.20 (Daphnia magna Reproduction Test)	NOEC (21 d): 0.9 mg/L test mat. (meas. (TWA)) based on: immobilisation 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	2 (reliable with restrictions) Read-across from supporting substance (structural analogue or surrogate) **Test material (EC number): 432-430-3**
Algae and aquatic plants		
Chlorella vulgaris (algae) freshwater static EU Method C.3 (Algal Inhibition test)	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.)	3 (not reliable) Experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diyl bis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]ethyl]octadecanamide; N,N'-ethane-1,2-diyl bis(12-hydroxyocta decanamide)**
Chlorella vulgaris CCAP 211/12 (algae) freshwater static OECD 201 (1984)	EL50 (72 h): > 1000 loading rate WAF test mat. (nominal) based on: growth rate and biomass	3 (not reliable) Experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diyl bis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]ethyl]octadecanamide; N,N'-ethane-1,2-diyl bis(12-hydroxyocta decanamide)**
Skeletonema costatum (algae) saltwater static ISO 10253 (Water quality - Marine Algal Growth Inhibition Test with Skeletonema costatum and Phaeodactylum tricornutum)	EC50 (72 h): 0.004 mg/L test mat. (nominal) based on: biomass (95% CL 0.0030 - 0.0040 mg/l) EC50 (72 h): 0.005 mg/L test mat. (nominal) based	1 (reliable without restriction) **Key study** Experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diyl bis(decanamide); 12-hydroxy-N-[2-[1-**

	on: growth rate (not possible to calculate 95% confidence limits) NOEC (72 h): 0.001 mg/L test mat. (nominal) based on: biomass NOEC (72 h): 0.0029 mg/L test mat. (meas.) based on: growth rate	**oxydecyl)amino]eth yl]octadecanamide; N,N'-ethane-1,2-diy lbis(12-hydroxyocta decanamide)** Form: powder
Skeletonema costatum (algae) saltwater static ISO 10253 (Water quality - Marine Algal Growth Inhibition Test with Skeletonema costatum and Phaeodactylum tricornutum)	EC50 (72 h): 4.08 mg/L loading rate, water accomodated fraction (nominal) based on: growth rate	2 (reliable with restrictions) Supporting study Experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diy lbis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]eth yl]octadecanamide; N,N'-ethane-1,2-diy lbis(12-hydroxyocta decanamide)**
Sediment organisms		
Corophium volutator saltwater short-term toxicity (laboratory study) static PARCOM 190.5	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) Key study Experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diy lbis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]eth yl]octadecanamide; N,N'-ethane-1,2-diy lbis(12-hydroxyocta decanamide)**

ECOSAR QSAR model predictions were performed for the constituents of the substance using both the measured and predicted log Kow values as input. It is noted that the predicted log Kow values of the constituents B and C seem to be within the range of the applicability domain of the model (Maximun log Kow > 8.0).

Table 20

Predicted chronic toxicity values of the constituents in fish, daphnia and algae using ECOSAR QSAR model (ECOSAR Class: Amides)				
Constituent	**Log Kow (measured/ predicted)**	**Fish**	**Daphnia**	**Algae**
A	5.4 6.1	0.005 0.002	0.050 0.017	0.105 0.051
B	6.0 8.5	0.004 0.0003	0.028 0.0007	0.077 0.006
C	6.6 11.3	0.003 <0.0001	0.015 <0.0001	0.053 0.0005

7.8.1.1. **Fish**

One acute study following OECD 203 is available for the registered substance. 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, the study is not considered reliabie.

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. Based on ECOSAR QSAR model predictions, all the main constituents may have chronic toxicity values below 0.01 mg/L.

7.8.1.2. **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 clear 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. 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.

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). According to the registration information, read across from this study was agreed with the UK CA during the former notification of new substances (NONS) procedure. The study resulted in a 21d-NOEC of 0.90 mg/L for reproduction and immobilisation based on time weighted average measured concentration.

The ECOSAR QSAR model predicts chronic toxicity values in Daphnia above or close to 0.01 mg/L for all the constituents based on the measured log Kow values. When using the predicted log Kow values as input, the model predicts chronic values below 0.01 mg/L for the constituents B and C. The log Kow values seem to be within the applicability domain of the model (maximum log Kow > 8.0).

7.8.1.3. **Algae and aquatic plants**

Two marine algal growth inhibition tests with *Skeletonema costatum* performed according to ISO 10253 are available for the substance. The key study was requested by UK Competent Authority during the former notification of new substances (NONS)procedure to provide a usable NOEC. The study was requested to use lower loading rates than previously performed marine test.

In the key study, *Skeletonema costatum* was exposed to an aqueous solution of the test material under static conditions for 72 hours. A 72-h ErC50 of 0.0054 mg/L (nominal concentration) and a 72-h NOErC of 0.0029 mg/L (measured concentration) were

determined for growth rate.

In the supporting marine algal study, 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 two fresh water 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 there is no information on measured concentrations.

The ECOSAR QSAR model predicts chronic toxicity values in algae above 0.01 mg/L for all the constituents based on the measured log Kow values. When using the predicted log Kow values as input, the model predicts chronic values below 0.01 mg/L for the constituents B and C. The the log Kow values seems to be within the applicability domain of the model (maximum log Kow >8.0).

7.8.1.4. **Sediment 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.

7.8.1.5. **Other aquatic organisms**

No relevant information available.

7.8.2. Terrestrial compartment

Table 21

OVERVIEW OF AVAILABLE TERRESTRIAL TOXICITY STUDIES		
Method and test species	**Results**	**Remarks**
Soil macro-organisms		
Eisenia fetida (annelids) short-term toxicity (laboratory study) Substrate: artificial soil OECD Guideline 207 (Earthworm, Acute Toxicity Tests)	NOEC (14 d): 1000 mg/kg soil test mat. (nominal) based on: mortality LC50 (14 d): > 1000 mg/kg soil test mat. (nominal) based on: mortality	2 (reliable with restrictions) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material (EC number): 432-430-3** Form: powder

Terrestrial plants		
Glycine max, Cucumis sativa and Allium cepa short-term toxicity (laboratory study) seedling emergence toxicity and seedling growth toxicity OECD Guideline 208 (Terrestrial Plants Test: Seedling Emergence and Seedling Growth Test)	*Glycine max, cucumis sativa and allium cepa*: LC50 (21 d): > 1000 mg/kg test mat. (nominal) based on: seedling emergence *Glycine max, cucumis sativa and allium cepa*: NOEC (21 d): 1000 mg/kg test mat. (nominal) based on: seedling emergence *Glycine max, cucumis sativa and allium cepa*: EC50 (21 d): > 1000 mg/kg test mat. (nominal) based on: growth *Glycine max, cucumis sativa and allium cepa*: NOEC (21 d): 1000 mg/kg test mat. (nominal) based on: growth	2 (reliable with restrictions) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material (EC number): 430-430-3** Form: powder
Soil micro-organisms		
Species/Inoculum: soil OECD Guideline 216 (Soil Microorganisms: Nitrogen Transformation Test)	NOEC (28 d): 1000 mg/kg soil dw test mat. (nominal) EC50 (28 d): > 1000 mg/kg soil dw test mat. (nominal)	2 (reliable with restrictions) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material (EC number): 432-430-3** Form: powder
Species/Inoculum: soil OECD Guideline 217 (Soil Microorganisms: Carbon Transformation Test)	NOEC (28 d): 1000 mg/kg soil dw test mat. (nominal) EC50 (28 d): > 1000 mg/kg soil dw test mat. (nominal)	2 (reliable with restrictions) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material (EC number): 432-430-3** Form: powder

There are no toxicity studies on terrestrial organisms available for Thixatrol Plus. For the similar substance Thixatrol Max four studies are available: one on invertebrates, one on plants and two on microorganisms. During the former notification of new substances (NONS)procedure it was agreed by the UK CA that the OECD 208 test conducted with Thixatrol Max can be read-across to support Thixatrol Plus. Therefore, also the studies on plants and soil microorganisms can be used as supporting information.

In an acute earthworm (*Eisenia fetida*) toxicity study with Thixatrol Max following OECD 207 guideline, no mortality was observed at the only tested concentration of 1000 mg/kg soil.

A study according to OECD 208 was performed to assess the effects of Thixatrol Max on the emergence and growth of three plant species: *Glycine max, Cucumis sativa* and *Allium cepa*. The seeds were exposed to concentrations up to 1000 mg/kg dry soil. The number of seedlings emerged and any mortalities and/or morphological abnormalities were determined daily for 21 days after 50% emergence in the control for each species.

The LC50 (emergence) and EC50 (growth) for *Glycine max, Cucumis sativa* and *Allium cepa* based on nominal test concentrations were greater than 1000 mg/kg.

The effect of Thixatrol Max on the nitrogen transformation activity of soil micro-organisms was investigated in a study according to OECD 216 resulting in an 28-d EC50 value of greater than 1000 mg/kg and a 28-d NOEC of 1000 mg/kg. In a study following OECD 217, the effect on the carbon transformation activity of soil micro-organisms was investigated over a 28 day period and gave an EC50 value of greater than 1000 mg/kg and a NOEC of 1000 mg/kg.

7.8.3. Microbiological activity in sewage treatment systems

Table 22

OVERVIEW OF AVAILABLE STUDIES ON TOXICTY TO SEWAGE TREATMENT SYSTEM MICRO-ORGANISMS		
Method and test species	**Results**	**Remarks**
activated sludge aerobic OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	NOEC (3 h): 1000 mg/L test mat. (nominal) based on: respiration rate EC50 (3 h): > 1000 mg/L test mat. (nominal) based on: respiration rate	1 (reliable without restriction) key study experimental result **Test material (EC name): A mixture of: N,N'-ethane-1,2-diy lbis(decanamide); 12-hydroxy-N-[2-[1-oxydecyl)amino]eth yl]octadecanamide; N,N'-ethane-1,2-diy lbis(12-hydroxyocta decanamide)** Form: powder

In a study according to OECD 209, the effect of Thixatrol Plus on the respiration of activated sewage sludge was assessed. The test material was aerated for a period of 3 h in the presence of activated sewage sludge with the addition of a synthetic sewage as a respiratory substrate. The rate of respiration was determined after 3 h contact time. The test substance did not cause any significant effects on activated sludge respiration at any concentration tested (up to 1000 mg/l).

7.8.4. PNEC derivation and other hazard conclusions

Table 23

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	Hazard assessment conclusion (freshwater): PNEC aqua (freshwater) 0.058 µg/L	Assessment factor: 50 Reliable chronic data on algae is available for the substance. Furthermore, reliable chronic data on *Daphnia magna* is available for the read across substance Thixatrol Max. Therefore, an assessment factor of 50 is applied to the lowest chronic value, i.e. the 72-h NOErC of 0.0029 mg/L determined for *Skeletonema costatum.*
Marine water	Hazard assessment conclusion (marine): PNEC aqua (marine) 0.0058 µg/L	Assessment factor: 500 Reliable chronic data on algae is available for the substance. Furthermore, reliable chronic data on *Daphnia magna* is available for the read across substance Thixatrol Max. Therefore, an assessment factor of 500 is applied to the lowest chronic value, i.e. the 72-h NOEC of 0.0029 mg/L determined for *Skeletonema costatum.*
Intermittent releases to water	Hazard assessment conclusion (intermittent releases): PNEC (intermittent releases) 0.054 µg/L	Assessment factor: 100 An assessment factor of 100 is used for the lowest available acute value, the 72h ErC50 of 0.0054 mg/L determined for *Skeletonema costatum*.
Sediments (freshwater)	Hazard assessment conclusion (sediment freshwater): PNECsed (freshwater) 1 mg/kg sediment dw (assessment factor)	Assessment factor: 1000 One acute 10 day study with the marine amphipod crustacean *Corophium volutator* is available

	PNECsed (freshwater) 576 mg/kg sediment ww (2.65 mg/kg sediment dw) (EPM)	for the substance. An assessment factor of 1000 is used for the lowest value (NOEC of 1000 mg/kg dw). This results in PNECsed of 1 mg/kg dw. Extrapolation method: According to ECHA Guidance R.10 (May 2008), if only acute data on sediment organisms exists, PNECsed should also be calculated using the EPM. Based on the PNECaq of 0.000058 mg/L and Koc of 457088, a PNECsed of 0.576 mg/kg wet weigth is calculated. This is converted to a PNECsed of 2.65 mg/kg dry weight.
Sediments (marine water)	Hazard assessment conclusion (sediment marine water): PNECsed (marine) 0.1 mg/kg sediment dw (assessment factor) PNECsed (marine) 0.0576 mg/kg sediment ww (0.265 mg/kg sediment dw) (EPM)	Assessment factor: 10000 One acute 10 day study with the marine amphipod crustacean *Corophium volutator* is available for the substance. An assessment factor of 1000 is used for the lowest value (NOEC of 1000 mg/kg dw). This results in PNECsed of 1 mg/kg dw. Extrapolation method: According to ECHA Guidance R.10 (May 2008), if only acute data on sediment organisms exists, PNECsed should also be calculated using the EPM. Based on the PNECaq of 0.0000058 mg/L and Koc of 457088, a PNECsed of 0.0576 mg/kg wet weigth is calculated. This is converted to a PNECsed of 0.265 mg/kg dry weight.
Sewage treatment plant	Hazard assessment conclusion (STP): PNECstp 100 mg/L	Assessment factor: 10 A respiration inhibition test (OECD 209) with the substance is available. No significant effects were observed at any of the tested concentrations, up to 1000 mg/L. An assessment factor of 10 is applied to the 3h NOEC of 1000 mg/L.

Soil	Hazard assessment conclusion (soil): PNECsoil 1 mg/kg soil dw (assessment factor) PNECsoil 0.69 mg/kg soil ww (0.78 mg/kg soil dw) (EPM)	Assessment factor: 1000 Soil toxicity data is not available on the substance. However, test data is available for the read across substance Thixatrol Max on earthworms (OECD 207), three plant species (OECD 208) and carbon and nitrogen metabolisation of soil microflora (OECD 216 and 217). In all tests the EC50/LC50 values were 1000 mg/kg dry soil. This data can be used as supporting information. Using an assessment factor of 1000 gives a PNECsoil of 1 mg/kg dry soil. Extrapolation method: Since there is no experimental information on the substance itself, EPM was also used to determine PNECsoil. Based on the PNECaq of 0.000058 mg/L, Koc of 457088 and H of , 5.7 x 10^{-7} Pa*m3/mole, a PNECsoil of 0.69 mg/kg soil wet weigth is calculated. This is converted to a PNECsoil of 0.78 mg/kg soil dry weight.
Air	No hazard identified.	No hazard identified.
Secondary poisoning	Hazard assessment conclusion (secondary poisoning): PNEC oral: 33.3 mg/kg food	Assessment factor: 300 A NOAEL of1000mg/kgbw/day is available from a 28 day repeat dose study with rats. A conversion factor of 10 is used to convert the NOAEL to NOEC (assuming age of 6 weeks or younger for the rats as worst case as in the corresponding summary of the study no age of the test organîsms indicated), Assessment factor of 300 is used based on the study duration of 28 days.

7.8.5. Conclusions for classification and labelling

Thixatrol Plus has a harmonised classification for aquatic hazards as Aquatic Chronic 2 in the Annex VI to CLP (Index number 616-127-00-5). However, the available aquatic toxicity data justifies a more stringent classification.

The lowest available LC/EC50 value is the 72h ErC50 of 0.0054 mg/L determined for the marine algae *Skeletonema costatum.* This results in an acute classification as Aquatic Acute 1 with M-factor of 100. It is noted that there is no reliable acute data on fish or aquatic invertebrates. The substance receives the most stringent classification category for acute aquatic hazards based on the available acute data for algae. The M-factor could potentially be affected if further acute data on fish or aquatic invertebrates was available. However, due to the low solubility of the substance, potential long-term effects are expected to be more relevant for these organisms.

Chronic data for Thixatrol Plus is only available on algae. In addition, chronic data on *Daphnia magna* is available for the similar substance Thixatrol Max. Since chronic data is not available for all three trophic levels, the substance should be classified for chronic hazards according to both Tables 4.1.0. (b) (i) or (ii) and 4.1.0. (b) (iii) of CLP and the most stringent outcome is selected.

The substance is considered rapidly degradable for classification purposes as the pass level was reached after 28 days in the OECD 301 B test with the substance. The 10 days window criteria was not met but according to CLP this is not required in case of complex multiconstituent substances consisting of structurally similar constituents, which is considered to be the case of Thixatrol Plus. The lowest available chronic value is the 72h NOErC of 0.0029 mg/L for *Skeletonema costatum.* This justifies classification as Aquatic Chronic 1 with M-factor of 1.

As mentioned above reliable acute data is only available for algae, and hence, this is used in the surrogate approach for chronic classification. Based on the lowest acute value, the 72h ErC50 of 0.0054 mg/L, and considering the substance as bioaccumulative for classification purposes (based on log Kow > 4), classification as Aquatic Chronic 1 with M-factor of 100 is considered justified. This is the most stringent outcome and is selected for classification.

It is noted that the available aquatic data leads to a more stringent classification than the current harmonised classification of the substance.

7.9. Human Health hazard assessment

Regarding the human health data, only information relevant for the bioaccumulation endpoint was evaluated.

7.9.1. Toxicokinetics

No toxicokinetics studies are available for the substance. Relevant information available from other studies on mammals was reviewed.

Absorption

In a 28-day repeat dose oral toxicity study (OECD 407) in rat conducted on the substance Thixatrol Plus significant changes were noted in the bodyweight of female rats at the higest dose levels (1000 and 150 mg/kg/day). Minor changes in some haematological parameters for both sexes and in blood clinical chemistry for males were also observed but considered of no toxicological signifigance.

In an oral gavage One-Generation Reproduction Study in rat with evaluation of subchronic toxicity (OECD 415) conducted on the structurally similar substance Thixatrol Max, no significant effects related to the treatment were observed at the dose levels up to 1000 mg/kg/day.

Hence, these studies suggest that Thixatrol Plus may possess low toxicity following oral administration, but they don't provide any clear information regarding absorption. However, as some effects were observed in the study with the substance, uptake of at least some of the constituents of the substance from the gastrointestinal tract is possible.

Clear evidence of dermal absorption is provided by the positive sensitisation result in the guinea pig study. This indicates that at least some constituents of Thixatrol Plus penetrated through skin and drained into the lymphatic system to the lymph nodes. This is consistent with the structures, molecular weights and Log Kow values of components present in Thixatrol Plus, although the lower molecular weight molecules would be expected to show better absorption characteristics. Similarly, it is expected that any substance absorbed across the gastrointestinal mucosa, following oral administration, may drain directly into the lymphatic system.

Any absorption following inhalation exposure is likely to be low, since only 3.62% of the substance was classed as inhalable, based upon particle size analysis, and less than 0.08% was considered to be respirable.

Distribution

The positive response in a skin sensitisation study suggests the substance may bind to carrier proteins in the circulatory system, potentially facilitating distribution. Once absorbed, some partitioning of the absorbed dose into fat deposits would be expected, based on the high log Kow values.

Metabolism and excretion

No relevant information available.

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3. Sensitisation

Not evaluated.

7.9.4. Repeated dose toxicity

Not evaluated.

7.9.5. Mutagenicity

Not evaluated.

7.9.6. Carcinogenicity

Not evaluated.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Not evaluated.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not evaluated.

7.10. Assessment of endocrine disrupting (ED) properties

Not evaluated.

7.11. PBT and VPVB assessment

Persistence

There is no information available on abiotic degradation. In a ready biodegradation screening test (OECD 301B) with the registered substance, 69 % degradation was observed after 28 days but the 10-d window was not met. 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*".

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 implies total degradation (assumes 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 remains 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, and considering that the constituents are structurally relatively similar (they mainly differ in the length of the linear alkyl chains), it can be assumed that the three constituents have degraded either almost completely or at least to a significant extent.

The substance EC 907-495-0, which has the same main constituents A, B and C as Thixatrol Plus, reached a 63% degradation after 28 days in an OECD 301D test and 52 % degradation after 28 days, 61 % after 42 days and 67 % after 60 days in an enhanced OECD 301D test. This supports the result of the OECD 301B study with Thixatrol Plus.

Based on the BIOWIN QSAR models the constituent A is readily biodegradable and the constituents B and C do not fulfil the screening criteria for P/vP according to ECHA Guidance R.11 either because the BIOWIN 2 and 6 model results are well above 0.5. It is noted that the results of the BIOWIN 3 model for these two constituents are in the range of 2.25-2.75 and hence they are close to meeting the screening criterion defined for this QSAR model in the ECHA Guidance R.11 (ECHA 2017a). However, in the case of constituent B the value is just in the borderline (2.749), and hence, it can be considered to screen ready biodegradable based on the BIOWIN models. Therefore, the BIOWIN QSAR models support the results of the OECD 301B test with Thixatrol Plus although some uncertainty remains on the degradation of the constituent C.

Some uncertainty arises from the results of ready biodegradation tests with Thixatrol Max and other similar substances. In these tests low degradation was observed after 28 days. In addition, only 22 % degradation after 28 days and 37 % after 60 days was observed in an OECD 301D study with the UVCB substance EC 309-629-8 that consists mainly of constituents that are equal or very similar to the constituent C of Thixatrol Plus. However, none of these tests shows a lack of degradation and a continuous degradation over prolonged exposure times is observed. According to ECHA Guidance Document R.7b (ECHA, 2017b), given that ready biodegradability tests may sometime fail because of the stringent test conditions, positive test results should generally supersede negative test results. The low degradation observed in the tests with similar substances may be due to low bioavailablity of the substances to the microorganisms as high initial concentrations compared with the water solubilities of the substances were used, especially in the OECD 301B tests. Therefore, the lower degradation observed in the tests with some of the similar substances is not considered to override the results of the screening tests with Thixatrol Plus and the substance EC 907-495-0 (which has the same main constituents as Thixatrol Plus).

In conclusion, considering all the available test data and QSAR predictions in a weight-of-evidence analysis, it can be concluded that all constituents of Thixatrol Plus are non-persistent.

Bioaccumulation

There is no experimental information on the bioaccumulation potential of the constituents of the substance or on similar substances. The predicted and measured log Kow values of the constituents meet the screening criterion for bioaccumulation in aquatic organisms. There is some uncertainty in the measured log Kow values (5.4-6.6) as the HPLC method is applicable only for log Kow values up to 6 and the predicted values are in the range of 6.12-11.31. It is also noted that the predicted log Kow of the constituent C is above 10 which may, together with its high molecular size, indicate hindered uptake and low potential for bioaccumulation.

The BCFBAF QSAR model predicts low bioaccumulation potential for all constituents based on the regression method and Arnot-Gobas method including biotransformation estimate. High BCF values are predicted only by the Arnot-Gobas method when assuming biotransformation rate of zero for the constituent A and also for the constituent B and C if the measured log Kow values are used as input.

The constituents also meet the screening criteria for bioaccumulation in terrestrial organisms based on predicted log Koa and log Kow values. Furthermore, there are some indications of potential binding to proteins. Based on the positive response of the substance observed in a skin sensitisation study (see section 7.9.1), at least some of the constituents/impurities of the substance may bind to carrier proteins in the circulatory system. In addition, QSAR-ToolBox indicates that the necessary conditions for eliciting direct or indirect protein interaction, described in a general mechanistic profile, are met by the main constituents (Protein binding OASIS v1.4).

Since there is no experimental information on the bioaccumulation potential and based on the log Kow values the constituents screen B/vB, a firm conclusion on the bioaccumulation of the constituents cannot be drawn. However, since the substance and its constituents are concluded to be non-persistent, no further information is needed on the bioaccumulation to conclude on the PBT asessment.

Toxicity

The substance and its constituents are not classified as CMR or STOT RE.

The whole substance meets the criterion for T based on the 72-h NOEC of 0.003 mg/L determined for *Skeletonema costatum* in a study following ISO 10253 guideline. However, as Thixatrol Plus is a multiconstituent substance and the fate and effects of the constituents may differ, the PBT status of the different constituents should be assessed.

In order to assess which of the constituents may have caused the effects observed in the algae study, ECOSAR QSAR models were performed. Based on the ECOSAR QSAR model, the constituent C has the highest predicted chronic toxicity in algae and constituent A the lowest toxicity. However, the predicted chronic toxicities of the constituents in algae are relatively similar when using the measured log Kow values as input and they are all above 0.01 mgL. When using the predicted log Kow values as input the predicted toxicities of the constituents in algae differ by orders of magnitude and the constituents B and C have values below 0.01 mg/L. It should be verified if the predicted log Kow values of these constituents are outside/within the applicability domain of the model (Maximum log Kow >8.0).

Additionally, the ECOSAR defines the ChV, or Chronic Value, as the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). Therefore, the NOEC would be a lower value.

Hence, based on the available information it seems likely that at least the constituent C fulfills the criterion for T based on algal toxicity, and possibly the constituent B as well. There may be more uncertainty as to whether the constituent A also meets the criterion. However, it is noted that there is no long-term information on toxicity to fish but the predicted chronic values in fish are below 0.01 mg/L for all the constituents.

Overall conclusion

Based on the available information and using a weight-of-evidence analysis, the constituents of the substance are concluded to be non-persistent. The constituents fulfill the screening criterion for B/vB and based on the available information it is not possible to draw a firm definite conclusion on the bioaccumulation. The whole substance meets the criterion for T based on aquatic toxicity but there is some uncertainty as to whether all the main constituents fulfill the criterion.

In conclusion, the substance and its constituents are not PBT/vPvB according to Annex XIII of REACH.

7.12. Exposure assessment

The ECHA Guidande documents on use descriptors (ECHA, 2015) and exposure assessment (ECHA, 2016) have been used for the assessement, but refinement have also been applied for the environmental exposure assessment based on Specific Environmental Release Categories (CEPE, 2010), ECETOC TRA v3 (ECETOC, 2012) for environmental exposure assessment, OECD ESD 22 (OECD, 2009) and EUSES parameters (EC, 2003) for exposure estimations.

All derivations require mandatory justifications, which are documented in the Chemical Safety Report to assure full transpoarency of the calculation and underlying assumptions.

7.12.1. Human health

7.12.1.1. Worker

Not evaluated.

7.12.1.2. Consumer

Not evaluated.

7.12.2. Environment

The substance Thixatrol Plus is manufactured outside the EU and imported to the European area but the information on volume is confidential. The substance is used as a viscosity adjuster in solvent-based paints. Paints containing Thixatrol Plus are used in different industrial settings and by professional workers.

Exposure scenarios have been developed on the basis of the latest versions of the ECHA REACH Guidance chapters R12 (2015), R14 (2012), R15 and R 16 (2016) and the EUSES programme.

The results of this assessment have been calculated by the eMSCA considering the information provided by the registrant(s) in the registration dossier. Confidential information has been included in the Confidential Annex.

In the next sections are summarised the characteristics of the three exposure scenarios considered. Additional information on the exposure assessment is provided in the confidential annex.

ES 1. FORMULATION

Formulation takes place in a multi-stage batch closed process. The composition of the products and the overall process are such that there are no discharges of raw materials or products to waste-water or to soil from the formulation plant.

Indirect emissions a) via dust deposition and subsequent wet cleaning of surfaces and b) via equipment cleaning are collected and disposed of by a professional waste disposal company.

This exposure scenario is defined based on the conditions described by the only registrant and written in site-specific terms according to CEPE SpERC 2.1c.v1, which covers the whole process of formulation of organic solvent borne liquid coatings and inks (solids).

Table 24. Duration, frequency and volume for Formulation.

Information type	Generic scenario	Explanation
Used amount of substance per day	Confidential information	This is based on a specific formulation volume.
Annual amount used per site	Confidential information	This is based on a specific formulation volume.
Emission days per site	Confidential information	Specific number of days

Environmental surrounding characteristics

Environmental surrounding characteristics are considered for both fresh water and marine water as follows:

Fresh water flow rate: 18,000 m^3/d (default value),

Municipal Sewage Treatment plant discharge: $2 \cdot 10^3$ m^3/d (defaul value).

Marine water flow rate: A default dilution factor for discharges to a coastal zone (marine environment) of 100 is assumed to be representative for a realistic worst case.

Operational conditions

The following specific characteristics are considered for the SpERC 2.1c.v1.

Release fraction to air from process	0.01%
Release fraction to wastewater from process	0%
Release fraction to soil from process	0%
Fraction tonnage to region	10%
Fraction used at main source	100%

According to CEPE SpERC 2.1c.v1 there is no emissions to soil, however as application of STP sludge on agricultural soil can not be excluded, default values have been applied for calculations for the soil compartment.

Risk management Measures

In Table 25 are summarized the the Risk Management measures applied and their effectivity.

Table 25. Risk Management Measures applied for Formulation.

Environmental compartment	Measure	Effectivity
Risk management measures (air)	Bag and cyclone filters	99%
Risk management measures (water)	Professional waste disposal company	100% 0.005% wil be present in cleaning organic solvents are re-used. All waste from equipment cleaning is collected and disposed of by a professional waste disposal company.
Risk management measures (soil)	-	-

ES 2. USE AT INDUSTRIAL SITE

This scenario covers the industrial use of the substance contained in final paint products. During chemical curing, the substance reacts with other substances in the matrix and thus is chemically transformed. The cured material contains only trace amounts of the substance. Therefore, direct emission to wastewater and soil is generally not expected.

Liquid waste-water from surface preparations, overspray control or equipment cleaning are collected and disposed of by a professional waste disposal company.

The exposure scenario is defined based on the conditions described by the only registrant and written in site-specific terms according to CEPE SpERC 4.1b.v1, /5.1(2)a.v1 for industrial application of coatings and inks by spraying and OECD ESD 22 (OECD 2009).

Table 26 Duration, frequency and volume for Use at industrial site.

Information type	Generic scenario	Explanation
Used amount of substance per day	Confidential information	This is based on a specific estimated volume.
Annual amount used per site	Confidential information	This is based on a specific estimated volume.
Emission days per site	Confidential information	Specific number of days

Environmental surrounding characteristics

Environmental surrounding characteristics are considered for both fresh water and marine water as follows:

Fresh water flow rate: 18,000 m^3/d (default value)

Municipal Sewage Treatment plant discharge: $2 \cdot 10^3$ m^3/d (defaul value).

Marine water flow rate: A default dilution factor for discharges to a coastal zone (marine environment) of 100 is assumed to be representative for a realistic worst case.

Operational conditions

The following specific characteristics are considered for the SpERCs:

Release fraction to air from process	0% (CEPE SpERC 5.1a.v1)
Release fraction to wastewater from process	0% (CEPE SpERC 4.1b.v1)
Release fraction to soil from process	0% (CEPE SpERC 4.1b.v1)
Fraction tonnage to region	10%
Fraction used at main source	100%

According to CEPE SpERC 4.1b.v1 there is no emissions to soil, however as application of STP sludge on agricultural soil can not be excluded, default values have been applied for calculations for the soil compartment.

Risk management Measures

In Table 27 are summarised the the Risk Management measures applied and their effectivity.

Table 27 Risk Management Measures applied for Use at industrial site.

Environmental compartment	Measure	Efectivity
Risk management measures (air)	Wet scrubber or filtration	99%
Risk management measures (water)	Professional waste disposal company	100% According to CEPE SpERC 2% will be present in spray booth scrubber water. All waste from spray booth scrubber water is collected and disposed of by a professional waste disposal company.
Risk management measures (soil)	-	-

ES 3. PROFESSIONAL USE

This scenario covers the professional use in paints and coatings in non-industrial settings but in skilled trade premises.

Environmental release during application of paint by brushing or rolling to water is not expected. However, losses to sewers from application equipment cleaning cannot be excluded.

This exposure scenario is defined based on the conditions described by the only registrant and written in site-specific terms according to CEPE SpERC 8f.2a.v1 for professional application of coatings and inks by brush or roller.

Table 28.Duration, frequency and volume for Professional use.

Information type	Generic scenario	Explanation
Used amount of substance per day	Confidential information	This is based on a specific formulation volume. Daily wide dispersive use.
Annual amount used per site	Confidential information	This is based on a specific formulation volume. Daily wide dispersive use.
Emission days per site	Confidential information	Specific number of days

Environmental surrounding characteristics

Environmental surrounding characteristics are considered for both fresh water and marine water as follows:

Fresh water flow rate: 18,000 m^3/d (default value),

Municipal Sewage Treatment plant discharge: $2 \cdot 10^3$ m^3/d (default value). Effectiveness water 91.1%. Therefore fractions of emissions from SWTP have been modified accordingly to water and sludge, 4.1% and 4.7%, respectively)

Marine water flow rate: A default dilution factor for discharges to a coastal zone (marine environment) of 100 is assumed to be representative for a realistic worst case.

Operational conditions

The following specific characteristics are considered for the SpERC 8f.2a.v1:

Release fraction to air from process 0%

Release fraction to wastewater from process 1%
(according to OECD ESD 22 1% of the initial solid fraction of the paint will be lost as brush residues and then properly disposed of by the painter, an estimated 3% of the initial coaintng will be left unused in paint cans while the remaining 96% will be deposited on the coated product).

Release fraction to soil from process 0.05%

Fraction tonnage to region 10%

According to CEPE SpERC 8f.2a.v1 there is no emissions to soil, however as application of STP sludge on agricultural soil can not be excluded, default values have been applied for calculations for the soil compartment.

7.12.2.1. **Aquatic compartment (including sediment)**

In Table 29 are included the aquatic PECs calculated for ES1 (Formulation), ES2 (Industrial use) and ES3 (Professional use) scenarios.

Table 29. PECs for the aquatic compartment and the different scenarios considered.

Protection target	ES1	ES2	ES3
Fresh water (mg/L)	2.53E-08	2.53E-08	2.06E-06
Fresh water sediment (mg/kgwwt)	2.37E-06	2.37E-06	1.28E-06
Marine water (mg/L)	2.39E-09	2.39E-09	2.06E-07
Marine sediment (mg/kgwwwt)	2.24E-07	2.24E-07	1.93E-05
Sewage Treatment Plant (mg/L)	0	0	2.05E-05

7.12.2.2. **Terrestrial compartment**

In Table 30 are included the terrestrial PECs calculated for ES1 (Formulation), ES2 (Industrial use) and ES3 (Professional use) scenarios.

Table 30. PECs for the terrestrial compostment and the different scenarios considered.

Protection target	ES1	ES2	ES3
Agricultural soil (mg/kgwwt)	7.49E-05	2.09E-07	8.33E-05

7.12.2.3. **Atmospheric compartment**

Not relevant for this assessment.

7.12.3. Combined exposure assessment

See regional RCRs for for the environmental compartments in section 7.13.

7.13. Risk characterisation

In Table 31 and Table 32 are presented the local RCRs calculated by the eMSCA for the aquatic and terrestrial compartments, respectively and the scenarios (ES1 (Formulation), ES2 (Industrial use) and ES3 (Professional use)) referred.

Table 31. Local RCRs for the aquatic compartment and the different scenarios considered.

Protection target	ES1	ES2	ES3
Fresh water	4.36E-04	4.36E-04	3.55E-02
Fresh water sediment	4.11E-05	4.11E-05	4.11E-05
Marine water	4.12E-04	4.12E-04	3.55E-02
Marine sediment	3.89E-05	3.89E-05	3.35E-03
Sewage Treatment Plant	0	0	2.05E-07

Table 32. Local RCRs for the terrestial compartment and the different scenarios considered.

Protection target	ES1	ES2	ES3
Agricultural soil	1.09E-03	3.03E-06	1.21E-03

In Table 33 are presented the regional RCRs estimated by the eMSCA for the relevant environmental compartments.

Table 33. Regional RCRs for the aquatic and terrestial compartments.

Protection target	Regional
Fresh water	4.59E-04
Fresh water sediment	4.07E-06
Marine water	4.5E-03
Marine sediment	3.38E-06
Agricultural soil	8.81E-8

The assessment results in RCS <1 for all local and regional environmental compartments.

Therefore, the eMSCA concludes that there is no need for further actions or risk management measures to be implemented.

7.14. References

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7.15. Abbreviations

B/vB	Bioaccumulative/very Bioaccumulative
BCF	Bioconcentration factor
C&L	Classification & Labelling
CMR	Carcinogenic, Mutagenic or Toxic to Reproduction
CoRAP	The Community rolling action plan
dw	dry weight
EAWAG	Swiss Federal Institute of Aquatic Science and Technology (Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz)
EC50	Median Effective Concentration
ECHA	European Chemicals Agency
EL50	Median Effective Loading rate
eMSCA	evaluating Member State Compentent Authority
EPM	Equilibrium Partitioning Method
ES	Exposure Scenario
GLP	Good Laboratory Practice
H	Henry´s Law Constant
Koa	Octanol-air partition coefficient
Koc	Organic carbon normalised adsorption coefficient
Kow	Octanol / water partition coefficient
LC50	Median Lethal Concentration
LL50	Median Lethal Loading rate
LOEC	Lowest Observed Effect Concentration
MCI	Molecular Connectivity Index
NOEC	No Observed Effect Concentration
NOELR	No Observed Effect Loading Rate

NONS	Notification of New Substances (under the Directive 67/548/EEC)
OECD	Organisation for Economic Co-operation and Development
P/vP	Persistent/very Persistent
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
QSAR	Quantitative Structure–Activity Relationship
RCR	Risk characterization ratio
SpERC	Specific Environmental Release Category
STOT RE	Specific Target Organ Toxicity - Repeated Exposure
SVHC	Substance of Very High Concern
T	Toxic
EPA	United States Environmental Protection Agency
UVCB	Substance of Unknown or Variable composition, Complex reaction products or Biological materials
vPvB	very Persistent and very Bioaccumulative
WAF	Water accommodated fraction
WS	Water solubility
ww	wet weight