Annex XV report

PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Name(s):	**5-sec-butyl-2-(2,4-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [1], 5-sec-butyl-2-(4,6-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [2] [covering any of the individual isomers of [1] and [2] or any combination thereof]**
EC Number(s):	**-**
CAS Number(s):	**-**
Submitted by:	**The Netherlands**
Date:	**25-02-2015**

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EC Number(s): -

CAS number(s): **-**

It is proposed to identify the substance(s) as very persistent and very bioaccumulative (vPvB) according to Article 57 (e) of Regulation (EC) No 1907/2006 (REACH).

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as vPvB. All available relevant information (such as the results of standard tests, modelling and (Q)SAR results) was considered together in a weight-of-evidence approach.

Persistence

The screening criterion for persistence (P) is fulfilled for the proposed substance. The results from three biodegradation studies showed that the proposed substance is neither readily nor inherently biodegradable. Hydrolysis of the proposed substance was shown to be at most very limited at environmentally relevant pH and temperature values. In a river water die-away test, the proposed substance substance degraded very slowly. This study was conducted with non-radio labelled substance. A best-case degradation half-life was estimated by attributing the observed dissipation to either biodegradation or hydrolysis, disregarding processes such as evaporation and binding of the proposed substance. At an environmentally relevant temperature of 12 °C this best-case degradation half-life corresponded to 74 days. Therefore, as the vP criterion of 60 days in freshwater is exceeded, the proposed substance should be regarded as fulfilling both the P and vP criterion

Bioaccumulation

The screening criterion for bioaccumulation (B) is fulfilled for the proposed substance. The proposed substance has a log K_{OW} of 6.3-7.3. Experimental bioaccumulation studies were conducted for the proposed substance in earthworm and fish yielding a $BSAF_k$ of 15.8 kg_{oc}/kg_{lipid} and a BCF_k of 9893 L/kg, respectively. Therefore, as the vB criterion of 5000 L/kg is exceeded, the proposed substance should be regarded as fulfilling both the B and vB criterion.

Toxicity

The proposed substance does not meet the toxicity (T) criterion of NOEC < 0.01 mg/L and is not classified as CMR and there is no evidence of chronic toxicity. The chronic NOECs for algae, daphnids and fish were 0.135, 0.096 and 0.03 mg/L, respectively. The

proposed substance has been classified in the ECHA's C&L inventory as a STOT RE2 substance. However, this classification is not a harmonised classification and therefore, the proposed substance cannot be considered T on this basis. The overall conclusion for the proposed substance is that regarding toxicity it is considered to be a borderline case

Conclusion

The proposed substance meets the criteria for a vPvB substance according to Article 57(e) REACH. In addition, it should be noted that the substance is considered to be borderline T.

Registration dossiers submitted for the substance? Yes

PART I

Justification

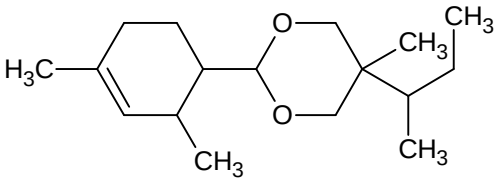
1. Identity of the substance and physical and chemical properties

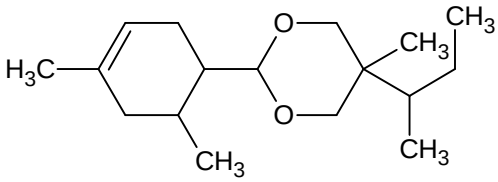
1.1. Name and other identifiers of the substance

Table 1: Substance identity

EC number:	-
EC name:	-
CAS number (in the EC inventory):	-
CAS number: **Deleted CAS numbers:**	-
CAS name:	-
IUPAC name:	5-sec-butyl-2-(2,4-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [1], 5-sec-butyl-2-(4,6-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [2] [covering any of the individual isomers of [1] and [2] or any combination thereof]
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	$C_{17}H_{30}O_2$
Molecular weight range:	266.43
Synonyms:	-

Structural formula:





1.2. Composition of the substance

Name: 5-sec-butyl-2-(2,4-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [1], 5-sec-butyl-2-(4,6-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [2] [covering any of the individual isomers of [1] and [2] or any combination thereof][1]

Description: group entry

Substance type: unspecific (group entry)

In Table 2 a list of example substances are given which are covered by the group entry.

The two following substances are registered:

- EC 413-720-9, trade name Karanal, identified by the Registrant as
  Reaction mass of
  5-sec-butyl-2-(2,4-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane
  and
  5-sec-butyl-2-(4,6-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane

  and

- The substance corresponding to
  Reaction mass of
  5-[(2R)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane
  and
  5-[(2R)-butan-2-yl]-2-[(1R,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane
  and
  5-[(2S)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane
  and
  5-[(2S)-butan-2-yl]-2-[(1S,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane
  and
  5-[(2S)-butan-2-yl]-2-[(1S,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane

  according to the information included in the registration dossier.

For substance 1,3-Dioxane, 2-(2,4-dimethyl-3-cyclohexen-1-yl)-5-methyl-5-(1-methylpropyl)- (CAS 117933-89-8) C&L notifications are submitted.

[1] anywhere the phrase "…the substance" or "… the proposed substance" (as appropriate) is used it refers to 5-sec-butyl-2-(2,4-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [1], 5-sec-butyl-2-(4,6-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane [2] [covering any of the individual isomers of [1] and [2] or any combination thereof].

The other substances are listed as examples. None of them is registered or a C&L notification (in IUCLID format) is submitted at the time of the submission of this A.XV report.

Table 2 provides a non-exhaustive list of examples of substances covered by the group name.

Table 2. Non-exhaustive list of substances covered by the group entry*

EC Name: CAS Name: IUPAC Name: Trade name:	EC Number	CAS Number
Reaction mass of 5-sec-butyl-2-(2,4-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane and 5-sec-butyl-2-(4,6-dimethylcyclohex-3-en-1-yl)-5-methyl-1,3-dioxane (Karanal)	413-720-9	
Reaction mass of 5-[(2R)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2R)-butan-2-yl]-2-[(1R,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane		
1,3-Dioxane, 2-(2,4-dimethyl-3-cyclohexen-1-yl)-5-methyl-5-(1-methylpropyl)-		117933-89-8
1,3-Dioxane, 2-[(1*R*,2*R*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *cis-rel*-		343934-04-3
1,3-Dioxane, 2-[(1*R*,2*R*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *trans-rel*-		343934-05-4
1,3-Dioxane, 2-[(1*S*,2*S*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *cis*-		676367-02-5
1,3-Dioxane, 2-[(1*S*,2*R*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *cis*-		676367-03-6
1,3-Dioxane, 2-[(1*R*,2*S*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *cis*-		676367-04-7
1,3-Dioxane, 2-[(1R,2R)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, cis-		676367-05-8
1,3-Dioxane, 2-[(1*S*,2*S*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *trans*-		676367-06-9
1,3-Dioxane, 2-[(1*S*,2*R*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *trans*-		676367-07-0
1,3-Dioxane, 2-[(1*R*,2*S*)-2,4-dimethyl-3-cyclohexen-1-yl]-5-methyl-5-(1-methylpropyl)-, *trans*-		676367-08-1
1,3-Dioxane, 2-[(1*R*,2*R*)-2,4-dimethyl-3-cyclohexen-		676367-09-2

1-yl]-5-methyl-5-(1-methylpropyl)-, *trans*-		
1,3-Dioxane, 2-(2,4-dimethyl-3-cyclohexen-1-yl)-5-methyl-5-(1-methylpropyl)-		186309-28-4

* This is a list of substances identified as covered by the generic substance description, however further substances not listed here may be covered as well.

1.3. Identity and composition of degradation products/metabolites relevant for the SVHC assessment

Not relevant for this dossier.

1.4. Identity and composition of structurally related substances (used in a grouping or read-across approach)

Not relevant for this dossier.

1.5. Physicochemical properties

Table 3: Overview of physicochemical properties

Property	Description of key information	Value	Reference/source of information
Physical state at 20°C and 101.3 kPa	Based on visual observations in GLP-compliant study reports	Clear colourless to pale yellow liquid	Several GLP-compliant study reports
Melting/freezing point	GLP-compliant according to OECD TG 102	< -50 °C	[1]
Boiling point	GLP-compliant according to OECD TG 103	> 250 °C at 101.3 kPa	[2]
Vapour pressure	GLP-compliant according to OECD TG 104	0.091 ± 0.01 Pa at 20 ºC 0.4 Pa at 38.8 °C	[3]
Density	GLP-compliant according to OECD TG 109	0.961 g/cm^3 at 20 °C	[4]
Water solubility	GLP-compliant according to OECD TG 105; column elution method	0.61 ± 0.06 mg/L at 20 ºC; pH 7.7-8.1	[5]
Partition coefficient n-octanol/water (log value)	GLP-compliant according to OECD TG 117; HPLC estimation	6.8 - 7.3 at 22 °C 6.3 - 6.7 at 35 °C	[6] [7]
Dissociation constant	-	Not relevant	-
Henry's Law Constant	calculated by evaluating MSCA	39.7 Pa x m^3 / mol at 20 °C	See section 3.2.2

2. Harmonised classification and labelling

The proposed substance is not listed in part 3 of Annex VI to the CLP Regulation. The self-classifications according to ECHA's C&L Inventory database (accessed 13.02.2015) are provided in Annex I to the report.

3. Environmental fate properties

3.1. Degradation

3.1.1. Abiotic degradation

3.1.1.1. Hydrolysis

The hydrolysis simulator of the OECD QSAR toolbox (v3.3) shows the possibility of acid catalysed hydrolysis, without providing any indication of a rate of hydrolysis. The predicted mechanism of hydrolysis is identical for both positional isomers with undefined stereochemistry. Therefore, the figures below depict only one of the two positional isomers. As shown in Figure 1, hydrolysis would start with the opening of the 1,3-dioxane ring and yields, after cleavage of the ring and subsequent dehydrogenation, hydrolysis product 4, which is a pre-registered substance known as 2,4-dimethylcyclohex-3-ene-1-carbaldehyde (CAS number 68039-49-6). Hydrolysis under neutral or basic conditions is not predicted by the hydrolysis simulator. Thus, based on these QSAR estimates hydrolysis of the proposed substance seems plausible at low pH values, but not likely under environmentally relevant pH values.

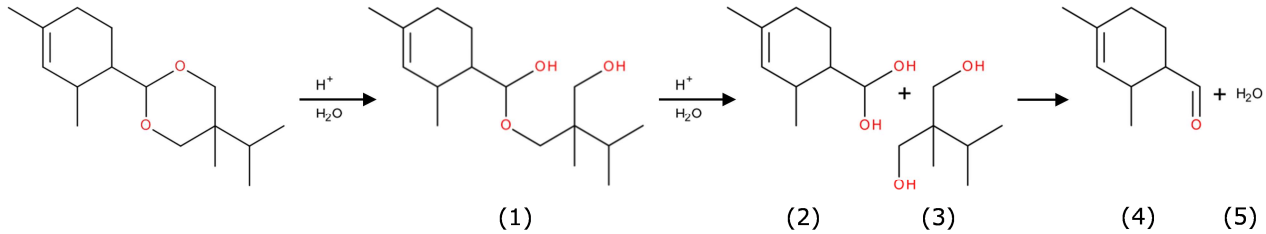


Figure 1. Proposed reaction pathway of the hydrolysis of the proposed substance under acid conditions

Two GLP-compliant studies are available that investigated hydrolysis of the proposed substance as a function of pH according to OECD TG 111. Both studies were conducted with non-radiolabelled substance. The registrant reported for the key study a half-life of 738.9 hours at pH 4 and 25 °C [8], and in the supporting study a half-life of > 275 < 830 hours at pH 4 and 25 °C [9].

The supporting study investigated the hydrolysis of non-radiolabelled substance in buffered aqueous solutions of pH 4, 7 and 9 at 50 and 55°C for up to 5 days [9]. Hydrolysis at pH 4 was additionally also investigated at 65°C for up to 42 hours. In all tests, a trend of time related decrease of the proposed substance was observed. For pH 7 and pH 9, a DT_{50} could not be calculated due to irregularities of the log (relative) concentration versus time curves. For pH 4, the registrant estimated a DT_{50} at 25 °C of 275-830 hours following extrapolation. However, inspection of the chromatograms by the evaluating MSCA showed that the peak corresponding to hydrolysis product 4 (see Figure 1) was absent in all samples. As non-radio-labelled material was used, a mass-balance could not be obtained, and dissipation by e.g. volatilisation cannot be ruled out. Considering the above remarks, this study is considered unreliable by the eMSCA, and is assigned a Klimisch score of 3.

The key study also investigated hydrolysis of non-radiolabelled substance in buffered aqueous solutions of pH 4, 7 and 9, but the test setup was more extensive as for each pH tests were conducted at 50, 60 and 70 °C for up to 11 days [8]. Furthermore, this

study also investigated hydrolysis in 0.22 µm filtered Rhône water (pH 8.2) that was collected upstream of Givaudan activity, at 50 °C for 11 days. In a non-GLP part of the study, hydrolysis products were identified for a single sample (pH 4, 60 °C, 144 h). Hydrolysis product 4 and another highly similar hydrolysis product were detected. Quantification of hydrolysis products (hydrolysis products 3 and 4 from Figure 1, as well as the reduction/ oxidation products of hydrolysis product 4 shown in Figure 2) was conducted for the test at pH 4 and 70 °C. Only hydrolysis product 4 could be detected. Concentration of hydrolysis product 4 increased limitedly with time, while the concentration of the proposed substance decreased rapidly especially at the start of the test. This shows that while hydrolysis under acid conditions occurs, other processes, such as binding to the test vessel and/or volatilization, also contributed to the reduced proposed substance levels in the aqueous phase. Consequently, hydrolysis of the proposed substance was most likely overestimated.

The registrant reported for the proposed substance a DT_{50} of 738.9 h at pH 4 following extrapolation to 25 °C. The evaluating MSCA reassessed the data and calculated a DT_{50} of 1830 h at pH 4 following extrapolation to 12 °C. This shows that even at acidic conditions hydrolysis of the proposed substance is very slow at a EU relevant environmental temperature. For pH 7 and 9, DT_{50} values could not be extrapolated to 12 °C, due to the irregularities of the log (relative) concentration versus time curves. For river water with pH 8.2, a DT_{50} of 241 h was calculated at 50 °C, demonstrating that at 50 °C dissipation in the hydrolysis test occurs almost a factor two slower at pH 8.2 compared to pH 4.

The key study strongly indicates that hydrolysis half-life at environmentally relevant pH and temperature will be considerably longer than 1830 hours. Thus, even though this study was not conducted with radiolabelled material, which most likely overestimated hydrolysis of the proposed substance, it has been demonstrated that hydrolysis of the proposed substance is at most very limited at relevant environmental conditions. The results from this study are considered reliable with restrictions, and are assigned a Klimisch score of 2.

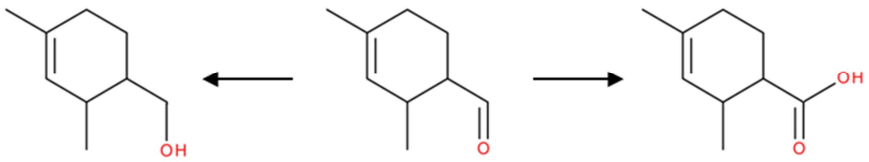


Figure 2. Proposed reaction pathways for the reduction/oxidation of hydrolysis product 4

Overall, the results from the two hydrolysis studies confirm the QSAR predictions that hydrolysis of the proposed substance can occur under acid conditions. However, this process is very slow. The DT_{50} at pH 4 and 12 °C is 1830 hours (76 days), but this dissipation may also include other losses such as volatilisation and sorption. The rate of hydrolysis under environmentally relevant pH and temperature could not be determined, most probably because hydrolysis of the proposed substance under these conditions is at best very limited. This was confirmed by the test with river water of pH 8.2 where dissipation in the hydrolysis test at 50 °C was almost a factor two slower than at pH 4. Therefore, the proposed substance is considered to be not hydrolysable, or at most hydrolysis has to be considered negligible under environmental conditions.

3.1.2. Biodegradation

3.1.2.1. Biodegradation in water

3.1.2.1.1. Estimated data

For both positional isomers with undefined stereochemistry, biodegradation and metabolism were estimated using the QSARs listed below. The obtained predictions did not differ. Therefore, the figures below depict only one of the two positional isomers.

EPIWIN

Biodegradation can be estimated by the EPIWIN program BioWin v.4.10. The outcome for the Biowin 2 model is 0.0001 and the outcome for the Biowin 6 model 0.0186, which means that the substance is predicted to be not inherently, nor readily degradable respectively. The outcome of the Biowin 3 model for ultimate biodegradation is 2.3809, which corresponds to an estimated mineralization half-life of weeks to months. The exact value would correspond to a half-life in water of 40 days (Aronson et al., 2006). ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.11, states that a substance is considered as potentially persistent if the value for Biowin 2 or 6 is below 0.5, and the value for Biowin 3 is below 2.25. Borderline cases with Biowin 3 between 2.25 and 2.75 should be carefully examined (ECHA, 2014b) .

CATABOL

Another program to assess biodegradability is CATABOL. This program estimates the percentage of mineralization in OECD TG 301C or 301F tests. The outcome of the model is 20.8% degradation after 28 days, which means that under the conditions of the OECD 301 tests the substance will only be partly degraded. However, it predicts that primary degradations occurs within 28 days, starting with epoxidation of the double bond. It should be noted that 20% mineralization as an experimental result in the OECD 302 series of test guidelines is considered as no mineralization (OECD, 2006). Values between 20-50% ThOD are considered partly mineralized (stable metabolites formed).

OECD QSAR Toolbox

The predictions for biotic degradation pathways (microbial) in the OECD QSAR Toolbox (v3.3) show a myriad of possibilities yielding up to 134 possible metabolites. The majority of these metabolites are formed following oxidation of the ring, and/or the alkyl substitution of carbon atoms at different positions. There is no indication of the likelihood of any of these possible transformations given in the Toolbox.

METEOR

METEOR is a rule-based expert system used to predict the metabolic fate of a query chemical structure (Marchant et al., 2008). METEOR predicts the oxidation (-OH) of the hexene-ring system and the methyl substituents as most probable (first pass) metabolites for the proposed substance (Figure 3), which is in line with the OECD QSAR Toolbox predictions for microbial metabolism. Dioxane ring opening, through hydrolysis, was not predicted. Plausible metabolites are indicated by a yellow header in the metabolic scheme below, and are for clarity also shown enlarged.

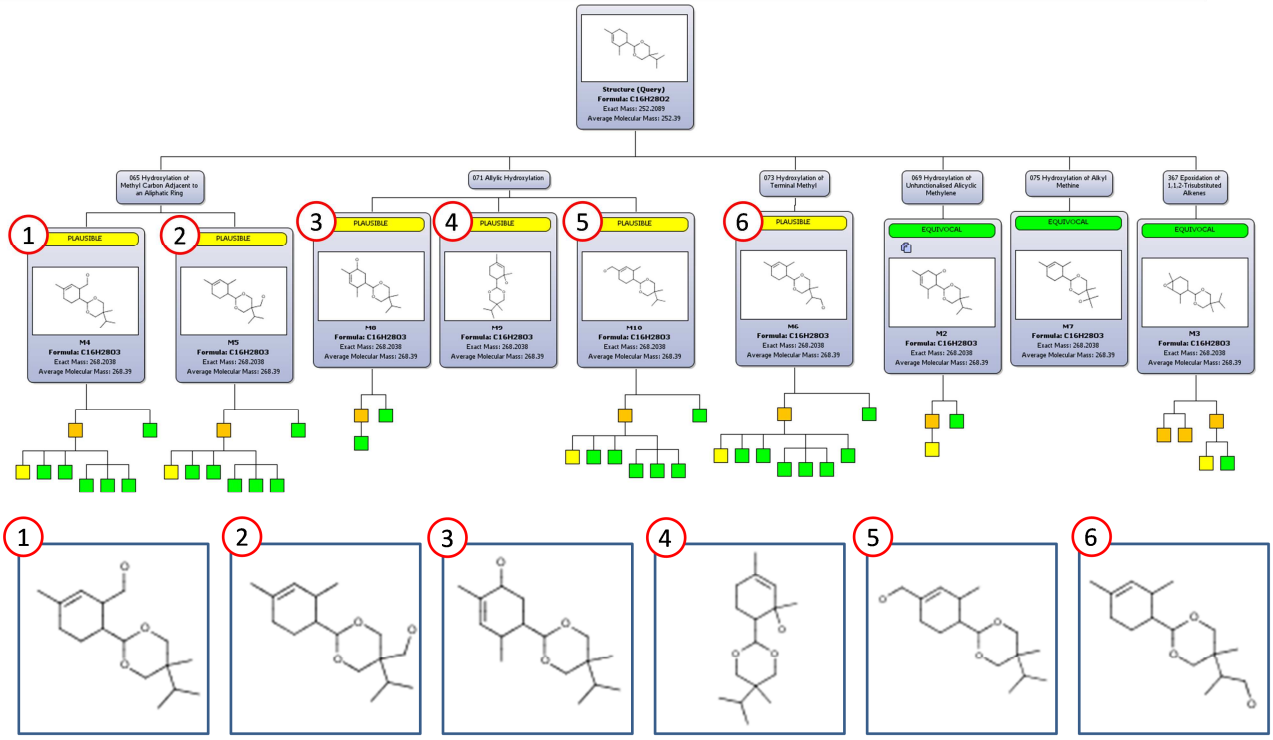


Figure 3. METEOR predicted metabolites of the proposed substance

EAWAG-BBD Pathway Prediction System

The most detailed prediction of microbial degradation is given by the Pathway Prediction System of the University of Minnesota Biodegradation and Bioremedation Database which now is now hosted at EAWAG, Switzerland (EAWAG-BBD PPS) (Gao et al., 2010). This prediction system shows the most likely (aerobic) degradation pathway to start with oxidation and subsequent ring opening of the 1,3-dioxane ring (Figure 4). The first step, i.e. oxidation in the *ortho* position to the ring oxygen, is thought to be the crucial (rate limiting) step, as the subsequent ring opening steps are considered to be more likely to occur.

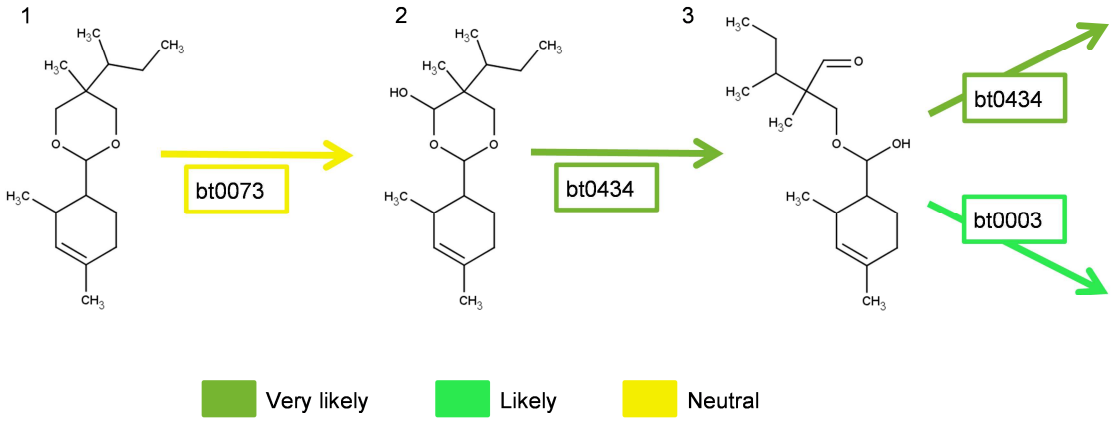


Figure 4. First three steps of the EAWAG-BBD Pathway Prediction System

The likelihood of the EAWAG-BBD PPS predicted first step is indicated to be "neutral". The prediction for the transformation of the 1,3-dioxane ring is predominantly based on the observed microbial transformation of 1,4-dioxane, as documented in Mahendra et al. (2007) (Figure 5). Although the mineralization in the laboratory of 1,4-dioxane is observed in "reinkultures" of monooxygenase expressing bacteria, both in growth supporting as well as co-metabolic mechanisms, this does not necessarily mean that this transformation will also occur in the environment.

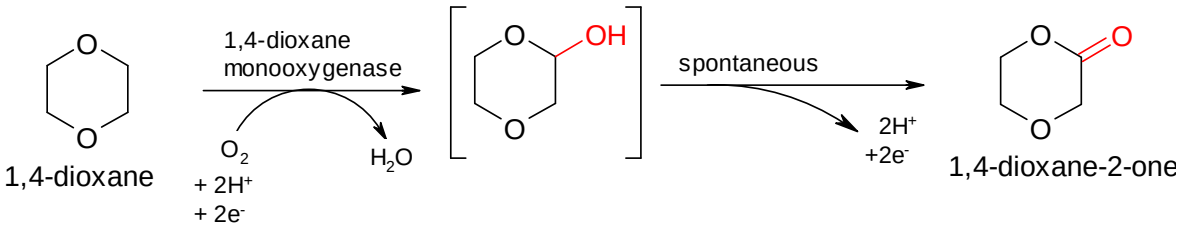


Figure 5. Reaction from 1,4-Dioxane to Dioxanone as predicted by EAWAG-BBD PPS (reacID#r1571)

On the ECHA dissemination website two ready biodegradability studies are available for 1,4-dioxane (CAS number 123-91-1; EC number 204-661-8). In the Manometric Respirometry test according to OECD TG 301F less than 10% of 1,4-dioxane degraded after 29 days based on oxygen consumption, while in the Headspace Test according to OECD TG 310 less than 5% degraded after 60 days based on CO_2 evolution. These studies are reliable, with the first study being assigned a Klimisch score of 2 by the registrants as it was not GLP-compliant, and the latter GLP-compliant test a Klimisch score of 1. From these studies, it can be concluded that 1,4-dioxane is not ready biodegradable. In fact, 1,4-dioxane appears to be poorly biodegradable even after prolonged test duration.

In a literature study, the biodegradation potential of 1,4-dioxane in river (n=4), soil (n=13) and activated sludge (n=3) samples was investigated (Sei et al., 2010). Biodegradation of 1,4-dioxane was observed in five out of six soil samples derived from the drainage area of a chemical factory producing 1,4-dioxane (<LOD within 33 days), and in one activated sludge sample via cometabolic degradation in the presence tetrahydrofuran (69% within 14 days). However, the majority of the samples, i.e. 14 out of 20, were not able to degrade 1,4-dioxane at all. Thus, it can be concluded that the

potential for 1,4-dioxane degradation is not ubiquitously distributed in natural environment.

Based on the above biodegradation studies with 1,4-dioxane, it seems likely that the same holds true for the biodegradation potential of 1,3-dioxane and substituted 1,3-dioxane substances like the proposed substance, as these are thought to biodegrade via the same pathways. Furthermore, the bulky branched alkane substituents at the 5-position (-5-methane-5-butan-2-yl) present in the proposed substance might also form a steric hindrance to the monoxygenase enzyme responsible for the first dioxane ring oxidation step.

The EAWAG-BBD PPS predicted next step is the actual ring opening catalyzed by an isomerase enzyme (not hydrolysis) of the 1,3-dioxane ring, which is indicated to be "very likely". Following the above described initial oxidation of the 1,3-dioxane ring (step 1), and subsequent ring opening (step 2), the next step (step 3) is another hemiacetal to alcohol and aldehyde transformation by another isomerase enzyme. The remains of the opened 1,3-dioxane ring are split off from the cyclohexene ring in this step. The predicted pathway for a similar structure is shown in Figure 6.

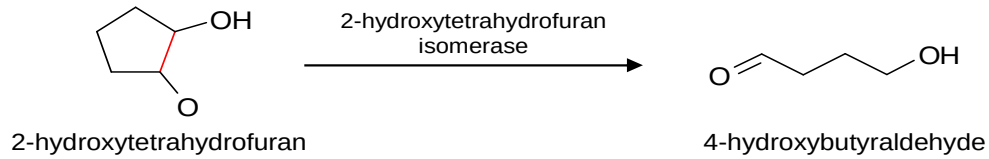


Figure 6. Reaction 2-hydroxytetrahydrofuran to 4-hydroxybutyraldehyde as predicted by EAWAG-BBD PPS (reacID#r0019)

Thus, the biodegradation pathway for the proposed substance is predicted to be as shown below (Figure 7).

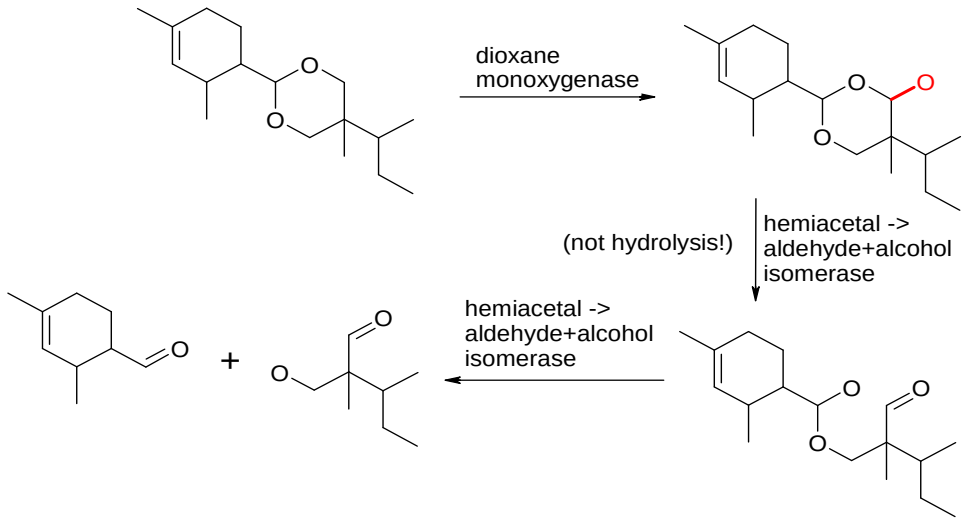


Figure 7. Biodegradation pathway for the proposed substance as predicted by EAWAG-BBD PPS

Based on the above discussion it is clear that although microbial degradation is possible and observed in lab-studies, the biodegradation studies for 1,4-dioxane show that the oxidation, and subsequent ring opening of cyclic ethers (such as 1,4-dioxane and 1,3-

dioxane, and the proposed substance) is not expected to occur in biodegradation simulation studies or to yield significant mineralization rates in the environment.

3.1.2.1.2. Screening tests

There are four biodegradation screening studies available for the proposed substance, of which three are available on the public dissemination site. All three studies were considered key studies by the registrant.

The GLP-compliant Modified Sturm (CO_2-evolution) study according to OECD TG 301B showed that the proposed substance is not-ready biodegradable [10]. In this ready biodegradability study with non-adapted activated sludge, degradation of the proposed substance amounted to 12 and 34% in the 10 and 20 mg/L treatments after 28 days, respectively. The results from this study are considered reliable without restrictions and are assigned a Klimisch score of 2.

The GLP-compliant Manometric Respirometry study according to the OECD TG 301F showed that the proposed substance is not ready biodegradable, with degradation after 50 days amounting to a maximum of 2% in spite of using enhanced substance application methods, and the prolonged study duration [11]. Study details are available on the ECHA dissemination website of the substance corresponding to the name Reaction mass of 5-[(2R)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2R)-butan-2-yl]-2-[(1R,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane. Inoculum was freshly activated sludge collected from a predominantly domestic WWTP that was washed three times prior to application, and had a suspended solid concentration of 30 mg/L (dry weight). Test concentration was 100 mg/L. Duplicate test flasks were prepared with direct addition, as well as two enhanced methods, i.e. the use of ultrasound to disperse the test substance and application of the test substance in silicone oil. Oxygen consumption was monitored daily and expressed as percentage of theoretical oxygen demand (ThOD). Since the proposed substance is volatile, evolved carbon dioxide was absorbed to soda lime pellets. Inoculum blank and a toxicity control with the reference substance sodium benzoate were included. The registrant of the substance corresponding to the name Reaction mass of 5-[(2R)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2R)-butan-2-yl]-2-[(1R,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane noted that an abiotic control was not needed, as the proposed substance is not expected to hydrolyse. The validity criteria were met. Degradation of the proposed substance amounted 1% after 50 days with direct addition. The use of ultrasound to disperse the proposed substance did not improve biodegradation after 28 days, and increased mineralization only marginally to a total of 2% after 50 days. Application of silicone oils also did not improve biodegradation (mineralization was decreased by 2%) after 28 and 50 days. There was no toxic effect to the inoculum, as sodium benzoate biodegradation was not altered by the addition of the proposed substance. The results from this study are considered reliable without restrictions and are assigned a Klimisch score of 1.

It should be noted that in addition to the two enhanced application methods discussed above [11], another three enhanced methods were applied using the same test setup, but reported in a separate study report [12]. This study was considered reliable without

restrictions, and showed that the three additional enhanced application methods also did not result in degradation of the proposed substance.

The fourth study, a GLP-compliant Manometric Respirometry study according to the OECD TG 302C showed that the proposed substance is not inherently biodegradable, with degradation amounting to 12 and 18% based on O_2 consumption, after 28 and 50 days, respectively. The test was conducted with non-adapted activated sludge from a domestic WWTP, and a test concentration of 30 mg/L. The results from this study are considered reliable without restrictions and are assigned a Klimisch score of 1.

Concluding, the screening and inherent biodegradation tests showed that the proposed substance is neither readily nor inherently biodegradable.

3.1.2.1.3. Simulation tests (water and sediments)

A GLP-compliant aerobic mineralisation study in surface water according to OECD TG 309 is available for the proposed substance [13]. This 60-day study was conducted with natural water and an initial nominal test concentration of 60 µg/L at 22 °C. The registrant reported a half-life of 395 h for the proposed substance at 22 °C, based on (pseudo-) first order kinetics. The registrant did not indicate the reliability of the study.

The evaluating MSCA conducted a thorough reassessment of this study, as several deviations from OECD TG 309 were observed.

According to the original study report, the initial nominal test concentration was 50 µg/L, and not 60 µg/L as reported on the public dissemination site. The OECD TG 309 recommends the use of two test concentrations that differ from each other by a factor of 5 to 10, with the higher and lower test concentration not exceeding 100 and 10 µg/L, respectively. As indicated in the test guideline, there is a risk when testing high concentrations that degradation will not follow first order kinetics and that the first order degradation constant and half-life cannot be estimated. Fortunately, in this study, degradation did seem to follow first order kinetics, and a half-life was derived for the proposed substance.

OECD TG 309 prescribes the inclusion of a blank control, solvent control, sterile control and reference control in the test design. The registrant only included a sterile control. Since the reference control was missing, the viability of the microbial community could not be determined. Considering that the water used in the study was sampled upstream of the registrants activities from an unpolluted river, that the only treatment of the natural water was filtration through a course filter of 100 µm and finally, that the natural water was used on the same day as sampled, the evaluating MSCA supposes that a viable microbial community was present in the water. The registrant also did not assess the toxicity of the proposed substance to the microbial community. Considering that in the above discussed GLP-compliant Manometric Respirometry study, the proposed substance displayed no toxic effects on the inoculum [11], this is not considered critical. The registrant also did not include a solvent control, but did indicate that the final concentration of the solvent carrier was 0.01% (v/v). Therefore, while the registrant did not include the necessary controls, careful consideration by the evaluating MSCA led to the conclusion that this does not invalidate the study, it merely lowers the reliability.

There are two issues that complicated the interpretation of this water simulation study. Firstly, the study was conducted at 22 °C, while the proposed substance is used and released within the context of the REACH Regulation in the EU. The temperature of 12 °C is a default value used in current risk assessment to reflect the average environmental conditions in the EU, and therefore the test should preferably be conducted at 12 °C.

Nevertheless, using the Arrhenius equation a DT_{50} at 12 °C can be extrapolated, and the results can be interpreted. Secondly, the registrant did not use radiolabelled test material. While this is not required by OECD TG 309, it is recommended as a mass-balance can be obtained. As no mass balance was available, it remains unclear to what extent the test substance degraded and to what extent it disappeared from the aqueous phase due to evaporation, binding to organic matter in the (turbid) aqueous phase and/or binding to the surface of the test vessels. The study did, however, meet the recovery criterion of 70-110% of nominal at the start of the experiment, and sterile controls were analysed throughout the experiment to quantify disappearance due to processes other than biodegradation. Comparison of the residual concentrations in the test vessels and sterile controls shows that they differed less than 10% for t = 0 h up to and including t = 504 h. This indicates that dissipation up to 504 h was predominantly abiotic. The recently conducted hydrolysis study [8] shows that hydrolysis of the proposed substance in water from the same source (pH 8.2) is slow, as the DT_{50} at 50 °C amounted to 241 h. Therefore, the evaluating MSCA concludes that the disappearance is most likely due to binding and/or volatilisation of the proposed substance, instead of abiotic degradation.

The registrant estimated a DT_{50} of 395 h by excluding data points as being in the lag and tailing phase, using only the measurements after 264, 504 and 696 h. This strategy is not supported by the data. Moreover, the tailing phase as referred to in the OECD TG 309, is only applicable to residual activity, in the case of the use of a radiotracer, because of the incorporation of labelled carbon into biomass. The application of this tailing phase to the disappearance of the parent compound is thus not in agreement with the guideline.

The evaluating MSCA plotted the natural logarithm of the concentration of the proposed substance in both the sterile controls and in the biotic test vessels against time (Figure 8). The data showed that only when t = 0 is included the plot was not linear for the biotic test vessels, indicating higher than average removal during the first day, i.e. no lag phase. Further, Figure 8 shows that there is no lag phase and no tailing phase. Linear regression with GraphPad Prism (v.6.04) of the data from day 1 until day 60 yielded a *k* of 0.022 (r^2 = 0.92) for the biotic test vessels, which corresponds to a DT_{50} of 31 days at 22°C. The data for the abiotic controls were rather irregular, however, a *k* of 0.010 could be determined (r^2 = 0.68), which corresponds to a DT_{50} of 68 days at 22°C. For both abiotic and biotic test vessels, half-lives are increasing if data from the beginning of the test are omitted from the regression. If the rate constant for the abiotic controls is subtracted from the rate constant for the biotic test, a value of 0.012 remains, which corresponds to a DT_{50} of 56 days at 22 °C estimated to be only due to biodegradation. Thus, even if all dissipation observed in the test could be attributed to either biotic or abiotic degradation, it is highly likely that at a relevant temperature of 12 °C the DT_{50} would exceed the vP criterion of 60 days. The median value for an extrapolation of half-lives of pesticides in soil to a temperature 10 °C lower is a factor of 2.58 (EFSA, 2007). Applying this default factor yields an estimated DT_{50} in freshwater due to degradation of 145 days at 12 °C.

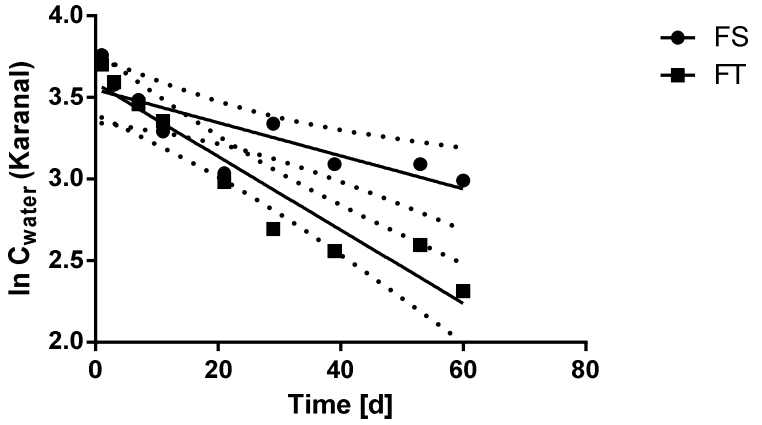


Figure 8. Semi-logarithmic plot of the residual concentrations of the proposed substance in the test vessels (FT) and the residual concentrations in the sterile controls (FS), as a function of time

The above DT_{50} of 145 days at 12 °C is a realistic estimation for biodegradation in freshwater. However, this DT_{50} should be considered indicative, since this study had several limitations, i.e. degradation was monitored only at one test concentration, distinction between degradation and dissipation was hampered by the use of non-labelled test material, and the experiment was conducted at 22 °C. Therefore, in addition to the realistic DT_{50} the evaluating MSCA also calculated a best-case $DegT_{50}$ in freshwater for the proposed substance, including the possibility of abiotic hydrolysis.

Firstly, the half-life for hydrolysis at 12 °C was calculated. For the process of hydrolysis, a default factor of 2.2 has been proposed for extrapolation to a 10 °C lower temperature, the corresponding formula being: t½ (X°C) = t½ * e $^{(0.08\ (T\ -\ X))}$) (ECHA, 2014a). Using this default factor and assuming that all of the loss processes in the abiotic controls are accounted for by hydrolysis, the half-life for hydrolysis at 12 °C was estimated to be 152 days compared to 68 days at 22 °C. The degradation in the biotic vessels was subsequently obtained by summing the rate constant for biodegradation at 12 °C (0.0048 d^{-1}) and hydrolysis (0.0046 d^{-1}) yielding an overall rate constant of 0.0093 d^{-1}. This corresponds to a best-case degradation half-life of 74 days at 12 °C. It should be noted that the assumption that all abiotic loss processes are due to hydrolysis is most probably a strong overestimation of the real hydrolysis rate. Another indication for this, comes from the chromatograms presented in the report, that do not show the appearance of the chromatographic peaks of any of the hydrolysis products, probably indicating the complete absence of hydrolysis at ambient temperature and at neutral pH.

Concluding, the evaluating MSCA reassessed the water simulation study, and while deviations from OECD TG 309 were detected, these were not considered sufficiently critical to invalidate the study results. The results are considered valid with restrictions and are assigned a Klimisch score of 2. The realistic DT_{50} in freshwater for the proposed substance was estimated to be 145 days at 12 °C, while the best-case DT_{50} in freshwater was estimated to be 74 days at 12 °C.

3.1.3. Summary and discussion of degradation

Hydrolysis of the proposed substance is at the best very slow, with an estimated DT_{50} of 76 days at pH 4 and 12.5 °C. Hydrolysis at pH 7 and 9 showed an irregular pattern and no DT_{50} could be derived for 12 °C. An additional test showed that at 50 °C dissipation in a hydrolysis test at an environmentally relevant pH of 8.2 (river water) was almost a

factor 2 slower than at pH 4. It should be noted that the presented hydrolysis study had shortcomings, as the test material was not radio-labelled. Consequently, hydrolysis was most likely overestimated. This was evident from the test conducted at pH 4 and 70 °C where the proposed substance concentration rapidly decreased in the first 24 hours, while the concentration of the proposed hydrolysis product 4 (see Figure 1) only limitedly increased in the same time period. The substance is therefore considered to be only slightly hydrolysable and most likely restricted to acidic conditions only.

Although some biodegradation is predicted by QSAR models, the experimental data show that this partial degradation is very limited. The highest percentage mineralization was observed in the OECD 301B test, but the amount was rather variable (12 to 34% after 28 days). However, it should be realised that this test is in itself not very suitable for a relatively volatile substance such as the proposed substance, although in principle this would result in a lower observed amount of CO_2-evolution. In a more recent OECD 301F test including bioavailability enhancements, the amount of mineralization after both 28 and 50 days, is virtually zero, varying between -2 and +2%. In an inherent test according to OECD guideline 302C there was 12% mineralization after 28 days and 18% mineralization after 50 days. These tests show that if mineralization occurs it will still be rather limited, even under the more favourable conditions of the inherent degradability test. The proposed substance is therefore considered to meet the screening criteria for P/vP substances.

Aerobic degradation of the proposed substance was studied in river water with a pH of 8.2. This study has several shortcomings, i.e. some controls were missing, only one concentration was tested, distinction between biodegradation and dissipation was difficult as the test material was not radio-labelled, and the experiment was conducted at 22 °C. Since the proposed substance is used and released within the context of the REACH Regulation in the EU, the test should preferably be conducted at 12 °C, which is considered to reflect the average environmental conditions in the EU. Nevertheless, a thorough reassessment by the evaluating MSCA showed that the results from this study are reliable with restrictions. This study showed that the proposed substance is slowly biodegraded with the DT_{50} at 22 °C amounting to 56 days. Extrapolated to an EU relevant environmental temperature of 12 °C a realistic DT_{50} of 145 days was obtained. Even though there were no indications of any significant hydrolysis in this test with river water at slightly alkaline conditions and ambient temperature, a best-case DT_{50} was also calculated by assuming that all dissipation is caused by either biodegradation or hydrolysis. This yielded a best-case DT_{50} at 12 °C of 74 days.

3.2. Environmental distribution

3.2.1. Adsorption/desorption

Adsorption of the proposed substance has been estimated at 30 °C using the HPLC method as described in guideline Annex V C.19 [14]. This GLP-compliant study reported a log K_{oc} of 3.61 for the proposed substance. The results are considered reliable, and are assigned a Klimisch score of 1.

QSAR-based estimates using default input parameters, yielded comparable values, i.e. log K_{oc} values of 3.53 and 4.00 using the MCI and K_{ow} based methods (KOCWIN v2.00), respectively.

The results of the HPLC and QSAR estimations of the adsorption behavior lead to the conclusion that the proposed substance will adsorb to organic material.

3.2.2. Volatilisation

Calculation of the Henry constant using the equation HENRY=VP*MOLW/SOL from the Guidance document on information requirements and chemical safety assessment (ECHA, 2012), and the substance properties from Table 3 results in a Henry constant of 39.7 Pa x m³/mol. This indicates that the substance is volatile from water. Due to the high adsorption potential to organic matter, volatilisation from soil and sludge is expected to be lower.

The distribution coefficient $K_{air,water}$ (Henry coefficient) was calculated from the Henry constant using the equation $K_{air,water}$ = HENRY/R*TEMP, and default values for R (8.314 Pa x m^3/mol x K) and TEMP (285 K) (ECHA, 2012). This yielded for the proposed substance a $K_{air,water}$ of 0.017, which indicates that the proposed substance is volatile from water surface.

3.2.3. Distribution modelling

The proposed substance has a high potential for adsorption to organic matter in soils, as indicated by a level III fugacity model calculation (LEV3EPI in EPIsuite) (Table 4). However, when the entry route is water or air/water, the largest fraction remains in the water, while only a limited part (10-15%) adsorbs to the sediment. Consequently, soil and water, and to a lesser extent sediment, are the primary compartments in which the substance will reside at steady state following release. These are the most important compartments in terms of the relevance of degradation.

Table 4: Level III fugacity modelling for the proposed substance

Release [a]	**Predicted environmental distribution**			
	Air	**Water**	**Sediment**	**Soil**
Equal emission to air, water and soil	0.0373% (0.0325%)	11.2% (13.5%)	1.96% (1.59%)	86.8% (84.9%)
100% emission to air	82.2% (81.2%)	4.95% (6.11%)	0.870% (0.720%)	11.9% (11.9%)
100% emission to water	0.0841% (0.0488%)	85.0% (89.4%)	14.9% (10.5%)	0.0122% (0.00716%)
100% emission to soil	0.00157% (0.00127%)	0.0994% (0.103%)	0.0175% (0.0122%)	99.9% (99.9%)
Equal emission to air and water	0.274% (0.209%)	84.8% (89.2%)	14.9% (10.5%)	0.0398% (0.0307%)
Equal emission to air and soil	0.0302% (0.0296%)	0.0101% (0.106%)	0.0178% (0.0124%)	99.9% (99.9%)
Equal emission to water and soil	0.0124% (0.00841%)	11.2% (13.5%)	1.96% (1.59%)	86.8% (84.9%)

[a] All calculations were based on a release of 1000 kg/hour to each compartment. The input parameters were a vapour pressure of 0.091 Pa (at 20 ºC), water solubility of 0.61 mg/L (at 20°C), Henry Law constant of 39.75 Pa*m^3/mol, log K_{oc} of 3.61 and a log K_{ow} of 6.3, respectively, 7.3 (the latter values is given between brackets). EPA default half-life values (based on BIOWIN (ultimate) and AOPWIN estimates) were used for all compartments.

3.2.4. Summary and discussion of environmental distribution

The proposed substance has as a high potential for adsorption to organic matter, as indicated by the HPLC and QSAR estimations that yielded log K_{oc} values in the range 3.53 to 4.00. The proposed substance was shown to be volatile from water, as indicated by the Henry constant of 39.7 Pa x m³/mol, and the distribution coefficient $K_{air,water}$ of 0.017. By performing a level III fugacity model calculation (Table 4) a more complete picture of the distribution of the proposed substance over the different compartments was obtained. It was shown that depending on the entry route(s) the proposed substance will reside at steady state in soil, water and to a lesser extent in sediment. Therefore, soil and water are considered to be the most important compartments in terms of the relevance of degradation.

3.3. Data indicating potential for long-range transport

Not relevant for this dossier.

3.4. Bioaccumulation

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

Bioconcentration of the proposed substance was studied in rainbow trout (*Oncorhynchus mykiss*) in a GLP-compliant study according to OECD TG 305 [15]. The study had a flow-through study design with an uptake phase of 47 days, followed by a depuration phase of 35 days. A solvent control was included. Two nominal concentrations (0.003 and 0.03 mg/L) were tested. Concentrations in water and fish were determined regularly. The study was conducted properly. It was noted that the standard deviation of the actual water concentrations in the lower test concentration was considerably higher than the validity criterion of 20%. Therefore, the results from the lower test concentration are considered less reliable, and are assigned a Klimisch score of 2 (= reliable with restrictions). The results from the higher test concentration are considered reliable without restrictions, and are assigned a Klimisch score of 1.

The results showed that steady state was achieved within the exposure period and that there was a steady decrease of the concentrations in fish during the depuration phase. At the end of the depuration phase, 99% and 94% of the test substance was eliminated from the fish in the lower and higher concentrations, respectively. The registrant reported a Bioconcentration Factor at steady state (BCF_{ss}) of 2114 and 8653 L/kg for the lower and higher test concentrations, respectively, and a Kinetic Bioconcentration Factor (BCF_k) of 2487 and 10778 L/kg.

The evaluating MSCA recalculated the BCF_k by refitting the data of both the uptake phase and the depuration simultaneously to a non-linear first-order kinetic model, contrary to the registrant that only considered the uptake phase in his calculations. This yielded BCF_k values of 2171 and 9406 L/kg for the lower and higher test concentrations, respectively. These values were subsequently corrected for the growth rate of the juvenile fish, a procedure that is prescribed by the recently updated OECD TG 305 (OECD, 2012), and normalized to a lipid content of 5%. This yielded growth corrected and lipid normalized BCF_k values of 1892 and 9893 L/kg for the lower and higher test concentrations, respectively.

3.4.1.1. Bioaccumulation estimation

Considering that it was experimentally demonstrated that the proposed substance

strongly bioaccumulates in fish, QSAR estimations of BCF values are only presented to provide a complete picture. Log K_{ow} of the proposed substance was estimated with the HPLC method as being between 6.8 and 7.3 at 22 ºC [6], and more recently between 6.3 and 6.7 at 35 ºC [7]. The first study was submitted by the registrant, but replaced during a recent update from the IUCLID dossier. Study details of the first study are available on the ECHA dissemination website of the substance corresponding to the name: Reaction mass of 5-[(2R)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2R)-butan-2-yl]-2-[(1R,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane. It was reported that the proposed substance had three peaks with retention times (RT) of 21.09, 22.22 and 23.24 minutes, which exceeded the RT of 15.3 minutes obtained with the reference substance 2,4'-DDT. It was therefore concluded that the log K_{ow} of the substance is >6.2. It should be noted that the OECD TG 117 from 1989 reports a log K_{ow} of 6.2 for DDT, while in the OECD TG 117 from 2004 the log K_{ow} of DDT has been corrected to 6.5. Therefore, the evaluating MSCA considers the log K_{ow} to be >6.5. In the second study, the RT of the peaks of the proposed substance (36 to 46 minutes) also considerably exceeded the RT of the DDT peak (24 minutes). Nevertheless, the registrant reported log K_{ow} values of 6.3 to 6.7 for the proposed substance, the reason being that inclusion of a reference substance with log K_{ow} of 7.1 (not recommended by OECD TG 117) affected the slope of the log k versus log K_{ow} regression line. Based on the available data, the evaluating MSCA considers it more appropriate to conclude that the log K_{ow} of the proposed substance is >6.5. Considering that in both studies extrapolation was done outside the range of 0 to 6 that is covered by the HPLC method (OECD, 2004), these results are considered reliable with restrictions (Klimisch score of 2).

Regression based estimates of the BCF were calculated with BCFBAF (v3.01) using the range of reported log K_{ow} values, and amounted to 6664, 14240 and 9484 L/kg for log K_{ow} 6.3, 6.8 and 7.3, respectively. The latter value, is in good agreement with the experimentally derived BCF for *O. mykiss* discussed above.

3.4.2. Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates)

Bioaccumulation of the proposed substance by the earthworm *Eisenia fetida* was studied in a GLP-compliant study according to OECD TG 317 [16]. The uptake phase and the depuration phase each lasted 21 days. Concentrations in soil and worms were determined regularly. The study was conducted properly and the validity criteria were met. Therefore, the results are considered reliable without restrictions and were assigned a Klimisch score of 1.

A kinetic bioaccumulation factor (BAF_k) of 9.91 kg soil/kg worm was calculated from the uptake phase. Following correction for lipid and organic carbon content, a Biota-Sediment Accumulation Factor ($BSAF_k$) of 15.76 kg OC/kg lipid was obtained. The results from this study clearly show that the proposed substance strongly bioaccumulates in earthworms.

3.4.3. Summary and discussion of bioaccumulation

The bioaccumulative potential of the proposed substance was experimentally determined in fish and earthworms. For the fish *Oncorhynchus mykiss* whole-body BCF_{ss} were reported as 2214 and 8653 L/kg at concentrations that are well below the water solubility limit of the substance (0.6 mg/L). Following growth correction and lipid

normalisation, the evaluating MSCA obtained BCF_k values of 1892 and 9893 L/kg for the lower and higher test concentration, respectively. Since the water measurements at the lower test concentration showed considerable variation, these results are considered less reliable. QSAR estimates agree with the higher BCF value, i.e. 6664, 14240 and 9484 L/kg when using a log K_{ow} of 6.3, 6.8 and 7.3, respectively. For the earthworm *Eisenia fetida* a lipid and organic carbon corrected $BSAF_k$ was reported of 15.76 kg OC/kg lipid. These results show that the proposed substance is strongly accumulating in fish and terrestrial oligochaetes.

4. Human health hazard assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (e) of REACH.

5. Environmental hazard assessment

5.1. Aquatic compartment (including sediment)

5.1.1. Fish

5.1.1.1. Short-term toxicity to fish

A GLP-compliant fish acute toxicity study according to OECD TG 203 is available for the proposed substance [17]. This 96-hour semi-static study was conducted with common carp (*Cyprinus caprio*). The registrant reported a 96-hour LC50 of ca. 0.3 mg/L for survival, based on nominal concentrations. Given the high hydrophobicity and moderate volatility of the proposed substance, the use of nominal concentrations invalidates the test. Therefore, the results are considered unreliable, and are assigned a Klimisch score of 3.

5.1.1.2. Long-term toxicity to fish

A GLP-compliant fish early-life stage study according to OECD TG 210 is available for the proposed substance [18]. This 32-day semi-static study was conducted with fathead minnow (*Pimephales promelas*). The registrant reported two NOECs with lowest being 0.054 mg/L for mortality, hatching of eggs, swim-up, survival, length and weight of surviving fish, based on time-weighted average (TWA) concentrations.

Following re-evaluation, it was concluded by the evaluating MSCA that based on mortality the NOEC should be 0.030 mg/L (TWA concentration). The results from this study are considered reliable without restrictions, and are assigned a Klimisch score of 1.

5.1.2. Aquatic invertebrates

5.1.2.1. Short-term toxicity to aquatic invertebrates

A GLP-compliant *Daphnia magna* immobilisation test according to OECD TG 202 is available for the proposed substance [19]. The registrant reported for this static test, a 48-hour EC50 of ca. 5.35 mg/L for mobility, based on nominal concentrations. Given that the actual test concentrations were not determined and that the reported NOEC greatly exceeds the water solubility of the proposed substance, i.e. 0.61 mg/L, the results from this study are considered unreliable, and are assigned a Klimisch score of 3.

5.1.2.2. Long-term toxicity to aquatic invertebrates

A GLP-compliant *Daphnia magna* reproduction test according to OECD TG 211 (2012) is available for the proposed substance [20]. The registrant reported for this 21-day semi-static test, a NOEC of 0.096 mg/L for reproduction, based on TWA measured concentrations. This study was evaluated as reliable without restrictions, and the results are assigned a Klimisch score of 1.

5.1.3. Algae and aquatic plants

Two algal inhibition studies are available for the proposed substance. Both studies are GLP-compliant.

The key study was conducted according to OECD TG 211 (version 2012) [21]. This 72-hour static test with *Pseudokirchnerella subcapitata* reported a number of effect

concentrations based on geometric mean measured concentrations. The EC50 and NOEC amounted for growth rate to >0.336 and 0.135 mg/L, respectively, and for yield to 0.306 and 0.135 mg/L. In the study report, EC10 values were also reported and they amounted for growth rate and yield 0.286 and 0.121 mg/L, respectively. This study was evaluated as reliable without restrictions, and the results are assigned a Klimisch score of 1.

The supporting study was conducted according to OECD TG 211 (version 1984) [22]. This 72-hour static test with *Scenedesmus subspicatus* (new name: *Desmodesmus subspicatus*) was conducted as a limit test. An EC50 and NOEC of >0.5 mg/L were reported based on geometric mean measured concentrations. This study was evaluated as reliable without restrictions, and the results are assigned a Klimisch score of 1.

The NOEC of 0.135 mg/L obtained from the key study with the more sensitive algal species is used in this annex XV report.

5.1.4. Sediment organisms

No data available.

5.1.5. Other aquatic organisms

No data available.

5.2. Terrestrial compartment

No data available.

5.3. Atmospheric compartment

No data available.

5.4. Microbiological activity in sewage treatment systems

No data available.

5.5. Toxicity to birds

No data available.

5.6. Summary and discussion of toxic effects

No toxicity data were available on microorganisms, sediment organisms and organisms in the terrestrial compartment. For the aquatic compartment, EC50 and LC50 values of the proposed substance were found to be >0.34, 5.35 and 0.3 mg/L, for algae, daphnids and fish, respectively. Only the algal EC50 was based on geometric mean measured concentrations, while the daphnid and fish EC50 were based on nominal concentrations. It should be noted that the EC50 for *Daphnia* exceeds the limit of water solubility by approximately a factor of 10. Chronic data were available for all three aquatic taxa. The NOECs were based on geometric mean measured concentrations and amounted to 0.135, 0.096 and 0.03 mg/L for algae, daphnids and fish, respectively. In concordance with the ecotoxicity data, the proposed substance has been classified by the industry in the ECHA C&L inventory as an aquatic acute 1 and aquatic chronic 1 substance.

6. Conclusions on the SVHC Properties

6.1. CMR assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (e) of REACH.

6.2. PBT and vPvB assessment

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as vPvB. All available relevant information (such as the results of standard tests, modelling and (Q)SAR results) was considered together in a weight-of-evidence approach.

6.2.1. Assessment of PBT/vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the proposed substance as PBT/vPvB. All available information (such as the results of standard tests, monitoring and modelling, information from the application of the category and analogue approach (grouping, read-across) and (Q)SAR results) was considered together in a weight-of-evidence approach.

6.2.1.1. Persistence

The proposed substance is considered hardly hydrolysable at environmentally relevant conditions, with an estimated DT_{50} of at least 76 days at pH 4 and 12 °C. The proposed substance was shown to be not readily biodegradable, as only 12-34% degraded within 28 days in a Modified Sturm (CO_2-evolution) test and only 1% degraded after 50 days in a Manometric Respirometry Test including several options to enhance biodegradation. The results of the inherent biodegradability study, where biodegradability was assessed under more favourable conditions than in the ready biodegradability tests, showed that the proposed substance is also not inherently biodegradable, with 12% biodegradation after 28 days and 18% after 50 days. These findings meet the screening criteria for persistence. The simulation test that investigated the aerobic degradation of the proposed substance in river water (pH 8.2) was considered reliable with restrictions in spite of several shortcomings. This study showed that even at 22 °C biodegradation is slow with a DT_{50} of at least 56 days. Extrapolation to a EU relevant temperature of 12 °C yields a realistic DT_{50} of 145 days at 12°C, which amply exceeds the vP criterion of DT_{50} >60 days for freshwater. The simulation study did not provide any indications of hydrolysis. However, even if abiotic dissipation would be fully assigned to hydrolysis, the overall degradation half-life extrapolated to 12 °C, would be 74 days and would thus still exceed the vP criterion in freshwater. Therefore, the proposed substance meets the criteria for very persistent substances as stated in Annex XIII of REACH, and the proposed substance is considered vP.

6.2.1.2. Bioaccumulation

The bioaccumulative potential of the proposed substance was experimentally determined in fish and earthworms. For the fish *Oncorhynchus mykiss* whole-body BCF_s were reported of 2214 and 8653 L/kg at concentrations that are well below the water solubility limit of the substance (0.6 mg/L). Following growth correction and lipid normalization, the evaluating MSCA obtained BCF_k values of 1892 and 9893 L/kg for the lower and higher test concentration, respectively. Since the water measurements at the lower test concentration showed considerable variation, these results are considered less

reliable. QSAR estimates agree with the higher BCF value, i.e. 14240 and 9484 L/kg when using a log K_{ow} of 6.8, respectively, 7.3. For the earthworm *Eisenia fetida* a lipid and organic carbon corrected $BSAF_k$ was reported of 15.76 kg OC/kg lipid. These results show that the proposed substance is strongly accumulating in fish and terrestrial oligochaetes. Therefore, the proposed substance meets the criteria for very bioaccumulative substances as stated in Annex XIII of REACH, and the proposed substance is considered vB.

6.2.1.3. Toxicity

A bacterial reverse mutation assay with four *Salmonella typhimurium* strains and an in vitro chromosome aberration test with human lymphocytes showed that the proposed substance is not a mutagen. There was no toxicological data available on the carcinogenicity or reproductive toxicity of this substance. Concerning the proposed substance's toxicity to the environment, several tests were conducted with aquatic organisms. The EC50 and LC50 values of the proposed substance were found to be >0.34, 5.35 and 0.3 mg/L, for algae, daphnids and fish, respectively. Only the algal EC50 was based on geometric mean measured concentrations, while the daphnid and fish EC50 values were based on nominal concentrations. In addition, the EC50 for daphnids exceeds the limit of the water solubility by approximately a factor of 10. NOECs based on geometric mean concentrations were available for all three taxa and amounted to 0.135, 0.096 and 0.03 mg/L for algae, daphnids and fish, respectively. The reported NOECs do not meet the T criterion (NOEC < 0.01 mg/L) that is stated in Annex XIII of REACH. The proposed substance has been self-classified by many notifiers in the ECHA C&L inventory as a STOT RE2 substance having specific target organ toxicity after repeated exposure. This would suffice as evidence of chronic toxicity, if the classification was harmonised. The evaluating MSCA concluded that the available repeated dose toxicity study would most likely be insufficient to pursue a harmonised classification of the proposed substance as STOT RE 2. Therefore, the proposed substance cannot be considered T based on the available data. Thus while there are indications that the proposed substance is toxic, the T assessment for the proposed substance is inconclusive.

6.2.2. Summary and overall conclusions on the PBT and vPvB properties

In conclusion, the proposed substance is identified as a vPvB substance according to Art. 57(e) of REACH by comparing all relevant and available information listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination. In addition, it should be noted that the proposed substance is considered to be borderline T.

Part II

7. Manufacture, import and export

Information on manufacture, import and export is confidential and therefore it cannot be provided.

8. Information on uses of the substance

Information on uses is confidential and therefore it cannot be provided.

9. Release and exposure from uses

Information on releases and exposure is confidential and therefore it cannot be provided.

10. Current knowledge on alternatives

Information on alternatives is confidential and therefore it cannot be provided.

11. Existing EU legislation

Not applicable.

12. Previous assessments by other authorities

None.

13. Executive summary of information on manufacture, use, exposure and alternatives

Not applicable.

REFERENCES

Due to confidentiality issues, references have been replaced in the main text by annotations such as [1], when deemed appropriate. The references reported in Sections ”References for Part I” and ”References for Part II” below only refer to non-confidential references.

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References for Part II

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Annex I - Self-Classifications of the proposed substance

The proposed substance is not listed in part 3 of Annex VI to the CLP Regulation.

There are nine aggregated notifications, corresponding to 967 notifiers, for the substance with EC number 413-720-9 that is covered by the group entry. The self-classifications according to Regulation (EC) No. 1272/2008 from ECHA's C&L Inventory database (accessed 13.02.2015) are provided below to give some indications on the hazards of the substance.

Table 5: Self-classifications of the substance with EC number 413-720-9

Hazard Class and Category Code(s)	**Hazard statement Code(s)**
STOT RE 2	H373: May cause damage to organs through prolonged or repeated exposure
Acute Tox 4	H302: Harmful if swallowed
Aquatic Acute 1	H400: Very toxic to aquatic life
Aquatic Chronic 1	H410: Very toxic to aquatic life with long lasting effects

The substance with CAS number 117933-89-8 that is covered by the group entry, is pre-registered (envisaged registration deadline 31/05/2018) has no self classifications in ECHA's C&L Inventory database.

> The substance Reaction mass of 5-[(2R)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2R)-butan-2-yl]-2-[(1R,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane

that is also covered by the group entry, is registered for 1-10 tonnes per annum by IFF Benicarlo, S.L. (Spain). There are no self classifications in ECHA's C&L Inventory database. However, in the registration dossier a more comprehensive self-classification is provided (Table 6).

> Table 6. Self classification of substance Reaction mass of 5-[(2R)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2R)-butan-2-yl]-2-[(1R,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1R,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,2R)-2,4-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane and 5-[(2S)-butan-2-yl]-2-[(1S,6R)-4,6-dimethylcyclohex-3-en-1-yl]-5-methyl-1,3-dioxane

Hazard Class and Category Code(s)	**Hazard statement Code(s)**	**Specific Concentration limits, M-Factors**
STOT RE 2	H373: May cause damage to organs through prolonged or repeated exposure. Affected organs: kidney and liver. Route of exposure: oral.	
Aquatic Acute 1	H400: Very toxic to aquatic life	M=1
Aquatic Chronic 1	H410: Very toxic to aquatic life with long lasting effects	M(chronic)=1