SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Resin acids and Rosin acids, hydrogenated, esters with glycerol

EC No 266-042-9

CAS No 65997-13-9

Evaluating Member State(s): Finland

Dated: 8 July 2021

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2015

Before concluding the substance evaluation a Decision to request further information was issued on 7 February 2017

Further information on registered substances here:

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

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

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

Resin acids and Rosin acids, hydrogenated, esters with glycerol ("HRGE"), i.e. the Substance, was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Wide dispersive use
- Exposure of environment
- Consumer use
- High (aggregated) tonnage

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Dossier evaluation and testing proposals under REACH

Extended One Generation Reproductive Toxicity Study (EOGRTS)

- Initially there was a testing proposal decision (ECHA, 2014) to request an OECD TG 416 (old two-generation reproductive toxicity study guideline). All requests for an OECD TG 416 studies were converted by the Commission (COM Decision, 2018) to an OECD TG 443 (EOGRTS). EOGRTS has not been requested under ECHA's testing proposal or compliance check decision.

Prenatal developmental toxicity test

- Under a testing proposal decision (ECHA 2014) the following has been requested:

  Pre-natal developmental toxicity study in rats or rabbits, oral route (Annex IX, 8.7.2.; test method: EU B.31/OECD 414) to be carried out using the analogue substances Resin acids and Rosin acids, methyl esters (CAS No. 68186-14-1); Resin acids and Rosin acids, esters with ethylene glycol (CAS No. 68512-65-2); Resin acids and Rosin acids, esters with triethylene glycol (CAS No. 8050-25-7); Resin acids and Rosin acids, esters with glycerol (CAS No. 8050-31-5); and Resin acids and Rosin acids, esters with pentaerythriol (CAS No. 8050-26-8).

  Follow-up evaluation is ongoing.

Repeated dose toxicity and screening for reproductive toxicity

- The dossier has been updated with several Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD TG 422) and Repeated Dose 90-Day Oral Toxicity studies (OECD TG 408) conducted with read across substances. Screening studies or 90-d study have not been requested under ECHA's TPE or CCH decision. A testing proposal that proposed 90-d studies (OECD Guideline 408, rat, oral route) to be performed on Resin acids and Rosin acids, esters with ethylene glycol (CAS No. 68512-65-2); Resin acids and Rosin acids, esters with triethylene glycol (CAS No. 8050-25-7); and an additional sub-chronic toxicity study to be conducted on Esters of rosin oligomers with pentaerythritol (CAS No. 65997-12-8) was rejected.

Evaluation under regulation on food additives

- Re-evaluation of glycerol esters of wood rosin (E 445) (CAS No. 8050-31-5) as a food additive Annex II of Regulation (EC) No 1333/2008 (EFSA ANS Panel 2018) [This is a read across substance]. The Panel recommended the European Commission to consider: "requesting the provision of a reproductive and developmental toxicity study, in accordance with the applicable OECD test guidelines, using a test material which is representative of the food additive present on the market and taking into account the above recommendations for the update of the specifications."

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State (eMSCA) to the following conclusions, as 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	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level[a]	✓

[a] Currently no need for regulatory follow-up action at EU level. See Section 5.

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

Not applicable.

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

Not applicaple.

4.1.3. Restriction

Not applicaple.

4.1.4. Other EU-wide regulatory risk management measures

Not applicaple.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

Currently there is no need for regulatory follow-up action at EU level. However, this will be reconsidered outside this substance evaluation and taking into account information foreseen from another ongoing regulatory process. See this section, and Sections 6 and 7.11. for further information.

Table 2.

REASON FOR REMOVED CONCERN The concern could be removed because	Tick box
Clarification of hazard properties/exposure	✓
Actions by the registrants to ensure safety, as reflected in the registration dossiers(e.g. change in supported uses, applied risk management measures, etc.)	

Based on the available data the eMSCA concluded that the majority of the constituents of the Substance are not PBT and not vPvB, under aerobic conditions. However, the concern for PBT/vPvB could still not be excluded due to incomplete information for one of the constituent fractions and for transformation products which may be formed under anaerobic conditions from resin acids and rosin acids. Resin acids and rosin acids are present in the Substance and are also potential transformation products of the ester constituents of the Substance. Further information requests under the current substance evaluation process are, however, not warranted. The eMSCA will further assess these PBT/vPvB concerns outside of substance evaluation.

5.2. Other actions

The eMSCA will review whether the information requested under compliance check on Turpentine, oil (EC number 232-350-7) is relevant for the PBT assessment of the Substance, as explained in Section 7.11.4. The requested information on Turpentine, oil, must be provided by 25 May 2025. The PBT/vPvB status of the Substance and the need for risk management measures should then be reconsidered based on the new data, if necessary.

The eMSCA will clarify whether there is a risk associated with the degradation/transformation products with potential PBT/vPvB properties which may be produced under anaerobic conditions from resin acids and rosin acids. Resin acids and rosin acids are constituents of the Substance and they may also be produced from precursors present in the Substance (including at least the mono-, di-, and triesters with glycerol). If a risk is confirmed, the need for risk management measures should be assessed. It should be noted that the same transformation products may be procuded from all substances containing resin acids and rosin acids or their precursors. Therefore, the same risk may arise also from other substances containing resin acids and rosin acids or their precursors.

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

Table 3.

FOLLOW-UP Follow-up action	Date for intention	Actor
Review whether information generated in an ongoing compliance check evaluation of Turpentine, oil (EC number 232-350-7) is relevant for the PBT assessment of the Substance. Consider whether this has implications to the PBT/vPvB conclusion of the Substance and risk management.	2025-2026	The eMSCA, i.e. Finnish Safety and Chemicals Agency (Tukes)
Consider whether there is a risk with transformation products with potential PBT/vPvB properties which may be produced under anaerobic conditions from resin acids and rosin acids. Resin acids and rosin acids are constituents of the Substance and they may also be produced from precursors present in the Substance (the mono-, di-, and triesters with glycerol and potentially also constituents of the heavy ends fraction).	2024	The eMSCA, i.e. Finnish Safety and Chemicals Agency (Tukes)
Under TPE the following has been requested: Pre-natal developmental toxicity study in rats or rabbits, oral route (Annex IX, 8.7.2.; test method: EU B.31/OECD 414) to be carried out using the analogue substances Resin acids and Rosin acids, methyl esters (CAS No. 68186-14-1); Resin acids and Rosin acids, esters with ethylene glycol (CAS No. 68512-65-2); Resin acids and Rosin acids, esters with triethylene glycol (CAS No. 8050-25-7); Resin acids and Rosin acids, esters with glycerol (CAS No. 8050-31-5); and Resin acids and Rosin acids, esters with pentaerythriol (CAS No. 8050-26-8). Follow-up evaluation is ongoing. The Commission decision requested the addressees to update their registration dossiers (several options given): new testing proposal for EOGRTS, or as an alternative to a testing proposal, the registrant(s) can provide an adequate justification for adaptation of the standard testing regime (column 2 adaptation at Annex IX or X, or Annex XI adaptation), or a robust study symmary of an existing study fulfilling the information requirement. EOGRTS has not been requested under ECHA's TPE or CCH decision (at least so far).	Ongoing	ECHA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Resin acids and Rosin acids, hydrogenated, esters with glycerol (HRGE), i.e. the Substance, was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB;
- Wide dispersive use;
- Exposure of environment;
- Consumer use;
- High (aggregated) tonnage.

Table 4.

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
PBT/vPvB	Most of the constituents of the Substance are considered not PBT and not vPvB, under aerobic conditions. However, information regarding one of the constituent fractions (the light ends fraction) is insufficient to conclude on its PBT/vPvB properties. Further information requests regarding the light ends fraction under substance evaluation are not warranted due to the ongoing compliance check on another substance Turpentine, oil (EC 230-352-7), which is foreseen to produce potentially relevant information. Therefore, this will be assessed and addressed outside the substance evaluation. In addition, resin acids and rosin acids, which are constituents of the Substance and which may also be produced from the precursors present in the Substance (the mono-, di-, and triesters with glycerol and potentially also constituents of the heavy ends fraction) may have the potential for biotransforming into potential PBT/vPvB degradation/transformation products under anaerobic conditions. This will be assessed and addressed outside this substance evaluation. See Section 7.11.4 for details.
Exposure/Wide dispersive use, exposure of environment, consumer use	Exposure of humans and the environment to the substance is expected. Currently no hazards have been identified and therefore exposure assessment is not considered relevant.

7.2. Procedure

Category and analogue approach

For the purpose of REACH registration, the registrant(s) presented a testing strategy comprising of categorizing rosin substances, testing of representative substances and applying read-across to other analogue substances.

The Substance belongs to a category of 12 chemically related rosin esters:

Name	**Abbreviations**	**CAS Number**
Rosin, methyl ester	RME	68186-14-1
Rosin, hydrogenated, methyl ester	HRME	8050-15-5
Rosin, ethylene glycol ester	REGE	68512-65-2
Rosin, diethylene glycol ester		68153-38-8
Rosin, triethylene glycol ester		8050-25-7
Rosin, hydrogenated triethylene glycol ester		68648-53-3
Rosin, glycerol ester	RGE	8050-31-5
Rosin, hydrogenated, glycerol ester (the Substance)	**HRGE**	65997-13-9
Rosin, pentaerythritol ester	RPE	8050-26-8
Rosin, hydrogenated, pentaerythritol ester	HRPE	64365-17-9
Rosin, oligomers, glycerol ester		68475-37-6
Rosin, oligomers, pentaerythritol ester		65997-12-8

PBT/vPvB screening

A PBT/vPvB screening was conducted for the Substance in 2012-2013, together with a structural analogue 'Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol (HRPE), EC No 264-848-5' and a PBT factsheet was compiled based on the evaluation (No 37 and 38, May 23, 2014). The assessment was discussed in ECHA's PBT Expert Group in 15 November 2012 and in 12-13 March 2013.

The Substance and HRPE are UVCB substances and based on a screening level assessment, it was concluded that some of the ester constituents of these substances may have PBT/vPvB properties, depending on the level of esterification. The conclusion was, however, based on limited information.

For the screening assessment, experimental data was available only for the UVCB substances as such (ready biodegradation tests, acute ecotoxicity tests and toxicity tests). There was no experimental data on bioaccumulation. QSAR predictions (and log Kow and water solubility values) on the constituents of the UVCB substances showed that the PBT-properties of the constituents may differ significantly.

Based on molecular size and log Kow values, it could be concluded that the larger molecules are unlikely to bioaccumulate (log Kow > 10; Dmax aver > 1.7 nm), whereas the smaller molecules (monoesters) may have bioaccumulation potential (log Kow 5.22 for the monoester constituents of the Substance). On the other hand QSAR results indicated that the monoesters may be more easily biodegraded. Nevertheless, it was considered not possible to conclude on the PBT-properties without more information on the constituents. Therefore, the substances (HRGE and HRPE) were proposed for Substance Evaluation in 2015.

Substance evaluation procedure

The substance evaluation was initiated in 2015 and it focussed on PBT/vPvB properties. Also the potential need for exposure assessment was considered. The initial substance evaluation was based on the data from the original dossier, QSAR modelling by the eMSCA, and publically available literature. The eMSCA submitted a draft SEv decision in 2016, and the final SEv decision was issued by ECHA on 7 February 2017 (ECHA 2017a). The decision included a sequential testing strategy to resolve the PBT/vPvB properties. The registrant(s) conducted the first requested test, a ready biodegradation study, and uptaded their dossier, concluding that the substance is not P/vP. The eMSCA assessed the study and considered that the study was incomplete to resolve the P/vP concern and additional information was still considered necessary to assess the biodegradability. Therefore, the registrant(s) conducted another biodegradability study. The eMSCA considered the SEV information request fulfilled on 29th May 2019, conducted a follow-up assessment and submitted a substance evaluation conclusion document on 29th May 2020.

During the substance evaluation process, additional information has been submitted to the eMSCA by the registrant(s). This information has been used for the assessment by the eMSCA but is not necessarily available in the registration dossier. The substance evaluation was discussed in ECHA's PBT Expert Group during the initial evaluation year (meeting on November 17-18, 2015) as well as at the follow-up evaluation phase (meeting on 8-9 May, 2018). In addition, a written procedure on the draft assessment report was organized for PBT Expert Group in April-May 2020. The feedback from the PBT Expert Group was taken into account in the assessment.

Assessment approach

Some parts related to the assessment of persistence, bioaccumulation and toxicity of the Substance and HRPE and their constituents were combined in the substance evaluation reports of both of the substances. This parallel treatment was considered beneficial for the assessment and efficient use of all available data as the substances belong to the same category of structurally analogous rosin esters, and read-across within this category has been applied also in the registration dossiers, i.e. the same experimental studies have been exploited in the dossiers of both substances.

The guidance on PBT/vPvB assessment (ECHA, 2017b) considers applicable approaches for the assessment of different types of substances. The PBT assessment of a monoconstituent substance would generally proceed stepwise with the assessment of potential persistence addressed first, followed by bioaccumulation (if the P criteria is met) and then toxicity testing (if both P and B are met). Instead, for multiconstituent substances and UVCBs the assessment strategy may need to be evaluated and treated on a case-by-case basis, depending upon the ease and cost of generating new data and animal welfare considerations.

Based on the PBT screening level information it was decided to focus the substance evaluation primarily on the monoester constituents of the Substance and HRPE and try to solve the concerns related to P and B properties at first. The available toxicological and ecotoxicological information was evaluated as well.

The Substance and HRPE contain different types of monoesters, with variation in the resin acid moiety of the ester. Also the level of hydrogenation varies. For the monoesters with glycerol, two positional isomers exist (See Annex 2). For the PBT assessment of the Substance, glycerol monoesters with dihydroabietic acid (DHAA-mono-GE) and tetrahydroabietic acid (THAA-mono-GE) were identified as most relevant constituents. Composition information of the Substance has been considered in the selection of the representative constituents; however, information supporting the selection is available also in published sources. Dihydroabietic acid has been demonstrated to be the major component in hydrogenated rosin which is used to produce the esters, and the presence of tetrahydroabietic acid may also be significant (Environment Canada 2011). Tetrahydroabietic acid monoesters with glycerol were chosen for the assessment as tetrahydroabietic acid represents the most hydrogenated form of the resin acids. DHAA

monoesters with glycerol and pentaerythritol represents a partially hydrogenated rosin. THAA-mono-GE is considered to present the most persistent of the monoesters present in HRGE as hydrogenation generally increases stability.

The SMILES codes of the studied constituents are shown in **Annex 1** and the structural formulas in **Annex 2**.

7.3. Identity of the substance

Table 5.

SUBSTANCE IDENTITY	
Public name:	Resin acids and Rosin acids, hydrogenated, esters with glycerol
EC number:	266-042-9
CAS number:	65997-13-9
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	UVCB
Molecular weight range:	-
Synonyms:	Rosin, hydrogenated, esters with glycerol, HRGE

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

Structural formula: UVCB, unspecified

Table 6.

MAIN CONSTITUENTS	
Constituents	**Typical concentration**
Resin acids, hydrogenated, tri-esters with glycerol	Confidential
Resin acids, hydrogenated, di-esters with glycerol	Confidential
Mixture of dimerised esters, acids and polyol	Confidential

7.4. Physico-chemical properties

Table 7.

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Solid amber coloured pellets
Vapour pressure	< 1 mBar (<100 Pa) (20 °C)
Water solubility	(1) 44 mg/L (EU A.6) (2) 0.15 mg/L (20 °C) (OECD 105)
Partition coefficient n-octanol/water (Log Kow)	(1) 3.28 (4.55 < pH < 5.07) (EU A.8) (2) 4.7 - 5.8 (unbuffered) (OECD 117)
Flammability	Not highly flammable (EU A.10)
Explosive properties	No chemical groups present associated with explosive or self reactive properties
Oxidising properties	Not capable of reacting exothermically with combustible materials based on chemical structures
Granulometry	N/A
Stability in organic solvents and identity of relevant degradation products	N/A
Dissociation constant	N/A

The water solubilities (Table 8) decrease and logKow values (Table 9) decrease remarkably as the size of the molecule grows (from monoesterified to di- and triesterified structures).

Table 8. Water solubility predicted by EPI Suite for representative ester constituents.

WATER SOLUBILITY				
Para-meter	**THAA-mono- GE**	**DHAA-mono- GE**	**DHAA-di-GE**	**DHAA- tri-GE**
Water solubilitymg/L WSKOW	0.1194	0.1453	6.365 e-009	9.753 e-018
Water solubilitymg/L WatSol	1.8891	2.5357	1.2995 e-006	9.5148 e-007

Table 9. Log Kow values predicted by EPI Suite KOWWIN for representative ester constituents of HRGE.

PARTITION COEFFICIENT				
Parameter	**THAA- mono- GE**	**DHAA- mono- GE**	**DHAA- di-GE**	**DHAA- tri-GE**
Log Kow (KOWWIN)	**5.30**	**5.22**	12.28	19.74

7.5. Manufacture and uses

7.5.1. Quantities

Table 10.

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

* last update of the annual tonnage band in the registration dossiers in 2013/2014

7.5.2. Overview of uses

Table 11.

USES	Use(s)
Uses as intermediate	N/A
Formulation	Use in closed or batch processes, transfer of substance to containers, production of preparations by tabletting, compression, extrusion, pelletisation
Uses at industrial sites	Use as reactive prosessing aid, inclusion into or onto a matrix, use as monomers for manufacture of thermoplastics, use in coatings, cleaning agents, binders, release agents, adhesives, use in rubber production and processing
Uses by professional workers	Roller application and spraying, treatment of articles by dipping and pouring, use as laboratory reagent, hand-mixing, use in cleaning agents, binders and realese agents and adhesives, use in road and construction applications, use in agrochemicals
Consumer Uses	Use in coatings, adhesives, sealants, anti-freeze and de-icing products, biocidal products, paints, thinners, paint removers, fillers, putties, plasters, modelling clay, finger paints, non-metal surface treatment products, ink and toners, leather tanning, dye, finishing, impregnation, lubricants, greases, polishes, wax blends, textile dyes, cleaning products, welding and soldering products, fragrances, cosmetics, agrochemicals
Article service life	Articles containing adhesives and sealants

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

The Substance has no harmonized classification.

7.6.2. Self-classification

- In the registration(s)*:*

Based on the registrated substance factsheet, the Substance has not been self-classified in the registration.

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

Aquatic Chronic 4 (H413)

7.7. Environmental fate properties

7.7.1. Degradation

As the concern under evaluation was PBT/vPvB properties, the degradation assessment is focused to aspects relevant to the PBT/vPvB assessment, i.e. comparison to the P/vP criteria. An UVCB substance can be identified as a PBT/vPvB substance when the PBT or vPvB criteria are fulfilled for the same constituent present in relevant concentrations (generally ≥ 0.1%). Estimations show that glycerol monoesters of hydrogenated resin

acids have the highest likelihood to fulfill the B criterion compared to the respective glycerol di- and triesters (see 7.7.3). The persistence assessment was focused on the persistence of the glycerol monoesters of hydrogenated resin acids in relation to the P/vP criteria.

For the PBT assessment, glycerol monoesters with dihydroabietic acid (DHAA-mono-GE) and tetrahydroabietic acid (THAA-mono-GE) were identified as most relevant constituents (see 0)

The SMILES codes of the studied constituents are shown in Annex 1 and the structural formulas in Annex 2.

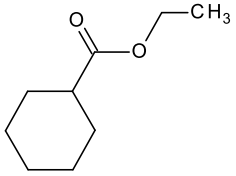
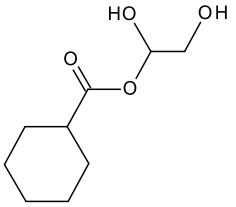
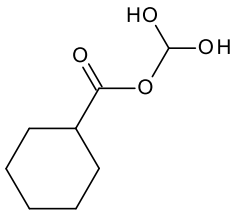
7.7.1.1. Abiotic degradation

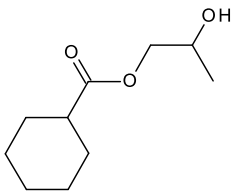
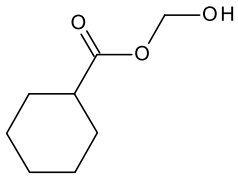
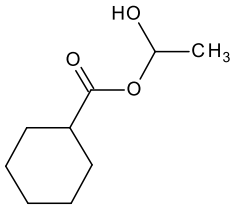
7.7.1.1.1 Hydrolysis

There is no hydrolysis data available in the registrated substance factsheet for the Substance ("data waiving" is mentioned for hydrolysis). However, the registration dossier includes a non-guideline hydrolysis test. In addition, the possibility to use HYDROWIN QSAR model to estimate the hydrolysis of the selected monoesters of resin acids with glycerol was explored by the eMSCA.

The HYDROWIN v2.00 (U.S. Environmental Protection Agency, 2010) results are presented in Table 12. The molecular fragments of the selected monoesters were not identified by HYDROWIN and therefore the program used substitute fragments for the prediction. HYDROWIN guidance mentions that "the substitute selected by the program may not be the best substitute selection". In this case, HYDROWIN used a substitute fragment [-CH2-CH3] for the glycerol part of the monoestersand, [-cyclohexyl] for the resin acid part. These substitute fragments clearly differ from the studied constituents. For example, the [-CH2-CH3] fragment does not have any hydroxy (OH-) groups unlike the glycerol part of the monoesters. HYDROWIN estimations using substitute fragments with hydroxyl group(s) give clearly higher hydrolysis rates (Table 12). However, these substitute fragments are still different from the monoesters of resin acids with glycerol and therefore the hydrolysis half-lives obtained are consireded not reliable. It can still be noteworthy that the shorter hydrolysis half-lives with structures with hydroxyl groups suggest that hydrolysis rates of glycerol monoesters may be higher than those predicted by the default substitute fragments selected by the model.

Table 12. Hydrowin modelling

HYDROWIN MODELLING					
Model run number	**Substitute fragments (R1; R2)[a]; SMILES strings used for the modelling**	**Structural formula**	**Kb Half-Life at pH 8**	**Kb Half-Life at pH 7**	**Remarks**
1	-cyclohexyl; -CH2-CH3 (default fragments given by the model for DHAA-mono-GE and THAA-mono-GE (alpha and beta isomers)); SMILES strings used: DHAA-mono-GE, alpha and beta isomers; THAA-mono-GE, alpha and beta isomers (Annex 2)		1.919 years	19.191 years	R1 substantially differs from the studied constituents. R2 fragment does not include any OH groups.
2	-cyclohexyl; -CH(OH)-CH2-OH; SMILES: OCC(O)OC(=O)C1CCCCC1		30.548 days	305.480 days	R1 substantially differs from the studied constituents. R2 group includes 2 OH groups which are at the same positions as in the glycerol moiety of the alpha isomers of DHAA-mono-GE and THAA-mono-GE
3	-cyclohexyl; -CH(-OH)2; SMILES: OC(O)OC(=O)C1CCCCC1		16.978 hours	7.074 days	R1 substantially differs from the studied constituents. R2 group is two carbons smaller than glycerol and includes 2 OH groups which are located at the same carbon (unlike in glycerol).

HYDROWIN MODELLING					
Model run number	**Substitute fragments (R1; R2)[a]; SMILES strings used for the modelling**	**Structural formula**	**Kb Half-Life at pH 8**	**Kb Half-Life at pH 7**	**Remarks**
4	-cyclohexyl;-CH2-CH(OH)-CH3; SMILES: CC(O)COC(=O)C1CCCCC1)		1.919 years	19.191 years	Same results as for the model run 1 (fragments not recognized although R2 fragment is included in model library)
5	-cyclohexyl; -CH2-OH; SMILES: OCOC(=O)C1CCCCC1		25.362 days	253.619 days	R1 substantially differs from the studied constituents. R2 group is two carbons smaller than glycerol and includes 1 OH group.
6	-cyclohexyl; -CH(OH)-CH3; SMILES: CC(O)OC(=O)C1CCCCC1		59.181 days	1.620 years	R1 substantially differs from the studied constituents. R2 group is one carbon smaller than glycerol and includes 1 OH group.

[a] ESTER: R1-C(=O)-O-R2). Substitute fragments were used because fragments on the studied constituents are not available from the fragment library of the model.

The registration dossier includes a report on investigations on the hydrolytic stability of esters of resin acids and rosin acids (e.g., with glycerol). No significant hydrolysis was detected in any of the test conditions and it is concluded in the report that the results proofed the esters' hydrolytic stability. It is not possible to obtain information on hydrolysis specifically for the monoesters from this study.

There is a published non-guideline study on degradation of polymerized rosin esters of glycerol and pentaerythritol in water solution (Fulzele *et al.* 2007). From molecular weight estimations it can be concluded that degradation took place. The reduction in molecular weight during 3 months was 43% or 39% of the initial molecular weight of the polymerized resin acids esterified with glycerol and pentaerythritol, respectively, whereas for polymerized rosin (which does not include ester bonds), the decrease was 27% (percentage reductions calculated by the eMSCA from data presented in Table 1 and Table 2 in Fulzele *et al.* (2007).

Sahu *et al.* (1999) studied *in vitro* hydrolytic degradation of rosin-glycerol ester derivative in phosphate buffered saline solutions (pH 4.4, 7.4, and 10.5) with 0.03% sodium azide, at 37±1°C. Buffer solutions were refreshed once the pH change was greater than 1.0. The maximum percentage weight loss occurred at pH 10.5 (7.54% after 90 days). The authors

mention that rosin glycerol ester is hydrophobic in nature and hence may not be expected to undergo significant hydrolytic degradation without the involvement of a biological source.

The relevance of the published studies (Fulzele *et al.* 2007, Sahu *et al.* 1999) to the present assessment is rather low as the substances studied were esters of polymerized rosins (Fulzele *et al.* 2007), the representativeness of the samples (e.g., pre-treatment) for the present assessment is questionable or not known, and the concentrations of the constituents and degradation products were not identified but degradation was only followed based on decrease in molecular weight or percentage weight loss.

In conclusion, no reliable data is available for concluding on abiotic hydrolysis for the Substance or for the selected constituents. QSARs (HYDROWIN) provide indications that hydrolysis is a possible degradation mechanism for the monoesters of resin acids with glycerol. However, the predicted (HYDROWIN) and observed rates of hydrolysis suggest that it is highly unlikely that hydrolysis rates in environmentally relevant conditions would be sufficiently high to rule out PBT/vPvB concern.

7.7.1.1.2 Phototransformation and photolysis

In the registrated substance factsheet no information is included for photodegradation. Photodegradation in air was estimated for the selected components by the eMSCA (Table 13). Based on a QSAR calculation using AOPWIN v1.92, the selected monoester constituents are susceptible to indirect photodegradation in air. The estimated half time for the reaction with OH-radicals is 0.09 days for DHAA-mono-GE and THAA-mono-GE (Table 13). Therefore, indirect photodegradation may be an important environmental fate process for these constituents. The predicted half-lives in air are below the criterion for persistent organic pollutants (POP) (2 d) as defined in the Annex D of the Stockholm convention (Stockholm Convention, 2009) and therefore the constituents are not expected to have long-range transport potential.

Phototransformation and photolysis in water and in soil were not included in this assessment.

Table 13. AOPWIN modelling of DHAA-mono-GE and THAA-mono-GE.

AOPWIN MODELLING		
Constituent	**Half-life (OH) (25 °C)**	**Half-life (Ozone) (25 °C)**
DHAA-mono-GE	0.085 days (12-hr day; 1.5E6 OH/cm^3)	0.027 days (at 7E11 mol/cm3)
THAA-mono-GE	0.237 days (12-hr day; 1.5E6 OH/cm^3)	Not estimated by the program

7.7.1.2. Biodegradation in water – estimated data

The eMSCA used BIOWIN QSAR models to estimate the biodegradability of the selected monoesters of resin acids with glycerol. The modelling was conducted using BIOWIN 4.10 (U.S. Environmental Protection Agency, 2010). The eMSCA estimated the applicability of the BIOWIN models by considering the ability of the models to recognize the molecular fragments of the structures. The molecular weights of the selected constituents are within the range of the training set compounds used for the Biowin models 1-6.

For the glycerol monoesters (DHAA-mono-GE , THAA-mono-GE) the BIOWIN model 1-4 results (Table 15) suggest that the biodegration would be relatively fast. However, the

molecular fragments included in the models 1-4 cover only a part of the structural formulas of THAA-mono-GE and DHAA-mono-GE and therefore these models are poorly applicable for these constituents. It is further noted that, the BIOWIN 3 result (2.39) is between 2.25 and 2.75, which indicates that more degradation relevant information is generally warranted (ECHA 2017b).

It is also noted that the molecular fragments employed by the BIOWIN 1-4 models do not include the differences in the fragments between DHAA-mono-GE and THAA-mono-GE (i.e., the presence/absence of -C=CH [alkenyl hydrogen], and the number of -CH2- [cyclic] and -CH - [cyclic] fragments. There is a minor difference in the BIOWIN 1-4 results between DHAA-mono-GE and THAA-mono-GE and this difference is explained solely by the difference in molecular weights.

Concerning BIOWIN models 5 and 6, the fragments included in the models completely cover the structural formulas of THAA-mono-GE and DHAA-mono-GE and therefore BIOWIN 5 and 6 can be considered applicable for these constituents. For BIOWIN 6, the result indicates "Not readily biodegradable". For BIOWIN 5, the result is "readily bioderadable"; however the values are close to the cut-off value of 0.5 and therefore the BIOWIN 5 predictions are not strong[2].

The overall predictions ("Ready Biodegradability prediction: YES or NO ") given by the BIOWIN outputs, suggest that THAA-mono-GE and DHAA-mono-GE are readily biodegradable. However, it should be noted that the prediction employs the BIOWIN 3 result which is poorly applicable in this case.

The screening criteria for P and vP based on BIOWIN models (ECHA 2017b) are not fulfilled. However, it should be noted that the BIOWIN screening criteria have only a low weighting in the assessment as BIOWIN models 2 and 3 are poorly applicable for these constituents.

In summary, no strong conclusions on the biodegradability or persistence of DHAA-mono-GE and THAA-mono-GE can be made on the basis of BIOWIN predictions. BIOWIN 5 and 6, which are more applicable for these constituents, give somewhat conflicting results.

[2] According to ECHA guidance borderline predictions which are close to the cut-off between ready and not ready biodegradability should be interpreted with caution. It has for example been proposed not using BioWIN 1, 2, 5, 4 or 6 model predictions with a biodegradability probability score between 0.4 and 0.6. (because the cut off point is 0.5). Such a strategy seems, according to an analysis done by RIVM on the SIDS data set included in OECD 2004, ENV/JM/TG/(2004)26Rev1, to increase the level of predictability (Rorije, 2005). (ECHA 2017a)

Table 14. BIOWIN modelling of DHAA-mono-GE.

BIOWIN MODELLING (DHAA-mono-GE)			
BIOWIN model	**Fragments used in model prediction**	**Result (the same results apply to both alpha and beta isomers of DHAA-mono-GE)**	**Remarks**
1	Aliphatic alcohol [-OH], Ester [-C(=O)-O-C], Carbon with 4 single bonds & no hydrogens	0.6911 (Biodegrades fast)	The fragments included in the model cover only a part of the structural formula. Therefore, the model is poorly applicable for the purpose.
2	as for BIOWIN 1	0.6229 (Biodegrades fast)	as for BIOWIN 1
3	as for BIOWIN 1	2.3985 (Weeks-Months)	as for BIOWIN 1
4	as for BIOWIN 1	3.4826 (Days-Weeks)	as for BIOWIN 1
5	Aliphatic alcohol [-OH], Ester [-C(=O)-O-C], Carbon with 4 single bonds & no hydrogens, Methyl [-CH3], -CH2- [linear], -CH- [linear], -CH2- [cyclic] , -CH - [cyclic], -C=CH [alkenyl hydrogen]	0.5679 (Readily degradable)	The fragments included in the model cover the structural formula completely. Therefore, the model is considered applicable for the purpose. However, the value is close to the cut-off value of 0.5 and therefore the prediction is not strong.
6	as for BIOWIN 5	0.2465 (Not Readily Degradable)	The fragments included in the model cover the structural formula completely. Therefore, the model is considered applicable for the purpose.

Table 15. BIOWIN modelling of THAA-mono-GE.

BIOWIN MODELLING (THAA-mono-GE)			
BIOWIN model	**Fragments used in model prediction**	**Result (the same results apply to both alpha and beta isomers of THAA-mono-GE)**	**Remarks**
1	Aliphatic alcohol [-OH], Ester [-C(=O)-O-C], Carbon with 4 single bonds & no hydrogens	0.6901 (Biodegrades fast)	The fragments included in the model cover only a part of the structural formula. Therefore, the model is not applicable for the purpose.
2	as for BIOWIN 1	0.6162 (Biodegrades fast)	as for BIOWIN 1
3	as for BIOWIN 1	2.3941 (Weeks-Months)	as for BIOWIN 1
4	as for BIOWIN 1	3.4796 (Days-Weeks)	as for BIOWIN 1

5	Aliphatic alcohol [-OH], Ester [-C(=O)-O-C], Carbon with 4 single bonds & no hydrogens, Methyl [-CH3], -CH2- [linear], -CH- [linear], -CH2- [cyclic] , -CH - [cyclic],	0.5879 (Readily degradable)	The fragments included in the model cover the structural formula completely. Therefore, the model is considered applicable for the purpose. However, the value is close to the cut-off value of 0.5 and therefore the prediction is not strong.
6	as for BIOWIN 5	0.2503 (Not Readily Degradable)	The fragments included in the model cover the structural formula completely. Therefore, the model is considered applicable for the purpose

7.7.1.3. Biodegradation in water – screening tests

7.7.1.3.1. Overview of ready biodegradation studies

There are several ready biodegradability tests available in the registration dossiers of the Substance. The tests are summarized in Table 16 whereas more detailed information is available in Table 17. The biodegradation percentages are presented also in Figure 1. Biodegradation percentages of different resin esters in ready biodegradation tests (OECD TG 301 B). Data points present mean or single values. Error bars present the minimum and maximum degradation results. The numbering of the rosin esters is explained in Table 16**Error! Reference source not found.**.

The range of degradation percentages for the Substance was 4.4-47.3%. In addition, there is data for other substances belonging to the same category; for those the degradation percentages range from 0 to 50.7%.

The Substance is thus not readily biodegradable and therefore fulfills the screening criterion for persistence. There is no information available for the degradation of the individual constituents in these tests conducted on UVCB substances. As the PBT assessment is focused on the selected constituents, these ready biodegradation tests do not provide exact information needed for PBT assessment. Therefore, these ready biodegradation tests were not evaluated in more detail for the present assessment.

In a decision on a testing proposal concerning the Substance (ECHA 2014) the categorization of different rosin esters by the registrant(s) is cited:

"The alcohol used in the esterification process influences both the 3-D structure and molecular weight of the products that are formed, and supports informal differentiation of the category members into simple, linear and bulky esters. The former appear potentially susceptible to enzyme breakdown with possible release of the parent reactants, while steric hindrance may lead to internalization of ester bonds in the latter suggesting they may resist enzymatic attack; the linear esters appear intermediate. Their chemical and biological properties may therefore vary in a regular and predictable manner that reflects the underlying structural features present."

It is further mentioned that the "*members of this category are formed by the esterification of rosin with methanol, mono-, di- or triethyleneglycol, glycerol and pentaerythritol*" and that "*they are composed primarily of esterified resin acids, with non-esterified acids generally accounting for less than ▪% of the total*".

The substances studied in the ready biodegradation tests have been differentiated into "simple", "linear", and "bulky" esters (Table 16) based on the following approach mentioned in the ECHA decision (ECHA 2014):

"*Depending on which alcohol that is used for the esterification the resulting ester will be "simple" (i.e. methanol ester), "linear" (i.e. mono-, di- or tri-ethyleneglycol esters), or "bulky" (i.e. glycerol or pentaerythritol esters).*"
The comparison indicates that the "simple" esters (i.e. methanol esters) seem to be more biodegradable compared to the "bulky esters" (such as the Substance) in ready biodegradation tests. Structurally the monoesters in the Substance are more complex than the studied methyl esters but less complex than the di-, tri- or tetraesters in the Substance. Therefore, the apparent differences in degradability may be linked to the differences in structural complexity and suspectibility to enzymatic attack.

Table 16. Summary table of screening tests for biodegradation in water. Tests are performed according to OECD guideline 301 B, with the exception of the two OECD 310 tests (the first rows of the table) that were conducted in response to the substance evaluation decision. Detailed information on these tests is included in Table 19. Results and reliability scores are as given in the REACH registered substance fact sheets)

BIODEGRADATION IN WATER			
Test material	**Degradation (CO_2 production) % after 28 d**	**Ester type[a] (and numbering used in Figure 1)**	**Reference; Registration dossier(s) in which the test is used (the Substance; HRPE)**
Test material (EC name): Test item produced by modifying rosin with mono esters as main products[b]	34	"bulky"[b]	Study report 2017a; the Substance, HRPE
Test material (EC name): Test item produced by modifying rosin with mono esters as main products[b]	37-52	"bulky"	Study report 2019a (the Substance, HRPE)
Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with glycerol; i.e. the Substance	47.3	"bulky" (6)	Study report 2002; the Substance, HRPE (in the HRPE dossier this test is used as read-across)
Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with glycerol; i.e. the Substance	4.4-13	"bulky" (7)	Study report 1988; the Substance, HRPE (in the HRPE dossier this test is used as read-across)
Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol	3	"bulky" (8)	Study report 2002, HRPE, the Substance (in the dossier for the Substance, this test is used as read-across)
Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol	4.9-8.7	"bulky" (9)	Study report 1988a, HRPE, the Substance (in the dossier for the Substance, this test is used as read-across)
Test material (CAS number): 8050-15-5 (See endpoint summary for justification of read-across) "Resin and rosin acids,	17.7-28.3	"simple" (1)	Study report 1988b; the Substance, HRPE

BIODEGRADATION IN WATER			
Test material	**Degradation (CO_2 production) % after 28 d**	**Ester type[a] (and numbering used in Figure 1)**	**Reference; Registration dossier(s) in which the test is used (the Substance; HRPE)**
hydrogenated, ME esters"			
Test material (CAS number): 68186-14-1 (See endpoint summary for justification of read-across) "Resin acids and rosin acids, Methyl esters"	40	”simple” (2)	Study report 2012; the Substance, HRPE
Test material (CAS number): 68186-14-1 (See endpoint summary for justification of read-across) "Resin acids and rosin acids, Methyl esters"	50.7	”simple” (3)	Study report 2002; the Substance, HRPE
Test material (CAS number): 8050-26-8 (See endpoint summary for justification of read-across) "Resin acids and Rosin acids, esters with pentaerythritol"	0	“bulky” (5)	Study report 2002; the Substance, HRPE
Test material (CAS number): 68153-38-8 (See endpoint summary for justification of read-across) "Resin acids and rosin acids esters with diethyleneglycol"	19.7	“linear” (4)	Study report 2002; the Substance, HRPE
Test material (CAS number): 8050-31-5 (See endpoint summary for justification of read-across) "Resin acids and Rosin acids, esters with glycerol"	0	“bulky” (10)	Study report 2002; the Substance, HRPE

[a] Ester type assigned based on information presented by ECHA (2014c) as described in the text above.
[b] Test substance composition indicated in Table 18.

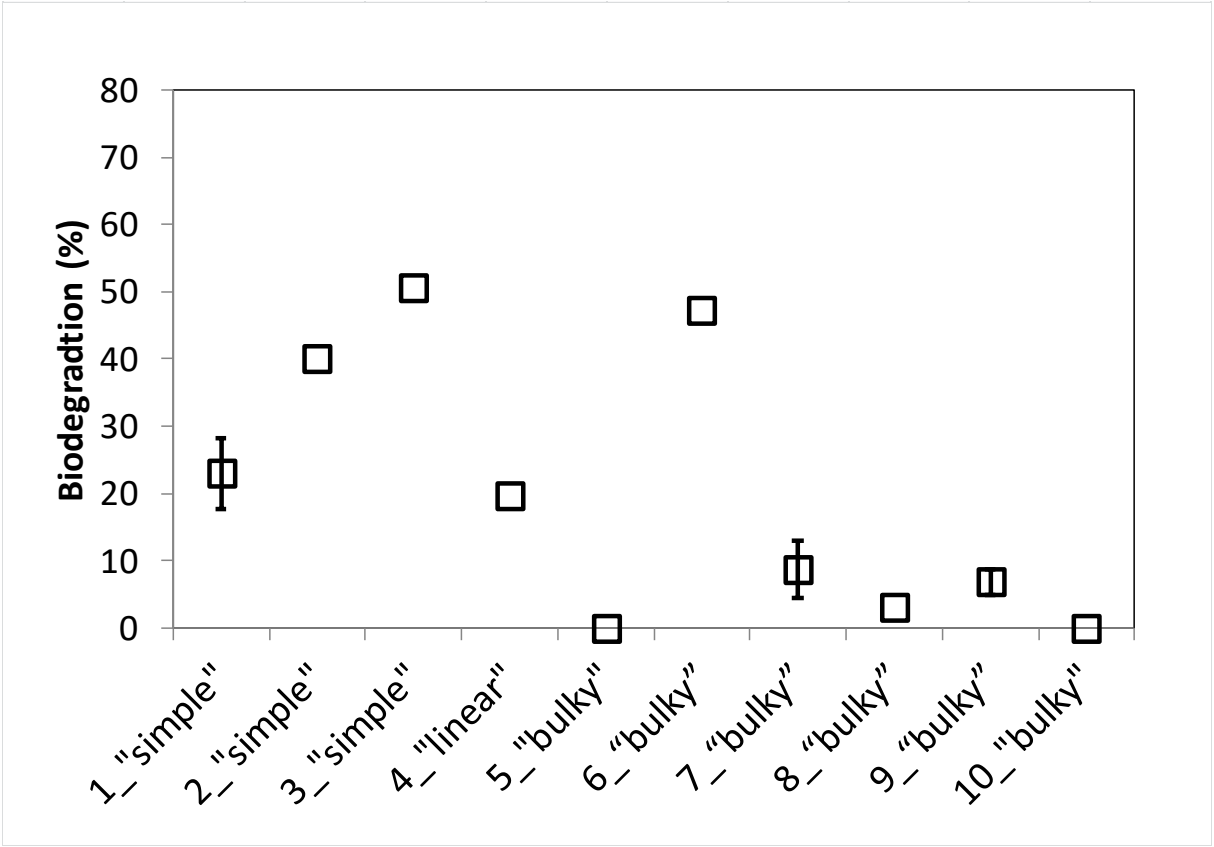


Figure 1. Biodegradation percentages of different resin esters in ready biodegradation tests (OECD TG 301 B). Data points present mean or single values. Error bars present the minimum and maximum degradation results. The numbering of the rosin esters is explained in Table 16. The OECD 310 tests (Study report 2017a, Study report 2019a) are not included in the graph.

Table 17. Detailed information on screening tests for biodegradation in water. Tests are performed according to OECD TG 301 B, with the exception of the two OECD 310 tests (two first rows of the table) that were conducted in response to the substance evaluation decision. Results and reliability scores are as given in the registrated substance factsheet.

SCREENING TEST INFORMATION			
Method	**Results**	**Remarks**	**Reference**
Test type: ready biodegradability activated sludge and standard soilnon-adapted OECD Guideline 310 (Ready Biodegradability - CO_2 in Sealed Vessels (Headspace Test)	Not readily biodegradable % Degradation of test substance: 34% after 28 d (CO_2 evolution) 35% after 60 d (CO_2 evolution) 89% after 28 d (test mat. analysis) 95% after 60 d (test mat. analysis)	1 (reliable without restriction) key study experimental study Test material: Composition of test item (area determined by size exclusion chromatography): 6.2% Tri-ester of Glycerol 10.3% Di-ester of Glycerol 70.7% Mono-ester of Glycerol 12.1% Rosin acids <0.1% Light ends TOC of substance components (as a proportion of test item): 5.0% Tri-ester of Glycerol 8.1% Di-ester of Glycerol 51.9% Mono-ester of Glycerol 9.6% Rosin acids 0.0% Light ends 74.6% Total TOC (calculated) In this test, separate analyses were performed for metabolite identification (see Study report 2017b and Study report 2017c.	Study report 2017a
Test type: ready biodegradability mixture of activated sludge and standard soil, non-adapted OECD Guideline 310 (Ready Biodegradability - CO_2 in Sealed Vessels (Headspace Test)	Not readily biodegradable 37% after 28 d (CO_2 evolution) (old test item) 42% after 45 d (CO_2 evolution) (old test item) 96% after 28 d (test mat. analysis) (old test item) 93% after 45 d (test mat. analysis) (old test item) 52% after 28 d (CO_2 evolution) (new test item) 67% after 60 d (CO_2 evolution) (new test item)	1 (reliable without restriction) key study experimental study Test material (old test item): Monoesters of hydrogenated rosin with glycerol (old test substance): 68% mono rosin ester Test material (old test item): Monoesters of hydrogenated rosin with glycerol (new test substance): 75% mono rosin ester	Study report 2019a

SCREENING TEST INFORMATION			
Method	**Results**	**Remarks**	**Reference**
	83% after 28 d (test mat. analysis) (new test item) 92% after 60 d (test mat. analysis) (new test item		
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	Not readily biodegradable % Degradation of test substance: 17.7 after 28 d (CO_2 evolution) (Low (10 mg/L addition)) 28.3 after 28 d (CO_2 evolution) (High (20 mg/L addition))	2 (reliable with restrictions) key study read-across based on grouping of substances (category approach) **Test material (CAS number): 8050-15-5 ; Resin and rosin acids, hydrogenated, ME esters**	Study report 1988b
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	Not readily biodegradable % Degradation of test substance: 40 after 28 d (CO_2 evolution)	1 (reliable without restriction) key study read-across based on grouping of substances (category approach) **Test material (CAS number): 68186-14-1; "Resin acids and rosin acids, Methyl esters"**	Study report 2012a
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test) EU Method C.4-C (Determination of the "Ready" Biodegradability - Carbon Dioxide Evolution Test)	50.7% biodegradation over 28 days % Degradation of test substance: 50.7 after 28 d (CO_2 evolution)	1 (reliable without restriction) supporting study read-across based on grouping of substances (category approach) **Test material (CAS number): 68186-14-1; "Resin acids and rosin acids, Methyl esters"**	Study report 2002
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	Not readily biodegradable % Degradation of test substance: 47.3 after 28 d (CO_2 evolution)	1 (reliable without restriction) weight of evidence experimental result **Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with glycerol**	Study report 2002

SCREENING TEST INFORMATION			
Method	**Results**	**Remarks**	**Reference**
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	Not readily biodegradable % Degradation of test substance: 13 after 28 d (CO_2 evolution) (Low (10 mg/L addition)) 4.4 after 28 d (CO_2 evolution) (High (20 mg/L addition))	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with glycerol**	Study report 1988a
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	under test conditions no biodegradation observed % Degradation of test substance: 3 after 28 d (CO_2 evolution)	1 (reliable without restriction) weight of evidence read-across based on grouping of substances (category approach) **Test material (CAS number): 64365-17-9; Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol**	Study report 2002
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	not readily biodegradable % Degradation of test substance: 8.7 after 28 d (CO_2 evolution) (Low (10 mg/L addition)) 4.9 after 28 d (CO_2 evolution) (High (20 mg/L addition))	2 (reliable with restrictions) weight of evidence read-across based on grouping of substances (category approach) **Test material (CAS number): 64365-17-9; Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol**	Study report 1988c
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	under test conditions no biodegradation observed % Degradation of test substance: 0 after 28 d (CO_2 evolution)	1 (reliable without restriction) weight of evidence read-across based on grouping of substances (category approach) **Test material (CAS number): 8050-26-8; "Resin acids and Rosin acids, esters with pentaerythritol"**	Study report 2002
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	not readily biodegradable % Degradation of test substance: 19.7 after 28 d (CO_2 evolution)	1 (reliable without restriction) weight of evidence read-across based on grouping of substances (category approach) **Test material (CAS number): 68153-38-8; "Resin acids and rosin acids esters with diethyleneglycol**	Study report 2002

SCREENING TEST INFORMATION			
Method	**Results**	**Remarks**	**Reference**
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	under test conditions no biodegradation observed % Degradation of test substance: 0 after 28 d (CO_2 evolution)	1 (reliable without restriction) weight of evidence read-across based on grouping of substances (category approach) **Test material (CAS number): 8050-31-5; "Resin acids and Rosin acids, esters with glycerol"**	Study report 2002
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	under test conditions no biodegradation observed % Degradation of test substance: 3 after 28 d (CO_2 evolution)	1 (reliable without restriction) weight of evidence experimental result **Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol**	Study report 2002
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	Not readily biodegradable % Degradation of test substance: 8.7 after 28 d (CO_2 evolution) (Low (10 mg/L addition)) 4.9 after 28 d (CO_2 evolution) (High (20 mg/L addition))	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol**	Study report 1988a
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	not readily biodegradable % Degradation of test substance: 47.3 after 28 d (CO_2 evolution)	1 (reliable without restriction) weight of evidence read-across based on grouping of substances (category approach) **Test material (CAS number): 65997-13-9 ; Resin acids and Rosin acids, hydrogenated, esters with glycerol**	Study report 2002
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test)	not readily biodegradable % Degradation of test substance: 13 after 28 d (CO_2 evolution) (Low (10 mg/L addition)) 4.4 after 28 d (CO_2 evolution) (High (20 mg/L addition))	2 (reliable with restrictions) weight of evidence read-across based on grouping of substances (category approach) **Test material (CAS number): 65997-13-9; Resin acids and Rosin acids, hydrogenated, esters with glycerol**	Study report 1988c

7.7.1.3.2. OECD TG 310 studies on monoesters of hydrogenated resin acids with glycerol

Two OECD TG 310 studies were conducted in response to the SEv decision (ECHA 2017a), referred to here as the 2017 study and the 2019 study, respectively. The 2017 study indicated a CO_2 production of 34%, and a significant decrease in monoester concentration was observed. The study was not fully in compliance with the specific requirements in the SEv decision. Due to the relatively low level of ultimate degradation there was a concern that the decrease in monoesters could be partially due to physical removal, such as adsorption, and the extent of primary degradation remained uncertain. The registrant(s) conducted a new OECD TG 310 study (test reports dated 2019) to address this uncertainty.

These studies are described in the following study reports:

OECD TG 310 study (2017):

- Study report (2017a): Main study and GC-MS analyses of monoesters.
- Study report (2017b): GC-MS analyses of resin acids and monoesters.
- Study report (2017c): HPLC analyses of mono- di- and triesters, resin acids, and heavy ends.

OECD TG 310 study (2019):

- Study report (2019a): Main study and GC-MS analyses of monoesters.
- Study report (2019b): Monoester analysis method validation.

These studies are presented and discussed here in parallel.

Test substances

Both OECD TG 310 studies were conducted on test substances which were produced by modifying rosin in order to enrich the monoester fraction. In the 2017 study one test substance was used whereas in the 2019 study two different test substances were used. The test substances of the 2019 study are referred to as the "old test substance" and the "new test substance". Regarding the origin of the test substances, the registrant(s) have provided the following information[3]:

- These names ("old" and "new" test substance) in the 2019 study refer to the different batches of glycerol monoesters of hydrogenated rosin before purification, which were used to produce the test substances.

- The same batch was used to produce the sample tested in the 2017 study and the old test substance in the 2019 study.

- A different batch was used to produce the new test substance in the 2019 study.

The compositions of the test substances are summarized in Table 18. Size exclusion chromatograms were available for the assessment[4]. The concentrations of each fraction are assumed to be equal to the relative proportion of each peak of the total area of the peaks.

It is noted that the compositions of the test substance used for the 2017 study and the old test substance in the 2019 study were relatively similar whereas the new test substance in the 2019 study differed from those two substances. The main differences of the new

[3] E-mail from the registrant(s) to the evaluating MSCA on 24 February 2020
[4] Size exclusion chromatograms for the test substance of the 2017 study are in Study report 2017a and for the test substanes of the 2019 study in additional documents submitted by the registrant(s) to the evaluating MSCA (e-mail on 24 February 2020)

test substance were the higher concentrations of the monoesters, resin acids, and the light ends, and the lower concentrations of di- and triesters. It is also noted that for the old test substance of the 2019, there was no information of the concentrations of di- and triesters specifically but only a sum of the concentrations of these two fractions was reported. The sum of di- and triesters was relatively similar (16.5-19.0 %) in the 2017 study and the old test substance of the 2019 study and lower (5.4 %) in the new test substance of the 2019 study.

There is uncertainty regarding the composition of the light ends fraction and regarding the concentration of glycerol in the test substances. It is stated in the registration dossier that a representative constituent for TOC calculation of the light ends fraction for the OECD TG 310 (2017) study is glycerol. However, in further communications the registrant(s) have indicated that the light ends fraction obtained by GPC covers compounds described as neutrals: neutral monoterpenes, and neutral diterpenes[5]. Regarding the glycerol concentration in the test substances, the registrant(s) provided the following additional information[5]:

- Glycerol was found to be present at 0.7 % in the sample used for the 2017 study.
- The concentration of glycerol in the sample tested in the 2019 study is unknown since this was not analyzed for free glycerol.
- It is expected that the amount of free glycerol in the test item would be very low.
- When the substance was refined to produce the test item it was extracted with water/hexane repeatedly and most of the free glycerol will have dissolved into the water fraction and would have been removed from the sample.

Thus, there is contradictory information regarding the glycerol concentration in the test substance used for the OECD TG 310 (2017) study. It has not been reported how the registrant(s) determined the 0.7% glycerol concentration.

Based on the information above it seems likely that the light ends fraction consisted of monoterpenes and diterpenes rather than of glycerol. Even though the proportion of the light ends fraction in the test substances (0-1.6 %w/w, or 0-3.5 %TOC) is relatively low, it affects the calculations which are based on TOC and therefore this uncertainty was considered in the assessment.

For the test substance in the 2017 study, TOC could not be reliably measured and consequently a calculated TOC was used for the biodegradation calculations (Table 18, Table 19). Due to the low concentration of the light ends fraction (< 0.1% w/w) in the 2017 study the way of calculating the TOC for the light ends (i.e. the light ends assumed to be either glycerol or terpenes) has an insignificant effect on the total TOC (74.60% vs. 74.64%) or proportions of the different fractions of the total TOC (Table 18). Therefore the eMSCA calculated the biodegradation results using the same TOC as used in the registration dossier (74.60%). However, additional calculation was performed by the eMSCA on the assumption that there was 0.0% of terpenes and 0.7% w/w glycerol (total TOC 74.92%).

For the 2019 study, the concentration of the light ends fraction was 0.8-3.0 % w/w and thus it has more effect on the calculated TOC compared to the 2017 study. For the 2019 study, a measured TOC was used for calculating the ultimate biodegradation result in relation to the whole test substance (IC production as a percentage of ThIC of test substance). However, for the estimation of the ultimate degradation of the monoesters it was necessary to calculate the proportions of the different fractions of the TOC so that the IC production per each fraction could be calculated. As the composition affects the TOC

[5] E-mail from the registrant(s) to the evaluating MSCA on 7 May 2020

proportions, the results were calculated in both ways (i.e., light ends assumed either as terpenes or as glycerol).

Test conditions and methods

Test conditions and other methodological information are summarized in Table 19. The duration of the test was 60 and 45 days for the 2017 and 2019 studies, respectively. Thus both studies were extended beyond the standard duration of 28 days. Mineralisation of the test item was followed by total inorganic carbon (TIC) analyses to assess CO_2 production. In both studies the concentrations of the monoester fraction in the test medium were determined. In the 2017 study, also resin acids, diesters, triesters and heavy ends were measured.

Comparison with the requirements of the SEv decision

In Table 20, the tests are compared with the specifications and recommendations in the SEv decision (ECHA 2017a). A sterile control was not included in the 2017 study even though according to the decision it was a mandatory requirement when primary degradation results are used for the conclusion. The 2019 study included a sterile control conducted in accordance with the test guideline. The other specifications set in the the SEV decision are considered either fulfilled or partially fulfilled and no critical deficiencies were found.

Recalculations of the CO_2 production results by the eMSCA

Some of the CO_2 production results of the 2019 study were recalculated by the eMSCA. The reasons are indicated in Table 21.

Regarding the correction for the sterile control, the OECD TG 310 (paragraph 59) states "*If there has been a significant increase in the TIC content of the sterile controls (FS) over the test period, then it may be concluded that abiotic degradation of the test substance has occurred and this must be taken into account in the calculation of D in Equation [2]*". The test guideline (paragraph 65) states "*If in flask FS (abiotic) a significant increase (>10%) in the amount of TIC is observed, abiotic degradation processes may have occurred.*"

It is not known whether the abiotic degradation was similar in sterile controls and active tests. However, the eMSCA considers that if the test substance degrades to CO_2 in the conditions of the test, for the purpose of the current tests there is no need have a certainty on whether the degradation is completely biotic or whether part of it can be abiotic. Thus if abiotic degradation to CO_2 has occurred in the sterile control, it may be occurring also in the active test and in that case it does not need to be differentiated from the degradation in the biotic test. On the other hand, if the abiotic degradation to CO_2 occurred only in the sterile controls (e.g. if formaldehyde is oxidized to CO_2) it should not be taken into account for calculating the biodegradation. Therefore, no correction for sterile control was performed for the CO_2 production in the active tests.

Validity criteria and toxicity controls

Both OECD TG 310 studies fulfill the validity criteria (Table 22), with the exception that for the 2019 study the validity criterion regarding TIC was not met for the day 45 measurement; however, based on the value in a NaOH check vessel it was considered that the TIC in the blanks was <3 mg/L, therefore fulfilling the validity criterion also on day 45.

In toxicity controls CO_2 production was 106% and 120% of the theoretical maximum inorganic carbon production (ThIC) of the reference substance in the 2017 study and 99% and 119% in the 2019 study, after 14 and 28 days, respectively. The corresponding values obtained from equation $[(D_{FC}^1 - D_{FI}^2)/D_{FC}] \times 100$ (OECD TG 310) on day 28 were -32% in the 2017 study and -72% in the 2019 study. As the values were below 25%, it can be concluded that based on the toxicity controls no inhibition by test substance was indicated

in either of the studies. For the toxicity controls, test substance was introduced with solvent in the 2017 study and by direct addition in the 2019 study. For the 2019 study toxicity control was only conducted with the new test substance but not with the old test substance.

GC-MS measurements of monoesters: methods

In both studies the concentrations of the monoester fraction in the test medium were determined by GC-MS analysis of representative monoester constituents after liquid-liquid extraction using dichloromethane (DCM) and derivatisation with trifluoroacetamide (TFAA) (Table 23). In the 2017 study, the monoester analyses were conducted from combined fractions of test medium and solvent (acetone) rinse of the test bottle. Whereas in the 2019 study, the test medium and solvent rinse were analysed separately. The sum of the peak areas of m/z values corresponding the TFAA derivatives of the monoesters were used to quantify the monoester concentration (see Table 23 for more details) (Study report 2017a, Study report 2019a). Three (2019 study) or four (2017 study) peaks were used for the quantification. Each of these peaks consisted of a group of monoesters. In the 2017 study, one group of derivatives was quantified using the m/z value 239 and the three other groups using the m/z values 243, 555, and 570 (Study report 2017a). In the 2019 study, one group of derivatives was quantified using the m/z value 239 and the two other groups using the m/z value of 243 (Study report 2019a). It is not indicated why different amounts of monoester derivatives were used for the quantification in the two studies (2017 and 2019) or to what extent the monoesters studied were the same in both studies. For the 2017, the results were reported also separately for two different fractions of the monoesters (Study report 2017b).

When a resin acid monoester with glycerol is transformed e.g. by hydrolysis, the resin and rosin backbone may still remain (e.g. as resin acids). Therefore, resin acids could interfere with the monoester analysis if co-elution occurred, due to the potential formation of the same ions in the MS detector. It is not stated in the study reports whether co-elution occurred. However, according to the registrant(s) the resin acids and monoesters are not thought to co-elute[6]. It is also stated (Study report 2017b) that different derivatisation method was used for the analysis of both monoesters and resin acids compared to the main study (Study report 2017a) where only the monoesters were quantified. There were also differences in the ions used for the quantification. Comparable results were obtained for the monoesters by both methods. Therefore, the eMSCA considers that co-elution of resin acids and monoesters did not occur and that the use of different ions for the quantification of the monoesters in the 2017 and 2019 studies did not significantly affect the results or the comparability of the 2017 and 2019 studies with each other. The assessment of the degradation of the monoesters in the 2017 study was based on the results from the Study report 2017a, unless otherwise specified.

Two fractions of monoesters were analysed in the 2017 study, reported to represent the monoesters of dehydroabietic acid (DeHAA) and the monoesters of hydrogenated resin acids, respectively (Study report 2017b). Based on the m/z values and structures presented (Table 23) there was a difference in the level of hydrogenation between the two fractions. Based on the proportions of the peak areas, ca. 59% of the studied monoesters in the test substance belonged to the monoesters of DeHAA, and ca. 41% to the monoesters of hydrogenated resin acids.

The registrant(s) have informed that with the GC-MS only hydrogenated resin acids and DeHAA and their monoesters have been analysed of the 2017 study[7]. The other remaining resin acids (and presumably also the other remaining monoesters) were not taken into account in the GC-MS analysis but were included in the resin acid (or monoester) fraction seen on the size exclusion chromatography results (Table 18). It is not known what proportion of the total monoester fraction the monoesters analysed in the GC-MS

[6] E-mail from the registrant(s) to the evaluating MSCA on 7 May 2020.
[7] E-mail from the registrant(s) to the evaluating MSCA on 21 November 2017.

measurements accounted for. The registrant(s) have informed that, in theory, the abietane group (Figure 2) represents 60-80% of the monoesters in the test substance used for the 2017 study (based on the generally 60-80% proportion of abietane group in the type of rosin used for the synthesis of the test substance)[7]. It is unclear whether the compounds analysed by GC-MS included only abietane group rosin structures or whether other types of structures were also included. The structures presented in the 2017 study report only include abietane structures. However, it was not indicated how reliable and accurate the presented structures were and whether the peaks could also have included other structures with the same m/z value. For example, some resin acids belonging to different structural groups have the same molecular weight and therefore could potentially result in same m/z values.[8]

For the 2019 study no calibration curve was derived with the old test substance and the results of the old test substance were calculated based on calibration curve obtained with the new test substance. The reason was that due to the small quantity of monoesters of the test substance available it was not possible to perform the GC-MS analysis using standards made with the old test substance. As indicated in the test report, the GC-MS analysis of the monoesters for the old test substance is "*more indicative than quantitative*". The quantification of the monoesters was based on the sum of the areas of the selected peaks and therefore the eMSCA considers that the calibration curve based on the sum of the main peaks in the new test substance should be to some extent applicable also to the old test substance. In the 2019 study it was reported that there was an additional peak in the old test substance that was not observed in the new test substance (Study report 2019a). The retention time and size of the additional peak were not specified. The eMSCA considers that the presence of additional peak means that there may have been some monoesters present in the old test substance (and also in the test substance for the 2017 study, which was produced using the same batch) which were not present in the new test substance. From the chromatograms (Study report 2019a) for the active tests and sterile controls on day 3, when no significant primary degradation had occurred yet, the following observations were made by the eMSCA:

- The number of peaks with retention times <15 min was not higher in the old test substance compared to the new test substance, despite the higher dilution of the samples for the new test substance.

- Peaks were observed mostly at the same retention times in both test substances, but the relative sizes of the peaks varied between the test substances.

- Three peaks were observed only with the old test substance[9]. However, these peaks were quantitatively not significant[10].

Therefore, the peaks chosen for quantification of the monoesters appear to include the main peaks of the chosen m/z values in both test substances and thus the presence of additional peaks does not significantly weaken the reliability of the concentration measurements based on the selected m/z values. The peaks which were not considered for the quantification in the 2019 study (some of them were present only in one of the two test substances) were most likely not significant for the primary degradation measurement. However, the response of the compounds forming the different peaks in the GC-MS analysis

[8] For example for abietic, isopimaric, neoabietic, palustric, and pimaric acids MW = 302.5 and for dehydroabietic and dihydropimaric acids MW = 304.5.
[9] Peak 1: retention time (RT) approx. 12.3 min (observed in the active test); peak 2 and peak 3 RT approx. 9.3 min. and 12.4 min. (observed in the active test and sterile control)
[10] Peak sizes were not reported in numeric form. The eMSCA made a rough estimation based on the chromatograms, which showed that the heights of the peaks present only in the old test substance were < 4% of the height of the highest peak used for the quantification of the monoesters within the same sample. It is noted that peak height is not accurate indicator of concentrations as the width of the peaks also varied. The difference of concentrations may be higher than the difference of peak heights.

is not necessarily the same, and therefore the differences in the proportions of the peaks may influence the quantification. Therefore, the primary degradation results for the old test substance are associated with increased uncertainty due to the issue with the calibration.

Presentation of the results

The results are presented in **Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, Figure 8, Figure 9, Figure 10,**

Table 24**, and Table 25.** The results are presented and discussed in parallel for both studies (2017 and 2019).

CO_2 production in active tests

In both studies (2017 and 2019) biodegradation (based on % ThIC) in the first 3 days was highest in the 2019 study with the new test substance (2.6% of ThIC) and lower in the 2019 study with the old test substance and in the 2017 study (1-1.5%) (Figure 4). Biodegradation proceeded in the new test substance (2019 study) at the same rate until day 10 and after that some increase in rate was observed until day 21. Biodegradation curve became less steep thereafter, attaining 54% after 28 days and 67% after 45 days, but a plateau was not reached. In the 2017 study and in the 2019 study with the old test substance, a fairly similar degradation was obtained for both, with lower biodegradation (34% after 28 days, 35-36% after 42-45 days). The 2017 study was continued for 60 days and the biodegradation was 35% at the end of the study.

The differences in the degradation curves particularly between the old and new test substances in the 2019 study can potentially be explained by the composition of the test substances and the different methods of introducing the test substance into the test vessels (discussed below under "*Discussion on selected issues important for P/vP assessment*").

CO_2 production in sterile controls

Sterile controls were included in the 2019 study but not in the 2017 study. Figure 3 shows that the IC production in both sterile controls (old and new test substance) increased during the study roughly in the same way as in the solvent control and inoculum blank. The CO_2 production in the sterile control with the old test substance was mostly lower than in the solvent control (or inoculum blank) (Figure 3) but it is noted that on day 0 CO_2 production was highest in the sterile control. The CO_2 production in the sterile control with the new test substance was lower than in the inoculum blank for the first 21 days but higher on days 28-45. The CO_2 production of the sterile control with the old test substance was lower than in the sterile control with the new test substance for the first 14 days.

The fact that CO_2 production in the sterile controls was mostly lower than in the inoculum blank or in solvent control indicates that in the sterile control not only the mineralization of the test substance was inhibited but apparently also CO_2 production from the inoculum was inhibited. This means that correcting the sterile control CO_2 production with the solvent control/inoculum blank (as was done in the test report) results mostly in negative biodegradation values indicating that this correction is not relevant. These results are still presented in Figure 4 for completeness (note that the negative values are converted to zero in Figure 4). These results indicate that in sterile controls, when corrected for the solvent control or inoculum blank (as appropriate), the biodegradation was 0-2% of ThIC after 28 days and 0-0.5% after 45 days in both test substances studied in the 2019 study (Figure 4).

Thus, there was some CO_2 production in the sterile controls, increasing during the study. The test guideline (OECD TG 310) states that "*If in flask FS (abiotic) a significant increase (>10%) in the amount of TIC is observed, abiotic degradation processes may have occurred.*". Accordingly, in the test report, it can be concluded that abiotic degradation may have occurred as the difference in the TIC between day 45 and day 0 was greater than 10%. The increase in the TIC compared to day 0 is greater than 10% on day 45 (and also e.g. on day 28), as can also be seen from Figure 3.

The eMSCA is not aware of other ready biodegradation tests with sterile controls using formaldehyde as toxicant and therefore it is not possible to evaluate whether this is a typical level of IC production in sterile controls with formaldehyde. However, there was a clear difference in the IC production between the active tests and sterile controls (Figure 3), indicating that mineralization of the test substance in the sterile controls was mostly inhibited by the toxicant (formaldehyde).

The OECD TG 310 test guideline does not specifically mention formaldehyde or its suitable concentration as sterilizing agent in sterile controls. However, in another test guideline for degradation (OECD TG 309) it is indicated that biological activity can be stopped by adding formalin at 100 mg/L. In the present study, the concentration of formaldehyde was higher (18 500 mg/L). Formaldehyde is an organic substance that can be degraded to CO_2 (biotically or abiotically) and could therefore contribute to the IC production. Therefore, the eMSCA considers that it cannot be ruled out that a part of the IC produced in the sterile controls was originating from formaldehyde. In the sterile controls formaldehyde contributed for most of the organic C content and thus even degradation of a small proportion of formaldehyde could cause a significant error in the determination of abiotic CO_2 production.

The eMSCA considers that whilst some CO_2 was produced in the sterile controls, it cannot be confirmed whether or not this represents degradation of the test substance. Also, if abiotic degradation of the test substance occurred in the sterile controls, it can occur also in the active tests (and thus does not need to be corrected). If the CO_2 production in sterile controls was due to formaldehyde, it must not be used for correcting the active test result. (See also Table 21).

For the above-mentioned reasons, the eMSCA considers that it is not appropriate in this case to correct the IC production of the active tests for the IC production in sterile controls.

GC-MS measurements of the monoesters: results

In the active tests, the monoester concentration at day 0 was 93, 81, and 93% of the applied concentration (AC), for the 2017 study, and the old and new test substance in the 2019 study, respectively. In the sterile controls, the concentration at day 0 was lower (72 and 82% of AC for the old and new test substances, respectively).

In the active tests, there was a small increase in monoester concentration in the first 3 days in all the tests (Figure 5). This was followed by a steep decrease from day 3 to day 10 in the 2017 study and in the 2019 study with the old test substance (to 8-16% of the AC), followed by further decrease (to 4-12% of AC at day 28, and 6-7% at end of study, i.e. day 45 or 60). In the new test substance, the concentration remained similar for the first 7 days and thereafter a decrease was observed. This decrease was not as steep as with the other test substances and the monoester concentration reached 17% of AC after 28 days and 8% of AC after 45 days (Figure 5).

In the 2017 study the different monoester fractions decreased at different rates. The main study (Study report 2017a) reported the results only using the sum of the studied monoesters; however, it was noted in the report that the main primary degradation was observed from the derivatives with m/z of 239, 551, and 566 (one derivative, peak ID 4) (Table 23), which corresponds to the monoesters of DeHAA. Study report 2017b includes the results for the two different fractions of monoesters as chromatogram peak sizes. Absolute concentrations were not determined. The summed peak area of the monoesters of DeHAA decreased faster than of the monoesters of hydrogenated resin acids. Based on the peak areas only 12% of the initial amount of the monoesters of DeHAA were present on day 7 based on peak areas on day 0 and day 7. In contrast, 65% of the monoesters of hydrogenated resin acids were still present on day 7. The peak areas of monoesters of DeHAA decreased to 2% and the monoesters of hydrogenated resin acids decreased to 28% in 28 days.[11] Figure 9 shows the summed peak areas for the studied groups of monoesters. The measurements of the two different fractions of the monoesters were available for the first 28 days but not for the later part of the study. The results for the

[11] In Study report (2017b) it is indicated that "*By day 28, the concentrations of both groups of monoesters were decreased by approximately 90% or more.*". This statement in the study report is incorrect. The eMSCA noticed an error in the day 28 values (the peak heights were used instead of peak areas). Corrected data indicates that 90% decrease in 28 days was observed only for the monoesters of DeHAA.

total monoester fraction were available until day 60 and from that it can be calculated that the decrease in the hydrogenated resin acids fraction was 90 % after 60 days.[12] For the other test substances the decrease was reported only for the sum of the measured monoesters. Assuming that the ratio of the two monoester fractions was similar in all test substances and that the ratio in the monoesters remaining on day 28 in the 2019 study was similar as observed in the 2017 study, the monoesters of hydrogenated resin acids were decreased by 73-94% after 28 days and 86-89% after 45 days with the two samples studied in the 2019 study, whereas the monoesters of DeHAA were decreased by 98-99% after 28 days and 99-100% after 45 days.

In the sterile controls (included only in the 2019 study), the concentration of the monoester started to decrease after 3-7 days, similarly as in the active tests (Figure 6). With the old test substance, the decrease occurred only until day 7, the monoester concentration attaining the level of 46% of AC. After this, the concentration varied and attained 57% of AC at day 45. With the new test substance the monoester concentration in the sterile control showed a fairly similar decrease as in the active test until day 21 whereas after that there was a high increase in the sterile control (up to 64% of AC at day 45).

The reason for the differences between the day 0 concentrations in the different tests, and particularly between the active tests and sterile controls for the same test substance, is not known.

The analysis of monoesters in the 2019 study included also blank controls for which it is reported that "*additional single replicate was analysed at each chemical analysis point for use as recovery check- it was dosed then underwent the same extraction procedure as other bottles*" (Study report 2019a). The percentage recovery for these blank controls (also referred to as spike samples in the report) varied 82-106% for the measurement days. Thus, in the active tests as well as in the sterile control with the new test substance the monoester concentrations on the results at day 0 were within the range of the spike controls, whereas for the sterile control in the old test substance the concentration was below the spike control range.

When normalized to the concentration at day 0, the monoester concentrations for the 2017 study and for the old test substance in the 2019 study were more similar to each other. When normalized, also the concentrations for the active test and sterile control were more similar to each other for the first 3 or 21 days for the old and new test substance, respectively.

GC-MS measurements of resin acids, diesters, and triesters

In the 2017 study, resin acids (DeHAA, hydrogenated resin acids), and the monoesters, were analysed by GC-MS (Study report 2017b). The results for the monoesters have been presented above under "*Measurement of the monoesters: results".* The results were presented only as chromatogram peak sizes and absolute concentrations were not determined.

On day 0, the ratio of resin acids to monoesters was 0.036 (based on summed peak areas), which is significantly lower than expected based on the ratio of resin acids to monoesters in the test substance (0.17). The registrant(s) have indicated that the reason for this is

[12] Based on the assumption that on day 60 the relative proportions of the two monoester fractions were either the same as on day 28 or that all remaining monoesters were monoesters of hydrogenated resin acids, These two calculations resulted in degradation of monoesters of hydrogenated resin acids of 90.4% and 89.8%, respectively.

not known but could be related e.g. to differences of the analytical methods used[13]. The eMSCA considers that this may mean that the measured resin acids cover a relatively low proportion of the total resin acids in the test bottles and that the representativeness of the measured resin acids of the total resin acids in the test substance may be lower than for the monoesters. Therefore, these results should not be used for estimating total concentrations of resin acids or their changes during the study, or for a fully quantitative comparison of the concentrations of monoesters and resin acids. However, the results show clear changes in the concentrations of the measured resin acids during the study[14].

There was a decline in resin acids (sum of hydrogenated resin acids and DeHAA) between day 0 and day 3 (to 27% of the initial level) whereas an increase was observed on day 7 (to 56 %). The amount of resin acids decreased between day 7 and day 10 (to 16 %), remaining at 10-17% for the rest of the study (measurements on days 14, 21, and 28). There was a considerable difference between the changes in DeHAA and the hydrogenated resin acids. On day 3, DeHAA was decreased to 6% of its initial level, increased to 15% on day 7, and then declined to 13% on day 10 and to 3-4% on days 14, 21, and 28. The hydrogenated resin acids decreased to 57% of their initial level on day 3, then increased (113% on day 7), and decreased again (21% on day 10) and remained 21-38% on days 14, 21, and 28.

HPLC measurements of resin acids, monoesters, diesters, triesters, and heavy ends

In the 2017 study, monoesters, diesters, triesters, resin acids, and heavy ends were analysed by HPLC (Study report 2017c). The results were presented only as chromatogram peak sizes and concentrations were not determined, with the exception of monoesters. Two sets of samples were analysed: P1 (day 0 and day 28) and P2 (day 0 and day 28) and the percentage changes on day 28 compared to day 0 were -75% and -68% for the peak including monoesters and internal standard, -52% and -66% for the diester peak, -12% and -53% for the triester peak, -11% and +12% for the rosin acids peak, and +93% and -3% for the heavy ends peak. According to the Study report (2017b) P1 samples showed a reduction in the diester constituents over the course of the study, with the triester constituents decreasing slightly, showing that the diester constituents appear to degrade and the triester constituents may degrade, but to a lesser extent. P2 results were considered less clear for these constituents as the peaks were less distinct, but appeared according to the report to show a reduction in diester constituents between day 0 and day 28 and a possible reduction in triester constituents as well. It was stated that the P1 samples showed that the amount of resin acids decreased between day 0 and day 28, and the P2 samples showed a slight increase in resin acids between the two time points. The report stated that the resin acid signal is complex, as resin acids are likely to degrade, but will also be produced as degradation products following degradation of other constituents within the test item. The peaks for the resin acids in the chromatograms were also considered difficult to interpret and it was reported that this may contribute to the differences between the two sets of results.

The Study report (2017c) further states that the analysis of two sets of samples from day 0 and day 28 of the ready biodegradation study showed a reduction in the monoester fraction, consistent with that shown in Study report 2017b. This was reported to indicate significant primary degradation of the monoester fraction. The monoester peak co-eluted with internal standard which was added in different amounts in day 0 and day 28 samples. With a number of assumptions the decrease in monoester concentration was calculated as 74% and 67% in P1 and P2, respectively. Analysis of the diester fraction was considered to show that this fraction decreased between 0 and 28 days. Peaks for the triester constituents were less clear, but it was reported that there may be a reduction in these

[13] E-mail from the registrant(s) to the eMSCA on 21 November 2017.
[14] In the study report the results were presented as areas of the individual peaks, and as a percentage of peak area of the resin acids of the initial sum of the monoester peak areas. The eMSCA calculated the results (for the two fractions and their sum) as areas of the respective peaks in percentage of the areas of the same peaks on day 0.

constituents over the course of the study as well. The resin acid signal was not consistent, as P1 samples showed a slight decrease and P2 samples a slight increase between 0 and 28 days.

It was stated (Study report 2017c) that although some assumptions have had to be made in the quantification, the % change results confirmed the significant reduction in monoester constituents, reduction in diester constituents and some reduction in triester constituents between 0 and 28 days.

It is unclear what is the composition of the peak named "heavy ends" in this study as this fraction was not indicated in the composition of the test substance.

Primary degradation of monoesters: approach

The OECD TG 310 test guideline does not include specific instructions whether and how the sterile control should be used for determination of the primary degradation in the active tests. Whilst there was a considerable decrease in the monoester concentration in the sterile controls, it cannot be confirmed whether and to what extent this was due to primary degradation. It is also not known whether the abiotic primary degradation of the monoesters was similar in the active tests and sterile controls. However, the eMSCA considers that if the monoesters undergo primary degradation in the conditions of the active test, for the purpose of the current tests there is no need have a certainty on whether the degradation is completely biotic or whether part of it can be abiotic. For example, it is possible that there were enzymes present in the inoculum which were still able to catalyse the hydrolysis of the monoesters, despite the presence of formaldehyde. However, this was not confirmed as there were no controls without inoculum (these were not required by the test guideline or by the SEv decision). If the decrease in sterile controls was due to enzyme activity, at least the same amount of hydrolysis would be expected to occur also in the active test. Thus, if abiotic primary degradation has occurred in the sterile control, it may be occurring also in the active test and in that case it does not need to be differentiated from the primary degradation observed in the biotic test. On the other hand, if the abiotic primary degradation/transformation of the monoesters occurred only in the sterile controls (e.g. due to reaction with formaldehyde) it should not be taken into account for calculating the primary degradation in the active test. Therefore, no correction for sterile control was performed for the primary degradation of the monoesters in the active tests.

The concentration of the monoesters was lower in the sterile controls on day 0. The reason for this is not known. The main difference between the biotic tests and sterile controls was the addition of formaldehyde in the sterile controls. The concentration of formaldehyde used in the present study was high. Formaldehyde is used as a fixative of tissues samples and reacts with biological material. Chemical reactions between formaldehyde and monoesters are unlikely to occur in the conditions of biodegradation tests. There were microorganisms and organic matter in the test medium which might react with formaldehyde. However, there is no evidence of any effect of formaldehyde in the present test conditions which could decrease the concentration or extractability of the monoesters. If formaldehyde had an effect on the (actual or measured) monoester concentration in the sterile controls, the same effect would not occur in the active study. Therefore, there is no need to correct the results of the active study.

The fact that in the sterile controls with the new test substance the monoester concentration first had a significant decrease but increased again to a substantial level (64% AC) indicates that the monoester concentrations in the sterile controls may have been affected by dissipation phenomena such as adsorption. Therefore, it cannot be confirmed that abiotic degradation of the monoesters occurred in the sterile controls. It is possible that there was even more non-degraded monoester constituents present in the sterile controls which was not exctractable with the method used.

The results from the sterile controls suggest that the concentrations may be affected by non-degradative phenomena also in the active tests. Therefore, no conclusions on the primary degradation of the monoesters can be done only based on the concentration measurements in the active tests. However, the eMSCA considers that the decrease of the monoesters in the active tests was due to biotransformation, as indicated by the following additional evidence:

- CO_2 production and the decrease in monoester concentration were considerably higher in the active tests compared to the sterile controls.

- In the active tests, the decrease in the monoester concentration was mostly consistent and non-reversible during the study period in the active tests. The concentrations either remained similar or decreased in the course of the experiment, with only a few exceptions where the concentration increased. At maximum, an increase of 5 percentage points (from 8% to 13% AC) was seen (2019 study, new test substance) (Figure 5), which is relatively low compared to the overall decrease from 81% to <10% AC during the study). No increasing trends in the monoester concentration were observed in the active tests.

- The liquid-liquid extraction (LLE) and solvent rinse (SR) of the test bottles in the 2019 study (data not shown) showed that in the active tests the amount of the monoesters in the test medium (determined by LLE) was mostly below the detection limit and the majority of the monoesters were detected in the solvent rinse. The amount of the monoesters on the inside of the bottle (determined by the SR) decreased faster in the active tests with both test substances. In the sterile controls, there was an increasing trend in the amount of the monoesters in the test medium. The results suggest that in the active tests, once released to the test medium, the monoesters were removed fast, whereas in the sterile controls there was no such removal or the removal was slower.

Therefore, the primary degradation in the active tests was estimated based on the concentration measurements, on the assumption that the decrease in the monoester concentration was solely due to degradation/transformation and not e.g. due to adsorption.

Primary degradation of monoesters: results

The estimated primary degradation is presented in

Table 24. The primary degradation was 83-96% after 28 days, and 92-94% after 45-60 days in both studies (2017 and 2019). It should be noted that these values may be underestimated as monoesters can be produced during the study from the degradation of the di- and triesters.

Table 24 also presents primary degradation percentages corrected for the production of monoesters from a complete hydrolysis of the di- and triesters (i.e., one mole of monoester is produced per each mole of di- or triester). These are likely to be overestimates (as also indicated by the >100% values obtained in some cases) as a complete hydrolysis of the di- and triesters is unlikely.

Low solubility of the monoesters limited the primary degradation, as indicated by the LLE+SR results.

Estimation of ultimate degradation (% ThIC) of the monoesters

The degradation of monoesters (% of ThIC) is not necessarily the same as the degradation of the test substance (% of ThIC) because the test substances included also other constituents which may have degraded to a varying extent. Therefore, information on test substance composition were used for the estimation of ultimate degradation of the monoesters. The estimated biodegradation using different calculation scenarios is presented in **Table 25 and in Figure 8.**

For the new test substance in the 2019 study, the scenarios 1, 3, and 4 gave monoester degradation of 51-59 % ThIC after 28 days and 67-75% ThIC after 45 days. Whereas for the other test substances, the degradation of the monoesters by these scenarios was lower and there was more variability, particularly the Scenario 4 indicates a lower biodegradation result. The low variability of the results with the new test substance can be explained by the differences in the test substances as the new test substance had a higher concentration of monoesters and a lower concentration of di- and triesters compared to the other test substances. Scenario 2 gave the highest biodegradability results with each of the test substances.

The eMSCA considers that the degradation of the monoesters is most likely between the predictions of Scenarios 3 and 4. For the new test substance, these scenarios gave relatively similar results (51-59% after 28 days, 70-75% after 45 days). For the other two test substances, the variability between scenarios 3 and 4 was high, due to the higher proportion of di- and triesters.

Based on three of the four scenarios, for each three test substances, the biodegradation of the monoesters was below 60% during 28 days. Only Scenario 2 gave a biodegradation ≥60%. However, this is considered an overestimate as Scenario 2 assumes that all CO_2 is from monoesters. Therefore, it can be concluded that the degradation of the monoesters did not fulfill the criterion for ready biodegradability (≥60% biodegradation based on ThIC) during 28 days. The degradation of the monoesters was 67% or higher for the new test substance after 45 days based on all four scenarios.

The biodegradation results (% ThIC) for the test substance (Figure 4) in the 2017 study have been calculated using calculated TOC whereas in the 2019 study they are based on measured TOC. In the 2017 study, measured and calculated TOC differed (Table 18, Table 19). In the 2017 study, it is mentioned that the measured TOC values were not considered to be accurate and the calculated values were used in the report. It is also stated that the use of a significantly lower TOC value would result in false positive biodegradation results and to avoid this, the conservative approach to use the higher theoretical values was chosen. As the calculations for ultimate degradation of the monoesters for the 2017 study are also based on the calculated TOC, the results (Table 25, Figure 8) may be underestimated compared to what would be obtained if the same calculations were performed with a successfully measured TOC. This should be considered when comparing the results obtained with the 2017 and 2019 studies. However, it is still not possible to conclude that with a correct TOC, the pass level of 60% would have been reached in the 2017 study.

For the 2019 study, the calculations for the monoester biodegradation (Table 25) were based on both measured and calculated TOCs (with the exception of Scenario 1, which for

the 2019 study was based on measured TOC). The calculated TOC was used for estimating the relative proportions of TOC of each fraction for the Scenarios 2, 3, and 4. For the calculation of the amount of CO_2 produced from each fraction, also the measured TOC was used. It was done by multiplying the relative TOC proportion of the fraction by the expected biodegradation (% ThIC) of the fraction and the measured TOC of the test substance (mg/L). In the 2019 study, no difficulties in the TOC analysis were reported and the measured TOC is considered valid. In the 2019 study, the calculated TOC differed from the measured TOC (Table 19), but as the calculated TOC was used only to derive the relative TOC proportions of the fractions, the potential error caused by this is probably lower than in the 2017 study. It is not possible to estimate whether it would lead to under- or overestimation of the biodegradation of the monoesters.

The uncertainties related to the test substance compositions (glycerol and terpene concentrations), and consequently on the calculated TOC of the test substance and on the calculated TOC proportions of the fractions, were taken into account by additional calculations based on the different assumed compositions of the test substance. For each test substance, the estimated degradation of the monoesters was very similar between the assumed test substance compositions (See footnotes in Table 25) and therefore this uncertainty is not considered significant.

Overall, the uncertainties related to TOC content or test substance composition (glycerol and terpene concentrations) do not affect the conclusions on whether or not the ultimate degradation of the monoesters achieved the level 60 %ThIC.

Discussion on selected issues important for P/vP assessment

There were differences in the primary degradation and ultimate degradation of the monoesters observed with the three test substances. It is important to assess the potential reasons for these differences to understand the relevance of the results for P/vP assessment of the monoesters. The eMSCA considered four main issues in this context:

- *Representativeness of monoester measurements*

The high levels of primary and ultimate degradation of the monoesters with the new test substance in the 2019 study indicate that the monoesters measured with GC-MS were well representative of the degradation of the whole monoester fraction in that test substance. In contrast, in the 2017 study and the old test substance in the 2019 study, the ultimate degradation of the monoesters was clearly below 60 % ThIC, whereas the primary degradation of the monoesters was high (88-96%). These results could mean either that a significant part of the monoesters which underwent primary degradation could not be mineralised, or, that a significant part of the monoesters did not undergo degradation at all and were not covered by the GC-MS measurements. If the latter case was true, the reported primary degradation results of the monoesters would be overestimated for these two test substances.

The eMSCA considered whether poor representativeness of the monoester measurements could be a plausible explanation. This was a relevant concern as the measured monoesters either did not cover the complete monoester fraction of the test substance (2017 study) or this issue was not clarified (2019 study) and also considering that different monoesters may have different degradability, as also indicated by the 2017 study (Study report 2017b). Therefore, the observed primary degradation could depend on the selection of the m/z values used for the quantification of the monoesters. Consequently the selection of monoesters for analysis could also affect the differences in the primary degradation observed between the different test substances studied, if their monoester compositions differs.

The eMSCA considers that the measured monoesters were most likely representative of the monoesters of the test substance, considering that for one test substance both primary and ultimate degradation achieved high levels, and that all the three test substances were

manufactured using the same type of rosin (Table 19) and therefore similarity of the monoester fractions is expected. There were no indications that the monoesters in the three test substances would markedly differ e.g. in their proportions of types of resin acids (abietane/pimarane/labdane). In addition, the quantification of the monoesters was considered comparable between the three test substances, with certain reservations (discussed above under *"GC-MS measurements of monoesters: methods")*. Considering the above, the eMSCA concluded that for all three test substances the observed high primary degradation was representative of the primary degradation of total monoester fraction.

- *Solubility constraints affected biodegradation kinetics*

All three test substances attained a relatively high primary degradation of the monoesters during the study but there were differences in the rates of primary degradation. The primary degradation of the monoesters was fastest with the solvent dosed test substances. The relatively short duration of the lag phase (ca. 5-8 days, based on 10% decrease of initial conc.) before the start of the primary degradation of the monoesters, and the fast decrease right after the lag phase, suggest that the microorganisms in the inocula either had a capability of primary degradation of the monoesters already at the start of the exposure, or that this capability was developed or activated soon after the start of the exposure. Inhibition of microbial activity by test substance is unlikely to explain the lag phase (discussed below).

The low solubility of the monoesters was limiting the rate of primary degradation with all three test substances. This is indicated by the measurements from the test medium and from the solvent rinse of the bottles, and is in accordance with the predicted low solubility of the monoesters of rosin acids with glycerol **(Table 8,**

Table 26). The slower primary degradation of the monoesters and the longer lag phase observed with the new test substance in the 2019 study suggest that the bioavailability limitation was highest with the new test substance. This is most likely due to the fact that the new test substance was introduced by direct addition (no solvents were used), also considering that the same inoculum was used for both test substances in the 2019 study. This is in accordance with the perception that direct addition will usually provide the most conservative estimate of biodegradation (ECHA 2017c, Appendix R.7.9—3). In the solvent dosed bottles, the test substance was most likely better in contact with enzymes and microorganisms compared to the direct weighed samples.[15]

- *Inconsistency of primary and ultimate degradation in two of the test substances*

Particularly with the solvent dosed test substances, the ultimate degradation curve was flatter than the primary degradation curve of the monoesters. This indicates that the kinetics of primary and ultimate degradation differed and that the further degradation of the primary degradation products to CO_2 did not proceed at the same rate as the primary degradation step. A possible explanation is that the primary degradation kinetics likely reflect the rate of a single enzyme-catalysed reaction step whereas mineralisation of a molecule to CO_2 requires a number of reactions occurring in a series. Mineralisation of the

[15] In the solvent dosed samples, 100 µl (2017 study) or 112 µl (2019 study) of stock solution of the test item in acetone was pipetted around the wall of the test vessel (2017 study) or swirled/shaken around to coat the surface of the bottle then rolled on a rock/roller or shaken on an orbital shaker for approximately an hour (2019 study). In both studies the acetone was allowed to evaporate for three days. In the 2017 study it was reported that during preliminary investigations it was observed that when applied as described the test substance was forming a film on the glass wall surface (approximately two millimetres wide). Also in the 2019 study it was reported that evaporation of acetone left a coating of compound on the inside of the bottle. The 2017 study further reports that the film was detached from the wall after the test solutions were filled in and was floating in the solution. In the sample added by direct weighing (2019 study) the bottles were prepared by directly weighing the required quantity of the test item (viscous liquid) to the relevant test bottles into each bottle on a glass slip.

primary transformation products of the monoesters under the conditions of ready biodegradation testing may therefore require more time for acclimation and growth of microorganisms capable of mineralising the rosin structures. This is supported also by information available on biodegradation of resin acids, which are likely intermediate transformation products in the ultimate degradation of the monoesters. Resin-acid-degrading microorganisms are widely distributed in the environment. However, in most cases they are probably found at low abundance (Martin *et al.* 1999). In most environments, resin acids are found in low concentrations and account only for a very small portion of the total organic matter present (Martin *et al.* 1999). While some microorganisms are able to mineralize resin acids, there are also microorganisms which are capable of only partial degradation of resin acids (Martin *et al.* 1999, Cheremnykh *et al.* 2018). In the ready biodegradation tests reported under the REACH registration of Rosin, the range of biodegradation was variable (see Section 7.7.1.6 Prediction of microbial transformation of selected monoester constituents).

The relatively low ultimate degradation of the monoesters in the solvent dosed test substances indicates that the transformation products of the monoesters were not fully mineralised. The two test substances in the 2019 study were tested with the same inoculum. Therefore, the differences in ultimate degradation between those test substances were most likely due to the method of test substance application or the test substance composition. Both of these factors affect the availability of the monoesters and other compounds to microorganisms, and therefore may affect the development of the microbial community during the study, which may have consequences for biodegradation. Solubility constraints of the monoesters or of the transformation products seem to be an unlikely explanation for the differences in ultimate degradation, because in the direct weighed sample, high ultimate degradation was obtained despite the higher solubility constraint.

As the ultimate degradation was higher with the direct weighed test substance, which had a slower primary degradation, it was considered whether the limited ultimate degradation observed with the solvent dosed test substances could be due to inhibition of microbial activity by resin acids. Some resin acids are toxic (Cheremnykh *et al.* 2017, Peng and Roberts 2000) and some resin acids and their derivatives have antimicrobial activity (Savluchinske-Feio *et al. 2006)*. Fast primary degradation of the monoesters may have resulted in higher levels of resin acids in the solvent dosed samples, as supported by the measurements in the 2017 study where an increase in resin acids was observed when the primary degradation of the monoesters was fastest. However, there were no clear indications of inhibition of microbial activity by resin acids. The observed mineralisation indicates that microorganisms in the test systems were able to metabolise and mineralize at least a significant part of these resin molecules. The toxicity controls did not suggest inhibition. The 2017 study showed that the levels of the measured resin acids were highest in the beginning of the study but CO_2 production and primary degradation of monoesters still occurred. However, the measured resin acids appeared to cover only a relatively low proportion of the carbon present in the test substance[16] and absolute concentrations of the resin acids were not determined. Thus, it remains uncertain what proportion of the initial monoester amount was converted to resin acids during the study and how the total resin acid concentration developed during the study. It is also unknown whether some of the resin acids were inhibitory or persistent during the study.

A possible reason for the limited ultimate degradation is differences in the degradation of different resin acids. The measurements of the two fractions of resin acids (2017 study) suggest that DeHAA was more degradable than hydrogenated resin acids under the conditions of the tests, even if the interpretation of resin acid degradation is complicated due to the production of resin acids from the degradation of the monoesters. The faster decrease of DeHAA seems to be in line with the results by Hemingway and Greaves (1973).

[16] The resin acid/monoester ratio on day 0 was lower than expected based on test substance composition. Resin acid peak areas on days 10-28 were 0.37-0.64% of the initial monoester peak area

They reported differences in degradability of sodium salts of resin acids by microflora of river waters and activated sludge, in a study where several resin acids were tested together in liquid shake cultures with total resin acid conc. of 49 ppm. They observed that the salt of DeHAA (and the salt of levopimaric/palustric acid) was most readily degraded, followed by abietic acid (AA) and neoabietic acid salts. Pimaric and isopimaric acid salts were most resistant to biodegradation. The study by Hemingway and Greaves (1973) did not include the same resin acids that were included in the hydrogenated resin acid fraction of the current OECD TG 310 studies **(Table 23)**. All resin acids studied by them were less hydrogenated than the hydrogenated resin acids fraction in the OECD TG 310 studies, and DeHAA was the least hydrogenated one[17]. The eMSCA is not aware of any previous biodegradation studies on individual resin acids at the same level of hydrogenation as in the current OECD TG 310 study. For hydrogenated rosin substances there are three OECD TG 301B ready biodegradation tests available which showed degradation (based on CO_2 production in 28 days) of 66 and 56% for resin acids and rosin acids, potassium salts, respectively, and 0.98% for rosin, hydrogenated (ECHA 2021a). For salt forms of non-hydrogenated rosin substances, biodegradation of 80, 89, 71, and 71% have been reported in OECD TG 301B/301D tests (based on CO_2 production or O_2 consumption) (resin acids and rosin acids, calcium zinc salt; resin acids and rosin acids, magnesium salts; resin acids and rosin acids, sodium salt, tall oil rosin sodium salt, respectively) (ECHA 2021a). For rosin, biodegradation of 58, 89, and 13.6-13.9% have been reported in OECD TG 301B studies (ECHA 2021a). Thus, the hydrogenated forms have shown a lower degradation in ready biodegradation tests compared to the non-hydrogenated forms of these rosin substances. This is in line with the observed results for the two resin acid fractions in the OECD TG 310 study even though it should be noted that differences in methods may contribute to the differences in the cited ready biodegradation tests and that there is no data on the same salt form for both hydrogenated and non-hydrogenated rosins.

It is also possible that in the current OECD TG 310 studies, a significant part of the resin acids produced were further biotransformed to unknown transformation products. In a study of wastewater treatment plant treating effluent from a bleached kraft pulp and paper mill (including natural resin acids), it was suggested that DeHAA and 7-oxodehydroabietic acid were transformation products from the oxidation of AA (Stuthridge *et al.* 1991 as cited by Liss *et al.* 1997). The same study reported the appearance of 13-hydroxyabietinic acid, abietanic acid (same as tetrahydroabietic acid (THAA), based on the molecular formula presented), kinleithic acid (13-hydroxyabietanic acid) and dehydroabietin (Liss *et al.* 1997). In bacterial cultures, 7-oxo-dehydroabietic acid and 7-oxo-palustric acid (Luchnikova *et al.* 2019 and references therein) and 5-hydroxy-abieta-8,11,13-triene-18-oat (Cheremnykh *et al.* 2018) have been detected as transformation products of resin acids. Thus, one potential fate pathway of resin acids is biotransformation to other resin acids, which are not necessarily detected with the GC-MS method used in the current OECD TG 310 studies.

The eMSCA considers that even if the toxicity control did not indicate inhibition, this still does not exclude the possibility of inhibition occurring later during the study e.g. due to transformation products. In the toxicity control, the degradation of the reference substance likely occurred before the primary degradation of the monoesters had started[18] and therefore the transformation products were not yet present.

The reason for the limited ultimate degradation and the apparent accumulation of transformation products in the solvent dosed bottles remains unknown but may be related to differences in the development of the microbial communities during the study, inhibition by resin acids or other transformation products, or persistence of resin acids or other

[17] Molecular formulas: DeHAA $C_{20}H_{28}O_2$, other resin acids studied by Hemingway and Greaves (1973) $C_{20}H_{30}O_2$, DHAA $C_{20}H_{32}O_2$

[18] In the 2019 study, degradation of the reference substance in the procedural control was 78 %ThIC by day 3. In the 2017 study, degradation of the reference substance in the procedural control was 79 %ThIC by day 7 (there were no measurements between day 0 and day 7). For the toxicity controls there were no measurements between day 0 and day 14.

transformation products under test conditions. It is also unknown why such a limitation occurred only with the solvent dosed samples and not with the direct weighed test substance.

The test substance composition is not considered to explain the observed differences in primary degradation of the monoesters but it may explain some of the differences in ultimate degradation. The new test substance could be more favourable for biodegradation due to its higher monoester concentration and due to higher biodegradability of the other constituents[19]. The higher proportion of resin acids (and possibly also the light ends fraction) in the new test substance may have favoured the growth of rosin-degrading microorganisms even before the hydrolysis of the monoesters has occurred, and consequently the microbial community in the new test substance may have been more capable of metabolizing the resin acids produced from the monoesters.

As there was no testing on the same test substance with different methods of test substance application, no definitive conclusions can be drawn on the factors affecting the differences. The different inocula and methods of TOC determination[20] may also have contributed to the differences between the results of the 2017 and 2019 studies.

- *Differences in primary degradation of different monoester constituents*

A part of the monoesters still remained after the study with all three test substances. In the 2017 study, a difference was observed in the degradability of the two monoester fractions. Most of the monoesters remaining after the study consisted of the fraction "monoesters of hydrogenated resin acids". During the study, the rate of decrease of this fraction slowed down. The quantification of the monoesters of hydrogenated resin acids was based on the sum of two different integrated peaks[21]. The eMSCA compared the decrease of each of the two peaks separately. These two peaks showed a very similar degradation rate in relation to their initial concentrations (Figure 10), indicating that in general, the monoesters forming these two peaks had a similar degradability. The comparison of the chromatograms for the two peaks (representing the monoesters of hydrogenated resin acids) on days 0 and 28 (Study report 2017b) was performed by the eMSCA. In general, the shapes of both peaks appear to be relatively similar on day 0 and day 28. However, there were also differences in the peak shapes. Some of the differences can be explained by the lower concentration on Day 28, and consequently better separation of the complex mixture by the column. Some of the differences between day 0 and day 28 peaks suggest differences in the degradation rates of the compounds forming the peaks. It was not possible to derive information of the concentrations or concentration differences of individual compounds forming these peaks from the available data.

In a mixture of compounds, biodegradation can be influenced e.g. by different solubilities and toxicities of the compounds, or by the selective utilization of some of the compounds by the microorganisms. Certain structural features are considered important for the

[19] The eMSCA considers that the monoesters are more degradable compared to the di- and triesters, which have more steric hindrance due to the more complex structures, as also suggested by the results of the current OECD TG 310 on the enriched monoester substances and of the ready biodegradation studies on the Substance. The light ends fraction is expected to include constituents with varying biodegradability (see 7.1.1) and resin acids degraded to > 45% in 10 of the available 12 ready biodegradation tests (see 7.7.1.2).

[20] A calculated TOC was used in the 2017 study and a measured TOC in the 2019 study. It is possible that the concentration of the test substance in the 2017 study is overestimated. Consequently, the ultimate biodegradation results for the 2017 study could be underestimated.

[21] It is not indicated in the test report why there were two different groups of peaks for the monoesters of hydrogenated resin acids. The same was observed also for the other group, the monoesters of DeHAA. However, as the identification was based on the same ions for both peaks, the rosin acid parts seem to be similar in both cases, and therefore it seems possible that the difference was in the glycerol moiety of the esters, or in the position of the ester bond in the glycerol molecule. Therefore, the two separate groups of peaks observed for each group of monoesters could possibly represent the two different positional isomers of the monoesters of resin acids with glycerol.

biodegradation of resin acids (Martin *et al*. 1999, Luchnikova *et al.* 2018). The same features may be relevant for the ultimate degradation of the monoesters, which is expected to occur via resin acid intermediates. However, it is not known whether the same features are also important for the primary degradation of the monoesters.

Based on the structural formulas **(Table 23)**, the difference in primary degradability of the two monoester fractions may be linked to the structural differences such as the level of hydrogenation of the rosin moiety. The eMSCA considered whether there could be a difference also in solubility between the two fractions. For the monoesters of resin acids with glycerol, there was no experimental water solubility data available. However, the relative solubilities of the different monoesters of resin acids with glycerol can be generally expected to follow a similar pattern observed for the respective resin acids, as the rosin moiety forms a major part of the monoester molecule. This is supported by the QSAR predictions for AA, DeHAA, dihydroabietic acid (DHAA), and tetrahydroabietic acid (THAA) and their monoesters with glycerol **(**

Table 26). According to Peng and Roberts (2000), DeHAA was the most soluble (pH 7 ± 0.2, temperature 20 °C) compared to other studied resin acids (i.e. isopimaric, sandaracopimaric, pimaric, palustric, neoabietic, levopimaric, and AA). The WSKOW model predicts that AA and DeHAA are more soluble than DHAA and THAA (range 0.06-0.09 mg/L for the four compounds, when predictions based on estimated log Kow are used). According to the Watsol model, DeHAA has the lowest solubility (range 0.13-0.28 mg/L for the four compounds). In DeHAA, one of the fused rings is aromatic whereas in palustric and levopimaric acids the corresponding ring has 2 double bonds and there are no aromatic rings **(Figure 2)**[22]. The measured solubility of DeHAA (5.11 mg/L) was higher compared to palustric (2.41mg/L) and levopimaric (2.54 mg/L) acids (Peng and Roberts 2000). As the solubilities of monoesters or resin acids with glycerol are expected to follow a similar pattern as the solubilities of resin acids, the monoesters of DeHAA may have a relatively high solubility among the monoesters followed in the OECD TG 310 studies. Therefore, solubilities may have contributed to the faster primary degradation of the DeHAA monoesters.

Reliability

Regarding the 2017 study, a reliability score of 2 (reliable with restrictions) is assigned by the eMSCA. The study was conducted according to GLP and no deviations from test guideline were indicated. The results reported were reproducible by the eMSCA from the raw data presented with only insignificant deviations in some of the values. The eMSCA noted the following deficiencies:

- The lack of a measured TOC value, and consequent uncertainty (potential underestimation) of the ultimate biodegradation result
- The limited reliability of the primary degradation determination due to the lack of sterile control (it is noted that a sterile control is not an obligatory requirement in the test guideline and does not affect the reliability of the ultimate degradation results)
- The primary degradation measurement of the monoesters was based on the analysis of only a part of the monoester fraction of the test substance; it was not explained why the measured monoesters were assumed to be representative of the whole monoester fraction of the test substance

[22] Molecular formulas: DeHAA $C_{20}H_{28}O_2$, other resin acids studied by Peng and Roberts (2000) $C_{20}H_{30}O_2$, DHAA $C_{20}H_{32}O_2$, THAA $C_{20}H_{34}O_2$

Regarding the 2019 study, a reliability score of 2 (reliable with restrictions) is assigned by the eMSCA. The study was conducted according to GLP. The eMSCA noted the following deficiencies:

- The storage time of the soil used for the inoculum was 38 days, whereas according to the test guideline the maximum time is one month.
- Some of the calculations were not in accordance with the test guideline such as the subtraction of the sterile control (instead of solvent control or inoculum blank) from the results of the test substance bottles; however, this does not prevent the use of the study for the assessment as recalculation was possible from the raw data. It was not indicated whether the measured monoesters covered the complete monoester fraction of the test substance or only a part of it.
- For the GC-MS analysis of the monoesters, there was no calibration curve for the old test substance and the results were based on the calibration curve obtained with the new test substance.

Regarding the primary degradation determination, the reliability of the 2017 study is supported by the results of the 2019 study, particularly for the old test substance which had a composition close to that in the 2017 study and the same method of introducing the test substance. The CO_2 production and monoester concentration curves were relatively similar between these two studies (although the results are not fully comparable due e.g. to differences in inocula and in calibration of the analytical method). The 2019 study, which included sterile controls, and monoester analyses from both the test medium and solvent rinse of the bottles, allows to conclude that the decrease in the monoester concentration also in the 2017 study was due to primary degradation, as explained above in 'Primary degradation results'.

Relevance

Both OECD TG 310 studies are considered relevant for the purpose of P and vP assessment of the Substance. This is based on the following considerations:

- The tests were performed in general accordance with the test guideline and SEV decision.

- All three test substances are considered relevant for the assessment.

The relevance of the test substances was considered based on the following three points:

1) The primary degradation results are representative of the monoesters of the test substance, as discussed above under "*Discussion on selected issues important for P/vP assessment"*

2) The monoesters of the test substances are considered representative of the monoesters of the Substance.

Regarding the representativeness of the monoesters in the test substances of the monoesters in the Substance, it should be noted that the exact composition of the monoester fractions is not known. There may be differences between the composition of the monoester fractions of the test substances and the monoester fraction of the Substance, due to different production processes and starting materials. The registrant(s) have indicated that the type of rosin used to produce the test materials represents one of the most common types of rosin on the market. Therefore, the eMSCA considers that it is reasonable to expect that the monoester composition of the test substances was representative to the Substance.

3) The test substances are suitable for assessing the degradability of the monoesters of Substance.

Regarding the potential influence of the other constituents of the test substance, the eMSCA paid attention in particular to the fractions which are considered potentially more degradable than the monoesters and which could influence the degradation of the monoesters either positively or neatively (discussed above under "*Discussion on selected issues important for P/vP assessment*"). This is relevant because the presence of the other constituents in the test substance was unwanted but unavoidable due to technical difficulty of producing a pure monoester fraction. Exposure to a pure monoester fraction may be also an environmentally relevant scenario because after release to the environment the different constituents of an UVCB substance may have different fate and distribution. Therefore, organisms may be exposed to the constituents/fractions in proportions differing from the initial composition of the substance. The eMSCA considers that the test substance composition may potentially explain some of the differences in the biodegradation of the monoesters. However, this could not be confirmed as the differences in test conditions and methods may also have an influence. Despite the uncertainty regarding the influence of test substance composition, the data for all three test substances are considered applicable for the assessment of the degradability of the monoesters of the Substance. In addition, the fact that the highest ultimate degradation of the monoesters was seen with the test substance with the highest monoester concentration should be taken into account.

The composition of the test substances was also compared to the composition of the Substance, as in some cases the environmental exposure may be to the whole UVCB substance, or to several constituents representing different fractions, rather than to an individual constituent or fraction. This included comparison of the concentrations of certain fractions. Also the available information on biodegradability of the relevant fractions was used for this comparison. Details of this comparison are not presented in this report due to confidentiality of the composition of the Substance. Based on this comparison, the eMSCA considers that it cannot be concluded that the test substance compositions would be significantly more favourable or unfavourable for the biodegradation of the monoesters compared to the composition of the Substance.

Summary of the OECD TG 310 studies on the monoesters

Two OECD TG 310 studies are available and both are considered reliable (with restricions) and relevant for PBT assessment. The studies included testing on three different test substances enriched in monoesters of resin acids with glycerol.

The estimated degradation of the monoesters was below 60% (% ThIC) with all three test substances after 28 days whereas with one of the test substances it was above 60% after 45 days. The primary degradation of the monoesters was 83-96% after 28 days, and 92-94% after 45-60 days with all three test substances. A part of the monoesters still remained after the study. Based on the study where more detailed analytical information was available, the remaining monoesters mostly belonged to the fraction "monoesters of hydrogenated resin acids".

The primary degradation of the monoesters was thus high already in 28 days, whereas mineralisation was relatively low with two test substances and higher with one of them. Potential explanations to the different biodegradation results were considered[23] to

[23] The ECHA guidance (ECHA 2017) states that "*Realising that ready biodegradability tests may sometime fail because of the stringent test conditions, positive test results should generally supersede negative test results.*". The guidance further mentions that when conflicting test results are reported, possible differences in the test conditions and design should be investigated. In particular the origin of the inocula should be examined in order to verify whether or not there are differences in the adaptation of the inocula which may explain the differences in the results (OECD, 2006b). The guidance further mentions that when faced with conflicting results using different ready

understand the relevance of the results for P/vP assessment. The eMSCA did not find notable differences in the inoculum or its pre-treatment, in test conditions, or reliability of the data between the two studies or between the tests with the different test substances that would affect their relevance. However, there were differences in the test substance compositions and the methods of introducing the test substance, which may explain some of the differences. In all tests there were indications that bioavailability was limiting the rate of degradation.

The primary degradation of the monoesters may be explained by microbial hydrolysis of the ester bond, yielding resin acids and glycerol. Alternatively, if other reactions on the glycerol moiety occured first, hydrolysis of the ester bond is expected to have occurred after that, resulting in resin acids and three- or two-carbon compounds derived from the glycerol moiety (See 7.7.1.6). The limited ultimate degradation observed with two of the three test substances indicate that some of the primary transformation products of the monoesters, such as the resin acids (released in the hydrolysis of the monoesters), or their further transformation products, could not be further metabolized under the conditions of these ready biodegradation tests and therefore accumulated in the test medium.

Table 18. Test substance compositions used in the OECD TG 310 studies (2017 and 2019) with monoesters of hydrogenated rosin with glycerol. Data for the 2017 study are from Study report 2017a. Data for the 2019 study are from additional documents submitted by the registrant(s) to the eMSCA (e-mail on 24 February 2020).

TEST SUBSTANCE COMPOSITION						
	OECD TG 310 (2017)	**OECD TG 310 (2019) old test substance**	**OECD TG 310 (2019) new test substance**	**OECD TG 310 (2017)** [e]	**OECD TG 310 (2019) old test substance**[f]	**OECD TG 310 (2019) new test substance**[g]
Conc.	% (weight / weight)	% (weight/ weight)	% (weight/ weight)	% (C/total organic C)[a]	% (C/total organic C)[a]	% (C/total organic C)[a]
Tri-ester of glycerol	6.2	not reported (sum of di-ester and tri-ester: 19.0)	1.5	6.65	10.09	1.60
Di-ester of glycerol	10.3	not reported (sum of di-ester and tri-ester: 19.0)	3.9	10.88	9.93	4.09
Mono-ester of glycerol	70.7	68.0	74.8	69.54	66.190	73.04
Resin acids / Rosin acids	12.1	12.2	16.8	12.88	12.86	17.76

biodegradability test methods, it is also important to consider the test substance concentration, pre-treatment of the inoculum, the test conditions, substance properties and reliability of the data.

Light ends	<0.1[c]	0.8	3.0	<0.1 [d]	0.94	3.52
Glycerol	<0.1	0.0	0.0	0.0	0.0	0.0
Sum	99.3 (100.0)	100.0	100.0	100.0	100.0	100.0

[a]For concentrations based on % TOC, the concentrations (w/w) of each fraction and the estimated C content for each fraction were used. The C contents were based on the molecular formulas of the mono-, di-, and triesters of abietic acid, ($C_{23}H_{36}O_4$, $C_{49}H_{78}O_5$, and $C_{63}H_{92}O_6$) (for the mono-, di-, and triester fractions, respectively), abietic acid (for the resin acids / rosin acids fraction), and glycerol ($C_3H_8O_3$), as was also used in the registration dossier in the context of the 2017 study. In addition, the general C:H ratio for terpene compounds (C_5H_8) was used for the light ends fraction. For the old test substance in the 2019 study for which the di- and triesters were not quantified separately but only as a sum, the TOC percentages were calculated based on assumed ratio of di- and triesters 1:1 (w/w).

[c]The value 0.1 % w/w was used for calculating the sum of the concentrations and for calculating the % (C/total organic C)

[d]The value 0.052 % (C/total organic C) (corresponding to the concentration of 0.1 w/w) was used for the calculations of the ultimate degradation of the monoesters.

[e]The values in the table are based on the assumption that light ends fraction consists of glycerol. Additional calculations on the assumption that the light ends fraction represents terpenes and there is no glycerol at all indicated a total TOC of 74.64% and the C proportions of the fractions are 6.65, 10.87, 69.49, 12.87, 0.12, and 0.00 % C/TOC for triesters, diesters, monoesters, resin acids / rosin acids , light ends, and glycerol, respectively. If calculated on the assumption that the the concentration of glycerol is 0.7% w/w and of terpenes 0.0 w/w, the TOC is 74.83% and the C proportions of the fractions are are 6.63, 10.84, 69.32, 12.84, 0.00, and 0.37 % C/TOC for triesters, diesters, monoesters, resin acids / rosin acids, light ends, and glycerol, respectively.

[f]The values in the table are based on the assumption that light ends fraction consists of terpenes. The calculated TOC was 75.37%. Additional calculations on the assumption that the light ends fraction represents glycerol indicated that the total TOC is 74.98% and that the C proportions of the fractions are 10.14, 9.98, 66.54, 12.92, 0.00, and 0.42 % C/TOC for the triesters, diesters, monoesters, resin acids / rosin acids, light ends, and glycerol, respectively.

[g]The values in the table are based on the assumption that light ends fraction consists of terpenes. The calculated TOC was 75.14%. Additional calculations on the assumption that the light ends fraction represents glycerol indicated that the total TOC is 73.67% and that the C proportions of the fractions are 1.63, 4.17, 74.49, 18.11, 0.00, and 1.59 % C/TOC for triesters, diesters, monoesters, resin acids / rosin acids, light ends, and glycerol, respectively.

Table 19. Summary of test conditions and other relevant information in OECD TG 310 tests (2017, 2019) with monoesters of hydrogenated rosin with glycerol

TEST CONDITIONS	OECD TG 310 study (2017)	OECD TG 310 study (2019) old test substance	OECD TG 310 study (2019) new test substance
Source of rosin used	Chinese gum rosin	Chinese gum rosin	Chinese gum rosin
Batch (glycerol monoesters of hydrogenated rosin) used for purification of test item	Batch 1	Batch 1	Batch 2
Test substance analysis date	Not reported. Receipt to test laboratory 10 December 2015	11 July 2018	20 July 2018
Experimental period	27 June 2016 – 7 August 2016	15 November 2018 – 04	15 November 2018 – 04

TEST CONDITIONS	OECD TG 310 study (2017)	OECD TG 310 study (2019) old test substance	OECD TG 310 study (2019) new test substance
		January 2019	January 2019
Incubation conditions	Shaker (150-200 rpm), temperature 20 ± 1 °C, actual 19.3-20.9 °C, low light conditions	Orbital shaker (150 -200 rpm), temperature 20 ± 1°C (except on a few occasions when there was a maximum temperature deviation of 0.5°C. On five occasions no maximum or minimum temperature was recorded due to the batteries failing in the thermometers. This is not believed to have adversely affected the outcome of this study as the study was performed in incubators designed to hold a set temperature.). The study was performed in an incubator in the dark - when the incubator was opened on sampling occasions the bottles were exposed to light for a brief period (this was less than 5 minutes per sampling occasion)[a]	Orbital shaker (150 -200 rpm), temperature 20 ± 1°C (except on a few occasions when there was a maximum temperature deviation of 0.5°C. On five occasions no maximum or minimum temperature was recorded due to the batteries failing in the thermometers. This is not believed to have adversely affected the outcome of this study as the study was performed in incubators designed to hold a set temperature.). The study was performed in an incubator in the dark - when the incubator was opened on sampling occasions the bottles were exposed to light for a brief period (this was less than 5 minutes per sampling occasion).[a]

TEST CONDITIONS	OECD TG 310 study (2017)	OECD TG 310 study (2019) old test substance	OECD TG 310 study (2019) new test substance
Deviations from the guideline	It is reported in the test report and in the registered substance factsheet that there were no deviations from the guideline.	It is reported in the registered substance factsheet that there were no deviations from the guideline. However, it is noted that the storage of the soil used for the inoculum was 38 days, whereas according to the test guideline the maximum time is one month.	It is reported in the registered substance factsheet that there were no deviations from the guideline. However, it is noted that the storage time of the soil used for the inoculum was 38 days, whereas according to the test guideline the maximum time is one month.
Analytical method for ultimate degradation determination	CO_2 production	CO_2 production	CO_2 production
Constituents/transformation products analysed (analytical method)	resin acids (GC-MS, HPLC), monoesters (GC-MS, HPLC), diesters (HPLC), triesters (HPLC)	monoesters (GC-MS)	monoesters (GC-MS)
Initial test substance conc. (mg/L)	33 mg/L	28.8 mg/L (calculated by eMSCA based on TOC)	31.3 (calculated by eMSCA based on TOC)
Initial test substance conc., (mgC/L)	24.6	20	20

TEST CONDITIONS	OECD TG 310 study (2017)	OECD TG 310 study (2019) old test substance	OECD TG 310 study (2019) new test substance
TOC of test substance	74.6% (calculated using size-exclusion chromatography peak areas of the different fractions, relative to the total peak area, and TOC of representative compounds for each fraction). Measured TOC was about 60.8% and was not considered accurate. Measurement of TOC was attempted but "unrealistically low TOC values" were obtained which was attributed to the viscous test item and therefore the calculated TOC was used to avoid false positive biodegradation values.	69.4 % (measured); 75.37 % (calculated)	64.0 % (measured); 75.14 % (calculated)
Intial monoester conc. (mg/L)	23.3 mg/L	18.04 mg/L (calculated by eMSCA based on TOC proportion of monoesters and C content of monoesters of 73.37%)	19.91 mg/L (calculated by eMSCA based on TOC proportion of monoesters and C content of monoesters of 73.37%)
Controls	solvent control, toxicity control,	solvent control, sterile control	toxicity control, sterile control
Dosing method	solvent (acetone)	solvent (acetone)	direct weighting
Inoculum	a mixture of the aqueous phase of non-adapted activated sludge (sewage plant receiving "predominantly domestic sewage and hardly any industrial chemical waste") and pre-treated, non-adapted standard soil) LUFA, Speyer, Germany).	a mixture of activated sludge (a WWTP treating sewage of predominantly domestic origin, UK) and soil (LUFA, Speyer, Germany)	a mixture of activated sludge (a WWTP treating sewage of predominantly domestic origin, UK) and soil (LUFA, Speyer, Germany)

TEST CONDITIONS	OECD TG 310 study (2017)	OECD TG 310 study (2019) old test substance	OECD TG 310 study (2019) new test substance
Inoculum concentration	10 mL/L of activated sludge supernatant and 7 mL/L of soil supernatant were added to test bottles. Sludge solids concentration is not reported.	4.0 mg sludge solids/L (obtained by adding 4.4 mg/L activated sludge dry weight and 10 mL/L soil supernatant to the vessels)	4.0 mg sludge solids/L (obtained by adding 4.4 mg/L activated sludge dry weight and 10 mL/L soil supernatant to the vessels)
Inoculum storage conditions	Activated sludge	Activated sludge sampled obtained on 16 November 2018. Soil was stored at at 2-4°C for 38 days. The soil was stored for slightly longer than the OECD 310 test guideline recommendation. It is stated that the microbial activity of the soil was not believed to have been adversely affected as the CFU measurement of the inoculum was within the expected range.	Activated sludge sampled obtained on 16 November 2018. Soil was stored at at 2-4°C for 38 days. The soil was stored for slightly longer than the OECD 310 test guideline recommendation. It is stated that the microbial activity of the soil was not believed to have been adversely affected as the CFU measurement of the inoculum was within the expected range.
Colony forming units in test solution (day 0)	The test report has contradictory information. In one part of the report, it is indicated: "*10^5-10^8 CFU/L in test solution.*" *In another part of the report it is indiated "1.5 10^{10} CFU/L for the inoculum of the aqueous phase non-adapted activated sludge (corresponds to approx. 1.5 x 10^8 CFU/L in the final test solution) and 1.1 x 10^{10} CFU/L for the non-adapted standard soil (corresponds to approx. 1.1 x 10^8 CFU/L in the final tests solution)*". However, both results are within the range of 10^2 to 10^5 CFU/mL considered optimal (OECD TG 310)	The CFU observed was within the expected range 10^2 to 10^5 CFU/mL (10^5 to 10^8 CFU/L) given in the 310 TG.	The CFU observed was within the expected range 10^2 to 10^5 CFU/mL (10^5 to 10^8 CFU/L) given in the 310 TG.

[a] Source of information on the light condition: e-mail from the registrant(s) to the eMSCA on 7 May 2020.

Table 20. Comparison of the OECD TG 310 ready biodegradability tests on the enriched glycerol monoesters with the requirements/recommendations of the SEV decision

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
1	"Ready biodegradability; test method: C02 in sealed vessels (Headspace Test), OECD 310…"	Yes.	Yes.	**Both studies:** The tests were in accordance with OECD 310.
2	"…using the test substance as specified in Appendix 3."	Yes.	Yes.	**Both studies:** The test substances were in accordance with Appendix 3 of the SEV-DD; however it is noted that the concentrations are based on percentage of each of the peaks of the total total peak area. In principle, it is possible that the response of the analytical method can vary between the different groups of constituents. Therefore, the peak areas relative to total peak areas are not necessarily directly comparable to mass concentrations. In this case, due to structural similarity of the test substance constituents (resin acids and their derivatives) it is considered that the response factor of each constituent can be assumed to be similar, with the possible exception of the light ends fraction and glycerol . The test substance concentration is not critical in determination of the relative reduction in monoester concentration (to estimate primary degradation); however, the uncertainty in test substance composition should be kept in mind when estimating the contribution of the different

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
				constituents to the CO_2 production.
3	"The concentrations of the test substance shall be analytically monitored during the test to verify the degradation."	Yes.	Partially.	**OECD TG 310 study (2017):** The concentrations of monoesters and resin acids were analytically determined during the test at days 0, 3, 7, 10, 14, 21, 28 and the concentration of monoesters was measured additionally on day 60. The concentrations of diesters, triesters, and resin acids were analytically determined on days 0 and 28.

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
				OECD TG 310 study (2019): The concentrations of monoesters were analytically determined at days 0, 3, 7, 10, 14, 21, 28, and 45.
4	"Test substance: monoesterified glycerol constituents of HRGE."	Partially.	Partially.	**Both studies:** concentration of the mono-ester fraction of the test item was 68.0-74.8% (based on relative peak areas in size exclusion chromatography with refractometer). The test substances are considered acceptable for the purpose.
5	"You may consider extension of the duration of the ready test up to 60 days and techniques to determine the biodegradability of poorly water-soluble chemicals in accordance with ECHA guidance"	Yes	Yes	These were not mandatory requirements but options that can be considered. **OECD TG 310 study (2017)**: The test duration was 60 days and the results are presented at 0, 3, 7, 10, 14, 21, 28, and 60 days **OECD TG 310 study (2019)**: The test duration was 45 days and the results are presented at 0, 3, 7, 10, 14, 21, 28, and 45 days.
6	"When performing, documenting and interpreting the test you need to consider the likely situation that the test substance is not a pure monoester fraction but it may contain constituents (e.g., rosin acids, glycerol), which can be also produced in the degradation of the monoesters."	Yes.	Partially.	**OECD TG 310 study (2017)**: The initial composition of test substance was measured. In addition to CO_2 production also the concentrations of the other constituents were measured during the test (see also item #3). **OECD TG 310 study (2019)**: The initial composition of test substance was measured. In addition to CO^2 production also the concentrations of the monoesters were measured during the test (see also item #3).
7	"To support the use of ultimate degradation result of	Not applicable.	Not applicable.	**Both studies:** Calculation of ultimate degradation of monoesters are not

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
	the test substance for the persistence assessment of the monoesters, you can present a justification why they consider it can be concluded that >60% ThIC production (or >70% DOC removal) of the monoesters is achieved based on the test results even though the inorganic carbon produced from the monoesters (or DOC removal due to the degradation of the monoesters) cannot be analytically separated from the inorganic carbon (or DOC removal) resulting from the degradation of the other constituents."			included in the dossier update. This was not a mandatory requirement in the decision.
8	"In case it cannot be demonstrated that >60°h of the ThIC production (or >70% DOC removal) of the monoesters has been achieved, their primary degradation measurements can be used to evaluate whether the substance does not screen as P/vP as the final hydrolysis products of the monoesters are not PBT/vPvB (footnote 4)."	Yes.	Yes.	**Both studies:** Primary degradation measurements have been used for the conclusion.
9	"If primary degradation rate is used to conclude on the further testing needs on P/vP, information on transformation products is necessary to verify that the decrease in concentration of the	Partially.	No.	**OECD TG 310 study (2017):** Information on transformation products is used to justify that monoesters degraded. **OECD TG 310 study (2019):** Chemical analysis was performed for the monoesters only.

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
	monoesters is really due to degradation and that there is no PBT/vPvB concern with the degradates."			
10	"The transformation products would then need to be analysed to the extent needed to demonstrate that there is no PBT/vPvB concern (i.e. that primary degradation does not lead to transformation products with PBT/vPvB properties)."	Yes.	No.	**OECD TG 310 study (2017):** The identity of transformation products has been reported and there are no indications of other transformation products of monoesters than resin acids. **OECD TG 310 study (2019):** Chemical analysis was only performed for the monoesters only.
11	"To quantify the amount of the relevant transformation products produced during the test, in particular the known hydrolysis products of rosin esters, their initial concentrations in the test substance need to be known."	Partially.	Partially.	**OECD TG 310 study (2017):** Information on these transformation products (fractions) in the test substance is provided. The uncertainty in test substance composition determination is discussed above (item #2). The concenrations of these fractions were measured also during the study. **OECD TG 310 study (2019):** Information on these transformation products in the test substance is provided but only the monoesters were measured during the study.
12	"In addition, if primary degradation measurement is used for the conclusion, sterile control experiment is necessary to verify the contribution of abiotic phenomena including adsorption processes."	No.	Yes.	**OECD TG 310 study (2017):** Sterile control was not included. **OECD TG 310 study (2019):** Sterile controls were included.

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
13	"A toxicity control should be included and if inhibition by test substance is suspected the test can be repeated as instructed in the test guideline, using, e.g., a lower test substance concentration."	Yes.	Partially.	**OECD TG 310 study (2017):** Toxicity control was included and according to the test report it did not indicate inhibition by test substance. **OECD TG 310 study (2019):** Toxicity control was included for the new test substance and according to the test report it did not indicate inhibition by test substance. Toxicity control was not included for the old test substance.
14	"The analytical techniques used need to have a sufficient sensitivity to analyse and quantify the monoesterified glycerol constituents as well as any other constituents relevant for determining the degradation of the monoester constituents and to identify and quantify possible transformation products relevant for PBT/vPvB assessment."	Partially.	Partially.	**Both studies:** The analytical techniques seem to be appropriate.
Appendix 3 of the SEv decision:				
16	"In relation to the required experimental studies, the sample of the substance to be used shall represent the monoesterified glycerol constituents of HRGE. In practise, the sample to be tested can be a fraction of the registered (UVCB) substance enriched for monoesterified glycerol constituents as far as technically possible. Based on communication with you it is not	Yes.	Yes.	**OECD TG 310 study (2019):** It is noted that information of the test substance composition is not included in the study report but has been provided to the eMSCA as additional document (e-mail from the registrant(s) to the evaluating MSCA on 24 February 2020).

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
	technically possible to synthesize or isolate a pure rosin monoestercompound, but an enrichment of a monoester fraction to at least 70% is achievable. It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material."			
17	"The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation."	Yes.	Yes.	**OECD TG 310 study (2019):** It is noted that information of the test substance composition is not included in the study report but has been provided to the eMSCA as additional document, including the concentrations of resin acids, light ends, and tri-, di-, and monoesters of glycerol.
18	"You have in your comments submitted composition data of a test substance that has been used for preliminary testing (the relative chromatogram peak areas of rosin acids, light ends, and tri, di-, and monoesters of glycerol). You note that, due to the complexity of the substance, further identification of the constituents in each group is impossible. ECHA considers that for the purpose of the present decision the concentrations of rosin acids, light	Yes.	Yes.	**OECD TG 310 study (2017):** The concentrations of resin acids, light ends, and tri-, di-, and monoesters of glycerol are presented. **OECD TG 310 study (2019):** It is noted that information of the test substance composition is not included in the study report but has been provided to the eMSCA as additional document, including the concentrations of resin acids, light ends, and tri-, di-, and monoesters of glycerol.

COMPARISON TO SEV DECISION				
Item #	**Requirement in the SEv decision**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2017)**	**Requirement fulfilled (Yes/No/Partially)** **OECD TG 310 (2019)**	**Remarks**
	ends, and tri-, di-, and monoesters of glycerol are necessary information."			
19	"In addition, the concentrations of any other constituents or fractions of constituents that are present in similar concentrations or otherwise considered relevant by you should be determined."	Not applicable.	Not applicable.	**Both studies:** no other relevant constituents or fractions of constituents were indicated.
20	"When submitting the test data you need to submit the test substance composition(to the level specified above) specific for the test substance used for the actual testing. In case that the same test substance sample used for preliminary testing is used also for the final testing and the composition is unchanged, this must be indicated."	Yes.	Yes.	**OECD TG 310 study (2019):** It is noted that information of the test substance composition is not included in the study report but has been provided to the eMSCA as additional document, including the concentrations of resin acids, light ends, and tri-, di-, and monoesters of glycerol.
	Appendix 5 of the SEv decision:			
21	"If DOC and/or primary degradation measurement is used for the conclusion, sterile control experiment is necessary to verify the contribution of abiotic phenomena including adsorption processes"	No.	Yes.	**OECD TG 310 study (2017):** Sterile control was not included. **OECD TG 310 study (2019):** Sterile control was included.

Table 21. Corrections applied to CO_2 production results in the OECD TG 310 (2019) test as used in the test report or by the eMSCA

CORRECTIONS TO CO_2 PRODUCTION			
Test	**Correction used (test report)**	**Correction used (eMSCA)**	**Remarks by eMSCA**
Old test substance	sterile control (old test substance)	solvent control	According to the test guideline, blank controls used for the calculation must include also "any chemicals, solvents, agents or glass fibre filters used to introduce the test substance into test vessels". It is also noted that in this case, correction with sterile control would lead to overestimated degradation of the test substance because TIC production was lower in sterile control compared solvent control.
New test substance	sterile control (new test substance)	blank control	According to the test guideline, the results must be corrected for the IC production in the blank control.
Old test substance	sterile control (old test substance)	solvent control	According to the test guideline, blank controls used for the calculation must include also "any chemicals, solvents, agents or glass fibre filters used to introduce the test substance into test vessels". It is also noted that in this case, correction with sterile control would lead to overestimated degradation of the test substance because TIC production was lower in sterile control compared solvent control.
New test substance	sterile control (new test substance)	blank control	According to the test guideline, the results must be corrected for the IC production in the blank control.
Sterile control, old test substance	solvent control	solvent control/ correction for initial IC	The test guideline does not indicate any corrections for sterile controls. However, the eMSCA considers that different corrections may give different information. Solvent control is used as the sterile controls were solvent dosed. However, CO_2 production in the sterile control with the old test substance was mostly lower in the sterile control than in the solvent control (therefore the correction leads to negative CO_2 production values, which are presented as zero in the graph (Figure 4). Therefore, it is expected that biological activity was inhibited in the sterile control. The correction for solvent control may therefore underestimate the abiotic degradation of the test substance. It is noted that on day 0 the CO_2 level was higher in the sterile control than in the active test. This is considered unlikely to be due to degradation of test substance and therefore degradation percentage based on no correction is likely to overestimate the

CORRECTIONS TO CO_2 PRODUCTION			
Test	**Correction used (test report)**	**Correction used (eMSCA)**	**Remarks by eMSCA**
			degradation of test substance in the sterile control. Therefore, eMSCA has presented the results also with a correction for initial IC, to indicate the increase in CO_2 during the study.
Sterile control, new test substance	blank control	blank control/correction for initial IC	The test guideline does not indicate any corrections for sterile controls. However, the eMSCA considers that different corrections may give different information. Blank control is used as the new test substance was introduced via direct weighing. However, CO_2 production in the sterile control with the old test substance was lower in the sterile control than in the blank control (therefore the correction leads to negative CO_2 production values, which are presented as zero in the graph (Figure 4), with the exception of days 28 and 45 where the CO_2 production from the sterile control was higher than in blank control. Therefore, it is expected that biological activity was inhibited in the sterile control. The correction for blank control may therefore underestimate the abiotic degradation of the test substance. It is noted that on day 0 the CO_2 level was higher in the sterile control than in the active test. This is considered unlikely to be due to degradation of test substance and therefore degradation percentage based on no correction is likely to overestimate the degradation of test substance in the sterile control. Therefore, eMSCA has presented the results also with a correction for initial IC, to indicate the increase in CO_2 during the study.
New test substance toxicity control	sterile control (new test substance)	blank control	The results need to be corrected for the TIC production of the inoculum. Therefore, blank control is used. According to the test guideline, sterile control does not need to be considered in the case of toxicity control.
Reference substance	blank control	blank control	

Table 22. Comparison of the OECD TG 310 ready biodegradability tests on monoesters of hydrogenated rosin with glycerol (2017 and 2019) test to the validity criteria of OECD TG 310

VALIDITY CRITERIA			
Validity criterion	**Criterion fulfilled (Yes/no)** **OECD TG 310 study (2017)**	**Criterion fulfilled (Yes/no)** **OECD TG 310 study (2019)**	**Remarks**
The mean percentage degradation in vessels FC containing the reference substance is >60% by the 14th day of incubation;	Yes	Yes	**OECD TG 310 study (2017):** 81% after 14 days **OECD TG 310 study (2019):** 75% after 7 days; 83% after 14 days (excluding outlier); 69% after 28 days
The mean amount of TIC present in the blank controls FB at the end of the test is <3mg C/L.	Yes	Yes (28 days); No (45 days)	**OECD TG 310 study (2017):** The maximum amount of total inorganic carbon (TIC) produced in the inoculum controls until the end of the test was 2.60 mg C/L (inoculum control after 28 days). **OECD TG 310 study (2019):** The mean IC content of the inoculum blanks after 28 days was 2.84 mg/L. For day 45, the following is mentioned in the test report "*The mean IC content of the inoculum blanks after 45 days was 3.54 mg/L, however there was 2.24 mg/L in a NaOH check vessel (100 mL ROW spiked with the same volume of 10 M NaOH (1 mL) used to convert CO_2 to carbonate in the test vessels) analysed with the day 45 samples, therefore it is deemed that the mean IC content in the inoculum blanks was less than 3 mg/L. Therefore, this test has satisfied all the validity criteria*."

Table 23. GC-MS methods used for the analysis of the monoesters in the OECD TG 310 studies (2017 and 2019) (Study report 2017a, Study report 2019a)

<table>
<tr><th colspan="3">GC-MS METHODS FOR MONOESTER ANALYSIS</th></tr>
<tr><th></th><th>2017 study</th><th>2019 study</th></tr>
<tr><td>Derivatisation</td><td>Derivatized with trifluoroacetanhydride (TFAA) for GC-MS</td><td>Derivatized with TFAA for GC-MS</td></tr>
<tr><td>GC-MS scanmethod</td><td>Single Ion Monitoring (SIM)</td><td>Selected Ion Monitoring (SIM)</td></tr>
<tr><td>Approach to monoester analysis</td><td>Four predominant derivatives monoesters were considered. These derivatives had a molecular weight of m/z = 374 (one derivative) and m/z = 378 (three derivatives) respectively. These derivatives were derivatized with TFAA prior to GC-MS analysis.</td><td>The concentration of Monoesters of hydrogenated rosin with glycerol was measured by GC-MS by monitoring the derivatives of the test substance after derivatisation by trifluoroacetic acid (TFAA).</td></tr>
<tr><td></td><td>1: TFAA derivative of glycerol monoester of dehydroabietic acid; m/z: 239, 551, 566 (one derivative, peak ID 4)

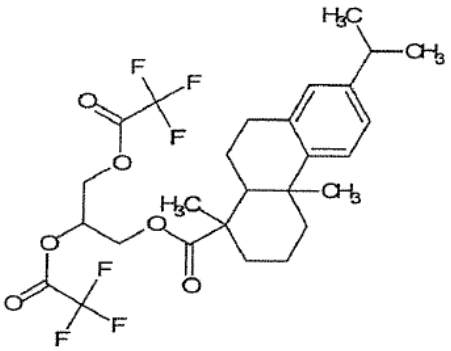
2: TFAA derivatives of hydrogenated resin acids (e.g., dihydroabietic acid) monoester m/z: 243, 555, 570 (three derivatives)

<table><tr><th>ID #</th><th>Quantification ion m/z</th><th>Reference ions m/z</th></tr><tr><td>1</td><td>555</td><td>243; 570</td></tr><tr><td>2</td><td>555</td><td>243; 570</td></tr><tr><td>3</td><td>243</td><td>555; 570</td></tr><tr><td>4</td><td>239</td><td>551; 566</td></tr></table></td><td>Three monoester derivatives were monitored according to their specific mass and retention times.

<table><tr><th>Group</th><th>Quantitative ion m/z</th><th>Plot Ions m/z</th></tr><tr><td>1</td><td>243</td><td>243, 555, 570</td></tr><tr><td>2</td><td>243</td><td>243, 555, 570</td></tr><tr><td>3</td><td>239</td><td>239, 551, 566</td></tr></table></td></tr>
<tr><td>Quantification of the monoesters</td><td>Quantification of the test item was calculated by peak area (sum of four peaks) based on the test item as external standard.</td><td>Quantitation is achieved using the total area of the peaks corresponding to the derivatives of Monoesters of hydrogenated rosin (new) from SIM ion chromatograms of the quantitative ion. Each group corresponds to a different retention time which will be identified on integration.</td></tr>
<tr><td>Remarks</td><td>The ions m/z= 566 and 570 correspond to the molecular ion</td><td>Due to the small quantity of Monoesters of hydrogenated</td></tr>
</table>

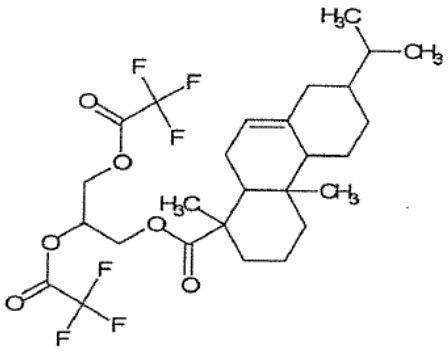
GC-MS METHODS FOR MONOESTER ANALYSIS		
	2017 study	**2019 study**
	(M+) of the resulting TFAA derivatives. The ions m/z 551 and 555 are formed by a cleavage of a methyl group. The ions m/z = 239 and 243 correspond to the resin and rosin backbone of the molecules.	rosin with glycerol (old) test substance available it is not possible to perform the GC-MS analysis using standards made with the old test substance, therefore the GC-MS analysis of the Monoesters of hydrogenated rosin with glycerol (old) is more indicative than quantitative, as the method is quantified against Monoesters of hydrogenated rosin with glycerol (new). There is an additional peak present in the old test substance that is not accounted for in the method as it is not observed in the new test substance.

Molecular formulas of TFAA derivatives of two monoesters of resin acids with glycerol (from the 2017 study) (1. monoester of dehydroabietic acid; 2. monoester of an isomer of dihydroabietic acid (different location of the double bond compare to dihydroabietic acid):



Molecular Formula: $C_{27}H_{32}F_6O_6$
Formula Weight: 566.5297992

1



Molecular Formula: $C_{27}H_{36}F_6O_6$
Formula Weight: 570.5615592

2

Table 24. Primary degradation of the monoesters in the OECD TG 310 studies as calculated based on the monoester concentration measurements (Study report 2017a, Study report 2019a). The values in parentheses are calculated on the assumptions that complete hydrolysis of di- and triesters occurred by day 28 and the monoesters formed were not further degraded during the study.

PRIMARY DEGRADATION OF MONOESTERS			
Measurement time (d)	**2017 study[a]**	**2019 study old test substance [a]**	**2019 study new test substance [a]**
28	88 (99)	96 (109)	83 (86)
42/45	n.d.	93 (106)	92 (95)
60	94 (105)	n.d.	n.d.

[a]The formation of monoesters was calculated from the proportions of the different fractions of TOC of test substance, on the basis that 1 mole of monoester was formed per mole of di- or triester. For the old test substance of the 2019 study as there was only a combined concentration of di- and triesters, the ratio of C of di- and triesters in the test substance was assumed to be 1:1. The amount of carbon in the monoesters formed from the di- and triesters was calculated, and the corrected primary degradation of the monoesters was then calculated (based on amounts of carbon in each fraction) by subtracting the calculated amount of formed monoesters from the observed residual amount in the monoesters.

Table 25. Estimated ultimate degradation (% ThIC) of the monoesters based on different calculation scenarios. (n.d.= not determined)

ULTIMATE DEGRADATION OF MONOESTERS					
		Monoester degradation (% ThIC)[a]			
Scenario[b]	**Measurement time (d)**	**2017 study**	**2019 study old TS**	**2019 study new TS**	**Remarks**
1	28	34.26	34.47	53.53	May under- or overestimate degradation of monoesters, depending on the extent of degradation of the monoesters and the other constituents.
1	42/45	36.10	35.44	67.37	
1	60	34.57	n.d.	n.d.	
2	28	49.27	52.07	73.30	Overestimates the degradation of the monoesters, as CO_2 is produced also from the other constituents.
2	42/45	51.91	53.54	92.24	
2	60	49.72	n.d.	n.d.	
3	28	43.77	45.60	59.06	May overestimate the degradation of monoesters, as it is assumed in this scenario that no CO_2 is produced from di- and triesters.
3	42/45	n.d.	46.85	74.76	
3	60	44.22	n.d.	n.d.	
4	28	22.99	21.43	51.15	May underestimate the CO_2 production from the monoesters, depending on
4	42/45	25.63	22.89	70.09	
4	60	23.43	n.d.	n.d.	

					degradability of other constituents.[c]

[a]The results in the table are based on the assumption that the light ends fraction of the 2017 study consisted of glycerol whereas the light ends fractions of the 2019 study samples consisted of terpenes. Due to the uncertainty regarding the glycerol content of the test substances, additional calculations for each scenario were performed in order to see the effect of this uncertainty on the estimated ultimate degradation of the monoesters.

For the 2017 study, assuming a 0.7 %w/w glycerol concentration and 0.0 %w/w terpenes concentration the ultimate degradation of the monoesters was, based on the different scenarios on days 28, 42, and 60, respectively:

Scenario 1: 34.16, 35.98, and 34.46 % ThIC; Scenario 2: 49.33, 51.97, and 49.78% ThIC; Scenario 3: 43.80, n.d., and 44.25% ThIC; Scenario 4: 22.67, 25.31, and 23.12% ThIC.

For the old test substance in the 2019 study, assuming that the light ends fraction consisted solely of glycerol, the ultimate degradation of the monoesters was, based on the different scenarios on days 28 and 45, respectively:

Scenario 1: recalculation not applicable (the uncertainty of composition did not affect this Scenario as it is based on measured TOC); Scenario 2: 51.80 and 53.26 % ThIC; Scenario 3: 45.75 and 46.99 % ThIC; Scenario 4: 21.63 and 23.08 % ThIC.

For the new test substance in the 2019 study, assuming that the light ends fraction consisted solely of glycerol, the ultimate degradation of the monoesters was, based on the different scenarios on days 28 and 45, respectively:

Scenario 1: recalculation not applicable (the uncertainty of composition did not affect this Scenario as it is based on measured TOC); Scenario 2: 71.86 and 90.43 % ThIC; Scenario 3: 58.97 and 74.31 % ThIC; Scenario 4: 51.32% and 69.89 % ThIC.

[b]**Scenario 1:** It is assumed that CO_2 originates from the monoesters as well as the other constituents of the test item and that degradation of monoesters (% ThIC) is equal to the degradation (% ThIC) of the rest of the test substance. Therefore, the degradation of the test substance (% ThiC of the test substance) is expected to be equal to the degradation of the monoester fraction (% ThIC of the monoester fraction)

Scenario 2: It is assumed that all CO_2 production of the test substance originates from the monoesters, i.e. that the other constituents do not produce CO_2 at all (calculation: total IC produced divided by amount of monoester x 100)

Scenario 3: It is assumed that CO_2 originates from monoesters, resin acids, light ends, and glycerol, but not from di- and triesters. The CO_2 production from monoesters was calculated by subtracting the CO_2 production (TIC) from resin acids, the light ends, and glycerol (using the results for the respective days) from the total TIC production. Note that "resin acids_intitial" and "glycerol_initial" refer to the resin acids and glycerol initially present in the test substance, thus excluding the resin acids and glycerol produced from the biodegradation of the monoesters.

$$(TIC_{monoester}\ mg/L) = (TIC_{test\_substance}\ mg/L) - (TIC_{resin\_acids\_initial}\ mg/L) - TIC_{light\_ends}\ (mg/L) - (TIC_{glycerol\_initial} mg/L)$$

$$\%\ ThIC_{monoester} = TIC_{monoester}\ (mg/L) / TOC_{monoester}\ (mg/L) \times 100$$

It is assumed that the CO_2 production of all constituents of the test substance occurs via resin acid intermediates, with the exception of glycerol, light ends, and compounds derived from the glycerol moiety of the esters. Therefore, the TIC of the resin acids is expected to be equal to the TIC of the test substance subtracted by the TIC from the degradation of glycerol, light ends, and the compounds derived from the glycerol moiety of the monoesters.

It was assumed that glycerol (or any other compound derived from the glycerol moiety of the monoesters) degrades to % 60 ThIC by the respective measurement day (day 28, 42/45, or 60). Degradation to % 60 ThIC is considered to practically represent a complete ultimate degradation of the compound as the remaining fraction of 40% of the

test substance is assumed to be assimilated by the biomass or present as products of biosynthesis (OECD, 2006).

The CO_2 production from the light ends was calculated as follows:

$$(TIC_{light\_ends})\ (mg/L) = (\%\ TOC_{light\_ends}) \times 0.6 \times TOC_{test\_substance}\ (mg/L)$$

The CO_2 production from glycerol ($TIC_{glycerol}$) was calculated using the same approach as for the light ends.

TIC from compounds derived from the glycerol moiety of the monoesters (assumed to degrade to 60% ThIC) was calculated as:

$$(TIC_{glycerol\_moiety}, mg/L) = (\%\ primary\_degradation_{monoester}/100) \times (\%\ TOC_{monoester}/100) \times (3/23) \times 0.6 \times (TOC_{test\_substance}, mg/L)$$

where (3/23) is the ratio of carbon atoms in the glycerol moiety and in the monoester.

The TIC originating from resin acids (either the resin acids present in the test substance, or those released from the monoesters) was calculated as follows:

$$TIC_{resin\_acids}\ (mg/L) = (TOC_{test\_substance,}\ mg/L) - (TIC_{glycerol\_initial,}\ mg/L) - (TIC_{light\_ends,}\ mg/L) - (TIC_{glycerol\_moiety,}\ mg/L)$$

Biodegradation (% ThIC) of resin acids was then calculated as:

$$\%\ ThIC_{resin\_acids} = (TIC_{resin\_acids}\ (mg/L) / TOC_{test\_substance}\ (mg/L)) \times 100$$

The TIC of free resin acids was calculated as:

$$TIC_{resin\_acids\_initial}\ (mg/L) = (\%\ ThIC_{resin\_acids} / 100) \times \%TOC_{resin\_acids\_initial} \times TOC_{test\_substance,}\ mg/L$$

In this calculation it is assumed that the light ends are degraded to 60 % ThIC by day 28. This is based on the average degradation of 59.6% based on O_2 consumption in OECD TG 301 studies for some monoterpenes (ECHA 2020b) and sesquiterpenes (Jenner *et al.* 2011), as calculated by the evaluating MSCA (See 7.11.1). It should be noted that the degradation of the individual terpene compounds varied from 19 to 81% and the exact composition of the light ends fraction is unknown. In addition, the light ends fraction of the test substance was mentioned to consist of monoterpenes and diterpenes. The evaluating MSCA is not aware of any standard biodegradation tests on diterpenes (other information on biotransformation of diterpenes is available (de Sousa *et al.* 2018)). If the degradation of the light ends is lower than 60%, this calculation would overestimate the degradation of the light ends and underestimate the degradation of the monoesters. However, as the proportion of the light ends fraction is low (≤3.52 % of TOC), the effect of the potential error on the calculations is relatively low.

Scenario 4: It is assumed that CO_2 originates from the monoesters and other constituents and that CO_2 production from the other constituents is 60 % of the summed ThIC of the other constituents by the same day. Calculated TIC of the other constituents is subtracted from the observed TIC, and the difference is assumed to represent the TIC from the monoesters, which is then divided by the TOC of the monoesters and multiplied by 100 to obtain biodegradation (% ThIC) of the monoesters.

Regarding scenarios 2, 3, and 4: For the old test substance of the 2019 study, as there was only a combined concentration of di- and triesters, the ratio of C of di- and triesters in the test substance was assumed to be 1:1.

[c]This scenario assumes a relatively high CO_2 production from the other constituents. The composition of the test substances likely affected the CO_2 production as the constituents are likely to differ in their biodegradabilities[19]. Therefore, this scenario may underestimate the degradation of the monoesters (due to the overestimation of degradation of the other constituents) with the old test substance of the 2019 study and the 2017 study whereas for the new test substance of the 2019 study this Scenario seems more accurate.

Example calculations

Scenario 3: 2019 study, new test substance, active test, day 28

$TOC_{test\_substance}$ mg/L = 20 mgC/L

$TOC_{monoester}$ = 0.7304 x 20 mgC/L = 14.61 mgC/L

$TOC_{free\_glycerol}$ = 0.0 x 20 mgC/L = 0.0 mgC/L

TOC_{light_ends} = 0.035 x 20 mgC/L = 0.700 mgC/L

$TIC_{test\_substance}$ (mg/L) = 10.71 mgC/L

$primary\_degradation_{monoester}$ = 83 %

(TIC_{light_ends}) (mg/L) = 0.035 x 0.6 x 20 mgC/L = 0.420

($TIC_{glycerol\_moiety}$, mg/L) = (83%/100) x (0.730 x 20 mg/L) x (3/23) x 0.6 mg/L =0.948 mgC/L

TIC_{resin_acids} (mg/L) = 10.71 mgC/L - 0.420 mgC/L – 0.95 mgC/L = 9.34 mgC/L

% $ThIC_{resin\_acids}$ = 9.54 mgC/L / 20 mgC/L = 46.7 % ThIC

$TIC_{resin\_acids\_initial}$ (mg/L) = (46.7%/100) x (17.76/100) x 20 mgC/L = 1.66 mg/L

$TIC_{monoester}$ mg/L = (10.7 – 0.19- 1.73) mg/L = 8.63 mgC/L

% $ThIC_{monoester}$ = (8.63 mgC/L) / (0.730 x 20 mgC/L) x 100 = 59.1 %

Scenario 4: 2019 study, new test substance, active test, day 28

$TOC_{test\_substance}$ mg/L = 20 mgC/L

$TOC_{monoester}$ = 0.7304 x 20 mgC/L = 14.608 mgC/L

$TOC_{test\_substance\_excluding\_monoesters}$ =0.2696 x 20 mgC/L = 5.392 mgC/L

% $ThIC_{test\_substance\_excluding\_monoesters}$ = 60 %

$TIC_{test\_substance}$ (mg/L) = 10.707 mgC/L

$TIC_{test\_substance\_excluding\_monoesters}$ (mgC/L) = 5.392 mgC/L x (60/100) = 3.235 mgC/L

$TIC_{monoester}$ mg/L = (10.707 mgC/L) – (3.235 mgC/L) = 7.471 mgC/L

% $ThIC_{monoester}$ = (7.471 mgC/L) / (14.608 mgC/L) x 100 = 51.15 %

Table 26. Water solubility and octanol-water partition coefficient (Log Kow) of selected resin acids and their monoesters with glycerol, based on QSAR predictions by the eMSCA and as indicated in the literature

WATER SOLUBILITY AND Log Kow					
Compound[a]	**Solubility (WSKOW) mg/L**	**Solubility (Watsol) mg/L**	**Solubility (measured) mg/L[b]**	**Log Kow (KOWWIN)**	**Log Kow (exp)**
AA	0.0896	0.27663	2.75-4.3	6.46	no data available
DeHAA	2.412[c]	0.13468	5.11-6.6	6.52	4.80
DHAA	0.07374	0.20636	no data available	6.55	no data available
THAA	0.06068	0.15394	no data available	6.63	no data available
AA-mono-GE, alpha isomer	0.1768	3.4035	no data available	5.13	no data available
DeHAA-mono-GE, beta isomer	0.1612	1.6592	no data available	5.19	no data available

DHAA-mono-GE, alpha isomer	0.1453	2.5357	no data available	5.22	no data available
THAA-mono-GE, alpha isomer	0.1194	1.8891	no data available	5.30	no data available

[a]DeHAA=Dehydroabietic acid; AA=Abietic acid, DHAA=Dihydroabietic acid; THAA=tetra-hydroabietic acid; AA-mono-GE = Abietic acid, monoester with glycerol; DeHAA-mono-GE= Dehydroabietic acid, monoester with glycerol

[b]Source: Nyrén and Back (1958), Peng and Roberts (2000)

[c]The prediction for DeHAA is based on experimental log Kow of 4.80, whereas for all other compounds, the predictions in this table are based on estimated log Kow. The model uses as the experimental value as a default parameter for DeHAA but predicted values as default parametres for the other studied componds. If an estimated log Kow (6.5220, Kowwin v1.68) is used for DeHAA (as user entered value), the WSKOW predicted water solubility for DeHAA is 0.08161 mg/L.

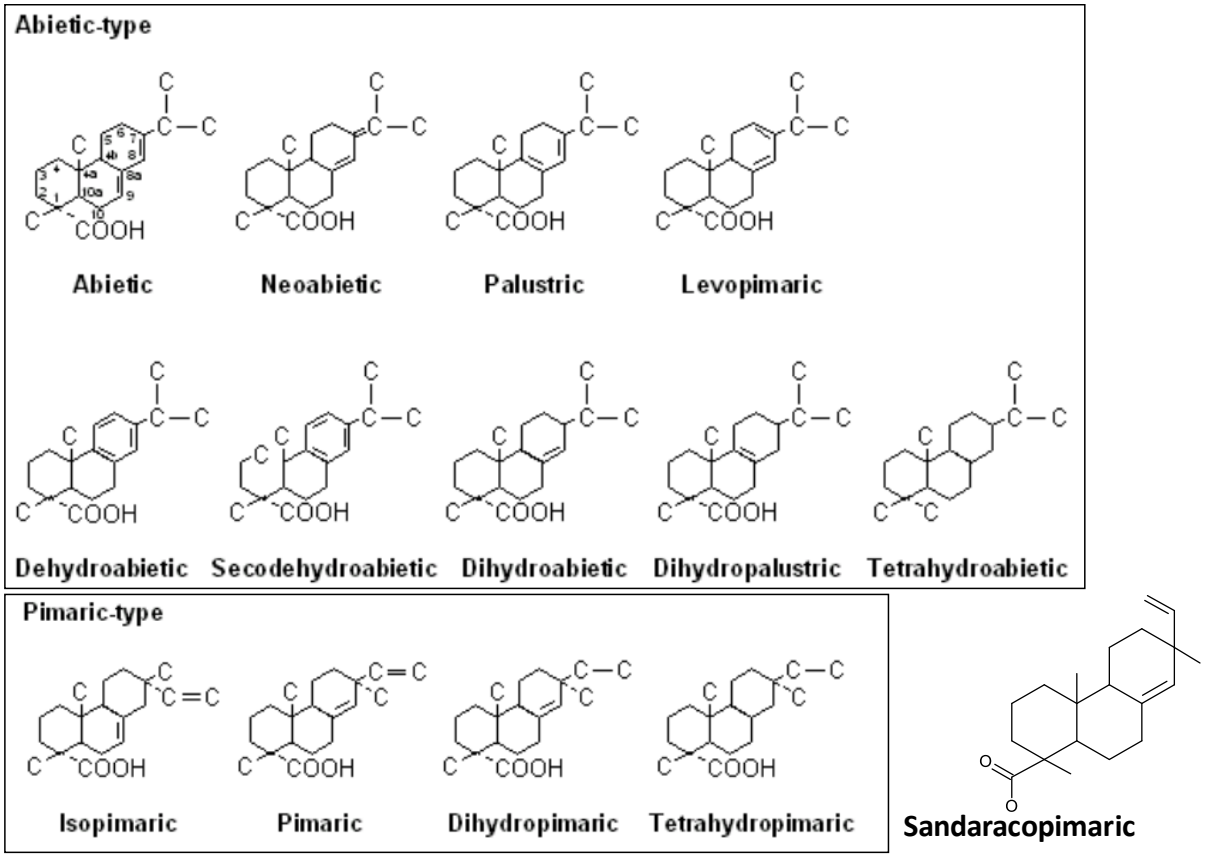


Figure 2. Structural formulas of resin acids (modified from http://www.eastman.com/Online_Publications/WA79/wa7903.htm) (accessed January 2016)

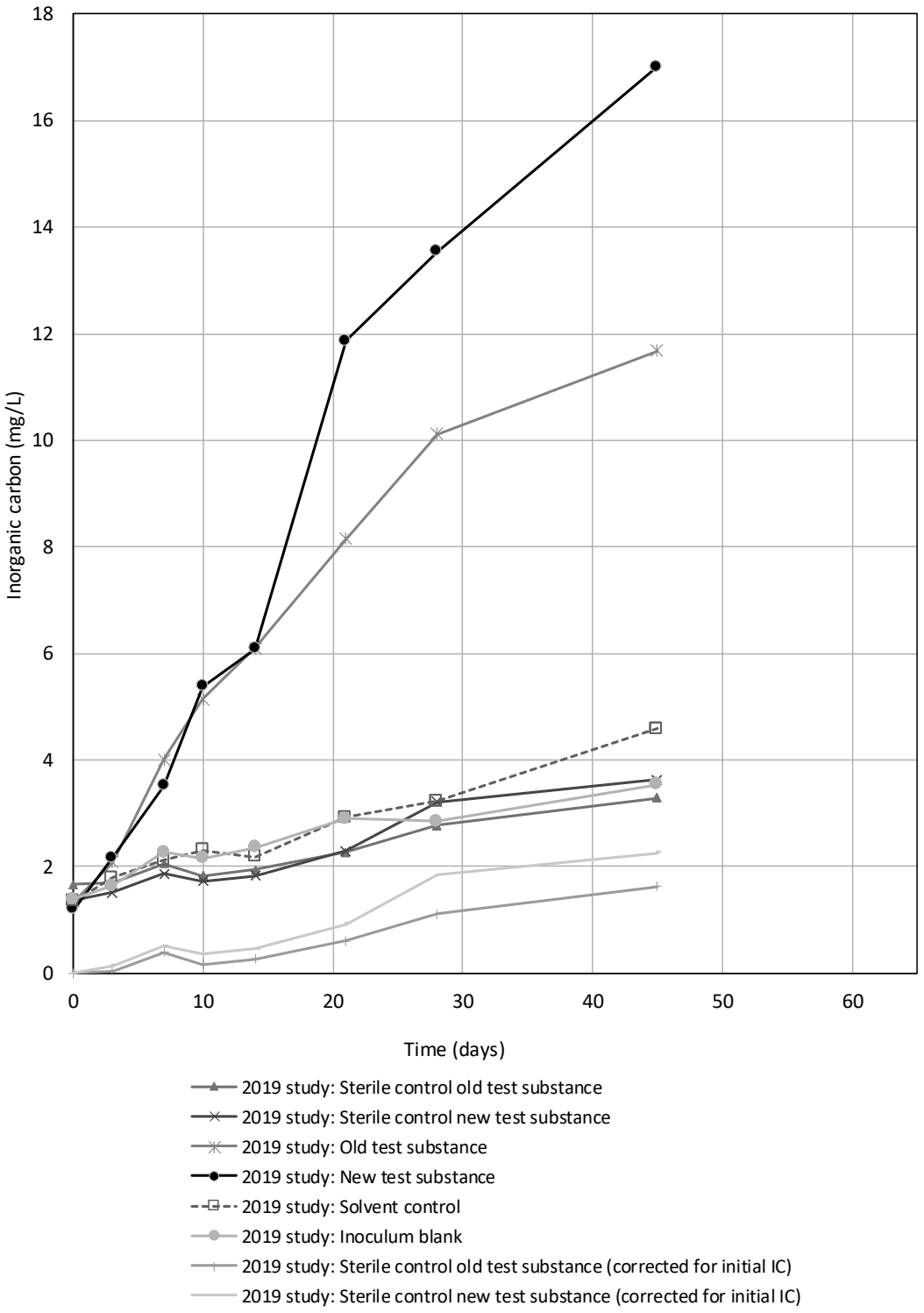


Figure 3. Inorganic carbon concentrations in the tests with "old" and "new" test substance, solvent control, blank control, and in sterile controls in OECD TG 310 study (2019). This is the raw data with no corrections (with the excpetion that the sterile controls are presented, in addition to non-corrected data, also by subtracting the amount of IC at the start of the experiment). Graph produced by the eMSCA based on the data in the Study report 2019a.

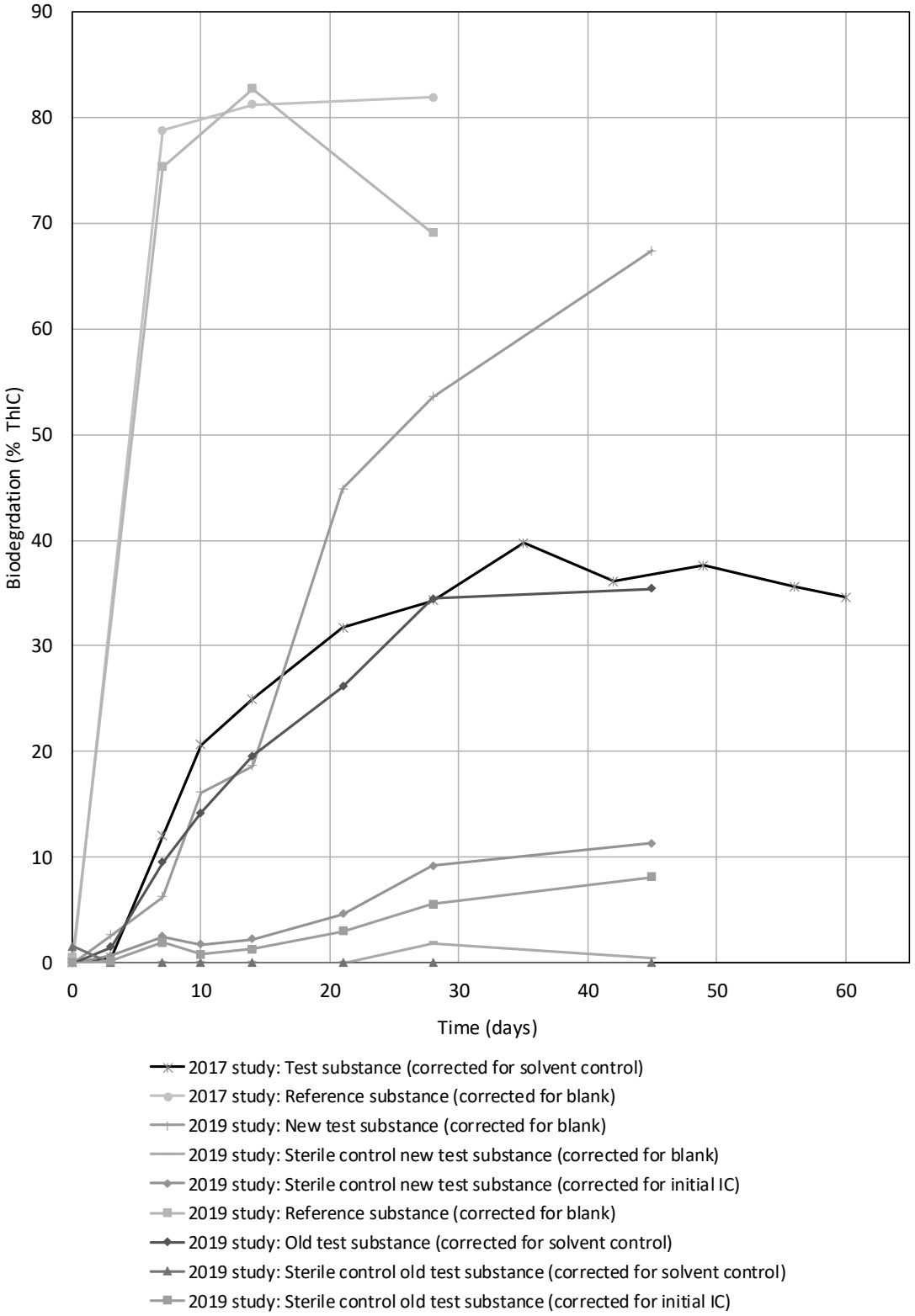


Figure 4. Biodegradation (% ThIC) of the test substances (TS) and reference substance in the two OECD TG 310 studies (2017 and 2019). Graph produced by the eMSCA based on the data in Study report 2017a and Study report 2019a.

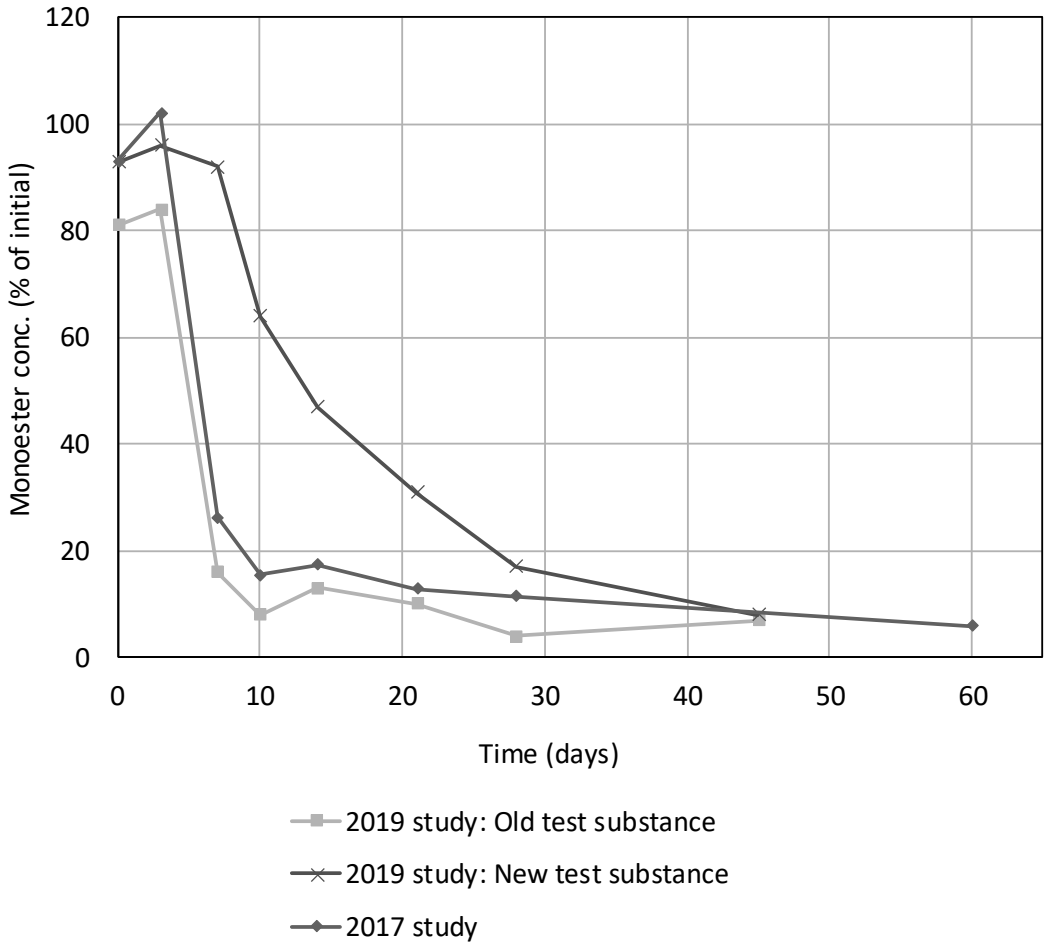


Figure 5. Concentration of the monoesters of hydrogenated rosin with glycerol in OECD TG 310 studies (2017 and 2019) in the active tests. Graph produced by the eMSCA based on the data in Study report 2017a and Study report 2019a.

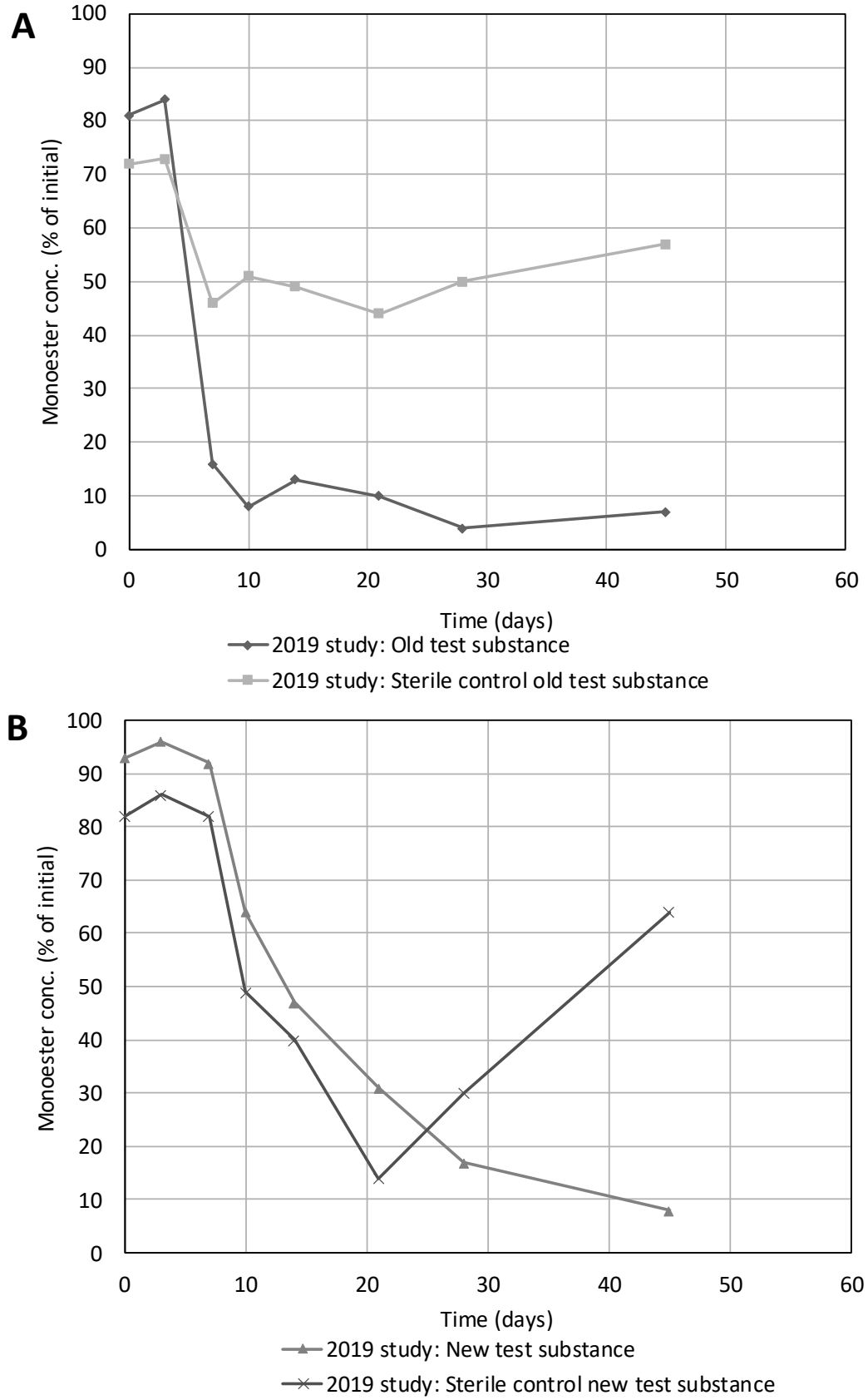


Figure 6. Concentration of the monoesters of hydrogenated rosin with glycerol in OECD TG 310 (2019) study with the "old test substance" (A) and "new test substance" (B) in the active tests and sterile controls. Graph produced by the eMSCA based on the data in Study report 2019a.

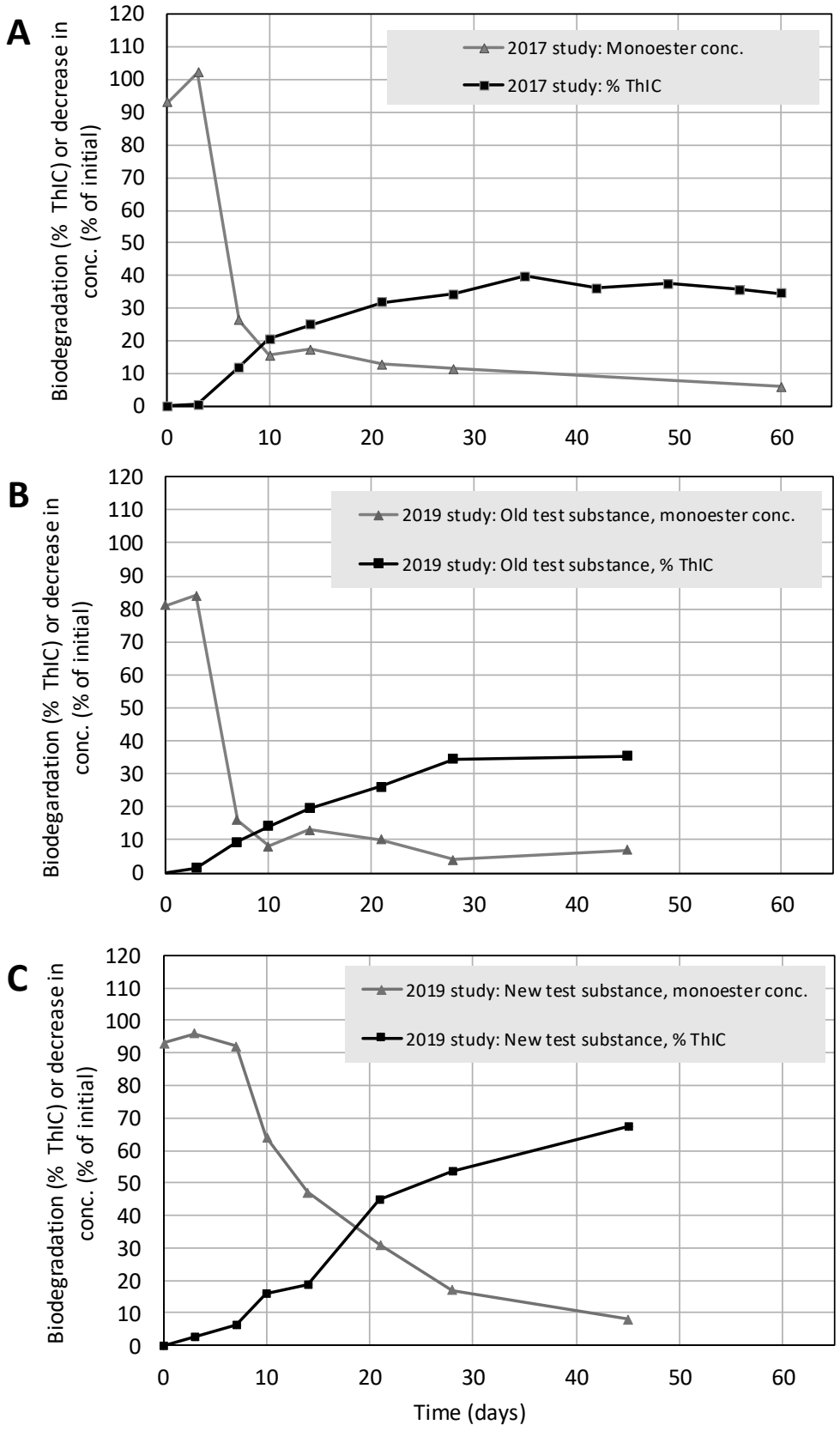


Figure 7. Concentration of the monoesters of hydrogenated rosin with glycerol and CO_2 production of the test substances in OECD TG 310 (2017) study (A) and in OECD TG 310 (2019) study with the "old test substance" (B) and "new test substance" (C) in the active tests. Graph produced by the eMSCA based on the data in Study report 2017a and Study report 2019a.

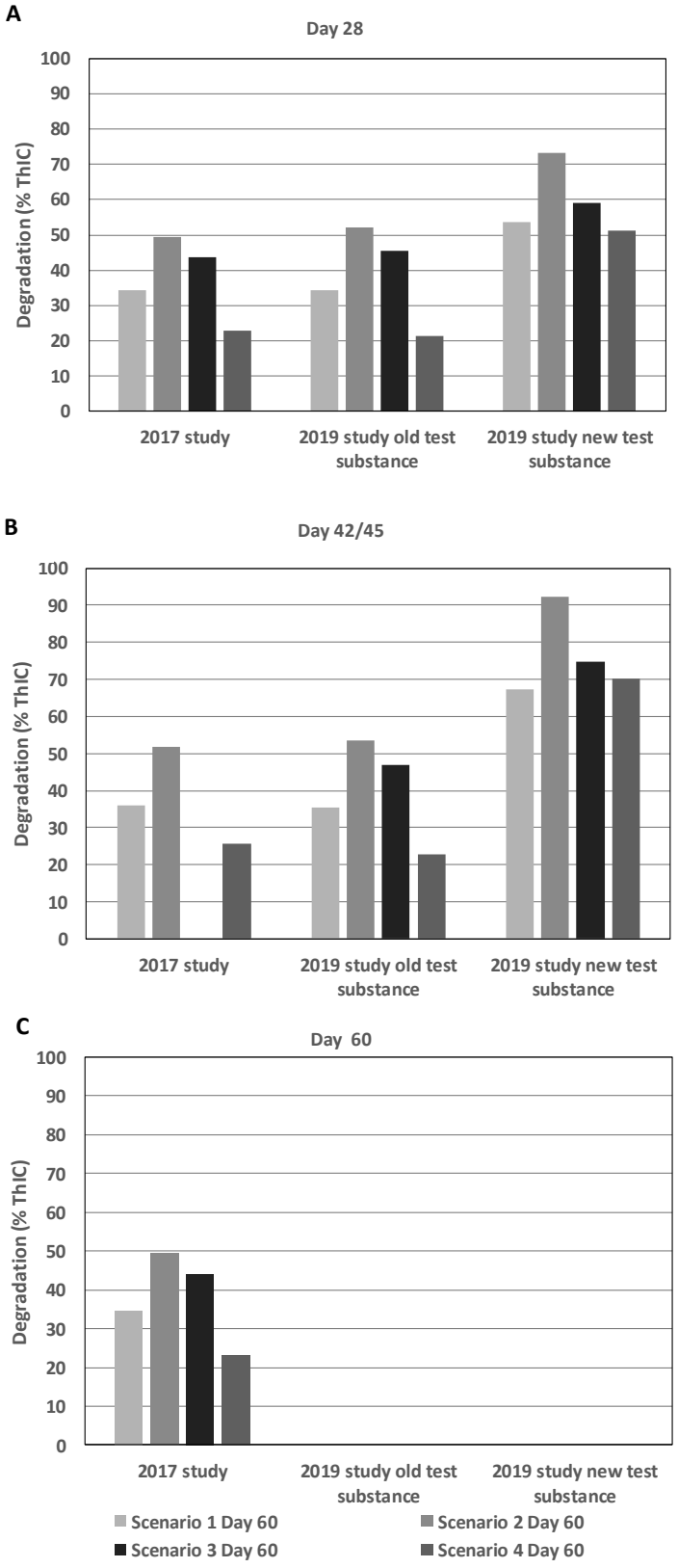


Figure 8. Estimated ultimate degradation (% ThIC) of the monoesters based on different calculation scenarios after days 28 (A), 42-45 days (B) and 60 days (C) in the OECD TG 310 studies (2017 and 2019). For the 2017 study for day 42/45, no results is available for Scenario 3 (due to the lack of primary degradation measurement). For the 2019 study, there are no results for day 60, due to the shorter duration of the study. Calculations by the eMSCA based on the data in Study report 2017a and Study report 2019b.

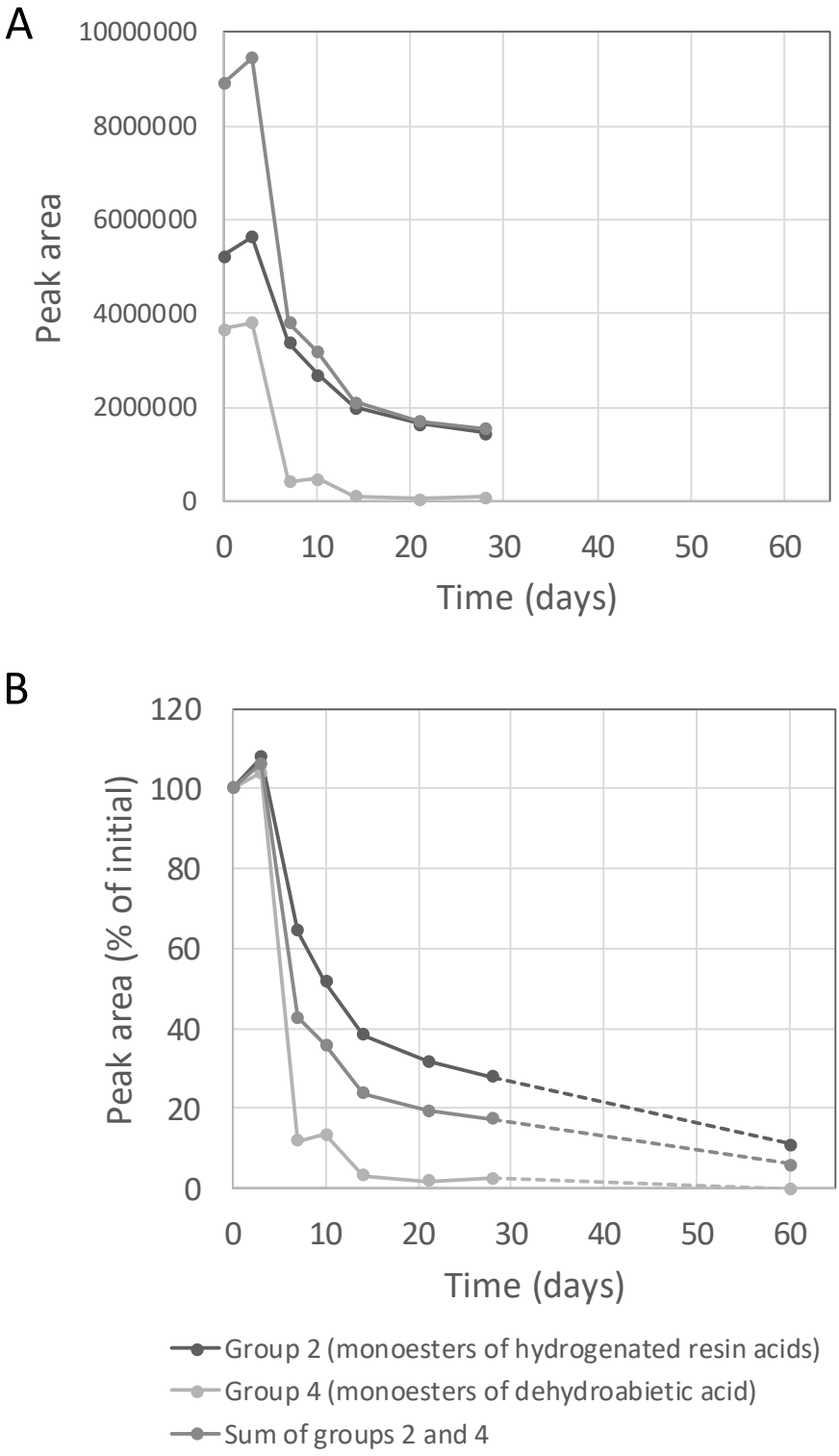


Figure 9. **Results for the two groups of monoesters studied in the OECD TG 310 (2017) study as peak areas (A) and peak areas in proportion to peak areas at day 0 (B). Graph produced by the eMSCA based on the data from Study report 2017b (days 0-28), Study report 2017a (day 60, sum of groups 2 and 4), and calculation by eMSCA based on data from Study report 2017a and 2017b (day 60, group 2 and group 4). For the two groups of monoesters the day 60 values were calculated on the assumptions that the monoesters on day 60 consisted only of Group 2 and that the ratio of the monoester concentrations on day 60 and day 0 in Study report 2017a equalled to the ratio of the monoester peak sizes on the corresponding days in Study report 2017b**.

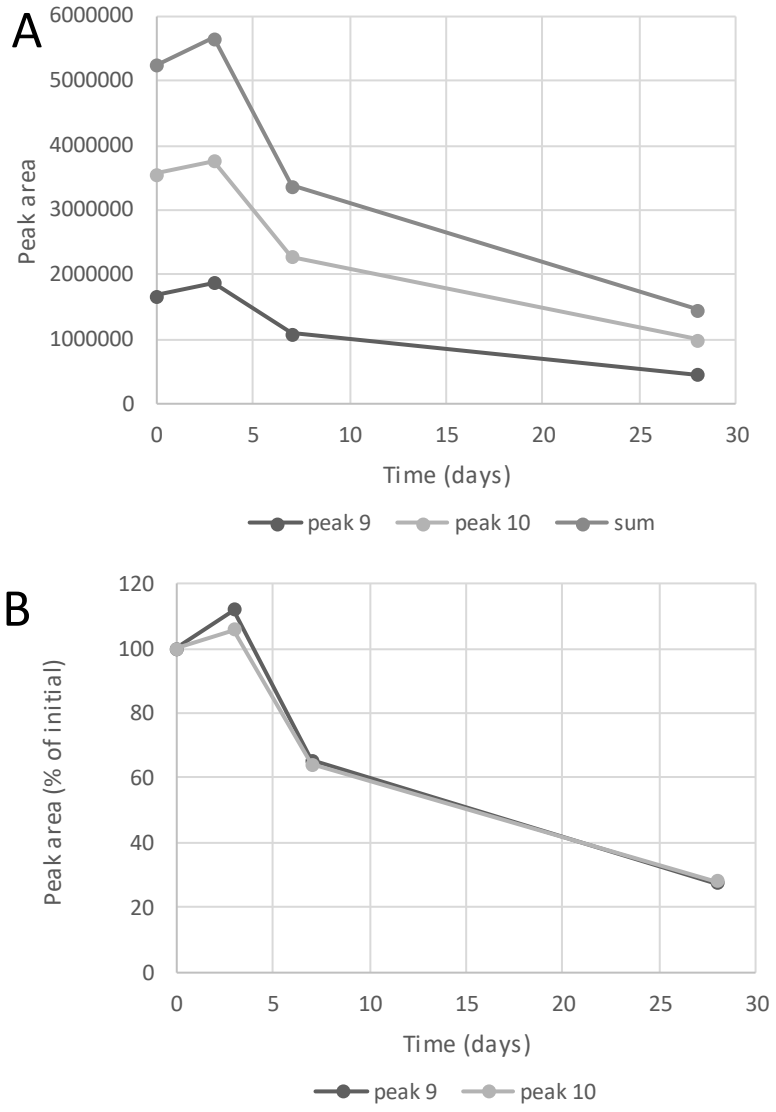


Figure 10. Results for the two integrated GC-MS peaks (peaks 9 and 10) consisting the "monoesters of hydrogenated resin acids" fraction in the OECD TG 310 (2017) study, presented as peak areas (A) and peak areas relative to day 0 (B). Graph produced by the eMSCA based on the data in Study report 2017b.

7.7.1.4 Biodegradation in water – simulation tests (water and sediment)

For the rosin esters, there are no simulation tests available. For resin acids, there are simulation tests in sediment. These are described in Section 7.7.1.7.

7.7.1.5. Biodegradation in soil

No data available.

7.7.1.6 Prediction of microbial transformation of selected monoester constituents

The biodegradation of the selected monoester constituents was investigated by the Pathway Prediction System (PPS) of the EAWAG Aquatic Research

Biocatalysis/Biodegradation Database (http://eawag-bbd.ethz.ch/predict/). Possible biodegradation metabolites predicted by the PPS are presented in Figure 11 and Figure 12 and the degradation routes are shown in Table 27.

For the glycerol monoesters, one predicted transformation route is the formation of glycerol and a resin acid (DHAA or THAA). The other predicted primary transformations are the oxidation of either of the hydroxy groups of the glycerol part (to a carbonyl group) and the resulting transformation products still include the ester bond. These can be further transformed (through a different number of transformations) to resin acids and the two- or three-carbon compounds glyceric acid, hydroxypyruvic acid, tartronic acid, or acetic acid.

In case that a primary degradation rate is used for P/vP assessment it is necessary to consider whether the transformation products have PBT/vPvB properties.

DHAA and **THAA** belong to the most hydrogenated resin acids; THAA being the fully saturated resin acid and DHAA containing one double bond. A hazard assessment has been conducted for Rosin, hydrogenated, of which resin acids are the predominant components (> 85%). The outcome was that the substance is not considered to meet the PBT/vPvB criteria based on the available, mainly screening level, information (Tukes 2015).

Regarding persistence, it is stated for Rosin, hydrogenated (Tukes 2015): "*In the registration dossiers, results from 13 ready biodegradation tests are available. The test materials used are rosin and rosin acids, their K-, Ca- and Zn- salts and hydrogenated forms of rosin acids. In four tests the substance degraded to the extent that the criteria for ready biodegradation were fulfilled. In two tests, the substance was readily biodegradable, but failed the 10-day window. In six tests, the substance was not readily biodegradable. Of these, in four tests degradation was > 45 %; in two tests degradation was 13.6 % and 0.9 %. In an OECD 302B inherent test, 73.3 % of the substance (rosin, K-salt) degraded within 28 days.*

The Episuite Biowin predictions for biodegradation indicate that individual constituents of the substance are not readily biodegradable. However, the results do not allow a screening assignment (P) in accordance with ECHA PBT guidance (R.11) Table R. 11-2.

In conclusion it can be stated that no final conclusion on P is possible based on the available data. Nevertheless, taking into account the percentages of degradation in the ready and inherent biodegradation test results, it seems unlikely that the P/vP-criterion would be fulfilled. However, no definitive conclusion can be made based on the available data."

Regarding bioaccumulation it is stated for Rosin, hydrogenated (Tukes 2015) that "*In conclusion it can be stated that, based on measured BCF-values in fish and mussels, the substance does not fulfil the B/vB-criteria.*".

It should be noted that there are indications that transformation products with potential PBT/vPvB properties may be produced from resin acids and rosin acids under anaerobic conditions (see 7.7.1.7). This was not considered in the previous assessment (Tukes 2015).

It can be concluded that the resin acid acid constituents of the Substance are not PBT and not vPvB, under aerobic conditions. However, further assessment is needed regarding transformation products that may be formed from resin acids and rosin acids under anaerobic conditions.

Glycerol is readily biodegradable according to the information on the REACH registered substance factsheet (ECHA 2020a) and according to OECD (2005).

Based on the above, the eMSCA considers that glycerol is not a PBT/vPvB substance.

The eMSCA considers that there is no PBT/vPvB concern with the other transformation products predicted to be derived from the glycerol moiety of the monoesters (Table 27).

If the primary degradation half-life of the monoesters would be shorter than the P criterion half-life and if the transformation products would only consist of glycerol, other compounds derived from the glycerol moiety, and DHAA/THAA, then DHAA-mono-GE and THAA-mono-GE could be considered as not P/ and not vP. Identification of further transformation products that may be produced from DHAA/THAA or other resin acids under aerobic conditions is not considered relevant for the present assessment (as they are not considered to be PBT/vPvB) and thus these were not investigated.

Some of the predicted transformation products of the monoesters (by Routes G1 and G3 in Table 27) still have the ester bond. For these products, PBT/vPvB assessment have not been previously conducted.

Information on the reaction rates and half-lives cannot be obtained from PPS predictions. However, the "Aerobic likelihood" indicated in the PPS predictions can be used for relative comparison of reactions. It is noted that for each of the predicted transformation products which have more than one possible further transformation routes according to the PPS, the ester hydrolysis route is always equally or more likely than the other possibility. Moreover, even if a non-hydrolytic reaction occurs first, it is predicted to be followed by a hydrolysis reaction (with or without intermediate steps), eventually leading to resin acid and glycerol or other derivative from the glycerol moiety.

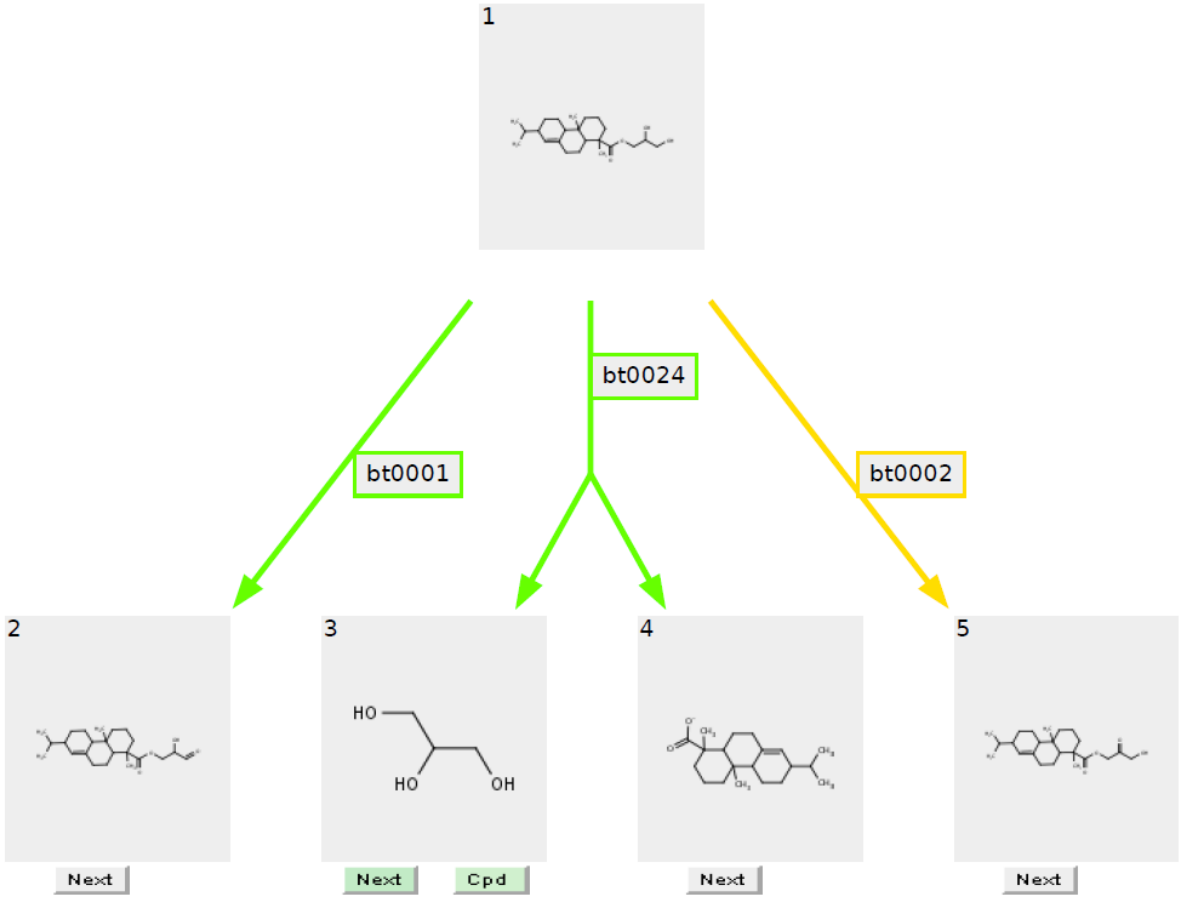
It should be noted that the current predictions using the PPS were performed using the default option, showing aerobic biotransformations only.

Table 27. Microbial transformation reactions based on EAWAG Aquatic Research Biocatalysis/Biodegradation Database (products after hydrolysis in bold). The prediction was performed with the default setting "Show biotransformations: Aerobic", which means that only those biotransformations are seen which are more likely to occur exposed to air (aerobic likelihood "neutral" or above).

MICROBIAL TRANSFORMATIONS Glycerol monoesters (DHAA-mono-GE, THAA-mono-GE)			
Route	**Reaction**	**Aerobic likelihood**	**Products and their further transformations**
G1[a]	primary Alcohol -> Aldehyde (Rule bt0001)	Likely	Product otherwise similar as the parent compound but terminal hydroxy group replaced by an aldehyde group; the aldehyde group is further predicted to transform to carboxyl group (Rule bt0003; Aerobic likelihood: likely); this product can further either split into **glyceric acid** (i.e., 2,3-dihydroxypropanoic acid) and the respective **resin acid** (Rule bt0024; Aerobic likelihood: likely) or transform to a product with a carbonyl group and a carboxyl group in the original glycerol moiety (Rule bt0002; Aerobic likelihood: neutral for the alpha isomer, likely for the beta isomer), which is predicted to either to split into the respective **resin acid** and **3-hydroxypyruvic acid** (i.e., 3-Hydroxy-2-oxopropanoic acid) (Aerobic likelihood: likely) or, alternatively, if originating from the alpha isomer, one carbon is released as CO_2 and the remaining part of the molecule is with a carboxyl group in the original glycerol moiety (Aerobic likelihood: neutral) or, if originating from the beta isomer, the remaining hydroxy group is oxidised to carbonyl (no release of CO_2)[d] (bt0001, Aerobic likelihood: likely)
G2[a]	Ester –> Alcohol + Carboxylate (Rule bt0024)	Likely	**glycerol** and the respective **resin acid**
G3[b]	secondary alcohol -> Ketone secondary alcohol -> Ester (Rule bt0002)	Neutral	Product otherwise simillar as the parent compound but a subterminal hydroxy group replaced by a carbonyl group; the product is further predicted to be either split into the respective **resin acid** and **dihydroxyacetone** (i.e., 1,3-dihydroxypropan-2-one) (bt0024; Aerobic likelihood: likely) or, alternatively, transform so that the terminal hydroxy group is oxidised to a carbonyl group (bt0001; Aerobic likelihood: likely) and further to carboxylate group (bt0003; Aerobic likelihood: likely); this product (containing a carbonyl group adjacent to the carboxyl group) is predicted to transform either (bt0024, Aerobic likelihood: likely) to the respective **resin acid** and to **3-hydroxypyruvic acid** (i.e., 3-Hydroxy-2-oxopropanoic acid) or, one carbon is released as CO_2 and the

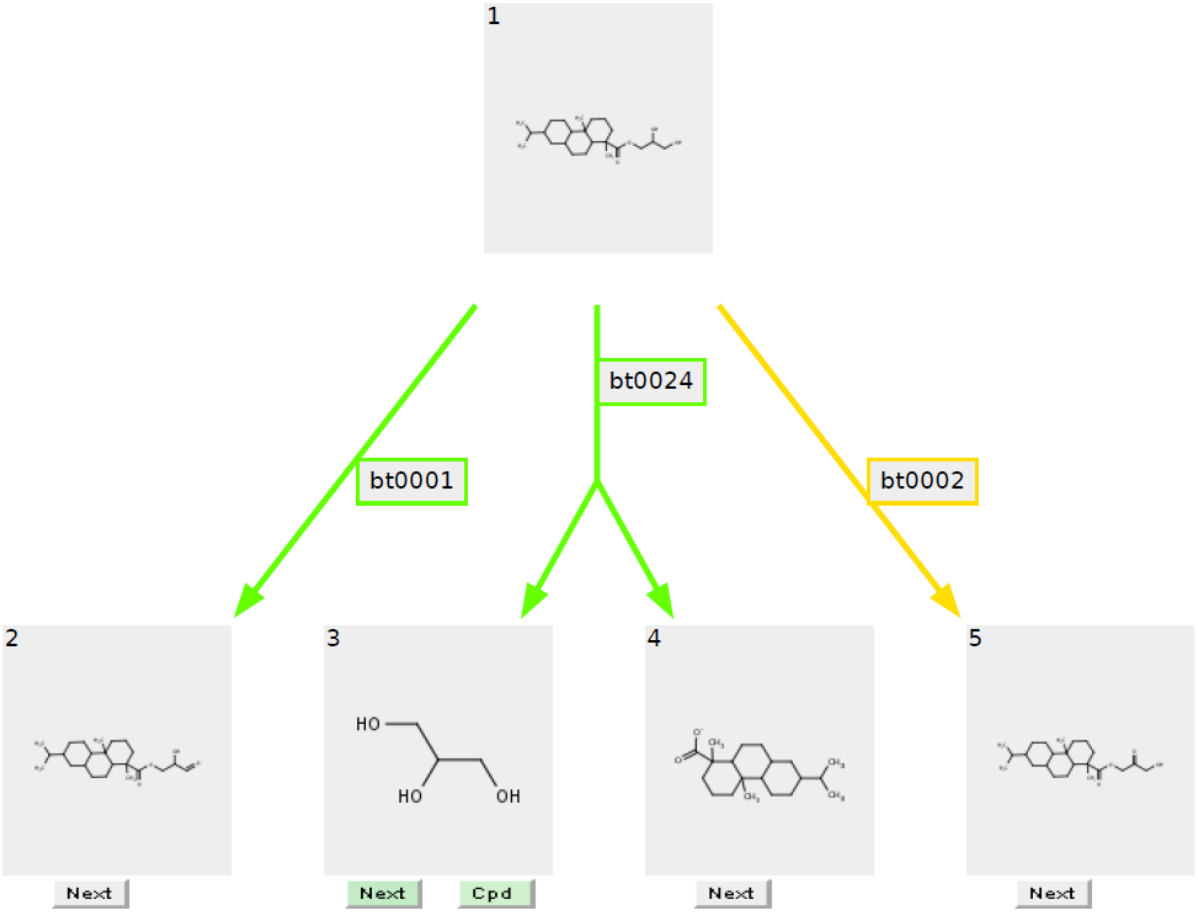
			remaining part of the molecule has a carboxyl group in the original glycerol moiety[c] (bt0082, Aerobic likelihood: neutral)

[a]Route predicted for both alpha and beta isomers
[b]Route predicted for the alpha isomer only
[c]This product is predicted to be further transformed (through bt0024; Aerobic likelihood: likely) to the respective **resin acid**. The EAWAG prediction does not indicate other products. However, based on the rule bt0024, **acetic acid** is likely to be formed.
[d]This product is predicted to be further transformed (bt0003 and bt0024; Aerobic likelihood for each reaction: likely) to the respective **resin acid** and **tartronic acid** (i.e., 2-hydroxypropanedioic acid).



Pathway prediction results from EAWAG-PPS, http://umbbd.ethz.ch/predict/ (JobID 2015.06.30-02.30.16-10)

Figure 11. EAWAG-PPS predicted pathways for DHAA-mono-GE (alpha isomer). The prediction was performed with the default setting "Show biotransformations: Aerobic".



Pathway prediction results from EAWAG-PPS, http://umbbd.ethz.ch/predict/ (JobID 2015.06.30-02.36.25-78)

Figure 12. EAWAG-PPS predicted pathways for THAA-mono-GE (alpha isomer). The prediction was performed with the default setting "Show biotransformations: Aerobic".

7.7.1.7 Biotransformation of resin acids under anoxic/anaerobic conditions

Regarding biotransformation of resin acids in anoxic conditions, the following observations from a mini-review by Martin *et al.* (1999), are considered relevant for the current assessment:

- Under anoxic conditions, resin acids can be biotransformed, but there is no conclusive evidence that their carbon skeletons are degraded. Furthermore, these anaerobic transformations have been observed only in complex microbial communities such as freshwater sediments and bioreactors.

- Resin acids are recalcitrant under a variety of anoxic conditions (Mohn *et al.* 1999 a, as cited in Martin *et al.* 1999), and no pure cultures have been found to use resin acids as a source of carbon or energy. Because of the hydrophobic nature of resin acids, they sorb to suspended solids and settle into environments devoid of oxygen such as sediments.

- A biological transformation pathway for DeHAA in anoxic sediments has been described (Tavendale *et al.* 1997a as cited in Martin *et al.* 1999). Deuterated DeHAA was incubated with anaerobic sediments and compared to a parallel autoclaved control sample. In a 264-day incubation period, dehydroabietin and tetrahydroretene were minor and major transformation products of DeHAA, respectively. These compounds had been previously observed in lake sediments (Wakeham *et al.* 1980 as cited in Martin *et al.* 1999) and were considered by Martin *et al.* (1999) to probably represent the principal path of anaerobic transformation of resin acids.

- Tetrahydroretene may be formed in a one-step reaction or may involve the formation of 20-norabietapentaenoic acid (simonellite), a short-lived intermediate measured at very low concentration. A very small percentage of tetrahydroretene was converted to retene (1.1%) and methyltetrahydrophenathrene, but the majority of the tetrahydroretene was transformed to unidentified compound(s). The time scale of these experiments clearly indicates that anaerobic transformation of resin acids is slow relative to aerobic degradation. The evidence for the anaerobic biotransformation of pimerane-type resin acids is less conclusive. Anaerobic incubation of lake sediments receiving bleached kraft mill effluent has been found to significantly reduce pimaric and isopimaric acids as compared to autoclaved control samples (Tavendale *et al.* 1997b as cited in Martin *et al.* 1999).

- The studies on the anaerobic fate of resin acids indicate that aromatization and decarboxylation to alkylated polycyclic aromatic hydrocarbons occurs and that the resulting compounds persist in the environment, as evidenced by the presence of retene and pimanthrene in dated samples. However, nitrate-reducing bacteria have recently been isolated with monoterpenes and cholesterol as sole carbon sources (Harder and Probian 1997 as cited in Martin *et al.* 1999; Foss and Harder 1998 as cited in Martin *et al.* 1999). It has been found that these organisms mineralize the organic substrates to carbon dioxide during anaerobic growth. These results suggest that resin acids may be mineralized under the appropriate anoxic culture conditions.

Resin acids and their degradation product retene have been found in sediments, especially in sediments adjacent to pulp and paper industries (Leppänen and Oikari 2001, Lahdelma and Oikari 2005). Also, the distribution modelling (Section 7.7.2) suggests that resin acids as well as other constituents of the Substance may end up in sediment.

Thus, the available information indicate that resin acids and their precursors may end up in anoxic/anaerobic sediments and may be transformed to e.g. aromatic three-ring compounds such as retene and tetrahydroretene. Several structurally relatively similar substances, such as anthracene and phenanthrene, have been identified as PBT or vPvB substances. Therefore, these transformation products form a potential PBT/vPvB concern that should be assessed further.

The Substance has uses with release to the environment. This together with the properties of the constituents (e.g., low water solubility, adsorption potential, not readily biodegradable nature) indicates that some of the constituents might reach sediments and be exposed to anaerobic conditions.

It should be noted that resin acids are constituents of many REACH registered substances. There is currently no clear guidance on how the transformation products which are formed only under anaerobic conditions should be assessed. Therefore, the eMSCA considers that this concern for the anaerobic transformation products should be also be discussed on a more generic level to assess the need for further action. Therefore, this concern is not addressed in detail in the present substance evaluation. It is noted that information on the relative proportions of the transformation products formed is available (e.g. Tavendale *et al*. 1997a, 1997b), which may be important for assessing the relevance of these products under REACH. It should also be noted that the studies by Tavendale *et al.* were conducted on a sediment exposed to bleached kraft mill effluent and therefore the sediment was likely

to be exposed to high concentrations of resin acids, which may affect the adaptation of microorganisms and biotransformation rates. For assessing the potential concern of the anaerobic transformation products, it may also be important to assess whether this would have a significant effect for the levels of these compounds (e.g., retene) in the environment.

7.7.1.8 Other studies on degradation of rosin substances

Some published studies on biodegradation of rosin ester substances (Sahu *et al.* 1999, Fulzele *et al.* 2003, Fulzele *et al.* 2007, Satturwar *et al.* 2003) are available but their significance for this assessment is considered low. These studies are mainly dealing with degradation of resin substances in animal tissues (e.g. studies on biopolymers for delivery of drugs) and not microbial degradation.

The article by Sahu *et al* (1999) includes biodegradation studies with a fungus *Aspergillus niger*, mixed culture of compost and garden soil, and sewage sludge. The rosin glycerol ester was the sole carbon source for the microorganisms. An abundant growth of *A. niger* was observed and the authors concluded that the rosin glycerol ester was utilized as a carbon source. However, it is mentioned that contamination of the agar with other microorganisms was observed along with *A. niger.* The authors mention that the contamination may be due to the fact that the rosin derivative material itself carried some microorganisms. Growth was also detected in cultures isolated from the garden soil. It is noted that degradation was monitored only by percentage weight loss, visual observations, and microscopy. Neither the concentrations of the constituents of the test substance nor the degradates were studied. The weight loss was 40-44% in 90 days in the different cultures (*A.niger*, compost elute:garden soil 50:50, and sewage sludge). It is not reported whether a sterile control experiment was included in the biodegradation studies to differentiate abiotic degradation.

The relevance of the results by Sahu *et al*. (1999) for the present assessment is considered rather low because the representativeness of the samples (e.g., pre-treatment) for the present assessment are questionable or not known, the concentrations of the constituents and degradation products were not identified, and the only quantitative parameter used for determination of degradation was percentage decrease in weight loss. The study set-up is not comparable, e.g., to ready biodegradation tests or simulation tests; nevertheless it is noted that the rate of degradation (40-44% decrease in weight in 90 days) is in line with potential persistence of the substance. Although the conclusion that the rosin glycerol ester was degraded and utilized as a microbial carbon source is interesting, no quantitative results for microbial growth (e.g. microbial biomass, cell counts) or mineralization are presented.

7.7.2. Distribution

In the registrated substance factsheet for the Substance, under the Chapter Environmental fate/Transport and distribution, there is information on adsorption but no other information on distribution in the environment.

The eMSCA has conducted distribution modelling for selected resin acid and monoester constituents (Table 29, Table 30).

With the default emission pattern of the software, i.e., equal emissions to air, water, and soil, the following observations were made:

- For the selected resin acids, the distribution was approximately 14% to water, 15% to sediment, 71% to soil, and 0.02-0.3% to air.

- For the selected monoesters, the distribution was 16-18% to water, 2% to sediment, 80-82% to soil, and 0.02-0.2% to air.
- For the selected diesters, the distribution was 6% to water, 2-3% to sediment, 91-93% to soil, and 0.003-0.3% to air.
- For the selected triesters, the distribution was 6% to water, 0 % to sediment, 94% to soil, and 0.002-0.02% to air.

Assuming that emissions are only to the water compartment, the following observations were made:

- For the selected resin acids, the distribution was approximately 48-49% % to water, 51-52% to sediment, 0.01-0.15% to soil, and 0.002-0.03% to air.
- For the selected monoesters, the distribution was 90% to water, 10-11% to sediment, 0% to soil, and 0% to air.
- For the selected diesters, the distribution was 70-78% to water, 22-30% to sediment, 0% to soil, and 0% to air.
- For the selected triesters, the distribution was 100% to water and 0% to sediment, soil, and air.

It should be noted that the distribution modelling was performed by using the default settings of the model. This means that the half-life was derived from the BIOWIN program using the approach described in Fugacity model guidance (available in the EPI Suite software). The half-lives used for the modelling are indicated inTable 28. It should also be noted that the conclusion of the present assessment is that the monoester constituents are not P and notvP, under aerobic conditions (see 7.11.1). The default sediment half-life used for the monoesters, which is above the P criterion for sediment, therefore seems to be not in accordance with this conclusion. According to the Fugacity model guidance, the sediment half-life is derived using a conversion factor which is based on the assumption that sediments are anaerobic and that the rate of ultimate biodegradation in sediment is on average one-ninth (1/9) of that in the water column (which is assumed to be aerobic). The eMSCA is not aware of any studies on anaerobic degradation of the monoesters. Therefore it is not possible to assess the relevance of the model-derived half-life in this case. The half-lives and the P/vP status of resin acids, diesters, and triesters are also unknown (see 7.11.1) and therefore the relevance of the default half-lives for these constituents is not known.

The predicted distribution to the sediment was lower for the triesters than for the diesters. This result should be used with caution as the triesters are predicted to be less water soluble than the diesters, and therefore could be expected to have a higher partitioning to the sediment. The reason for these potentially contradictory results has not been explored further in the current assessment.

Table 28. Half-lives used as input parametres for the distribution modelling

HALF-LIVES FOR MODELLING				
Compound / Compartment	**Air**	**Water**	**Soil**	**Sediment**
Resin acids	Half-life (hours)			
DHAA	0.502	900	1.8e+003	8.1e+003

THAA	0.304	900	1.8e+003	8.1e+003
Glycerol esters				
DHAA-mono-GE, alpha isomer	0.487	900	1.8e+003	8.1e+003
DHAA-mono-GE, beta isomer	0.49	900	1.8e+003	8.1e+003
THAA-mono-GE, alpha isomer	5.68	900	1.8e+003	8.1e+003
THAA-mono-GE, beta isomer	6.14	900	1.8e+003	8.1e+003
DHAA-di-GE, 1,2-isomer	0.249	4.32e+003	8.64e+003	3.89e+004
THAA-di-GE, 1,2-isomer	3.76	4.32e+003	8.64e+003	3.89e+004
DHAA-di-GE, 1,3-isomer	0.248	4.32e+003	8.64e+003	3.89e+004
THAA-di-GE, 1,3-isomer	3.58	4.32e+003	8.64e+003	3.89e+004
DHAA-tri-GE	0.167	4.32e+003	8.64e+003	3.89e+004
THAA-tri-GE	2.71	4.32e+003	8.64e+003	3.89e+004

Table 29. Environmental distribution of selected constituents of the Substance as predicted by Level III fugacity model in EPI Suite v.4.11. The input parameters were according to the default settings of the software (including the emission values air 1000 kg/hr, water 1000 kg/hr, soil 1000 kg/hr).

ENVIRONMENTAL DISTRIBUTION, DEFAULT EMISSIONS				
Compound / Compartment	**Air**	**Water**	**Soil**	**Sediment**
Resin acids	Distribution, mass amount (%)			
DHAA	0.0203	14.1	70.9	15
THAA	0.304	14	71.2	14.5
Glycerol esters				
DHAA-mono-GE, alpha isomer	0.0211	17.6	80.4	1.96
DHAA-mono-GE, beta isomer	0.0212	17.5	80.4	2.05
THAA-mono-GE, alpha isomer	0.169	15.9	82.1	1.77
THAA-mono-GE, beta isomer	0.177	15.8	82.2	1.84
DHAA-di-GE, 1,2-isomer	0.00257	6.18	91.1	2.68

THAA-di-GE, 1,2-isomer	0.0296	5.75	92.5	1.69
DHAA-di-GE, 1,3-isomer	0.00256	6.19	91.2	2.56
THAA-di-GE, 1,3-isomer	0.0285	5.77	92.6	1.62
DHAA-tri-GE	0.00178	6.45	93.6	0.00000304
THAA-tri-GE	0.0234	6.03	94	0.00000156

Table 30. Environmental distribution of selected constituents of rosin esters, hydrogenated as predicted by Level III fugacity model in EPI Suite v.4.11. The input parameters were according to the default settings of the software except considering emissions to water only (emission values air 0 kg/hr, water 1000 kg/hr, soil 0 kg/hr).

ENVIRONMENTAL DISTRIBUTION, EMISSIONS TO WATER ONLY				
Compound / Compartment	**Air**	**Water**	**Soil**	**Sediment**
DHAA	0.0*0227*	48.4	0.0*123*	51.6
THAA	0.0*328*	49	0.1*48*	50.8
Glycerol esters				
DHAA-mono-GE, alpha isomer	03.75e-006	90	0.000264	10
DHAA-mono-GE, beta isomer	3.76e-006	89.5	0.000263	10.5
THAA-mono-GE, alpha isomer	3.25e-005	90	0.00229	10
THAA-mono-GE, beta isomer	3.43e-005	89.6	0.00241	10.4
DHAA-di-GE, 1,2-isomer	1.51e-011	69.8	5.09e-009	30.2
THAA-di-GE, 1,2-isomer	1.33e-010	77.3	4.46e-008	22.7
DHAA-di-GE, 1,3-isomer	1.53e-011	70.7	5.14e-009	29.3
THAA-di-GE, 1,3-isomer	1.29e-010	78.1	4.33e-008	21.9
DHAA-tri-GE	4.73e-016	100	1.59e-013	4.72e-005
THAA-tri-GE	3.3e-015	100	1.11e-012	2.59e-005

7.7.3. Bioaccumulation

7.7.3.1 Experimental data

There are no experimental data available on the bioaccumulation of rosin esters.

7.7.3.2 Partition coefficients

Octanol-water partition coefficients (log Kow) can be applied as a screening criterion for bioaccumulation. A log Kow greater than 4.5 indicates potential for bioaccumulation, based on the assumption that the uptake of an organic substance is driven by its hydrophobicity. For organic substances with a log Kow value below 4.5 it is assumed that the affinity for the lipids of an organism is insufficient to exceed the B criterion, i.e. a BCF value of 2000 L/kg (based on wet weight of the organism, which refers to fish in most cases). A very high lipophilicity and a very low water solubility may also limit the bioavailability of substances and hence the potential for bioaccumulation. Log Kow > 10 can be used as weight-of-evidence information for indicating that a substance is not B (ECHA 2017b).

In general, bioaccumulation of acids increases with decreasing pH, and highest toxicity and bioaccumulation will be found where the compounds are the most neutral, at the isoelectric point (the point in the middle of the two pKa values) (Rendal et al. 2011). Based on modelling results (ACD Labs, ACD/Percepta 14.0.0) on the selected esters of resin acids with glycerol, the mono-, di- and triester constituents of have no dissociation constant (pKa, acid or base) or the pKa acid is extremely high (pKa > 13), meaning that these constituents are in their neutral forms at all relevant pH ranges. The predicted log D (distribution coefficient corresponding to log Kow at varying pH) values are the same as log Kow values, meaning that these ester constituents are not expected to have ionized forms. Hence the differences in log Kow for the Substance at different conditions (pH and buffering) (Table 7) were most likely not affected by variation between neutral and ionized form of the ester constituents.

Based on the log Kow values predicted by KOWWIN model for the selected individual structures (Table 9) (see Annex 2 for structural formulas), it can be stated that monoesterified glycerol constituents have potential for bioaccumulation, whereas the structures with a higher degree of esterification (di- and triesters of glycerol) have log Kow values exceeding 10 and indicating lower potential.

Also the experimental log Kow results support that these UVCB substances have bioaccumulation potential, although it is not possible to draw conclusions on individual constituents as the analytical peaks have not been identified (Table 31). It is noted that the measured log Kow values range from 4.6 – 7.3. Therefore, it seems that the HPLC method did not identify components with log Kow > 10. This might be explained by the fact that the used reference substances covered a log Kow range of 1 – 6.2. Thus, the method is not calibrated for log Kow values > 6.2.

Octanol-air partition coefficient (log Koa) can be used to estimate the potential of a substance to bioaccumulate in air breathing organisms (Table 32). Based on JRC (2014) non-metabolised hydrophobic organic substances with log Kow > 2 and log Koa > 5 can have potential to biomagnify in non-aquatic food chains.

Table 31. Experimental log Kow values for the Substance.

EXPERIMENTAL Log Kow			
Method	**Test substance**	**Log Kow**	**Reference**
HPLC method (OECD 117) Range of logKow covered by reference substances: 1 – 6.2. GLP	Rosin acids, hydrogenated esters with glycerol (unbuffered media)	4.7 and 5.8 (two peaks)	Study report 2003a

EU Method A.8 (Partition Coefficient)	Rosin acids, hydrogenated esters with glycerol	3.28	Study report 2010a

Table 32. Log Koa values predicted by EPISuite KOAWIN for representative constituents of the Substance.

Para-meter	THAA-mono-GE	DHAA-mono-GE	DHAA-di-GE	DHAA-tri-GE
Log Koa (KOAWIN v.1.0)	11.924	11.828	18.910	23.392

Estimation of the applicability and accuracy of KOWWIN predictions to the Substance

According to EPI Suite help, *"The KOWWIN model is built on a training set of 2447 compounds. The model has been tested on an external validation dataset of 10 946 compounds (compounds not included in the training set). The validation set includes a diverse selection of chemical structures that rigorously test the predictive accuracy of any model. It contains many chemicals that are similar in structure to chemicals in the training set, but also many chemicals that are different from and structurally more complex than chemicals in the training set. The average molecular weight of compounds in the validation set is 258.98 versus 199.98 for the training set. "The correlation between the experimental and predicted logKow values in the validation set is r^2 = 0.943, std = 0.479."*

"Currently there is no universally accepted definition of model domain. However, users may wish to consider the possibility that log Kow estimates are less accurate for compounds outside the MW range of the training set compounds (18.02 – 719.92), and/or that have more instances of a given fragment than the maximum for all training set compounds. It is also possible that a compound may have a functional group(s) or other structural features not represented in the training set, and for which no fragment coefficient was developed. These points should be taken into consideration when interpreting model results."

In general, the correlation between the experimental and predicted log Kow values in the validation set is considered quite good by the eMSCA (r^2 = 0.943, std = 0.479).

Regarding the representative mono- and diester constituents of the Substance the model identifies several fragments and applies two correction factors (see Table 33). The triesterified compounds exceed the MW range of the training set compounds (18.02 – 719.92). Nevertheless, the accuracy of the predictions for validation set substances that exceeded the molecular weight domain is considered reasonably good by the eMSCA (number of substances 103, r^2= 0.879 and std 0.815).

In conclusion, it can be stated that the applicability and accuracy is best for the monoesterified constituents, whereas for the diesters, and especially triesters more uncertainty is expected for the predictions. Nevertheless, it seems reasonable to estimate that the log Kow values exceed 10 for the di- and triesters.

Table 33. Fragments and correction factors applied by KOWWIN

KOWWIN					
	Fragment / factor	**Maximum number of instances in any substance in the training set**	**Number of fragments in analyzed constituents (in bold when exceeding the maximum)**		
			DHAA-mono-GE	DHAA-di-GE	DHAA-tri-GE
Frag	-CH3 [aliphatic carbon]	13	4	8	12
Frag	-CH2- [aliphatic carbon]	18	9	16	**23**
Frag	-CH [aliphatic carbon]	16	5	9	13
Frag	=CH- or =C< [olefinic carbon]	10	2	4	6
frag	-OH [hydroxy, aliphatic attach]	6	2	1	0
Frag	-C(=O)O [ester, aliphatic attach]	3	1	2	3
Frag	- tert Carbon [3 or more carbon attach]	4	2	4	**6**
Factor	Multi-alcohol-correction	1	1	0	0
Factor	Fused aliphatic ring unit correction	8	4	8	**12**

7.7.3.3 Predicted bioconcentration factors (BCF) and bioaccumulation factors (BAF)

Measures of BAF would be the preferred metric for assessing bioaccumulation potential of HRGE type of substances (Table 34). This is because BCF may not adequately account for the bioaccumulation potential of substances via the diet, which predominates for substances with log Kow > ~ 4.0 (Arnot and Gobas 2003). However, for the comparison of REACH Annex XIII criteria, the BCF-values are the preferred metric.

Table 34. BAF values predicted by EPISuite Arnot-Gobas (upper trophic*) for representative consituents of the Substance.

Para-meter/ EPISuite BCFBAF model	THAA-mono-GE	DHAA-mono-GE	DHAA-di-GE	DHAA-tri-GE
BAF L/kg wet-wt*	229	214	280	0.893
BAF L/kg wet-wt**	328100	242300	2292	0.8931

*) including biotransformation rate estimates

**) zero biotranformation

The predicted BCF values suggest, in general, that the lower the level of esterification is the higher the potential for bioaccumulation is (**Error! Reference source not found.**). For di- and triesterified structures even the worst case estimation (Arnot Gobas assuming zero biotransformation) predicts BCF values that are well below the B criterion.

Table 35. BCF values predicted by EPI Suite BCFBAF for representative constituents of the Substance, L/kg (In bold when exceeding B criterion).

Para-meter/ EPI Suite BCFBAF model	THAA-mono-GE	DHAA-mono-GE	DHAA-di-GE	DHAA-tri-GE
regression based	1465	1287	34.57	3.16
Arnot-Gobas, upper trophic, 10.7 % lipids*	227.7	213.4	0.9966	0.893
(Arnot-Gobas, upper trophic, 5 % lipids*	106.4	99.7	0.466	0.417
Arnot-Gobas, upper trophic, 10.6 % lipids**	**12420**	**11060**	1.344	0.893
Arnot-Gobas, upper trophic, 5 % lipids**	**5804**	**5168**	0.628	0.417
Biotrans-formation half-life normalized to 10 g fish (days)	0.56	0.52	128	4139

*) including biotransformation rate estimates

**) zero biotranformation

7.7.3.3.1 Estimation of the applicability of EPI Suite BCFBAF regression model to the Substance

According to EPI Suite help, the BCFBAF regression model uses the non-ionic regression models to predict BCF values for the ester constituents of the Substance. Considering the pKa values and that the ester constituents are expected to occur in the neutral form in relevant pH values this seems reasonable. The non-ionic training dataset includes 466 compounds. The dataset is divided into three groups based on log Kow values (log

Kow < 1.0, log Kow 1.0 to 7.0 and log Kow > 7.0). For each group a "best-fit" straight line has been derived by common statistical regression methodology. The regression methodology includes derivation of correction factors based on specific structural features (ketone, phosphate ester, multi-halogenated biphenyl/PAH, aromatic ring-CH-OH, aromatic sym-triazine ring, ter-butyl ortho-phenol type, phenanthrene ring, cyclopropyl-C(=O)-O-ester, alkyl chains, disulfide, multihalogenated phenol). These correction factors are not applicable to ester constituents of the Substance. Therefore, the model predictions are based on the regression equations without structure related correction factors. The following equations are used to predict the BCF-values:

Log BCF = -0.49 log Kow + 7.554 (log Kow > 7; no applicable correction factor)

According to EPI Suite help, *"there is currently no universally accepted definition of model domain. However, users may wish to consider the possibility that bioconcentration factor estimates are less accurate for compounds outside the MW and logKow ranges of the training set compounds, and/or that have more instances of a given correction factor than the maximum for all training set compounds. It is also possible that a compound may have a functional group(s) or other structural features not represented in the training set, and for which no fragment coefficient was developed; and that a compound has none of the fragments in the model's fragment library. In the latter case, predictions are based on molecular weight alone. These points should be taken into consideration when interpreting model results."*

The minimum and maximum values for molecular weight and log Kow for the training set are listed below:

Molecular Weight:

Minimum MW: 68.08 (Furan)
Maximum MW: 959.17 (Benzene, 1,1 -oxybis[2,3,4,5,6-pentabromo-)
Average MW: 244.00

Log Kow:

Minimum Log Kow: -1.37 (1,3,5-Triazine-2,4,6-triamine)
Maximum Log Kow: 11.26 (Benzenamine, ar-octyl-N-(octylphenyl)-)

The mono-GE compounds are within the set boundaries for molecular weight and log Kow values, whereas the di- and tri-GE compounds are outside the upper limit of log Kow value. In conclusion, it can be stated that the applicability and accuracy is best for the monoesterified constituents, whereas for the di-and triesters more uncertainty is expected for the predictions.

7.7.3.3.2 Estimation of the applicability of EPI Suite BCFBAF Arnot-Gobas model to the ester constituents of the Substance

According to EPI Suite help, *"the Arnot-Gobas model estimates steady-state bioconcentration factor (BCF; L/kg) and bioaccumulation factor (BAF; L/kg) values for non-ionic organic chemicals in three general trophic levels of fish (i.e., lower, middle and upper) in temperate environments. The model calculations represent general trophic levels (i.e., not for a particular fish species) and are derived for "representative" environmental conditions (e.g., dissolved and particulate organic carbon content in the water column, water temperature). Thus, it provides general estimates for these conditions in absence of site-specific measurements or estimates. The default temperature for the BCF and BAF calculations is 10°C."*

There is no specific description of model applicability or accuracy. However, *"model predictions may be highly uncertain for chemicals that have estimated log K_{OW} values > 9."*

It is further noted, that the Arnot-Gobas model assumes default lipid contents of 10.7%, 6.85% and 5.98% for the upper, middle and lower trophic levels. Since the laboratory studies from which most data in the measured BCF database were derived typically used fish with 3-5% lipid content, this may help to explain why the regression-based BCF model typically yields estimated BCF values lower than from the Arnot-Gobas model. Therefore the values predicted for the Substance have been normalized to 5% lipids (**Error! Reference source not found.**).

In conclusion, the predictions of the model for di- and triesterified constituents should be considered highly uncertain as they have log Kow values > 9.

7.7.3.3.3 The whole body primary biotransformation rate constant (k_M) model for fish

According to EPI Suite help, *"Biotransformation is defined as the change of the parent substance to another molecule or a conjugated form of the parent substance. The model calculates k_M as a whole body value, namely the fraction of the mass in the whole body biotransformed per unit of time. The model does not provide predictions for the formation of specific biotransformation products (some of which may be more toxic than the parent compound), nor does it identify specific pathways for the biotransformation process (Phase I oxidations or reductions or Phase II conjugations). If formed metabolites are known, these can be re-introduced to the model as a distinct substance for novel predictions. The model assumes first-order processes and cannot estimate biotransformation rates that may occur under non-first order conditions (e.g., enzyme saturation).*

A dataset of 632 experimental kM biotransformation rates in fish (compiled in units of log biotransformation half-lives in days) was divided into a training set of 421 compounds for model derivation and validation set of 211 compounds for model testing.

Initially, each individual compound in the training set was divided into structural fragments based on the same fragments used by the BIOWIN Program (biodegradation probability) and BioHCwin Program (biodegradation of hydrocarbons). Fragments not occurring in any training set compound were excluded from the model derivation. Initial regressions were used to identify fragments having no statistical significance (with coefficient values having little or no effect on results), and these fragments were excluded from the final regression. Several new fragments were added based on structural similarities of the training set compounds. The final multiple-linear regression was performed on a matrix containing the number of occurrences of each fragment in each compound plus the logKow and molecular weight of each compound.

The final multiple-linear regression-derived equation (which is used by the BCFBAF program to estimate the kM Biotransformation Half-Life) is:

$$\text{Log kM/Half-Life (in days)} = 0.30734215*\text{LogKow} - 0.0025643319*\text{MolWt} - 1.53706847 + \boldsymbol{\Sigma}(F_i*n_i)$$

where Log Kow is the log octanol-water partition coefficient, MolWt is the Molecular Weight, and $\boldsymbol{\Sigma}(F_i*n_i)$ is the summation of the individual Fragment coefficient values (F_i) times the number of times the individual fragment occurs in the structure (n_i). The -1.53706847 is the equation constant.

Currently there is no universally accepted definition of model domain. However, users may wish to consider the possibility that biotransformation estimates are less accurate for compounds outside the MW and log Kow ranges of the training set compounds, and/or that have more instances of a given fragment than the maximum for all training set compounds. It is also possible that a compound may have a functional group(s) or other

structural features not represented in the training set, and for which no fragment coefficient was developed; and that a compound has none of the fragments in the model's fragment library. These points should be taken into consideration when interpreting model results."

Training Set (421 Compounds):

Molecular Weight:

Minimum MW: 68.08 (Furan)

Maximum MW: 959.17 (Decabromodiphenyl ether)

Average MW: 259.75

Log Kow:

Minimum LogKow: 0.31 (Benzenesulfonamide)

Maximum LogKow: 8.70 (Decabromodiphenyl ether)

Regarding the ester constituents of the Substance, it can be stated that monoester constituents (which are most relevant for B assessment as they fulfill the screening criterion for log Kow), fit the range of molecular weights and log Kow values of the training set. The fragments of these constituents seem to be reasonably well identified by the model (Table 36).

Table 36. Fragments and correction factors applied by Arnot-Gobas biotransformation rate constant (k_M) model

Fragment	Maximum number of instances in any substance in the training set	THAA-mono-GE	DHAA-mono-GE
Aliphatic alcohol [-OH]	3	2	2
Ester [-C(=O)-O-C]	2	1	1
Carbon with 4 single bonds & no hydrogens	10	2	2
Methyl [-CH3]	12	4	4
-CH2- [linear]	28	2	2
-CH- [linear]	2	2	2
-CH2- [cyclic]	12	8	7
-CH - [cyclic]	17	4	3
-C=CH [alkenyl hydrogen]	6	0	1
Number of fused acyclic rings	5	1	1
Polycyclic -CH3 (3 fused rings or less)	na	2	2

7.7.3.4 Molecular size as a limiting factor for bioaccumulation

ECHA's Guidance on information requirements and chemical safety assessment (Chapter R.11 PBT assessment) (2017b) presents indicators that can be used to strengthen the evidence for limited bioaccumulation potential of a substance, such as information on molecular size (maximum molecular length (MML), average maximum diameter (Dmax aver) or molecular weight (MW) of a substance).

The size related screening criteria indicating limited possibility of the molecules to penetrate through the cell membranes are:

Not B: Dmax aver > 1.7 nm and MW > 1100

Not vB: Dmax aver > 1.7 nm and MW > 700

The strict cut-off values for molecular weight and size in predicting bioaccumulation potential have been under scientific evaluation in 2000's (e.g. Dimitrov *et al.* 2005, Sakuratani *et al.* 2008, Arnot *et al.* 2010). The overall conclusion is that size parameters should be applied with caution in predicting bioaccumulation potential, as, in some cases, large molecules with slow uptake rates may show even slower elimination rates, which could lead to bioaccumulation. It has also been stated that the length of a needle shape large molecule has little limiting effect on the cell uptake rate and hence the shape of molecule should be carefully considered. Here, the molecules of the ester constituents of the Substance are branched and especially the higher esters have large cross-sectional diameters, which imply that size limiters can be applied with some reliability.

The molecular sizes and molecular weights presented in Table 37 indicate that the more complete the esterification of the rosin esters is, the more reduced bioaccumulation potential can be expected for these substances due to the slower uptake rates of the molecules.

Based on the molecular size estimates, triesters have probably low potential for bioaccumulation, diesters are borderline cases and monoesters can be potentially bioaccumulable.

The (Q)SAR estimates for BCF-values also indicate that the bioaccumulation decreases with increasing rate of esterification (and concurrently with increasing molecular size).

Table 37. Molecular sizes established for the Substance (*)

MOLECULAR SIZES		
Molecular weight (g/mol)	monoester diester triester	378.6 665.0 951.5
Molecular size, min-max Dmax (nm)	monoester diester triester	1.27 - 1.90 1.59 - 3.05 1.98 - 2.72

*) Canadian POPs Model, 2008 in Environment Canada (2011)

7.7.3.5 Bioaccumulation potential of the starting materials (resin acids and alcohol)

The starting alcohol, glycerol (EC 200-289-5) does not have potential to bioaccumulate, based on the measured partition coefficient value, log Kow -1.75 (ECHA, Registered substances database), which is well below the screening criterion of bioaccumulation (log Kow ≥ 4.5).

The starting component hydrogenated resin (HR) (EC 266-041-3) is also not bioaccumulable according to QSAR evaluation (EPI Suite BSFBAF v3.00). The estimated log BCF is 0.5 (regression based method) and log BAF is 2.4 (Arnot-Gobas upper trophic method). The measured value of log Kow 3.42 supports this estimate.

In the registration dossiers, one experimental bioaccumulation study on resin acids is available, which shows that resin acids (abietic, dehydroabietic, chlorohydroabietic, dichlorohydroabietic, neoabietic, pimaric, isopimaric, sandracopimaric, palustric acids) are unlikely to bioaccumulate in fish (Niimi and Lee 1992). The relevance of this study in assessing the bioaccumalation potential of the corresponding esters is considered low, as there is no indication that rosin esters would significantly hydrolyse into the acid and alcohol.

7.7.3.6 Summary and conclusions

Experimental results

Experimental study results are not available for the estimation of bioconcentration or bioaccumulation of hydrogenated rosin esters in fish. The only available experimental study results concerned bioaccumulation of single resin acids in fish, which is considered of limited relevance for the assessment of rosin esters.

Modelling

The estimation of bioaccumulation potential of the Substance is based on partition coefficients and predicted values obtained by modelling. Moreover, information on the molecular sizes of the substances was taken into account in comparison with the screening criteria.

Bioconcentration/bioaccumulation has been estimated using (Q)SAR estimation software (EPI Suite BCFBAF v3.01), in accordance with REACH Annex XI.

The (Q)SAR estimates show variation in bioaccumulation in relation to the level of esterification of the substances. In general, the higher the level of esterification, the lower the bioaccumulation potential, which is supported also by the water solubility estimates and is in line with the values of log Kow > 10 for di- and triesters (Table 9) and the molecular sizes (Table 37). The results suggest that the B/vB criteria (BCF > 2000/5000) are probably not fulfilled for the ester constituents of the Substance, when the biotransformation rate estimates are taken into account (**Error! Reference source not found.**). This is supported by the experimental results of bioaccumulation potential and short depuration half-lives of single resin acids (Niimi and Lee 1992).

The predicted BCF/BAF values contain uncertainties for poorly soluble substances and large molecules, and hence the actual B/vB estimation should be based on experimental data.

7.8. Environmental hazard assessment

The evaluation of potential environmental toxicity of the Substnace is based on short-term aquatic toxicity available for the Substance and/or structural analogues of the same category of rosin esters. The experimental information concerned only the registered UVCB substances as such and no constituent related information was available.

The experimental toxicity data was compared to the PBT/vPvB criteria given in the PBT guidance (ECHA 2017b).

The screening criteria for toxicity are the following:

Type of screening information	*Criterion*	*Conclusion*
Short-term aquatic toxicity (algae, daphnia, fish)	EC50 or LC50 < 0.01 mg/L	T, criterion considered to be definitely fulfilled
Short-term aquatic toxicity (algae, daphnia, fish)	EC50 or LC50 < 0.1 mg/L	Potentially T

The given definitive criterion for environmental toxicity is:

Property	*PBT-criterion*
Toxicity	NOEC (long-term) < 0.01 mg/L for marine or freshwater organisms,

In addition, model predictions were compiled for the representative constituents of the Substance, but the applicability of the model was restricted by the low water solubility and high lipophilicity of these substances.

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1.1 Short-term toxicity to fish

Five experimental studies were available on short-term toxicity to fish for several chemically related rosin esters belonging to the same structural category, of which two were considered not reliable (Klimisch 3) in the registration information, because the exposure solutions were prepared as dilutions of single water accommodated fraction (WAF), instead of preparing individual WAF for each concentration as required in the test guidance.

The results showed no short-term toxicity to fish caused by rosin esters (see table below), and the short-term toxicity screening criterion for PBT (E/LC50 < 0.1 mg/L) was not met in any of the studies.

Table 38. Overview of short-term effects on fish (reliability rating by the registrant(s))

SHORT-TERM EFFECTS ON FISH				
Method	**Results**	**Remarks**	**Test material**	**Reference**
Oncorhynchus mykiss Freshwater Semi-static OECD Guideline 203 (Fish, Acute Toxicity Test)	LL50 (96 h): > 100 mg/L WAF (nominal) based on: mortality NOELR (96 h): 100 mg/L WAF (nominal) based on: mortality	1 (reliable without restriction) key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with ethylene glycol** CAS 68512-65-2	Study report 2014a
Pimephales promelas Freshwater Static OECD Guideline 203 (Fish, Acute Toxicity Test)	LL50 (96 h): > 1000 mg/L WAF (nominal) based on: mortality NOELR (96 h): 1000 mg/L WAF (nominal) based on: mortality	2 (reliable with restrictions) key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, hydrogenated, esters with methyl** CAS 8050-15-5	Study report 2001a
Pimephales promelas Freshwater Static OECD Guideline 203 (Fish, Acute Toxicity Test)	NOELR (96 h): ≥ 1000 mg/L dissolved (nominal) LL50 (96 h): > 1000 mg/L dissolved (nominal)	2 (reliable with restrictions) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with pentaery-thritol** CAS 8050-26-8	Study report 2001b
Brachydanio rerio (new name: Danio rerio) Freshwater Static Sample administration: single WAF Equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test)	LC50 (96 h): > 400 mg/L Dissolved (nominal) based on: mortality	3 (not reliable) *Disregarded study* Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with glycerol** CAS 8050-31-5	Study report 1993a
Brachydanio rerio (new name: Danio rerio) Freshwater Static Sample administration: single WAF Equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test)	LC50 (96 h): > 400 Dissolved (nominal) based on: mortality	3 (not reliable) *Disregarded study* Read-across based on grouping of substances (category approach)	**Resin acids and Rosin acids, esters with pentaerythritol** CAS 8050-26-8	Study report 1993b

The applied nominal test concentrations varied from 1 to 1000 mg/L in the fish short-term tests, the higher concentrations being way over the water solubility level of the test substances.

The Klimisch 1 rated study (Study report 2014a) with rainbow trout and with 'Resin and rosin acids, esters with ethylene glycol (REGE)' was conducted as a limit test with single loading rate WAF of 100 mg/L. WAF was prepared by magnetic stirring with vortex 23 h (2.2 g test item/22 l) and then settling. WAF was removed with siphoning and microscopic inspection of WAF showed no micro-dispersions or undissolved test item.

The actual concentration of test item (consisting of rosin mono- and diesters with ethylene glycol and unknown constituents) in WAF was measured with HPLC-MS using tetrahydrofuran as solvent. The test item gave a chromatographic profile consisting of a single peak although it is a UVCB substance with several constituents.

Additionally the measured concentrations decreased significantly during the 96 h exposure:

WAF initial	0 h	0.153 mg/L
WAF initial	72 h	0.0332 mg/L
WAF old	24 h	0.116 mg/L
WAF old	96 h	0.0110 mg/L

It was concluded in the study results that the dissolved test item may have been one or several components of the test item. Given that toxicity cannot be attributed to a single component or mixture of components but to the test item as a whole, the results were based on nominal loading rates only.

Clearly the analytical control of WAF of this type of UVCB substances is very unreliable and the actual test concentration of the test item remains uncertain. The substance also seems to be unstable in the test solution, probably due adsorption following low water solubility. In the registration information (CAS 68512-65-2) water solubility ≤0.0033 - 0.0037 mg/L and log Kow of 5.65 - >6.5 were given for the test substance.

Predicted (EpiWin/KowWin) estimates of water solubility and lipophilicity (log Kow) for the constituents of the Substance under evaluation show a clear tendency of decreasing water solubility and increasing lipophilicity with the rate of esterification. Slight water solubility is predicted for the monoester constituents of the Substance (e.g., 0.145 mg/L for DHAA-mono-GE), but for higher esters water solubility is practically non-existent. This supports the assumption that the WAF fraction of the test item contains probably only monoesters and some constituents other than rosin esters.

However, despite of the difficulties in measuring and/or maintaining the dissolved concentrations of constituents in the test solutions, it may be expected that acute toxic effects would have been observed with this kind of test setup if such existed in low substance concentrations.

7.8.1.1.2 Long-term toxicity to fish

No information available.

7.8.1.2. Aquatic invertebrates

7.8.1.2.1 Short-term toxicity to aquatic invertebrates

Seven experimental studies were available on short-term toxicity to aquatic invertebrates, of which two were considered not reliable (Klimisch 3) by the registrant(s), because the exposure solutions were prepared as dilutions of single water accommodated fraction (WAF), instead of preparing individual WAF for each test concentration as required in the test guidance.

The results showed some short-term toxicity to Daphnia in one study (Study report 2001), (Table 39), caused by 'Resin and rosin acids, hydrogenated, esters with methyl (HRME)' (EC50 (48 h) 27 mg/L, WAF, nominal concentration), which is the most simple molecule of the category substances. However, the water solubility given in the registration information for the substance in its entirety as a complex mixture is only 2.10 mg/L and the water solubility of the major components of HRME is 0.42 mg/L. In another study with a very high loading rate (10 g/L) the water solubility was ≤6.3 mg/L.

In four other reliable studies, including esters of (unhydrogenated) resin acids and rosin acids with glycerol/pentaerythritol as test items (**Table 39),** no short-term toxicity to Daphnia was discovered and hence the short-term toxicity screening criterion for PBT (E/LC50 < 0.1 mg/L) was not met in any of the studies.

Table 39. Overview of short-term toxicity to aquatic invertebrates (reliability rating by the registrant(s))

SHORT-TERM TOXICITY TO AQUATIC INVERTEBRATES				
Method	**Results**	**Remarks**	**Test material**	**Reference**
Daphnia magna Freshwater Static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EL50 (48 h): > 100 mg/L WAF (nominal); based on: mobility (95% CL not stated) NOELR (48 H): 100 mg/L WAF (nominal); based on: mobility (95% CL not stated)	1 (reliable without restriction) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with trimethylolpropane**	Study report 2017d
Daphnia magna Freshwater Static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): > 100 mg/L WAF (nominal); based on: mobility (95% CL not stated) NOELR (48 h): 100 mg/L WAF (nominal); based on: mobility (95% CL not stated)	1 (reliable without restriction) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with glycerol** CAS 8050-31-5	Study report 2010b
Daphnia magna Freshwater Static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): > 100 mg/L WAF (nominal); based on: mobility (95% CL not stated) NOELR (48 h): 100 mg/L WAF (nominal); based on: mobility (95% CL not stated)	1 (reliable without restriction) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with ethylene glycol** CAS 68512-65-2	Study report 2014b
Daphnia magna Freshwater Static OECD Guideline 202 (Daphnia sp.	EC50 (48 h): 27 mg/L WAF (nominal); based on: mobility (95% CL 22-32 mg/L) NOELR (48 h): < 19 mg/L	2 (reliable with restrictions) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, hydrogenated, esters with methyl** CAS 8050-15-5	Study report 2001c

SHORT-TERM TOXICITY TO AQUATIC INVERTEBRATES				
Method	**Results**	**Remarks**	**Test material**	**Reference**
Acute Immobilisation Test	WAF (nominal); based on: mobility (95% CL not stated)			
Daphnia magna Freshwater Static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EL50 (48 h): > 1000 mg/L WAF (nominal); based on: mobility NOELR (48 h): ≥ 1000 mg/L WAF (nominal); based on: mobility	2 (reliable with restrictions) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with pentaery-thritol** CAS 8050-26-8	Study report 2001d
Daphnia magna Freshwater Static Equivalent or similar to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): 259 mg/L Dissolved (nominal); based on: mobility (189-353 mg/L)	3 (not reliable) *Disregarded study* Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with glycerol** CAS 8050-31-5	Study report 1993c
Daphnia magna Freshwater Static Equivalent or similar to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): 166 mg/L Dissolved (nominal); based on: mobility (117-225 mg/L)	3 (not reliable) *Disregarded study* Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with pentaery-thritol** CAS 8050-26-8	Study report 1993d

The Klimisch 1 rated studies (Study report 2010b and Study report 2014,) with 'Resin and rosin acids, esters with glycerol (RGE)' and with 'Resin and rosin acids, esters with ethylene glycol (REGE)' and ´Resin and Rosin acids, esters with trimethylolpropane´ were conducted as limit tests with single loading rate WAFs of 100 mg/L. WAFs were prepared by magnetic stirring with vortex for 23 h followed by 1 h settling. WAFs were removed with siphoning. Microscopic inspection of WAF showed no micro-dispersions or undissolved REGE, but dispersed RGE was present in the WAF, which was removed by filtering through a glass wool plug.

The actual concentration of RGE in WAF was measured with HPLC method and of REGE with HPLC-MS method using tetrahydrofuran as solvent. The test standards and test items of RGE and REGE were reported to give chromatographic profiles consisting of a single peak for both substances, although they are UVCB substances with several constituents. The concentration of RGE during the 48 h test decreased ca. 50% and the concentration of REGE ca. 45-87%, respectively.

It was concluded in the study results that the dissolved test item may have been one or several components of the test item. Given that toxicity cannot be attributed to a single component or mixture of components but to the test item as a whole, the results were based on nominal loading rates only.

Altogether, the applied nominal test concentrations in the studies varied from 1 to 2000 mg/L, the higher concentrations being way over the water solubility level of the test substances. As with short-term tests with fish, despite of the difficulties in measuring and/or maintaining the dissolved concentrations of constituents in the test solutions, it may be expected that acute toxic effects would have been observed with this kind of test setup if such existed in low substance concentrations.

7.8.1.2.2 Long-term toxicity to aquatic invertebrates

No information currently available. The registrant(s) has proposed a *Daphnia magna* Reproduction Test (OECD Guideline 211).

7.8.1.3 Algae and aquatic plants

Five experimental studies were available on toxicity to aquatic plants, of which two were considered not reliable (Klimisch 3), because the exposure solutions were prepared as dilutions of single WAF, instead of preparing individual WAF for each test concentration as required in the test guidance.

In three other reliable studies, including unhydrogenated form of HRPE as test item, no toxicity to algae was discovered, and hence the short-term toxicity screening criterion for PBT (E/LC50 < 0.1 mg/L) was not met in any of the studies (see table below).

Table 40. Overview of toxicity to algae and aquatic plants (reliability rating by the registrant(s))

TOXICITY TO ALGAE AND AQUATIC PLANTS				
Method	**Results**	**Remarks**	**Test material**	**Reference**
Pseudokirchne-riella subcapitata Freshwater Static OECD Guideline 201 (Alga, Growth Inhibition Test)	EL10 (72 h): > 100 mg/L WAF (nominal) based on: growth rate EL50 (72 h): > 100 mg/L WAF (nominal) based on: growth rate NOELR (72 h): 100 mg/L WAF (nominal) based on: growth rate EL10 (72 h): > 100 mg/L WAF (nominal) based on: inhibition of yield EL50 (72 h): > 100 mg/L WAF (nominal) based on: inhibition of yield NOELR (72 h): 100 mg/L WAF (nominal) based on: inhibition of yield	1 (reliable without restriction) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with ethylene glycol** CAS 68512-65-2	Study report 2014c
Selenastrum capricornutum (new name: *Pseudokirchn-*	NOELR (72 h): 1000 mg/L WAF (nominal) based on: growth rate	2 (reliable with restrictions) Key study	**Resin and rosin acids, hydrogenated, esters with**	Study report 2001e

TOXICITY TO ALGAE AND AQUATIC PLANTS				
Method	**Results**	**Remarks**	**Test material**	**Reference**
eriella subcapitata) Freshwater Static OECD Guideline 201 (Alga, Growth Inhibition Test)	NOELR (72 h): 1000 mg/L WAF (nominal) based on: biomass EL50 (72 h): > 1000 mg/L WAF (nominal) based on: growth rate EL50 (72 h): > 1000 mg/L WAF (nominal) based on: biomass	Read-across based on grouping of substances (category approach)	**methyl** CAS 8050-15-5	
Selenastrum capricornutum (new name: *Pseudokirchne-riella subcapitata*) Freshwater Static OECD Guideline 201 (Alga, Growth Inhibition Test)	NOELR (72 h): 1000 mg/L WAF (nominal) based on: growth rate NOELR (72 h): 1000 mg/L WAF (nominal) based on: biomass EL50 (72 h): > 1000 mg/L WAF (nominal) based on: growth rate EL50 (72 h): > 1000 mg/L WAF (nominal) based on: biomass	2 (reliable with restrictions) Key study Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with pentaery-thritol** CAS 8050-26-8	Study report 2001f
Selenastrum capricornutum (new name: *Pseudokirchne-riella subcapitata*) Freshwater Static Sample administration: single WAF Equivalent or similar to OECD Guideline 201 (Alga, Growth Inhibition Test)	EC50 (72 h): > 1000 mg/L Dissolved (nominal) based on: growth rate EC50 (72 h): > 1000 mg/L Dissolved (nominal) based on: biomass	3 (not reliable) *Disregarded study* Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with glycerol** CAS 8050-31-5	Study report 1993e
Selenastrum capricornutum (new name: *Pseudokirchne-riella subcapitata*) Freshwater Static Sample administration: single WAF Equivalent or similar to OECD Guideline 201 (Alga, Growth Inhibition Test)	EC50 (72 h): > 1000 mg/L Dissolved (nominal) based on: growth rate EC50 (72 h): > 1000 mg/L Dissolved (nominal) based on: biomass	3 (not reliable) *Disregarded study* Read-across based on grouping of substances (category approach)	**Resin and rosin acids, esters with pentaery-thritol** CAS 8050-26-8	Study report 1993f

The Klimisch 1 rated study (Study report 2014) with algae and with 'Resin and rosin acids, esters with ethylene glycol (REGE)' was conducted as a limit test with single loading rate WAF of 100 mg/L. WAF was prepared by magnetic stirring with vortex 23 h and then 1 h settling. WAF was removed with siphoning and microscopic inspection of WAF showed that dispersed REGE was present in the WAF, which was removed by filtering through a glass wool plug.

The actual concentration of REGE in WAF was measured with HPLC-MS method using tetrahydrofuran as solvent. The test standard and the test item REGE were reported to give chromatographic profiles consisting of a single peak, although REGE is a UVCB substance with several constituents. The measured concentration of REGE in WAF during the 72 h test decreased from 0.000679 mg/L to less than the limit of quantification (0.000089 mg/L).

It was concluded in the study results that the dissolved test item may have been one or several components of the test item. Given that toxicity cannot be attributed to a single component or mixture of components but to the test item as a whole, the results were based on nominal loading rates only.

The applied nominal test concentrations varied from 1 to 1000 mg/L, the higher concentrations being way over the water solubility level of the test substances. As with short-term tests with fish and Daphnia, despite of the difficulties in measuring and/or maintaining the dissolved concentrations of constituents in the test solutions, it may be expected that acute toxic effects would have been observed with this kind of test setup if such existed in low substance concentrations.

7.8.1.4 Estimated data

ECOSAR (v1.11) QSAR predictions offer very limited possibilities for predicting the ecotoxicity of the Substance as the applicability is restricted by low water solubility and high lipophilicity of the constituents. Only monoesterified constituents (mono-HRGE) fit the ECOSAR model (class esters) for some endpoints (
Table **41**). For these endpoints the results are under the PBT screening criterion of 0.1 mg/L. The lowest ChV values for fish (mono-HRGE) are close to the T criterion for long-term aquatic toxicity (NOEC < 0.01 mg/L).

Of the ester constituents of the Substance, only monoesters are slightly water soluble (0.1 - 2.5 mg/L for the selected monoesters of HRGE), according to modelling results with EPISuite/WSKOW and WatSol) and hence potentially more bioavailable than di-, tri-, and tetraesters, which are practically not water soluble. This is also seen with other rosin ester analogues: only monoesters are slightly water soluble according to modelling results. Therefore, it can be estimated that monoesterified rosin ester structures are potentially the most toxic rosin ester constituents.

Table 41. Model predictions of ecotoxicity for monoesterified HRGE (ECOSAR (v1.11), Class: esters)

ECOSAR PREDICTION				
	EC/LC50 (mg/L)		ChV (mg/L)	
	DHAA-mono-GE (log Kow 5.2)	THAA-mono-GE (log Kow 5.3)	DHAA-mono-GE (log Kow 5.2)	THAA-mono-GE (log Kow 5.3)
Algae	0.252*	0.221*	0.183*	0.165*
Daphnid	0.984*	0.874*	0.267*	0.231*
Fish	0.668*	0.599*	0.026	0.023

*Above the estimated water solubilty (WSKowwin v1.43) of substance.

7.8.1.5 Summary of short-term aquatic toxicity

It is stated in ECHA's Guidance on information requirements and chemical safety assessment, Chapter R.11, PBT/vPvB assessment (2014) that:

"Toxicity is defined via a concentration response (Mackay et al, 2001) and is dependent on the bioavailability of the individual constituents in an MCS or an UVCB test substance. This may make interpretation for some substances very difficult. For example, the physical form may prevent the dissolution of the individual constituents of such a substance to any significant extent where the whole substance is applied directly to the test medium. The consequence of this would be that toxicity may not be seen in the test system (e.g. coal tar pitch), whereas in the real world the toxic constituents would be released into the environment in a manner that meant they were no longer confined by the physico-chemical structure of the substance as a whole and hence could cause toxic effects."

Esters with (hydrogenated) rosins are typical UVCB substances consisting of constituents with differing solubility/lipophilicity/bioavailability properties. The available studies, applying water WAF of several rosin substances belonging to the same rosin substance category for short-term aquatic toxicity testing, showed no toxic effects (with the exception of one Daphnia test, EC50 27 mg/L).

However, only monoesters of the known constituents are slightly water soluble and hence potentially better bioavailable. Even their stability in the test solution seems to be rather poor and the concentration decreases significantly during the exposure, the mechanism of which has not been studied. The analytical control measurements may not be reliable for UVCB substances as the quantification in the available reliable studies have been based only on one single chromatographic peak. Hence the actual composition and concentration of these test items in toxicity tests remains more or less unknown.

It can be concluded that the evaluated UVCB substances are not considered to cause any short-term ecotoxic effects, with the provision that the actual test substances and concentrations are uncertain.

7.8.1.6 Sediment organisms

No information available.

7.8.1.7 Other aquatic organisms

No information available.

7.8.2. Terrestrial compartment

No information available.

7.8.3. Microbiological activity in sewage treatment systems

Not evaluated.

7.8.4. PNEC derivation and other hazard conclusions

Not evaluated.

7.8.5. Conclusions for classification and labelling

Not evaluated.

7.9 Human Health hazard assessment

The human health hazard assessment is based on available information on a number of studies on toxicokinetics (rat), repeated dose toxicity (rat), mutagenicity/genotoxicity (bacteria, mammalian cells) and toxicity to reproduction (rat). No carcinogenicity studies were available, and a pre-natal developmental toxicity study (PNDT) is under way. The UVCB substances tested belonged to the rosin ester category and were applied as such.

The experimental information was compared to the toxicity criteria for human health given in the PBT guidance (ECHA 2017b) for PBT assessment.

The PBT-criteria for human health toxicity are the following:

Property	*PBT-criteria*
Toxicity	– substance is classified as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2), or
	– there is other evidence of chronic toxicity, as identified by the classifications: STOT (repeated exposure), category 1 (oral, dermal, inhalation of gases/vapours, inhalation of dust/mist/fume) or category 2 (oral, dermal, inhalation of gases/vapours, inhalation of dust/mist/fume) according to the CLP Regulation.

7.9.1 Toxicokinetics

Table 42. Studies on absorption, metabolism, distribution and elimination (information obtained from the registrated substance factsheet, reliability rating by the registrant(s))

TOXICOKINETICS			
Study	**Remarks**	**Results**	**Reference**
Fischer 344 rat, male/female Administaration: oral Exposure regime: Dietary exposure: ad libitum for 18 hours -10 days followed by administration of a single dose of radiolabelled test material administered by oral gavage. Sample collection following administration of radiolabelled test substance: 120 hours Doses/conc.: Dietary exposure (Phases I, II, and IV): 14000 ppm Oral gavage of radiolabelled material: 200 mg/kg bw The method contained the essential elements outlined in OECD Guideline 417.	1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with glycerol	Metabolites identified: yes Details on metabolites: No metabolites were specifically identified in this study. Absorption/Disposition of radioactivity (Phase I): The results of the present study indicate that only a small percentage of the administered dose was absorbed by male rats given a single oral dose of approximately 200 mg/kg bw of [C^{14}]ester gum. Only a small percentage of the administered dose appeared to be hydrolyzed in the gastrointestinal tract. The levels of radioactivity recovered in either expired CO_2, urine or the cage rinses each accounted for only 1% or less of the dose. Between 94.7-105.7% of the dose was eliminated in the feces during the 120-hour sample collection interval. Of the total dose accounted for in the various sample types collected, 96.4-107.7% was eliminated within 48 hours after dose administration. Absorption/Disposition of radioactivity (Phase II): The disposition of radioactivity was similar to that observed for Phase I male rats which indicates that a 10-day dietary administration of ester gum did not affect the absorption and/or disposition of the test substance, as compared with 1-day dietary administration. Absorption/Disposition of radioactivity (Phase III): These results also indicate that only low levels of radioactivity were absorbed by rats following oral administration of a single dose of approximately 200 mg/kg bw of [C^{14}]ester gum and the radioactive species excreted in bile appeared to be a hydrolyzed product of [C^{14}]ester gum. Radioactivity was excreted in bile within 4 hours after dosing and was detectable in all samples collected through 24 hours post dose. The total amount of radioactivity excreted in bile during the 24-hour collection period ranged from 1.6-2.9% of the dose among the individual animals.	Study report 1996

		Absorption/Disposition of radioactivity (Phase IV): The results indicated that, similar to male rats in Phase I, female rats absorbed less than 2% of the administered radioactivity within a 120-hour interval following oral administration of a single dose of approximately 200 mg/kg bw of [C^{14}]ester gum. Only 1% or less of the dose was recovered in either expired CO_2, urine or the cage rinses.	

The toxicokinetic study indicates that Resin acids and Rosin acids, esters with glycerol are only slightly absorbed and completely or almost completely excreted in feces within the 120-hour following oral administration. Resin acids and Rosin acids, esters with glycerol did not indicate bioaccumulation potential after repeated exposure up to 10 days.

Additional remark

In ECHA's decision on a testing proposal (ECHA 2014) it is mentioned "*The Registrant has committed to provide ex vivo absorption data on all members of the category. Absorption information is to be generated using an "everted gut-sac model". ECHA considers that this model is currently not validated for this type of substances, and that the Registrant has not demonstrated that the ex vivo absorption observed accurately predicts in vivo gastrointestinal absorption and ultimately correlates to the systemic toxicity observed in available toxicity studies. These uncertainties will have to be addressed by the Registrant. Nevertheless, ECHA considers that information on bioavailability is useful to strengthen the read-across argumentation and considers it to be an essential condition for the ultimate acceptance and use of read-across for the category.*"

7.9.2 Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3 Sensitisation

Not evaluated.

7.9.4 Repeated dose toxicity

Table 43. Studies on repeated dose toxicity after oral administration (information obtained from the registrated substance factsheet, reliability rating by the registrant(s))

REPEATED DOSE TOXICITY			
Study	**Remarks**	**Results**	**Reference**
Sprague-Dawley rat, male/female subchronic (oral: feed) 0.2% (nominal in diet) 0.5% (nominal in diet) 1.0% (nominal in diet) Exposure: 13 weeks (Daily, ad libitum) equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol	NOAEL: 1 % (w/w), nominal in diet (male/female) based on: test mat. (No test substance-related effects were observed in any of the study parameters evaluated. All changes that occurred did not occur in a dose-dependent manner or were within normal ranges for this strain and age of rat and were not considered related to the test substance.)	Study report 1985a
Sprague-Dawley rat, male/female subchronic (oral: feed) 2000 ppm (nominal in diet) 5000 ppm (nominal in diet) 10000 ppm (nominal in water) Exposure: up to 90 days (Daily, ad libitum) OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	1 (reliable without restriction) key study experimental result Test material (EC Name): Resin acids and Rosin acids, hydrogenated, esters with glycerol	NOAEL: 10000 ppm (male/female) based on: test mat. (No test substance-related effects were noted in male and female rats when Staybelite Ester 5 was administered in the diet for a period of 90 days at a dosage level of 10000 ppm.)	Study report 1987
Sprague-Dawley rat, male/female subchronic (oral: feed) Hercolyn DE 100, 500, 2000, and 10000 ppm (eq. to received doses of 8-9, 39-43, 154-177 and 777-901 mg/kg bw/day) (nominal in diet) Exposure: 13 weeks (Daily) U. S. Food and Drug Administration 'Red Book' Guidelines. (Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives used in Food, US FDA Bureau of Foods. 1982). equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	NOAEL (Hercolyn DE): 500 ppm, equivalent to an overall received dose of 39-43 mg/kg bw/d (male/female) based on: test mat. (No treatment-related changes were noted following administration at a dietary concentration of 500 ppm. Abnormally shaped livers in both sexes (present in controls also), increased relative liver weights in females only, and hepatocyte hypertrophy in 2 males (no other relevant histopathology) were observed at a dietary concentration of 2000 ppm)	Study report 1994

REPEATED DOSE TOXICITY			
Study	**Remarks**	**Results**	**Reference**
Sprague-Dawley rat, male/female subchronic (oral: feed) Hercolyn DR 100, 500, 2000, and 10000 ppm (eq. to received doses of 8-9, 39-45, 155-178 and 782-871 mg/kg bw/day) (nominal in diet) Exposure: 13 weeks (Daily) U. S. Food and Drug Administration 'Red Book' Guidelines. (Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives used in Food, US FDA Bureau of Foods. 1982). equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	NOAEL (Hercolyn DR): 500 ppm, equivalent to an overall received dose of 39-45 mg/kg bw/d (male/female) based on: test mat. (No treatment-related changes were noted following administration at a dietary concentration of 500 ppm. Abnormally shaped livers in both sexes (present in controls also), increased relative liver weights in females only, and hepatocyte hypertrophy in 1 animal of each sex (no other relevant histopathology were observed at a dietary concentration of 2000 ppm.)	Study report 1994
Sprague-Dawley rat, male/female subchronic (oral: feed) 2000 ppm Ester Gum PGR or Ester Gum CGR (nominal in diet) 5000 ppm Ester Gum PGR or Ester Gum CGR (nominal in diet) 10000 ppm Ester Gum PGR or Ester Gum CGR (nominal in diet) Exposure: up to 90 days (Daily, ad libitum) OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	1 (reliable without restriction) supporting study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with glycerol	NOAEL Ester Gum-CGR: 10000 ppm, which corresponds to 714.0 and 831.0 mg/kg bw/day in males and females, respectively (male/female) based on: test mat. (No test substance-related effects were noted in male and female rats when Ester Gum-CGR was administered in the diet for a period of 90 days at a dosage level of 10000 ppm.) NOAEL Ester Gum-PGR: 10000 ppm, which corresponds to 713.5 and 815.0 mg/kg bw/day in males and females, respectively (male/female) based on: test mat. (No test substance-related effects were noted in male and female rats when Ester Gum-PGR was administered in the diet for a period of 90 days at a dosage level of 10000 ppm.)	Study report 1989
Sprague-Dawley rat, male/female subacute (oral: feed) 0.2% (nominal in diet) 1.0% (nominal in diet) Exposure: 28 days (Daily, ad libitum) Observations included clinical signs, morbidity/mortality, body weight and food consumption. Animals were subject to gross necropsy and microscopic examination	2 (reliable with restrictions) supporting study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with glycerol	NOAEL: 1 % (w/w), nominal in diet (male/female) based on: test mat. (No treatment related effects were observed in clinical observations, body weights, food consumption, postmortem examination and microscopic examination in any animal.)	Study report 1985b

REPEATED DOSE TOXICITY			
Study	**Remarks**	**Results**	**Reference**
of selected tissues.			
Fischer 344 rat, male/female subchronic (oral: feed) 625 mg/kg/day (nominal in diet) 1250 mg/kg/day (nominal in diet) 2500 mg/kg/day (nominal in diet) Exposure: 90 days (Daily, ad libitum) equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	1 (reliable without restriction) supporting study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with glycerol	NOAEL: 2500 mg/kg diet/day, nominal (male/female) based on: test mat. (There were no adverse effects observed when male and female rats were offered diets at dosage levels of up to 2500 mg/kg diet/day Ester Gum 8BG for 13 weeks.)	Study report 1991
Sprague-Dawley rat, male/female subchronic (oral: feed) 0.2% (nominal in diet) 1.0% (nominal in diet) 5.0% (nominal in diet) Exposure: 90 days (Daily, ad libitum) Observations included clinical signs, morbidity/mortality, body weight, food consumption, feed utilization, clinical chemistry, hematology and fecal examinations. Animals were subject to gross necropsy (including organ weights) and microscopic examination of selected tissues.	2 (reliable with restrictions) supporting study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with glycerol	NOAEL: 1 % (w/w), nominal in diet (male/female) based on: test mat. Exposure to the test substance at a concentration of 5.0% (w/w) in the diet resulted in decreased food consumption during the initial 3-5 weeks of dosing in males and females, increased liver weights in females that were also associated with very slight or slight periportal hepatic vacuolation, and increased relative liver-to-body weight in males.	Study report 1982
Sprague-Dawley rat, male/female subacute (oral: feed) 0.2% (eq. to a received dose of 177-183 mg/kg bw/d) (nominal in diet) 1.0% (eq. a received dose of to 877-918 mg/kg bw/d) (nominal in diet) Exposure: 28 days (Daily) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	4 (not assignable) supporting study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	NOAEL: 1 % in diet, equivalent to an overall mean received dose of 877-918 mg/kg bw/d (male/female) based on: test mat. (No morbidity or mortality or gross abnormalities at necropsy. Microscopic changes limited to slight hypertrophy of cells of the zona glomerulosa of the adrenal cortex in high dose group, considered either spontaneous or non-adverse by study pathologist.)	Study report 1985c

REPEATED DOSE TOXICITY			
Study	**Remarks**	**Results**	**Reference**
Sprague-Dawley rat, male/female combined repeated dose and reproduction / developmental screening (oral: feed) 5000 ppm (eq. to 405 mg/kg bw/d for males and 476 mg/kg bw/d for females) (nominal in diet) 10000 ppm (eq. to 769 mg/kg bw/d for males and 915 mg/kg bw/d for females) (nominal in diet) 20000 ppm (eq. to 1579 mg/kg bw/d for males and 1553 mg/kg bw/d for females) (nominal in diet) Exposure: Females: 57-60 days Males: 28 days (Daily, ad libitum) OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	1 (reliable without restriction) supporting study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	LOAEL: 5000 ppm (male/female) based on: test mat. (Equivalent to received dose of 405-476 mg/kg bw/d. Dose-related increases in liver weights were noted in both sexes at all dose levels. This was accompanied by hepatocellular hypertrophy at all dose levels.)	Study report 2003b
Benchmark Dose Modeling was conducted using software developed by US-EPA (2011). Dose-response data for hepatocyte hypertrophy noted in Toxicol (1994) were analysed separately for males and females.Each model was tested for goodness-of-fit, and the BMDL calculated using the profile likelihood method, where the BMDL refers to the 95% lower confidence limit on the BMD (US-EPA 2010).	2 (reliable with restrictions) supporting study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	BMDL10: 150 mg/kg bw/day (nominal) (male) based on: test mat. (Based on hepatocyte hypertrophy in male rats following sub-chronic administration of Hercolyn DR via diet.) BMDL10: 141 mg/kg bw/day (nominal) (female) based on: test mat. (Based on hepatocyte hypertrophy in female rats following sub-chronic administration of Hercolyn DR via diet.) BMDL10: 93.9 mg/kg bw/day (nominal) (male) based on: test mat. (Based on hepatocyte hypertrophy in male rats following sub-chronic administration of Hercolyn DE via diet.) BMDL10: 222 mg/kg bw/day (nominal) (female) based on: test mat. (Based on hepatocyte hypertrophy in female rats following sub-chronic administration of Hercolyn DE via diet.) BMDL10: 151.7 mg/kg bw/day (nominal) (male/female) based on: test mat. (Overall mean BMDL10 for hepatocyte	Study report 2012b US-EPA 2010 US-EPA 2011

REPEATED DOSE TOXICITY			
Study	**Remarks**	**Results**	**Reference**
		hypertrophy in male and female rats administered Hercolyn DR and Hercolyn DE via diet for 90-days.)	
Sprague-Dawley rat, male/female subchronic (oral: feed) Exposure: 90 days (Daily, ad libitum) Groups of ten animals/sex/group were fed diets containing the test material at concentrations of up to 5.0 wt% for 90 days.		4 (not assignable) disregarded study read-across based on grouping of substances (category approach) Test material (Common name): Resin acids and rosin acids, esters (various)	Study report 1960a Study report 1960b Study report 1960c Study report 1960d Study report 1960e Study report 1967

The Substance has not been classified for specific target organ toxicity – repeated exposure. The repeated dose toxicity studies are all conducted in rats via oral route. No severe treatment-related findings were observed in any of these studies. Some studies did report slight and likely adaptive changes in the liver, such as increased liver weight and hepatocellular hypertrophy, thus the relevance of these of these findings to humans can be doubted. Two studies conducted on Resin acids and Rosin acids, hydrogenated, Me esters (CAS No. 8050-15-5) found out NOAEL values of 39 - 45 mg/kg bw/day and 39 - 43 mg/kg bw/day were derived based on increased liver weight and hepatocellular hypertrophy. A Benchmark Modelling was performed to these two studies, and a BMDL10 of 151.7 mg/kg/ was established. It needs to be pointed out that Resin acids and Rosin acids, hydrogenated, Me esters are low molecular weight esters, and no systemic toxicity was observed in higher molecular weight esters below 10000 ppm.

7.9.5 Mutagenicity

7.9.5.1 Non-human information

7.9.5.1.1 In vitro data

Table 44. *In vitro* genotoxicity studies (information obtained from the registrated substance factsheet, reliability rating by the registrant(s))

IN VITRO GENOTOXICITY			
Study	**Remarks**	**Results**	**Reference**
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 TA 100 and TA 1538 (with and without metabolic activation) Test concentrations: Preliminary and Mutation assay: 0, 50, 150, 500, 1500, and 5000 µg/plate OECD Guideline 471 (Bacterial Reverse Mutation Assay) EU Method B.13/14 (Mutagenicity - Reverse Mutation Test Using Bacteria)	Reliability: 1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with diethylene glycol(EC Name): Resin acids and Rosin acids, esters with diethylene glycol	Evaluation of results: negative with metabolic activation negative without metabolic activation Test results: negative in all strains/cell types tested ; met. act.: with and without; cytotoxicity: no, but tested up to precipitating concentrations ; vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	Study report 1997
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 1538 (with and without metabolic activation) Test concentrations: Preliminary Screening and Main Assay: with and without metabolic activation: 100, 333, 1000, 3333, and 10000 µg/plate Internal test laboratory protocol SOP 301	Reliability:1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with pentaerythritol	Evaluation of results: negative with metabolic activation negative without metabolic activation Test results: negative in all strains/cell types tested ; met. act.: with and without ; cytotoxicity: no, but tested up to limit concentrations ; vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	Study report 1982
Mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (with and without metabolic activation) Test concentrations: The maximum dose level used in the mutagenicity tests was limited by the onset of aggregated precipitate effectively reducing the	Reliability: 1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with pentaerythritol	Evaluation of results: negative (Non-mutagenic) Test results: negative (non-mutagenic) for mouse lymphoma L5178Y cells(strain/cell type: TK +/-, locus of the L5178Y mouse lymphoma cell line) ; met. act.: with and without ; cytotoxicity: yes ; vehicle controls valid: yes; negative controls valid: not applicable;	Study report 2009

IN VITRO GENOTOXICITY			
Study	**Remarks**	**Results**	**Reference**
exposure of the test material to the cells in the preliminary toxicity test, as indicated by the %RSG values Vehicle and positive controls were used in parallel with the test material. Solvent (Acetone) treatment groups were used as the vehicle controls. Ethylmethanesulphonate (EMS was used as the positive control in the absence of metabolic activation. Cyclophosphamide (CP) at 2 µg/mL was used as the positive control in the presence of metabolic activation. OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test) EU Method B.17 (Mutagenicity - In vitro Mammalian Cell Gene Mutation Test)		positive controls valid: yes	
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (with and without metabolic activation) E. coli WP2 uvr A (met. act.: with and without) Test concentrations: Toxicity and Mutation Assays: 17, 50, 167, 500, 1667 and 5000 µg per plate. OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Reliability: 1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	Evaluation of results: negative with metabolic activation negative without metabolic activation Test results: negative in all strains/cell types tested; met. act.: with and without ; cytotoxicity: no; tested up to and including precipitating concentrations. The two highest concentrations tested caused some precipitation but lawns were assumed to be normal. ; vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	Study report 2001a

IN VITRO GENOTOXICITY			
Study	**Remarks**	**Results**	**Reference**
Mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (with and without metabolic activation) Test concentrations: The maximum dose level used was limited by test material induced toxicity. The dose range for Experiment 1 was 2.5 to 40 µg/ml in the absence of metabolic activation and 10 to 60 µg/ml in the presence of metabolic activation. The dose range for Experiment 2 was 5 to 50 µg/ml in the absence of metabolic activation, and 10 to 60 µg/ml in the presence of metabolic activation. Vehicle and positive controls were used in parallel with the test material. Solvent (Acetone) treatment groups were used as the vehicle controls. Ethylmethanesulphonate (EMS) at 400 µg/ml for Experiment 1, and at 150 µg/ml for Experiment 2, was used as the positive control in the absence of metabolic activation. Cyclophosphamide (CP) at 2 µg/ml was used as the positive control in the presence of metabolic activation. OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	Reliability: 1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	Evaluation of results: Non-mutagenic Test results: negative (non-mutagenic) for mouse lymphoma L5178Y cells(strain/cell type: TK +/-, locus of the L5178Y mouse lymphoma cell line) ; met. act.: with and without ; cytotoxicity: yes ; vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	Study report 2010c
In vitro mammalian chromosome aberration test (chromosome aberration) lymphocytes: (met. act.: with and without) Test concentrations: 4 hour without S9: 0, 2.5, 5, 10, 20, 40, 80 ug/mL 4 hour with S9: 0, 2.5, 5, 10, 20, 40, 80 ug/mL 24 hour without S9: 0. 2.5, 5, 10, 20, 40, 80 ug/mL OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	Reliability: 1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with pentaerythritol	Test results: negative for lymphocytes: ; met. act.: with and without ; cytotoxicity: no, but tested up to precipitating concentrations ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	Study report 2011

IN VITRO GENOTOXICITY			
Study	**Remarks**	**Results**	**Reference**
In vitro mammalian chromosome aberration test (chromosome aberration) Chinese hamster Ovary (CHO) (met. act.: with and without) Test concentrations: Preliminary experiment: Nine dose concentrations with the highest dose being 5000 µg/mL and subsequent dose levels were conducted by halving the previous dilution but the test was stopped due to excessive toxicity. Test 1: Presence and absence of S9 mix: 1.25, 2.5, 5, 10, 20, 30, and 40 µg/mL Test 2: Presence of S9 mix: 5, 10, 20, 30, and 40 µg/mL Absence of S9 mix: 2.5, 5, 10, 12.5, 15, 17.5, and 20 µg/mL OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	Reliability: 1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	Evaluation of results: negative with metabolic activation negative without metabolic activation Test results: negative for Chinese hamster Ovary (CHO)(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: yes ; vehicle controls valid: yes; negative controls valid: not applicable;	Study report 2001b

The Substance has not been classified for mutagenicity. Test compounds were not observed to be mutagenic or clastogenic in bacterial or mammalian cells *in vitro* with or without metabolic activation.

7.9.5.1.2 In vivo data

No *in vivo* data was available.

7.9.5.2 Human data

No human data was available.

7.9.6 Carcinogenicity

No carcinogenicity studies are provided. However, Rosin esters are not expected to be carcinogenic, based upon an absence of mutagenic and clastogenic activity *in vitro* and no evidence of hyperplasia and/or pre-neoplastic lesions following repeated sub-chronic exposure.

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

7.9.7.1 Non-human information

Table 45. Studies on reproductive toxicity (information obtained from the registrated substance factsheet, reliability rating by the registrant(s))

REPRODUCTIVE TOXICITY			
Study	**Remarks**	**Results**	**Reference**
Sprague-Dawley rat, male/female screening oral: feed 1000 ppm, 5000 ppm, 20000 ppm (nominal in diet) Exposure: Females: 57-60 days Males: 28 days (Daily) OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)	1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC Name): Resin acids and Rosin acids, esters with pentaerythritol	NOAEL (reproductive) (P): 20000 ppm (nominal) (male/female) (No effects related to test substance administration were noted on reproductive performance or other parameters evaluated in the study.) NOAEL (developmental toxicity) (F1): 20000 ppm (nominal) (male/female) (No effects related to test substance administration were noted on pups).	Study report 2003b
Sprague-Dawley rat, male/female screening oral: feed 5000 ppm (eq. to 405 mg/kg bw/d for males and 476 mg/kg bw/d for females) (nominal in diet) 10000 ppm (eq. to 769 mg/kg bw/d for males and 915 mg/kg bw/d for females) (nominal in diet) 20000 ppm (eq. to 1579 mg/kg bw/d for males and 1553 mg/kg bw/d for females) (nominal in diet) Exposure: Females: 57-60 days Males: 28 days (Daily, ad libitum) OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	1 (reliable without restriction) key study read-across based on grouping of substances (category approach) Test material (EC name): Resin acids and Rosin acids, hydrogenated, Me esters	LOAEL (parental) (P): 5000 ppm (male/female) based on: test mat. (Equivalent to received dose of 405-476 mg/kg bw/d. Dose-related increases in liver weights were noted in both sexes at all dose levels. This was accompanied by hepatocellular hypertrophy at all dose levels.) LOAEL (offspring) (F1): 5000 ppm (male/female) (Equivalent to a maternal received dose of 476 mg/kg bw/d. Mean pup weights and mean litter weights were initially reduced in all test groups relative to the controls, likely due to maternal toxicity. At 5000 and 10000 ppm, body weights were initially reduced but were unaffected when adjusted based on maternal weight. There were no effects of treatment on litter survival in the two lower dose groups.) NOAEL (for reproductive toxicity) (P): 20000 ppm (male/female) (Equivalent to a received dose of 1553-1579 mg/kg bw/d. Mating performance and reproductive organs were not affected by treatment.) NOEL (for reproductive toxicity) (P): 10000 ppm	Study report 2003b

REPRODUCTIVE TOXICITY			
Study	**Remarks**	**Results**	**Reference**
		(female) (Equivalent to a received dose of 915 mg/kg bw/d. Slight decrease in the mean number of implant sites per pregnancy in the 20000 ppm dams, although litter size at birth was similar to the control group.)	

Reproductive/developmental toxicity screening tests according to OECD TG 421 and 422 in rats have been provided. The OECD 421 study was conducted on Resin acids and Rosin acids, esters with pentaerythritol (CAS No. 8050-26-8) and no treatment related reproductive/developmental effects were observed in concentrations up to 20000 ppm.

Resin acids and Rosin acids, hydrogenated, Me esters (CAS No. 8050-15-5) was used in the OECD 422 study. Dose-related increases in liver weights, accompanied by hepatocellular hypertrophy was observed in both sexes at all doses, as well as mean pup weights and mean litter weights were initially reduced in all test groups compared to the controls. Thus these findings could be due to maternal toxicity. There were no treatment related effects on mating performance and reproductive organs were not affected by treatment. At females receiving 20000 ppm (approx. 1553 mg/kg bw/d) the mean number of implant sites per pregnancy were slightly decreased, although there were no effects on litter size at birth compared to the control group. This study reports a NOAEL for reproductive effects of 20000 ppm (1579 mg/kg bw/d) in males and 10000 ppm (915 mg/kg bw/d) in females, and a NOAEL for developmental effects at 5000 ppm (405-476 mg/kg bw/d). However, it should be noted that the effects were observed in the low molecular weight esters Recin acids and Rosin acids, hydrogenated, Me esters (GAS No. 8050-15-5).

7.9.7.2 Human data

No human data was available.

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.

7.9.10 Conclusions of the human health hazard assessment and related classification and labelling

No severe treatment-related findings were observed in any of the repeated dose toxicity studies. Neither were treatment-related reproductive/developmental effects observed in concentrations up to 20 000 ppm. The tested substances were not found to be mutagenic or glastogenic in bacteria or mammalian cells *in vitro* with or without metabolic activation. Therefore, the available toxicological information does not justify classification of the

category substances as mutagenic or toxic for reproduction. No other evidence of chronic toxicity was observed in the experimental data either that would justify STOT RE classification.

7.10 Assessment of endocrine disrupting (ED) properties

Not evaluated.

7.10.1 Endocrine disruption – Environment

Not evaluated.

7.10.2 Endocrine disruption - Human health

Not evaluated.

7.10.3 Conclusion on endocrine disrupting properties (combined/separate)

Not evaluated.

7.11 PBT and vPvB assessment

Assessment approach

The PBT/vPvB assessment shall take into account of the PBT/vPvB-properties of relevant constituents of a substance and relevant transformation and/or degradation products. In general, constituents and transformation/degradation products are considered relevant for the PBT/vPvB assessment when they are present in concentration of ≥ 0.1% (w/w).

The Substance is a UVCB and includes mono-, di-, and triesters of hydrogenated resin acids with glycerol, as well as the fractions named as "light ends" (mono- and sesquiterpenes) and "heavy ends" (a mixture of dimerised esters, acids and polyol). All of these fractions are present at concentrations of ≥0.1 w/w.

Based on the PBT screening level results it was anticipated that, of the mono-, di-, and triesters probably only the monoesters had potential to bioaccumulate according to their bioavailability properties. Moreover, it was expected that there might be significant differences in biodegradability between the rosin constituents of different level of esterification.

Therefore, it was decided to focus the substance evaluation primarily on the monoester constituents of the Substance and try to solve the concerns related to B and P properties at first. Monoesters of dihydroabietic acid and tetrahydroabietic acid glycerol (DHAA-mono-GE, THAA-mono-GE) were identified as representative constituents for persistence assessment.

7.11.1 Persistence

Monoester constituents

The estimated half time for the photodegradation with OH-radicals is 0.09 days for DHAA-mono-GE and THAA-mono-GE. Therefore, indirect photodegradation may be an important environmental fate process for these constituents and the constituents do not have long-range transport potential.

No reliable data is available for concluding on abiotic hydrolysis for the Substance and its selected constituents. However, the hydrolysis rates predicted for DHAA-mono-GE and THAA-mono-GE and observed rates of hydrolysis of esters of resin acids and rosin acids (e.g., with glycerol) suggest that it is highly unlikely that abiotic hydrolysis rates in environmentally relevant conditions would be sufficiently high to rule out PBT/vPvB concern.

No strong conclusions can be made on the biodegradability of the selected monoester constituents on the basis of BIOWIN models. The screening criteria for P and vP based on BIOWIN models (ECHA 2017b) are not fulfilled. However, it should be noted that the BIOWIN results have only a low weighting in the assessment as BIOWIN 2 and 3 are poorly applicable and as BIOWIN 5 and 6, which are applicable, give somewhat conflicting results. For the glycerol monoesters DHAA-mono-GE and THAA-mono-GE the BIOWIN 5 and 6 results as a whole are somewhat more towards to the outcome "not readily biodegradable" than "readily biodegradable".

Based on the ready biodegradation tests, the Substance is not readily biodegradable (degradation was 4 - 47% during 28 days) and therefore fulfills the screening criterion for persistence. The concentrations of test substance constituents were not determined in these ready biodegradation tests and therefore degradation of individual constituents or fractions in these tests is not known. A comparison between analogous substances indicates that methyl esters of resin acids and rosin acids (belonging to "simple" esters) seem to be more biodegradable compared to "bulky" esters (such as the Substance) in ready biodegradation tests. Structurally the monoesters in the Substance are more complex than the methyl esters of resin acids and rosin acids but less complex than the di- or triesters in the Substance.

Two ready biodegradation studies (OECD TG 310) were conducted on enriched monoesters of resin acids and rosin acids with glycerol, in response to the SEv decision. Three different enriched monoester substances were tested in these studies. Degradation of the test substances (consisting of 68-75% of monoesters) was 34-54 % ThIC during 28 days and 34-67 % ThIC during 45-60 days.

Estimated ultimate degradation of the monoesters (based on two different calculation scenarios, Scenarios 3 and 4, See 7.7.1.3.2 and Table 25) was 21-59 % ThIC after 28 days and 23-75 % ThIC after 42-60 days. The estimated degradation of the monoesters was below 60 % ThIC after 28 days with each of the three test substances whereas with one of the test substances it was above 60% ThIC after 45 days. The highest ultimate degradation of the monoesters (51-59 % ThIC after 28 days and 70-75% % ThIC after 45 days) was obtained with the test substance which had a higher concentration of the monoesters (74.8%), resin acids and light ends, and a lower proportion of di- and triesters, compared to the other test substances. The primary degradation of the monoesters was 83-96% after 28 days, and 92-94% after 45-60 days with the three test substances. Therefore, 4-17% of the monoesters remained undegraded after 28 days and 6-8% after 45-60 days.[24]

The pass level of ≥60% ($ThCO_2$) of the monoesters was only reached with one of the three test substances and only during the extended test period. The guidance (ECHA 2017c) mentions that "*positive test results should generally supersede negative test results*". The positive test result means that the ultimate degradation of a test substance or a compound of concern exceeded the pass level for ready biodegradability or for screening as not P and not vP. However, according to the guidance (ECHA 2017b, ECHA 2017c) degradation achieving the pass level during extended test period can be used for P/vP assessment only in certain specific cases. One precondition for this is that there is "*some initial, slow but*

[24] It is noted that these values are based on the assumption that the di- and triesters were not degraded to monoesters during the study. The primary degradation of the monoesters may have been higher (and the amount of remaining monoesters lower) if monoesters were produced from the di- and triesters during the study.

steady, biodegradation but did not reach a plateau by the end of the ready biodegradability test, i.e. after 28 days". The guidance does not specify how to define whether there is "*some initial, slow but steady, biodegradation*". In addition, the eMSCA considers that this part of the guidance is not fully applicable in this case because in the present OECD TG 310 studies, the overall degradation kinetics of the test substance (based on % ThIC) reflect the degradation of several constituents and the exact ultimate degradation kinetics of the monoesters are not known. As in the present case also primary degradation was measured, the ultimate degradation results were not used on their own but in combination with primary degradation and other available data, to obtain a more complete view of the degradation occurring in these studies, for the purpose of P/vP assessment.

ECHA guidance documents do not include specific advice (such as pass levels) on how to use primary degradation in ready biodegradation tests for P assessment. Normally, a "not P and not vP" conclusion from ready biodegradation tests can be drawn only when the pass level for ultimate degradation is reached, which practically presents a complete ultimate degradation of the test substance according to OECD (2006).[25] It should also be considered that residual parent substance analysis is not a requirement in OECD TG 310 or in most of the other OECD ready biodegradation test guidelines and that the pass level for a "not P/vP" conclusion in ECHA guidance is based on ultimate degradation. The eMSCA considers that even if a pass level (e.g., ≥60% ThIC) is reached a small amount of the parent substance may still remain. Therefore, the eMSCA considers that the incomplete primary degradation of the monoesters in the OECD TG 310 studies should not be taken strictly as an indication of potential P/vP properties but all available information need to be assessed, including the potential reasons for the incomplete degradation.

The measurements of the monoesters from the test medium and from the solvent rinse of the test bottles indicated that the low water solubility limited the rate of primary degradation of the monoesters. In addition, with the test substance applied by direct weighing the monoesters were released to the test medium more slowly than with the solvent dosed test substances. This could explain the slower primary degradation of the monoesters in the test substance applied by direct weighing and could also be a reason why a degradation ≥60 %ThIC was reached only during the extended test period. The eMSCA considers that, in the case of the test substance applied with direct weighing, the use of the extended period for P/vP assessment is in general accordance with the guidance text[26], as the primary degradation, and consequently also the ultimate degradation, appeared to be limited by bioavailability constraints in this test. Even if the shape of the primary degradation curve does not necessarily comply with the precondition regarding the use of the extended test period, it should be noted that this precondition (ECHA 2017b, ECHA 2017c) does not consider primary degradation or the potential difference between primary and ultimate degradation kinetics, which in this case may be influenced by the presence of the ester functional group in the studied compounds.

The lower ultimate biodegradability of the monoesters observed in the solvent dosed test substances does not seem to be convincingly explained by solubility constraints, even considering that based on visual assessment the ultimate degradation curve could be interpreted as "slow but steady degradation". In these bottles, a high primary degradation of the monoesters occurred early and therefore primary transformation products were expected to be available for further degradation. The potential reasons for the incomplete

[25] According to OECD (2006) the pass levels of either 60% ThOD (or $ThCO_2$) or 70% DOC removal practically represent complete ultimate degradation of the test substance as the remaining fraction of 30-40% of the test substance is assumed to be assimilated by the biomass or present as products of biosynthesis.

[26] For example the purpose of the enhancements of the biodegradation screening tests, such as the prolongation of the test duration, should only be to compensate the poor bioavailability to the degrading microorganisms of poorly soluble and/or adsorptive substances, but should not be used to induce additional adaptation of the inoculum. Also, a late acceleration of biodegradation is likely to reflect an adaptation of the microorganisms and in that case the prolongation of the test duration should not be regarded as adequate for the P/vP assessment (ECHA 2017b).

degradation of the transformation products were considered but no firm conclusions could be drawn. The incomplete primary degradation obviously restricted also ultimate degradation but this explains only a relatively minor part of the lacking ultimate degradation in the solvent dosed bottles and it does not explain why the ultimate degradation in the solvent dosed samples remained lower than in the direct weighed sample, which had a similar or lower primary degradation.

In one of the OECD TG 310 studies there is more detailed analytical information available for the monoester fraction. The decrease in GC-MS peak areas of the monoesters of dehydroabietic acid and monoesters of hydrogenated resin acids were 98% and 72% after 28 days. At the study end (60 days) the monoesters of hydrogenated resin acids attained roughly a decrease of 90%. The reasons for the lower degradation of this fraction of monoesters are not known but may be related to differences in solubility. The constraints to the biodegradation are probably more pronounced in a ready biodegradation study as the test substance concentration is higher compared to the concentrations used in simulation tests or concentrations expected in the environment. Therefore, even if a part of the monoesters did not degrade, the reasons for this are possibly explained by the high test substance concentration in the ready biodegradation test. It is not possible from the current data to identify and quantify the individual remaining monoesters in more detail.

Microbial transformation prediction for the selected monoester constituents (DHAA-mono-E, THAA-mono-GE) using the Pathway Prediction System (PPS) of the EAWAG Aquatic Research Biocatalysis/Biodegradation Database predicts primary degradates that include DHAA/THAA, glycerol, and other three- or two-carbon compounds derived from the glycerol moiety, all of which are considered not PBT and not vPvB under aerobic conditions. Also transformation products which still include the ester bond may be formed based on the predictions and for these PBT/vPvB assessment has not been previously conducted. For the parent monoesters as well as each of the predicted transformation products which have more than one possible transformation routes according to the predictions, the ester hydrolysis route is always equally or more likely than the other routes. Moreover, even if a non-hydrolytic reaction occurs first, it is predicted to be followed by a hydrolysis reaction (with or without intermediate reaction steps), eventually leading to a resin acid and a three- or two-carbon compound.

The ultimate degradation of the monoesters is expected to occur through the hydrolysis of the ester bond. The relatively high ultimate degradation in one of the OECD TG 310 tests, and the fast primary degradation in all three OECD TG 310 tests, suggest that hydrolysis of the ester bond of the monoesters took place. Also taking into account the predicted likelihoods of ester hydrolysis and other transformations of the monoesters, it is unlikely that any of the predicted transformation products which still have an ester bond would be more persistent than the parent monoesters.

Primary degradation of the monoesters is therefore expected to proceed to non PBT/vPvB transformation products, at least under aerobic conditions. These transformation products include most likely resin acids, glycerol, or other three- or two-carbon compounds derived from the glycerol moiety. The compounds derived from the glycerol moiety are expected to mineralise relatively fast whereas resin acids may be either further biotransformed or mineralised, or they may accumulate, depending on the potential of the microbial communities to metabolise these compounds, or their sensitivity to inhibition by resin acids or other transformation products. It should be noted that the P/vP status of resin acids is not known. In addition, resin acids may have the potential for biotransforming into PBT/vPvB degradation/transformation products under anaerobic conditions (See 7.7.1.7 and 7.11.4).

The eMSCA considers that the high primary degradation of the monoesters observed with all three test substances and the significant ultimate degradation of the monoesters in the OECD TG 310 studies point to the conclusion that the monoesters are not P and not vP. It can be concluded that the monoesters undergo substantial and fast primary biodegradation under the conditions of ready biodegradability testing. Even if the primary degradation in

the OECD TG 310 studies was not complete it is noted that there were indications that primary degradation was limited by the low water solubility of the monoesters under the conditions of ready biodegradability testing. The part of the monoesters which showed a slower degration rate still showed a consistent decrease until the end of the study (Figure 9), suggesting that these monoesters continued to degrade. Hence the eMSCA considers that there is only a very low probability that the remaining monoesters in the OECD TG 310 study would be P or vP. The high primary degradation also suggests that the main reason why the ultimate degradation of the monoesters was below 60% ThIC with two of the test substances was the accumulation of transformation products of the monoesters and not the presence of undegraded monoesters.

Therefore, the monoester constituents of the Substance are considered to be not P and not vP under aerobic conditions, when the parent compounds are considered. It should be noted that transformation of the monoesters into P and/or vP transformation products could not be excluded. Resin acids, which are potentially P and potentially vP, were shown to be present throughout the study in the OECD TG 310 study (however, it has to be noted that resin acids were also constituents of the test substance). Resin acids are potentially P and potentially vP but they are not PBT and not vPvB under aerobic conditions (see 7.11.4).

Other constituents

Regarding the resin acid and rosin acid constituents of the Substance, the OECD TG 310 studies on enriched monoesters are to some extent relevant, as a significant part of the carbon in the test substances used consisted of the rosin backbone and as there were also resin acids and rosin acids in the test substances. The present results indicate that a significant part of the rosin moieties of the monoesters were biodegraded. However, the present results cannot be used to definitively assess the ready biodegradability or (non-) persistence of resin acids. It should be noted that the monoesters contain a glycerol moiety which is likely to be more biodegradable than the resin acid part. The previous assessment on Rosin, hydrogenated (Tukes 2015) concluded that no definitive conclusion on persistence can be made based on the available data. The eMSCA considers that this conclusion is still valid even after considering the new data. Therefore, resin acids and rosin acids are considered potentially P and potentially vP. In addition, it should be noted there is some remaining concern also for the potential transformation products formed from resin acids and rosin acids under anaerobic conditions, (See 7.7.1.7 and 7.11.4).

The di- and triesters are considered potentially P and potentially vP based on ready biodegradation tests conducted on the Substance. Di- and triesters were also present in the OECD TG 310 studies with enriched monoesters and in one of the studies di- and triesters were measured (Study report 2017c). Reduction in the peak areas of di- and triesters was reported. However, it is not known to what extent the reduction was due to degradation as the recovery rate of the extraction and analytical method was not determined and as there were no sterile controls. In addition, the remaining concern for anaerobic transformation products of resin acids and rosin acids is relevant also for the di- and triesters, as resin acids and rosin acids are potential transformation products of these constituents.

For the light ends fraction (mono- and sesquiterpenes), relevant information is available in the registration data for Turpentine, oil (EC 232-350-7) (ECHA 2021c). Turpentine, oil is composed primarily of the $C_{10}H_{16}$ terpene hydrocarbons: α-pinene, β-pinene, limonene, 3-carene, camphene (ECHA 2021c). It may contain other acyclic, monocyclic, or bicyclic terpenes, oxygenated terpenes, and anethole. For Turpentine, oil, two OECD TG 301 F studies are available and indicate 71.7-72.7% degradation in 28 days (ECHA 2021b). As noted in the CCH decision (ECHA 2020b), in the studies conducted with a multi-constituent substance the biodegradation may be related only for some constituents and the studies do not allow assessment of ready biodegradability of each relevant constituent of Turpentine, oil.

OECD TG 301 D studies for several individual terpene compounds are available and indicated the following degaradation percentages in 28 days: β-pinene 76 %, Delta-3-

carene 73.8 %, dipentene multi-constituent 80%, myrcene 76% , and terpinolene 81 %, (ECHA 2020b). Each of these compounds belongs to monoterpenes. The eMSCA considers that these data suggest a relatively high degradability of monoterpenes. Regarding sesquiterpenes, there are indications of potential persistence. Jenner *et al.* (2011) studied selected cyclic sesquiterpenes and reported that 60% degradation (% theoretical oxygen demand, ThOD) during 28 days in an OECD TG 301 F study was reached for alpha-humulene (64 % ThOD) beta-caryophyllene (70 %), and alpha-cedrene (78 %) whereas it was not reached for delta-cadinene (50 %), germarcrene D (19 %) , alpha-gurjunene (43 %), himalachines (alpha , beta, gamma) (38 %), longifolene (50%), and (−)-thujopsene (36 %). There is data for at least one sesquiterpene, caryophyllene, in the REACH registration database (ECHA 2021d). Caryophyllene attained a degradation percentage of -0.8, 3.2, 63.9, 54.4, 60.2, 58.8, 64.3, and 56 % after 0, 3, 5, 7, 10, 14, 21, and 28 days in an OECD TG 310 study. There was a fast degradation up to the pass level of ≥60% by day 5 of the study but then the degradation plateaued and the pass level was not consistently exceeded in the following measurement days.

Based on the available data the eMSCA considers that currently the light ends fraction should be considered potentially P and potentially vP, particularly due to the presence of sesquiterpenes. ECHA has requested further information on degradation of Turpentine, oil, in a compliance check (ECHA 2020b). When available, that information should be considered for its potential relevance for the light ends fraction of the Substance.

For the heavy ends fraction (a mixture of dimerised esters, acids and polyol), the eMSCA considers that at least the dimers of resin acids and rosin acids, as well as the dimerised esters, are potentially P and potentially vP. The dimers of resin acids and rosin acids include the (dimerised) rosin backbone but due to the dimerization have more complex structures and higher molecular weights than resin acids and rosin acids. According to the registration dossier for rosin, oligomers (consisting of, e.g., rosin dimers and trimers), the substance is readily biodegradable (ECHA 2021e). However, this conclusion is based on a category approach and no studies conducted on rosin, oligomers are presented (ECHA 2021e). The eMSCA assumes that "dimerised esters" refer to esters in which at least one of the acid groups is a rosin dimer and which have two glycerol moieties. These are considered potentially P and potentially vP due to the more complex structure and higher molecular weight compared to the dimers of resin acids and rosin acids. The same conclusion would apply also to glycerol esters including a dimerised rosin acid moiety and only one glycerol moiety although it is unclear whether these are present in the Substance.

Conclusion on persistence

The monoester constituents of the Substance should be considered not P and not vP under aerobic conditions, when considering the parent compounds only. It should be noted that the monoesters may transform into compounds that are potentially P and potentially vP, as specified above.

For the other constituents (resin acids and rosin acids, di- and triesters, light ends, and heavy ends) no definitive conclusion on the P/vP property can be drawn based on the available data and therefore these fractions are considered potentially P and potentially vP.

7.11.2 Bioaccumulation

Monoesters

The monoester constituents may have bioaccumulation potential as their log Kow values exceed 4.5 (5.3 and 5.2 for THAA-mono-GE and DHAA-mono-GE, respectively). BCF values predicted by BCFBAF regression model do not exceed the B criterion of 2000 for THAA-mono-GE and DHAA-mono-GE (1465 and 1287, respectively). The Arnot Gobas model predicts BCF and BAF levels well below the B criterion (< 300) when biotransformation rate estimation is included in the estimation. However, applying a worst case assessment

assuming zero biotranformation, the model predicts BCF values (5804 and 5168 for THAA-mono-GE and DHAA-mono-GE, respectively) exceeding B and vB criteria (5000). Therefore these constituents screen as potentially B and potentially vB.

The remaining concern for anaerobic transformation products of resin acids and rosin acids (discussed below) is relevant also for the monoesters, as resin acids and rosin acids are likely relevant transformation products of the monoesters.

Other constituents

Resin acids and rosin acids were considered to be not B/vB in the previous assessment on Rosin, hydrogenated (Tukes 2015). The eMSCA considers that this conclusion is still valid under aerobic conditions, whereas regarding the potential transformation products formed from resin acids and rosin acids under anaerobic conditions, there is some remaining concern (See 7.7.1.7 and 7.11.4).

The di- and triester constituents are expected to have rather low bioaccumulation potential due to their physical and chemical properties (i.e., high molecular weights, large cross-sectional diameters of some components), slow uptake potential and low predicted BCF values (< 40). These constituents have predicted log Kow > 10, which indicates reduced bioavailability and bioaccumulation for the parent constituents. Therefore, the di- and triesters are considered not B and not vB. However, the di- and triesters have the potential to be transformed into monoesters, which are potentially B and potentially vB. Also, the remaining concern for anaerobic transformation products of resin acids and rosin acids is relevant for the di-, and triesters as resin acids and rosin acids are potential transformation products of these constituents.

The constituents of the heavy ends fraction are considered to have a low bioaccumulation potential. According to the REACH registration for rosin, oligomers (ECHA 2021e), it is stated that "*log Kow based on OECD TG 117 study is* >6.5". The dossier also states "*QSAR predictions have been conducted for a representative structure of rosin dimers and based on the predictions these constituents are considered to have a low potential for bioaccumulation due to their large molecular size and very high log Kow (log Kow >10), meaning that they are unlikely to be taken up by organisms.*". The eMSCA considers that also dimerized esters, which may also be present in the heavy ends fraction (See 7.11.1), are likely to have a low bioaccumulation potential, as those have a larger molecular size compared to dimers of resin acids. As an example, a log Kow of 11.46 was obtained by the eMSCA for a dimer of resin acid monoesters with glycerol[27]. The same conclusion (low bioaccumulation potential) would apply also to glycerol esters including a dimerised rosin acid moiety and only one glycerol moiety. Therefore, the heavy ends are considered not B and not vB.

However, based on the description of the heavy ends fraction ("a mixture of dimerized esters, acids and polyol") the eMSCA considers that the "dimerized esters" may include constituents (dimerised mono-, di-, or triesters) including also (non-dimerised) resin acid moieties, in addition to the dimerized resin acid moieties. These compounds could potentially be biotransformed into other compounds, such as resin acids, monoesters of resin acids (which are potentially P and potentially vB), or diesters of resin acids/dimerized resin acids, through ester hydrolysis. The diesters of resin acids could then further transform into monoesters of resin acids. In addition, if resin acids are released, the above-described concern for the anaerobic transformation products applies also to the heavy ends fraction.

The light ends fraction may contain constituents which have bioaccumulation potential. For the monoterpenes alpha-pinene (ECHA 2021b), beta-pinene (ECHA 2021f), limonene

[27] Kowwin v1.68 in Epi Suite v4.11; SMILES: CC(C)C1CCC2C3C1C4CC5C(C)(CCCC5(C)C(=O)OCC(O)CO)C6CCC(C(C)C)C(=C46)C3=CC7C2(C)CCCC7(C)C(=O)OCC(O)CO

(ECHA 2021g), delta-carene (ECHA 2021h), and camphene (ECHA 2021i), the reported log Kow values are 4.22-4.48 and therefore below (but close to) the screening criterion for B/vB. The reported BCFs (mostly based on QSARs) are below the B criterion. The sesquiterpenes (alpha-humulene, delta-cadinene, germarcrene D, gurjunene, himalachines (alpha, beta, gamma), longifolene, and (−)-thujopsene) studied by Jenner *et al.* (2011) fulfill the screening criterion for B/vB as they have a log Kow of > 4.5 (range 5.48 to 7.12) based on QSAR predictions (KowWin version 1.67). For caryophyllene, REACH registration reports a measured log Kow of 6.23 (ECHA 2021d). ECHA has requested further information on bioaccumulation of Turpentine, oil, in a compliance check (ECHA 2020b). When available, that information should be considered for its potential relevance for the light ends fraction in the Substance.

Conclusion on bioaccumulation

The monoesters and the light ends are considered potentially B and potentially vB. For a definitive conclusion on B/vB for these fractions, further experimental information would be needed.

Resin acids and rosin acids are considered not B and not vB under aerobic conditions.

The diesters, triesters, and the heavy ends are considered not B and not vB under aerobic conditions, when considering the parent compounds only. It should be noted that these constituents may transform into monoesters, which are potentially P and potentially vB.

7.11.3 Toxicity

Only short-term toxicity studies were available for aquatic toxicity and no studies for terrestrial environment for the Substance and the analoguous rosin ester substances. Based on the short-term test results none of the studied rosin ester substance analogues showed such acute aquatic toxicity that would fulfil either the short-term screening criteria (EC50 < 0.1 mg/L) or the definite criteria (EC50 < 0.01 mg/L) for environmental toxicity in PBT assessment. Only the WAF fractions of the UVCB substances as such have been tested experimentally and there are no experimental studies for the individual constituents or fractions.

Of the ester constituents, ECOSAR predictions could be conducted only for monoesters of the Substance as these constituents fit the ECOSAR model. The lowest estimated ChV values for fish (0.023 and 0.026 mg/L for THAA-mono-GE and DHAA-mono-GE, respectively) are close to the T criterion for long-term aquatic toxicity (NOEC < 0.01 mg/L).

The Substance has not been classified according to the CLP Regulation. Therefore, the study findings were compared to the relevant guidance values for classifications (carcinogenic category 1A or 1B, germ cell mutagenic category 1A or 1B, toxic for reproduction category 1A, 1B or 2, or specific target organ toxicity 1 or 2) that would fulfil the toxicity criterion (T). No carcinogenicity or germ cell mutagenicity studies have been carried out, yet the studied rosin ester substance analogues were not observed to be mutagenic or clastogenic in bacterial or mammalian cells *in vitro* with or without metabolic activation. Studies on toxicity for reproduction and subchronic toxicity have been conducted, and the findings do not indicate that these rosin ester substances would fulfil the toxicity criteria.

Regarding resin acids and rosin acids, no definitive conclusion on the T criterion was drawn in the previous assessment for Rosin, hydrogenated (Tukes, 2015). The toxicity/ecotoxicity of resin acids and rosin acids has not been assessed further in the substance evaluation. The eMSCA considers that resin acids and rosin acids are potentially T.

Regarding the light ends, no toxicity/ecotoxicity assessment has been conducted in the current substance evaluation. ECHA has requested further information on toxicity and exotoxicity of Turpentine, oil, in a compliance check (ECHA 2020b). When available, that

information should be considered for its potential relevance for the light ends fraction in the Substance.

Regarding the heavy ends, no toxicity/ecotoxicity assessment has been conducted in the current substance evaluation. In the registration dossier for rosin, oligomers (ECHA 2021e), the registrant(s) consider that the T criterion is not fulfilled, based on a grouping approach. However, the eMSCA is not aware of any toxicity or ecotoxicity studies on the dimerized or polymerized rosin or dimerized or polymerized rosin esters.

7.11.4 Overall conclusion

The conclusions regarding persistence, bioaccumulation, and toxicity per each fraction of the constituents, are summarised in Table 46.

The mono-, di-, and triester fractions, the heavy ends fraction, and the resin acids and rosin acids fraction of the Substance are considered not PBT and not vPvB under aerobic conditions.

For the monoester fraction, ready biodegradation testing with determination of primary degradation was conducted in response to the Substance Evaluation decision. Based on the requested data and other available data it is concluded that the monoester constituents of the Substance should be considered not P and not vP under aerobic conditions, when considering the parent compounds only. It was shown that the monoesters have a potential to fulfill the B and/or the vB criterion. No definitive conclusion on B/vB can be done based on the available data. Some of the transformation products of the monoesters are potentially P and potentially vP. However, as neither the parent monoester compounds nor the transformation products of the monoesters are PBT or vPvB under aerobic conditions, there is no PBT/vPvB concern for the monoesters under aerobic conditions.

For the di- and triester fractions, the heavy ends fraction, and the resin acids and rosin acids fraction, the parent compounds are not PBT and not vPvB under aerobic conditions and there are no indications of PBT/vPvB transformation products under aerobic conditions. Therefore, there is no PBT/vPvB concern for these constituent fractions under aerobic conditions.

Regarding the light ends fraction, a concern still exists for PBT/vPvB properties, and relevant information is expected to be produced in the ongoing compliance check for turpentine, oil (EC 232-350-7). When that information is available, its relevance for the Substance will be assessed, outside this substance evaluation. The PBT/vPvB conclusions of the Substance will then be updated, if necessary.

It should be noted that resin acids and rosin acids may have the potential for biotransforming into potential PBT/vPvB degradation/transformation products under anaerobic conditions (See 7.7.1.7). Further assessment of the transformation products derived from resin acids and rosin acids under anaerobic conditions is therefore warranted. Resin acids and rosin acids are also transformation products of the mono-, di-, and triesters as resin acids and rosin acids may be released in the hydrolysis of the ester bonds. Therefore, some uncertainty remains regarding the PBT/vPvB conclusions of resin acids and rosin acids as well as of the mono-, di-, and triesters, when anaerobic transformation is taken into account. The same may apply also to the heavy ends fraction, as the presence of ester constituents with (non-dimerised) resin acid moieties in this fraction could not be excluded.

The concern regarding the anaerobic transformation products of resin acids and rosin acids was not mentioned in the registration dossier for the Substance and neither was it mentioned in the previous assessments on the rosin substances (e.g., Tukes 2015). The eMSCA notes that there are currently open questions on how the degradation/transformation products formed only under anaerobic conditions should be

addressed under REACH PBT assessment. The eMSCA considers that these general questions should be discussed between the authorities responsible for REACH implementation before taking action on specific substances, to ensure consistent assessment and regulation of substances. As most of the fractions of the Substance are considered not PBT and not vPvB under aerobic conditions, and as there is an ongoing compliance check for Turpentine, oil, the eMSCA considers that further information requests under this substance evaluation to clarify the PBT/vPvB properties of the Substance or its constituents are not justified. The concern regarding the transformation products formed under anaerobic conditions will be evaluated further outside this substance evaluation.

It is noted that the concern regarding the transformation products formed under anaerobic conditions is valid not only for the Substance but for substances containing resin acids and rosin acids, or their precursors. Therefore, the eMSCA considers that, if this issue of degradation/ transformation products produced under anaerobic conditions is considered relevant under REACH, it is preferable to consider this concern first for the whole group of relevant substances (i.e., in this case, the substances containing resin acids and rosin acids or their precursors) rather than only for a single substance such as the Substance. If the concern for PBT/vPvB transformation products under anaerobic conditions is confirmed for the Substance based on a further assessment, this could potentially warrant changes in the PBT/vPvB conclusion of the Substance.

Table 46. Summary and conclusions of PBT/vPvB properties for the relevant fractions of the Substance (EC 266-042-9). "Pot." = "potentially" (meaning that there is a concern with the endpoint but no sufficient information is available on the constituent/substance for a definitive conclusion). Note that the conclusions "Not P", "Not vP", "Pot. B", "Pot. vB", etc., concern the parent compounds, meaning that, "Not P" is indicated when there is no concern with the parent constituent and "Pot. P" is indicated only when there is a concern with the parent constituent. The concerns relevant for transformation products are indicated by the respective footnotes (i.e., the footnotes b and c).

SUMMARY OF PBT AND vPvB PROPERTIES							
	Resin acids and rosin acids	**Mono-esters**	**Diesters**	**Triesters**	**Light ends**	**Heavy ends**	**Substance**
End-point	**Outcome/conclusion**						
P/vP	Pot. P[a] Pot. vP[a]	Not P[b,c] Not vP[b,c]	Pot. P Pot. vP	Pot. P Pot. vP	Pot. P Pot. vP	Pot. P Pot. vP	Pot. P[b,d] Pot. vP[b,d]
B/vB	Not B[a,b] Not vB[a,b]	Pot. B Pot. vB	Not B[b,c] Not vB[b,c]	Not B[b,c] Not vB[b,c]	Pot. B Pot. vB	Not B[b,c] Not vB[b,c]	Pot. B[b,d] Pot. vB[b,d]
T	Pot. T[e]	Pot. T	Pot. T	Pot. T	Not assessed	Not assessed[b, c]	Pot. T[b,d]
PBT/ vPvB	Not PBT[b] Not PvB[b]	Not PBT[b] Not vPvB[b]	Not PBT[b] Not vPvB[b]	Not PBT[b] Not PvB[b]	PBT not assessed Pot. vPvB	Not PBT[b] Not vPvB[b]	Pot. PBT[b,d] Pot. vPvB[b,d]

[a]Conclusion based on previous assessment (Tukes 2015).
[b]Conclusion is pending further assessment regarding the transformation products with potential PBT/vPvB properties which may be produced under anaerobic conditions from resin acids and rosin acids.
[c]It is noted that the constituent may potentially transform into compounds for which there is insufficient information regarding the respective endpoint (P/B/T), but the information is sufficient to consider that neither the parent constituent nor the transformation products are PBT/vPvB under aerobic conditions.
[d]Conclusion is pending further assessment regarding the light ends fraction.
[e]Based on screening level data for isopimaric acid (reviewed in Tukes 2015).

7.12 Exposure assessment

The Substance has industrial, professional as well as consumer use (See 7.5.2).

The Substance does not have harmonised classification according to the CLP Regulation (1272/2008/EC) nor has the registrant(s) self-classified the Substance.

Regarding environmental classification, most of the available experimental studies have been performed with the UVCB substances (rosin ester substance analogues) as such (ready biodegradation tests [also with the Substance] and acute ecotoxicity tests). In addition, there are two ready biodegradability studies on test substances with enriched glycerol monoesters of rosin (See 7.7.1.3). No studies for terrestrial environment have been submitted. The lowest EC50 from available acute aquatic toxicity studies is 27 mg/L from a Daphnia study with an analogue substance resin acids and rosin acids, hydrogenated, methyl ester. No ecotoxicity studies performed exactly with the Substance are available. The lowest test result (27 mg/L) with an analogue substance has been used in the CSR for PNEC derivation. Resin acids and rosin acids, Me esters is self-classified to Chronic Category 3 as the lowest EC50 value 27 mg/l is > 10 and < 100 mg/l, and it is considered not readily biodegradable substance. However, the registrant(s) has not used a read-across approach for the classification of the Substance as no self-classification is applied.

Acknowledging the identified short-comings in the acute ecotoxicity studies (see 7.11.3) based on the available information the Substance does not meet the criteria for classification as hazardous to the aquatic environment according to CLP (1272/2008/EC). Therefore, the criteria of article 14(4) REACH are currently not fulfilled based on environmental hazards and the exposure assessment including the generation of exposure scenarios and exposure estimation, and also risk characterisation for the environment is not warranted. However, as the available Chemical safety report for the Substance includes also exposure assessment for the environment, this brief review of the environmental risk characterisation values was conducted. It is noticed that based on the available information all RCRs are presently below 1, and no risk for any environment compartment has been identified.

No hazards have been identified, therefore no exposure assessment and risk characterisation regarding workers and consumers are needed, Annex I: 0.6.2./0.6.3.).

It is noted that the need for exposure assessment of the Substance should be reviewed in case relevant new experimental information becomes available (See Section 2, Section 7.11.4).

7.12.1 Human health

Worker

See above (section 7.12.).

Consumer

See above (section 7.12.).

7.12.2 Environment

Aquatic compartment (incl. sediment)

See above (section 7.12.).

Terrestrial compartment

See above (section 7.12.).

Atmospheric compartment

See above (section 7.12.).

7.12.3 Combined exposure assessment

No hazards have been identified, therefore assessment of combined exposure is not considered relevant.

7.13 Risk characterisation

Not evaluated.

7.14 References

Arnot J, Arnot M, Mackay D, Couillard Y, MacDonald D, Bonnell M, Doyle P (2009). Molecular size cutoff criteria for screening bioaccumulation potential: fact or fiction? Integ. Env. Ass. Manag. 6(2), 210-224.

Bornscheuer UT (2002). Microbial carboxyl esterases: classi¢cation, properties and application in biocatalysis FEMS Microbiology Reviews 26 (2002) 73-81

Burggraaf S, Langdon AG, Alistair LW, Roper DS (1996). Accumulation and depuration of resin acids and fichtelite by the freshwater mussel *Hyridella Menziesi*. Environ Toxicol Chem 15, 369–375.

Cheremnykh KM, Grishko VV, Ivshina IB (2017). Bacterial degradation of ecotoxic dehydroabietic acid. Catalysis in Industry 9, 331–338.

Cheremnykh KM, Luchnikova NA, Grishko VV, Ivshina IB (2018). Bioconversion of ecotoxic dehydroabietic acid using *Rhodococcus* a ctinobacteria. Journal of Hazardous Materials 346, 103–112.

Commission implementing decision, C(2017) 5834 final.

de Sousa IP, Sousa Teixeira MV, Jacometti Cardoso Furtado NA (2018). An overview of biotransformation and toxicity of diterpenes. Molecules 23, 1387.

Dimitrov S, Dimitrova N, Parkerton T, Comber M Bonnell M, Mekenyan O (2005). Base-line model for identifying the bioaccumulation potential of chemicals. SAR and QSAR in Env. Res. 16 (6), 531-554.

ECHA (2011). Decision on a compliance check of a registration pursuant to article 41(3) of regulation (EC) No 1907/2006. For Resin acids and rosin acids, hydrogenated, esters with glycerol. Helsinki 04/11/2011. European Chemicals Agency. Available at: https://echa.europa.eu/fi/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.060.020 (accessed January 2021).

ECHA 2014. Decision on a testing proposal set out in a registration pursuant to article 40(3) (EC No 266-042-9). Decision number: TPE-D-0000004925-66-05/F. Helsinki, 14 August 2014. European Chemicals Agency. Available at: https://echa.europa.eu/fi/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.060.020 (accessed March 2021)

ECHA 2017a. Decision on substance evaluation. Resin acids and rosin acids, hydrogenated, esters with glycerol. EC 266-042-9, CAS 65997-13-9. Helsinki,7 February 2017. European Chemicals Agency. Available at: https://echa.europa.eu/fi/information-on-

chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807e9747 (ccessed January 2021).

ECHA 2017b. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment Version 3.0 June 2017. European Chemicals Agency.

ECHA 2017c. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance Version 4.0 June 2017. European Chemicals Agency.

ECHA 2020a. REACH Registrated substance fact sheet. Glycerol (EC 200-289-5), https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/14481 (accessed May 2020)

ECHA (2020b). Decision on a compliance check. Turpentine, oil (EC 232-350-7, CAS 8006-64-2. Helsinki, 3 August 2020. European Chemicals Agency. Available at: https://echa.europa.eu/fi/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.029.407 (accessed January 2021).

ECHA (2021a). Registrated substance fact sheet. Rosin, hydrogenated (EC 266-041-3). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/2086 (accessed April 2021)

ECHA (2021b). Registrated substance fact sheet. Alpha-pinene (EC 201-291-9). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/14724 (accessed January 2021)

ECHA (2021c). Registrated substance fact sheet. Turpentine, oil (EC 232-350-7). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/15489 (accessed January 2021)

ECHA (2021d). Registrated substance fact sheet. Caryophyllene (EC 201-746-1). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/20872 (accessed January 2021)

ECHA (2021e). Registrated substance fact sheet. Rosin, oligomers (EC 500-163-2). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/13162 (accessed January 2021)

ECHA (2021f). Registrated substance fact sheet. Beta-pinene (EC 242-060-2). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/15934 (accessed January 2021)

ECHA (2021g). Registrated substance fact sheet. Limonene (EC 227-813-5). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/15256 (accessed January 2021)

ECHA (2021h). Registrated substance fact sheet. Delta-3-carene (EC 207-856-6). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/24165 (accessed January 2021)

ECHA (2021i). Registrated substance fact sheet. Camphene (EC 201-234-8). https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/14290 (accessed January 2021)

FSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Younes M, Aggett P, Aguilar F, Crebelli R, Filipic M, Frutos MJ, Galtier P, Gott D, Gundert-Remy U,

Kuhnle GG, Lambre C, Leblanc J-C, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Briemer L, Pasquale M, Christodoulidou A, Horvath Zs, Lodi F, Tard A and Dusemund B, 2018. Scienti□c Opinion on the re-evaluation of glycerol esters of wood rosin (E 445) as a food additive. EFSA Journal 2018;16(7):5370, 47 pp. https://doi.org/10.2903/j.efsa.2018.5370

FSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Younes M, Aggett P, Aguilar F, Crebelli R, Filipic M, Frutos MJ, Galtier P, Gott D, Gundert-Remy U,

Kuhnle GG, Lambre C, Leblanc J-C, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Briemer L, Pasquale M, Christodoulidou A, Horvath Zs, Lodi F, Tard A and Dusemund B, 2018. Scienti□c Opinion on the re-evaluation of glycerol esters of wood rosin (E 445) as a food additive. EFSA Journal 2018;16(7):5370, 47 pp. https://doi.org/10.2903/j.efsa.2018.5370

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food) (2018). Scientifc Opinion on the re-evaluation of glycerol esters of wood rosin (E 445) as a food additive. EFSA Journal 16(7): 5370, 47

Environment Canada (2011). Screening Assessment for the Challenge - Resin, hydrogenated; Resin acids and rosin acids, hydrogenated, esters with pentaerythritol; Resin acids and rosin acids, hydrogenated, esters with glycerol; Resin acids and rosin acids, hydrogenated, esters triethylene glycol. available at: https://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=8E8373E7-1

Foss S, Harder J (1998). *Thauera linaloolentis* sp. nov. and *Thauera terpenica* sp. nov. isolated on oxygen-containing monoterpenes (linalool, menthol and eucalyptol) and nitate. Systematic and Applied Microbiology 21, 365–373.

Fulzele SV, Satturwar PM, Dorle AK (2003). Study of the biodegradation and in vivo biocompatibility of novel biomaterials. European Journal of Pharmaceutical Sciences 20, 53-61.

Fulzele SV, Satturwar PM, Dorle AK (2007). Novel biopolymers as implant matrix for the delivery of ciprofloxacin: Biocompatibility, degradation, and in vitro antibiotic release. Journal of Pharmaceutical Sciences 96, 132–144.

Harder J, Probian C (1997). Anaerobic mineralization of cholesterol by a novel type of denitrifying bacterium. Archives of Microbiology 167, 269–274.

Hemingway RW, Greaves H (1973). Biodegradation of resin acid sodium salts. Tappi 56(12), 189-192.

JRC (2014). Gottardo, S., Hartmann, N. and Sokull-Kluettgen, B. Review of available criteria for non-aquatic organisms within PBT/vPvB frameworks. JRC Science and Policy Report. European Commisison. Report EUR 26802 EN.

Lahdelma I, Oikari A (2005). Resin Acids and Retene in Sediments Adjacent to Pulp and Paper Industries (8 pp). Journal of Soils and Sediments, 5(2), 74–81.

McLeay DJ, Walden CC, Munro JR (1979). Effect of pH on toxicity of kraft pulp and paper mill effluent to Salmonid fish in fresh and saltwater. Water Res 13, 249–254.

Leppänen H, Oikari A (2001). Retene and resin acid concentrations in sediment profiles of a lake recovering from exposure to pulp mill effluents *Journal of Paleolimnology, 25(3), 367–374.*

Liss, SN, Bicho, PA, Saddled, JN (1997). Microbiology and biodegradation of resin acids in pulp mill effluents: a minireview. Can J Microbiol 75, 599-611.

Luchnikova NA, Ivanova KM, Tarasova EV, Grishko VV, Ivshina IB (2019). Microbial Conversion of Toxic Resin Acids. Molecules 24, 4121.

Martin VJJ, Yu Z, Mohn WW (1999). Recent advances in understanding resin acid biodegradation:microbial diversity and metabolism. Arch Microbiol (1999) 172 :131–138.

Mattson FH and Volpenhein RA (1972). Hydrolysis of fully esterified alcohols containing from one to eight hydroxyl groups by the lipolytic enzymes of rat pancreatic juice. J Lipid Res. 1972 May;13(3):325-8.

Mohn WW, Wilson AE, Bicho P, Moore ERB (1999) Physiological and phylogenetic diversity of bacteria growing on resin acids. Syst Appl Microbiol 22:68–78

National Center for Biotechnology Information (2021a). PubChem Compound Summary for CID 10569, Abietic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Abietic-acid. (accessed February 2021)

National Center for Biotechnology Information (2021b). PubChem Compound Summary for CID 94391, Dehydroabietic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Dehydroabietate. (accessed February 2021)

National Center for Biotechnology Information (2021c). PubChem Compound Summary for CID 227277, Dihydroabietic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Dihydroabietic-acid. (accessed February 2021)

National Center for Biotechnology Information (2021d). PubChem Compound Summary for CID 72941537, Tetrahydroabietic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Tetrahydroabietic-acid. (accessed February 2021)

National Center for Biotechnology Information (2021e). PubChem Compound Summary for CID 11852355, Glyceryl 1-abietate. https://pubchem.ncbi.nlm.nih.gov/compound/Glyceryl-1-abietate. (accessed February 2021)

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 124081842, Glyceryl 2-dehydroabietate. https://pubchem.ncbi.nlm.nih.gov/compound/Glyceryl-2-dehydroabietate. (accessed February 2021)

Niimi AJ and Lee HB (1992). Free and Conjugated concentrations of nine resin acids in rainbow trout (Oncorhynchus mykiss) following waterborne exposure. Environmental Toxicology and Chemistry 11, 1403-1407.

Nyrén V and Back E (1958). The ionisation constant, solubility product and solubility of abietic and dehydroabietic acid. Acta Chemica Scandinavica 12, 1516-1520.

OECD (2006). Guidelines for the testing of chemicals. Revised introduction to the OECD guidelines for testing of chemicals, Section 3 Part 1: Principles and strategies related to the testing of degradation of organic chemicals degradation of organic chemicals.

Savluchinske-Feio S, Curto MJM, Gigante B, Roseiro JC (2006). Antimicrobial activity of resin acid derivatives. Appl Microbiol Biotechnol (2006) 72:430–436

Study report (1960a). Ninety Day Subacute Oral Toxicity of Pentalyn A Resin. Unpublished report.

Study report (1960b). Ninety Day Subacute Oral Toxicity of Ester Gum 8D. Unpublished report.

Study report (1960c). Ninety Day Subacute Oral Toxicity of Pentalyn H. Unpublished report.

Study report (1960d). Ninety-day Subacute Oral Toxicity of Poly-pale Ester 10. Unpublished report.

Study report (1960e). Ninety Day Subacute Oral Toxicity of Staybelite Ester 3. Unpublished report.

Study report (1967). 90-Day Subacute Oral Toxicity of Staybelite Ester 5 (HT-355-62-2) XA 112-128 Albino Rats. Unpublished report.

Study report (1982a). Three Month Toxicity Study on Ester Gum 8D-SP Given Orally (Diet) to Rats. Unpublishedreport.

Study report (1982b). Ames Salmonella/Microsome Plate Test PC 12-99. Unpublished report.

Study report (1985a). 91-Day Subchronic Dietary Toxicity Study in the Rat with Pentalyn HD-CGR. Unpublished report.

Study report (1985b). 28-Day Dietary Screening Study for Rosin Derivative, Ester Gum 8D. Unpublished report.

Study report (1985c). 28-Day Dietary Screening Study for Rosin Derivative, Hercolyn D. Study number: Hercules Project No. 83-21. Unpublished report.

Study report (1987). 90-Day Dietary Study in Rats with Staybelite Ester 5 (X24343-83-1, CGR). Unpublished report.

Study report (1988a). Biodegradability study of pentalyn HE in the modified sturm test. . Unpublished report.

Study report (1988b). Biodegradability study of Hercolyn D in the modified sturm test. . Unpublished report.

Study report (1988c). Biodegradability study of staybelite ester 10E in the modified sturm test. Unpublished report.

Study report (1989). 90-Day Dietary Study in Rats with Ester Gum-CGR and Ester Gum-PGR. . Unpublished report.

Study report (1991). 13-Week Dietary Toxicity Study in Rats. Unpublished report.

Study report (1993a). Fish Acute Toxicity Test of Glycerol Ester of Rosin with Zebrafish (Brachydanio rerio). Unpublished report.

Study report (1993b). Fish Acute Toxicity Test of Pentaerythritol Ester of Rosin with Zebrafish (Brachydanio rerio). Unpublished report.

Study report (1993c). Immobilization Test of Glycerol Ester of Rosin with the crustacean Daphnia magna. Unpublished report.

Study report (1993d). Immobilization Test of Pentaerythritol Ester of Rosin with the crustacean Daphnia magna. Unpublished report.

Study report (1993e). Growth inhibition test of Glycerol Ester of Rosin with the micro algae Selenastrum capricornutum. Unpublished report.

Study report (1993f). Growth inhibition test of Pentaerythritol Ester of Rosin with the micro algae Selenastrum capricornutum. Unpublished report.

Study report (1994). Hercules Incorporated-Hercolyn DR and Hercolyn DE, 13-Week Oral (Dietary) Toxicity Study in the Rat. Unpublished report).

Study report (1996). Pharmacokinetic Study of Ester Gum 8BG in Rats. Unpublished report.

Study report (1997). ZONESTER® 25: Acute Oral Toxicity (Limit Test) in the Rat. Unpublished report

Study report (2001a). Rosin, partially hydrogenated, methyl ester, CAS No. 8050-15-5 Determination of Acute Toxicity (LL50) to Fathead Minnows (96 h, Static). Unpublished report.

Study report (2001b). Rosin esters with Pentaerythritol, CAS No. 8050-26-8 Determination of Acute Toxicity (LL50) to Fathead Minnows (96 h, Static). Unpublished report.

Study report (2001c). Rosin, partially hydrogenated, methyl ester, CAS No. 8050-15-5 Determination of Acute Toxicity (EL50) to Daphnia (48 h, Static). Unpublished report.

Study report (2001d). Rosin, esters with Pentaerythritol, CAS No. 8050-26-8 Determination of Acute Toxicity (EL50) to Daphnia (48 h, Static). Unpublished report.

Study report (2001e). Rosin, partially hydrogenated, methyl ester, CAS No. 8050-15-5 Alga, Growth Inhibition Test (72 h, EL50). Unpublished report.

Study report (2001f). Rosin, esters with Pentaerythritol, CAS No. 8050-26-8 Alga, Growth Inhibition Test (72 h, EL50). Unpublished report.

Study report (2002). Determination of Ready Biodegradability of Six Rosin Esters by the Modified Sturm Test. Unpublished report.

Study report (2003a). Determination of the Partition Coefficient of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters. Unpublished report.

Study report (2003b). Rosin, PHME (Cas No. 8050-15-5) Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test. Unpublished report.

Study report (2009). CAS No 8050-26-8 Resin acids and rosin acids, esters with pentaerythritol: L5178Y TK +/- MOUSE LYMPHOMA ASSAY. Unpublished report.

Study report (2010a). Phys/Chem Testing on a Sample of Glycerol Ester of Hydrogenated Rosin (CAS 65997-13-9). Unpublished report.

Study report (2010b). CAS No 8050-31-5 Resin and rosin acids, esters with glycerol, Acute toxicity to Daphnia magna. Unpublished report.

Study report (2010c). CAS No 8050-15-5 Resin acids and rosin acids, hydrogenated, methyl esters: L5178Y TK +/- MOUSE LYMPHOMA ASSAY. Unpublished report.

Study report (2011). Chromosome Aberration Test in Human Lymphocytes in vitro. Unpublished report.

Study report (2012a). Resin acids and rosin acids, Me esters 68186-14-1: Assessment of ready biodegradability; CO_2 evolution test. Unpublished report.

Study report (2012b). Benchmark dose modelling of Hercolyn DR and Hercolyn DE. Unpublished report.

Study report (2014a). Rosin, ethylene glycol ester CAS No. 68512-65-2: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss). Unpublished report.

Study report (2014b). Rosin, ethylene glycol ester CAS No. 68512-65-2: Daphnia sp., 48-Hour Acute Immobilization Test. Unpublished report.

Study report (2014c). Rosin, ethylene glycol ester CAS No. 68512-65-2: Algal Growth Inhibition Test. Unpublished report.

Study report (2017a). Monoesters of hydrogenated rosin with glycerol: Ready biodegradability CO_2 in sealed vessels (headspace test). Unpublished report.

Study report (2017b). Monoesters of hydrogenated rosin with glycerol: Ready biodegradability CO_2 in sealed vessels (headspace test) – Metabolite identification. Unpublished report.

Study report (2017c). Analysis of extracts from the ready biodegradability study with Mono-esters of hydrogenated rosin with glycerol. Unpublished report.

Study report (2017d). Short-term toxicity to aquatic invertebrates. Unpublished report.

Study report (2019a). Monoesters of hydrogenated rosin with glycerol: Determination of ready biodegradability - CO_2 in sealed vessels (Headspace Test). Unpublished report.

Study report (2019b). Monoesters of hydrogenated rosin with glycerol: Analytical method validation in support of TG 310, Ready Biodegradability – CO2 in sealed vessels (Headspace Test). Unpublished report.

OECD (2005). SIDS Glycerol. CAS N°: 56-81-5. UNEP Publications. https://hpvchemicals.oecd.org/UI/handler.axd?id=4b0a2d87-3183-40d4-84f5-0e118c647b19 (accessed May 2020)

OECD (2002). SIDS Pentaerytritol. CAS N°: 115-77-5. UNEP Publications. https://hpvchemicals.oecd.org/UI/handler.axd?id=612aeade-0c0d-4fb7-ab3d-c1b117780e7e (accessed May 2020)

OECD (2006). Guidelines for the testing of chemicals. Revised introduction to the OECD guidelines for testing of chemicals, Section 3 Part 1: Principles and strategies related to the testing of degradation of organic chemicals degradation of organic chemicals

Peng G and Roberts JC (2000). Wat. Res. Vol. 34, No. 10, pp. 2779 -2785, 2000

Rendall C, Kusk KO, Trapp S (2011). Optimal choice of pH for toxicity and bioaccumulation studies of ionizing organic chemicals. Env. Tox. Chem. 30 (11), 2395-2409.

Sahu NH, Mandaogade PM, Deshmukh AM, Meghre VS, Dorle AK (1999). Biodegradation studies of rosin-glycerol ester derivative. Journal of Bioactive and Compatible Polymers 14, 344-360.

Satturwar PM, Fulzele SV, Dorle AK (2003). Biodegradation and in vivo biocompatibility of rosin: a natural film-forming polymer. AAPS PharmSciTech 4, 434-439.S

Sakuratani Y, Noguchi Y, Kobayashi K, Yamada J, Nishihara T (2008). Molecular size as a limiting characteristic for bioconcentration in fish. J. Env. Biol. 29(1), 89-92.Stockholm Convention, 2009. Stockholm Convention on Persistent Organic Pollutants (POPs) as amended in 2009. Text and Annexes. Available at: http://chm.pops.int/TheConvention/Overview/TextoftheConvention/tabid/2232/Default.aspx

Stuthridge TR, Campin DN, Langdon AG, Mackie KL, McFarlane PN, Wilkins AL (1991). Treatability of bleached kraft pulp and paper mill wastewaters in a New Zealand aerated lagoon treatment system. Water Sci. Technol. 24(314), 309-317.

Tavendale MH, Farlane PN, Mackie KL, Wilkins AL, Langdon AG (1997a). The fate of resin acids-1. The biotransformation and degradation of deuterium labelled dehydroabietic acid in anaerobic sediments. Chemosphere, vol.35, No.10, pp 2137-2151.

Tavendale MH, Farlane PN, Mackie KL, Wilkins AL, Langdon AG (1997b). The fate of resin acids-2. The fate of resin acids and resin acid derived neutral compounds in anaerobic sediments. Chemosphere Vo.35, No. 10, pp. 2153-2166.

Tukes 2015. Hazard Assessment Outcome Document for Rosin, hydrogenated. EC No 266-041-3 CAS No 65977-06-0. 30 March 2015. Version 1.2. Finnish Safety and Chemicals Agency. Available at: https://echa.europa.eu/fi/pact?p_p_id=disspact_WAR_disspactportlet&p_p_lifecycle=0&_disspact_WAR_disspactportlet_substanceId=100.029.518&_disspact_WAR_disspactportlet_jspPage=%2Fhtml%2Fportlet%2Fdisspact%2FdetailsPage%2Fview_detailsPage.jsp (accessed March 2021)

US-EPA (2010). Benchmark Dose Technical Guidance Document. Available from: http://cfpub. epa. gov/ncea/cfm/recordisplay. cfm?deid=22506.

US-EPA (2011). Benchmark Dose Software (BMDS) Version 2.2 R67 [Build: 12/08/2011]. Available from: http: /www. epa. gov/NCEA/bmds/index. html.

Wakeham SG, Schaffner C, Giger W (1980). Polycyclic aromatic hydrocarbons in recent lake sediments. 2. Compounds derived from biogenic precursors during early diagenesis. Geochimica et Cosmochimica Acta 44, 415–429.

7.15 Abbreviations

AA	abietic acid
HRPE	hydrogenated rosin pentaerythritol ester
HRGE	hydrogenated rosin glycerol ester
DeHAA	dehydroabietic acid
DHAA	dihydroabietic acid
THAA	tetrahydroabietic acid
DHAA-mono-GE	dihydroabietic acid, monoester with glycerol

DHAA-mono-GE, alpha isomer	dihydroabietic acid, monoester with glycerol (an isomer where the ester bond is formed with a carbon atom with a primary hydroxyl group (C-1 or C-3 carbon, i.e., the alpha, or alpha´-position))
DHAA-mono-GE, beta isomer	dihydroabietic acid, monoester with glycerol (an isomer where the ester bond is formed with the carbon atom with a secondary hydroxyl group (C-2 carbon, i.e., the beta position))
DHAA-di-GE	dihydroabietic acid, diester with glycerol
DHAA-tri-GE	dihydroabietic acid, triester with glycerol
THAA-mono-GE	tetrahydroabietic acid, monoester with glycerol
THAA-mono-GE , alpha isomer	tetrahydroabietic acid, monoester with glycerol (an isomer where the ester bond is formed with a carbon atom with a primary hydroxyl group (C-1 or C-3 carbon, i.e., the alpha, or alpha´-position))
THAA-mono-GE, beta isomer	tetrahydroabietic acid, monoester with glycerol (an isomer where the ester bond is formed with the carbon atom with a secondary hydroxyl group (C-2 carbon, i.e., the beta position))
THAA-di-GE	tetrahydroabietic acid, diester with glycerol
THAA-tri-GE	tetrahydroabietic acid, triester with glycerol

Annex 1:

Molecular formulas, molecular weights, and SMILES codes of compounds used for modelling (SMILES codes obtained using Accelrys Draw software, unless otherwise specified):

Compound	Molecular formula	Molecular weight	SMILES
AA[a]	C20 H30 O2	302.5	CC(C)C1=CC2=CCC3C(C2CC1)(CCCC3(C)C(=O)O)C
DeHAA[a]	C20 H28 O2	300.44	CC(C)C1=CC2=C(C=C1)[C@]3(CCC[C@@]([C@@H]3CC2)(C)C(=O)O)C
DHAA[a]	C20 H32 O2	304.5	CC(C)C1CCC2C(=C1)CCC3C2(CCCC3(C)C(=O)O)C
THAA[a]	C20 H34 O2	306.5	CC(C)C1CCC2C(C1)CCC3C2(CCCC3(C)C(=O)O)C
AA-mono-GE, alpha isomer[a]	C23 H36 O4	376.54	CC(C)C1=CC2=CC[C@@H]3[C@@]([C@H]2CC1)(CCC[C@@]3(C)C(=O)OCC(CO)O)C
DeHAA-mono-GE, beta isomer[a]	C23 H34 O4	374.5	CC(C)C1=CC2=C(C=C1)[C@]3(CCC[C@@]([C@@H]3CC2)(C)C(=O)OC(CO)CO)C
DHAA-mono-GE, alpha isomer	C23 H38 O4	378.56	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OCC(O)CO)=C1
DHAA-mono-GE, beta isomer	C23 H38 O4	378.56	CC(C)C1CCC2C(=C1)CCC3C2(C)CCCC3(C)C(=O)OC(CO)CO
THAA-mono-GE, alpha isomer	C23 H40 O4	380.57	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OCC(O)CO)C1
THAA-mono-GE, beta isomer	C23 H40 O4	380.57	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OC(CO)CO)C1
DHAA-di-GE (1,3-isomer)	C43 H68 O5	665.0	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OCC(O)COC(=O)C2(C)CCCC3(C)C4CCC(C=C4CCC23)C(C)C)=C1
DHAA-di-GE (1,2-isomer)	C43 H68 O5	665.0	CC(C)C1CCC2C(=C1)CCC3C2(C)CCCC3(C)C(=O)OCC(CO)OC(=O)C4(C)CCCC5(C)C6CCC(C=C6CCC45)C(C)C
THAA-di-GE (1,3-	C43 H72	669.03	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OCC(O)COC(=O)C4(C)CCCC5(C)C6CCC(CC6C

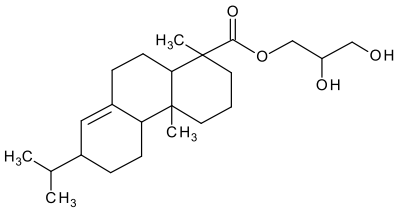
Compound	Molecular formula	Molecular weight	SMILES
isomer)	O5		CC45)C(C)C)C1
THAA-di-GE (1,2-isomer)	C43 H72 O5	669.03	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OCC(CO)OC(=O)C4(C)CCCC5(C)C6CCC(CC6CCC45)C(C)C)C1
DHAA-tri-GE	C63 H98 O6	951.5	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OCC(COC(=O)C2(C)CCCC3(C)C4CCC(C=C4CCC23)C(C)C)OC(=O)C2(C)CCCC3(C)C4CCC(C=C4CCC23)C(C)C)=C1
THAA-tri-GE	C63 H104 O6	957.5	CC(C)C1CCC2C(CCC3C2(C)CCCC3(C)C(=O)OCC(COC(=O)C4(C)CCCC5(C)C6CCC(CC6CCC45)C(C)C)OC(=O)C7(C)CCCC8(C)C9CCC(CC9CCC78)C(C)C)C1

[a]Data Sources: AA, National Center for Biotechnology Information (2021a); DeHAA, Epi Suite v4.11 National Center for Biotechnology Information (2021b); DHAA, National Center for Biotechnology Information (2021c); THAA, National Center for Biotechnology Information (2021d); AA-mono-GE, alpha isomer, National Center for Biotechnology Information (2021e); DeHAA-mono-GE, beta isomer, National Center for Biotechnology Information (2021f).

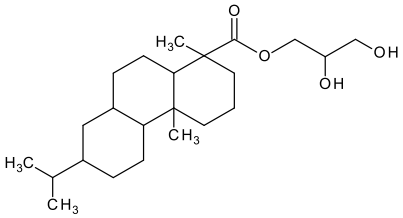
Annex 2.

Structural formulas of selected compounds relevant for the assessment.

DHAA-monoGE (alpha isomer)



THAA-monoGE (alpha isomer)



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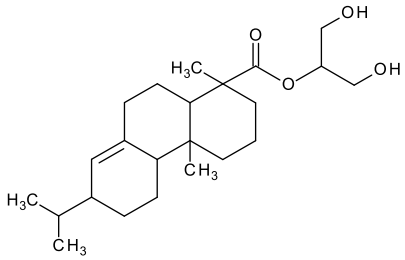
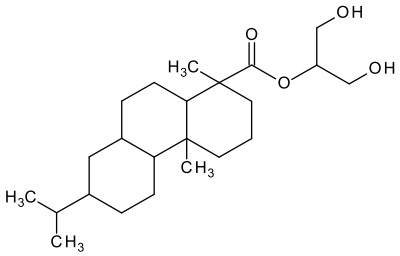
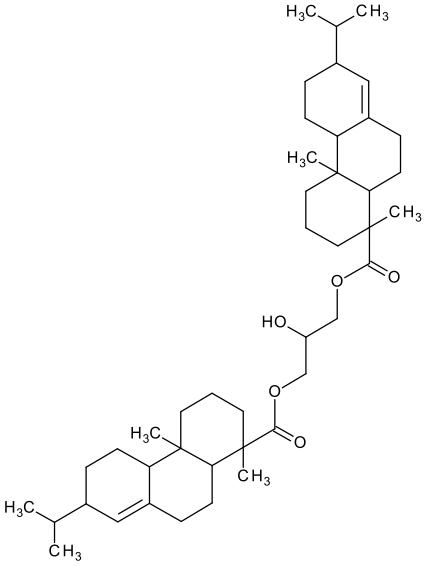


THAA-monoGE (beta isomer)



DHAA-diGE (1,3-isomer)



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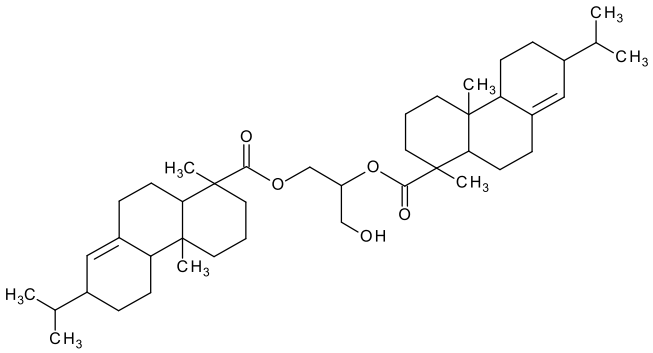
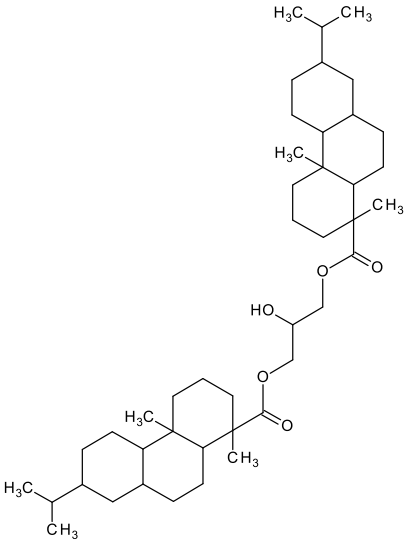


THAA-diGE (1,3-isomer)



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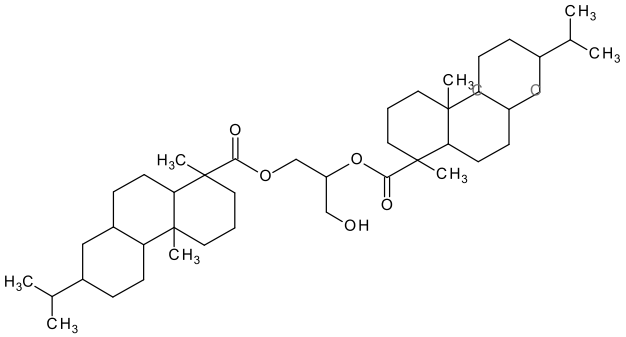
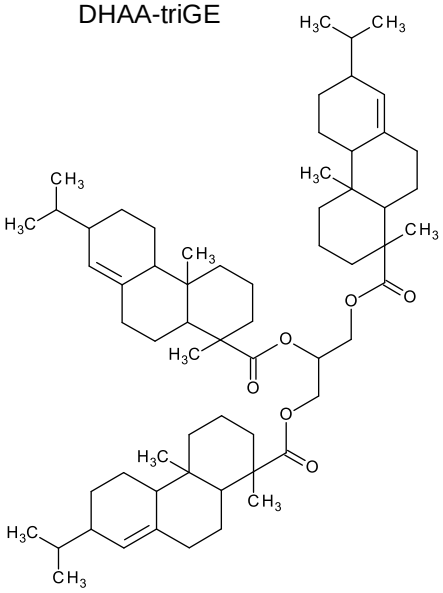


DHAA-triGE



THAA-triGE