SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

A mixture of: 4-(2,2,3-trimethylcyclopent-3-en-1-yl)-1-methyl-2-oxabicyclo[2.2.2]octane; 1-(2,2,3-trimethylcyclopent-3-en-1-yl)-5-methyl-6-oxabicyclo[3.2.1]octane; spiro[cyclohex-3-en-1-yl-[(4,5,6,6a-tetrahydro-3,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]furan]; spiro[cyclohex-3-en-1-yl-[4,5,6,6a-tetrahydro-4,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]furan]

EC No. 422-040-1

CAS RN 426218-78-2

Evaluating Member State: Spain

Dated: 4 July 2023

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2016

Before concluding the substance evaluation, a Decision to request further information was issued on: 12 April 2018

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 7
1. CONCERN(S) SUBJECT TO EVALUATION 7
2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION 7
3. CONCLUSION OF SUBSTANCE EVALUATION 7
4. FOLLOW-UP AT EU LEVEL 8
4.1. Need for follow-up regulatory action at EU level 8
4.1.1. Harmonised Classification and Labelling 8
4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation) 8
4.1.3. Restriction 8
4.1.4. Other EU-wide regulatory risk management measures 8
5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL 8
5.1. No need for regulatory follow-up at EU level 8
5.2. Other actions 9
6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY) 9
Part B. Substance evaluation 10
7. EVALUATION REPORT 10
7.1. Overview of the substance evaluation performed 10
7.2. Procedure 10
7.3. Identity of the substance 11
7.4. Physico-chemical properties 15
7.5. Manufacture and uses 16
7.5.1. Quantities 16
7.5.2. Overview of uses 16
7.6. Classification and Labelling 16
7.6.1. Harmonised Classification (Annex VI of CLP) 16
7.6.2. Self-classification 17
7.7. Environmental fate properties 17
7.7.1. Degradation 17
7.7.2. Environmental distribution 32
7.7.3. Bioaccumulation 33
7.8. Environmental hazard assessment 41
7.8.1. Aquatic compartment (including sediment) 41
7.8.2. Terrestrial compartment 43
7.8.3. Microbiological activity in sewage treatment systems 43
7.8.4. PNEC derivation and other hazard conclusions 44
7.8.5. Conclusions for classification and labelling 45
7.9. Human Health hazard assessment 45
7.9.1. Toxicokinetics 45
7.9.2. Acute toxicity and Corrosion/Irritation 46
7.9.3. Sensitisation 46

7.9.4. Repeated dose toxicity 46
7.9.5. Mutagenicity 46
7.9.6. Carcinogenicity 46
7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity) 46
7.9.8. Hazard assessment of physico-chemical properties 46
7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects 46
7.9.10. Conclusions of the human health hazard assessment and related classification and labelling 46
7.10. Assessment of endocrine disrupting (ED) properties 46
7.11. PBT and vPvB assessment 47
7.11.1. Persistence 47
7.11.2. Bioaccumulation 47
7.11.3. Toxicity 48
7.11.4. Overall conclusion 48
7.12. Exposure assessment 49
7.12.1. Human health 52
7.12.2. Environment 52
7.12.3. Combined exposure assessment 59
7.13. Risk characterisation 59
7.14. References 60
7.15. Abbreviations 61
Annex 1 Volatilisation correction for DT50 values of the constituents in the OECD TG 309 study performed by the eMSCA 62
Annex 2 Kinetic analysis performed by the eMSCA for the data from the OECD TG 305 study. 70
Constituent 1 70
Constituent 2 74
Constituent 3 78
Constituent 4 82

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

A mixture of: 4-(2,2,3-trimethylcyclopent-3-en-1-yl)-1-methyl-2-oxabicyclo[2.2.2]octane; 1-(2,2,3-trimethylcyclopent-3-en-1-yl)-5-methyl-6-oxabicyclo[3.2.1]octane; spiro[cyclohex-3-en-1-yl-[(4,5,6,6a-tetrahydro-3,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]furan]; spiro[cyclohex-3-en-1-yl-[4,5,6,6a-tetrahydro-4,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]furan] (hereafter named 'Cassiffix') was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Exposure of environment
- High RCR

During the evaluation no additional concerns were identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

The substance was notified and assessed under the NONS procedure. This procedure was closed since additional information for removing the concerns listed above could not be requested via a NONS decision.

Cassiffix is included in the Annex VI of the Regulation (EC) No. 1272/2008 (CLP Regulation) with the index number 601-074-00-2.

A dossier evaluation decision under REACH has been performed in 2016 (CCH-D-2114324394-53-01/F) regarding the name or other identifier of the substance. The deadline to provide this information was July 2016.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State 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	**X**

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 applicable

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

Table 2

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

Cassiffix was originally selected for substance evaluation to clarify concerns about:

- Suspected PBT/vPvB: after a SEV decision, no firm conclusion on the persistency of the constituents and impurities of Cassiffix can be drawn. However, the constituents and impurities are concluded to be not B and therefore, the substance is concluded to be not PBT/vPvB.

- Exposure of environment and high RCRs: information on tonnage data at the initial of the assessment (2016) was in the range of 10 – 100 tonnes/y. During the Substance Evaluation period, the annual tonnage significantly decreased to the current (2022) tonnage within the range of 1 to 10 tonnes/y[2]. Additionally, the manufacturing step was removed from the registered uses shortly after the start of the Substance Evaluation. Therefore, in this SEv Conclusion Document (2023), only emissions from formulation, use at industrial sites and uses by professional workers and consumer are included considering the last updates of the registration dossier. The scenario applied for the exposure assessment considers the EU registered tonnage. The significant decrease in the annual tonnage and the elimination of the

[2] Additional information on annual tonnage decrease can be consulted at the Confidential Annex.

manufacturing step have resulted in acceptable RCRs for the environment. However, some actions are needed by the registrants (see next section).

5.2. Other actions

- Regarding soil compartment:
    - o confirm that the sludge of the industrial and municipal STPs at the formulation and industrial uses, respectively, are managed appropriately and confirm that sludge is not applied as soil amendments,
    - o and/or decrease the tonnage used per site in formulation and at industrial sites to a maximum of acceptable RCR (see confidential annex),
    - o and/or refine the PNECsoil by providing additional information on long-term toxicity tests.
- Additionally, it must be considered that any increase in annual tonnage range needs to be revised since it will potentially result in RCRs above 1 for the aquatic compartment.

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

Not applicable

Table 3

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Not applicable		

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Cassiffix was originally selected for substance evaluation to clarify concerns about:

- Suspected PBT/vPvB
- Exposure of environment
- High RCR

During the evaluation no other concerns were identified.

Table 4. Evaluated endpoints related to initial concerns.

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Suspected PBT/vPvB	**No concern**. Based on the results of a recent OECD TG 305 study, the constituents of the Substance do not fulfil the B criterion. Thus, the Substance is not PBT/vPvB
Persistency	**Concern unresolved**. Based on the available data, a firm conclusion on the persistence of the constituents cannot be drawn. P/vP cannot be excluded.
Bioaccumulation	**No concern**. Based on the results of a recent OECD TG 305 study, the constituents of the Substance do not fulfil the B criterion.
Toxicity	**Concern unresolved**. There is no long-term toxicity data on fish or invertebrates.
RCRs for soil	**No concern**. RCR < 1
Exposure of environment	**No concern**. A decrease in the aggregated tonnage of above 50 % in the last years, from the range of 10-100 t/y to the range 1-10 t/y, results in acceptable RCRs. Additionally Manufacturing step has been removed. Only emissions from formulation, uses at industrial sites and uses by professional workers and consumers are included in the last updates of the registration dossier.

7.2. Procedure

The substance was notified to the Spanish CA and assessed under the NONS procedure. Additional non-standard information was needed to remove the following identified concerns: potential PBT/vPvB properties, high RCR for soil compartment and exposure of environment.

This information could not be requested via NONS decision. Therefore, the NONS procedure was closed, and the substance was included in the Community Rolling Action Plan (CoRAP) on 17 March 2015 to be evaluated in 2016 by Spain. On 22 March 2016 the updated CoRAP list was published on the ECHA website, and the evaluation was officially started.

According to the initial identified concerns, the scope of the evaluation is focused on environmental aspects. In addition, those human health endpoints that could be relevant for the PBT assessment have been reviewed.

On 7th February 2017, registrant provided data on log Kow (Unpublished, 2017), based on which the constituents of the substance screened B. Based on the evaluation of the available information, the eMSCA concluded that it was necessary to request new data.

On 12 April 2018 ECHA adopted a Substance Evaluation decision including a tiered testing strategy to clarify the PBT/vPvB concern. The first tier of testing was an OECD TG 309 test with the registered substance but determining the degradation half-lives for the relevant fractions of the substance. The following tiers included conditional requests for OECD TG 305, OECD TG 211, and OECD TG 210 tests.

The registrants submitted OECD TG 309 test on 29.05.2020. The eMSCA concluded that the concern on P could not be excluded. Therefore, following the conditional testing strategy included in the SEV decision, an OECD TG 305 test was triggered.

The registrants provided the requested OECD TG 305 test on 31 March 2022.

7.3. Identity of the substance

Table 5

SUBSTANCE IDENTITY	
Public name:	a mixture of: 4-(2,2,3-trimethylcyclopent-3-en-1-yl)-1-methyl-2-oxabicyclo[2.2.2]octane; 1-(2,2,3-trimethylcyclopent-3-en-1-yl)-5-methyl-6-oxabicyclo[3.2.1]octane; spiro[cyclohex-3-en-1-yl-[(4,5,6,6a-tetrahydro-3,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]furan]; spiro[cyclohex-3-en-1-yl-[4,5,6,6a-tetrahydro-4,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]furan]
EC number:	422-040-1
CAS number:	426218-78-2
Index number in Annex VI of the CLP Regulation:	601-074-00-2
Molecular formula:	C16H26O
Molecular weight range:	234.377
Synonyms:	Cassiffix 3-cyclohexene-1-methanol,3(or 4)-methyl-1-(2,2,3-trimethyl-3-cyclopenten-1-yl)-,acid-isomerized Reaction mass of (1R)-5-methyl-1-[(1R)-2,2,3-trimethylcyclopent-3-en-1-yl]-6-oxabicyclo[3.2.1]octane and (1S)-3,6',6',6a'-tetramethyl-4',5',6',6a'-tetrahydro-3a'H-spiro[cyclohex-3-ene-1,3'-cyclopenta[b]furan] and (1S)-5-methyl-1-[(1R)-2,2,3-trimethylcyclopent-3-en-1-yl]-6-

	oxabicyclo[3.2.1]octane and 4-methyl-1-[(1R)-2,2,3-trimethylcyclopent-3-en-1-yl]-2-oxabicyclo[2.2.2]octane Reaction mass of: 4-(2,2,3-trimethylcyclopent-3-en-1-yl)-1-methyl-2-oxabicyclo[2.2.2]octane 1-(2,2,3-trimethylcyclopent-3-en-1-yl)-5-methyl-6-oxabicyclo[3.2.1]octane spiro[cyclohex-3-en-1-yl-[(4,5,6,6a-tetrahydro-3,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]furan] spiro[cyclohex-3-en-1-yl-[4,5,6,6a-tetrahydro-4,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]furan]

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

Structural formula: See structural formulas of the main constituents below.

The name Cassiffix is used for the substance throughout this report.

Degree of purity: ≥ 85.0 - ≤100.0 (w/w)

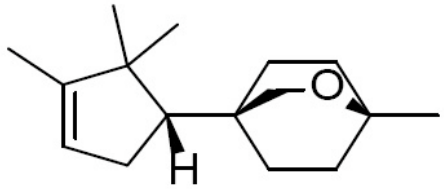
Multi-constituent / UVCB substance / others

The registered substance is a multi-constituent substance consisting of four main constituents (presented in Tables 6-9) and several impurities (further information in the confidential Annex). Being either different enantiomers or other closely related isomers of the same structures, they are all very similar with each other.

Table 6

Constituent	
Public name:	(1S,4s)-1-methyl-4-[(R)-2,2,3-trimethylcyclopent-3-en-1-yl]-2-oxabicyclo[2.2.2]octane
EC number:	-
CAS number:	139539-67-6
Index number in Annex VI of the CLP Regulation:	-
Smiles:	CC3=CC[C@@H]([C@]12CCC(C)(CC1)OC2)C3(C)C
Molecular formula:	C16H26O
Molecular weight range:	234.38
Synonyms:	

Structural formula:



SID is not clear as this constituent is not reported in the EC name nor in the earlier NONS notification of Cassiffix. Instead, a constituent 4-(2,2,3-trimethylcyclopent-3-en-1-yl)-1-methyl-2-oxabicyclo[2.2.2]octane is reported in these as well as referred to in many endpoints in the CSR. But as the two constituents are very similar, this uncertainty does not affect the conclusions of the current assessment.

Table 7

Constituent	
Public name:	(1R, 5S)-5-methyl-1-((R)-2,2,3-trimethylcyclopent-3-en-1-yl)-6-oxabicyclo[3.2.1]octane
EC number:	-
CAS number:	139539-66-5
Index number in Annex VI of the CLP Regulation:	-
Smiles:	CC3=CC[C@H]([C@]12CCCC(C)(C1)OC2)C3(C)C
Molecular formula:	C16H26O
Molecular weight range:	234.38

Structural formula:

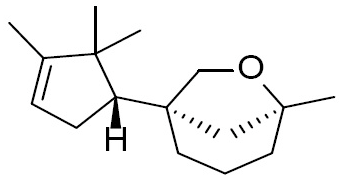


Table 8

Constituent	
Public name:	(1S,5R)-5-methyl-1-[((R)-2,2,3-trimethylcyclopent-3-en-1-yl]-6-oxabicyclo[3.2.1]octane
EC number:	-
CAS number:	139539-66-5
Index number in Annex VI of the CLP Regulation:	-
Smiles:	CC3=CC[C@H]([C@@]12CCCC(C)(C1)OC2)C3(C)C

Molecular formula:	C16H26O
Molecular weight range:	234.38

Structural formula:

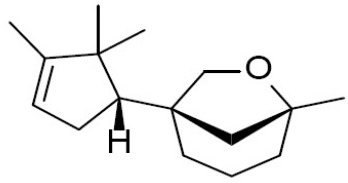
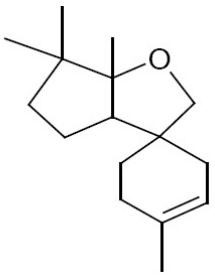


Table 9

Constituent	
Public name:	4,6',6',6a'-tetramethyl-4',5',6',6a'-tetrahydro-3a'H-spiro[cyclohex-3-ene-1,3'-cyclopenta[b]furan]
EC number:	-
CAS number:	142169-44-6
Index number in Annex VI of the CLP Regulation:	-
Smiles:	CC1=CCC3(CC1)COC2(C)C3CCC2(C)C
Molecular formula:	C16H26O
Molecular weight range:	234.38

Structural formula:



7.4. Physico-chemical properties

The available information on the physico-chemical properties of the registered substance is summarised below.

Table 10

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	liquid
Vapour pressure	1.5 Pa at 25°C (OECD TG 104)
Water solubility	11.1 mg/L at 20°C (OECD TG 105) For the constituents and impurities of the substance, the WSKOW v1.42 QSAR model predicts a water solubility of 6.39 and 3.37mg/L when the lowest (4.71) and highest (5.01) measured log Kow values (and the melting point of -25°C) are used as input. WATERNT v1.01 QSAR model predicts water solubility of 4.30 mg/L for the main constituents and most of the impurities, and 6.79 mg/L for two of the impurities.
Surface tension	54.7 mN/m at 19.5°C (OECD TG 115 and EU Method A.5) The surface tension for a 90% saturated aqueous solution of Cassiffix is 54.7 mN/m at 19.5°C. This is just below 60 mN/m which is the criterion for surface active substances according to this EU method. This could affect the interpretation of some endpoints, e.g., bioaccumulation, log Kow, log Koc. However, as the surface tension of Cassiffix is close to the threshold value, any effect is expected to be low.
Partition coefficient n-octanol/water (Log Kow)	4.72 at 25°C (OECD TG 123) Based on the results of the study, the weighted average log Kow of the whole substance is 4.79 (at 25°C) (Unpublished, 2017). At least seven peaks were identified in the chromatograms of the study and the log Kow values determined for these peaks ranged from 4.71 to 5.01. The registrant has used the formula from the EPISuite WSKOWWIN QSAR to back-calculate the log Kow from the measured WS (Log WS (mol/l) = 0.796 - 0.854 Log Kow - 0.00728 MW (+ Correction factor)). This resulted in log Kow of 4. EpiSuite KOWWIN QSAR model using the smiles of the main constituents predicts a log Kow of 5.70.
Flammability	Non flammable (EU Methods A.12 and A.13)
Explosive properties	Non explosive (EU Method A.14)
Oxidising properties	Waived. The substance does not possess oxygen or halogen atoms that are chemically bound to nitrogen or oxygen atoms.

7.5. Manufacture and uses

7.5.1. Quantities

Table 11

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

7.5.2. Overview of uses

Table 12

USES	
	Use(s)
Uses as intermediate	-
Formulation	Formulation of fragrance compounds and end-products
Uses at industrial sites	Industrial end-use of washing and cleaning products
Uses by professional workers	Professional end-use of polishes, wax blends, cleaning and washing products
Consumer Uses	Consumer end-use of polishes, wax blends, cleaning and washing products, air care products, cosmetics, and biocides
Article service life	-

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 13

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
601-074-00-2	reaction mass of 4-(2,2,3-trimethylcyclopent-3-en-1-yl)-1-methyl-2-oxabicyclo [2.2.2]octane 1-(2,2,3-trimethylcyclopent-3-en-1-yl)-5-methyl-6-oxabicyclo[3.2.1]octane	422-040-1	-	Skin Irrit. 2 Eye Irrit. 2 Aquatic Chronic 2	H315 H319 H411	-	-

	spiro[cyclohex-3-en-1-yl-[(4,5,6,6a-tetrahydro-3,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]furan] spiro[cyclohex-3-en-1-yl-[4,5,6,6a-tetrahydro-4,6',6',6'a-tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]furan]						

7.6.2. Self-classification

- In the registration(s):

Same as the harmonised classification.

- 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

Table 14

OVERVIEW OF AVAILABLE DEGRADATION STUDIES		
Method	**Results**	**Remarks**
EU Method C.7 (Degradation: Abiotic Degradation: Hydrolysis as a Function of pH); equivalent or similar to OECD Guideline 111 (Hydrolysis as a Function of pH)	Half-life (DT50): At pH 4: 710 h at 25°C At pH 7: 520 h at 25°C At pH 9: 830 h at 25°C At pH 7 the data points did not fall on a straight line indicating that the hydrolysis reaction was not a pseudo-first order reaction. At pH 4 and 9 the data points were inconclusive. Transformation products: not specified.	1 (reliable without restriction) key study experimental study **Test material** A mixture of: 4-(2,2,3-trimethylcyclopent- 3-en-1-yl)-1-methyl- 2-oxabicyclo[2.2.2]oct ane; 1-(2,2,3- trimethylcyclopent- 3-en-1-yl)-5-methyl- 6-oxabicyclo[3.2.1]oct ane; spiro[cyclohex- 3-en-1-yl-[(4,5,6,6a- tetrahydro-3,6',6',6'a- tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]fu ran]; spiro[cyclohex-3- en-1-yl-[4,5,6,6a- tetrahydro-4,6',6',6'a- tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]f uran]

		Form: Colourless liquid
OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test); EU Method C.4-E (Determination of the Ready Biodegradability - Closed Bottle Test)	% Degradation of test substance: 3 after 28d (% of ThOD N03)	1 (reliable without restriction) key study experimental study **Test material** A mixture of: 4-(2,2,3-trimethylcyclopent- 3-en-1-yl)-1-methyl- 2-oxabicyclo[2.2.2]oct ane; 1-(2,2,3- trimethylcyclopent- 3-en-1-yl)-5-methyl- 6-oxabicyclo[3.2.1]oct ane; spiro[cyclohex- 3-en-1-yl-[(4,5,6,6a- tetrahydro-3,6',6',6'a- tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]fu ran]; spiro[cyclohex-3- en-1-yl-[4,5,6,6a- tetrahydro-4,6',6',6'a- tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]f uran] Form: Colourless liquid
OECD Guideline 309 (Aerobic Mineralisation in Surface Water - Simulation Biodegradation Test)	High volatilisation of the test substance occurred during the test. The reported DT50 values are not representative of DegT50 of the main constituents as significant dissipation through volatilisation occurred. Some of the volatilisation corrected DegT50 are above 40 days, although there is high uncertainty in these values. See Section 7.7.1.2.1.3 for further information.	2 (reliable with restrictions) key study experimental study **Test material** A mixture of: 4-(2,2,3-trimethylcyclopent- 3-en-1-yl)-1-methyl- 2-oxabicyclo[2.2.2]oct ane; 1-(2,2,3- trimethylcyclopent- 3-en-1-yl)-5-methyl- 6-oxabicyclo[3.2.1]oct ane; spiro[cyclohex- 3-en-1-yl-[(4,5,6,6a- tetrahydro-3,6',6',6'a- tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]fu ran]; spiro[cyclohex-3- en-1-yl-[4,5,6,6a- tetrahydro-4,6',6',6'a- tetramethyl)-1,3'(3'aH)-[2H]cyclopenta[b]]f uran] Form: Colourless liquid

7.7.1.1. **Abiotic degradation**

7.7.1.1.1. Hydrolysis

A study on the hydrolysis of Cassiffix in accordance with OECD TG 111 is available. The hydrolysis did not show a first order kinetic and therefore the DT50s were read from the graphs. The DT50 at pH 4, 7 and 9 were 710 hours (30 days), 520 hours (22 days) and 830 hours (35 days), respectively, at 25°C. Volatilisation cannot be fully excluded because the Henry's Law constant is circa 30 Pa m^3/mol and some volatilisation from water can therefore be expected.

7.7.1.1.2. Phototransformation / photolysis

Based on EPIWIN AOPWIN, the half-lives in air for the reaction with OH-radicals and ozone are 1.18 or 3.72 hours, respectively, for the main constituents of Cassiffix. This half-life is < 2 days, which is the cut-off for potential long-range transport (http://www.unece.org/fileadmin/DAM/env/documents/2000/ece/eb/ece%20eb%20air.60.e.pdf). The substance does not have an ozone depletion potential because it does not contain halogens and does not have the potential to reach the stratosphere.

No information available on phototransformation in water or soil.

7.7.1.2. **Biodegradation**

7.7.1.2.1. Biodegradation in water

7.7.1.2.1.1. Estimated data

Estimations for persistency have been carried out (EPISuite BIOWIN - v4.10) for the main constituents and impurities of Cassiffix.

According to the REACH Guidance R.11: PBT/vPvB assessment, the output of the models BIOWIN 2, BIOWIN 3 and BIOWIN 6 of the software BIOWIN can be used to give a screening assessment of persistence. The following outcome indicates that a substance may be persistent: BIOWIN 2 <0.5 and BIOWIN 3 <2.2 or BIOWIN 6 <0.5 and BIOWIN 3 <2.2. According to similar structures, EPISuite BIOWIN v4.10 models for the main constituents and impurities of Cassiffix result in the following predictions:

BIOWIN 2 = 0.0001

BIOWIN 3 = 2.0362

BIOWIN 6 =0.1474 - 0.1950

Based on the above BIOWIN predictions, the constituents, and impurities of Cassiffix are considered to fulfil the screening criteria for P and vP.

7.7.1.2.1.2. Screening tests

The ready biodegradability test of Cassiffix was conducted in accordance with OECD TG 301D (Closed Bottle test). Sealed bottles containing the test substance (adsorbed onto glass filter paper) and inorganic nutrient medium were inoculated with activated sewage sludge bacteria and incubated for up to 28 days at 20 ± 1° C. Percentage biodegradation values were determined by comparing the extent of oxygen depletion with the Theoretical Oxygen Demand (6.02 mg O2/l). Only 3% of the test substance was biodegraded after 28 days.

Hence, the substance is considered not readily biodegradable. The substance was not found to be inhibitory to activated sewage sludge bacteria under the conditions of this test.

7.7.1.2.1.3. Simulation tests (water and sediments)

In May 2020 the registrants submitted an Aerobic Mineralization test in surface water according to OECD TG 309. The simulation study included a mass-balance calculation and was performed at 12°C for 59 days, using radio-labelled test substance. A degradation product TP-1 was detected in surface water under aerobic conditions.

However, although registrants were requested to determine degradation half-lives, they have only provided dissipation DT50 values that are influenced by high volatilization observed in the study. The eMSCA has recalculated the degradation DT50s for the four main constituents of the parent substance. Correction procedures to account for dissipation by volatilization have been applied according to Appendix 11 of FOCUS guidance and approaches found in published literature.

Test item

^{14}C-labelled Cassiffix, consisting of four constituents. The four major constituents of the Substance were regarded as parent compounds. The radiochemical purity of the spike solution on the day of spiking was 93.14%, based on four main constituents as determined by LC. The four constituents, numbered as 1,2,3 and 4, were present at 38.46 %, 9.57 %, 24.24 %, and 20.87 %, respectively. Other Cassiffix constituents or impurities remained below the limit of quantification. There is no information to allow the identification of the constituents used in the simulation test.

Test conditions

During the total equilibration and incubation period, the temperature was within the range 12.0 – 12.7 °C. During incubation, the pH ranged between 8.0 and 8.3 (mean 8.2) indicating stable slightly alkaline conditions. The dissolved oxygen concentration in the water layer fluctuated between 7.8 and 8.8 mg/L (mean 8.1 mg/L) indicating aerobic conditions.

Ten flasks were prepared including a sterile control. Two initial measured concentrations of Cassiffix were spiked to water phase: 20 µg/L (low measured concentration 20.2 µg/L) and 100 µg/L (high measured concentration 96.8 µg/L). Spike solutions for the low and high concentration were prepared by dissolving radiolabelled Cassiffix in acetonitrile. Benzoic acid (50 µg/L) was used as a reference control to assess microbial viability. The reference spiked solution was prepared by diluting a solution of 14C-labelled benzoic acid in ethanol with acetonitrile. The spike solutions were prepared on the day of spiking. The radiochemical purity was determined by LC on the same day.

Immediately after spiking, the metabolism flasks were placed in a climate room in the dark followed by 59 days of incubation. Volatiles, test item and/or transformation products, were trapped by polyurethane foam (PUF), ethylene glycol monoethyl ether (EGME) and NaOH traps.

During incubation aeration took place continuously. The ingoing air was allowed to flow above the surface water. Dissolved oxygen and pH were determined every week in the water of an untreated flask. Samples were taken on day 0, 1, 3, 7, 14, 28, 42 and 59 after spiking.

Analysis

The water surface was directly analysed by Liquid Scintillation Counting (LSC). Concentrated extracts of surface water and PUF traps were analysed by LSC and Liquid Chromatography (LC). The limit of detection in water was in range 2.5-3.2% of applied radioactivity and the limit of quantification in water was in the range 2.9-3.7% of applied radioactivity.

The mass balance consisted of volatiles (PUF + EGME + NaOH) + water layer (DCM fraction + water residue) + flushed flasks.

The “water layer” was extracted two times with dichloromethane (DCM). The DCM extracts were combined, weighed and total radioactivity was determined in a 200µL weighed aliquot. After addition of 200 µL keeper solution (1% glycerol in acetone), the DCM extract was evaporated on a rotary evaporator (40°C). The residue was dissolved in 1 mL acetonitrile, radioactivity was determined in a 200 µL weighed aliquot. Radioactivity in the residual aqueous phase after extraction with DCM was determined by LSC in a 1 mL weighed aliquot. Although a keeper solution was added this process, which includes high temperature, could also contribute to the volatilization out of the system.

To measure residual radioactivity, flasks were rinsed with 5 ml of acetonitrile, radioactivity was determined by LSC in a 1 mL weighed aliquot. If radioactivity was less than 5% LC analysis was not performed.

At Days 28, 42 and 59 (non-sterile) water residue was concentrated by evaporation of water on a rotary evaporator (40°C). Radioactivity was determined in a 200 µL weighed aliquot and 50 µL was analysed on LC in case the recovered activity was >5% of applied activity.

Radioactivity from polyurethane foam (PUF) plugs were extracted with 50 mL acetonitrile. After addition of 200 µL keeper solution (1% glycerol in acetone), the acetonitrile extract was evaporated on a rotary evaporator (40°C). The residue was dissolved in 1 mL acetonitrile, radioactivity was determined in a 100 µL weighed aliquot and 50 µL was analysed on LC. If the radioactivity was less than 5% LC analysis was not performed.

Validity of the test

A decrease in total amount of radioactivity was observed for the reference control. Measurements with the reference control showed significant degradation of sodium benzoate within 14 days in one of the replicates, which is in line with the validity criterion (see Table 15). However, in the other replicate with the reference substance there was a lag phase until day 7 and only from day 14 onwards degradation was observed and the $^{14}CO2$ reached only 23 % of AR on day 28. Furthermore, the radioactivity measured on day 28 in the water phase in that replicate is high (76%) indicating that low degradation had occurred.

Table 15. Recoveries of applied radioactivity in the reference control

Distribution of Radioactivity in Test System with Reference Control (% of Applied Radioactivity)

Time (days)	Flasks	NaOH	Water layer	Summed
1	20/21	0.0 / 0.5	na	0 / 1
3	20/21	0.2 / 18.0	na	0 / 19
7	20/21	0.9 / 28.1	na	1 / 47
14	20/21	18.7 / 12.7	na	20 / 59
28	20/21	3.6 / 11.1	na	23 / 70
	20/21	3.3 / 0.1	76 / 7.9	34 / 78

na: not applicable

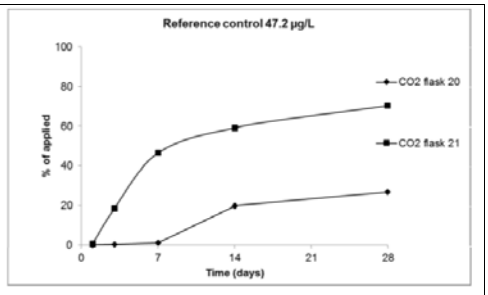


Figure 4
CO_2 Production in Test System Spiked with Reference Control

There are some deviations from the OECD guidance 309 that make the interpretation of the results difficult. According to OECD TG 309, Henry’s law constants less than about 1 Pa m^3 /mol can be regarded as non-volatile in practice. For substances with higher HLC biometer type flasks or closed flasks with a headspace are recommended to reduce volatilization of test substance. In the case of Cassiffix, based on the HLC of 14 Pa·m^3/mol

at 12°C, calculated by EUSES, the substance should be considered slightly volatile, and therefore, measures to reduce volatilization should have been applied.

In the test vessels with Cassiffix, the mass balances during the second half of the whole study (from day 28) are below the recommendation of 90-100% for radiolabelled test item. Based on the radioactivity measured in the PUF traps, volatilization is a major route of dissipation for Cassiffix. Potential volatilization out of the system could be related with the incomplete mass balance.

Mass balance in the sterile controls at both concentrations was 69.8 % after 59 days of incubation. Together, these results would indicate that the presence of volatile constituents or volatile transformation products leads to losses from the test system, which are only partially trapped by the PUF traps.

Results

- Mass balance

The recoveries of the applied radioactivity in the different experimental parts are shown in Table 16 and Table 17 (low and high concentration, respectively) and in Table 15 (reference control).

Table 16. Recoveries of radioactivity in the Surface Water system at Low concentration

Low Concentration: Distribution of Radioactivity in SW Water System

Time [days]	Percentage of applied radioactivity [%]							Mass Balance[1]
	Volatiles			Water layer				
	PUF	EGME	NaOH	DCM fraction	Concentrated water extract	Water residue	Flush Container	
1	11.3	0.1	0.0	85.2	82.4	1.6	0.1	98
3	17.5	0.0	0.0	75.1	74.6	1.3	0.3	94
7	35.4	0.0	0.1	35.9[2]	30.7	1.2	0.0	73
14	29.5	0.0	0.0	65.0	55.4	4.9	1.3	101
28	28.8	0.0	0.3	31.7	29.3	19.7	2.0	82
42	34.9	0.0	2.4	27.0	28.5	20.7	1.0	86
59	37.5	0.0	0.4	27.8	28.9	22.4	0.2	88
59 (sterile)	43.5	0.0	0.0	24.9	26.6	0.4	0.9	70

PUF: polyurethane foam trap; EGME: Ethylene glycol mono methyl ether trap; NaOH: Sodium hydroxide traps
[1] Mass balance = PUF + EGME + NaOH + DCM fraction + water residue + flush container
[2] Part of the sample was lost during processing

Table 17. Recoveries of radioactivity in the Surface Water system at High concentration

High Concentration: Distribution of Radioactivity in SW Water System

Time [days]	Percentage of applied radioactivity [%]							Mass Balance[1]
	Volatiles			Water layer				
	PUF	EGME	NaOH	DCM fraction	Concentrated water extract	Water Residue	Flush container	
1	10.2	0.0	0.0	82.7	81.5	6.5	0.0	99
3	31.2	0.0	0.0	61.9	62.2	1.7	0.0	95
7	17.7	0.0	0.0	75.5	68.8	2.1	0.0	95
14	33.1	0.0	0.0	52.8	49.3	3.5	0.6	90
28	36.4	0.0	0.1	35.7	32.2	14.2	0.4	87
42	37.9	0.0	0.1	32.2	30.8	10.3	0.1	80
59	42.3	0.0	0.1	15.5	15.6	14.6	0.2	73
59 (sterile)	55.0	0.0	0.0	13.4	13.4	0.9	0.4	70

PUF: polyurethane foam trap; EGME: Ethylene glycol mono methyl ether trap; NaOH: Sodium hydroxide traps
[1] Mass balance = PUF + EGME + NaOH + DCM fraction + water residue + flush container
[2] Part of the sample was lost during processing

Mass balance was calculated by summation of the recovered radioactivity in the various fractions and reported as % of the applied radioactivity: PUF + EGME + NaOH + DCM fraction + water residue + flushed flasks.

At the low test concentration, the mass balance was not constant and decreased over time (98.3 % on Day 1, 88.4 % on Day 59), with one outlier after 7 days of incubation (72.6 %) (see figures below). Similarly, at the high-test concentration, the mass balance also seems to decrease over time (99.4 % on Day 1, 72.5 % on Day 59). As indicated in the footnote in Table 15, part of the sample of low concentration collected at day 7 in the water layer was lost during processing with DCM. This results in a high decrease in the radioactivity of this sample resulting in a mass balance of 73% for this point. Therefore, this sample, has been considered an outlier and has not been considered in the kinetic analysis made by the eMSCA.

- Volatilised radioactivity

Radioactivity measured in the PUF traps can be considered as radioactivity dissipated into the atmospheric compartment. As can be seen in Figure 1, significant amounts of radioactivity were detected in the PUF traps. No radioactivity was found in the EGME traps, indicating that volatile components potentially escaping the PUF traps were not trapped by EGME.

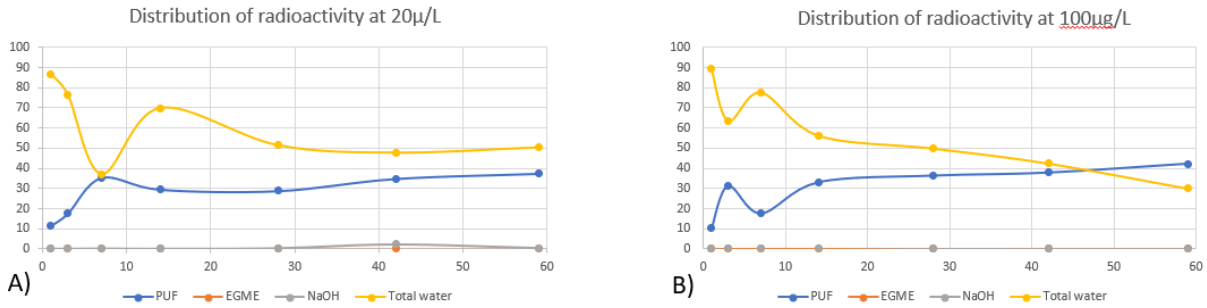


Figure 1. Distribution of radioactivity in the test system at low (A) and high (B)concentrations.

In the NaOH traps connected to the test systems negligible percentage of applied radioactivity (<0.1 %) was recovered in both, low and high concentrations, with the exception of the low concentration on Day 42 (2.4 %) (see Table 16). This means that mineralisation was negligible.

The results of the PUF traps in Table 16 and Table 17 indicate that at least 37.5 % (low concentration) to 42.3 % (high concentration) dissipates from the aqueous phase through volatilization. The incomplete mass balance suggests that dissipation through volatilization could be even higher. The constituents volatilised from both low and high concentrations very quickly during the first 7 days, and after that the concentrations in the PUF traps remained relatively constant until the end of the study (Figure 2).

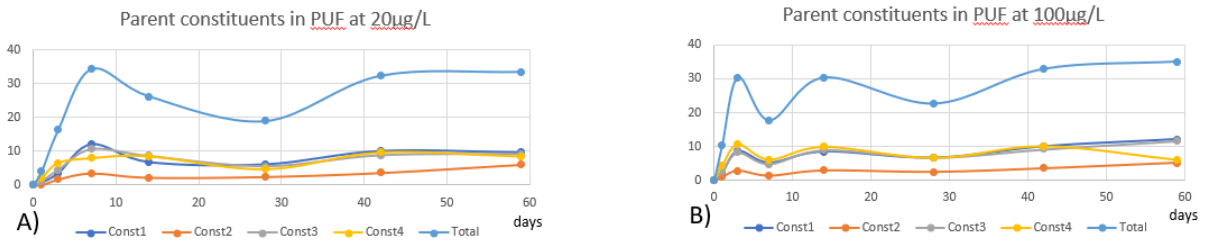


Figure 2. Distribution of parent constituents' radioactivity in PUFs at low (A) and high (B) concentrations.

Additionally, in the sterile controls, approximately 25% (low concentration) and 14% (high concentration) of applied radioactivity was recovered in the water phase and 41% and 54 %, respectively, in the PUF traps after 59 days (Table 18). The concentrations of the four main constituents were low on the last day of the study (day 59), especially in the high concentration treatments, the concentrations in the sterile controls decreased to very low levels similar as in the active bottle. Also, the concentrations measured in the PUF traps were similar between the sterile controls and active bottles on the day 59. In the sterile controls no metabolites were detected, and hence, the disappearance of the constituents from the water phase was not due to degradation. This indicates that the volatilisation was very high during the study, and in case of some of the constituents almost all the amount present in the water phase at the beginning of the test may have volatilised.

Table 18. % of applied radioactivity of parent constituents in sterile controls.

Time (days)	Sample	Constituent 1	Constituent 2	Constituent 3	Constituent 4	TP-1
Low concentration						
59	Water	13.0	2.1	4.5	1.7	-
59	PUF	13.9	3.4	12.0	11.8	-
High concentration						
59	Water	3.3	0.0	0.8	0.5	-
59	PUF	22.0	5.6	13.3	9.4	-

- : Not detected

- Water phase radioactivity

Radioactivity of parent constituents in water phase at different times is shown in

Table 19. As can be seen also in Figure 3, all the constituents disappeared very quickly from water. At the low concentration, the amount of radioactivity in the surface water decreased to 51.4% by day 28 and then remained stable towards the day 59. Regarding the high concentration, radioactivity in surface water decreased to 49.9% of AR after 28 days and decreased to 30.1% at day 59. However, the decreasing mass balance suggests that not all the volatilised radioactivity was registered in PUFs and that potential volatilization out of the system occurred.

Table 19. Radioactivity of Parent constituents in aqueous phase at low and high concentration.

Parent Constituents in Aqueous Phase at Low Test Concentration (% of Applied Radioactivity)

Time (days)	Constituent 1	Constituent 2	Constituent 3	Constituent 4
0	38.69	9.29	23.21	21.08
1	36.29	8.37	20.33	13.04
3	35.20	7.91	18.83	12.68
7	16.17	3.59	7.77	1.94
14	22.74	5.06	10.66	2.62
28	6.06	3.33	4.73	3.11
42	3.56	0.00	2.95	2.18
59	6.77	0.00	3.19	1.00

Parent Constituents in Aqueous Phase at High Test Concentration (% of Applied Radioactivity)

Time (days)	Constituent 1	Constituent 2	Constituent 3	Constituent 4
0	38.69	9.29	23.21	21.08
1	34.20	8.46	19.61	13.58
3	29.32	6.36	13.41	7.43
7	23.99	7.23	17.87	17.70
14	23.36	4.88	11.16	4.41
28	8.78	2.42	5.85	1.88
42	11.56	2.93	5.14	1.51
59	1.40	0.00	0.69	0.00

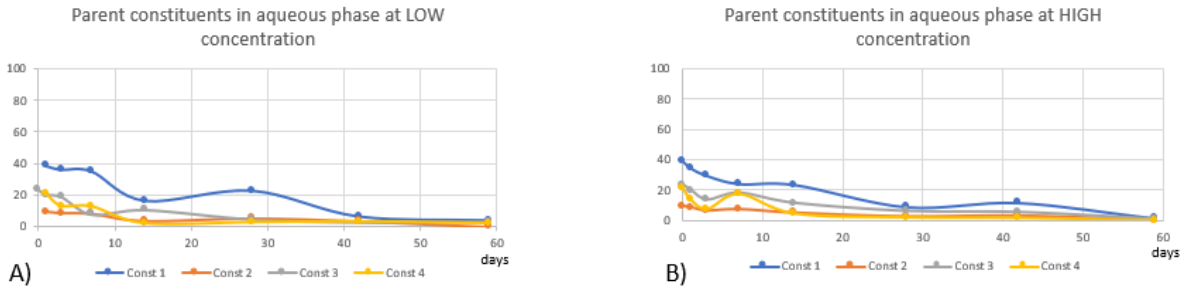


Figure 3. Radioactivity of parent constituents at low (A) and high (B) concentrations.

- Transformation products

One major transformation product (TP-1), a Cassiffix-lactone, was detected in the aqueous phase above 10%, at 14.9% and 13.4% of applied radioactivity in the low and high concentration, respectively (see

Table 20 and Table 21). It appeared after 28 days of incubation in the high concentration and at day 59 in the low concentration system. It was not detected in the PUF traps.

TP-1 was not detected in the sterile controls after 59 days (see above Table 18) which confirms that it is a biodegradation product.

TP-1 is most likely the result of oxidation to ultimately form a ketone in combination with a reduction. The most likely chemical structure is represented in Figure 4 (see the Confidential Annex), however, it is not possible to determine the exact position of the reduction. The Log Kow of the TP-1 was estimated to be between 2.5 and 3.

(As this structure is not publicly available at the ECHA´s dissemination website it has been included in the Confidential annex)

Figure 4. Structure of degradation product (TP-1) proposed by registrants.

The difference in the day of appearance in the low and high concentrations (day 59 and day 28, respectively) could be explained by the decrease of test concentrations due to volatilization. It could be that the remaining concentration of the parent substance in the aqueous phase, especially in the low concentration, was so low that TP-1 was not formed at detectable levels until day 59.

Table 20. Parent and transformation products in aqueous system at low concentration.

Low Concentration: Parent and Transformation Products in SW Water (% of Applied Radioactivity)

Time [days]	Water		PUF		Water residue, concentrated		
	Parent	Others	Parent	Others	Parent	TP-1	Others
1	78.0	4.4	4.2	6.9	-	-	-
3	74.6	0.0	16.5	0.0	-	-	-
7	29.5	1.2	34.3	0.7	-	-	-
14	41.1	14.3	26.2	1.1	-	-	-
28	17.2	12.0	18.9	2.1	0.0	-	0.0
42	8.7	19.8	32.4	1.5	0.0	-	13.5
59	11.0	18.0	33.7	2.3	0.0	14.9	6.9
59 (sterile)	21.3	5.3	41.1	0.0	-	-	-

Table 21. Parent and transformation products in aqueous system at high concentration

High Concentration: Parent and Transformation Products in SW Water (% of Applied Radioactivity)

Time [days]	Water		PUF		Water residue, concentrated		
	Parent	Others	Parent	Others	Parent	TP-1	Others
1	75.9	5.6	10.4	0.0	-	-	-
3	56.5	5.7	30.3	0.0	-	-	-
7	66.8	2.0	17.7	0.5	-	-	-
14	43.8	5.5	30.3	0.3	-	-	-
28	18.9	13.3	22.7	4.1	0.0	9.4	4.4
42	21.1	9.7	32.9	2.7	0.0	9.6	0.6
59	2.1	13.5	35.0	6.0	0.0	13.4	1.1
59 (sterile)	4.5	8.9	50.4	3.7	-	-	-

LC analysis of concentrated water residue reflect TP-1, with a retention time of 4.20 minutes. No other major transformation products exceeding 10 % were formed. Analysis of the NaOH traps indicated minimal conversion of Cassiffix constituents to CO2. No Cassiffix or transformation products were found in the EGME traps.

It is also observed that other components appeared in the mass balance (Table 20 and Table 21). The fraction 'others' contains peaks with an RT in the range of approximately 3.50 min - 5.50 min and a second range around 26.50 min - 29.50 min. These peaks also appeared in the measurement of test item solution, in a comparable absolute value (Figure 5). These signals were regarded by registrants as non-volatile minor constituent of the test item. However, there is some indication that some metabolites could also be included in the "others" fraction. It can be observed in Table 20 and Table 21 an increase with time of the fraction "others" in PUFs which would indicate generation during the test. Especially the "others" detected in the "water residue, concentrated" samples are likely to be metabolites as they only appear in the later part of the test and are not detected in the sterile controls. In the "water" fraction, which refers to the DCM extract, "others" were present already from the beginning of the test, and they were also observed in sterile controls at the end of the study, and hence, these are likely to be minor constituents. However, their concentration also increases during the test which could indicate formation of some transformation products. Additionally, this signal does not appear so clear in the sterile controls (Figure 6).

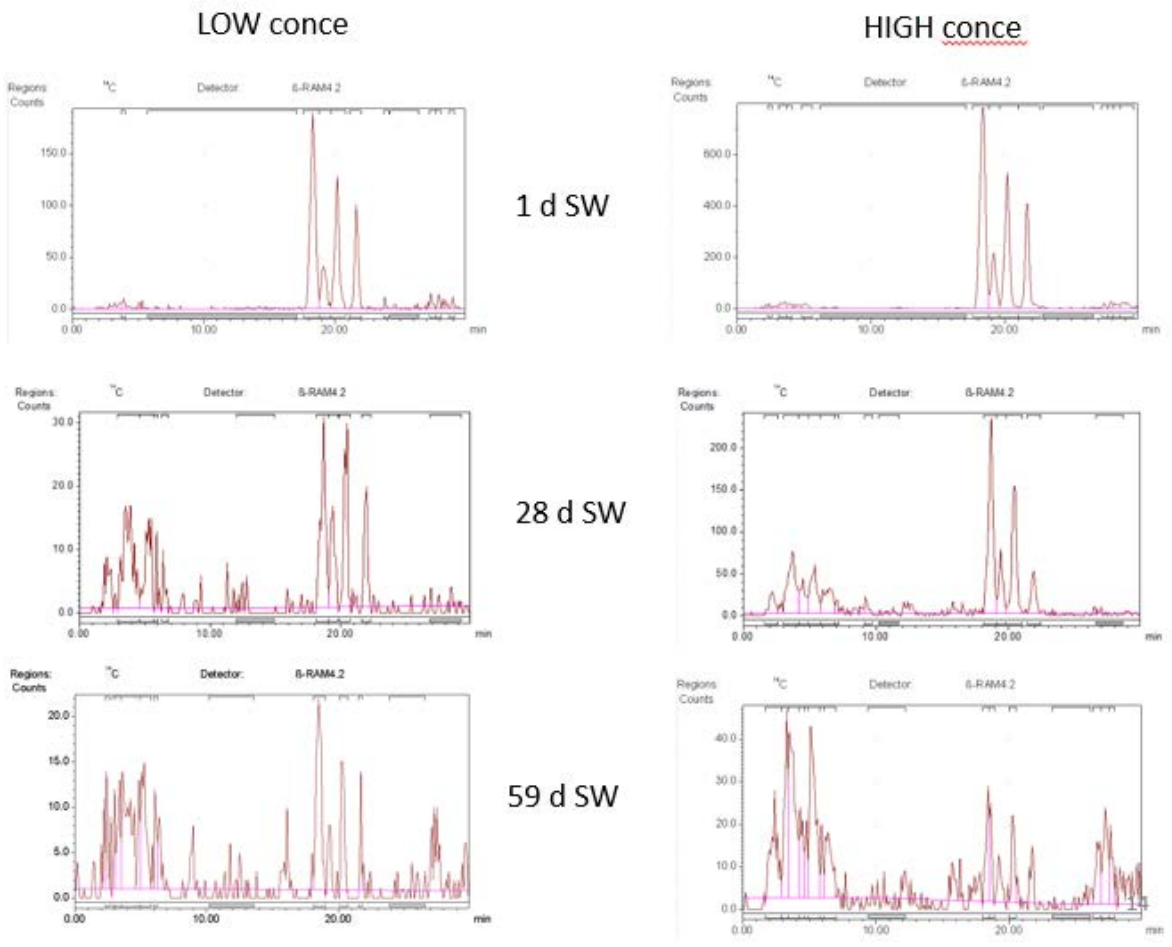
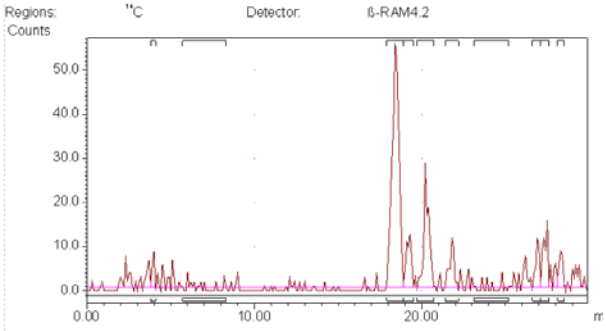
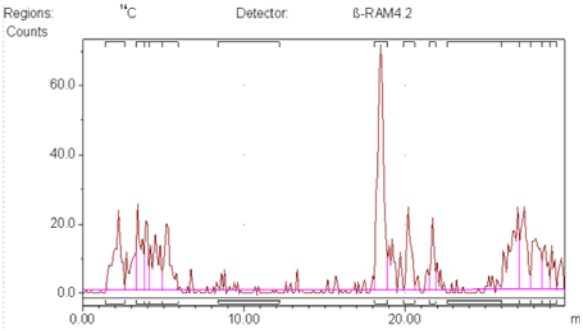


Figure 5. Evolution of "Other" products with time in surface water.



LC Chromatogram SW Water Low Concentration Sterile 59 Days of Incubation



LC Chromatogram SW Water High Concentration Sterile 59 Days of Incubation

Figure 6. LC chromatograms of sterile controls at low and high concentration.

- DT50deg

DT50s presented for the four main constituents by the registrants (Table 22) correspond to dissipation and are highly influenced by volatilization.

Table 22. DT50s and DT90s estimated by registrants.

DT_{50} and DT_{90} of Cassiffix in Surface Water (Aqueous Phase)

Compartment	Initial test concentration	Kinetics	DT_{50} (days)	DT_{90} (days)
Parent – low concentration				
Constituent 1	20 µg/L	SFO	12	41
Constituent 2	20 µg/L	SFO	13	42
Constituent 3	20 µg/L	DFOP	6.6	61
Constituent 4	20 µg/L	HS	2.9	41
Parent – high concentration				
Constituent 1	100 µg/L	SFO	18	58
Constituent 2	100 µg/L	SFO	18	59
Constituent 3	100 µg/L	SFO	17	55
Constituent 4	100 µg/L	SFO	10	34

In conclusion, the DT_{50} of Cassiffix in water is <20 days, using the conservative value of Constituent 1 and 2 at 100 µg/L.

Considering the observed volatilization, the eMSCA includes a tentative calculation of the degradation DT50 for each constituent including a volatilization correction. Therefore, degradation kinetics were re-modelled considering the volatilisation.

Appendix 11 of the FOCUS Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (2014) includes correction procedures to account for dissipation by volatilisation in the kinetic analysis.

According to Appendix 11 *"the correction procedures are most straightforwardly derived by considering a parent compound that is subject to an overall rate of loss from the water-sediment system by degradation and volatilisation, and that each loss process is described by SFO kinetics. In this case, there are three SFO rate constants that can be used to describe different aspects of the loss process: kTOT, kVOL and kDEG for the total overall loss from the water-sediment system, and the losses by volatilisation and by degradation, respectively. Assuming that:*

$k_{TOT} = k_{VOL} + k_{DEG}$ (Equation 1)

then k_{DEG} can be estimated simply from the difference between these two parameters by re-arranging the above equation."

According to the discussion document "ECHA note: Options to assess persistence of volatile substances in regulatory PBT assessment" (ECHA, 2022), the approach indicated in the FOCUS Guidance to estimate kvol is incorrect and may lead to underestimation of kdeg. Therefore, this approach is not used for Cassiffix. Other two approaches were found in the open literature. These approaches are also based on the above rationale presented in the FOCUS Guidance Appendix 11, and assume that the data follow first order kinetics, but the modelling of the parameters is done in a different way than in the FOCUS Guidance. Hence, the eMSCA used two different approaches to obtain an estimate of the volatilisation corrected kDEG and DegT50.

A) Modelling of k_{TOT} and k_{VOL} simultaneously using ModelMaker following the approach used in Jene B. 2007b, in RIVM (2018)
B) Modelling of k_{TOT} and k_{VOL} simultaneously using CAKE following Shrestha et al (2019)

The above correction approaches found in the open literature were used for water-sediment and soil studies but as they refer to processes in the total system, the same correction procedures can be applied to systems including only the water phase, too.

More information on the approaches and fits obtained with the approaches can be found in Annex 1.

Before performing the kinetic analyses, the FOCUS Guidance (2014) recommendations for handling measurements below the limit of quantification (LOQ) or the limit of detection (LOD) were applied to the data. Furthermore, for the low concentration treatment, the data of the water phase on day 7 was removed as an outlier from the analyses as part of the water sample was lost during the processing and the mass balance was low (72.6 %) on that day.

In both volatilisation correction approaches presented above the DT50 values estimated for the total dissipation of the constituents from the water phase are very similar (see Table 23). This could be expected because the water phase data, except for constituent 4, fit well to the SFO kinetics.

The volatilisation corrected DegT50 values calculated for the constituents differ significantly between the two approaches (Table 23). The DegT50 values estimated for the constituent 2 are consistently above 40 days while for the other constituents some of the values are above 40 days and others below 40 days.

In the study with Cassiffix, the PUF traps data had a poor fit to SFO kinetics, and hence, the volatilisation corrections based on SFO kinetics have high uncertainty. Based on the concentrations measured in the PUF traps, the volatilisation was high during the first days of the study and after that remained relatively stable. However, it is noted that the mass balance decreased towards the end of the study (being 88% and 73 % of AR on day 59 at low and high concentration treatments, respectively) which could indicate that part of the volatilised constituents (or transformation products) was not captured by the PUF traps and were lost from the system. The low mass balances (70%) observed in the sterile controls support this. Hence, the volatilisation was very high during the study, and in case of some of the constituents almost all the amount present in the water phase at the beginning of the test may have volatilised. This makes the estimation of reliable DegT50 very difficult or impossible as the constituents were not available for the degrading microorganisms in water.

Table 23 Summary of the DegT50 and $DT50_{TOT}$ values calculated using both approaches (A-B) presented in this document. The values above the P-criterion (40 days) are highlighted in red and the values close to the criterion in orange. The values in yellow are considered highly uncertain because the model predicted that all or almost all parent constituent volatilised and consequently estimation of degradation rate is not possible.

Constituent	DegT50 (days)		$DT50_{TOT}$ (days)	
	Approach A	Approach B	Approach A	Approach B
HIGH Concentration				
C1	27.9	39.61	17.4	17.9
C2	52.5	189.32	19.0	20.2
C3	38.1	273.62	15.3	16.8
C4	14.8	>10000	7.0	7.75
LOW Concentration				
C1	19.5	29.28	13.7	14.2
C2	49.9	50.44	18.3	18.4
C3	21.2	>10000	11.3	13.7
C4	5.6	5.33	3.2	2.96

Considering that the constituents are structurally related isomers, relatively similar degradation behaviour could be expected. Since some of the four main constituents had low initial concentrations and high volatilisation leading to high uncertainty in the data, the eMSCA performed the DT50 calculations and volatilisation correction also on data considering the four main constituents as one. Hence, the concentrations of the four constituents in water and in PUFs were summed up and the total concentrations were used in the kinetic analysis. For the volatilisation correction of the total parent data, only the approach B (Shrestha et al 2019 approach) was applied. This resulted in a volatilisation corrected DegT50 value of 38 days for the low concentration treatment and 112 days for the high concentration treatment.

In order to further estimate the extent of primary degradation, the eMSCA made some additional calculations considering a "best case scenario" for degradation where the "others" fraction found in water residue samples as well as part of the "others" fraction found in the Water (DCM) sample are considered as transformation products (see Table 20 and Table 21). The "others" fraction found in PUFs are not considered transformation products as they could be minor constituents since they are detected in the PUF already on day 1 in the low concentration and are also observed in one of the sterile controls.

The following scenarios are assumed for the whole substance (best case considering higher percentage of metabolites):

- Based on the PUF data, the substance has high and rapid volatilisation in the test, ca. 30% of the AR already during the first week of the study. So, during the test only the remaining ca. 70% of Cassiffix could be considered as Measurable Radioactivity in water (MR).
- Cassiffix goes to primary degradation up to a maximum of 35.4 % AR in the LOW treatment after 59d (14.9% TP-1 + 6.9 % "others" fraction in Water residue + 13.6[3] % "others" fraction in Water). That would mean that 50.6 % of the "Measurable radioactivity in water" has been degraded after 59 days.
- Similarly, primary degradation up to 22.4 % AR in the HIGH treatment after 59d (13.4% TP-1 + 1.1 % "others" fraction in Water residue + 7.9 % "others" fraction in Water). This would correspond to 32 % of the Measurable radioactivity in water.
- It is noted that these calculations (Figure 7) can overestimate the primary degradation as it is not fully clear whether or to what extent the "others" fraction observed in the water sample are transformation products or minor constituents. On the other hand, based on the decreasing mass balance, the volatilisation was higher than that measured in the PUF traps. Consequently, the amount of the test substance available for microorganisms in water was probably lower than 70 % AR for most part of the test. This could potentially lead to underestimation of the primary degradation.

[3] From Table 14 (LOW), %AR measured on day 59 - % AR measured on day 1 (18.0-4.4 = 13.6)

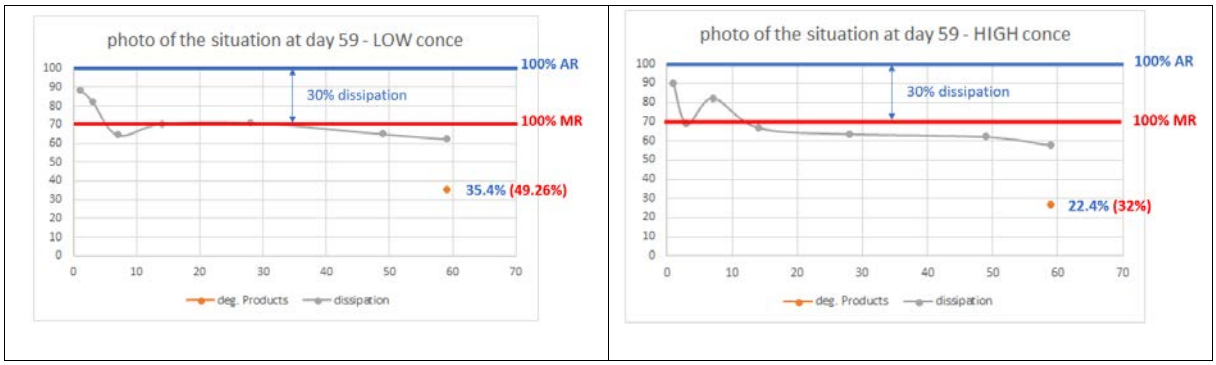


Figure 7 Maximum degradation at LOW and HIGH concentrations considering all potential degradation products (TP-1 plus "others" fraction) in water phase at 100% AR and a maximum of 70% MR during the test. MR – real Measurable Radioactivity.

Based on the results presented in this document, some of the constituents have DegT50s > 40 days when applying volatilisation correction according to the different approaches presented in this document. However, there is high uncertainty in the calculations of the volatilisation correction. The volatilisation was high and fast during the first days of the study, and hence a significant part of the test substance was not bioavailable to the microoganisms. Additionally, kVOL estimations result in low reliability due to the poor fit of the PUF data to SFO kinetics, and because part of the volatilised constituents was probably not captured by the PUF traps.

Based on the formation of one major metabolite and possibly some other minor metabolites, some primary degradation of the test substance occurred during the study. The mineralisation was negligible. Due to the high volatilisation, the DT50 values calculated for the dissipation from water cannot be used for comparison with the P/vP criteria. Some of the volatilisation corrected DegT50 are above 40 days, although there is high uncertainty in these values.

Therefore, the eMSCA thinks that based on the results of the study, although some primary degradation occurs, it cannot be excluded that some of the constituents of the substance could be P/vP. Consequently, based on the available information, it is not possible to conclude that the substance is not P.

7.7.1.2.2. Biodegradation in soil

No relevant information available.

7.7.1.3. **Summary and discussion on degradation**

Hydrolytic half-lives of Cassiffix at pH 4, 7 and 9 were 30, 22 and 35 days at 25 °C, respectively, in a study according to OECD TG 111. There is no information on the degradation products.

In a ready biodegradability study according to OECD TG 301D, 3% biodegradation of Cassiffix was observed after 28 days. Furthermore, EPISuite BIOWIN models predict that the constituents and impurities of the substance are not readily biodegradable.

Based on the available information and predictions, Cassiffix as the whole substance as well as its constituents and impurities fulfil the screening criteria for persistent (P) and very persistent (vP) substances.

In the OECD TG 309 study performed following the Substance Evaluation Decision, high volatilisation of the test substance and decreasing mass balance was observed which made the estimation of reliable DegT50 values difficult. Based on the formation of one major metabolite and possibly some other minor metabolites, some primary degradation of the

test substance occurred during the study. The mineralisation was negligible. Due to the high volatilisation, the DT50 values calculated for the dissipation from water cannot be used for comparison with the P/vP criteria. Some of the volatilisation corrected DegT50 are above 40 days, although there is high uncertainty in these values.

Therefore, the eMSCA thinks that based on the results of the study, although some primary degradation occurs, it cannot be excluded that some of the constituents of the substance could be P/vP. Consequently, based on the available information, it is not possible to conclude that the substance is not P.

7.7.2. Environmental distribution

7.7.2.1. **Adsorption/desorption**

The log Koc of Cassiffix was determined using the HPLC method according to OECD TG 121 for each of the four main constituents. The log Koc were determined as: 4.02, 4.12, 4.19. 4.25. The weighted average value of all 4 constituents (5, 55, 20 and 20%, respectively) were used to derive the log Koc for Cassiffix of 4.2.

In the registration dossier the Koc is calculated using the QSAR model “predominantly hydrophobics” (Koc = 1.26 x Kow^0.81) from EUSES. Using the latest measured log Kow of 4.72 as input (Unpublished, 2017), the model results in a Koc of 8 386 and log Koc of 3.92 at 20 °C.

EPISuite KOCWIN v.2.00 using the smiles of the main constituents and the measured log Kow of 4.72 as input predicts the following values: Koc 2 788 L/kg and log Koc 3.45 L/kg.

7.7.2.2. **Volatilisation**

The Henry’s law constant is calculated using the equation from EUSES. Using a molecular weight of 234 g/mol, and the experimentally determined vapour pressure of 1.5 Pa (at 25 °C) and water solubility of 11.1 mg/L (at 20 °C) the Henry’s law constant at 25 °C and 12 °C is calculated to be 29.5 and 14 Pa m^3/mol, respectively. Hence, some volatilisation from water can be expected.

7.7.2.3. **Distribution modelling**

Based on Level III distribution modelling in EPISUITE (assuming equal and continuous releases to air, water and soil) using the smiles of the main constituents and the measured physico-chemical parameters of Cassiffix as input, it is estimated that the majority of the substance released to the environment will partition into soil (88%) with smaller amounts to water (8%) and sediment (4%), and only very small amounts will partition to air (< 0.1%).

The registrant has used The SimpleTreat model, which is incorporated in EUSES, to simulate the distribution of the substance in a Sewage Treatment Plant based on vapour pressure, water solubility, log Kow of 4.72 and biodegradability. The model predicts that 0 % of the substance will biodegrade, 60% will partition to water, 25% to sewage sludge and 15% to air.

The EPISuite STP Fugacity Model using the smiles of the main constituents and the measured physico-chemical parameters as input predicts that 66 % will partition to sludge, 0.49 % to air, 34 % to effluent water and < 1 % is biodegraded.

7.7.3. Bioaccumulation

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

An OECD 305 aqueous exposure test (GLP compliant) was performed for Cassiffix in accordance with the Substance evaluation decision adopted in 2018. Common carp (*Cyprinus carpio*) were exposed to the test substance under flow-through conditions for 28 days uptake phase, followed by a 7-day depuration phase. It is noted that in some parts of the full study report, a duration of 4 days is incorrectly indicated for the depuration phase. According to the registrant, this incorrect duration was indicated by the study authors because the sampling was done only in the last 4 days of the depuration phase (Days 32-35 of the study). However, the fish were transferred into clean water without the test substance after the last day of the uptake phase, and hence, Day 29 was the first day of the depuration phase. According to OECD TG 305, the depuration phase should start immediately after the end of the uptake phase.

Only one test concentration (20 µg/L) was used in the test as in the preliminary test conducted at two test concentrations of 2 and 20 µg/L no concentration dependence of the BCF was observed. The nominal exposure concentrations of the four main constituents in the definitive test were 6.478, 3.540, 2.088 and 1.494 µg/L for Constituents 1, 2, 3 and 4 (see Figure 8 in the Confidential Annex), respectively.

N,N-dimethylformamide (DMF) was used as solvent in the preparation of the stock solution. The stock solution was prepared by dissolving 800 mg of test sample in 1L of DMF. The solvent (without the test substance) was also included in the test water of the control group during the uptake phase.

The concentration of the solvent in the final test solution is not clearly indicated in the study report. According to the full study report, flow rates of 0.04 ml/min and 1600 mL/min were used for the stock solution (pure solvent in case of control vessels) and the dilution water, respectively. Therefore, the eMSCA estimated a final concentration of DMF of 23.75 mg/L (density of DMF of 0.95 g/ml) in the test solution. This concentration is well below the 100 mg/L indicated as the maximum concentration for solvents in the OECD 305. However, the guideline also states that the concentration of solvent should not exceed the corresponding toxicity thresholds determined for the solvent under the test conditions. According to the OECD guidance document nº23 (2019), the concentration of the solvent in the final test medium should be at least one order of magnitude below the appropriate no-observed effect concentration (NOEC) depending on the test species and the length/type of toxicity test or in any case below 100 mg/l (or 0.1ml/l).

At the ECHA dissemination website the available information on long-term toxicity of DMF (EC 200-679-5) to fish include an OECD TG 204 test and a two-generation study with two fish species performed according to US EPA guidelines in the 1980s. In the OECD TG 204 study with *Oryzias latipes* a 21d-NOEC of >102 mg/L is reported for growth as no effects were observed. It is noted that according to ECHA Guidance 7b, OECD TG 204 test is not considered suitable for studying chronic effects as the test is done with adult fish, and hence, sensitive life cycle forms are not tested. In the two-generation test with *Salvelinus fontinalis*, DMF had no effect on the F0-generation at the tested concentrations (up to 98.2 mg/L) but the concentration of 42.8 mg/L and higher resulted in reduced survival of the F1-generation. The maximal acceptable toxic concentration (MATC) was reported to be 42.8 - 98.2 mg/L. MATC is the geometric mean of the NOEC and the LOEC. In the two-generation test with *Pimephales promelas*, a MATC between 5 to 11 mg/L is reported. In

the F1 generation the fish sizes became reduced, depending on the DMF concentrations after a 1-month exposure period and mortality increased after 2 months exposure. Based on the ECHA dissemination site, the registrants of DMF state that the two-generation study is only available as draft with hand-written corrections, and that the report provided limited information which was not sufficient to evaluate the study. Furthermore, it is mentioned that according to information from US-EPA the study was never finalised.

Hence, based on the results of the two-generation tests, the concentration of the solvent DMF in the OECD TG 305 study could have been above or close to its NOEC value in fish. However, as the validity of the two-generation studies is not verified, there is some uncertainty regarding the NOECs. The eMSCA did not find further published information on chronic toxicity of DMF in fish. The mean lipid content of the control fish (exposed to DMF) decreased 20% during the uptake phase (see below), and this raises some concern on potential toxic effects of the solvent in the test fish.

(As these structures are not publicly available at the ECHA´s dissemination website, they have been included in the Confidential annex)

Figure 8 The structures of the four main constituents analysed in the OECD TG 305 study.

One-year-old common carp (weight 4.42-4.86 g, and length 7.1-9.2 cm at the start of the uptake phase) were used as test fish. The exposure group included 56 fish and the control group 26 fish. 70-L glass tanks were used as test vessels. Feeding (amount corresponding to 2 % of total body weight) of the fish was done daily. Before sampling the fish were starved for 24 hours. Excreta and dirt were removed from the test tanks approximately once a day.

Water temperature, pH, dissolved oxygen, total organic carbon, hardness of the water and flow rate were monitored during the test as indicated in the OECD TG 305. The temperature of the test water ranged from 24.2 to 25.8 °C and pH was 7.6-7.8. Concentration of dissolved oxygen was always above 60% saturation.

Test water of the exposure group was sampled before the start of the uptake phase and six times during the uptake phase. In the control group test water was analysed only at the end of the uptake phase. In the depuration phase, both exposure and control groups were sampled only once, at the same time as the first fish sampling, i.e., on 4th day of the depuration phase.

In the exposure group, fish were sampled five times during the uptake phase and four times during the depuration phase. In the control group fish were sampled only at the end of the uptake and depuration phases. Four individuals were sampled in each sampling event, and they were pooled into two groups of two fish as one fish was not enough for the analytical sensitivity of the test item (LOQ range 3.8-5.7 ng/g for the different peaks). In addition, at the end of the uptake phase, two fish from the exposure group were separated in edible and non-edible fractions and the test substance concentration was measured separately in these two fractions. The sampled fish were weighed before performing the analysis.

Test substance concentrations in water and fish were measured using GC-MS analysis. The concentrations of the four main constituents were determined based on the peaks corresponding to each constituent. The concentrations in water and fish measured with

GC-MS were corrected with the recovery rates determined for each constituent in the recovery test.

Fish lipid content was only measured from six control fish before and after the uptake phase as well as after the depuration phase. The mean measured lipid content was 3.92% at the start of the test, 3.12% at the end of the uptake phase and 3.05% at the end of the depuration phase. The average lipid content during the test was 3.36%. Hence, there was a reduction of 20 % in the mean lipid content during the uptake phase. As indicated above the final concentration of the solvent DMF may have been above its chronic toxicity value, and therefore, a potential effect of DMF in the lipid content reduction cannot be excluded. There was no marked difference between the lipid content at the end of the uptake phase (3.12%) and end of the depuration phase (3.05%) when the fish were not exposed to the solvent.

The average measured concentrations of the main constituents in water were 5.77, 3.10, 1.87 and 1.28 µg/L for Constituent 1, 2, 3 and 4, respectively. The concentrations remained stable and were within ± 20% of the mean of the measured values during the uptake phase.

According to Annex 2 of OECD TG 305 the maximum acceptable value for total organic carbon (TOC) of the dilution water is 2 mg/L. According to the study report the dilution water had TOC of 0.6 mg/L. However, before and during the uptake phase TOC was measured to be in the range of 12.3-13-1 mg/L both in the treatment and control vessels. In the depuration phase the TOC was 1.0 mg/L or lower. Hence, the higher TOC during the uptake phase is assumed to be due to the solvent used. The concentration of DMF in the test solution was 23.75 mg/L and this corresponds to a carbon content of 11.64 mg/L (based on the molecular weights of DMF and carbon of 73.9 g/mol and 12.01, respectively). This is in accordance with the indications of OECD TG 305 stating that throughout the test, the concentration of TOC in the test vessels should not exceed the concentration of organic carbon originating from the test substance, and solvent or solubilising agent if used, by more than 10 mg/L (± 20%).

Although, no mortality nor loss of fish body weight were observed during the test, uncertainty on the potential adverse effects of DMF on lipid content have been observed by the eMSCA. Nevertheless, as other validity criteria of the OECD TG 305 were fulfilled, and the possible effects of DMF cannot be confirmed, the study can be accepted and considered valid with restrictions.

For all four constituents, the mean concentrations in fish on days 18, 20, 26 and 28 of the uptake phases were within ±20 % of each other and there was no significant increase in the concentrations between the days 18 and 28. Therefore, it is considered that the steady state was reached for all constituents. The BCF values calculated for the pooled samples on the different sampling days of the uptake phase and the steady-state BCF (BCF_{SS}) and 5% lipid normalised BCF_{ss} are shown in the Table 24. The lipid normalisation was done based on the average lipid concentration at the end of the uptake (3.12%).

Table 24 BCF values (L/kg) calculated for the pooled samples on different sampling dates during the uptake phase, the steady state BCF (BCF_{ss}) and 5 % lipid normalised BCF_{ss}

Constituent	Day 11	Day 18	Day 20	Day 26	Day 28	BCF_{ss}	BCF_{ssL}
1	330 280	350 190	220 260	230 240	190 280	250	390
2	300 280	360 190	230 280	260 240	210 290	260	420
3	320 290	400 190	230 280	250 240	230 300	270	430
4	390 430	600 290	410 460	470 350	320 440	420	670

The two fish that were separated in fractions before analysis at the end of the uptake phase had mean BCF values in fillets of 190-210 L/kg for Constituents 1-3 and 260 L/kg for Constituent 4; and BCF values in viscera of 380-410 L/kg for Constituents 1-3 and 540 L/kg for Constituent 4.

In the study report kinetic BCF values are also reported. The k_1 and k_2 values were estimated using a sequential method. A linear regression of ln(Cf) versus time was performed to calculate k_2. To calculate k_1, a computer program was used to fit the below equation.

$C_f(t) = \overline{C_w} \times k_1 / k_2 \times (1 - e^{-k2 \times t})$ Uptake phase (Day 0 to 28)

$C_f(t) = C_{0,d} \times e^{-k2 \times (t - tc)}$ Depuration phase (Day 32 to 35)

$C_f(t)$: Test item concentration in test fish at sampling time t (ng/g)

$\overline{C_w}$: Average test item concentration in test water during uptake phase (µg/L)

k_1 : Uptake rate constant (L/kg/day)

k_2 : Depuration rate constant (/day)

t : Sampling time (day)

$C_{0,d}$: Test item concentration in test fish at beginning of depuration phase (ng/g)

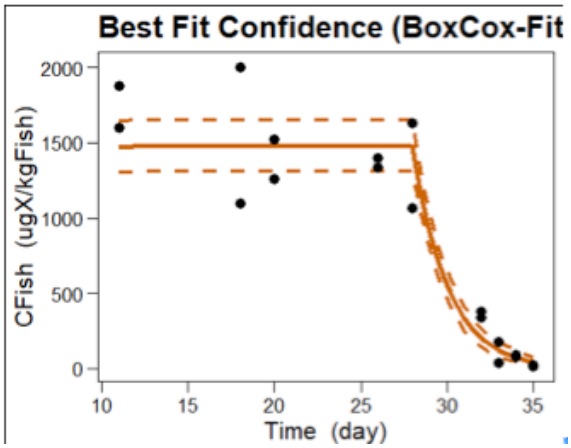
The eMSCA re-ran the kinetic analysis on the raw data available in the full study report using the bcmfR R-Package programme (Version 0.4-18). For constituent 3, one of the pooled samples on the last day of the depuration phase was below the LOQ (5.7 ng/g) and therefore it was removed from the analysis.

For constituents 1, 2 and 3 the results with the untransformed and Box-Cox transformed data were very similar. However, the residuals and Q-Q plot model diagnostics were slightly better for the Box-Cox transformed fit and hence that was selected as the best fit to calculate the results (Figure 9). The ln-transformed fit gave more weight to the last data points of the depuration phase than the Box-Cox and untransformed data fits. As sampling was done only in the last four days of the seven-day depuration phase, and the concentrations were quite low, the ln-transformed fit may overestimate the depuration rate.

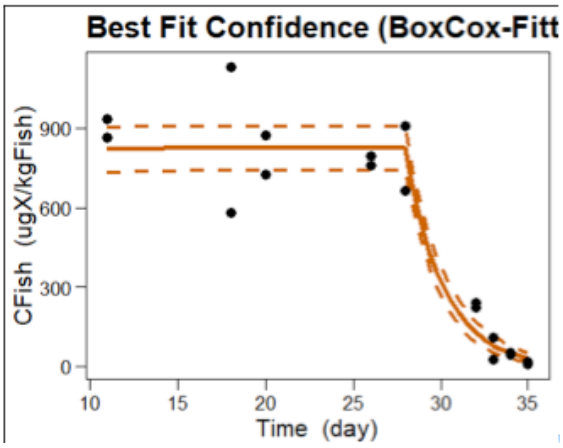
Also, for constituent 4 the untransformed data and Box-Cox transformed data gave very similar fits and results. However, in this case, the untransformed data seemed to give slightly better fit based on the model diagnostics and hence it was selected for the best fit (Figure 9).

See Annex 2 in this document for further information on the different fits and model diagnostics.

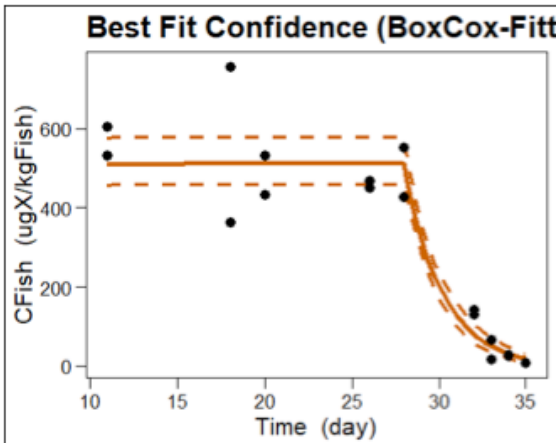
A) Constituent 1



B) Constituent 2



C) Constituent 3



D) Constituent 4

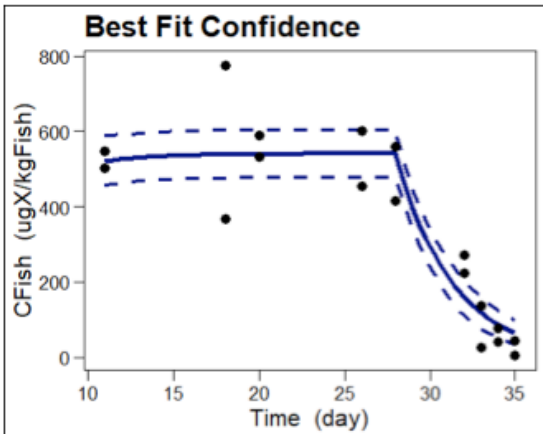


Figure 9 The measured concentrations in fish and the best fits (with 95% CI) for A) Constituent 1, B) Constituent 2, and C) Constituent 3 with Box-Cox transformed data (plotted on back-transformed normal scale), and for D) for Constituent 4 with untransformed data. See Annex 2 for further graphs of the fits.

It is noted that the growth rates calculated by the eMSCA differ from those indicated in the study report (see Table 25). The eMSCA calculated a k_g of 0.0118 day-1 based on the data on the fish weights of the treatment group during the whole study as there was no statistically significant difference ($p>0.05$ based on Student's T-test) between the k_g of the treatment group during the uptake and depuration phases. It is not known exactly why the growth rates reported in the study report are different, but based on the information in the full study report it may be that the study authors used only the fish weight data at the start and end of the uptake phase and at the end of the depuration phase. The eMSCA used the weight data collected at each sampling point.

Table 25 Growth rates, k_g, (day^{-1}) reported in the study report and calculated by the eMSCA.

	k_g, Study report		k_g, eMSCA	
	Treatment	Control	Treatment	Control
Uptake phase	0.0019	0.0117	0.0125	0.0138
Depuration phase	0.0236	0.0104	0.0187	0.0011
Whole study	Not reported	Not reported	0.0118	0.0118

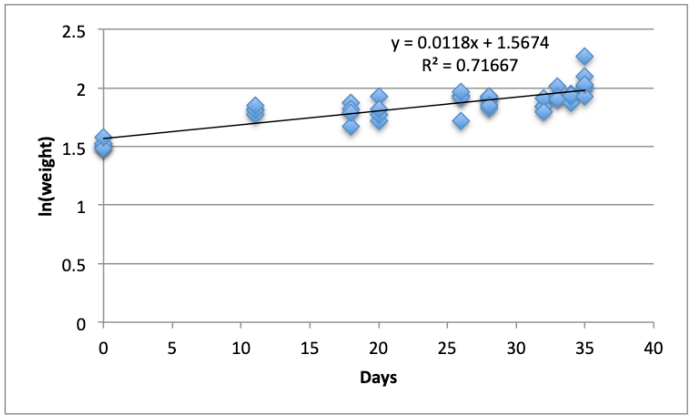


Figure 10 Natural logarithm of the fish weight data in the treatment group during the study and the fitted line to calculate the growth rate.

Also, the lipid content used for lipid normalisation of the kinetic BCF slightly differs between the registrant and the eMSCA. The registrant used the mean lipid content calculated for the whole study duration (3.36%) whereas the eMSCA used the mean lipid content at the end of the uptake phase, as recommended by the OECD TG 305 guideline since there was no marked difference between the lipid content at the end of the uptake phase (3.12%) and end of the depuration phase (3.05%).

The results of the kinetic analysis reported in the study report and re-calculated by the eMSCA are shown in Table 26 and Table 27, respectively. In the study report only lipid normalised BCF values are reported but no growth corrected k_2 or BCF values are calculated.

Table 26 Results of the kinetic analysis reported in the study report.

	Constituent 1	Constituent 2	Constituent 3	Constituent 4
K_1 (day^{-1})	231	243	230	351
K_2 (day^{-1})	0.899	0.916	0.838	0.842
BCF_k (L/kg)	260	270	270	420
BCF_{kL} (L/kg)	380	400	410	620
$t_{1/2}$ (days)	0.8	0.8	0.8	0.8

Table 27 Results of the kinetic analysis performed by the eMSCA.

	Constituent 1	Constituent 2	Constituent 3	Constituent 4
C_W (µg/L)	5.77	3.10	1.87	1.28
K_g (day^{-1})	0.0118			
K_1 (day^{-1}) (95%CI)	126.2 (93.9-158.5))	123.8 (92.6-155.0)	126.1 (92.9-159.2)	128.4 (75.7-181.1)
K_2 (day^{-1}) (95%CI)	0.493 (0.403-0.583)	0.466 (0.379-0.553)	0.462 (0.376-0.547)	0.304 (0.186-0.422)
K_{2g}(day^{-1})	0.481 (0.392-0.571)	0.454 (0.367-0.541)	0.450 (0.365-0.535)	0.292 (0.175-0.410)
BCF_k (L/kg) (95%CI)	256 (221-291)	266 (232-300)	273 (235-311)	422 (376-468)
BCF_{kLg} (L/kg) (95%CI)	420 (364-477)	437 (382-492)	449 (387-511)	704 (627-781)
$t_{1/2}$ growth corrected (days) (95%CI)	1.44 (1.17-1.71)	1.53 (1.24-1.82)	1.54 (1.25-1.83)	2.37 (1.41-3.33)

As can be seen in both the results calculated by the registrant and those by the eMSCA, the constituents 1, 2 and 3 have very similar growth corrected and/or lipid normalised BCF values around 400 L/kg. The constituent 4 has a bit higher BCF, around 600-700. The kinetic BCF values are very similar with the lipid-normalised steady-state BCF values, and hence, it seems that steady state was reached in the study for all constituents.

As there was no sampling of fish during the first days of the depuration phase, this adds some uncertainty to the k_2 estimation. However, as the BCF values are well below 2000 and the depuration was relatively fast, the kinetic results are considered acceptable. Furthermore, the lack of sampling at the first part of the depuration phase does not affect the steady state BCF values and it seems that steady state was reached.

Using the lowest log Kow of 4.71 measured in the OECD TG 123 study as input, the BCFBAF QSAR model predicts a BCF of 595 L/kg for the main constituents and impurities based on the regression method and BCF values in the range of 1439-1554 L/kg (upper, mid, and lower trophic level, including biotransformation rate estimations) based on the Arnot-Gobas BCF & BAF method. When using the highest measured log Kow of 5.01 as input, the regression-based model results in a BCF of 925 L/kg and the Arnot-Gobas BCF & BAF method results in BCF of 1918-2281 L/kg (upper, mid, and lower trophic level, including biotransformation rate estimations) for the main constituents and impurities.

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

No experimental data on bioaccumulation on terrestrial organisms is available.

According to the ECHA guidance R11, an efficiently absorbed, non-biotransformed neutral organic substance with a log Koa ≥ 5 in combination with a log Kow ≥ 2 has the potential to biomagnify in terrestrial food chains and air-breathing marine wildlife as well as in

humans. In an OECD TG 123 study, a weighted average log Kow of 4.72 was determined for the whole substance. Based on the available chromatograms in the study report, the constituents and impurities of the substance are expected to have log Kow values in the range of 4.71-5.01. The log Koa values of the constituents and impurities predicted by the KOAWIN (v1.10) QSAR model are 6.39 and 6.68 when the log Kow values of 4.71 and 5.01, respectively, are used as input. Hence, the measured log Kow and predicted log Koa values of the constituents and impurities of Cassiffix fulfill the criteria for potential accumulation in air-breathing organisms.

No experimental information on toxicokinetics in mammals is available. However, based on available toxicity studies in mammals and considering the physico-chemical properties, the constituents are readily taken up via inhalation, oral and dermal routes (see section 7.9.1). Based on the QSAR Toolbox predictions, metabolism in rats may occur in several positions of the constituents. OH groups may be attached to every methyl group and an acid may be formed. Also, the formation of an epoxide and a ketone group is predicted by the Toolbox. These metabolites are expected to be more water soluble, have a lower Log Kow value and will therefore be more easily excreted.

QSAR Toolbox profiling was also used for checking alerts on potential protein binding. The Toolbox found only one structural alert regarding moderate reactivity for potential covalent binding with the thiol group of gluthanione (GSH) for the main constituents 1, 2 and 3.

7.7.3.3. **Summary and discussion of bioaccumulation**

Based on the recently conducted OECD TG 123 study the log Kow of the constituents and impurities of Cassiffix are in the range of 4.71 to 5.01. Hence, the screening criteria for B/vB in aquatic organisms are met.

In the recent OECD TG 305 study, performed following a request in the Substance Evaluation decision, the growth-corrected and lipid normalised BCF_K values as well as the lipid normalised BCF_{SS} values of the four main constituents are around 400-700 L/kg. The study is valid and reliable for the assessment. Hence, the constituents are concluded to be not B/vB in aquatic organisms according to REACH Annex XIII.

Based on the predicted log Kow values and QSAR models on BCF values, it seems that there may be some differences in the bioaccumulation potential of the constituents and impurities. However, considering that all impurities and minor constituents are (or assumed to be) structurally very similar to the four main constituents, and since in the OECD TG 305 study all BCF values were well below 2000, it is expected that all constituents and impurities are also not-B in aquatic organisms.

Regarding potential accumulation in air-breathing organisms, the log Koa values of the constituents and impurities predicted by the KOAWIN (v1.10) QSAR model are 6.39 and 6.68 when the log Kow values of 4.71 and 5.01, respectively, are used as input. Hence, based on the log Kow and log Koa values, the constituents screen for potential accumulation in air-breathing organisms.

There is no experimental information on mammalian toxicokinetics. Based on available mammalian toxicity studies uptake via inhalation, oral and dermal routes is expected. Based on QSAR Toolbox predictions metabolism leading to more water-soluble metabolites may occur. The relatively rapid depuration observed in fish in the OECD TG 305 study (half-lives in the range of 1.4-2.4 days), suggests that the constituents are likely to be depurated relatively rapidly also in mammals, which usually have higher metabolic capacity than fish.

Therefore, although a firm conclusion cannot be drawn due to lack of experimental information in mammals, considering all available information, it is concluded that the constituents are not likely to be bioaccumulative in air-breathing organisms either.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

Available aquatic toxicity data on Cassiffix is presented in Table 12.

Table 12

OVERVIEW OF AVAILABLE AQUATIC TOXICITY STUDIES		
Test species and method	**Results**	**Remarks**
Oncorhynchus mykiss freshwater flow through OECD Guideline 203 (Fish, Acute Toxicity Test); EU Method C.1 (Acute Toxicity for Fish)	LC50 (96h): 3.8 mg/L test mat. (meas.) (95% CL 3.0-3.9 mg/l) based on mortality	1 (reliable without restriction) key study **Test material** A mixture of: 4-(2,2,3-trimethylcyclopent- 3-en-1-yl)-1-methyl- 2-oxabicyclo[2.2.2]oct ane; 1-(2,2,3- trimethylcyclopent- 3-en-1-yl)-5-methyl- 6-oxabicyclo[3.2.1]oct ane; spiro[cyclohex- 3-en-1-yl-[(4,5,6,6a- tetrahydro-3,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]furan]; spiro[cyclohex-3- en-1-yl-[4,5,6,6a- tetrahydro-4,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]]furan]
Daphnia magna freshwater static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test); EU Method C.2 (Acute Toxicity for Daphnia) GLP	EC50 (24h): 1.9 mg/L test mat. (meas.) (95% CL 1.5 - 2.5 mg/L) based on mortality EC50 (48h): 1.3 mg/L test mat. (meas.) (95% CL 1.0 - 1.6 mg/L) based on mortality	1 (reliable without restriction) key study **Test material** A mixture of: 4-(2,2,3-trimethylcyclopent- 3-en-1-yl)-1-methyl- 2-oxabicyclo[2.2.2]oct ane; 1-(2,2,3- trimethylcyclopent- 3-en-1-yl)-5-methyl- 6-oxabicyclo[3.2.1]oct ane; spiro[cyclohex- 3-en-1-yl-[(4,5,6,6a- tetrahydro-3,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]furan]; spiro[cyclohex-3- en-1-yl-[4,5,6,6a- tetrahydro-4,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]]furan]
Pseudokirchneriella subcapitata freshwater static	EbC50 (72h): 8.6 mg/L test mat. (meas.) based on biomass ErC50 (72h): 13 mg/L test mat. (meas.)	1 (reliable without restriction) key study **Test material**

OECD Guideline 201 (Alga, Growth Inhibition Test); according to EU Method C.3 (Algal Inhibition test)	based on growth rate NOErC (72h): 2.6 mg/L test mat. (meas.) based on growth rate	A mixture of: 4-(2,2,3-trimethylcyclopent- 3-en-1-yl)-1-methyl- 2-oxabicyclo[2.2.2]oct ane; 1-(2,2,3- trimethylcyclopent- 3-en-1-yl)-5-methyl- 6-oxabicyclo[3.2.1]oct ane; spiro[cyclohex- 3-en-1-yl-[(4,5,6,6a- tetrahydro-3,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]furan]; spiro[cyclohex-3- en-1-yl-[4,5,6,6a- tetrahydro-4,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]]furan]

7.8.1.1. **Fish**

Short-term toxicity to fish

A study on the acute toxicity of Cassiffix to rainbow trout (*Oncorhynchus mykiss*) conducted in accordance with OECD TG 203 is available. The test substance was dissolved in 10% Tween 80-acetone. Groups of ten juvenile fish were exposed to nominal concentrations of 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L of Cassiffix dissolved in water under flow-through conditions. Observations were made on the numbers of dead fish and the incidence of sub-lethal effects after 3, 6, 24-, 48-, 72- and 96-hours exposure. The test resulted in a 96h LC50 of 3.8 mg/L (95% confidence limit 3.0 – 4.9 mg/L), based on measured concentration. Measured concentrations ranged from 87 - 126% of nominal at 0 hours, 83 - 94% of nominal at 24 hours and 85 - 99% of nominal at 96 hours.

Long-term toxicity to fish

No experimental data on the long-term toxicity to fish is available.

The ECOSAR v1.11 QSAR model predicts a chronic fish toxicity value of 0.093 mg/L for the constituents and impurities of Cassiffix based on the lowest measured log Kow of 4.71. When using the highest measured log Kow of 5.01 as input, the model gives a chronic value of 0.052 mg/L for fish. The chronic values given by ECOSAR QSAR-model are geometric means of the predicted LOEC and NOEC, and hence, the predicted NOEC is lower than the given chronic value.

7.8.1.2. **Aquatic invertebrates**

Short-term toxicity to aquatic invertebrates

A study on the acute toxicity of Cassiffix to *Daphnia magna* conducted in accordance with OECD TG 202 is available. Groups of twenty, 1st instar daphnia (less than 24 hours old) were exposed for 48 hours to nine concentrations of the substance (nominal concentrations 0. 10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L) dispersed in test water under static conditions. The incidence of immobilisation was recorded for each test and control group at 24 hours and at 48 hours. The test resulted in a 48h EC50 value of 1.3 mg/L (95% confidence limit 1.0 – 1.6 mg/L) based on measured concentration (geometric mean of concentrations at 0 and 48 h). Measured concentrations ranged from 83 - 103 % of nominal at 0 hours and 69 - 111 % of nominal at 48 hours.

Long-term toxicity to aquatic invertebrates

No experimental data on the long-term toxicity to aquatic invertebrates is available.

The ECOSAR v1.11 QSAR model predicts a chronic daphnia toxicity value of 0.101 mg/L for the constituents and impurities of Cassiffix based on the lowest measured log Kow of 4.71. When using the highest measured log Kow of 5.01 as input, the model gives a chronic value of 0.060 mg/L for daphnia. As indicated above, the chronic values given by ECOSAR QSAR-model are geometric means of the predicted LOEC and NOEC, and hence, the predicted NOEC is lower than the given chronic value.

7.8.1.3. Algae and aquatic plants

A study on the toxicity of Cassiffix on the growth of the unicellular green alga *Selenastrum capricornutum* conducted in accordance with OECD TG 201 is available. Algal cultures exposed to 5 test concentrations of the substance (nominal concentrations: 3.125, 6.25, 12.5, 25 and 50 mg/L) plus one untreated control and one solvent control (100 µl auxiliary solvent per litre), were incubated on an orbital shaker under continuous illumination at 24 ± 1 QC for 72 hours. Growth was monitored daily by determining the cell density of each culture by direct counts. The following values were derived from the data: 72h EbC50 of 8.6 mg/L; 72h ErC50 of 13 mg/L and 72h NOErC of 2.6 mg/L. All results are based on measured concentrations (geometric mean of the concentrations at 0 and 72 h). The measured concentrations ranged from 77 - 91 % of nominal at 0 hours and 19 - 43 % of nominal at 72 hours.

The ECOSAR v1.11 QSAR model predicts a chronic algae toxicity value of 0.496 mg/L for the constituents and impurities of Cassiffix based on the lowest measured log Kow of 4.71. When using the highest measured log Kow of 5.01 as input, the model gives a chronic value of 0.327 mg/L for algae. As indicated in the previous paragraphs, the chronic values given by ECOSAR QSAR-model are geometric means of the predicted LOEC and NOEC, and hence, the predicted NOEC is lower than the given chronic value.

7.8.1.4. Sediment organisms

No relevant data available.

7.8.1.5. Other aquatic organisms

No relevant data available.

7.8.2. Terrestrial compartment

No relevant data available.

7.8.3. Microbiological activity in sewage treatment systems

Table 13

OVERVIEW OF AVAILABLE STUDIES ON TOXICITY TO MICROORGANISMS		
Test species and method	**Results**	**Remarks**
activated sludge of a predominantly domestic sewage freshwater static	NOEC (30min): 18 mg/L test mat. (nominal) EC50 (30min): >100 mg/L test mat. (nominal) based on inhibition of total respiration - respiration rate	1 (reliable without restriction) key study **Test material** A mixture of: 4-(2,2,3-trimethylcyclopent-3-en-1-yl)-1-methyl-2-

OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test	EC50 (3h): >100 mg/L test mat. (nominal) based on inhibition of total respiration - respiration rate	oxabicyclo[2.2.2]oct ane; 1-(2,2,3- trimethylcyclopent- 3-en-1-yl)-5-methyl- 6-oxabicyclo[3.2.1]oct ane; spiro[cyclohex- 3-en-1-yl-[(4,5,6,6a- tetrahydro-3,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]furan]; spiro[cyclohex-3- en-1-yl-[4,5,6,6a- tetrahydro-4,6',6',6'a- tetramethyl)-1,3'(3'aH)- [2H]cyclopenta[b]]furan]

A study on the inhibitory effect of Cassiffix on the respiration of activated sewage sludge in accordance with OECD TG 209 is available. Cultures of activated sewage sludge were incubated with synthetic sewage under vigorous aeration and in the presence of the test substance at nominal concentrations of 10, 18, 32, 56 and 100 mg/L. The respiration rates were measured electrochemically for each culture after 30 minutes and after 3 hours of aeration. Percentage inhibition of respiration was calculated for each culture by comparing oxygen depletion rates for the test substance with those for the negative control culture. A positive control (i.e., 3,5-dichlorophenol) was tested concurrently with the test substance to demonstrate the satisfactory performance of the procedure. The reported EC50 (respiration inhibition) values for the substance were > 100 mg/L for the 30-minute contact time and > 100 mg/L for the 3-hour contact time. At 30 min the NOEC was 18 mg/L. At 3 h the respiration inhibition at 10, 18 and 32 mg/l was 10, 12 and 1%, respectively. At 56 and 100 mg/L 17 and 16% respiration inhibition was seen, respectively. Thus, at the 3 h time point a clear dose effect relationship was not observed.

7.8.4. PNEC derivation and other hazard conclusions

The PNEC values calculated based on the available data are shown in Table 11. The values will be recalculated once the requested further ecotoxicity information is available.

Table 11

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks / Justification**
Freshwater	PNEC (freshwater): 0.0013 mg/L	Assessment factor: 1000
Marine water	PNEC (marine waters): 0.00013 mg/L	Assessment factor: 10000
Intermittent releases to water	not applicable	not applicable, no intermittent releases
Sediments (freshwater)	PNEC (sediment fw): 0.238 mg/kg sed ww	Extrapolation method, estimated by EUSES
Sediments (marine water)	PNEC (sediment marine) _ 0.024 mg/kg sed ww	Extrapolation method, estimated by EUSES
Sewage treatment plant	PNEC (STP): 1.8 mg/L	Assessment factor: 10

Soil	PNEC (soil) 0.193 mg/kg soil ww	Extrapolation method, estimated by EUSES
Air	not relevant for the environment	no oral nor dermal toxicity observed
Secondary poisoning	not relevant for the assessment	The substance is not bioaccumulative (BCF 700) it is expected not to bioamagnify

7.8.5. Conclusions for classification and labelling

The substance has a harmonised classification as Aquatic Chronic 2. The available information supports this classification.

7.9. Human Health hazard assessment

Human health endpoints were not evaluated. However, information relevant for the PBT assessment has been considered and is summarised below.

7.9.1. Toxicokinetics

There is no experimental data on the toxicokinetics of Cassiffix. However, some information can be inferred from other assays.

Adsorption

Adsorption through oral, dermal, and inhalation routes can be expected based on the effects observed in 28-day repeat oral dose (gavage) and oral (dietary) reproductive toxicity studies, classification as skin irritant, and physico-chemical properties (e.g., moderate water solubility and log Kow) of Cassiffix.

Based on this information, it can be concluded that the substance is likely to be orally absorbed > 50%; skin absorption is not expected to exceed 50% whereas it will be readily absorbed via the inhalation route close to 100%.

These results are in agreement with the log Kow suggesting that the substance would pass through the biological cell membrane.

Distribution and metabolism

Distribution: The moderate water solubility of the test substance would limit distribution in the body via the water channels. The log Kow would suggest that the substance would pass through the biological cell membrane. The log Kow of 4.72 indicates some bioaccumulation potential. However, due to the expected metabolisation the substance as such may have limited accumulation in the body fat.

Metabolism: In the registration dossier, the metabolisation of Cassiffix has been assessed using OECD Toolbox 3 liver metabolism simulator. According to the simulator predictions, OH-groups may be attached to every methyl group and an acid may be formed. Also, the formation of an epoxide and a ketone group is predicted by the Toolbox. These metabolites are expected to be more water soluble, have a lower Log Kow value and will therefore be more easily excreted.

The registrants also mention that, in the water simulation study (OECD TG 309) the metabolite Cassiffix-Lactone was found, which has an oxidised carbon next to the ether bond and an additional bond in the same ring. Because this is a simple oxidation (and reduction) such a product is also expected in mammalian systems. The lactone may be de-

esterified and turn into an acid or it may be reduced into an alcohol. Both are expected to be conjugated in the Phase 2 pathway.

7.9.2. Acute toxicity and Corrosion / Irritation

Not evaluated nor relevant for the assessment.

7.9.3. Sensitisation

Not evaluated nor relevant for the assessment.

7.9.4. Repeated dose toxicity

Not evaluated nor relevant for the assessment.

7.9.5. Mutagenicity

Not evaluated for the environmental assessment, but summarising the information provided in the registration dossier. Three studies on the mutagenicity of Cassiffix are available: *in vitro* gene mutation study in bacteria, *in vitro* mammalian chromosome aberration test and mammalian cell gene mutation assay. All studies were conducted according to relevant OECD guidelines and GLP. All tests gave a negative result for genotoxicity.

7.9.6. Carcinogenicity

Not evaluated for the environmental assessment. Summarising the information provided in the registration dossier and considering the information from studies assessing genotoxicity, no genotoxic carcinogenicity is expected, via oral, inhalation nor dermal route.

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

Not evaluated for the environmental assessment. Summarising the information provided in the registration dossier: there is one OECD TG 421 study available for the substance which resulted in no observed effects in the reproductive endpoints of rats at the tested concentrations of circa 70, 170 and 550 mg/kg bw. No adverse effects are observed in fertility nor developmental toxicity.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated nor relevant for this environmental assessment.

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

Not evaluated nor relevant for this environmental assessment.

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

Not evaluated nor relevant for this environmental assessment.

7.10. Assessment of endocrine disrupting (ED) properties

Not evaluated. According to available information endocrine disruption properties are not expected.

7.11. PBT and vPvB assessment

According to Annex XIII of REACH, the PBT assessment must take account of all relevant constituents of the substance. ECHA Guidance R.11 describes relevant constituents as all constituents, impurities and additives present in the substance at levels equal or above 0.1 % (w/w).

Cassiffix is a multi-constituent substance consisting of four main constituents and several impurities. The constituents and impurities are similar with each other as they are different enantiomers or other closely related isomers of the same structures. However, based on the log Kow values determined for the peaks identified in a chromatogram in the recent OECD TG 123 study, there seems to be some differences in the n-octanol affinity of the constituents and impurities of the substance. Consequently, also the PBT properties may differ, and this has been taken into consideration in the PBT assessment.

7.11.1. Persistence

Hydrolytic half-life of Cassiffix at pHs 4, 7 and 9 was 30, 22 and 35 days at 25 °C, respectively, in a study according to OECD TG 111. There is no information on the degradation products.

In a ready biodegradability study according to OECD TG 301D, 3% biodegradation of Cassiffix was observed after 28 days. Furthermore, EPISuite BIOWIN models predict that the constituents and impurities of the substance are not readily biodegradable. Thus, Cassiffix as well as its constituents and impurities fulfil the screening criteria for persistent (P) or very persistent (vP) substances.

In the OECD TG 309 study performed following the Substance Evaluation Decision, high volatilisation of the test substance and decreasing mass balance was observed which made the estimation of reliable DegT50 values difficult. Based on the formation of one major metabolite and possibly some other minor metabolites, some primary degradation of the test substance occurred during the study. The mineralisation was negligible. Due to the high volatilisation, the DT50 values calculated for the dissipation from water cannot be used for comparison with the P/vP criteria. Some of the volatilisation corrected DegT50 are above 40 days, although there is high uncertainty in these values. Therefore, the eMSCA thinks that based on the results of the study, although some primary degradation occurs, it cannot be excluded that some of the constituents of the substance could be P/vP. Consequently, based on the available information, it is not possible to conclude that the substance is not P.

In conclusion, a firm conclusion on the persistence of the constituents cannot be drawn. However, since the constituents are concluded to be not B according to Annex XIII of REACH, no further assessment of persistency is needed.

7.11.2. Bioaccumulation

Based on an OECD TG 123 study the log Kow of the constituents and impurities of Cassiffix are in the range of 4.71 to 5.01. Hence, the screening criteria for B/vB in aquatic organisms is met.

In the OECD TG 305 study performed following a request in the Substance Evaluation decision, the growth-corrected and lipid normalised BCF_K values as well as the lipid normalised BCF_{SS} values of the four main constituents are around 400-700 L/kg. The study is valid and reliable with restrictions for the assessment. Hence, the constituents are concluded to be not B/vB in aquatic organisms according to REACH Annex XIII.

Based on the predicted log Kow values and QSAR models on BCF values, it seems that there may be some differences in the bioaccumulation potential of the constituents and impurities. However, considering that all impurities and minor constituents are structurally

very similar to the four main constituents, and since in the OECD TG 305 study all BCF values were well below 2000, it is expected that all constituents and impurities are also not-B in aquatic organisms.

Regarding potential accumulation in air-breathing organisms, the log Koa values of the constituents and impurities predicted by the KOAWIN (v1.10) QSAR model are 6.39 and 6.68 when the log Kow values of 4.71 and 5.01, respectively, are used as input. Hence, based on the log Kow and log Koa values, the constituents screen for potential accumulation in air-breathing organisms.

There is no experimental information on mammalian toxicokinetics. However, based on available mammalian toxicity studies uptake of the Substance via inhalation, oral, and dermal routes is expected, and based on QSAR Toolbox predictions metabolism leading to more water-soluble metabolites may occur. Additionally, the relatively rapid depuration observed in fish in the OECD TG 305 study (half-lives in the range of 1.4-2.4 days) suggests that the constituents are likely to be depurated relatively rapidly also in mammals, which usually have higher metabolic capacity than fish.

Therefore, although a firm conclusion cannot be drawn, due to lack of experimental information in mammals. Considering all available information, it is concluded that the constituents are not likely to be bioaccumulative in air-breathing organisms either.

7.11.3. Toxicity

7.11.3.1. **Fulfilment of the T criterion based on human health classification:**

Cassiffix or its constituents are not classified as Carcinogenic 1A or 1B, Mutagenic 1A or 1B, Toxic to reproduction 1A, 1B or 2 or STOT RE 1 or 2.

7.11.3.2. **Fulfilment of the T criterion based on ecotoxicity data:**

There is only acute toxicity data on the whole substance available for fish and aquatic invertebrates. For algae EC50 and NOEC values are available for the whole substance.

The short-term aquatic E(L)C50 values for all three trophic levels are higher than the screening criterion of 0.1 mg/L (lowest EC50 value is 1.3 mg/L for aq. invertebrates). The chronic toxicity value for algae is above 0.01 mg/L (NOEC 2.6 mg/L).

ECOSAR QSAR model for the main constituents and impurities results in chronic toxicity values in the range of 0.05-0.1 mg/L for fish and aquatic invertebrates when the measured log Kow values of 4.7 and 5.0 are used as input. For algae the predicted chronic values are in the range of 0.3-0.5 mg/L.

There is no convincing evidence that chronic toxicity to fish and aquatic invertebrates could not be below the criterion of 0.01 mg/L in the Annex XIII of REACH. However, as the substance is to be not B/vB, and therefore not PBT, no further information on long-term aquatic toxicity is needed.

7.11.4. Overall conclusion

No firm conclusion on the persistency of the constituents and impurities of Cassiffix can be drawn. The eMSCA concluded that the constituents of the substance do not fulfil the criteria for B/vB in aquatic organisms according to REACH Annex XIII. Available information regarding potential bioaccumulation in air-breathing organisms did not indicate a concern for B/vB either. Therefore, the eMSCA concluded that the substance is not-B, and hence, not PBT/vPvB.

7.12. Exposure assessment

REACH requires, according to Article 14(4), exposure assessment and subsequent risk characterisation to be carried out for substances subject to registration, which are manufactured or imported in quantities equal to or greater than 10 tonnes/year, and where the substance meets any of the criteria to be classified as hazardous.

Currently the substance is imported in the range of 1 – 10 tonnes/y and no exposure assessment and risk characterisation are included in the last registration dossier updated 20 September 2022. However, before inclusion in the CoRAP the tonnage range was 10-100 tonnes/y. Therefore, the eMSCA has performed the exposure assessment for the currently registered uses, considering the currently registered tonnages.

No systemic adverse effects are observed in any of the human health endpoints therefore, no exposure assessment nor risk characterization is required for human health endpoints. Anyway, this is out of the scope of this environmental assessment.

Regarding the environment, the substance has a harmonised classification as Aquatic Chronic 2. The available information supports this classification of environmental hazard. Additionally, high RCRs were identified as a concern subject to evaluation in the previous tonnage range of 10 – 100 tonnes/y.

The current assessment performed by the eMSCA in 2023 is a generic assessment which covers the scenarios and conditions of the individual registrants according to the information provided (see Table 28) in the tonnage range of 1-10 tonnes/y. Annual aggregated tonnes corresponding to the latest year (2021) included in the most recent updated dossier submitted by the registrants in 2022 are considered in this assessment . Detailed information on tonnages has been included in the Confidential Annex.

As the number of registrants is low, PECs have been considered as confidential to avoid recalculations of tonnages.

For the exposure assessment, information on uses, tonnage and relevant spERCs included in Table 29 have been considered.

The scenario ES3 (uses at industrial sites) is not included in the CSR (dated 29/03/2022) included in the last updated the registration dossier (31/03/2022), however, the use is currently registered and included at the ECHA dissemination site, and therefore has been included by the eMSCA in the assessment.

Table 28. Description of the scenarios covered in the assessment

Scenario	Life-cycle stage	Description
ES1	Formulation	**GES1. FORMULATION OF FRAGRANCE COMPOUNDS (MIXING OF FRAGRANCE SUBSTANCES INTO FRAGRANCE COMPOUNDS**) at large, medium and small sites
ES2		**GES2. FORMULATION OF FRAGRANCED END-PRODUCTS**: a) AISE Granular & Low Viscosity Liquids at large, medium and small scale; b) AISE High Viscosity Liquids + CE/AISE Solid Products + CE Low Viscosity Liquids at large, médium and small scale;
ES3*	Uses at industrial sites	**GES3. INDUSTRIAL END-USE OF WASHING AND CLEANING PRODUCTS.** All scales
ES4	Use by professional workers	**GES4. PROFESSIONAL END-USE OF WASHING AND CLEANINGAGENTS**: Wide Dispersive Use in 'Down the Drain' cleaning and maintenance products (Consumers and Professionals)
ES5		**GES5. PROFESSIONAL END-USE OF POLISHES AND WAX BLENDS**: Wide Dispersive Use in 'Down the Drain' cleaning and maintenance products (Consumers and Professionals)
ES6	Consumer use	**GES6 CONSUMER END-USE OF WASHING AND CLEANING PRODUCTS**: Wide Dispersive Use in 'Down the Drain' cleaning and maintenance products (Consumers and Professionals)
ES7	Consumer use	**GES7 CONSUMER END-USE OF AIR CARE PRODUCTS**: Wide Dispersive Use in 'Down the Drain' cleaning and maintenance products (Consumers and Professionals)
ES8	Consumer use	**GES8 CONSUMER END-USE OF BIOCIDES**: Wide Dispersive Use in 'Down the Drain' cleaning and maintenance products (Consumers and Professionals)
ES9	Consumer use	**GES9 CONSUMER END-USE OF POLISHES AND WAX BLENDS**: Wide Dispersive Use in 'Down the Drain' cleaning and maintenance products (Consumers and Professionals)
ES10	Consumer use	**GES10 CONSUMER AND PROFESSIONAL END-USE OF COSMETICS**: Wide Dispersive Use in 'Down the Drain' cleaning and maintenance products (Consumers and Professionals)

*This scenario is not included in the registrant's CSR, but the use is currently registered and included at the ECHA dissemination site, and therefore included by the eMSCA in this assessment.

Table 29. Relevant information used for the environmental exposure assessment.

Exposure escenario	Description	Condition of use	Tn/y per use ****	days	ERC	SPERCs*	% range of EU tonnage	Release		
								Water	Air	Soil**
ES1	GES1	Large/medium	see conf annex - - - -	250	ERC2	IFRA 2.1a. v1	30-45	0.002	0.025	0
		Small scale				IFRA 2.1b. v1	30-45	0.005		
ES2	GES2a	Large scale				AISE 2.1 a, g	30-45	0.0001	0	
		Medium scale				AISE 2.1 b, h	10-18	0.001		
		Small scales				AISE 2.1 c, i	9-14	0.002		
	GES2b	Large scale				AISE 2.1 j	8-13	0.001		
		Medium scale				AISE 2.1 k	2-7	0.02		
		Small scale				AISE 2.1 l	2-7	0.004		
		All scales				CE 2.2 a-c	12-20	-		
		All scales				CE 2.1 d-j	1-2	0.02		
ES3	GES3	All scales		300	ERC4	AISE SPERC 4.1.v3***		1		
ES4	GES4	Under GES6		365	ERC8a	Under GES6		1		
ES5	GES5	Under GES6				Under GES6				
ES6	GES6					AISE 8a.1.a. v2				
ES7	GES7	Under GES6				Under GES6				
ES8	GES8	Under GES6				Under GES6				
ES9	GES9	Under GES6				Under GES6				
ES10	GES10	Under GES6				Under GES6				

*SPERCs scenarios from IFRA (2012).
**Release fraction to “industrial soil” is considered 0 as established in the SpERC IFRA 2.1a.v1. However, soil exposure was additionally calculated by EUSES by deposition of the fraction released to air and considering application of STP sludge to agricultural soil.
*** AISE (2021)
**** See confidential annex

7.12.1. Human health

7.12.1.1. Worker

Not evaluated nor relevant for this environmental assessment.

7.12.1.2. Consumer

Not evaluated nor relevant for this environmental assessment.

7.12.2. Environment

The substance is used by consumers in articles, by professional workers (widespread uses), in formulation or re-packing and at industrial sites.

According to the indicated uses and the above exposure scenarios, the following worst-case scenarios have been considered to cover all the indicated uses:

- ES1 formulation in large scale formulation sites, (although the release rate to water is lower than in small sites, the tonnage used in large sites is much higher leading to higher releases),
- ES3 for industrial end use of fragrance end-products and,
- ES6 for consumer use.

Large and medium compounding sites have been aggregated in a single spERC as no major differences in operating conditions and environmental release were observed in an industry survey (Haskoning 2008 in IFRA (2012)).

Physico-chemical data included in Table 10 has been included in the assessment. A log Kow of 4.72 measured for the whole substance has been used in calculations.

Additionally, for EUSES estimations, Cassiffix has been considered as a not biodegradable substance, predominantly hydrophobic with a BCF of 700.

Whenever possible, conditions included in the specific scenarios have been applied. Whenever no confirmation on the applied risk management measures is available, default EUSES conditions have been considered. The latest aggregated EU tonnage included in the updated dossier, notified for 2021, has been used in the calculations. Results and figures have been included in the confidential annex.

According to the information from this SpERC (IFRA, 2012), the concentration of fragrance substance in washing and cleaning end-products may be lower than the applicable concentration limit as listed in REACH 14.2. In practice this usually means <1% or <0.1% for substances classified as NR50/53 or N, R51/53 (equivalent to Aquatic Chronic cat 1 or Chronic cat 2). Therefore, in this case 0.1% concentration in fragrance end-product could be considered as a worst-case for emissions. See also confidential annex for additional information.

ES1. FORMULATION of fragrance compounds at large sites (ERC2 – formulation of preparations)

This scenario includes the specific ERC (spERC) for compounding of fragrance compounds, considering the conditions for the large size compounding sites, SpERC: IFRA 2.1.a.v1 (IFRA 2012). This scenario will cover also the operational conditions of ES2 for medium and small size compounding sites, considering the EU tonnage range of 1-2% for CE 2.1 d-j (see Table 29). Both SPECS result in emissions within the same order and magnitude, but it seems more reasonable to assume a worst case 100% EU tonnage for the E1.

The following has been considered in the assessment:

- **Regarding the size of the compounding sites**

Compounding sites have been confirmed by registrants as large-scale sites based on the overall volume of fragrance compounds being made on an annual basis. Therefore, formulating in large scale sites have been considered as a worst-case covering also the scenario of medium/small scale sites (ES2), because even though the medium/small scale sites have higher release rate to water according to the IFRA SpERCs, the tonnage used is much lower leading to lower exposure. So, based on this assumption the generic scenario included in Table 30 has been considered for the ES1 (Formulation).

Table 30. Duration, frequency, and volume for ES1. (See section 7.12.2. in the confidential annex for additional information)

Information type	Generic Scenario	Explanation
Amount of substance used per day (kg)	-	This is based on a generic volume for formulation.
Annual amount used per site (tonnes)	Confidential	This amount is based on the joint tonnage for 2021considered in the updated registration dossier
Emission days per site	250	number of days indicated in the formulation sites (IFRA 2.1a.v1)

Environmental surrounding characteristics

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

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

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

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

According to the information provided by the registrants the following operational conditions of use are applied corresponding to the large facilities:

Operational conditions

The following specific characteristics are considered for the exposure scenario ES1 according to the SpERC IFRA 2.1a.v1 for large scale formulating sites (IFRA, 2012):

Release fraction to air	2.5E-02
Release fraction to wastewater	2E-03
Release fraction to industrial soil	0.0 (EC, 2003, default EUSES[4])
Fraction of tonnage to region	100%
Fraction of the main source	1 (worst case)

Risk management measures

In the table below the Risk Management measures applied and their effectivity are summarised.

Table 31. Risk Management Measures applied for ES1.

Environmental compartment	Measure	Effectivity
Risk management measures (air)	none	EUSES default
Risk management measures (water)	It is a common practice the wastewater be treated in a physical-chemical system before it is discharged into a biological wastewater treatment plant on-site or in a municipal sewage treatment plant. Removal efficiencies are reported from 30 to 70% (IFRA, 2012). However, individual plants may vary and data collection will be needed to verify a particular treatment plant efficiency. Therefore, default EUSES abatement is considered as there is no clear information on the efficiency of this RMM.	Default EUSES organic abatement is considered for "Industrial" STPs at large formulating sites
Risk management measures (soil)	none	EUSES default

In the SpERC it is indicated that the solid waste is collected and that used packaging and spills are cleaned, but no mention on the incineration nor any other treatment of the sludge is confirmed. Therefore, in the condition of use application of the STP sludge to agricultural soil is considered as a default.

[4] Release fraction to "industrial soil" is considered 0 as established in the SpERC IFRA 2.1a.v1. However, soil exposure was additionally calculated by EUSES by deposition of the fraction released to air and considering application of sludge to agricultural soil, since the SpERC only mentions incineration or recycling regards to used packaging.

ES3. Industrial end-use of washing and cleaning products.

Fragranced end-products are used in an industrial setting for cleaning and maintenance of industrial process equipment. This use at industrial site of the products is part of the operational process.

Regarding the fraction of the main local source. This is not applicable for Industrial uses (TGD, 2003). A.I.S.E. and CEFIC accepted that it is industry's responsibility to ensure that emission standards are met at production and formulation plants. However, the local risk assessment for a plant is generally driven by specific local conditions, such as specific treatment facilities and dilution factors. Generic local scenarios are typically not applicable to the individual plant situations. Instead, environmental safety should be assessed on a case-by-case basis for individual plants and be compatible with local water quality management schemes. For this reason, it was decided not to include the local environmental risk assessments for these facilities within the scope of risk assessment (HERA, 2005) and considered "not applicable" when estimating the fraction of the main source in the TGD (TGD, 2003) and then EUSES apply a "fraction of the main source" as 0.

The updated SPERC, AISE SPERC 4.1.v3 included in background document for the Specific Release Categories (SPERCs) for the industrial use of Water borne processing aids (AISE, 2021[5]) has been considered for the estimation of the indicative use rates.

Considering the total registered tonnage of the Substance, small industrial laundries as described in this AISE SPERC for ERC4[6] were selected as a reasonable worst-case scenario with the following operational conditions:

- maximum amount of product used per day of 580 (kg/d).
- typical dilution of the product during operation (% of formulation) ranged from 0.06-0.5.

Following considerations have been applied in calculations of this ES3:

- averaged amount of substance estimated for the local emission to water (kg/day) estimated according to this SPERC (see confidential annex for calculations):

- 0.5% of formulation (AISE, 2021). This dilution has been applied by assuming an increment of the water, applied to the industrial scenario (laundries), during the washing process, previously to the release of the product to municipal STP.

Table 32. Duration, frequency, and volume for ES3. (See section 7.12.2. in the confidential annex for additional information)

Information type	Generic Scenario	Explanation
Amount of substance used per day (kg)	-	See calculations at the confidential. annex.
Annual amount used per site (tonnes)	Confidential	See calculations at the confidential annex.
Emission days per site	300	Number of days indicated in the formulation sites (AISE Sperc 4.1.v3)

[5] AISE, 2021. Specific Environmental Release Categries (SPERCs) for the Industrial use od Water-borne Processing Aids. www.aise.eu

[6] Use of non-reactive processing aids at industrial site (no inclusion into or into article)

Environmental surrounding characteristics

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

Fresh water flow rate: 36E+05 m^3/d (it has been modified to reflect the typical % of formulation after dilution during the washing processes, see above and AISE, 2021),

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

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

According to the information provided by the registrants the following operational conditions of use are applied corresponding to the large facilities:

Operational conditions

The following specific characteristics are considered for the exposure scenario ES3 according to the AISE 4.1.v3 (IFRA, 2012):

Release fraction to air	0
Release fraction to wastewater	1
Release fraction to industrial soil	0 (EC, 2003, default EUSES[7])
Fraction of tonnage to region	100%
Fraction of the main source	1 (worst-case assumption)

Risk management measures

No obligatory RMMs are included in the SPERC AISE 4.1.v3 (AISE, 2021). The table below summarises the Risk Management measures applied and their effectivity.

Table 33. Risk Management Measures applied for ES3.

Environmental compartment	Measure	Effectivity
Risk management measures (air)	none	EUSES default
Risk management measures (water)	None. No on-site STP is considered, as this use could be applied to small scale sites.	Default EUSES municipal STP is considered
Risk management measures (soil)	None	EUSES default

[7] Release fraction to "industrial soil" is considered 0 as established in the SpERC AISE 4.1.v3. However, soil exposure was additionally calculated by EUSES by deposition of the fraction released to air and considering application of sludge to agricultural soil, since the SpERC only mention incineration or recycling regards to used packaging.

ES6. Consumer use of AIB care products (ERC 8a – widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)).

This scenario covers all the registered wide dispersive uses of cleaning and maintenance products by consumers and professionals (these are scenarios ES4, ES5, and ES7 to ES10). The SpERC AISE SPERC 8a.1.a.v2 - IFRA (2012) has been applied based on sector specific knowledge.

Table 34. Duration, frequency, and volume for consumer use covering professionals. (For additional information see section 7.12.2. in the confidential annex)

Information type	Generic Scenario	Explanation
Used amount of substance per day (kg)	-	Based on calculation. See confidential annex.
Annual total tonnage for wide dispersive use. (tonnes)	Confidential	This amount is based on joint tonnage considered in the updated registration dossier for 2021
Emission days per site	365	number of days indicated in AISE SPERC 8a.1.a. v2

Environmental surrounding characteristics

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

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

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

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

A municipal STP is considered.

Operational conditions

The following specific characteristics are considered for the SpERC AISE 8a.1.a.v2

Release fraction to air from process	0.0%
Release fraction to wastewater from process	100%
Release fraction to soil from process	0.0% (EC, 2003, default EUSES[8])
Fraction of tonnage to region	10%
Fraction of the main source	0.002% (EUSES output)

[8] Release fraction to soil is considered 0 as established in the SpERC AISE 8a.1.a.v2. However, soil exposure was additionally calculated by EUSES by deposition of the fraction released to air and considering application of sludge to agricultural soil, since the SpERC only mention incineration or recycling regards to used packaging.

Risk management measures

On the table below are summarised the Risk Management measures applied and their effectivity.

Table 35. Risk Management Measures applied for uses by professional workers and consumers.

Environmental compartment	Measure	Effectivity
Risk management measures (air)	None	EUSES default
Risk management measures (water)	Wastewater is assumed to be discharged via public sewer system.	EUSES default by municipal STP
Risk management measures (soil)	None	EUSES default

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

Table 36. Local PECs for the aquatic compartment (see confidential annex for figures)

Scenario	Freshwater (mg/m3)	Freshwater Sed. (mg/kg ww)	Marine water (mg/m3)	Marine sediment (mg/kg ww)
ES-1 (formulation)	see confidential annex	see confidential annex	see confidential annex	see confidential annex
ES-3 (Industrial end-use)	see confidential annex	see confidential annex	see confidential annex	see confidential annex
ES-6 (consumer and professional uses)	see confidential annex	see confidential annex	see confidential annex	see confidential annex

7.12.2.2. **Terrestrial compartment**

Local PECs for the terrestrial compartment

Scenario	Agricultural soil (mg/kg ww)
ES-1 (formulation)	see confidential annex
ES-3 (Industrial end-use)	see confidential annex
ES-6 (consumer and professional uses)	see confidential annex

7.12.2.3. **Atmospheric compartment**

Not relevant for the assessment.

7.12.3. Combined exposure assessment

When information is available that a combination of several activities are taking place within one site, it is advisable to cover the combination of those activities in the assessment. However, in this case only formulation is taking place.

Additionally, as the regional exposure concentrations are one order of magnitude lower than the local PECs, no significative differences in the risk characterization is expected when considering the sum of local and regional exposure concentrations.

Regional PECs for the different environmental compartments

Regional PECs	Freshwater (mg/m3)	Freshwater Sed. (mg/kg ww)	Marine water (mg/m3)	Marine sediment (mg/kg ww)	Agricultural soil (mg/kg ww)
PECs	see confidential annex	see confidential annex	see confidential annex	see confidential annex	see confidential annex

7.13. Risk characterisation

The below table includes a summary of the local RCRs calculated by the eMSCA considering the aggregated EU tonnage in the updated registration dossier for 2021.

Scenario	Freshwater	Freshwater Sed.	Marine water	Marine sediment	Soil	STP
ES-1 (formulation)	RCR ≤1	RCR ≤1	RCR ≤1	RCR ≤1	RCR > 1	RCR ≤1
ES-3 (Industrial end-use)	RCR ≤1	RCR ≤1	RCR ≤1	RCR ≤1	RCR >>1	RCR ≤1
ES-6 (consumer and professional uses)	RCR ≤1	RCR ≤1	RCR ≤1	RCR ≤1	RCR ≤1	RCR ≤1

By using the registered tonnage, the model does not predict a safe use for the local terrestrial environment for the formulation step, although the RCR value is close to 1 in case of the formulation scenario.

These RCRs >1 are likely to be the result of the sludge application from the on-site “industrial STP” at the large-scale formulation sites and the sludge of the municipal STP for the industrial uses, to agricultural soils. This assumption is also considered in the SPERCs and assumed by the eMSCA in the assessment.

Therefore, registrants should,

- **Regarding soil compartment:**
    - **confirm that the sludge of the industrial and municipal STPs at the formulation and industrial uses, respectively, are managed appropriately and confirm that sludge is not applied as soil amendments,**
    - **and/or decrease the tonnage used per site in formulation and at industrial sites to a maximum of acceptable RCR (see confidential annex),**
    - **and/or refine the PNECsoil by providing additional information on long-term tests.**

- Additionally, it must be considered that **any increase in annual tonnage range need to be revised since it will potentially result in RCRs above 1 for the aquatic compartment.**

7.14. References

AISE, 2021. Specific Environmental Release Categries (SPERCs) for the Industrial use of Water-borne Processing Aids. Background document. at www.aise.eu

ECHA, 2017. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R7c: Endpoint specific guidance.

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Unpublished study,2017. Cassiffix: determination of partition coefficient (n-octanol/water) according to OCDE TG 123. February, 2017. Updated in the registration dossier. https://echa.europa.eu/en/registration-dossier/-/registered-dossier/10234/4/8

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7.15. Abbreviations

BPR	Biocidal products regulation (EU) 528/2012
CAS RN	CAS registry number
CCH	Compliance check
CLP	Classification, labelling and packaging
CoRAP	Community rolling action plan
CSR	Chemical Safety Report
DCM	Dichloromethane
DMEL	Derived minimal effect level
DMF	N,N-dimethylformamide
DNEL	Derived no-effect level
DegT50	Time for degradation 50% of substance
DT50	Time for disappearance 50% of substance
EC	European community
ECHA	European Chemicals Agency
ED	Endocrine disruption
EGME	Ethylene glycol monoethyl ether
eMSCA	Evaluating member state competent authority
EU	European Union
EUSES	European union system for the evaluation of substances
GLP	Good laboratory practice
LOD	Limit of detection
LOQ	Limit of quantification
LSC	Liquid Scintillation Counting
MSCA	Member state competent authority
NO(A)EC	No observed (adverse) effect concentration
NOAEL	No observed adverse effect level
NONs	Notification of new substances
OECD	Organisation for economic co-operation and development
PBT	Persistent, bioaccumulative and toxic
PMT	Persistent, mobile, and toxic
PNEC	Predicted no-effect concentration
POP	Persistent organic pollutants
PPP	Plant protection products regulation EC 1107/2009
QSAR	Quantitative structure-activity relationship
PUF	Poly Urethane Foam
RAR	Risk assessment report
RCR	Risk characterization ratio
REACH	Regulation No 1907/2006 concerning registration, evaluation, authorisation, and restriction of chemicals
SID	Substance identification dossier
STOT RE	Specific target organ toxicity – repeated exposure
STOT SE	Specific target organ toxicity – single exposure
SVHC	Substances of very high concern
TG	Test guideline
TGD	Technical guidance document
TPE	Testing proposal examination
UNEP	United nations environment program
UVCB	Unknown or variable composition, complex reaction products or of biological materials.
vPvB	Very persistent and very bioaccumulative
vPvM	Very persistent and very mobile

Annex 1 Volatilisation correction for DT50 values of the constituents in the OECD TG 309 study performed by the eMSCA

Approach A Simultaneous fitting of the water phase and volatilisation data using ModelMaker

In an OECD TG 308 study included in the CLH report of pendimethalin[9], ModelMaker (v3.0.4) was used to simultaneously fit the total residue data of the whole system and the cumulative volatilisation data to derive DegT50 that described the volatilisation corrected total degradation of the substance.

Similar modelling was performed for Cassiffix for the data on the four constituents in water and in PUF traps. A compartment model was set up to describe the total dissipation indicated by the total dissipation rate kTOT as sum of the degradation and the volatilisation indicated by the degradation rate kDEG and the volatilisation rate kVOL. A schematic diagram of the model is shown below (Figure 11). The model was implemented in ModelMaker (v4.0) and the Chi^2 error level was calculated using the FOCUS kinetics tool FOCUS_DEGKIN_v2.

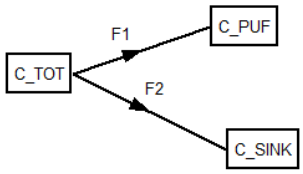


Figure 11 Compartment model for the parent constituents in water (C_TOT) including volatilisation (C_PUF) and sink (elimination compartment) implemented in ModelMaker.

The underlying differential equation system is given by:

$$\frac{\partial}{\partial t} C_{TOT} = -k_{DEG}\, C_{TOT} - k_{VOL}\, C_{TOT} = -k_{TOT}\, C_{TOT}$$

$$\frac{\partial}{\partial t} C_{VOL} = +k_{VOL}\, C_{TOT}$$

$$\frac{\partial}{\partial t} C_{SINK} = +k_{DEG}\, C_{TOT}$$

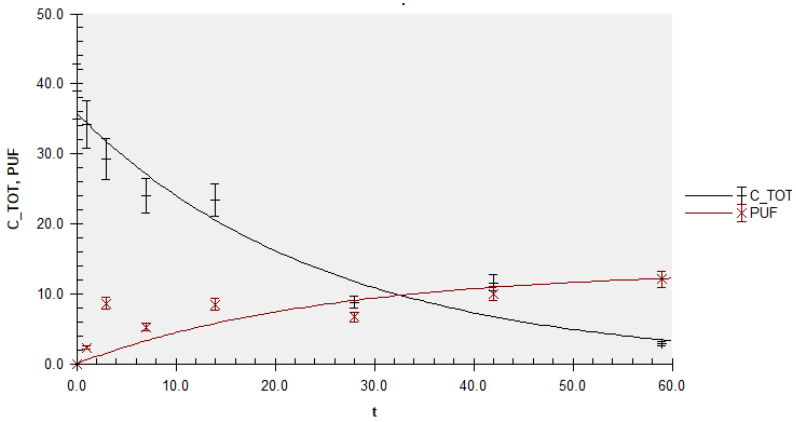
where

C_{TOT} = *total measured concentration in water*
C_{VOL} = *cumulative volatilisation*
C_{SINK} = *cumulative degradation products (and other elimination processes, e.g., NER)*
k_{DEG} = *degradation rate of the system*
k_{VOL} = *volatilisation rate of the system*
k_{TOT} = *total dissipation rate of the system*

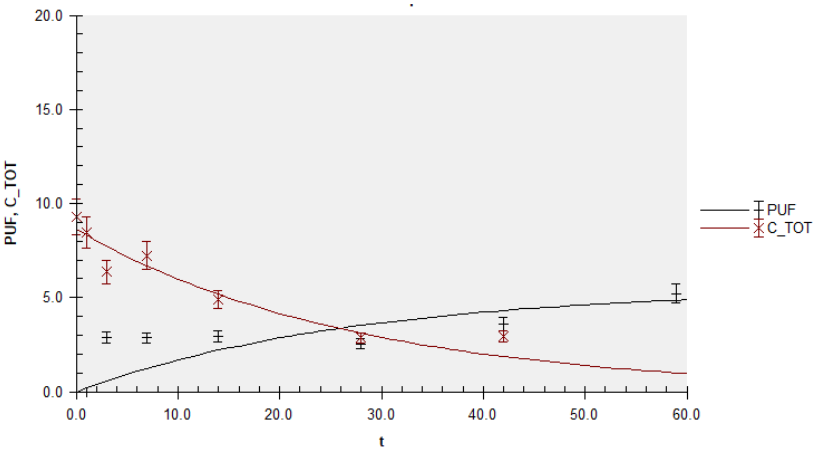
[9] STUDY 7.2.2.3/4
https://echa.europa.eu/documents/10162/17218/clh_rep_annex_pendimethalin_en.pdf/76b2443b-b1a3-802f-1b0d-ab04f42ff9e8

As can be seen in the Figure 12 and based on the Chi^2 error values, the parent constituents' data in water fit well to the predicted concentrations while the PUF data have poor fit.

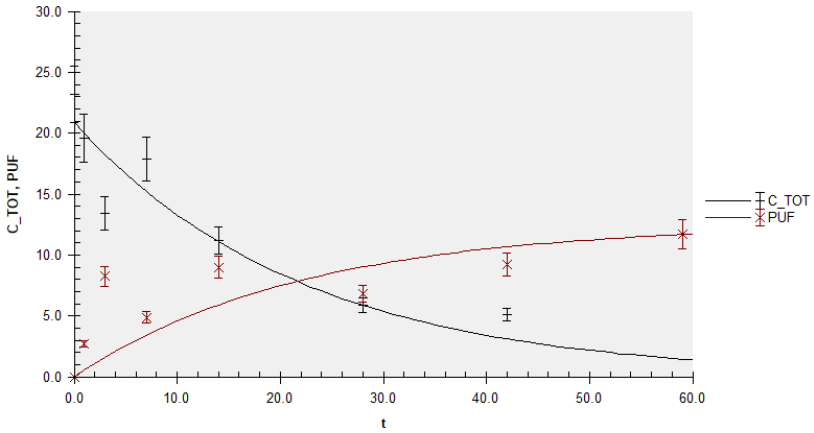
C1 HIGH



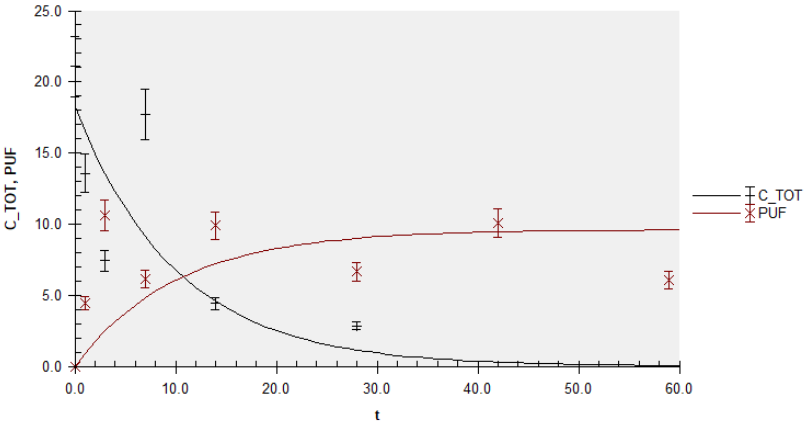
C2 HIGH



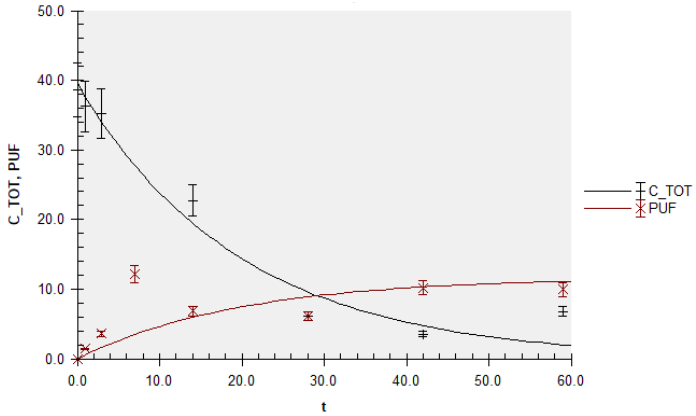
C3 HIGH



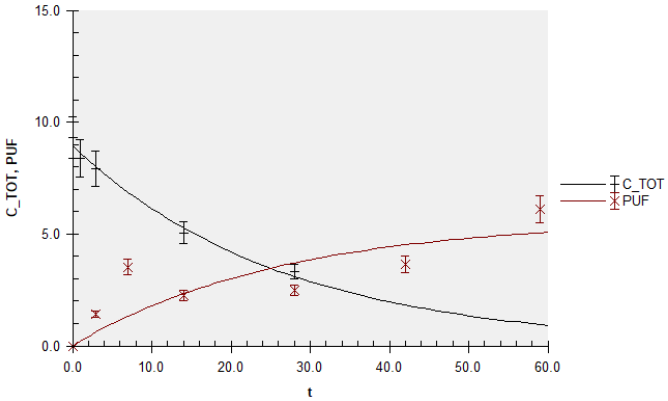
C4 HIGH



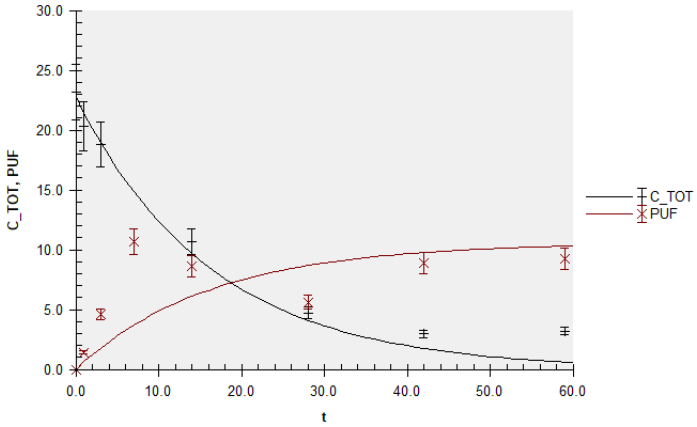
C1 LOW



C2 LOW



C3 LOW



C4 LOW

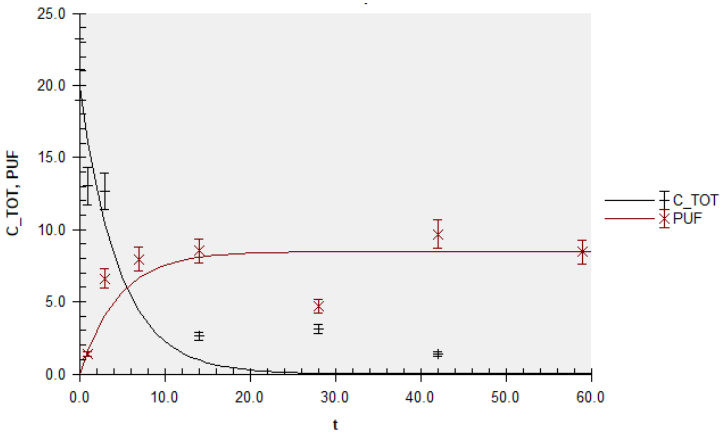


Figure 12. Observations and fitted models for the concentrations of the constituents (C1-C4) in water and in PUF traps in high and low concentration treatments modelled using ModelMaker. The error bars shown in the figures are the errors calculated by ModelMaker for the data points based on the default optimization error.

The model estimated DegT50 values above 40 days only for the constituent 2 in high and low concentration treatments (see Table 32). For the remaining constituents, the DegT50 values are below the P criterion, although the DegT50 of the constituent 3 in the high concentration treatment is close to the criterion.

Table 37 ModelMaker optimisation results for the parameters kVOL, kDEG and Cini (=Initial concentration in water), and the calculated kTOT (=kVOL+kDEG), DT50 and DegT50 for the constituents (C1-C4) at high and low concentration treatments.

	kVOL	kDEG	Cini	kTOT	DT50	DegT50
HIGH						
C1	0.01495	0.02482	35.78	0.03977	17.4	27.9
C2	0.02326	0.01320	8.62	0.03646	19.0	52.5
C3	0.02713	0.01818	20.91	0.04531	15.3	38.1
C4	0.05207	0.04682	18.24	0.09890	7.0	14.8
LOW						
C1	0.01495	0.03548	39.48	0.05043	13.7	19.5
C2	0.02395	0.01388	8.95	0.03783	18.3	49.9
C3	0.02838	0.03274	22.78	0.06111	11.3	21.2
C4	0.09206	0.12421	19.93	0.21627	3.2	5.6

Approach B: Simultaneous fitting of the water phase and volatilisation data in CAKE using a model developed by Shrestha et al 2019[10].

Shrestha et al (2019) presented an extended kinetic modelling to enable considering volatilization in the modelling of degradation kinetics in OECD TG 307 tests. In the model, the volatilization losses are considered as an additional product that neither decline nor repartition into the soil. The volatilization is thus treated as a separate sink for the parent compound, and it is considered to occur in parallel to the biodegradation. Therefore, in this extended model the degradation and the volatilization of the compound were considered as two processes and separated so that individual rate constants could be calculated for the volatilization process as well as the degradation process. In general, the model assumes first order kinetics with k as an overall dissipation rate and c the concentration of test chemical according to following equation:

$$\frac{dc}{dt} = -kc \qquad \textbf{(Equation 2)}$$

However, for the extended model it is assumed that k consists of two rate constants kV (volatilization rate) and kT (transformation rate):

[10] Shrestha P, Meisterjahn B, Klein M, Mayer P, Birch H, Hughes CB, Hennecke D. Biodegradation of Volatile Chemicals in Soil: Separating Volatilization and Degradation in an Improved Test Setup (OECD TG 307). Environ Sci Technol. 2019 Jan 2;53(1):20-28.

k = kT + kV (Equation 3)

To describe the ratio of the two parallel processes the model internally uses “fractions” FV (volatilization fraction) and FT (transformation fraction) which can be calculated based on the individual rates for volatilization and transformation together with the overall decline rate as follows:

$$F^T = \frac{k^T}{k}$$ **(Equation 4)**

$$F^V = \frac{k^V}{k}$$ **(Equation 5)**

When the model is run in CAKE, the values for the two fractions (FT and FV) are estimated by the tool. They describe how the optimization tool evaluate the importance of the respective processes, transformation, and volatilization, in the experiment. Based on the fraction for volatilization and the overall DT50 estimated by CAKE, half-lives for volatilisation (DT50, vol: half-life due to the volatilization of the compound) and for transformation (DegT50: half-life due to all (primary) transformation processes) can be calculated using the following equations:

$$DT^{50,vol} = \frac{DT^{50}}{F^V}$$ **(Equation 6)**

$$DegT^{50} = \frac{DT^{50}}{1-F^V}$$ **(Equation 7)**

It is noted that this DegT50 does not only include the formation of metabolites but also other processes (e.g., formation of NER or loss of the susbtance through adsorption to test vessel, leakage etc).

Similar modelling as the one presented in Shrestha et al (2019) was performed for the data from the OECD TG 309 study with Cassiffix, as it is assumed that changing the system from soil to water does not affect the applicability of the kinetic model. However, in the case of Cassiffix, only data on the parent constituent in water and the volatilisation data (PUF traps) were used in the model (Figure 13), and unlike in the Shrestha et al (2019), data on the metabolites could not be used. This was because the modelling was done separately for the different constituents, and it was not possible to assess from which constituents the different observed metabolites were formed. Therefore, CAKE estimated only the fraction for volatilisation (F^V), and not the fraction for transformation (F^T). But since in the calculations of DegT50 only the F^V is used (see Equation 7), this was not a problem for using the model.

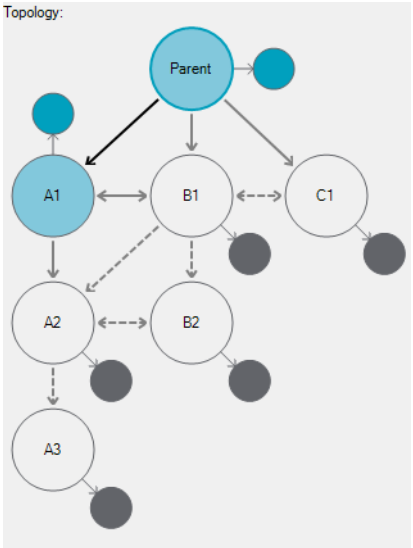


Figure 13 Structure of the model used in the CAKE tool. Parent: concentration of each constituent measured in the water phase, A1: concentration of each constituent measured in the PUF trap.

As seen also in the other modelling approaches presented in this document, based on the Chi^2 error (Table 33) and the graphs with observed and predicted concentrations (see Annex 1) the parent constituent data in water fits well to the SFO kinetics with the exception of constituent 4, while the volatilisation data has a poor fit.

The DegT50 values calculated using the Equation 7 above based on the F^V and DT50 total estimated by CAKE are very high for constituents 2, 3 and 4 at high concentration treatment and for constituent 3 at low concentration. This is because the F^V estimated by CAKE is 1 or close to 1. Hence, the tool found the best fit of the data to the model when it assumed that all or almost all the parent constituent volatilised. This means that no reliable DegT50 can be estimated with the model based on the available data for these constituents.

Table 38 CAKE results for K_{TOT}, F^V and $DT50_{TOT}$, and the $DT50_{VOL}$ and DegT50 calculated using the Equations 6 and 7 for the constituents (C1-C4) at high and low concentration treatments.

Constituent	Chi2 error % (df)		K_{TOT}	F^V	$DT50_{TOT}$	$DT50_{VOL}$	DegT50
	Parent	PUF					
HIGH							
C1	10.5 (6)	31.6(5)	0.03883	0.5481	17.9	48.1	39.61
C2	9.81(5)	30.1(4)	0.03391	0.8933	20.2	67.2	189.32
C3	13.6(5)	31.2(5)	0.04132	0.9386	16.8	43.1	273.62
C4	32.6(4)	32.9(5)	0.08949	1	7.75	26.27	>10000
LOW							
C1	10(5)	35.5(5)	0.04885	0.5151	14.2	31.1	29.28
C2	2.78(3)	29.7(4)	0.03777	0.6352	18.4	>10000	50.44
C3	6.42(5)	31.7(5)	0.05048	1	13.7	22.7	>10000
C4	20.3(4)	21(5)	0.234	0.4445	2.96	289	5.33

Approach B applied for the data of the four main constituents considered as one substance.

The concentrations of the four constituents in water and in PUFs were summed up and the total concentrations used in the kinetic analysis.

Table 39 Total concentrations of the four main constituents in water and PUFs used in the kinetic analyses.

	HIGH		LOW	
Days	**Total parents water phase (% AR)**	**Total parents PUF (% AR)**	**Total parents water phase (% AR)**	**Total parents PUF (% AR)**
0	92.27	0	92.27	0
1	75.85	9.39	78.03	4.2
3	56.52	30.34	74.62	16.18
7	66.79	19.12		34.27
14	43.81	30.32	41.08	26.19
28	20.33	22.66	17.23	18.93
42	19.63	32.85	7.91	32.37
59	2.85	35.01	9.96	33.7

Table 40 CAKE results for K_{TOT}, F^V and $DT50_{TOT}$, and the $DT50_{VOL}$ and DegT50 calculated using the Approach B for the total parent constituents at high and low concentration treatments.

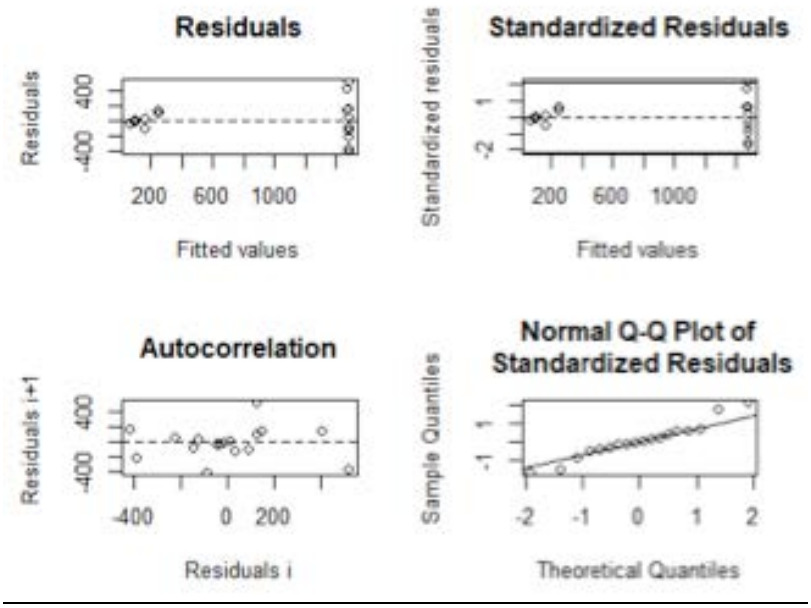
Treatment	Chi2 error %		K_{TOT}	F^V	$DT50_{TOT}$	$DT50_{VOL}$	DegT50
	Parent	PUF					
HIGH	12.8	31.7	0.04517	0.8639	15.3	17.7	112.4
LOW	6.28	31.4	0.05526	0.6723	12.5	18.6	38.1

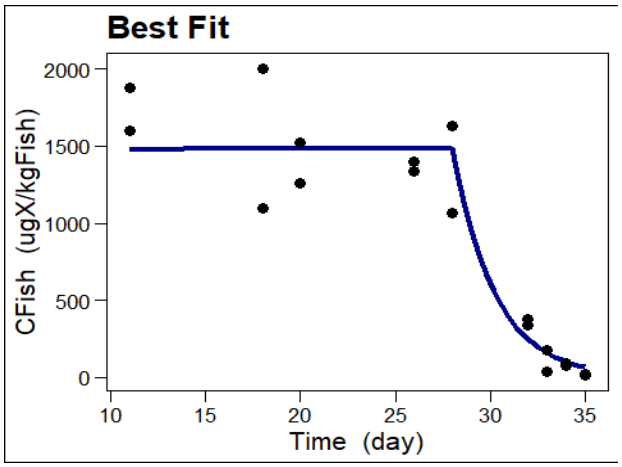
Annex 2 Kinetic analysis performed by the eMSCA for the data from the OECD TG 305 study.

Constituent 1

Untransformed data

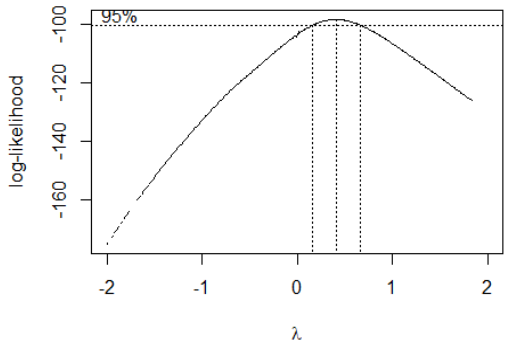
```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.95288, p-value = 0.472
##
##
## -------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = -0.93003, p-value = 0.3524
## alternative hypothesis: two.sided
```





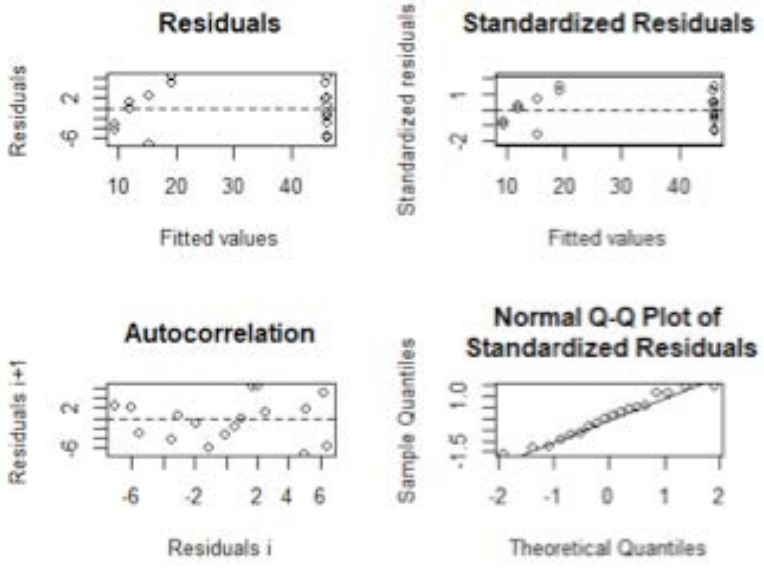
```
#----BCF SUMMARY TABLE
SummTable_Aqueous()

##          Estimate Std.Error    2.5%    97.5%        Unit
## k1       114.44    29.894     55.848   173.03    L/kgFish/day
## k2       0.445     0.11279    0.2239   0.6661       1/day
## k2g      0.4332    0.11279    0.2121   0.6543       1/day
## BCFK     257.16    13.078     231.53   282.79      L/kgFish
## BCFKg    264.17    13.458     237.79   290.54      L/kgFish
## tHalfg   1.6       0.41658    0.7835   2.4165        day
## BCFKgLip 423.34    21.568     381.07   465.62      L/kgFish
```

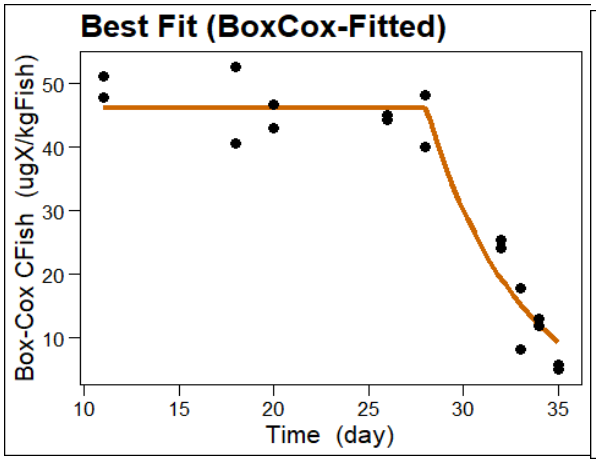
Box-Cox transformed data

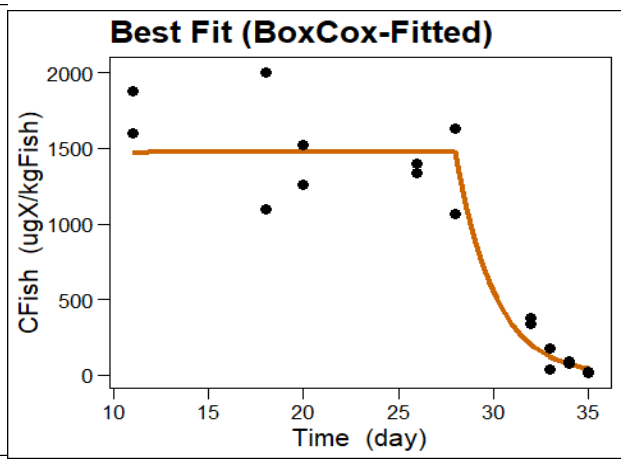



```
##       fit    conflo    confup
## 0.4100000 0.1647009 0.6590743
```

```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.95775, p-value = 0.5588
##
##
## ------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = -0.93003, p-value = 0.3524
## alternative hypothesis: two.sided
```



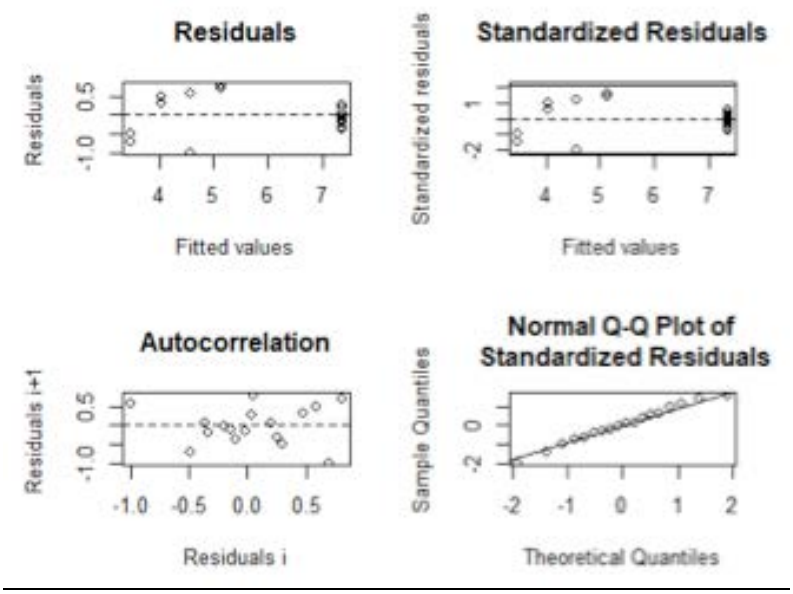


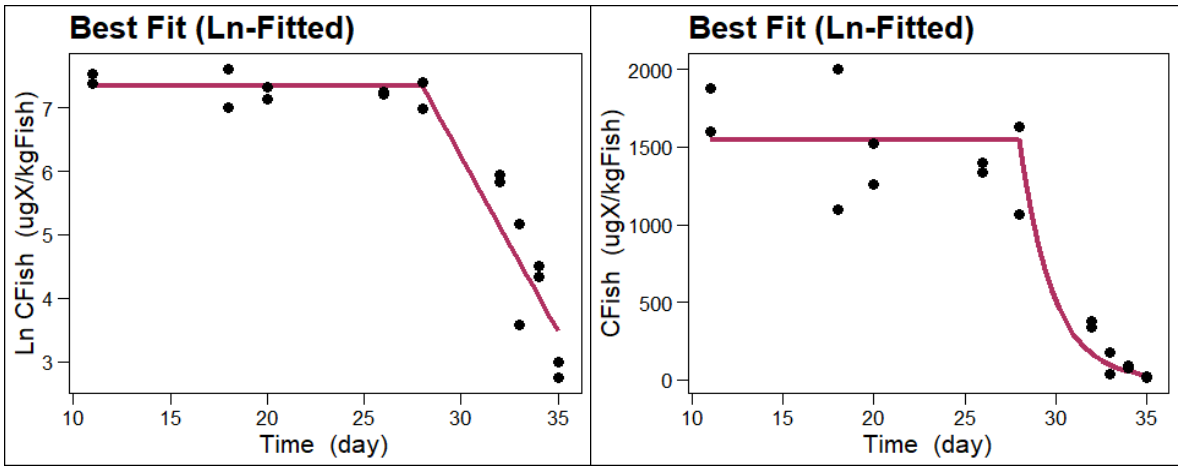



```
SummTable_Aqueous_BoxCox()

##          Estimate Std.Error     2.5%    97.5%         Unit
## k1       126.22    16.489     93.905   158.54    L/kgFish/day
## k2       0.493     0.045758   0.4033   0.5827        1/day
## k2g      0.4812    0.045758   0.3915   0.5709        1/day
## BCFK     256.04    17.751     221.25   290.83      L/kgFish
## BCFKg    262.32    18.026     226.99   297.65      L/kgFish
## tHalfg   1.4405    0.13699    1.172    1.709           day
## BCFKgLip 420.39    28.888     363.77   477.01      L/kgFish
```

Ln-transformed data

```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.98602, p-value = 0.9909
##
##
## -------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = -1.9437, p-value = 0.05194
## alternative hypothesis: two.sided
```



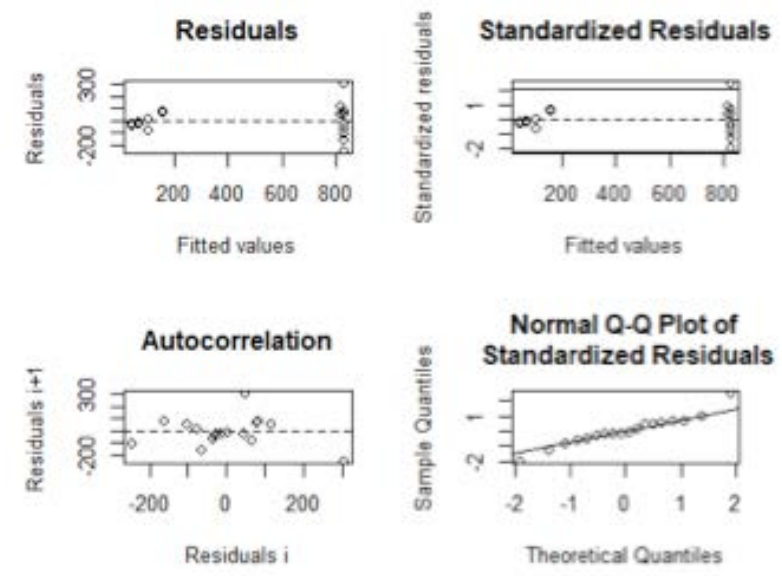


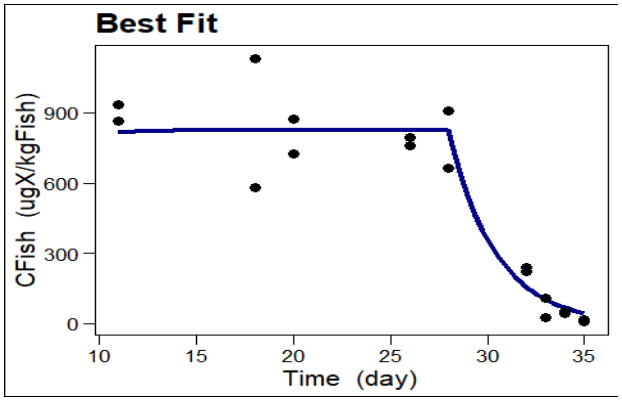

```
#----BCF SUMMARY TABLE
SummTable_Aqueous_Ln()

##..........Estimate Std.Error.....2.5%....97.5%.........Unit
## k1.......147.93....31.313....86.552...209.3....L/kgFish/day
## k2.........0.5517...0.04141...0.47055...0.6329.......1/day
## k2g........0.5399...0.04141...0.45875...0.6211.......1/day
## BCFK.....268.13....41.54....186.71....349.54.......L/kgFish
## BCFKg....273.99....42.156...191.36....356.61.......L/kgFish
## tHalfg.....1.2838...0.09846...1.0909....1.4768..........day
## BCFKgLip.439.08....67.558...306.67....571.49.......L/kgFish
```

Constituent 2

Untransformed data

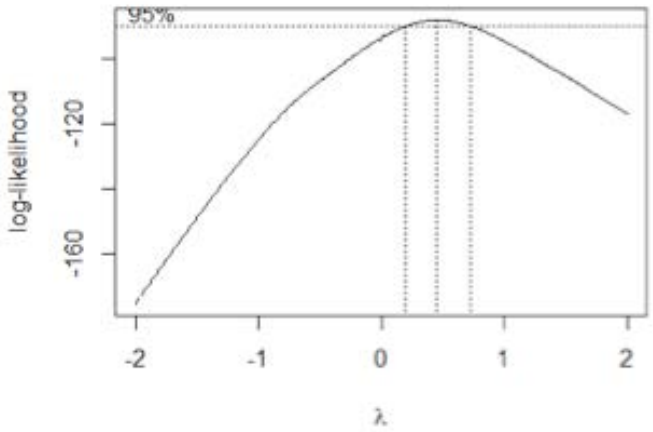
```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.9527, p-value = 0.4689
##
##
## -------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = -0.93003, p-value = 0.3524
## alternative hypothesis: two.sided
```





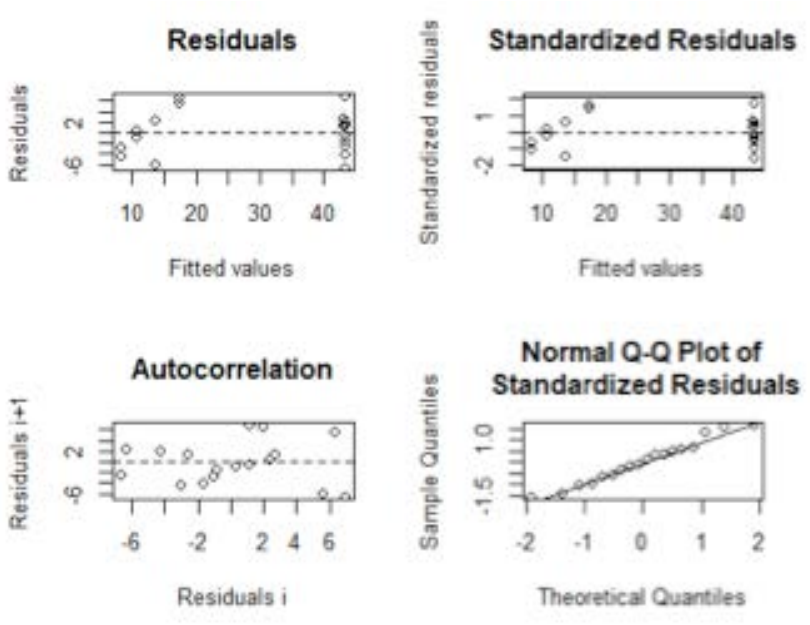
```
#----BCF SUMMARY TABLE
SummTable_Aqueous()

##..........Estimate Std.Error.....2.5%....97.5%.........Unit
##.k1........110.45...24.574......62.281...158.61....L/kgFish/day
##.k2..........0.4145..0.089036...0.24......0.589..........1/day
##.k2g.........0.4027..0.089036...0.2282....0.5772.........1/day
##.BCFK.....266.44....12.443.....242.05....290.82........L/kgFish
##.BCFKg....274.24....12.821.....249.11....299.37........L/kgFish
##.tHalfg.....1.7211..0.3805.....0.9753....2.4669..........day
##.BCFKgLip.439.49....20.547.....399.22....479.76........L/kgFish
```

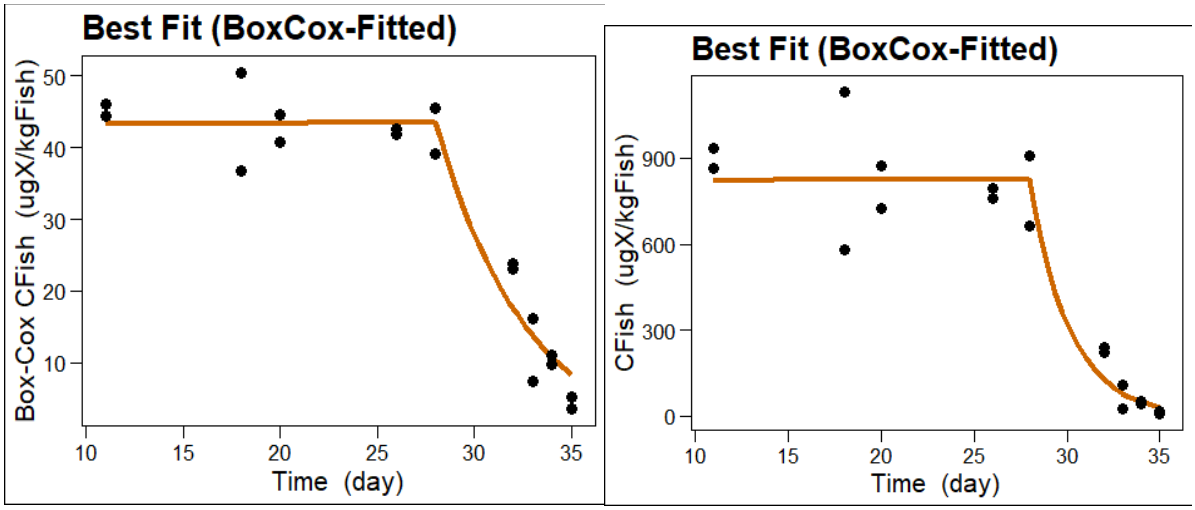
Box-Cox transformed




```
##........fit.....conflo.....confup.
##.0.4500000.0.1924300.0.7265842
```

```
##..Shapiro-Wilk.normality.test
##.
##.data:..stdres
##.W.=.0.96742,.p-value.=.0.7481
##.
##.
##.-------

##.
##..Runs.Test
##.
##.data:..as.factor(run)
##.Standard.Normal.=..-0.97183,.p-value.=.0.3311
##.alternative.hypothesis:.two.sided
```

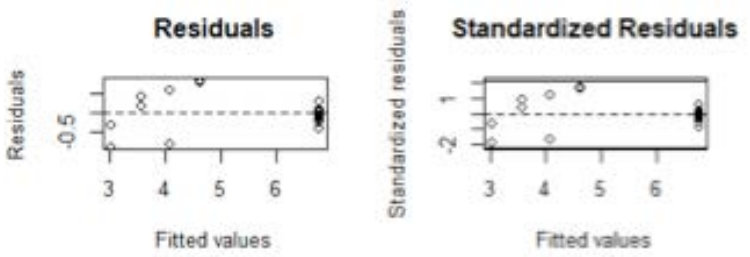


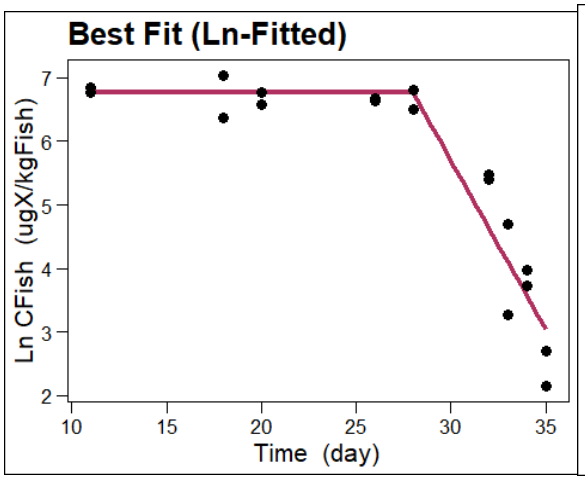


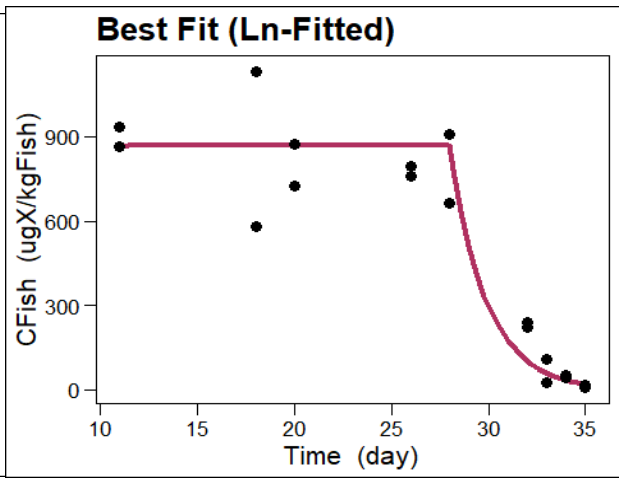

```
#----BCF SUMMARY TABLE
SummTable_Aqueous_BoxCox()

##          Estimate Std.Error     2.5%    97.5%        Unit
## k1       123.84    15.922     92.629   155.04    L/kgFish/day
## k2       0.4657   0.044263    0.379    0.5525       1/day
## k2g      0.4539   0.044263    0.3672   0.5407       1/day
## BCFK     265.89    17.179     232.22   299.56      L/kgFish
## BCFKg    272.8     17.455     238.59   307.01      L/kgFish
## tHalfg   1.527    0.14889     1.2351   1.8188        day
## BCFKgLip 437.18    27.973     382.36   492.01      L/kgFish
```

Ln-transformed data

```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.96851, p-value = 0.7692
##
##
## -------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = 0, p-value = 1
## alternative hypothesis: two.sided
```





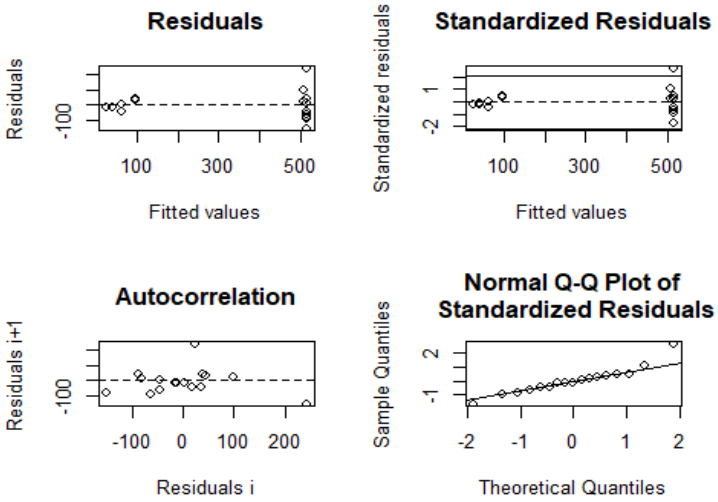


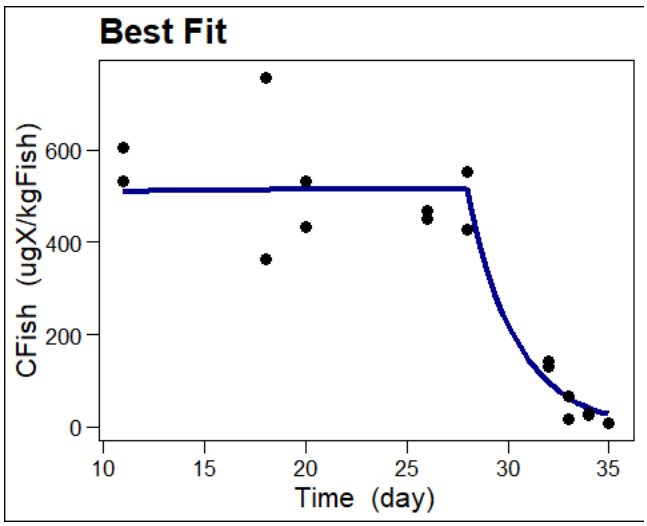
```
#----BCF SUMMARY TABLE
SummTable_Aqueous_Ln()

##..........Estimate Std.Error.....2.5%....97.5%.........Unit
##.k1.......149.21....31.188....88.079..210.34...L/kgFish/day
##.k2.........0.5322...0.04045...0.4529...0.6115.........1/day
##.k2g........0.5204...0.04045...0.4411...0.5997.........1/day
##.BCFK.....280.36....42.432...197.19...363.52........L/kgFish
##.BCFKg....286.71....43.074...202.29...371.14........L/kgFish
##.tHalfg.....1.3319...0.10353...1.129....1.5348..........day
##.BCFKgLip.459.47....69.028...324.18...594.77........L/kgFish
```

Constituent 3

Untransformed data

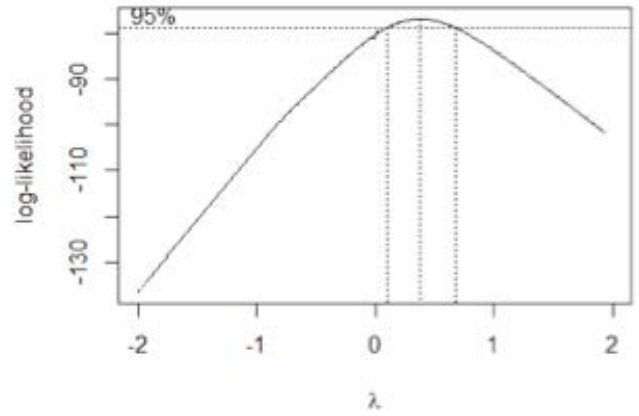
```
##..Shapiro-Wilk.normality.test
##.
##.data:..stdres
##.W.=.0.91714,.p-value.=.0.1321
##.
##.
##.-------

##.
##..Runs.Test
##.
##.data:..as.factor(run)
##.Standard.Normal.=.-0.73946,.p-value.=.0.4596
##.alternative.hypothesis:.two.sided
```



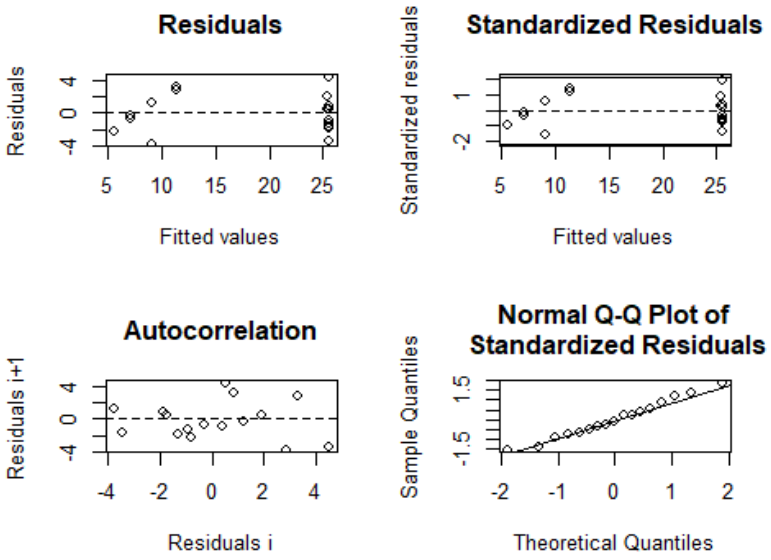



```
#----BCF SUMMARY TABLE
SummTable_Aqueous()

##          Estimate Std.Error     2.5%    97.5%        Unit
## k1        114.9     31.066     54.014   175.79    L/kgFish/day
## k2       0.4187    0.10955    0.2039    0.6334         1/day
## k2g      0.4069    0.10955    0.1921    0.6216         1/day
## BCFK     274.46     15.042    244.98    303.95       L/kgFish
## BCFKg    282.42     15.506    252.03    312.82       L/kgFish
## tHalfg   1.7037    0.45876    0.8045    2.6029           day
## BCFKgLip 452.6      24.849    403.9     501.31       L/kgFish
```

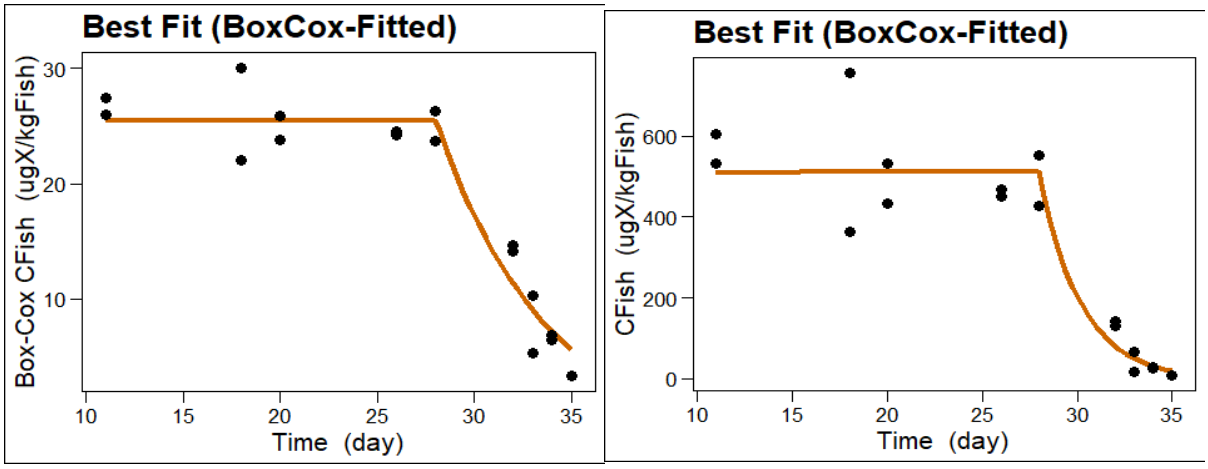
Box-Cox transformed data




```
##        fit     conflo     confup
## 0.38000000 0.09957289 0.67195020
```



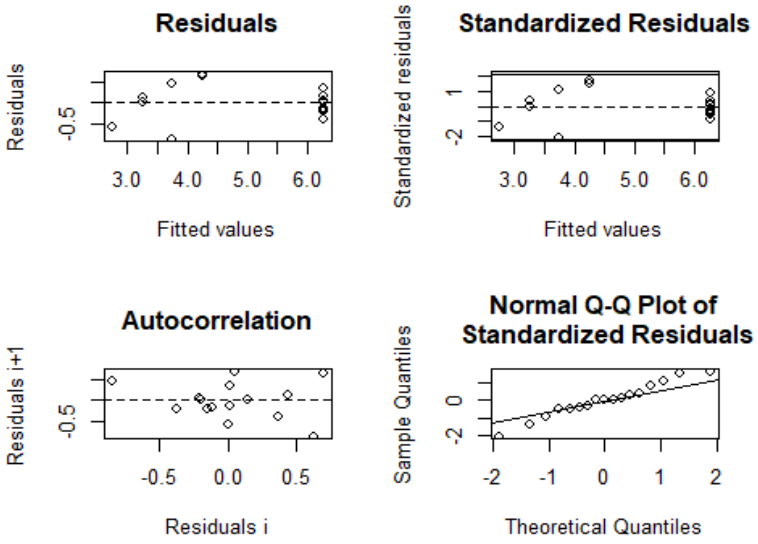

```
##  Shapiro-Wilk normality test
##
##  data:  stdres
##  W = 0.97887, p-value = 0.9461
##
##
##  -------

##
##  Runs Test
##
##  data:  as.factor(run)
##  Standard Normal = -0.73946, p-value = 0.4596
##  alternative hypothesis: two.sided
```

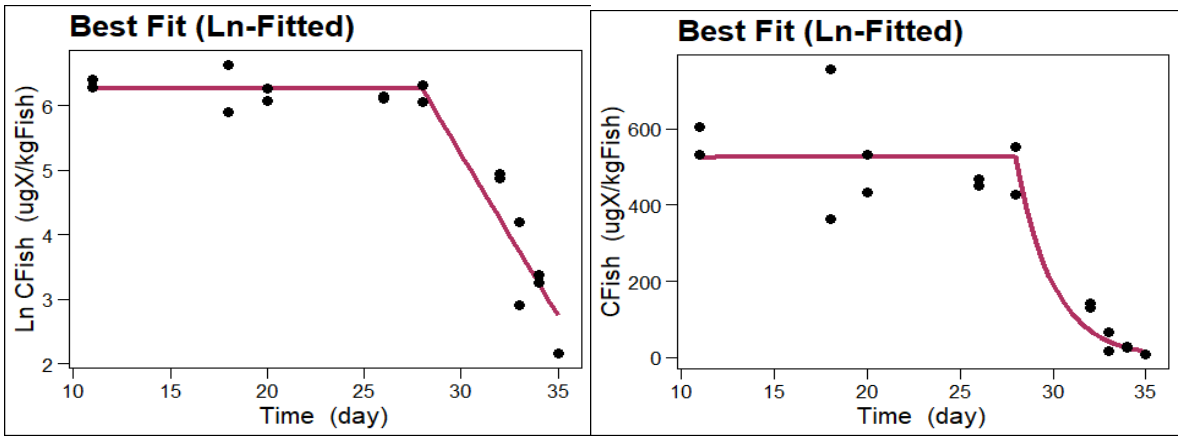


```
#----BCF SUMMARY TABLE
SummTable_Aqueous_BoxCox()

##          Estimate Std.Error    2.5%    97.5%       Unit
## k1       126.05    16.922     92.878   159.21    L/kgFish/day
## k2       0.4616    0.043526   0.3763   0.547        1/day
## k2g      0.4498    0.043526   0.3645   0.5352       1/day
## BCFK     273.04    19.324     235.16   310.91     L/kgFish
## BCFKg    280.2     19.628     241.73   318.67     L/kgFish
## tHalfg   1.5409    0.14909    1.2486   1.8331        day
## BCFKgLip 449.04    31.456     387.39   510.69     L/kgFish
```

Ln-transformed data

```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.97398, p-value = 0.8837
##
##
## -------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = -0.64012, p-value = 0.5221
## alternative hypothesis: two.sided
```



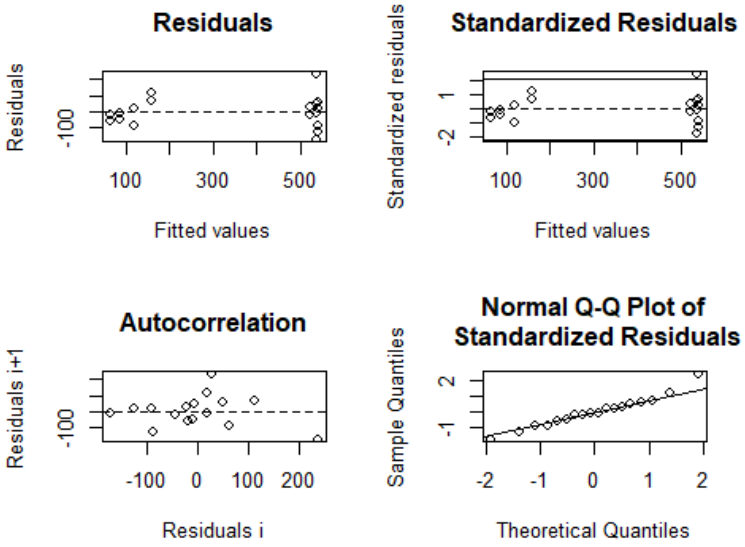


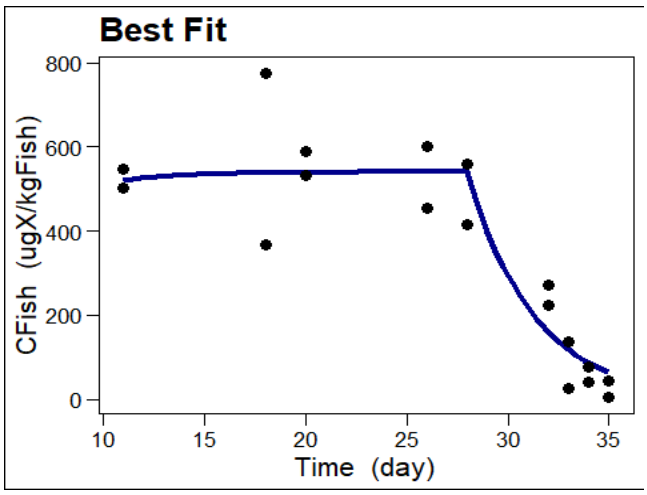
```
# ---- BCF SUMMARY TABLE
SummTable_Aqueous_Ln()

##           Estimate Std.Error     2.5%    97.5%        Unit
## k1         141.43    26.083    90.306   192.55   L/kgFish/day
## k2         0.5027   0.03723   0.4297   0.5757         1/day
## k2g        0.4909   0.03723   0.4179   0.5639         1/day
## BCFK       281.35    36.199    210.4    352.3      L/kgFish
## BCFKg      288.12    36.749   216.09   360.14      L/kgFish
## tHalfg     1.4121   0.1071    1.2021   1.622           day
## BCFKgLip  461.72    58.892   346.29   577.15      L/kgFish
```

Constituent 4

Untransformed data

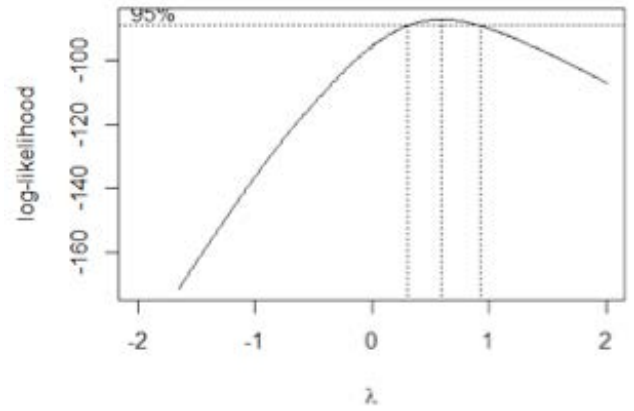
```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.9646, p-value = 0.6922
##
##
## ------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = -0.43766, p-value = 0.6616
## alternative hypothesis: two.sided
```





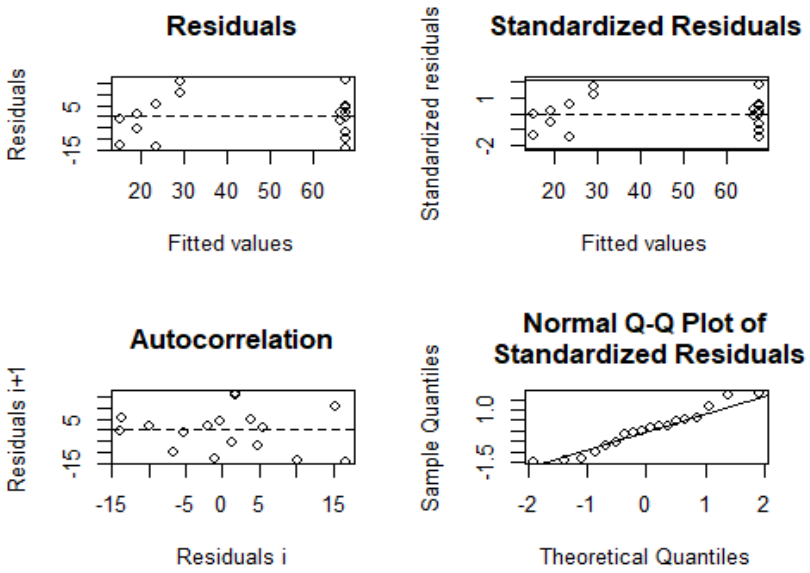

```
#----BCF SUMMARY TABLE
SummTable_Aqueous()

##          Estimate Std.Error     2.5%    97.5%         Unit
## k1        128.4     26.871    75.728   181.06    L/kgFish/day
## k2       0.3042    0.06017   0.1863   0.4221          1/day
## k2g      0.2924    0.06017   0.1745   0.4103          1/day
## BCFK     422.09     23.535   375.96   468.21        L/kgFish
## BCFKg    439.12     24.488   391.12   487.11        L/kgFish
## tHalfg    2.3706    0.48787   1.4144   3.3268             day
## BCFKgLip 703.72     39.243   626.8    780.63        L/kgFish
```

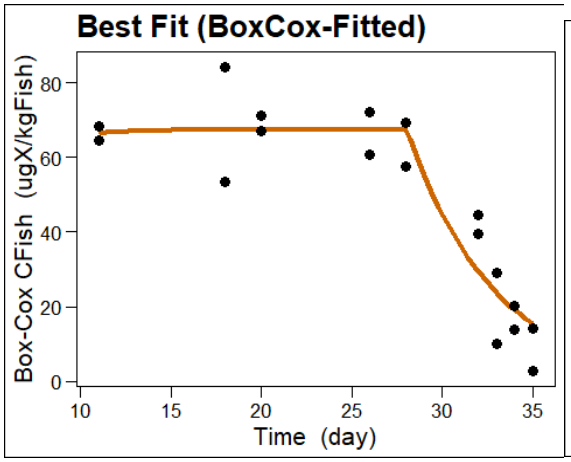
Box-Cox transformed data

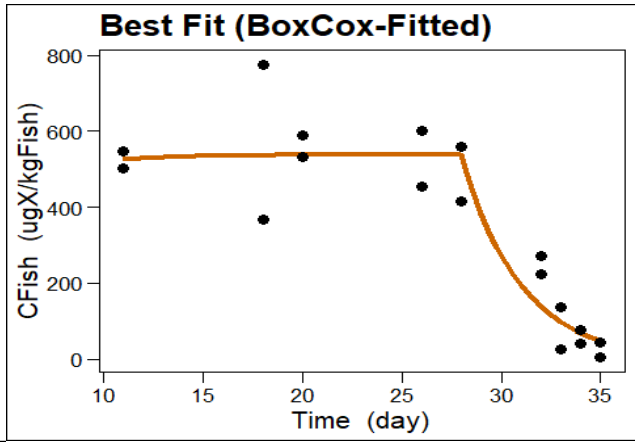



```
##       fit    conflo    confup
## 0.5900000 0.3042957 0.9212226
```

```
##  Shapiro-Wilk normality test
##
## data:  stdres
## W = 0.95785, p-value = 0.5608
##
##
## -------

##
##  Runs Test
##
## data:  as.factor(run)
## Standard Normal = -0.48591, p-value = 0.627
## alternative hypothesis: two.sided
```



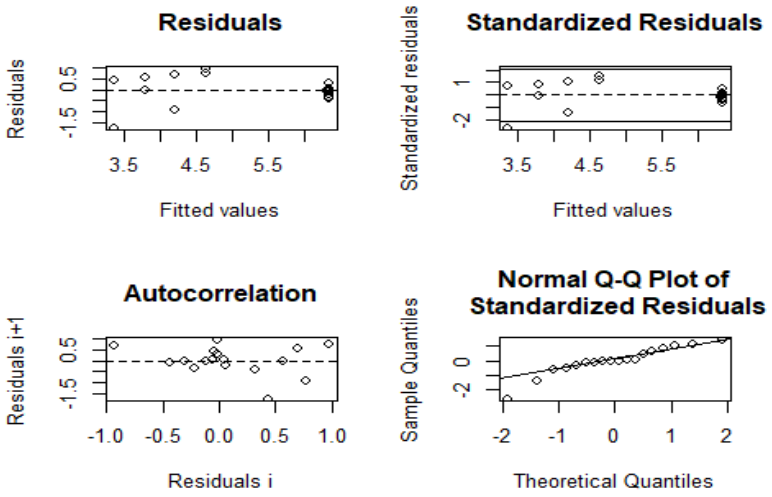


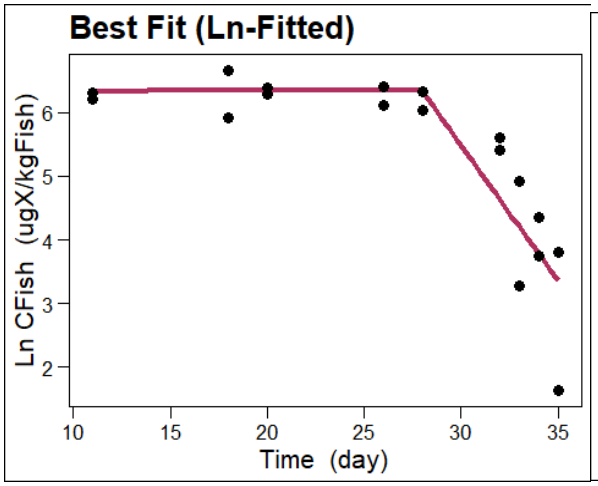


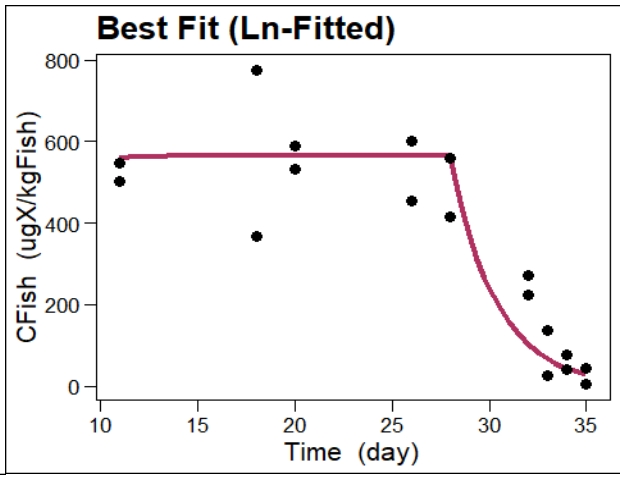

```
#----BCF SUMMARY TABLE
SummTable_Aqueous_BoxCox()

##          Estimate Std.Error    2.5%    97.5%        Unit
## k1       142.99    24.323    95.319   190.66    L/kgFish/day
## k2       0.3404    0.04674   0.2488   0.432         1/day
## k2g      0.3286    0.04674   0.237    0.4202        1/day
## BCFK     420.06    30.082    361.1    479.02    L/kgFish
## BCFKg    435.15    30.692    374.99   495.3     L/kgFish
## tHalfg   2.1094    0.30001   1.5213   2.6974        day
## BCFKgLip 697.35    49.185    600.95   793.75    L/kgFish
```

Ln-transformed data

```
##  Shapiro-Wilk normality test
##
##  data:  stdres
##  W = 0.92015, p-value = 0.13
##
##
##  -------

##
##  Runs Test
##
##  data:  as.factor(run)
##  Standard Normal = 0.54708, p-value = 0.5843
##  alternative hypothesis: two.sided
```







```
# ---- BCF SUMMARY TABLE
SummTable_Aqueous_Ln()

##           Estimate Std.Error     2.5%     97.5%         Unit
## k1         188.02    56.573    77.14    298.91  L/kgFish/day
## k2         0.4255   0.05408   0.3195    0.5315        1/day
## k2g        0.4137   0.05408   0.3077    0.5197        1/day
## BCFK       441.88    89.418   266.62    617.14      L/kgFish
## BCFKg      454.48    90.907   276.31    632.66      L/kgFish
## tHalfg     1.6754   0.21902   1.2462    2.1047          day
## BCFKgLip   728.34    145.68    442.8    1013.9      L/kgFish
```