Substance Name:
2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (UV-329)

EC Number: 221-573-5

CAS Number: 3147-75-9

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF

2-(2*H*-BENZOTRIAZOL-2-YL)-4-(1,1,3,3-TETRAMETHYLBUTYL)PHENOL (UV-329)

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS VPVB (ARTICLE 57(E)) PROPERTIES

Adopted on 13 December 2023

Note
The Annex XV dossier that was submitted by Germany covers both the substances UV-326 and UV-329. However, for each substance a separate support document has been created for their identification as a substance of very high concern. The present support document may therefore contain some redundant references to the other substance in the sections describing the underlying scientific data. Separating the documents resulted in removal of tables 5, 6 and 9.

This document has been prepared according to template: TEM-0049.04

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FIGURES

ABBREVIATIONS

AR: applied radioactivity
B: bioaccumulative (pertaining to REACH Annex XIII)
BAF: bioaccumulation factor
BCF: bioconcentration factor
BCF_K: kinetic bioconcentration factor
BCF_{KL}: lipid-normalised kinetic bioconcentration factor
BCF_{KgL}: lipid-normalised growth corrected kinetic bioconcentration factor
BCF_{ss}: steady-state bioconcentration factor
BCF_{ssL}: lipid-normalised steady-state bioconcentration factor
BMF: biomagnification factor
BMF_{Kg}: growth-corrected kinetic biomagnification factor
BOD: biochemical oxygen demand
bw: body weight
CAS RN: CAS registry number
C_f: test substance concentration in fish
C_w: test substance concentration in water
CLH: Harmonised classification and labelling
CLP: Classification, labelling, and packaging of substances
DF: detection frequency
DMEL: Derived minimum effect level
DNEL: Derived no-effect level
DT50: half-life
DegT50 degradation half-life
dw: dry weight
EC: effect concentration
es-BANK: Environmental Specimen Bank of Ehime University
GC-HRMS: gas chromatography/ high resolution mass spectrometry
GC/MS: gas chromatography/ mass spectrometry
GC-MS/MS: gas chromatography tandem mass spectrometry
GLP: good laboratory practice
H. Azteca: *Hyalella Azteca*
HPLC: high performance liquid chromatography
HYBIT: *Hyalella Azteca* Bioconcentration Test
IB: Iles des Boucherville
IUCN: International Union for Conservation of Nature
IV: Iles Vert
k_1: uptake rate constant
k_2: depuration rate constant
k_{2g}: growth corrected depuration rate constant
K: Kaveri River, India
K_{OA}: octanol-air partition coefficient
K_{OW}: octanol-water partition coefficient
LSL: Lake St. Louis, Montreal
LC50: Lethal concentration to 50% of test animals
LC-HRMS: liquid chromatography/ high resolution mass spectrometry
LC-MS/MS: liquid chromatography tandem mass spectrometry
LOD: limit of detection
LOEC: lowest observed effect concentration
LOQ: limit of quantification
lw: lipid weight
M1: 3-[3-(2*H*-1,2,3-benzotriazol-2-yl)-5-*tert*-butyl-4-hydroxyphenyl]propanoic acid (EC 630-348-4)
MDL: method detection limit
MSC: Member State Committee

MQL: method quantification limit
NA: not analysed
ND: not detected
NER: non-extractable residues
NOAEC: No observed adverse effect concentration
NO(A)EL: no observed (adverse) effect level
NOEC: no observed effect concentration
OEL: occupational exposure limit
P: Persistence (pertaining to REACH Annex XIII) or persistent
PBT: persistent, bioaccumulative, and toxic
PLE: pressurised liquid extraction
(Q)SAR: (quantitative) structure-activity relationship
R2: coefficient of determination
RAC: Committee for Risk Assessment
RMSE: Root Mean Square Error
SAR: structure-activity relationship
SEV: Substance Evaluation
SFO: Single First Order
SVHC: Substance of very high concern
STOT RE: Specific Target Organ Toxicity – Repeated exposure
T: Toxic (pertaining to REACH Annex XIII)
TG: Test guideline
TGR: Transgenic rodent
TMF: Trophic magnification factor
TOC: total organic carbon
TWA: time weighted average
UPLC-MS/MS: ultra-high performance liquid chromatography tandem mass spectrometry
UM-PPS: University of Minnesota Biocatalysis/Biodegradation Prediction System
UV-320: 2-benzotriazol-2-yl-4,6-di-*tert*-butylphenol (EC 223-346-6)
UV-326: Bumetrizole, 2-*tert*-butyl-6-(5-chloro-2*H*-benzotriazol-2-yl)-4-methylphenol (EC 223-445-4)
UV-327: 2,4-di-*tert*-butyl-6-(5-chlorobenzotriazol-2-yl)phenol (EC 223-383-8)
UV-328: 2-(2*H*-benzotriazol-2-yl)-4,6-ditertpentylphenol (EC 247-384-8)
UV-329: 2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (EC 221-573-5)
UV-350: 2-(2*H*-benzotriazol-2-yl)-4-(*tert*-butyl)-6-(sec-butyl)phenol (EC 253-037-1)
UV-384: A mixture of branched and linear C7-C9 alkyl 3-[3-(2*H*-benzotriazol-2-yl)-5-(1,1-dimethylethyl)-4-hydroxyphenyl]propionates (EC 407-000-3)
UV-P: 2-(2*H*-benzotriazol-2-yl)-p-cresol (EC 219-470-5)
V: Vellar River, India
vB: very bioaccumulative (pertaining to REACH Annex XIII)
vP: very persistent (pertaining to REACH Annex XIII)
vPvB: very persistent and very bioaccumulative (pertaining to Annex XIII REACH)
WoE: weight-of-evidence
ww wet weight

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

Substance name:

2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (UV-329)

EC number: 221-573-5

CAS number: 3147-75-9

- The substance is identified as very persistent and very bioaccumulative (vPvB) according to Article 57 (e) of Regulation (EC) No 1907/2006 (REACH).

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

A weight of evidence determination according to the provisions of Annex XIII of REACH has been used to identify UV-329 as a vPvB substance. All available relevant information (such as the results of standard tests, monitoring and modelling, information from the application of the read-across and (Q)SAR results) was considered together in a weight-of-evidence approach.

Persistence

The screening criterion for persistence (P) is fulfilled for UV-329. The results from the available screening study (reliable with restrictions) showed that this substance is not readily biodegradable. This is confirmed by the available (Q)SAR results with BIOWIN and CATALOGIC which indicate that UV-329 screens as potentially P or vP. The outcomes of the screening tests and the (Q)SARs predictions have been assigned a low weight in the weight-of-evidence approach (WoE) for the P assessment.

Hydrolysis of UV-329 is not expected due to the absence of functional groups susceptible to hydrolysis. As a conclusion, abiotic degradation of UV-329 is not considered to be a significant degradation pathway in the environment.

In a water-sediment simulation study for UV-329 at 20 °C (reliable with restrictions), no degradation was observed after 100 days. At an environmentally relevant temperature of 12 °C this corresponded to a half-life significantly larger than 212 days for UV-329 thus indicating its very persistent properties in sediment (DegT50>180 days). The outcome of this higher tier study is given a high weight in the WoE approach as it provides information directly comparable with the P and vP criteria set out in Annex XIII, points 1.1.1 (d) and 1.2.1 (b) of the REACH Regulation.

Faster dissipation of UV-329 in an aquifer test (reliable with restrictions) may be related to the different test conditions and the studied compartment compared to an OECD water-sediment simulation study. This study has been assigned a low weight in the WoE approach considering the studied compartment, the test conditions and the difficulty to derive an appropriate DT50.

UV-P (a substance with a particular high structural similarity to UV-329) and further phenolic benzotriazoles have been detected in sediment cores that date back years and even decades (starting from the 1960s), both in samples downstream from a former point source and in samples from urban estuaries. This information provides indirect evidence that UV-P and

potentially UV-329 (based on a read-across from UV-P) can persist in sediments for several decades. Monitoring data in sediment cores are used as supporting information in the WoE approach for UV-329. They are in line with the outcome of the water-sediment simulation study, the screening studies and the QSAR predictions as they point towards the potential persistence of UV-329 (based on results of the structural analogue UV-P) in sediments.

UV-329 is persistent (and potentially very persistent) in two soil dissipation studies (reliable with restrictions) (at least DegT50>120 days).

As an overall conclusion, based on the above information used in a weight-of-evidence-approach, it is concluded that UV-329 meets the 'persistence' criterion (P) and the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of its persistent and very persistent (P/vP) properties in sediment (DegT50 > 180 days). Furthermore, UV-329 meets the 'persistence' criterion (P) and potentially the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of its persistent and potentially its very persistent (P/vP) properties in soil (at least DegT50 > 120 days).

Bioaccumulation

UV-329 screens as potentially B/vB due to the available log K_{ow} values above the screening trigger value of 4.5.

The conclusion for UV-329 is based on a weight-of-evidence assessment using different pieces of information. The outcome of the *Hyalella azteca* bioconcentration test (HYBIT; reliable with restrictions) for UV-329 is given a high weight in the WoE approach with 3 %-lipid-normalised steady-state bioconcentration factor (BCF_{ssL}) and 3%-lipid-normalised kinetic bioconcentration factor (BCF_{kL}) values in the range of 11063—11876 L/Kg. This study provides information directly comparable with the B (BCF>2000) and vB criteria (BCF>5000) set out in Annex XIII. Re-calculated fish BCF_{kgL} >2000 derived from an OECD TG 305 study (aqueous exposure; reliable with restrictions) with rainbow trout (*Oncorhynchus mykiss*) supporting the bioaccumulation potential of UV-329. Monitoring data tend to confirm this conclusion as UV-329 has been found in human breast milk and in biota including in top predators such as the polar bears which are listed as vulnerable to extinction, according to the IUCN Red List. Based on the weight of evidence of the data available, it is concluded that UV-329 meets the 'bioaccumulation' criterion (B) and the 'very bioaccumulative' criterion (vB) in accordance with Annex XIII, points 1.1.2 and 1.2.2, of the REACH Regulation.

Conclusion

In conclusion, UV-329 is identified as a vPvB substance according to Art. 57(e) of REACH by comparing all relevant and available information listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

Registration dossiers submitted for the substance: Yes

Justification

1 Identity of the substance and physical and chemical properties

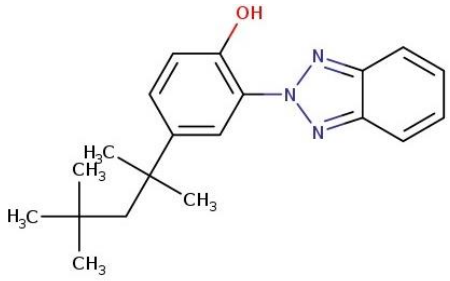
1.1 Name and other identifiers of the substance

Table 1: Substance identity for UV-329

(2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)

EC number:	221-573-5
EC name:	2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol
CAS number (in the EC inventory):	3147-75-9
CAS number:	3147-75-9
IUPAC name:	2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	$C_{20}H_{25}N_3O$
Molecular weight range:	323.4 g/mol
Synonyms:	2-(2*H*-*H*-1,2,3-benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol *H*-2-(2*H*-Benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol 2-(2*H*-Benzotriazol-2-yl)-4-*tert*-octylphenol 2-[2'-Hydroxy-5'-(1,1,3,3-tetramethylbutyl)phenyl]benzotriazole 2-(2-Hydroxy-5-t-octylphenyl)-2*H*-benzotriazole 2-(2-Hydroxy-5-t-octylphenyl)benzotriazole 2-(2-Hydroxy-5-*tert*-octylphenyl)-2*H*-benzotriazole 2-(2-Hydroxy-5-*tert*-octylphenyl)benzotriazole 2-(2'-Hydroxy-5'-*tert*-octylphenyl)benzotriazole 2-(5-t-Octyl-2-hydroxyphenyl)benzotriazole 2-(5'-*tert*-Octyl-2'-hydroxyphenyl)benzotriazole 2-Benzotriazolyl-4-*tert*-octylphenol UV-329 UV 5411

Structural formula:



1.2 Composition of the substance

Name: (2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (UV-329))

Substance type: mono-constituent

Table 2: Constituents other than impurities/additives (for UV-329)

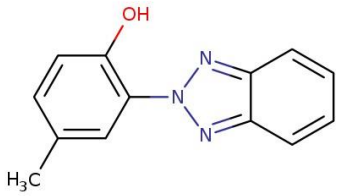
Constituents	Typical concentration	Concentration range	Remarks
2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (EC: 221-573-5)	≤100 %		

1.3 Identity and composition of structurally related substance UV-P (read-across approach)

Table 3: Structurally related substance identity of UV-P

EC number:	219-470-5
EC name:	2-(2*H*-benzotriazol-2-yl)-*p*-cresol
SMILES:	CC1=CC(=C(C=C1)O)N2N=C3C=CC=CC3=N2
CAS number (in the EC inventory):	2440-22-4
CAS number:	2440-22-4
IUPAC name:	2-(2*H*-1,2,3-benzotriazol-2-yl)-4-methylphenol
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	$C_{13}H_{11}N_3O$
Molecular weight range:	225.246 g/mol
Synonyms:	*2-(2H-benzotriazol-2-yl)-4-methylphenol* *2-(2H-benzotriazol-2-yl)-p-cresol* *2-(2'-hydroxy-5'-methylphenyl)-benzotriazole* *2-benzotriazol-2-yl-4-methylphenol* *UV-P*

Structural formula:



Substance type: mono-constituent

Table 4: Constituents of structurally related substance UV-P

Constituents	Typical concentration	Concentration range	Remarks
2-(2H-benzotriazol-2-yl)-p-cresol (EC: 219-470-5)	≤100 %		

1.4 Physicochemical properties

Table 5: Overview of physicochemical properties of UV-329 (2-(2H-*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)[1]

Property	Description of key information	Value [Unit]	Reference/source of information
Physical state at 20°C and 101.3 kPa	*Visual inspection*	*Solid* *White powder*	*ECHA dissemination page*
Melting/freezing point	*Differential Scanning Calorimetry (DSC) method*	*104.3 °C*	*ECHA dissemination page*
Boiling point	*TGA measurement*	*>225 °C (decomposition)*	*ECHA dissemination page*
Vapour pressure	*The measurement was based on heats of evaporation by DSC.*	*0.0000041 Pa (at 20 °C)*	*ECHA dissemination page*
Density	*Internal analytical method*	*1180 kg/m³ (at 20 °C)*	*ECHA dissemination page*

[1] Access date ECHA dissemination page for all information on 08.03.2023
https://echa.europa.eu/de/substance-information/-/substanceinfo/100.019.612

Water solubility	*OECD Guideline 105 (Water Solubility)* *Column elution method (HPLC)*	*2 µg/L (at 20 °C, pH 6.2 – 7.1)*	*ECHA dissemination page*
Partition coefficient n-octanol/water (log value)	*OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method)*	*6.8 – 7.4 (at 23 °C)* *≥6.5 (at 23 °C; pH 6.4)*	*ECHA dissemination page*
	Estimated using COSMOtherm	*6.4*	*COSMOconf*[2] *COSMOtherm*[3]
	Experimental	*6.91*	*Do et al. (2022)*
Dissociation constant	*The calculation was using the recommended software program SPARC v4.5*	*pKa = 8.9 (at 25 °C)*	*ECHA dissemination page*
	Calculated with Chemicalize	*Strongest acidic pKa = 9.4* *(at 25°C)*	*Chemicalize*[4]
	ACD Percepta prediction	*pKa = 10.2 (at pH 9, no temperature available)*	*ACD/Labs*[5]
Using ACD Percepta for the prediction of the dissociation constant resulted in a pKa of 10.3. At pH 9 in total 5 % of UV-329 is ionised (hydroxy group negatively charged). Although a higher pKa compared to 8,9 (ECHA dissemination page) is predicted by ACD Percepta, the computation is regarded as conclusive based on the chemical structure and the same stabilizing effect of the hydroxyl group as in the case of bumetrizole: For UV-329 ionisation can only occur at the hydroxyl group, which is the only functional group that could act as proton acceptor/donor. However, this group is stabilized by the aromatic system as well as via hydrogen bonds by the nitrogen atoms. Thus, dissociation is very unlikely to occur at environmentally relevant pH values of 4 – 9 and therefore in this pH range (4 – 9) the non-ionised form is dominant.			

Table 6: Overview of physicochemical properties of structurally related substance UV-P

Property	Description of key information	Value [Unit]	Reference/source of information
Physical state at 20°C and 101.3 kPa	*Visual inspection*	*Solid* *Slightly yellow powder*	*ECHA dissemination page*
Melting/freezing point	*DSC according to the ASTM E537*	*130 °C*	*ECHA dissemination page*

[2] COSMOconf conformer generation performed using the BP-TZVP-COSMO+GAS template; BIOVIA COSMOconf, Release 2021; Dassault Systèmes. http://www.3ds.com
[3] COSMOtherm property estimation performed using the BP_TZVP_21-parameterisation; BIOVIA COSMOtherm, Release 2021; Dassault Systèmes. http://www.3ds.com;
[4] 27.10. 2022, https://chemicalize.com/ developed by ChemAxon (http://www.chemaxon.com)
[5] ACD Percepta. ACD/Labs release 2019.2.1 (2019), Advanced Chemistry Development, Inc.

Boiling point	*DSC according to the ASTM E537*	*>398 °C (decomposition)*	*ECHA dissemination page*
Vapour pressure	*The measurement was based on heats of evaporation by DSC.*	*1.47 10^{-4} Pa (at 20 °C)*	*ECHA dissemination page*
Density	*OECD Guideline 109*	*1385 kg/m³ (at 20 °C)*	*ECHA dissemination page*
Water solubility	*OECD Guideline 105 (flask method)*	*173 µg/L (at 20 °C, pH 6.5)*	*ECHA dissemination page*
Partition coefficient n-octanol/water (log value)	*OECD Guideline 107 (shake flask method)*	*4.20 (at 25 °C, pH 6.3)*	*ECHA dissemination page*
	Estimated using COSMOtherm	*3.95*	*COSMOconf[6] COSMOtherm[7]*
Dissociation constant	*The calculation was using ACD/Labs software V8.14 (©1994 – 2010 ACD/Labs) cited in SciFinder Database*	*pKa = 8.15 (at 25 °C)*	*ECHA dissemination page*
	Calculated with Chemicalize	*Strongest acidic pKa = 9.53 (at 25°C)*	*Chemicalize[8]*
For UV-P ionisation can only occur at the hydroxyl group, as with is the only functional group that could act as proton acceptor/donor. However, this group is stabilized by the aromatic system as well as via hydrogen bonds by the nitrogen atoms. Thus, dissociation is very unlikely to occur at environmentally relevant pH values of 4 – 9. Although the pKa was computed to be 8.15, the dominant form in the pH range of 4 – 9 is the non-ionised molecule.			

[6] COSMOconf conformer generation performed using the BP-TZVP-COSMO+GAS template; BIOVIA COSMOconf, Release 2021; Dassault Systèmes. http://www.3ds.com
[7] COSMOtherm property estimation performed using the BP_TZVP_21-parameterisation; BIOVIA COSMOtherm, Release 2021; Dassault Systèmes. http://www.3ds.com;
[8] 27.10. 2022, https://chemicalize.com/ developed by ChemAxon (http://www.chemaxon.com)

2 Harmonised classification and labelling

2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (UV-329 (EC: 221-573-5; CAS 3147-75-9) has no harmonised classification in part 3 of Annex VI to the CLP Regulation.

The following hazard classes are contained in self-classifications notified to the CLP inventory[9]:

- Aquatic Chronic 4 (H413)
- Aquatic Chronic 3 (H412)
- Aquatic Chronic 1 (H410)
- Skin Irrit. 2 (H315)
- Eye Irrit. 2 (H319)
- STOT SE 3 (H335)
- Acute Tox. 4 (H312)
- Skin Sens. 1 (H412)

[9] https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/68877

3 Environmental fate properties

Considerations on chemical structure and properties and justification for read-across

UV-329 is part of a group of phenolic benzotriazoles which are also called UV-benzotriazoles. These substances share the 2-(2-hydroxyphenyl)-2*H*-benzotriazole moiety as a common structural feature. The intramolecular hydrogen bond between the hydroxy group and the nitrogen of the benzotriazole ring is essential for their function as UV absorbers (see Figure 1).

Information on some structurally related phenolic benzotriazoles is used in this dossier for a read-across or as supporting evidence. Thus, a brief general description of the group is given along with a more detailed discussion of the substances relevant for the assessment. A data matrix is given in the appendix to support the read-across.

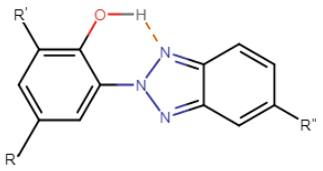


Figure 1: Structures of phenolic benzotriazoles

(R = alkyl, alkylphenyl; R‵ = H, alkyl, alkylphenyl; R‵‵ = H, Cl).

Due to the structural similarity of the substances, similar properties are expected which vary depending on the different substituents. In combination with the substituents described in Figure 1, the 2-(2-hydroxyphenyl)-2*H*-benzotriazole moiety common to all substances is associated with high stability and lipophilicity. Accordingly, water solubility is rather low and adsorption potential is expected to be high. This is confirmed by the available data (see Annex I and ECHA 2014a, 2014b, 2015a, 2015b). The substances are not volatile and volatilisation is not expected to impact degradation (see Annex I and ECHA 2014a, 2014b, 2015a, 2015b, ECHA 2022).

Generally, the alkyl substituents are considered to increase lipophilicity and to reduce water solubility – the larger the substituents, the more lipophilic the molecule. Furthermore, substituents in ortho position to the hydroxy group provide a steric stabilisation to the intramolecular hydrogen bond. Finally, the absence of substituents at the phenyl moiety might theoretically enable enzymatic attacks at the respective molecular sites.

The very high persistence and very high bioaccumulation of the structurally related substances UV-320, UV-328, UV-327 and UV-350 has previously been confirmed by their identification as SVHC substances due to their vPvB properties.

The main structural difference of UV-329 to the confirmed vPvB benzotriazoles is the lack of a substituent in ortho-position to the hydroxyl group. UV-P is another phenolic benzotriazole that shares this structural feature with UV-329. However, while the substituent in para position to the hydroxy group is a *tert*-octyl group for UV-329, it is a methyl group for UV-P. Thus, UV-P is less lipophilic than UV-329 and has a higher water solubility. It is expected to be adsorptive, but to a lesser extent than UV-329. The vapour pressure of UV-P indicates that volatilisation might occur in degradation tests or soil field studies (ECHA 2022). However, available data of a soil dissipation study do not support volatilisation of UV-P. In summary, bioavailability is expected to be slightly higher for UV-P than for UV-329.

According to their structural similarity, UV-329 and UV-P should have a comparable degradation behaviour. These expectations are supported by the available data, i.e. CATALOGIC predictions,

ready biodegradation tests and the soil dissipation study (see Annex I).[10] If the missing substituent had an impact on persistence, this impact would be expected to be similar for UV-329 and UV-P.

The impact of impurities is not considered relevant for a read-across from UV-P to UV-329: The data used for read-across refer to detection of UV-P in the environment.

As outlined above, the available data support the proposed similarity of UV-329 and UV-P in degradation properties (see Annex I).

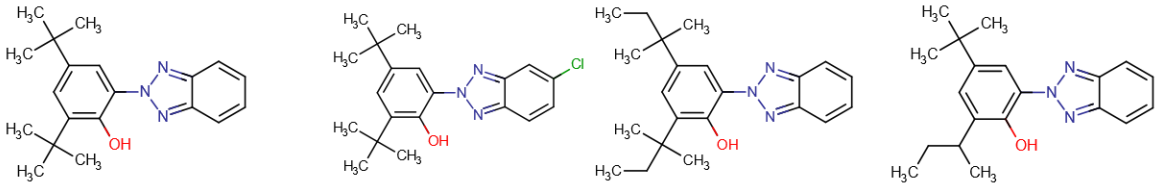


Figure 2: Structures the known SVHC substances UV-320, UV-328, UV-327 and UV-350 identified as vP.

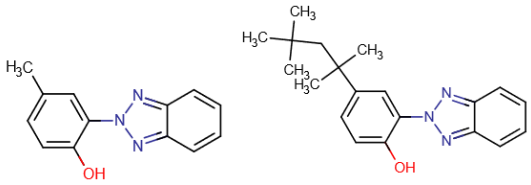


Figure 3: Structures of UV-329 and UV-P.

3.1 Degradation

A weight-of-evidence approach is applied for the assessment of persistence. The "Weight of Evidence/Uncertainty Analysis Template[11]" is applied to structure the data.

3.1.1 Problem Formulation

A weight of evidence determination according to the provisions of Annex XIII of REACH is used to assess if the substance meets the criteria for P/vP.

3.1.2 Documentation of search strategy & documentation/reporting of evidence

Information/evidence used in the approach includes:

- Experimental studies from ECHA dissemination site
- (Q)SAR results

[10] BIOWIN models even indicate a better degradability of UV-P. However, the other data are considered more reliable and more relevant.

[11] https://echa.europa.eu/documents/10162/17169198/template_for_weight_of_evidence_en.docx/eb183c2e-c360-cbce-7a58-ad2d1270e5bd

- Published Literature
- Research Project
- Information on structurally related substances

3.1.3 Collection and documentation of all information

In addition to the information from the registration dossiers, further studies were used for persistence assessment. These are documented in the Reference List.

The assessment includes field studies in sediment and soil. Various further monitoring data are available: UV-329 has been detected in wastewater treatment plants (Brorström-Lundén *et al.*, 2011; Casado *et al.*, 2013; Krzeminski *et al.*, 2017; Liu *et al.*, 2011; Liu *et al.*, 2012; Lu *et al.*, 2017; Montesdeoca-Esponda *et al.*, 2012; Montesdeoca-Esponda *et al.*, 2019; Ruan *et al.*, 2012; Thomas *et al.* 2014), in freshwater and marine sediments (for example Wick *et al.*, 2016a and 2016b[12]; Kameda *et al.*, 2011; Apel *et al.*, 2018; Brorström-Lundén *et al.*, 2011; Montesdeoca-Esponda *et al.*, 2019; Schlabach *et al.*, 2019), in surface water (for example Tashiro and Kameda, 2013; Khare *et al.*, 2023; Allan *et al.*, 2022, Brorström-Lundén *et al.*, 2011; Schlabach *et al.*, 2019; Allison *et al.*, 2018; Montesdeoca-Esponda *et al.*, 2019; Ruus *et al.,* 2022) and in soil (Brorström-Lundén *et al.*, 2011).

According to the PBT guidance, monitoring data may be indicative of persistence, but the impact of factors other than persistence needs to be taken into account (ECHA, 2017). In many cases, it is not clear to which extent the detection of a substance is due to slow degradation, and to which extent due to exposure. Actually, both factors are expected to have an impact for UV-329.

The available data from sediment cores (cf. section 3.1.4.2.4) and soil dissipation studies (cf. section 3.1.4.3.1) contain information on contamination after a certain time span. Thus, their informative value for persistence assessment is greater than that of the other monitoring studies. The other available studies are not considered further in the assessment because they contain a high degree of uncertainty and they are not considered to have a significant impact on the conclusion.

3.1.4 Assessment of quality of individual evidence

3.1.4.1 Abiotic Degradation

Table 7: Data on abiotic degradation for UV-329

Type of Evidence / Source Name - Reference	Relevance	Reliability	Adequacy
HYDROWIN[13]	The program reports that the substances do not belong to the substance classes for which hydrolysis is predicted. This result indicates the absence of "classical" hydrolysable groups, but does not prove that the substance is hydrolytically stable.	Phenolic benzotriazoles are outside the application domain; the program reports that they do not belong to the substance classes for which hydrolysis is predicted. Klimisch score 3	Adequacy to be considered within integration of evidence

[12] Wick et al. 2016a and 2016b contain both monitoring data and water sediment studies. While this section refers to the monitoring data, the water sediment studies are discussed below.
[13] 2017 U.S. Environmental Protection Agency. HYDROWIN v2.00 in EPISUITE v4.11. Result for all structures: The chemical structure does not contain typical functional groups that are susceptible to hydrolysis.

CATALOGIC Abiotic[14]	Screening information on aerobic abiotic degradation under OECD TG 301 C testing conditions. This model was calibrated on experimental data on parent chemicals and their transformation products. The model predicts abiotic degradation under the conditions of a ready biodegradability test, including hydrolysis as a major abiotic transformation pathway.[15]	CATALOGIC includes an automatic check of application domain.[16] The domain check accounts for molecular fragments and is stricter as compared to HYDROWIN. UV-329 is in the parameter range of the models. However, all substances are out of the applicability domain due to > 50% unknown structural fragments. Klimisch score 3	Adequacy to be considered within integration of evidence
Literature on hydrolysis of organic compounds	General publications on hydrolysis reactions of organic compounds without clear reference to phenolic benzotriazoles or benzotriazoles.	The publications indicate the absence of "classical" hydrolysable groups, but do not prove that the substance is hydrolytically stable. Klimisch score 2	Adequacy to be considered within integration of evidence

No experimental data on hydrolysis are available. The registration dossier for UV-329 cites literature that is not specific for UV-329, but deals with the hydrolysis of organic compounds in general. The HYDROWIN and CATALOGIC results are outside the applicability domain and thus considered as not reliable (Klimisch score 3).

The structure of UV-329 does not contain functional groups that are susceptible to hydrolysis, a finding that is supported by HYDROWIN results and by the general literature cited in the UV-329 registration dossier. The CATALOGIC Abiotic 301 C model predicts no abiotic transformation for UV-329 under the testing conditions of OECD TG 301 C. Thus, it is assumed that the substances are hydrolytically stable.

No data on photolysis or oxidation are available in the registration dossier for UV-329.

3.1.4.2 Biodegradation in aqueous media or aqueous environment

3.1.4.2.1 Estimated data

Table 8: Estimated data on biodegradation for UV-329

Type of Evidence / Source Name - Reference	Relevance	Reliability	Adequacy
BIOWIN[17]	Screening information on biodegradation. BIOWIN models 1,2,5,6 were calibrated based on screening tests for ready biodegradability, i.e., these models predict respective test results. BIOWIN 3 and 4 are based on results of an	The BIOWIN user guide recommends to assess the applicability domain using the Molecular Weight range and the fragment count, i.e., to check whether the occurrence of a given fragment in the predicted compound exceeds the maximum occurrence of that fragment per molecule in the training set. UV-329 is in the Molecular Weight range of the model's training sets.	Adequacy to be considered within integration of evidence

[14] OASIS CATALOGIC v.5.15.2.14. http://oasis-lmc.org/products/software/catalogic.aspx (November 2022); CATALOGIC Abiotic 301C v.01.08

[15] http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity.aspx (November 2022)

[16] Default setting tolerates a certain percentage of unknown fragments; adjusted setting: No unknown fragments accepted.

[17] 2017 U.S. Environmental Protection Agency. BIOWIN v4.11 in EPISUITE v4.11

	expert survey and predict the semi-quantitative timeframe for Ultimate and Primary Biodegradation, respectively. As these models were not calibrated to experimental data, but to an expert survey, BIOWIN 3 and 4 can be regarded to predict the result of a respective expert judgement. [18]	For UV-329, the molecular fragments used for calculation do not exceed the maximum number of such fragments per molecule observed in the training set. A search for structurally related structures in the available information on the training set was carried out: The training set for BIOWIN 1 and 2 does not contain benzotriazoles. BIOWIN 3 and 4 were trained on the structurally related substance 2-(2*H*-Benzotriazol-2-yl)-phenol[19] which enhances their applicability to the target substance. The predicted BIOWIN 3 and 4 values for this substance are in good agreement with the survey average. The training set (old) for BIOWIN 5 and 6 does not contain benzotriazoles, but 1*H*-Benzotriazole is in the validation set[20] and is correctly predicted as not readily biodegradable.	
CATALOGIC[21]	Screening information on biodegradation. The applied CATALOGIC biodegradation models were calibrated on data from OECD 301 test results and on information on biodegradation pathways. These models predict biodegradation under the conditions of a ready biodegradability test.[22]	CATALOGIC includes automatic check of application domain.[23] The domain check accounts for molecular fragments and is stricter as compared to BIOWIN. UV-329 is in the parameter range of the models. The structural domain check is stricter than that recommended for BIOWIN. All predictions for UV-329 are considered out of domain. However, the CATALOGIC 301C v12.17 model contains structurally related substances and if the applicability domain settings are adjusted to accept inert additions, UV-329 is in the domain of the model. It is noted that applying such a domain check to models like BIOWIN would result in all or almost all predictions being out of domain as well.	Adequacy to be considered within integration of evidence

3.1.4.2.1.1 BIOWIN

The BIOWIN software, as described in Table 8, yields the following results:

Table 9: BIOWIN results

Name	BIOWIN1 (Linear Model)	BIOWIN2 (Non-Linear Model)	BIOWIN3 ultimate	BIOWIN4 primary	BIOWIN5 (Linear MITI Model)	BIOWIN6 (Non-Linear MITI Model)	R11 screening
UV-329	0.3415	0.016	2.1165	3.1139	0.0704	0.0081	fulfilled

[18] 2017 U.S. Environmental Protection Agency. BIOWIN v4.11 in EPISUITE v4.11. User Guide.
[19] Substance no. 88, 2-(2H-Benzotriazol-2-yl)-phenol
[20] Substance no. 95147, 1H-Benzotriazole
[21] OASIS CATALOGIC v.5.15.2.14. http://oasis-lmc.org/products/software/catalogic.aspx (November 2022); CATABOL 301B v.02.07; CATABOL 301C v.02.08; CATALOGIC 301C v.12.17; CATALOGIC Kinetic 301B v.02.11; CATALOGIC Kinetic 301F v.15.18
[22] http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity.aspx (November 2022)
[23] Default setting tolerates a certain percentage of unknown fragments; adjusted setting: No unknown fragments accepted.

For the BIOWIN models 1, 2, 5 and 6 a result greater than or equal to 0.5 indicates that the substance is predicted to be readily biodegradable. A result below 0.5 indicates that the substance is not readily biodegradable. The BIOWIN user guide recommends to assess the applicability domain using the Molecular Weight range and the fragment count, i.e., to check whether the occurrence of a given fragment in the predicted compound exceeds the maximum occurrence of that fragment per molecule in the training set. UV-329 is in the Molecular Weight range of the model's training sets. For UV-329, the molecular fragments used for calculation do not exceed the maximum number of such fragments per molecule observed in the training set. While none of these models were trained on benzotriazoles[24], the validation set of BIOWIN 5 and 6 at least contained the structurally related 1*H*-Benzotriazole, which was predicted correctly as not readily biodegradable. The updated training and validation sets contain UV-320 (training set) and UV-P, UV-326 and 1H-Benzotriazole (all validation set). Therefore, BIOWIN 5 and 6 are considered more reliable than BIOWIN 1 and 2.

UV-329 is predicted to be not readily biodegradable by BIOWIN 1, 2, 5 and 6.

BIOWIN models 3 and 4 indicate the timeframe for ultimate and primary biodegradation, respectively. BIOWIN 3 and 4 were trained on the structurally related substance 2-(2*H*-Benzotriazol-2-yl)-phenol which enhances their applicability to the target substance. Predicted biodegradation timeframes for UV-329 are "Months" (ultimate) and "Weeks" (primary).

According to REACH Chapter R.11 (ECHA, 2017[25]), a substance is considered as potentially P or vP if the estimated probability value for Biowin 2 or 6 is below 0.5, and the estimated probability value for Biowin 3 is below 2.25 (to 2.75).

Based on the screening criteria from the guidance, UV-329 is considered to be potentially P or vP.

[24] Additional training set data from 2017 update to BIOWIN 5 and 6 were not available. If these contained benzotriazoles, they might improve the applicability of the model. In summary, this would not change the finding that BIOWIN 5 and 6 are assumed to be more reliable for these substances than BIOWIN 1 and 2.

[25] ECHA 2017. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Version 3.0.
https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f (accessed November 2022)

3.1.4.2.1.2 CATALOGIC

The CATALOGIC software includes an automatic applicability domain check. The following results were obtained:

Table 10: Results of CATALOGIC applicability domain check

Name	CATALOGIC 301C v12.17	CATABOL 301C v02.08	CATABOL 301B v02.07	CATALOGIC Kinetic 301B v02.11	CATALOGIC Kinetic 301F v15.18
UV-329	Out of Domain (5.26% unknown fragments)	Out of Domain (47.37% unknown fragments)	Out of Domain (63.16% unknown fragments)	Out of Domain (63.16% unknown fragments, out of mechanistic domain)	Out of Domain (57.89% unknown fragments)

If the default settings of the structural domain for the model CATALOGIC 301 C v12.17 are changed to allow for unknown fragments with inert additions (meaning fragments that are not expected to have an impact), then also UV-329 would be 100 % within structural domain. The training set of CATALOGIC 301 C v12.17 contains the structurally related phenolic benzotriazoles UV-P, UV-326, UV-327, UV-328, UV-350 and UV-320.[26] The training set of CATABOL 301 C v02.08 contains UV-327. In summary, CATALOGIC 301 C v12.17 is considered the preferred model , as its training set contains structurally related compounds and the substance shows the smallest deviation from the applicability domain.

The substance is predicted to not readily biodegradable. The CATALOGIC 301 C v12.17 model estimates 1% ultimate biodegradation in 28 days under OECD 301 C conditions.

The model additionally predicts ultimate and primary half-life values that are estimated based on extrapolated data from OECD 301 tests:

Table 11: CATALOGIC results for extrapolated ultimate and primary half-life values (expressed as days (d), months (m) and years (y))

Name	CATALOGIC 301C v12.17	
	Primary Half-life	Ultimate Half-life
UV-329	2m 28d	6y 9m 11d

Degradation kinetics observed in OECD 301 tests cannot be extrapolated to relevant conditions of REACH Annex XIII. Therefore, the respective results should be treated with caution. However, they may give an indication of the expected degradation kinetics under prolonged / extrapolated OECD 301 testing conditions.

CATALOGIC 301 C v12.17 predicts primary half-life values in the range of months and ultimate half-life values in the range of years for UV-329 thus indicating the substance screens as potentially P/vP.

[26] The training set results for UV-P, UV-326, UV-327, UV-328, UV-350 and UV-320 range from 0% to 8% ultimate biodegradation in 28 days. The respective predicted ultimate biodegradation in 28 days ranges from 0% to 1%.

3.1.4.2.2 Screening tests

Table 12: Screening tests on ready biodegradability

Type of Evidence / Source Name - Reference	Relevance	Reliability	Adequacy
Biodegradation Screening test OECD TG 301B (Testing Laboratory 1989) UV-329	Yes, according to test guideline protocol covering parameters required for assessment.	Klimisch score 2	Adequate study for biodegradation in water

A relevant and reliable screening test on ready biodegradability is available for UV-329. The OECD 301 B study from the registration dossier is considered as key study by the registrant. For the applied testing concentrations of 10.2 mg/L and 21.5 mg/L, 0 and 1% CO_2 evolution were observed after 28 days, respectively.

3.1.4.2.3 Simulation tests

Table 13: Water sediment simulation tests

Type of Evidence / Source Name - Reference	Relevance	Reliability	Adequacy
Water-sediment simulation study (non-GLP study) (Wick et al 2016a, Wick et al 2016b) UV-329	Yes, according to test guideline protocol covering parameters required for assessment.	Klimisch score 2	Adequate study for biodegradation in water sediment systems
Aquifer simulation study (Liu et al. 2013) UV-329	Relevant with restriction: Study design specifically targeted on aquifer systems, but study contains information on dissipation in a system of water and aquifer sediment. Methodology described, covering most parameters required for assessment	Klimisch score 2	Adequate study for biodegradation in aquifer systems

3.1.4.2.3.1 Study on UV-benzotriazoles in a river water-sediment system (Wick et al. 2016a, Wick et al 2016b)

Wick *et al.* (2016a, 2016b) investigated the biodegradation of UV-329 and other phenolic benzotriazoles[27] in an aerobic water-sediment study. This non-GLP study shows some variations to the OECD TG 308:

- Only one sediment was used with a relatively high TOC of 4.22% (fine texture, as required);
- the sediment was freshly collected at a site where previous contamination with organic chemicals may be expected[28], resulting in possible pre-adaptation of micro-organisms;
- volatiles were not collected.

Besides that, test conditions were in accordance with OECD TG 308: equilibration time 4 weeks; test temperature 20 °C; a pH range of 7.8 to 8.4 and O_2 concentrations of 8.8 to 9.1 mg/L. No information on redox potential is available. The test was conducted in 250 mL amber glass bottles that were filled with sediment and surface water at a ratio of 1:4 (w/w). 2 µg radioactively non-labelled test substance was dissolved in 100 µL methanol and spiked into the water phase. The initial test concentration for each individual benzotriazole was 10 µg/L in the supernatant, which was applied as a mixture.
Triplicate sampling was conducted at 0 (30 min), 2, 4, 8, 16, 25, 50 and 100 days after spiking; test duration was 100 days. Extraction of the freeze-dried sediment samples was done by pressurised liquid extraction (PLE) followed by silica clean-up. Analysis of the test substances was accomplished by LC-MS/MS measurements. An external standard calibration with 14 calibration points ranging from 0 to 100 µg/L and a linear fitting were used for quantification.

A parallel test with the reference substance Lenacil showed degradation of up to 50% on day 100 in the total system, thus demonstrating the microbial viability of the test system. Results for this test are presented in Figure 4.

For all analysed phenolic-benzotriazoles, a gap in the mass balance was observed at the beginning of the incubation period (until day 16), before the sorption equilibrium between water and sediment was reached (see Figure 4). This is probably due to an underdetermination of the dissolved concentrations due to a strong sorption of the substances on the vessel walls. In the subsequent period between day 16 and day 100, however, the recoveries were mostly within the range of the OECD Guideline 308 target (70-110%). From day 16 onwards, recoveries were relatively constant and the standard deviations were mostly below 20%.

The results confirmed the high sorption affinity of the phenolic-benzotriazoles. After 16 days, no substance could be detected in the water phase. Taking into account the quantification limits for the water phase[29,] it was found that >99.5% of the amount of UV-329 were sorbed

[27] This study also includes results for UV-327 and UV-350 that are discussed in the respective SVHC dossiers for these substances (ECHA 2015a, ECHA 2015b).
Different phenolic benzotriazoles were tested together in common test vessels. However, the impact of co-exposure to other phenolic benzotriazoles is expected to be negligible because:

- similar susceptibility to degradation is expected (see discussion on structure and properties above in section 3)
- no degradation was observed, i.e. enhanced biodegradation by co-metabolism was not significant under testing conditions
- no toxicity to microorganisms is expected based on the available data for the tested substances.

[28] River Rhine, Germany (Koblenz, harbor, river km 591.4)
[29] Limit of quantification in water (LOQ_{water}):
LOQ_{water} = 0.04 µg/L for UV-326, UV-329, UV-328 and UV-327 and UV-350
LOQ_{water} = 0.01 µg/L for UV-928 and UV-234

on the sediment. Due to the intensive extraction method, which was specifically designed to recover as much of the non-radioactive test substance as possible, there were practically no "non-extractable residues" (NER). No significant decrease in total concentration was observed for any of the phenolic benzotriazoles investigated during the 100 d incubation period. Therefore, no degradation kinetics could be modelled – any rate constants derived would probably not significantly differ from zero. The calculation of a half-life from such modelled rate constants would probably not be meaningful.

Consequently, the phenolic benzotriazoles were persistent in the experiment and the half-life was significantly higher than the observation period of 100 d. At an environmentally relevant temperature of 12 °C this corresponded to a half-life significantly larger than 212 days for UV-329.

The impact of the deviations from OECD TG 308 is not considered detrimental in this test because:

- the chosen sediment was appropriate for the test and as no biodegradation was observed, it can be considered as worst case;
- the possible pre-adaptation of micro-organisms did not lead to the observation of biodegradation;
- volatiles were not collected but recovery was sufficient and neither degradation nor volatilisation were observed.

This study is considered as reliable with restrictions.

In conclusion, the UV-329 rapidly and nearly completely adsorbs to sediment in a water/sediment system and hardly degrades over a period of 100 days (DegT50, sed. >>100 d).

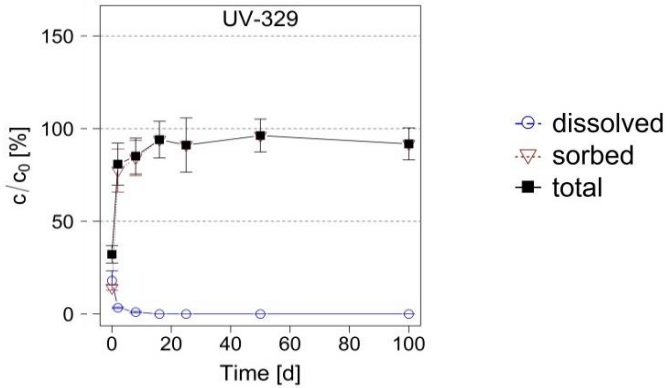


Figure 4: Average relative concentrations [% of initial concentration c0] of UV-benzotriazoles in sediment-water systems incubated for 100 d at 20 ± 1 °C in a climate cabinet (n=3). The error bars represent the standard deviation (taken from Wick et al., 2016a, supporting data).

3.1.4.2.3.2 Study on UV-329 in aquifers

Dissipation of a mixture of different UV-filters in an aquifer system was studied by Liu et al., 2013. Among the test substances there are UV-329 and another phenolic benzotriazole (UV-326); but also UV filters that are significantly more susceptible to biodegradation than

phenolic benzotriazoles, e.g. benzophenone-3.[30] All tested UV-filters were present in each treatment and the authors assume that interaction effects on degradation are negligible; however, co-metabolism could potentially have occurred. The study was conducted using in different treatments, one of which was aerobic. The other treatments referred to specific anaerobic conditions that are considered to have a lower relevance for persistence assessment. The aerobic treatment is reported in more detail as it is considered the most relevant part of the study for persistence assessment. The study appears to be well-conducted, but it simulates the fate of UV filters in aquifers rather than in ponds, rivers or in the sea. Hence, there are some characteristic differences to OECD TG 308:

- Only one sediment was tested (low TOC of 0.4%[31], coarse texture)
- Material for sediment was taken from the aquifer 5 m below the ground surface
- Groundwater sampled from a well nearby with a very low level of dissolved oxygen (0.4 mg/L)
- Smaller test systems & deviating water sediment ratio (5 g aquifer + 5 mL water)[32]
- The test system was not checked for transformation products.

As no information on equilibration time is available, it is uncertain whether and how equilibration was conducted. The aerobic treatment was conducted in a laminar flow chamber by opening the caps three times a day; however, no information on the level of dissolved oxygen during the test is available.

Anaerobic treatments were handled under exclusion of oxygen. These included an anaerobic control (i.e., anaerobic treatment without addition of reducing agents), and three reducing treatments. The reducing treatments were prepared by the addition of Na_2S (1 mM) and sodium lactate (10 mM). Either $NaNO_3$ (20 mM), Na_2SO_4 (20 mM), or Fe(III) citrate (20 mM) were added to generate nitrate-reducing, sulfate reducing or Fe(III) reducing conditions, respectively.

The incubation temperature was 20 °C and the initial concentration of each compound was given as 1 mg/L. A stock solution was prepared from the radioactively non-labelled test substances and methanol (concentration of each test substance 100 mg/L); this solution was used to apply the tests substances in the reaction tubes. The description indicates that the substances were pipetted into each test vessel with an applied concentration of 1 µg/g per substance in the aquifer sediment (50 µL stock solution). Triplicate sampling was conducted for each treatment at days 0, 7, 14, 21, 28, 35, 49, 63, and 77; test duration was 77 days. Extraction of the freeze-dried sediment samples was done by pressurised liquid extraction (PLE). Analysis of the test substances was accomplished by GC-MS/MS measurements. Based on sterile groundwater and aquifer material systems a recovery of 111% was determined for UV-329. Total numbers of culturable bacteria in each treatment were monitored on each sampling.

Sterile controls (aerobic and anaerobic) were autoclaved at 120 °C for 20 minutes on three consecutive days followed by treatment with sodium azide. The authors report that all tested UV-filters did not dissipate under sterile conditions.

The observed dissipation of UV-329 was faster in the aerobic treatments than in the anaerobic treatments.[33] For UV-329, the authors give

[30] According to the registration dossier, this substance is readily biodegradable but failing the 10 day window. It shows rapid dissipation in the aquifer test with a half-life of 5.3 days.
[31] This value is slightly below the range recommended in the OECD 308 TG.
[3232] OECD TG 308 outcome can be affected both by test vessel and system geometry and the associated water-sediment interface size (ECHA 2017). There is no specification of the vessel size or geometry in the test guideline, but the system geometry should be consistent with the range indicated in the OECD TG 308.
[33] For a discussion of the aerobic kinetics see below. The derivation of the anaerobic half-lives is not assessed further, but it is apparent from the graphs in the study that dissipation is indeed faster for the aerobic treatment. Potentially, another kinetic model might yield a better fit for the available data.

first-order dissipation half-lives of 34 days for the aerobic treatment. Dissipation half-lives for the anaerobic treatments are 51 days in the anaerobic control, 49 days under nitrate reducing conditions, 47 days under sulfate reducing conditions and 65 days under Fe(III) reducing conditions.

Aerobic treatment
In both the sterile and non-sterile samples, UV-329 was mainly present in sediment. In the sterile controls, the parent concentration in the test system remained constant during the test.

In the non-sterile treatments, a rapid growth of biomass was observed during the first 28 days, which might have been caused by the fast degradation of UV filters like benzophenone-3. During this initial phase of microbial growth, fast dissipation of UV-329 from the total system was observed, followed by a phase with significantly slower dissipation. The authors used first-order kinetics to calculate dissipation half-lives of 34 d for UV-329. A re-modelling conducted using the reported data (Cake 3.3, see Annex II) yielded acceptable statistics for some models but an unsatisfactory visual fit (see Annex II). The residuals confirm that a conclusion based on the data reported by this source should be made with caution as they are quite regularly distributed in every model. Due to these uncertainties, no modelled DT50 is preferred. Model results other than SFO reflect the slow dissipation observed after the initial phase of fast dissipation.[34] At an environmentally relevant temperature of 12 °C, this would correspond to half-life values that are larger, with temperature corrected slow phase DT50 values > 180 days.

As the substances were not radio-labelled, no information on the formation of non-extractable residues (NER) or transformation products is available. Based on the constant test concentrations in the sterile controls, no significant amounts of NER were formed in the sterile controls. As UV-329 does not dissipate in the sterile controls, its dissipation in the non-sterile tests appears to be associated with the presence of microorganisms.

The impact of the several deviations from the OECD TG 308 guideline on the study results is unclear. The following conditions may have contributed to the observed dissipation:

- In contrast to the study of Wick et al. (2016a, 2016b), this study has a sediment/aquifer with a low organic carbon content. A study on UV-384 shows rather fast dissipation of the structurally related metabolite M1 in the sediment with low organic carbon content, but DT50 >180 d for the sediment with high organic carbon content.[35] Thus, the type of sediment may impact the observed dissipation.
- Co-metabolism might have occurred due to the presence of further substances, some of which are more readily biodegradable than phenolic benzotriazoles.
- The formation of non-extractable residues (NER) could have occurred in the sediment phase in the presence of microorganisms. The

[34] Calculation results:

SFO DT50	DFOP		HS		FOMC	
DT50	DT50_fast	DT50_slow	DT50_fast	DT50_slow	DT50	DT90
34.5	25.8	>10,000	24.2	95.2	33.1	133

[35] A detailed description of this study is given in the SVHC support documents for UV-328, UV-320, UV-327 and UV-350 (ECHA 2014a, ECHA 2014b, ECHA 2015a, ECHA 2015b).

positive impact of microorganisms on NER formation is also reported in other studies (Botterweck *et al.*, 2014). The biomass growth promoted by other test substances might have contributed to this. No such effect was observed in the study of Wick et al. (2016a, 2016b). However, both studies show some characteristic differences.

- The test concentration of 1 mg/L or 1 µg/g per substance[36] seems rather high, compared e.g. to the water-sediment studies of Wick et al. (2016a, 2016b) (10 µg/L in the supernatant). This might require significant growth of microorganisms to occur in order to obtain as high a decrease in test substance by degradation as observed in the study. Growth based kinetics could potentially explain the unexpected results. As significant microbial growth has occurred, (pseudo) first-order kinetics may not apply and the study is not appropriate for derivation of a degradation half-life.
- The location from which aquifer and water were sampled possibly resulted in the use of a pre-adapted microbial population. Both were drawn in the vicinity of the waste water treatment plant of Bolivar, a district of Adelaide, Australia that feeds its water into reservoirs for irrigation use. Exposition of the withdrawal site with phenolic benzotriazoles may have occurred beforehand as a complete withholding of particles in a wastewater treatment plant seems unrealistic.
- Several deviations from the OECD TG 308 guideline may have an impact on the study results, although there is no straightforward interpretation to explain the observed results. However, as mentioned above, results of the re-modelling were not satisfactory and the derived DT50 values should be treated with caution.

This study is considered as reliable with restrictions. Derivation of DT50 values is challenging. Testing conditions were designed to simulate aquifers and the test system does not correspond to common water sediment studies.

[36] This corresponds to a total UV-filter concentration of 6 mg/L or 6 µg/g.

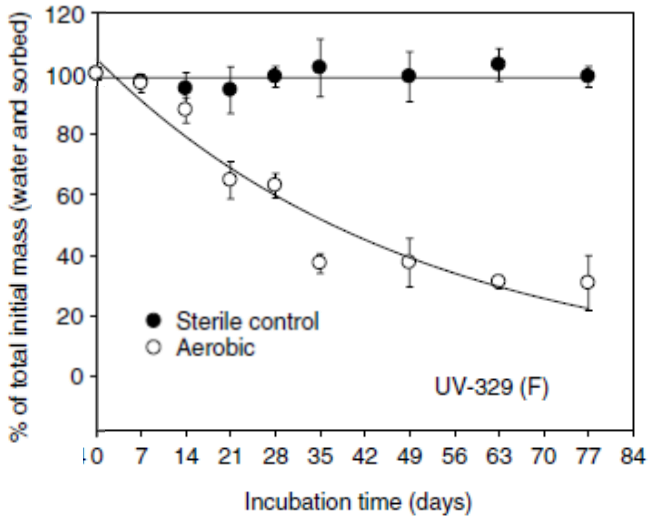


Figure 5: Dissipation of UV-329 (initial concentration of µg/g) in aerobic aquifer microcosms media. Error bars indicate standard deviations of the residual concentrations (n = 3) (Liu et al., 2013).

3.1.4.2.3.3

Not applicable.

3.1.4.2.4 Field data with information from sediment cores

Table 14: Field data with information from sediment cores and related studies

Type of Evidence / Source Name - Reference	Relevance	Reliability	Adequacy
Field study with dated sediment cores from Pearl River (Peng *et al.*, 2017)	Yes, covering parameters required for assessment.	Klimisch score 2	Adequate study for time profiles of sediment contamination.
Field study with dated sediment cores from Salem Sound & Narragansett Bay (Cantwell *et al.*, 2015)	Yes, covering parameters required for assessment.	Klimisch score 2	Adequate study for time profiles of sediment contamination with a structural analogue, indicative of persistence in sediments.
Field Study with sediment cores from Pawtuxet River (all) & Narragansett Bay (UV-P, UV-327, UV-328)	Yes, covering parameters required for assessment.	Klimisch score 2	Adequate study for time profiles of sediment contamination with a structural analogue, indicative of persistence in sediments.

(Reddy *et al.*, 2000)			
Field Study with sediment cores from Pawtuxet River (Lopez-Avila and Hites, 1980)	Supporting, covering some parameters required for assessment.	Klimisch score 2	Study for profiles of sediment contamination, adequacy limited because study is on structurally related substances and not on the target. Study supports findings of Cantwell *et al.*, 2015; Reddy *et al.*, 2000; White *et al.*, 2008.
Field Study with sediment cores from Narragansett Bay (Hartmann *et al*., 2005)	Supporting, covering some parameters required for assessment.	Klimisch score 2	Study for profiles of sediment contamination, adequacy limited because study is on structurally related substances and not on the target. Study supports findings of Cantwell *et al.*, 2015; Reddy *et al.*, 2000; White *et al.*, 2008.
Field Study with sediment cores from Providence River & Narragansett Bay (Pruell and Quinn, 1985)	Supporting, covering some parameters required for assessment.	Klimisch score 2	Study for profiles of sediment contamination, adequacy limited because study is on structurally related substances and not on the target. Study supports findings of Cantwell *et al.*, 2015; Reddy *et al.*, 2000; White *et al.*, 2008.
Field Study including sediment samples from Pawtuxet River (White *et al.*, 2008)	Supporting, covering some parameters required for assessment.	Klimisch score 2	Adequate study for sediment contamination with a structural analogue, supporting indication of persistence in sediments.
Field study including sediment cores from Providence River & Narragansett Bay but without profiles (Latimer and Quinn, 1996)	Supporting, covering some parameters required for assessment.	Klimisch score 2	Adequacy limited because study is on structurally related substances and not on the target. Measured data for phenolic benzotriazoles are used as markers but not given in the study; study includes data on production history of phenolic benzotriazoles in Cranston, Rhode Island. Study supports findings of Cantwell *et al.*, 2015; Reddy *et al.*, 2000; White *et al.*, 2008.
Field Study (Jungclaus *et al.*, 1978)	Supporting, covering some parameters required for assessment.	Klimisch score 2	Study for contamination of water and sediment by industrial wastewater, adequacy limited because study is on structurally related substances and not on the target. Study supports findings of Cantwell et al., 2015; Reddy et al., 2000; White et al., 2008.

Sediment cores and detections related to sediment cores include information on whether pollutants emitted in the past are still detectable after a certain time span. The original exposure is often unknown. Detection of a substance in sediment layers dating back years or decades ago can be considered indicative of high persistence. In these cases, the concentration profiles can be considered to reflect past exposure. The lack of detection of a substance does not necessarily mean that it is not persistent.

Data are available for the phenolic benzotriazoles UV-P, UV-326, UV-327, UV-328, and UV-320. UV-P is structurally very similar to UV-329 (see beginning of section 3 on structurally related substances).

Observed concentrations are particularly high near point sources like the former chemical production plant at Cranston in Rhode Island. While the lower concentrations influenced by diffuse entries are of higher environmental relevance, the contaminated sites in Rhode Island are well-examined by a variety of studies and the high concentrations allow to study the fate of the substances more comprehensively.

3.1.4.2.4.1 Sediment cores from Pearl River

The Pearl River Estuary in China is influenced by diffuse sources. Peng *et al.* (2017) sampled sediment cores and analysed them for several pollutants, including the phenolic benzotriazoles UV-329, UV-P, UV-326, UV-327 and UV-328. Available data on sedimentation rate were used to estimate the year of deposition.[37] Data on UV-329, UV-P and UV-327 were not reported in the publication. According to a personal communication with the study author, UV-329 was mostly not detected or close to the method quantification limit (MQL), whereas UV-P fluctuated at 0.41-1.45 ng/g dw in the first sediment core C1 and at 0.19-1.77 ng/g dw in the second sediment core C2, without obvious trends (Peng, 2023). As a comparison, the structurally related vP substances UV-327 and UV-328 were also detected. While UV-328 was reported in the publication as one of the more abundant UV absorbers, detected levels of UV-327 were about 0.74-1.84 ng/g dw along C1 and trace level in some layers of C2 (Peng 2023).

As historic exposure data are missing, the non-detection / low detection of UV-329 cannot be interpreted unambiguously. While all phenolic benzotriazoles are used as UV filters, their respective history of production and use in China is expected to differ. Available data from ready biodegradation tests and structural considerations do not indicate a significantly different biodegradation behaviour for UV-P and UV-329. Thus, it is assumed that exposure to UV-329 was lower than exposure to the other phenolic benzotriazoles, resulting in concentrations below or close to the analytical limits.

3.1.4.2.4.2 Sediment cores from Salem Sound

Cantwell *et al.* (2015) studied benzotriazole contamination in Salem Sound, Massachusetts, USA. Salem Sound is an urban estuary not influenced by nearby benzotriazole production sites; sediment cores were sampled in May 2010 near the South Essex Sewage District outfall pipe. Radiometric dating was used to develop an age model. One sediment core was analysed for several phenolic benzotriazoles, among them UV-P. Results are depicted in Figure 6. The sediment layers from 11 cm depth to the surface of the core are considered undisturbed, while deeper sediment layers are considered affected by physical disruption.[38] The sediment layers of detection of UV-P and other phenolic benzotriazoles date back several decades, indicating very high persistence in sediment.

[37] The available sedimentation rate is based on radiometric dating, see https://doi.org/10.1016/j.scitotenv.2007.05.043 .

[38] The presence of phenolic benzotriazoles in deeper sediment layers would correspond to decades prior to their production and thus, these findings indicate a past physical disruption of the sediment. However, based on radiometric data, there appears to be no significant post-sedimentation disturbance from 11 cm to the surface of the core.

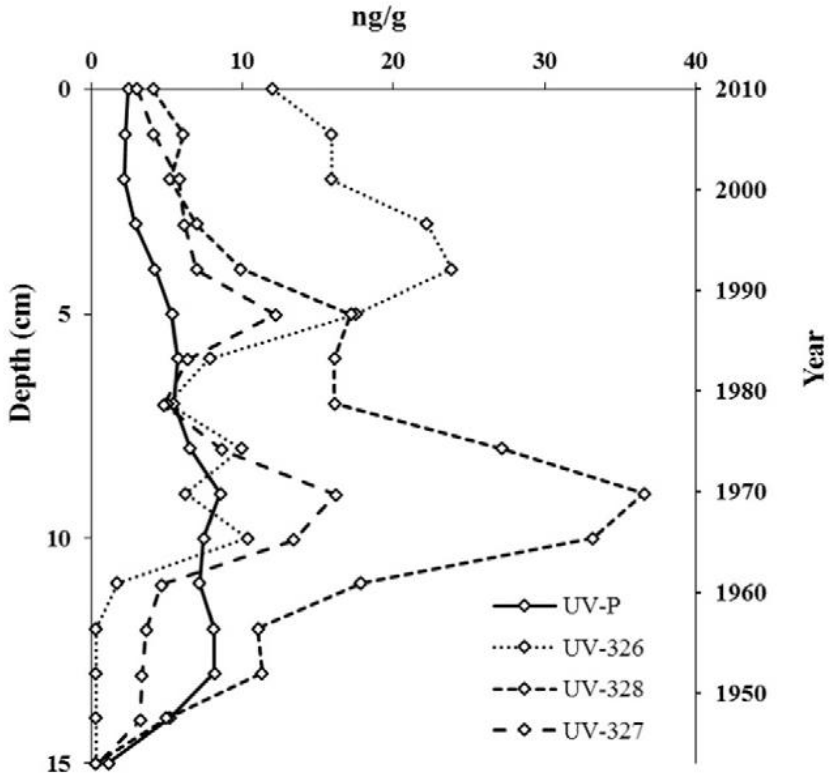


Figure 6: UV-Benzotriazoles in Salem Sound sediments (from Cantwell *et al.*, 2015).

3.1.4.2.4.3 Sediment cores and associated data from Pawtuxet River, Providence River and Narragansett Bay [39]

Until its closure in 1985 (Latimer and Quinn, 1996), a former chemical plant in Cranston, Rhode Island, USA discharged its industrial wastewaters in the Pawtuxet River. These wastewaters contained various chemicals produced at the plant, among them several phenolic benzotriazoles (Jungclaus *et al.*, 1978; Lopez-Avila and Hites, 1980). Consequently, contamination of Pawtuxet river sediments with phenolic benzotriazoles has been shown in several studies (Jungclaus *et al.*, 1978; Lopez-Avila and Hites, 1980; Reddy *et al.*, 2000; White *et al.*, 2008). Furthermore, these contaminated sediments from the Pawtuxet river are resuspended and transported, resulting in contamination of sediments downstream in the Providence River and the Narragansett Bay as well (Lopez-Avila and Hites, 1980; Pruell and Quinn, 1985; Latimer and Quinn, 1996, Reddy *et al.*, 2000; Hartmann *et al.*, 2005; Cantwell *et al.*, 2015).

[39] Except for the most recent study from Cantwell, these monitoring data have been discussed in the SVHC dossiers for UV-328, UV-320, UV-327 and UV-350 (ECHA 2014a, ECHA 2014b, ECHA 2015a, ECHA 2015b).

Based on this set of studies, environmental contamination of this area with phenolic benzotriazoles is quite well-documented. Hence, studies on sediment cores from this area can use a comprehensive data set to support the interpretation of the analytical results. Based on the available data, UV-P, UV-327 and UV-328 appear to be the most abundant (Reddy *et al.*, 2000).

UV-P, UV-327 and UV-328 were used as markers along with other characteristic contaminants; known data on start and stop of production were used to estimate the age of sediment core sections (Lopez-Avila and Hites, 1980; Pruell and Quinn, 1985; Latimer and Quinn, 1996; Hartmann *et al.*, 2005). While the dating of these sediment cores is partially based on the substances themselves, the agreement with other markers indicates that they are present in sediment layers deposited years / decades before sampling.

White and co-workers (2008) analysed sediments from Pawtuxet River that were sampled in 2003, i.e., about 18 years after closure of the chemical plant. No phenolic benzotriazoles were detected in the sample upstream the chemical plant, while several phenolic benzotriazoles were detected in the samples downstream, including UV-P.

Two studies on sediment cores from this area are of particular interest:

Sediment core from Narragansett Bay with radiometric dating

Cantwell et al. (2015) studied benzotriazole contamination in a sediment core from Narragansett Bay that was sampled in October 2007. Radiometric dating was used to develop an age model. The sediment core was analysed for several phenolic benzotriazoles, including UV-P (see Figure 9).[40] The results were compared with available data on production history: Sediment layers of detection of UV-P and other phenolic benzotriazoles date back to 1961 when first patents for some of these compounds were documented. Phenolic benzotriazoles are also detected in sediment layers that correspond to the years after the production stop in 1985. The authors explain this by the transport of resuspended sediments from Pawtuxet River to the Bay.

As expected, measured concentrations at Narragansett Bay were significantly higher as concentrations at the core from Salem Sound examined in the same paper and described above.

[40] UV-P, UV-326, UV-327, UV-328 and UV-320.

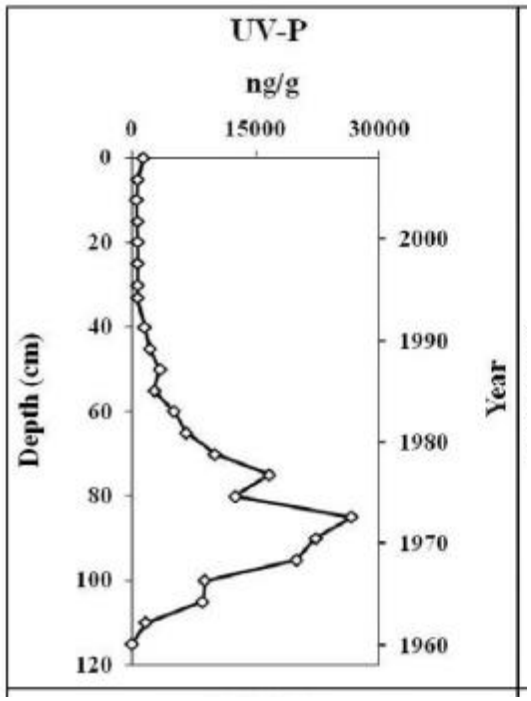


Figure 7: UV-P in Narragansett Bay(from Cantwell et al. (2015)).

Sediment cores from Narragansett Bay and Pawtuxet River

Reddy *et al.* (2000) analysed two sediment cores for UV-P and other phenolic benzotriazoles. Free and bound benzotriazoles were analysed and the ratio between free and bound benzotriazoles was discussed. The ratio of free to bound phenolic benzotriazoles varied depending on substance, sediment depth and location. The authors concluded that phenolic benzotriazoles without a substituent next to the hydroxyl group are more likely to bind to sediments. One of the cores had been sampled from Pawtuxet River and the other from Narragansett Bay. The core sections were not assigned to specific dates, but sedimentation rates of the sample locations were known and the authors estimate that the deepest core sections approximately date back to the start of phenolic benzotriazoles production, which they specify as about 1961-1970.

In the Narragansett Bay core, the most abundant phenolic benzotriazoles UV-P, UV-327 and UV-328 were significantly more concentrated than other phenolic benzotriazoles. UV-P was detected in all sections of the sediment core. The authors give a sedimentation rate of 0.3 cm/yr for the sampling location; the respective core was sampled in 1997. The deepest section of the core with a depth from 13 to 10 cm would thus correspond to the years from 1954 to 1964.

The sampling site at Pawtuxet River is closer to the chemical plant and the authors give a sedimentation rate of 2-3 cm/yr. The core was sampled in 1989. The deepest section with a depth of 52 cm to 50 cm would thus correspond to the years 1963-1964 (2 cm/yr) or to the year 1972 (3 cm/yr), respectively. UV-P was detected in all sections of the core.

Sediment core studies described above provide indirect evidence that UV-P and potentially UV-329 (based on a read-across from UV-P) can persist in sediments for several decades.

3.1.4.3 Biodegradation in soil

3.1.4.3.1 Soil dissipation studies

Table 15: Soil dissipation studies

Type of Evidence / Source Name - Reference	Relevance	Reliability	Adequacy
Soil dissipation study (Lai *et al.*, 2014a)	Yes, covering parameters required for assessment.	Klimisch score 2	Adequate study for dissipation in soil, indicative of persistence in soil
Soil dissipation study (Lai *et al.*, 2014b)	Yes, covering parameters required for assessment.	Klimisch score 2	Adequate study for dissipation in soil, indicative of persistence in soil

Lai and co-workers (Lai *et al.*, 2014a; 2014b) examined the dissipation behaviour of several phenolic benzotriazoles (UV-329, UV-P, UV-327, UV-328, and UV-326) in order to assess whether the application of biosolids as fertilisers in agricultural land might be a relevant pathway for environmental contamination.[41]

In the first study (Lai *et al.*, 2014a), dewatered sludge from a WWTP in Beijing was applied onto agricultural land in Shandong, China. The sludge was not further amended with reference substances or benzotriazoles meaning that all benzotriazoles were incorporated in it. In the first experiment (Treatment T1) this was done only once in May 2007 while in the second experiment (Treatment T2) application was repeated every year in October from 2007 until 2010. Each treatment consisted of application of the same dewatered sludge on four replicates. In addition, there was a control site where no treatments were conducted. In order to incorporate the sludge, the trial fields were ploughed to a depth of 20 cm. On the fields wheat and maize were cultivated.

Starting from October 2010 until October 2011, soil samples were taken monthly at a depth between 0 and 20 cm. Each sampling of the

[41] The first study (Lai *et al.*, 2014) has been discussed in the SVHC dossiers for UV-328, UV-320, UV-327 and UV-350 (ECHA 2014a, ECHA 2014b, ECHA 2015a, ECHA 2015b).

four replicates consisted of five subsamples that were mixed. Due to experimental problems this practice was stopped in winter and resumed in March 2011. The soil samples were extracted with methanol/dichloromethane (50:50, v/v) at 120 °C for 5 minutes in two cycles. Concentrations of the benzotriazoles were detected via GC-MS. The recovery in soil was 117% for UV-329. For UV-329 the limit of detection in soil was 0.25 ng/g and the limit of quantification in soil 0.84 ng/g.

At the beginning of the measurements (October 2010 to March 2011), considerable variability (i.e., a rise) of the concentrations was reported by Lai *et al*., 2014a. The authors attribute this to problems with obtaining a homogenous sample during the frost period or the degradation processes in samples during storage until extraction. No information is given if these were the reasons for the occurring variability and how this problem was finally solved. Beginning with March 2011 the problem was eliminated. In all the control samples only trace concentrations at the limit of quantification of UV-327 were detected, but other phenolic benzotriazoles were not found.

Due to the problem described above the authors performed a dynamic curve-fitting only between March 2011 and October 2011. They report the following times for field dissipation:

Table 16: Overview of reported DT50-values (dissipation in the field) by Lai *et al.* (2014a)

Substance	UV-329	
Treatment	**T1**	**T2**
DT50 [d]	129	98
Error [d]	28	16

The authors employed SFO-kinetics to derive DT50 values. As the treatments were carried out under highly similar environmental conditions, similar DT50-values are expected for T1 and T2. For UV-329, DT50 values of 129 days and 98 days were determined for treatment 1 and treatment 2, respectively.

A detailed discussion of this study is given in the SVHC support documents for UV-328, UV-320, UV-327 and UV-350 (ECHA 2014a, ECHA 2014b, ECHA 2015a, ECHA 2015b). .

A very similar study from the same authors on the same type of test soil at the same location is available (Lai *et al.*, 2014b). This study includes treatment groups with repeated biosolid applications every year (OT1, OT2, OT3, OT4), groups with biosolid applications only during the first year (NT2, NT3, NT4) and control sites. Field studies started in October 2006, with sampling conducted from October 2010 to October 2011.

The soil samples were extracted with methanol/dichloromethane (50:50, v/v) at 120 °C for 5 minutes in two cycles. Concentrations of the benzotriazoles were detected via GC-MS. The recovery in soil was 117% for UV-329. For UV-329 limit of detection was 0.09 ng/g and the limit of quantification was 0.30 ng/g.

The phenolic benzotriazoles were detected in all samples from sites with biosolid application, but not in the control groups. Concentrations of the target compounds increased from October 2010 to March 2011 – an effect observed in the other study (Lai *et al.*, 2014a) as well. Hence, in analogy to the approach from the related study, the authors performed dynamic curve fitting for the period of March 2011 to October 2011.

Table 17: Overview of reported DT50-values (dissipation in the field) by Lai *et al.* (2014b)

Treatment	Biosolid application [t ha^{-1}]	Substance UV-329
OT1	5 every year	91
OT2	10 every year	93
OT3	20 every year	97
OT4	40 every year	94
NT2	10 once	79
NT3	20 once	106
NT4	40 once	155

The observed dissipation shows variation with respect to the different treatments:

- For UV-329, modelled DT50 values in the different treatments ranged from 79 days to 155 days.

The results of these two soil dissipation studies have to be regarded as best cases for the disappearance in the environment as:

- they only reflect the warmer period of the year. Longer DT50s are expected during colder period of the year;[42]
- three (Lai et al 2014a) to four (Lai et al 2014b) years passed between (first) application and measurements, therefore potentially allowing microorganisms to adapt;
- only dissipation was monitored;
- NER were not considered at all.

[42] The average annual temperature of the site is 12.9°C but no information on seasonal variation is given. Furthermore, it is not specified whether the given temperature refers to air or soil. It can be assumed that the average temperature during the modelled timeframe was larger than 12°C and that a longer DT50 could be expected for the whole year.

The field studies of Lai *et al.* (2014a, 2014b) have some practical shortcomings: The concentrations of the different benzotriazoles in the sludge are missing and no initial concentration values for the different field trials after the first[43] applications of the biosolids are given. In addition, the limits of detection and quantification are quite high, at least compared to the concentrations found in some applications. To assess the method, it would also have been helpful to determine the level of NERs.[44] Furthermore, the concentration values during the sampling time varied: for unknown reasons there was a rise in concentration levels during the winter months. This was solved by not considering them in the kinetic simulation, which in turn lowers the number of data points for fitting. Finally, it would have been helpful to employ a substance with known DT50 value as a point of reference. A shortcoming for the use in this dossier is that the study gives information on primary disappearance only since none of the metabolites were determined.

Based on the above two field studies, it is concluded that UV-329 is persistent (and potentially very persistent) in soil (at least DT50 in soil >120 days).

3.1.5 Integration and Weighing of evidence (WoE analysis) and Application of Levels of Confidence

3.1.5.1 Abiotic Degradation

Based on general chemistry knowledge, UV-329 is not expected to be susceptible to hydrolysis. The registration dossier for UV-329 contains references to general texts about the hydrolysis of organic compounds that support this expectation. HYDROWIN does not find hydrolysable groups and CATALOGIC predicts no abiotic transformation under OECD 301 C testing conditions. Thus, all available lines of evidence support the assumption that the substance does not hydrolyse under environmental conditions.

Table 18: Integration and weighing of evidence for abiotic degradation

Type of Evidence	Consistency & Specificity	Likelihood/ Biological Plausibility	Temporality	Confidence / Strength of Evidence	Remaining Uncertainty
HYDROWIN	Prediction of hydrolysis, result consistent with general knowledge about hydrolysis of organic compounds	Plausible	Not relevant	low strength of evidence	Medium
CATALOGIC Abiotic	Prediction of abiotic transformation under OECD 301 C testing conditions; result consistent with general knowledge about hydrolysis of organic compounds	Plausible	Not relevant	low strength of evidence	Medium

[43] In case of repeated application, concentration values for the subsequent applications before October 2010 are missing as well.
[44] No NER were observed in the water sediment tests (Wick et al 2016a, 2016b). Reddy et al. (2000) analysed free and bound phenolic benzotriazoles in sediments. Their results indicate that phenolic benzotriazoles can bind to sediment and phenolic benzotriazoles without a substituent next to the hydroxyl group are more likely to form a bound fraction.
In summary, the sediment data indicate that NER might be relevant in some cases, especially for phenolic benzotriazoles like UV-329 that do not have a substituent next to the hydroxyl group.

References to Literature	General knowledge about hydrolysis of organic compounds	Plausible	Not relevant	High/Medium strength of evidence	Low
Conclusion from overall confidence	No hydrolysis under environmental conditions (High/Medium Confidence)				

The (Q)SAR predictions are not all valid as some of the predictions are not within the applicability domain. Thus, their overall weight is low. Based on general principles of chemistry, the chemical structure does not contain functional groups that are susceptible to hydrolysis. This finding is given the highest weight. The registrants of UV-329 included some general references on the hydrolysis of organic compounds which support this finding. In summary, it is concluded that the substances are expected to show no hydrolysis under environmental conditions with high confidence.

3.1.5.2 Biodegradation in aqueous media or aqueous environment

The (Q)SAR results can be considered as screening information and as prediction of ready biodegradability. Based on both EPISUITE and CATALOGIC results, the substances are not expected to be readily biodegradable.

While all CATALOGIC models predicted ultimate half-life values in the range of months or years, some predicted primary half-life values are in the range of days. However, the preferred model CATALOGIC 301 C v12.17 predicts primary half-life values in the range of months.

UV-329 was found to be not readily biodegradable in the available screening test.

For UV-329, no biodegradation after 100 days was observed in a water sediment study conducted at 20 °C, i.e., the respective DegT50 is >>100 days. The reference temperature for simulation tests is 12 °C[45], and degradation at 12 °C is expected to proceed even more slowly[46]. Application of the Arrhenius equation to extrapolate from 20 °C to 12 °C would result in a factor of about 2.12; i.e., the corresponding DegT50 at 12 °C would be >>212 days.

In an aquifer study (Liu, 2013) conducted at 20 °C, observed dissipation was larger than expected based on the other data. Application of first order kinetics yielded a dissipation half-life of 34 d for UV-329, respectively. Other kinetic models indicate lower dissipation in the slow

[45] ECHA 2017a. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Version 3.0, p. 59. https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f (accessed February 2022)
ECHA 2017b. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7b: Endpoint specific guidance. Version 4.0, p. 219, 221-222. https://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf/1a551efc-bd6a-4d1f-b719-16e0d3a01919 (accessed February 2022)
EC (European Commission). 2003. Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and Commission Directive (EC) 98/8 on biocides. 2nd Edition, Luxembourg: European Commission.EC (European Commission) 2006, p. 49 and 53.

[46] Application of the Arrhenius equation to extrapolate from 20 °C to 12 °C would result in a factor of about 2.12. This would mean that DT50 >>212 days.

phase. The corresponding DT50 values at 12°C would be higher.

UV-P is a substance with a particular high structural similarity to UV-329. UV-P and further phenolic benzotriazoles have been detected in sediment sections that date back years or even decades, both in samples downstream from a former point source and in samples from urban estuaries.
18 years after closure of a chemical plant UV-P was still detected in sediments sampled downstream the site, but not in sediment samples from upstream the site.

With exception of the aquifer study, all available lines of evidence support the assumption that UV-329 is very persistent in sediment.

Table 19: Integration and weighing of evidence for biodegradation in aqueous media or aqueous environment.

Type of Evidence	Consistency & Specificity	Likelihood/ Biological Plausibility	Temporality	Confidence / Strength of Evidence	Remaining Uncertainty
BIOWIN	Consistent predictions indicating the substances are not readily biodegradable	Plausible	Not relevant	Low strength of evidence	High
CATALOGIC	Consistent predictions indicating the substances are not readily biodegradable	Plausible	Not relevant	low strength of evidence	High
Biodegradation Screening test OECD TG 301B 1989, UV-329	Consistent experimental study indicating UV-329 is not readily biodegradable	Plausible	Not relevant	low strength of evidence	High
Water sediment simulation study (Wick A *et al.*, 2016a, 2016b)	Consistent experimental study indicating the substances are very persistent in sediment	Plausible	Not relevant	High strength of evidence	Low
Aquifer simulation study (Liu Y-S *et al.*, 2013)	Experimental study indicating the substance dissipates in an aquifer system under the specific conditions of the study	Limited plausibility: high stability of molecular structure and other	Not relevant	Low/Medium confidence; low strength of evidence	Medium / High

	, not consistent with other data; relevance for natural aquatic sediment systems questionable	experimental data on sediments show limited / no degradation			
Field study with dated sediment cores from Pearl River (Peng *et al.*, 2017)	Consistent monitoring study	Plausible	Not relevant	low strength of evidence	High
Field study with dated sediment cores from Salem Sound & Narragansett Bay (Cantwell MG *et al.*, 2015)	Consistent monitoring study indicating a structural analogue substance (UV-P) is very persistent in sediment	Plausible	Not relevant	medium strength of evidence	Medium
Field Study with sediment cores from Pawtuxet River (all) & Narragansett Bay (Reddy CM *et al.*, 2000)	Consistent monitoring study indicating a structural analogue substance (UV-P) is very persistent in sediment	Plausible	Not relevant	medium strength of evidence	Medium
Field Study with sediment cores from Pawtuxet River (Lopez-Avila V and Hites, RA 1980)	Consistent monitoring study indicating a structural analogue (UV-P) is very persistent in sediment	Plausible	Not relevant	low strength of evidence	Medium / High
Field Study with sediment cores from Narragansett Bay (Hartmann PC *et al.*, 2005)	Consistent monitoring study indicating a structural analogue (UV-P) is very persistent in sediment	Plausible	Not relevant	low strength of evidence	Medium / High
Field Study with sediment cores from Providence River & Narragansett Bay (Pruell RJ and Quinn, JG 1985)	Consistent monitoring study indicating a structural analogue (UV-P) is very persistent in sediment	Plausible	Not relevant	low strength of evidence	Medium / High
Field Study including sediment samples from Pawtuxet River (White *et al.*, 2008)	Consistent monitoring study indicating a structural analogue (UV-P) is very persistent in sediment	Plausible	Not relevant	low strength of evidence	Medium / High
Field study including	Consistent monitoring study indicating a	Plausible	Not relevant	low strength of evidence	Medium / High

sediment cores from Providence River & Narragansett Bay but without profiles (Latimer JS and Quinn, JG 1996)	structural analogue (UV-P) is very persistent in sediment				
Field Study (Jungclaus GA *et al.*, 1978)	Consistent monitoring study supporting findings from Cantwell *et al.* (2015) and Reddy *et al.* (2000)	Plausible	Not relevant	low strength of evidence	Medium / High
Conclusion from overall confidence	UV-329 is very persistent in sediment (High Confidence)				

As the (Q)SAR models are trained on test results on ready biodegradability or - in the case of BIOWIN 3 and 4 - on expert judgement, they are considered as screening information with a low weight in the WoE approach for the P assessment of UV-329. Their results are consistent with the other data. UV-329 could be considered in the BIOWIN domain when following the recommendations of the User Guide, though the models are not trained on phenolic benzotriazoles. The CATALOGIC models include an automatic determination of applicability domain, which is much stricter. UV-329 is not in the domain of any of the CATALOGIC models, though the preferred model includes some structurally related phenolic benzotriazoles in the training set.

The available screening test is considered valid and reliable. The results of this test are consistent with the other data. They are considered as screening information with a low weight in the WoE approach.

The water-sediment study for UV-329 shows slight deviations from OECD TG 308 but the impact of the deviations on the test is not considered detrimental. The results are consistent with the other data supporting the evidence that UV-329 is very persistent in sediment. The confidence level for this study is high and it is given the highest weight in the WoE evidence approach for the P assessment.

The aquifer study shows more dissipation than expected based on the other data. The study conditions represent aquifers rather than systems tested in common water sediment studies. Several deviations from the OECD TG 308 guideline may have an impact on the study results. Although there is no unambiguous interpretation to explain the observed results, results from a water-sediment simulation test on UV-384 and its degradation product M1 indicate that the applied sediment type may influence the observed dissipation. The value of the study for deriving information for water-sediment systems seems low. The confidence level for the aquifer study is low/medium and it is given a low weight in the WoE approach.

The confidence in the presence of UV-P in aged sediment sections that date back years or even decades is considered high. Monitoring data are used as supporting information in the WoE approach for UV-329. They are in line with the outcome of the water-sediment simulation study, the screening studies and the QSAR predictions as they point towards the persistence of UV-P and potentially UV-329 (based on results of the structural analogue UV-P) in sediments. Non-detection and low detection of UV-329 and UV-P in the Pearl River sediment cores is not considered as contradicting information, as it probably reflects lower historic exposure of the site. Furthermore, both substances were detected at least in low concentrations in some sections of the core.

In summary, based on a weight-of-evidence approach it is concluded that UV-329 fulfils the P and vP criteria of REACH Annex XIII for the sediment compartment (DegT50 in sediment>180 days).

3.1.5.3 Biodegradation in soil

3.1.5.3.1 Soil dissipation studies

The two soil dissipation studies indicate that both substances are persistent and potentially very persistent in soil. This is consistent with the available screening information, with data on hydrolysis and with data on biodegradation in water sediment systems.

Table 20: Integration and weighing of evidence for biodegradation in soil (see **Table 19** for screening data)

Type of Evidence	Consistency & Specificity	Likelihood/ Biological Plausibility	Temporality	Confidence / Strength of Evidence	Remaining Uncertainty
Soil dissipation study (Lai *et al.*, 2014a)	Consistent field study indicating the substances are persistent in soil	Plausible	Not relevant	Medium/high strength of evidence	Medium/Low
Soil dissipation study (Lai *et al.*, 2014b)	Consistent field study indicating the substances are persistent in soil	Plausible	Not relevant	Medium/high strength of evidence	Medium/Low
Conclusion from overall confidence	Persistent and potentially very persistent in soil (Medium Confidence)				

The ready biodegradability test and the QSARs show that UV-329 screens as P/vP (see Table 19 and section 3.1.5.2). These data are given a low weight.

The two soil studies are field studies and not equivalent to simulation tests. Their results describe dissipation. Confidence is medium/high and they are given a medium weight.

In summary, it is concluded that UV-329 fulfils the P criteria and potentially the vP criteria of REACH Annex XIII for the soil compartment (at least DegT50 in soil>120 days).

3.1.6 Uncertainty Analysis

No specific uncertainty analysis was considered necessary. The evidence was conclusive to decide on the vP properties of the substances.

The evidence was of good quality and confidence levels were high.

No additional information is considered necessary.

3.1.7 Conclusions: Summary and discussion of degradation

The screening criterion for persistence (P) is fulfilled for the substance. The results from the available screening study shows that this substance is not readily biodegradable. This is confirmed by the available (Q)SAR results.

Hydrolysis of the substance is not expected due to the absence of functional groups susceptible to hydrolysis.

In a water sediment simulation study at 20 °C, no degradation was observed after 100 days. At an environmentally relevant temperature of 12 °C this corresponded to a half-life significantly larger than 212 days.

Faster dissipation was observed in an aquifer test with specific conditions deviating from standard water sediment systems. The observed dissipation may be related to the different test conditions and the studied compartment compared to an OECD water-sediment simulation study.

UV-P is a substance with a particular high structural similarity to UV-329. UV-P and further phenolic benzotriazoles have been detected in sediment sections that date back years and even decades, both in samples downstream from a former point source and in samples from urban estuaries.

UV-329 is persistent (and potentially very persistent) in two soil dissipation studies.

As an overall conclusion, based on the above information used in a weight-of-evidence-approach, it is concluded that UV-329 meets the 'persistence' criterion (P) and the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of its persistent and very persistent (P/vP) properties in sediment (DegT50 > 180 days). Furthermore, UV-329 meets the 'persistence' criterion (P) and potentially the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of its persistent and potentially its very persistent (P/vP) properties in soil (at least DegT50 > 120 days).

3.2 Environmental distribution

3.2.1 Adsorption/desorption

The registration dossier for UV-329 contains (Q)SAR estimations of the adsorption potential. The KOCWIN program v2.00[47] was used to predict log K_{OC} values of 5.11 (MCI method) and 4.93 (log K_{OW} method) for UV-329. According to the information from the dossier, the substance is in the applicability domain of the models.

3.2.2 Volatilisation

Except for the vapour pressure given in section 1.4, no data are available on volatilisation. For screening purposes, the data matrix in Annex I contains QSAR predictions for Henry's Law constant. However, these QSAR results are not documented according to Anne XI of REACH and are the results should be treated with caution.
Based on the available data, volatilisation is not expected to impact degradation studies.

3.2.3 Distribution modelling

No data are available on distribution modelling.

3.2.4 Summary and discussion of environmental distribution

Based on the available log K_{OC} values, the substance is expected to adsorb to soil and sediment.

3.3 Data indicating potential for long-range transport

Not assessed.

3.4 Bioaccumulation

A weight-of-evidence assessment is carried out for bioaccumulation assessment. The "Weight-of-Evidence/Uncertainty Analysis Template[48]" is applied to structure the weight-of-evidence.

[47] KOCWIN v2.00 in US EPA EPISuite v4.11.
[48] https://echa.europa.eu/documents/10162/17169198/template_for_weight_of_evidence_en.docx/eb183c2e-c360-cbce-7a58-ad2d1270e5bd

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

3.4.1.1 Screening data

In addition to the available experimental data, the log K_{OW} of the substances was estimated using COSMOtherm. [49]

Table 21: Available log K_{OW} data of the substance

Log K_{ow}	Method
6.4	COSMOtherm
≥ 6.5	OECD TG 117
6.91	experimental, Do *et al.* (2022)

COSMOtherm is an implementation of the COSMO-RS method. This approach has proven useful to predict a wide range of physico-chemical properties like liquid/liquid equilibria or solubilities (Klamt, 2011). The method is not a (Q)SAR but combines quantum chemical calculations and statistical thermodynamics. Consequently, there is no applicability domain in the classical sense. It is applicable to a much broader range of substances and the applied parameters are not specific of functional groups or molecule types (Eckert and Klamt, 2002).

The log K_{ow} values are above the screening trigger value of 4.5 for bioaccumulation. Consequently, UV-329 screens as potentially B/vB.

3.4.1.2 Aquatic bioaccumulation data

3.4.1.2.1 Problem formulation

A weight of evidence determination according to the provisions of Annex XIII of REACH is used to identify if UV-329 meets the criteria for B/vB in aquatic organisms. For UV-326 a direct comparison with REACH Annex XIII criteria is possible due to the available standard information.

3.4.1.2.2 Collection and Documentation of all Information

Information/evidence used in the approach include:

- Experimental studies from endpoint study records from corresponding IUCLID Registration Dossiers and study reports
- Published Literature
- Research Project

[49] COSMOconf conformer generation performed using the BP-TZVP-COSMO+GAS template; COSMOtherm property estimation performed using the BP_TZVP_21-parameterisation;
BIOVIA COSMOtherm, Release 2021; Dassault Systèmes. http://www.3ds.com;
BIOVIA COSMOconf, Release 2021; Dassault Systèmes. http://www.3ds.com

Table 22: Collected information and documentation:

Source Name	Date of search	Type of information/evidence	Link/Reference	Keywords Searched	Reason for inclusion / exclusion from WoE approach
OECD TG 305-I UV-329 Study having medium weight	-	Experimental Study	ECHA Dissemination website and study report	-	N/A
Development of a bioaccumulation test using *Hyalella azteca* UV-329 Study having the highest weight	-	Experimental Study	Schlechtriem *et al.*, UBA TEXTE, no. 134/2022 https://www.umweltbundesamt.de/publikationen/development-of-a-bioaccumulation-test-using	-	N/A
"Bioaccumulation assessment of superhydrophobic substances." UV-329, Supporting Study	-	Estimated/Predicted	Goss and Ebert, UBA TEXTE, no. 40/ 2023 https://www.umweltbundesamt.de/publikationen/bioaccumulation-assessment-of-superhydrophobic	-	N/A
Published Literature: Several field studies UV-329: supporting evidence		Field Studies	See Table 26: UV-329 concentrations in aquatic biota samples from different field studies	-	N/A

3.4.1.2.3 Assessment of quality of individual evidence

Table 23: Assessment of quality of individual evidence

Type of Evidence / Source Name - Reference	Relevance	Reliability	Adequacy
OECD TG 305-I (aqueous exposure) UV-329 Study having medium weight	Study appropriate for investigation of aquatic bioaccumulation	Klimisch score 2 Reliable with restrictions (restrictions: experimentally derived uptake rate k_1 and steady-state not reliable and disregarded, k_2 values of low statistical confidence)	Adequate on basis of reliability and relevance
Development of a bioaccumulation test using *Hyalella azteca* UV-329 Study having the highest weight	New study protocol (A draft OECD TG for the *Hyalella azteca* bioconcentration test is currently under preparation and is scheduled to be adopted in 2024), appropriate for investigation of aquatic bioaccumulation	Klimisch score 2 Reliable with restrictions (restrictions: new test system, uncertainties regarding k_1)	Adequate on basis of reliability and relevance
"Bioaccumulation assessment of superhydrophobic substances." UV-329 Supporting Study	Model development to predict k_1 and BCF for *Hyalella Azteca*, Study appropriate for investigation of aquatic bioaccumulation	Klimisch score 2 Reliable with restrictions (restriction: further validation with experimental data necessary)	Adequate on basis of reliability and relevance
Published Literature: Several field studies UV-329: supporting evidence	Field study appropriate as supporting information to investigate the bioaccumulation	Klimisch score 2 Reliable with restrictions (limitations of field studies are mentioned in the respective section	Adequate on basis of reliability and relevance

3.4.1.2.3.1 Robust Study Summaries of Key Studies

OECD TG 305 with UV-329 (aqueous exposure)[50]

Study description and test conditions:

This study determined the bioconcentration potential of UV-329 in juvenile rainbow trout (*Oncorhynchus mykiss*) according to the guideline OECD 305-I (aqueous exposure). The fish were exposed via water in a flow through system at test concentration 0.2 µg/L and a dilution water control (no solvent control as all acetone was evaporated prior to adding water) over an uptake period of 28 days followed by a depuration period in clean water of 28 days. In the interest of animal welfare, this study used only one concentration of test substance to determine bioconcentration as recommended in the 2012 revised OECD 305 test guideline for non-polar organic chemicals. Scientific publications (Creton et al. 2013, Burden et al. 2014) provide compelling evidence that BCF values do not differ when multiple concentrations are tested. Concentrations in fish and water were determined on 7 occasions during uptake by measuring the total radioactivity. During

[50] ECHA Dissemination website and study report

depuration the concentrations in water were measured on 3 occasions and in fish on 6 occasions. No toxic effects (i.e. mortality) or changes in behaviour or appearance were observed in the test treatment organisms in comparison to the control group. The registrant resumes that over the entire test all water quality parameters were maintained within acceptable limits. However, differing from the guideline as TOC was only measured in the control group. The mean measured concentrations of the test substance during the uptake period in water was 0.19 ±0.01 µg/L. The water concentration was kept constant within ±20% of the nominal concentration, except on day 7 when the concentration briefly dropped to 0.079 µg/L due to a pump malfunction. As the test concentration drop was very brief it was not included in the mean measured calculation (Table 24). Total radioactive residues in fish were measured separately in edible (e.g., fillet) and non-edible (e.g., remaining carcass) portions and the whole-fish value was calculated from the weight-normalised sum of the individually measured portions. Measuring the concentrations in separate portions add uncertainties to the whole body BCF value. The bioconcentration factor was based on analyses of total radioactive residues in water and fish tissue thus also includes residues of possible metabolites of the test substance in the fish. The BCF calculated on the basis of these values therefore represents a worst-case assumption and might be lower if only the unmetabolized parent test substance is considered. Metabolite identification would certainly help to discriminate between metabolites, which may be slowly depurated (and thus contribute to the B-potential of the parent) and those that are rapidly excreted (e.g., glucuronide or sulfate derivates) from the body via liver, gall bladder or kidney (Arnot et al., 2018). UV 329 has a hydroxy group, which is relevant for phase II metabolism (i.e., conjugation with glucuronides and/or sulfates). This hydroxy group is less accessible as it forms a hydrogen bond to the nitrogen atom of the benzotriazole moiety. Substituted compounds may be less metabolized compared to unsubstituted phenolic benzotriazoles in rats (Waidyanatha et al., 2021). UV-329 is monosubstituted and metabolism in fish is expected to be in general lower than in rats. In conclusion, using the BCF values based on total radioactivity analysis for the B/vB assessment of UV-329 is considered acceptable as a worst-case-scenario.

Table 24: Test substance concentration in water and whole fish during uptake phase

Sampling time (day)	Concentration in water, mean value (µg/L)	Concentration in whole fish, mean value (µg/L)
0	0.181	-
3	0.188	20.0 ±4.7
7	0.218	46.8 ±12.0
10	0.188	46.2 ±6.3
14	0.181	32.1 ±6.1
21	0.201	35.8 ±2.4
24	0.180	46.8 ±4.0
28	0.181	71.8 ±5.2
Depuration		
1	0.004	55.9 ±10.8
3	0.001	23.4 ±5.4
7	0.001	10.4 ±2.6
14	-	8.2 ±1.5
21	-	4.7 ±2.5
28	-	3.3 ±1.1

Estimated results:

There was no statistically significant difference in fish growth rate between control and treatment group during the experiment, therefore data from both groups were combined to determine the overall growth rate (k_g) of 0.0132 day^{-1} for "growth-corrected" calculations. The lipid content of control fish sampled over the test period remained constant considering the variability of individual values and the lipid content from the end of the uptake period (4.1%) was used for lipid normalisation calculations.

The registrant concluded that the steady-state criterion in OECD TG 305 is fulfilled after 24 days and derived a lipid-normalised BCF_{ssL} of 461. According to the OECD TG 305 guidance a steady-state is reached in the plot internal body concentration (C_f) of the test substance in fish against time when the curve becomes parallel to the time axis and three successive analyses of C_f made on samples taken at intervals of at least two days are within ±20% of each other, and there is no significant increase of C_f in time between the first and last successive analysis. All this is only true if C_f of the last sampling point of the uptake period (day 28) is disregarded. However, the registrant concluded that the value must be accepted as plausible and therefore needs to be considered for steady-state derivation. Consequently, steady-state was not reached at the end of the uptake period as (1) there was an extreme intermediate drop in C_f between day 10 and day 20, (2) the three last C_f values of the uptake phase (day 21, 24, 28) are not within ±20% of each other, and (3) there is a significant increase between day 21 and day 28 curve. Therefore, the derived steady-state value is considered as not reliable.

The kinetic derived growth and lipid corrected BCF_{kgL} given in the study report is 458 L/kg. The corresponding experimentally derived uptake rate constant k_1 of 64 L/kg/day was below model expectation (Table 25) by an order of magnitude. Experimental artefacts were expected for the uptake period as there were problems maintaining solute concentration (further discussed by Goss & Ebert, 2023). TOC was only measured in the control group and not in the treatment group. Consequently, TOC concentration of the treatment group is unknown, which is not in accordance with the OECD TG 305 guideline. As UV-329 is a very hydrophobic substances TOC concentration has a strong influence on freely dissolved test concentration. Therefore, monitoring of TOC concentration during uptake phase is essential. Sorption of the test substance to organic matter may reduce its bioavailability and therewith result in an underestimation of the BCF. Consequently, a bioavailability issue could explain the extreme intermediate drop in C_f between day 10 and day 20. Therefore, the fitted k_1 value is considered as unreliable.

The depuration phase is considered to be reliable and subsequently derived k_2 values were used for BCF estimation together with estimated uptake rate k_1 (Goss *et al.*, 2018) using the OECD-BCF-Estimation-Tool. According to OECD 305 guidance document (OECD, 2017), it is recommended to use the tool to evaluate the results of fish feeding studies. The BCF estimation methods, however, are based on the depuration rate constant and are independent of the uptake rate and uptake route. Therefore, the tool can also be used to calculate BCF using depuration rates constants from studies with aqueous exposure. It should be noted that these calculated BCFs may be more uncertain than experimental BCFs due to the uncertainty in the k_1 prediction. The log Kow values of UV-329 (see section 3.4.1.1) are in the given range of the indicative applicability domain of method 1 (3.5 – 8.3) and method 2 (3 – 8.2) of the OECD-BCF-Estimation-Tool. Also, the used fish species is within the training set. For the prediction of a BCF it is not appropriate to take the mean value from all estimates derived in different ways. Therefore, the results of the OECD BCF estimation Tool (Version 2) are used in a weight of evidence approach by considering all results in a more general comparison with BCF trigger values. Growth corrected k_{2g} value of 0.168 day^{-1} (one compartment model) and 0.267 day^{-1} (two compartment model) were given in the study report. The report states "Since the depuration data demonstrate a biphasic pattern, the data were fit to a two-compartment kinetic model.". The different fits in Figure 8 support that observation. In the two-compartment model, however, additional parameters are used which leads to over-parametrisation if the sample size is not considerably increased. Furthermore, k_2 values from the study report are derived by a simultaneous fit of uptake and depuration phase. However, as

stated above the uptake phase in this BCF study is too uncertain to be used. Therefore, the data from the study report were only used for comparison. Based on the raw data k_2 was refitted by using the data from the depuration phase only. In dependence of the used method (one compartment model: untransformed, BoxCox transformed, ln transformed) k_2 value differ extremely and subsequently calculated lipid normalised and growth corrected BCF_{kgL} values ranged between 188 and 11782 (Table 25). The k_2 value based on BoxCox transformation reveals the best fit, related BCF values range around 2000 and indicate that the B criterion might be fulfilled. In summary, these BCF results are relatively uncertain as the statistical confidence of the k_2 fitting is in general low. This is due to the fact that the depuration phase is biphasic but a one compartment model was used for refitting. A biphasic model would not, however, led to higher confidence due to the low sample size, which would over-parametrisation. The log kow value 6.91 (Do *et al.*, 2022) was used for the calculation presented in Table 25. When using the remaining log K_{ow} values (Table 21) as input parameter the general picture did not change.

Table 25: k_1 and BCF_{kgL} estimates using OECD BCF Estimation Tool (Version 2) and experimentally derived k_2 values

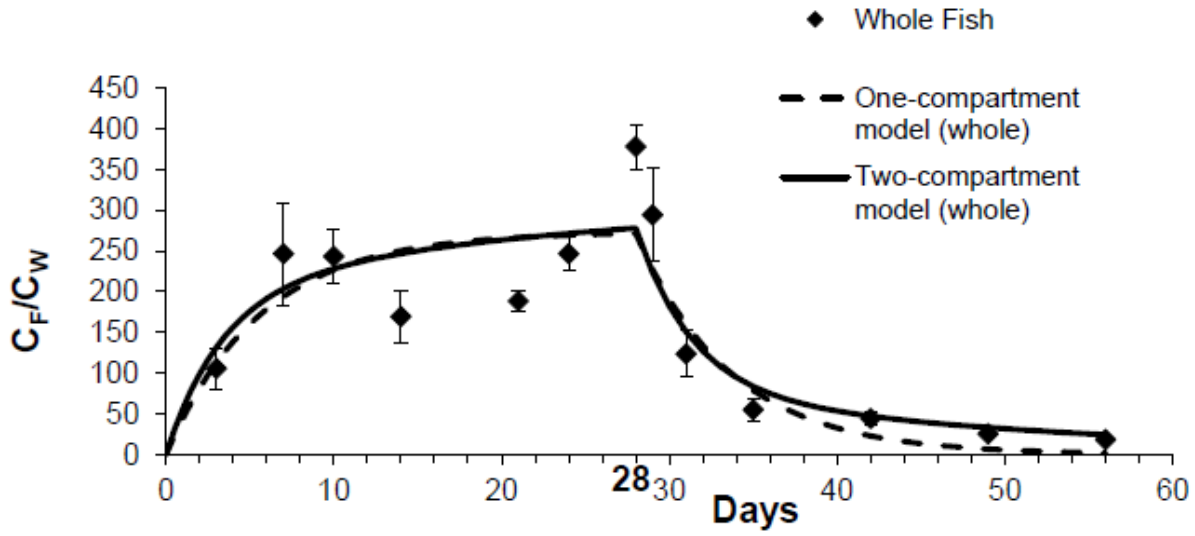
(Input parameter: mean weight at test start 2.24 g, uptake phase duration 28 days, Log K_{OW} 6.91 (Do *et al.*, 2022), growth rate 0.0132 day^{-1}, Mean fish lipid uptake end or depuration start (fraction) 4.1%, Mean fish lipid depuration end (fraction) 4.5%, Depuration phase duration 28 days).

K_{2g} (day^{-1})		**0.168***	**0.267****	**0.297*****	**0.092******	**Reference**
inputs for K_1	**K_1**	**BCF_{kgL}**				**Method 1**
weight	427	2957	1859	1438	4643	Hayton and Barron (1990)
weight	589	4079	2565	1984	6405	Erickson and McKim (1990a)
weight	584	4043	2542	1966	6348	Barber *et al.* (1991)
weight	377	2610	1641	1270	4098	Barber (2003) - observed
weight	605	4189	2634	2038	6578	Barber (2001)
weight	115	793	499	386	1245	Streit and Sire (1993)
weight	470	3255	2047	1583	5111	Erickson and McKim (1990b)
weight	397	2750	1729	1338	4318	Sijm *et al.* (1995)
weight	477	3300	2075	1605	5180	Barber (2003) - calibrated
log K_{ow}	1084	7501	4717	3650	11782	Tolls and Sijm (1995)
log K_{ow}	990	6854	4310	3334	10764	Spacie and Hamelink (1982)
weight, log K_{ow}	104	718	452	350	1128	Hendriks *et al.* (2001)
weight, log K_{ow}	56	386	243	188	606	Thomann (1989)

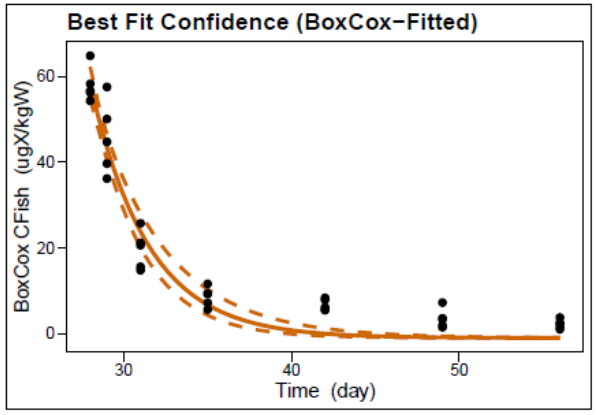
$K_{2\,g\,l}$						**Method 2**
0.168*	380	2631				Brookes and Crooke (2012)
0.267**	343		1493			
0.297***				1091		
0.092****					4568	

* one compartment model, untransformed, source: study report, ** two compartment model, source: study report *** depuration phase only, one compartment model, BoxCox transformed, source: recalculation provided by the NL CA following the public consultation, **** depuration phase only, one compartment model, ln transformed, source: recalculation by NL, see Figure 8

a)



b)



c)

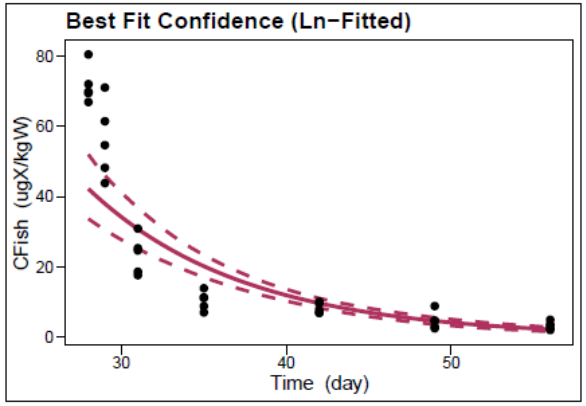


Figure 8: a) Plot of the one-and two-compartment uptake and depuration curves (source: study report), b) Best fit confidence of BoxCox transformed data from depuration phase only (source: recalculation by NL), c) Best fit confidence of ln transformed data from depuration phase only (source: recalculation by NL)

Conclusion:

This study is considered as reliable with restrictions.

BCF_{kgL} values for UV-329 were estimated based on experimental derived k_2 values and calculated k_1 values. Due to k_2 values of relatively low statistical confidence the calculated BCF values are uncertain. Beside this many BCF exceed the B criterion of 2000 and the study therefore indicates that UV-329 is bioaccumulative in fish.

"Development of a bioaccumulation test using Hyalella azteca" - Bioconcentration test with UV-329

This project was carried out to elucidate the suitability of the HYBIT test for testing an extended range of substance classes including difficult to test compounds and, if required, to further enhance the test concept. Among others, the bioaccumulation potential of highly lipophilic UV stabilisers were tested including UV-329. The solvent-facilitated and solvent-free application of the hydrophobic test compounds were compared.

Study description and test conditions:
This study by Schlechtriem et al., 2022 determined the bioconcentration potential of UV-329 in freshwater amphipods *Hyalella azteca* (HYBIT) using the test protocol as described by Schlechtriem et al. (2019). Male amphipods older than 2 months were exposed via water in a flow-through set-up. Solvent facilitated application of UV-329 was performed using a test media performed with a nominal concentration of 1 µg/L at a flow rate of 6 L/h. Uptake and depuration phases lasted 7 days. The test with solvent facilitated application was disregarded in the assessment due to significant mortality (> 20%) of the test animals.

In addition, a solvent free column-generated application of UV-329 was performed with a test substance concentration (time weighted average) of 0.208 µg/L at flow rate of 6 L/h, an uptake phase of phase of 5 days, and a depuration phase of 6 days. The proposed guideline suggests an extended exposure period of > 10 days for substances with high hydrophobicity (log K_{OW} above 6). The exposure period of the solvent free test with UV-329 was only 5 days because the test with solvent-facilitated application already provided clear indications that steady state conditions are reached already after 5 days of exposure. Mortality in this test was 15.9%, the water temperature was 23.8 to 24.9°C and the dissolved oxygen ranged from 98.1% to 102.8% (Schlechtriem, 2023; personal communication). Concentrations in amphipods and water were determined on 9 occasions during uptake phase and on 6 occasions during the depuration phase for the solvent-free application and analysed by LC-MS/MS.

The lipid content of the amphipods was determined at test start, end of the uptake phase and end of the depuration phase. A mean lipid content of 2.1 % was used for lipid normalisation calculations. In contrast to the BCF determination in fish, growth can be neglected in *H. azteca* BCF calculation due to the short duration of the study. The time-weighted average (TWA) concentrations of the test substance during the uptake period in water was 0.208±0.055 µg/L for UV-329.

Results:

The measured values of UV-329 in amphipods were within ±20% of each other from days 2 – 5, thus steady-state concentration was

reached and a BCF_{ss} of 7744 L/kg was derived. Normalised to 3% lipid the BCF_{ssL} was 11063 L/kg. The kinetic derived BCF_k value was 8352 L/kg, normalised to 3% lipid BCF_{kL} was 11876 L/kg. The corresponding experimentally derived uptake rate constant k_1 was 8288 L/kg/day and the derived depuration rate k_2 was 0.992 day^{-1}.

The test concentration in water was uncertain during the test as it was not always within the ±20% range (day 1: -27 %, day 5: +50 %). Even though, the tissue concentration reached steady-state already within the exposure period of five days. This is confirmed by the measurements (±20 % from day 2-5) and the high similarity of kinetic and steady state BCF of around 11000 L/kg. Therefore, no impact of the variability in water concentration and the shorter exposure period is expected.

To address remaining uncertainties especially for k_1 estimation from HYBIT test the project "Bioaccumulation assessment of superhydrophobic substances" was initiated (see study summary of Goss and Ebert (2023) below). Based on the project results we could conclude that the experimentally derived k_1 value of approximately 8000 L/kg/d for H. azteca and the subsequently derived H. azteca BCFs for UV-329 are plausible. This is also supported by k_1 values from HYBIT BCF tests with similar hydrophobic substances like e.g. Benzo(a)pyrene (k_1 of 6659 L/kg/d), PCB153 (k1 of 7884 - 14172 L/kg/d), PCB77 (k1 of 6618 L/kg/d; Schlechtriem et al., 2019).

Conclusion:

This study is considered as reliable with restrictions.

According to the PBT guidance, BCFs from *H. azteca* bioconcentration tests can be compared against the REACH Annex XIII criteria on B and vB properties (ECHA, 2023).

The experimentally derived BCF_{ssL} and BC_{FkL} values for *Hyalella azteca* are in the range of 11063—11876 L/Kg and thus exceed the vB criterion of 5000 in accordance with REACH Annex XIII.

3.4.1.2.3.2 Study summaries of supporting studies

"Bioaccumulation assessment of superhydrophobic substances"

This study of Goss and Ebert (2023) focuses on the highly hydrophobic UV stabilisers UV-234 and UV-329. These chemicals had been tested in *H. azteca* for their bioaccumulative potential before (Schlechtriem *et al.*, 2022), yet strong variations in the uptake rate constants k_1 were observed, not only between fish and *H. azteca*, but also between different experiments conducted in *H. azteca* for the same chemical. In *H. azteca*, k_1 for UV-329 ranged from 8288 Lw/kgorg/d (solvent-free test) to 66085 Lw/kgorg/d (solvent-facilitated test). Goss and Ebert (2023) consider the second value to be an experimental artefact due to the strong growth of biofilm in the solvent-facilitated test, which might have led to the uptake of contaminated diet. It was therefore not discussed any further in their study and not in this B assessment. To assess whether the increased k_1 as compared to fish are realistic, the authors developed a model to predict k_1 for *H. azteca* from the K_{ow} and molecular weight (MW). Furthermore, the authors evaluated the suitability of HYBIT for superhydrophobic substances.

A detailed literature search was undertaken to gather the physiological data necessary for model development, and to determine the relevant uptake/elimination processes. Data regarding the respiration rate and uptake efficiency of O_2 allowed the estimation of the ventilation rate constant, which resulted in quite similar values as estimated empirically for fish of the same weight. Empirical correlations developed for amphipods were used, or physiological data from similar amphipods were scaled down to estimate organ surface areas. Estimates for unstirred water layer thickness in water and blood correspond to assumptions made in literature, in case of blood assumed for fish. Although data on protein content in amphipods exist, binding kinetics and partition coefficients are yet unknown. For the calculations, the authors thus assumed proteins similar to albumin in fish, which might act as a carrier across the unstirred layer in blood for superhydrophobic compounds and thus facilitate transport. Having no data on blood flow in *H. azteca*, the authors simply assumed it to be insignificant for superhydrophobic chemicals due to facilitated transport by the albumin-like protein.

The authors also collected information on the test chemical UV-329. Experimental log K_{OW} from Do et al (2022) were not yet available and the other experimental data only indicated that log $K_{ow} \geq 6.5$. Predicted octanol/water partition coefficients varied widely between different prediction methods, resulting in a broad uncertainty in k_1 prediction. The authors decided to use the mean log K_{ow} for their calculations (6.5 for UV-329). These values are in very good agreement with new experimental data from Do et al (2022) (6.91 for UV-329).

The physiological data allowed the determination of relevant uptake processes: The amphipods were fed uncontaminated diet; therefore, the diet was excluded as a possible uptake path. The authors had a closer look at uptake via skin, because the area to volume ratio is higher for smaller animals. Yet, the total body area was estimated to be only marginally higher than gill area. Considering the chitin shell, additional cell layers, and an increased unstirred water layer as compared to the gills, this possible uptake path was deemed irrelevant. The authors thus identified uptake via gills as the important uptake path.

For the uptake via gills, another effect must be considered. The influence of chemical binding to organic matter (TOC) in water is very high for superhydrophobic chemicals. The bioavailable fraction may decrease by orders of magnitude, decreasing k_1 in turn. It is not yet clear whether for superhydrophobic chemicals, some fraction of chemical bound to TOC might still be bioavailable, i.e., whether de-/sorption kinetics are fast enough for the chemical to diffuse across the ventilation volume or unstirred water layer bound to TOC and then desorb before being absorbed by the gills. Yet, the authors assumed all chemical bound to TOC as not bioavailable. This assumption is considered appropriate as it is the usual approach (Arnot and Gobas, 2004).

Modelling k_1 revealed the unstirred layer in water and the ventilation rate as the main resistances for the uptake via gills. Within uncertainties, modelled k_1 values corresponded well to experimental values from literature, i.e. for UV-329 experimentally derived k_1 (solvent-free test) and modelled k_1 values are nearly the same. A slight overestimation of k_1 for chemicals with log K_{ow} below 6.5 (i.e. not for UV-329) was observed, which could be due to the absence of blood flow in the modelling, which might be a limiting factor for low K_{ow}. The resulting k_1 for H. *azteca* are indeed higher than k_1 values for fish, due to the increased ventilation rate per body weight in *H. azteca*.

The authors finally concluded that experimentally measured k_1 in *H. azteca* are quite plausible. However, they considered data in the superhydrophobic range as too sparse and K_{ow} uncertainties too high to conclusively validate the prediction method or the experimental data. Due to the availability of new measured log K_{OW} data for UV-329 (Do et al, 2022), at least the respective log K_{OW} uncertainty decreased

since the author's conclusion.

In summary, the project results confirm that (1) the experimentally derived k1 value of approximately 8000 for H. azteca is plausible, (2) k1 in H. azteca is expected to be higher than in fish due to increased ventilation rate, and (3) subsequently derived H. azteca BCF for UV-329 is plausible. Uncertainties of the developed prediction model due to limited availability of experimental data for validation need to be kept in mind.

Conclusion:

The project results support the reliability and plausibility of the experimentally derived BCF values for UV-329 with *Hyalella azteca* which are > 5000.

3.4.1.2.3.2.1 Field Data

Table 26 summarises several field studies providing measured organism concentration of UV-329. The study list is supposed to be not complete and more studies might be available. This overview, however, shows that UV-329 is found in several fish and some other species in different regions of the world. Partial high concentrations in muscle tissues give evidence, that UV-329 accumulates in fish in the environment. Partially, these studies did not detect UV-329 (e.g., Langford *et al.*, 2015), what is no contradicting evidence but rather indicates that UV-329 may so far not entered every region of the world. Further explanation for absence of detection can be that LOD/LOQ values may set too high or analytical issues.

The bioaccumulation potential of UV-329 is supported by BAF values for fish > 5000 from the study of Castilloux et al. (2022, see Table 30). The study is, however, of high uncertainty as water samples were taken 2 years after fish sampling. Fish BAF values from Wang et al. (2022), Vimalcumar et al. (2018) and Peng et al. (2020) are below 2000 (see Table 30) and therefore are supposed to not support a B or vB conclusion. Such field studies, however, are connected to lot of uncertainties as described below. Furthermore, UV-329 is a very hydrophobic substance why bioavailability may to some degree influence the observed BAF by overestimation of the water concentrations (total vs. dissolved). For the study of Peng et al. (2020) it should be noted that BAF values are based on muscle tissue and these have in general a rather low lipid content, whole body BAFs are therefore expected to be higher (> 2000).

Available TMF values (Table 27) again gave an unclear picture regarding bioaccumulation assessment. The TMF estimated in the study of Peng et al. (2017b) is > 1, two other TMFs do not significantly differ from 1 and the TMF from the study of Wang et al. (2022) is far below 1. These field studies are connected to the same uncertainties like the BAF studies (see next paragraph) and finally would in any case not override the conclusion based on standardized laboratory test like OECD 305 or HYBIT.

Uncertainties of these studies are considered to be medium and sometimes high mainly due to: (1) sampling of different species with differences in habitat, feeding, digestion, metabolism, age, sex, size, weight and lipid content (2) different locations with different fluctuating and unknown exposure scenarios under different environmental conditions (3) methodical differences in sampling, extraction and analysis

(4) incomplete documentation. Therefore, the measured values are not directly comparable with each other and therefore only used in a more general way as supporting evidence in the weight-of-evidence assessment.

Table 26: UV-329 concentrations in aquatic biota samples from different field studies

Reference	Species	Location	Date	Age	Sex	Size & weight	Sampling	Storage, extract, analysis,	Lipid content [%]	Concentration of UV-329	Frequency of detection [%]
Brorström-Lundén et al. (2011)	fish (perch)	2 urban sites and 2 background sites in Sweden	09/2009 (urban), 03-04/2010 (back-ground)	N/A	N/A	N/A	N/A	frozen (-18°C), freeze dried, thawed, extracted with methanol using a soxhlet apparatus; LC/LC-MS/MS	N/A	[µg/g dw] 1-2.5	75
Castilloux *et al.* (2022)	Lake sturgeon (n=15),	Lake St. Louis (LSL), Montreal	06/ 2018	16-25 years	M & F	94-129.5 cm, 4.2-12.5 kg	Fish tissues, on-sight (liver, muscle)	Frozen in field (-20 °C), ultrasonic assisted extraction with hexane/ dichloromethane, GC-MS	Liver: 1.9±1.1, muscle: 0.11-14.1	[ng/g ww] liver: ND, muscle: ND-41	Liver: 0 muscle: 20
	Northern pike (n=32)	Iles des Boucherville (IB), Iles Vert (IV) -> upstream and downstream of Montreal's WWTP	05/ 2016	2-7 years	M & F	43.5-93.0 cm, NA	Fish tissue, on-sight (liver, muscle, brain, blood plasma)		Liver (IB): 1.9±1.1, muscle (IB): 0.75±0.8, brain (IB): 1.65±1.3, Liver (IV): 2.29±1.55, muscle (IV): 0.27±0.2, brain (IV): 1.58±0.4	[ng/g ww] Liver (IB): ND-214, muscle (IB): ND-<MQL, brain (IB): 0, blood (IB): ND-19 (median: 5.5), liver (IV): ND, muscle (IV): ND-<MQL (median: 4.5), brain (IV): ND, blood (IV): ND-17 (median: 7.5)	Liver (IB): 38, muscle (IB): 44, brain (IB): 0, blood (IB): 88, liver (IV): 0, muscle (IV): 64, brain (IV): 0, blood (IV: 86
Kim *et al.*	20 fish	Manila Bay (local fish	01 &	N/A, juvenile	N/A	8.5-31.0	Fish tissues	polyethylene bags, transported on dry	0.13-2.6	[ng/g lw]	muscle:

(2011)	species (n=58)	markets)	06/2008	, adult		cm, 3.4-531 g	(muscle)	ice, frozen (-25 °C), High Speed Solvent Extractor, hexane/acetone, ultra-fast LC-MS/MS		Muscle: ND-39.4	41
Lu et al. (2018)	Lake trout (n=35),	Great Lakes of North America	2014	4.6-6.0 years	N/A	42.1-71.3 cm, 748-4815	fish were anesthetized in ice water, kept frozen until processed, whole carcasses homogenizing	ultrasonic assisted extraction with hexan/dichloromethane, UPLC-MS/MS	9.6-23.3	ND	ND
Lyu *et al.* (2022)	17 fish species	Lake Chaohu, China	09/2016	N/A	N/A	7.8-63.3 cm, 10.8-4178 g	Fish tissue (back muscle), whole body for small species	Aluminium foil, frozen in field, stored in low-temperature box for transport, freeze-dried, stored (-20 °C), extract in an ultrasonic bath, GC	7.6-18.0	[ng/g dw, mean] Muscle: 0.64 – 6.65	100
	2 shrimp species					N/A	Head and shell removed, rest reserved		9.2-10.4	[ng/g dw, mean] 1.03 – 2.19	
Langford *et al.* (2015)	Atlantic cod (n=15), northern shrimp (n=15 bulk samples of 50-60 individuals), common shore crab (n=15 bulk samples of 10-13 individual	Oslofjord, Norway	08/2016	N/A	Fish: M & F	Cod: 43-82 cm, 0.76-8.5 kg	Fish tissue (liver), shrimps were peeled	Frozen within 4 h of collection, stored at -20 °C, accelerated solvent extraction, hexane/dichloromethane, LC-HRMS & GC-HRMS	N/A	ND (LOQ: 25 ng/g ww)	0

	s)										
	burbot (n=15), perch (n=15), whitefish (n=15)	Lake Mjøsa, Norway	06-08/2016		N/A	N/A	Fish tissues (muscle, stomach, intestine, liver)		4.4, 1.6, 1.3	ND (LOQ: 25 ng/g ww)	0
Montesdeoca-Esponda *et al.* (2020)	3 fish species	3 different marine outfalls in Grand Canaria Island (Spain)	07/2016, 10/2026, 04/2017, 07/2017, 01/2018, 04/2018	N/A	N/A	N/A	Fish tissues (muscle, viscera	Freeze dried, microwave extraction, acetonitrile, UHPLC-MS/MS		[ng/g dw] Muscle: ND Viscera: 1.34-10.7	
Peng *et al.* (2017b)	17 fish species	Pearl River Estuary, south China	04/ & 10/ 2013	N/A	N/A	8.4-49.3 cm, 5-206 g	Fishes were skinned, only muscles used, 2-3 individuals were pooled for each species	aluminium foil and ziplock polyethylene bags, on ice during transport, freeze-dried, stored at -20 °C, ultrasonic assisted extraction with ethyl acetate/ cyclohexane, ultra-high liquid C-MS, stabile isotopes abundance of carbon & nitrogen	Muscle: 1.36-4.21	[ng/g lw] muscle: ND-27.6	Muscle: 88
Peng et al. (2015)	red snappers (mari-culture) 8 wild fish species, squilla, squid & whelk (wild)	Pearl River Estuary	04/2013 (wild species), N/A (maricult ure)	N/A	N/A	N/A	big red snappers: separate collection of filet and belly wild species: whole body (skinning of fishes & squids, deshelling of squillas, only carcasses used without heads and internal	frozen (-20°C), freeze-dried, ground, homogenized. extracted by ultrasonic-assisted extraction, purified by gel permeation chromatography (GPC) coupled with silica gel column fractionation, determined by ultra-high performance liquid chromatography coupled with tandem mass spectro metry	fish lipid content from 2.5 (arrow fish) to 12.0 (pomfret); 8.2 (goby); 19.7 (squid); 18.7 (squilla); 8.7 (whelk)	[ng/g dw] goby: 0.105 squilla: 0.225 not detected in other species	18 (wild species); 0 (mari-culture)

							organs)	(UHPLC–MS/MS)			
Peng et al. (2020)	9 fish species (n=174)	Pearl River Estuary, south China	Several sampling times between 10/2014 and09/2016	N/A	N/A	12.5-49.0 cm, 30.6-1232.4 g	Fishes were skinned, fish tissues were measured (muscle, belly fat, livers, swimming bladders, ovaries	aluminium foil and ziplock polyethylene bags, on ice during transport, freeze-dried, stored at -20 °C, ultrasonic assisted extraction with ethyl acetate/ cyclohexane, ultra-high liquid MS-MS, stabile isotopes abundance of carbon & nitrogen	N/A	N/A	85.9
Schlabach et al. (2022)	3 fish species (n=23)	Urban and remote arears in the Nordic countries	N/A	N/A	N/A	N/A	-	Aluminium foil, plastic bag, freeze at -18 °C samples were extracted twice with organic solvents in an ultrasonic bath, GC-MS	N/A	Only found in cod liver: <0.06-2.78	Cod liver: 60
Tang *et al.* (2019)	6 fish species (n=43)	Lake Chaohu, China	09/ 2016	N/A	N/A	24.6-68.0 cm, 0.32-5.45 kg	Fish tissue (gill, liver, muscle) samples from 2-3 individuals were pooled	Aluminium foil, frozen in field, thaw naturally in laboratory, freeze-dried, stored at -20 °C, ultrasonic assisted extraction with acetone/n-hexane, GC-MS	muscle7.6-18 liver: 9.2-25 gill: 16.8-30.4	[ng/g dw] muscle: <0.19-15.86 liver: 0.92-12.1 gill: 0.85-4.54	Muscle: 88
Vimalkumar *et al.* (2018)	6 fish species (n=14)	2 rivers (Kaveri (K), Vellar (V)), India	11/2015 (wet season), 05/2016 (dry season)	N/A	N/A	N/A	muscle	Frozen (-20 °C), liquid-liquid extraction, hexan/ dichloromethan, GC-MS	N/A	[ng/g ww] muscle: 0.6-28 (7.4±3.6)	K: 100 V: 100
Wang et al. (2022)	14 fish species, 6 benthic invertebrate species (10-20	South Korea	01-02/2016	N/A	N/A	Fish: 9.3-74.0 cm, 4.5-1376 g Invertebrates: 3.6-73.1 cm,	homogeneous composite sample was prepared for each species by mixing equal	extracted by ultrasonic-assisted extraction with dichloromethane and hexane, determined by HPLC-MS	fish: 5.02 (median) 1.5-27.6 (range) invertebrates: 2.38	[ng/g lw] magnitude 10^0 to 10^1, median ≈ 10 (fish and	>50

	individual organism per species)					6.1-192 g	amounts of each organism		(median), 1.1-5.9 (range)	benthic in-vertebrates, values only given in graph) (estimations from a figure as values are not given in numbers)	
Zhong et al. (2019)	carp, black carp	purchased from local supermarket (China)	N/A	N/A	N/A	N/A	carp muscle, black carp muscle, mixed liver sample	freeze-dried, ground, homogenized, stored (-20°C) extracted by ultrasonic-assisted extraction, purified by a combination of column fractionation, redissolution, and d-SPE, determined by GC–MS.	N/A	[ng/g dw] carp: ca. 2 black carp: ca. 1.5 liver: ca. 11 (estimations from a figure as values are not given in numbers)	N/A

Table 27: Selected bioaccumulation factors (BAF) and trophic magnification factor (TMF) of UV-329. For more study details see Table 26.

Reference	Species	C_w [ng/L]	BAF [L/kg]	BAF lipid normalised to 5%	TMF
Castilloux *et al.* (2022)	Northern pike (n=32)	IB: ND-31 IV: ND-4 (different sampling time 2018-2020)	Liver: 15848 Muscle: 2512 Plasma: 3162 (estimations from a figure as values are not given in numbers)	Liver: 37733 Muscle: 23287 (estimations from a figure as values are not given in numbers)	N/A
Lyu *et al.* (2022)	17 fish species 2 shrimp species	N/A	N/A	N/A	0.87[a] NS* (deduced from given equation)

Peng *et al.* (2017b)	17 fish species	N/A	N/A	N/A	1.70
Peng et al. (2020)	9 fish species	ND- 2.19	Muscle (mean): 1047 log BAF = 3.02±0.63	N/A	0.9±0.1*
Vimalkumar *et al.* (2018)	6 fish species (n=14)	1.7-29.3 (13.7±8.1)	Muscle (mean): 552	N/A	NA
Wang et al. (2022)	14 fish species, 6 benthic invertebrate species (10-20 individual organism per species)	0.7-0.83	Lipid (100%): fish: 1406815000 (median), 354800-354816000 (range) and for benthic invertebrates: 3162217000 (median), 70796900-5623476000 (estimations from a figure as values are not given in numbers)	N/A fish: 750 (median), 180-1800 (range) and for benthic invertebrates: 830 (median), 340-3800 (range)	0.398

[a]based on dry weight normalized concentration, *Not significantly deviating from value of 1

3.4.1.2.4 Integration and Weight of Evidence (WoE) analysis and Application of Level of Confidence

Table 28: Conclusion for aquatic bioaccumulation potential of UV-329

Type of Evidence	Consistency & Specificity	Likelihood/ Biological Plausibility	Temporality	Confidence / Strength of Evidence	Remaining Uncertainty
OECD TG 305-I with UV-329	Consistent with the other evidence if uptake period is disregarded and replaced by applicable estimation, high specificity as substance specific BCF value is the result of the study and enables direct comparison with B/vB criteria of Annex XIII	Plausible, if uptake phase disregarded and replaced by applicable estimations	Not relevant	medium for B conclusion,	medium for B conclusion,
HYBIT BCF with UV-329	Consistent with the other evidence, high specificity as substance specific BCF value is the result of the study and enables direct comparison with B/vB criteria of Annex XIII	Plausible	Not relevant	Medium to high for B/vB conclusion	Low/medium (new test system and uncertainties regarding k_1 -> to address these uncertainties the project "Bioaccumulation assessment of superhydrophobic substances." was initiated)
Project report "Bioaccumulation assessment of superhydrophobic substances."	Consistent with the other evidence, high specificity as substance specific calculated HYBIT BCF values enable direct comparison with B/vB criteria of Annex XIII	Plausible	Not relevant	Supporting evidence for B/vB conclusion	Low/medium (further validation with experimental data necessary)
Several field studies UV-329	Consistent with the other evidence, high specificity as substance specific results can be used in WoE to conclude on B/vB	Plausible	Not relevant	Supporting evidence for B/vB conclusion	Medium to high
Conclusion from overall confidence	The conclusion for UV-329 is based on a weight-of-evidence assessment using different pieces of information. The HYBIT BCF is given the highest weight. BCFs derived from HYBIT test are directly comparable with the numerical B and vB criteria of REACH Annex XIII. Considering all evidence together especially the HYBIT test in combination with the related model predictions, fish BCFs and the field data the confidence that UV-329 fulfills the B and vB criterion for aquatic organisms is high.				

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

The substance might screen as potentially B in terrestrial organisms. This potential is, however, not fully assessed. In the following, some studies are summarised as supporting information.

Each of the many steps involved in the process of performing environmental studies described below will have an impact on the overall uncertainty of the final results. This uncertainty begins with the design of the sampling regime and is compounded through the entire process to storage of samples, chemical analysis and data treatment. As it is difficult to estimate the absolute uncertainty for all steps in the process the results are used in a more general way as supporting evidence in the bioaccumulation assessment.

3.4.2.1 Detection in breast milk

Lee *et al.* (2015)

Breast milk samples (n=208) were collected from 87 lactating women in the Children's Health and Environmental Chemicals in Korea Panel, or CHECK Panel. In this study, breast milk samples were collected from five Korean university hospitals located in four cities including Seoul, Pyeongchon, Ansan and Jeju, from February to December in 2011. The breast milk samples were divided into four groups at the following timepoints after delivery: <7, 15, 30, and 90 days postpartum. Participants completed questionnaire about current and previous pregnancy histories, medical history, and demographic parameters (age, BMI, parity, gestational age at delivery, sex of newborn, and delivery mode). Breast milk samples were collected in polypropylene tubes and were frozen and transported on ice to the laboratory. Samples were stored in the laboratory at −70 °C until analysis. Breast milk samples (10 mL) were extracted with 2.5 mL of 8% potassium oxalate solution, 10 mL of ethanol, and 5 mL of diethyl ether for 30 min by mechanical shaking and analysed with GC/MS. Among the BUVSs [benzotriazole UV stabilisers] analysed, UV-328 was dominant in all the samples, with a detection rate of 98%. The concentrations of UV-329 ranged from <5 to 178 ng/g lipid wt. in human breast milk with detection rate of 8.7 %.

Kim *et al.* (2019)

Human breast milk samples (n=87) from primipara and multipara mothers were collected from Kanagawa Prefecture, Japan (n=20) in 2009–2011, Malate (n=19) and Payatas (n=22), the Philippines in 2008, and Hanoi (n=7), Bui Dau (n=10), and Trang Minh (n=9), Vietnam in 2008. Informed consent was obtained from all the donors. The general information in the questionnaire included each mother's age, height, occupation, dietary habits, body mass index, birth procedure, and breastfeeding period, as well as the age and weight of the baby (Table 1). From each participant, about 100 mL of breast milk was collected using a breast pump to express milk into prewashed glass containers prepared for every individual. The collected milk samples in the Philippines and Vietnam except for Japan were shipped frozen to Japan and were stored at −25 °C in the Environmental Specimen Bank (es-BANK) of Ehime University until chemical analysis. Approximately 10 g of human breast milk samples were freeze-dried and then were extracted by High Speed Solvent Extractor (hexane/acetone, 1:1, v/v). Total concentrations of the 8 benzotriazoles in breast milk ranged from MDL (method detection limit) to 1100 ng/g lipid wt. in present study. Among the 8 benzotriazoles compounds targeted, the highest concentration of UV-9 was found in breast milk samples collected from Vietnam. The concentrations of UV-329 ranged from <MDL to 26 ng/g lipid wt. in human breast milk. In the samples from the Philippines the detection frequency of UV-329 was 0% and in Hanoi 100%.

3.4.2.2 Detection in predators

Schlabach *et al.* (2018)

In this screening study about 90 different compounds with an array of physiochemical properties were measured in environmental samples. These samples included a selection of biota from localised hot-spot areas and from the most remote Arctic species. Biota samples were stored frozen (−20 °C) and extracted with organic solvents in an ultrasonic bath. UV-329 was detected in eggs of European shag (0.35 ng/g w.w., n=5, DF: 100%), blood of polar bear (0.5 ng/g w.w., n=5, DF: 10%), liver of American mink (0.35 ng/g w.w., n=5, DF: 100%). The UV filter UV-326 was found in Arctic hotspot biota. These findings suggest a potential to bioaccumulate and support earlier conclusions.

Lu *et al.* (2019)

Ringed seals were collected at Resolute Bay (n=3), Arviat (n=3), Sachs Harbour (n=3), and Lake Melville (n=5) by local hunters in 2016 and 2017 during subsistence harvesting. The livers of ringed seals were used for the present study. A mixture solvent of 5 mL 1:1 (v:v) hexane:dichloromethane was used to extract the sample. An ultra-performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS) system was used for sample analysis. UV-329 was detected in all samples from Resolute Bay and Sachs Harbour. The concentration of UV-329 ranged from <580 to 9411 pg/g ww. in seal liver samples with detection rate of 0-100 %. The detection suggests the presence of this contaminant in remote regions and resident species.

NILU reports: 20/2019 (Schlabach et al. 2022), 01/2023 (Heimstadt et al. 2023)
The monitoring programme on behalf of the NILU-Norwegian Institute for Air Research provided results for various inorganic and organic environmental pollutants measured in different media and biological samples in the greater area of Oslo. Regarding the biotic samples UV-329 was only detected in brown rat (01/2023: 3.1 ng/g). The absence of detection in monitoring studies is no contradicting evidence to the vB conclusion for the substances but rather may reflect low exposure in the area. Further explanation for absence of detection can be that LOD/LOQ values may be set too high or analytical issues.

3.4.3 Summary and discussion of bioaccumulation

UV-329 screens as potentially B/vB due to log Kow values above the screening trigger value of 4.5.

The conclusion for UV-329 is based on a weight-of-evidence assessment using different pieces of information. The HYBIT BCF of >5000 is given the highest weight. HYBIT is a new test system and therefore is connected to some uncertainties regarding less experience in judging the results especially k_1 values. To address these uncertainties especially for k_1 estimation from HYBIT test the project "Bioaccumulation assessment of superhydrophobic substances" was initiated which supports the HYBIT BCF. *H.azteca* is an aquatic organism to which the B and vB criteria of REACH annex XIII refers to. Considering all evidence together especially the HYBIT test in combination with the related model predictions, recalculated fish BCF >2000 and the field data as supporting evidence, it is concluded that UV-329 fulfils the B and vB criterion of REACH Annex XIII for aquatic organisms.

Furthermore, UV-329 is detected in human breast milk and predators. As it is difficult to estimate the absolute uncertainty for the results of these studies they are used in a more general way as supporting information.

4 Human health hazard assessment

Not assessed.

5 Environmental hazard assessment

Not assessed.

6 Conclusions on the SVHC Properties

6.1 vPvB assessment

6.1.1 Assessment of vPvB properties

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

6.1.1.1 Persistence

The screening criterion for persistence (P) is fulfilled for UV-329. The results from the available screening study show that this substance is not readily biodegradable. This is confirmed by the available (Q)SAR results.

Hydrolysis of the substance is not expected due to the absence of functional groups susceptible to hydrolysis.

In a water sediment simulation study at 20 °C, no degradation was observed after 100 days. At an environmentally relevant temperature of 12 °C this corresponded to a half-life significantly larger than 212 days.

Faster dissipation in an aquifer test may be related to the different sediment type tested or to the several deviations from standard conditions.

UV-P (a substance with a particular high structural similarity to UV-329) and further phenolic benzotriazoles have been detected in sediment sections that date back years and even decades, both in samples downstream from a former point source and in samples from urban estuaries.

UV-329 is persistent (and potentially very persistent) in two soil dissipation studies.

As an overall conclusion, based on the above information used in a weight-of-evidence-approach, it is concluded that UV-329 meets the 'persistence' criterion (P) and the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of its persistent and very persistent (P/vP) properties in sediment (DegT50 > 180 days). Furthermore, UV-329 meets the 'persistence' criterion (P) and potentially the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of its persistent and potentially their very persistent (P/vP) properties in soil (at least DegT50 > 120 days).

6.1.1.2 Bioaccumulation

UV-329 screens as potentially B/vB due to the available log K_{ow} values above the screening trigger value of 4.5.

The conclusion for UV-329 is based on a weight of evidence assessment using different pieces of information. The HYBIT BCF of >5000 is given the highest weight. HYBIT is a new test system and therefore is connected to some uncertainties regarding less experience in judging the results, especially k_1 values. To address these uncertainties especially for k_1 estimation from HYBIT test the project "Bioaccumulation assessment of superhydrophobic substances" was initiated, which supports the HYBIT BCF. *H.azteca* is an aquatic organism to which the B criteria of REACH Annex XIII refers to. Considering all evidence together especially the HYBIT test in combination with the related model predictions, recalculated fish BCF_{kgL} >2000 and the field data as supporting evidence UV-329 fulfils the B and vB criterion for aquatic organisms.

Furthermore, UV-329 is detected in human breast milk and top predators. This information is used in a more general way as supporting information.

6.1.2 Summary and overall conclusions on the vPvB properties

A weight of evidence determination according to the provisions of Annex XIII of REACH has been used to identify UV-329 as vPvB substance. All available relevant information (such as the results of standard tests, monitoring and modelling, information from the application of the read-across and (Q)SAR results) was considered together in a weight-of-evidence approach.

Persistence
The screening criterion for persistence (P) is fulfilled for UV-329. The results from the available screening studies (reliable with or without restrictions) showed that this substance is not readily biodegradable. This is confirmed by the available (Q)SAR results with BIOWIN and CATALOGIC which indicate that UV-329 screens as potentially P or vP. The outcomes of the screening test and the (Q)SARs predictions have been assigned a low weight in the weight-of-evidence approach (WoE) for the P assessment.

Hydrolysis of UV-329 is not expected due to the absence of functional groups susceptible

to hydrolysis. As a conclusion, abiotic degradation of UV-329 is not considered to be a significant degradation pathway in the environment.

In a water-sediment simulation study for UV-329 at 20 °C (reliable with restrictions), no degradation was observed after 100 days. At an environmentally relevant temperature of 12 °C this corresponded to a half-life significantly larger than 212 days for UV-329 thus indicating its very persistent properties in sediment (DegT50>180 days). The outcome of this higher tier study is given a high weight in the WoE approach as it provides information directly comparable with the P and vP criteria set out in Annex XIII, points 1.1.1 (d) and 1.2.1 (b) of the REACH Regulation.

Faster dissipation of UV-329 in an aquifer test (reliable with restrictions) may be related to the different test conditions and the studied compartment compared to an OECD water-sediment simulation study. This study has been assigned a low weight in the WoE approach considering the studied compartment, the test conditions and the difficulty to derive an appropriate DT50.

UV-P (a substance with a particular high structural similarity to UV-329) and further phenolic benzotriazoles have been detected in sediment cores that date back years and even decades (starting from the 1960s), both in samples downstream from a former point source and in samples from urban estuaries. This information provides indirect evidence that UV-P and potentially UV-329 (based on a read-across from UV-P) can persist in sediments for several decades. Monitoring data in sediment cores are used as supporting information in the WoE approach for UV-329. They are in line with the outcome of the water-sediment simulation study, the screening studies and the QSAR predictions as they point towards the potential persistence of UV-329 (based on results of the structural analogue UV-P) in sediments.

UV-329 is persistent (and potentially very persistent) in two soil dissipation studies (reliable with restrictions) (at least DegT50>120 days).

As an overall conclusion, based on the above information used in a weight-of-evidence-approach, it is concluded that UV-329 meets the 'persistence' criterion (P) and the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of their persistent and very persistent (P/vP) properties in sediment (DegT50 > 180 days). Furthermore, UV-329 meets the 'persistence' criterion (P) and potentially the 'very persistent' criterion (vP) in accordance with Annex XIII, points 1.1.1 and 1.2.1, of the REACH Regulation on the basis of their persistent and potentially their very persistent (P/vP) properties in soil (at least DegT50 > 120 days).

<u>Bioaccumulation</u>
UV-329 screens as potentially B/vB due to the available log K_{ow} values above the screening trigger value of 4.5.

The conclusion for UV-329 is based on a weight-of-evidence assessment using different pieces of information. The outcome of the *Hyalella azteca* bioconcentration test (HYBIT; reliable with restrictions) for UV-329 is given a high weight in the WoE approach with 3 %-lipid-normalised steady-state bioconcentration factor (BCF_{ssL}) and 3%-lipid-normalised kinetic bioconcentration factor (BCF_{kL}) values in the range of 11063—11876 L/Kg. This study provides information directly comparable with the B (BCF>2000) and vB criteria (BCF>5000) set out in Annex XIII. Re-calculated fish BCF_{kgL} >2000 derived from an OECD TG 305 study (aqueous exposure; reliable with restrictions) with rainbow trout (*Oncorhynchus mykiss*) support the bioaccumulation potential of UV-329. Monitoring data tend to confirm this conclusion as UV-329 has been found in human breast milk and in biota including in top predators such as the polar bears which are listed as vulnerable to extinction, according to the IUCN Red List. Based on the weight of evidence of the data available, it is concluded that UV-329 meets the 'bioaccumulation' criterion (B) and the

'very bioaccumulative' criterion (vB) in accordance with Annex XIII, points 1.1.2 and 1.2.2, of the REACH Regulation.

Conclusion

In conclusion, UV-329 is identified as a vPvB substance according to Art. 57(e) of REACH by comparing all relevant and available information listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

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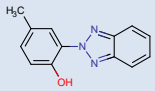
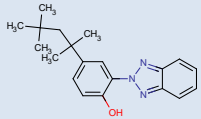
ANNEX I

Data matrix

Data matrix for UV-329 and UV-P.

Chemical name	UV-P	UV-329			
EC number	219-470-5	221-573-5			
Chemical structure					
Molecular weight	225.25	323.44			
Impurities	None registered	None registered			
Octanol water partition log K_{ow}	4.2 (OECD TG 107) 3.95 (COSMOtherm)	≥ 6.5 (OECD TG 107) 6.91 (Do et al. 2022) 6.4 (COSMOtherm)			
Water solubility in µg/L	173 (OECD TG 105)	2 (OECD TG 105)			
Estimated log K_{OC} (KOCWIN)	3.593	5.114			
Vapour pressure at 20°C in Pa	1.47 E-4 (exp)	4.1 E-6 (exp)			
HLC in atm-m3/mole (HENRYWIN)	6.12E-14	4.45E-13			
pK_a	9.53 (chemicalize) [51]	9.4 (chemicalize)			
Hydrolysis	not expected	not expected			
BIOWIN1 (Linear Model) Probability	0.8108	0.3415			
BIOWIN2 (Non-Linear Model) Probability	0.7851	0.016			

[51] 27.10. 2022, https://chemicalize.com/ developed by ChemAxon (http://www.chemaxon.com)

Chemical name	UV-P	UV-329			
BIOWIN3 numerical output	2.6829	2.1165			
BIOWIN4 numerical output	3.4939	3.1139			
BIOWIN5 (Linear MITI Model) Probability	0.2481	0.0704			
BIOWIN6 (Non-Linear MITI Model) Probability	0.1597	0.0081			
CATALOGIC_301C_v12-17 BOD [28 days]	0	0.01			
Ready biodegradability tests [28 days]	2% (OECD 301 B)	1% (OECD 301 B)			
Water sediment study (Wick et al 2016a, 2016b)	not tested	no degradation in 100 d			
Aquifer system DT50	not tested	34 d			
Rhode Island sediment cores (downstream Cranston chemical plant point source)	detected	not analysed			
Pear River Estuary sediment core (diffuse sources)	detected	not constantly detected, detections close to MQL			
Soil dissipation studies DT50	75-113 d; 85-157 d	98-129 d; 79-155 d			

CATALOGIC results
CATALOGIC 301C v12.17 is preferred because its training set contains the most phenolic benzotriazoles. The training set of CATALOGIC 301 C v12.17 contains UV-P and consequently, this substance is in domain.

BIOWIN results for UV-P

Some BIOWIN results are contradicting; however, the reliability of these predictions is considered lower than the reliability of CATALOGIC 301C v12.17.

K_{OC}

The dossiers for UV-329 contains KOCWIN predictions. For the sake of comparison, KOCWIN predictions[52] is given for UV-P as well. However, these predictions are not documented according to REACH Annex XI and hence, the respective results should be considered with caution. All considered substances are in the molecular weight range of training set and validation set. Correction factors for carboxylic acid groups and phenolic hydroxy groups were derived from training set substances with these structural features, i.e. though being dependent on pH, the ionic interactions of these groups may be covered by the model to a certain extent.
The registration dossier of UV-P contains further K_{OC} estimations. These were not included as they cannot be directly compared with estimations from the same methods for the other substances.

Henry's Law Constant

To estimate the expected impact of volatility on degradation studies, HENRYWIN predictions[53] are given for the substances. Note that these predictions are not documented according to REACH Annex XI and hence, the respective results should be considered with caution. All considered substances are in the molecular weight range of training set. Both UV-P and UV-329 are in the Henry's Law constant range of the training set.

[52]2010 U.S. Environmental Protection Agency. KOCWIN v2.01 in EPISUITE v4.11.
[53]2010 U.S. Environmental Protection Agency. HENRYWIN v3.21 in EPISUITE v4.11. Bond method was applied; no results were obtained for the group method.

ANNEX II

Kinetic modelling for aquifer study Liu et al. 2013: UV-329

CAKE Kinetic Evaluation Report

Experiment 1 (SFO)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final

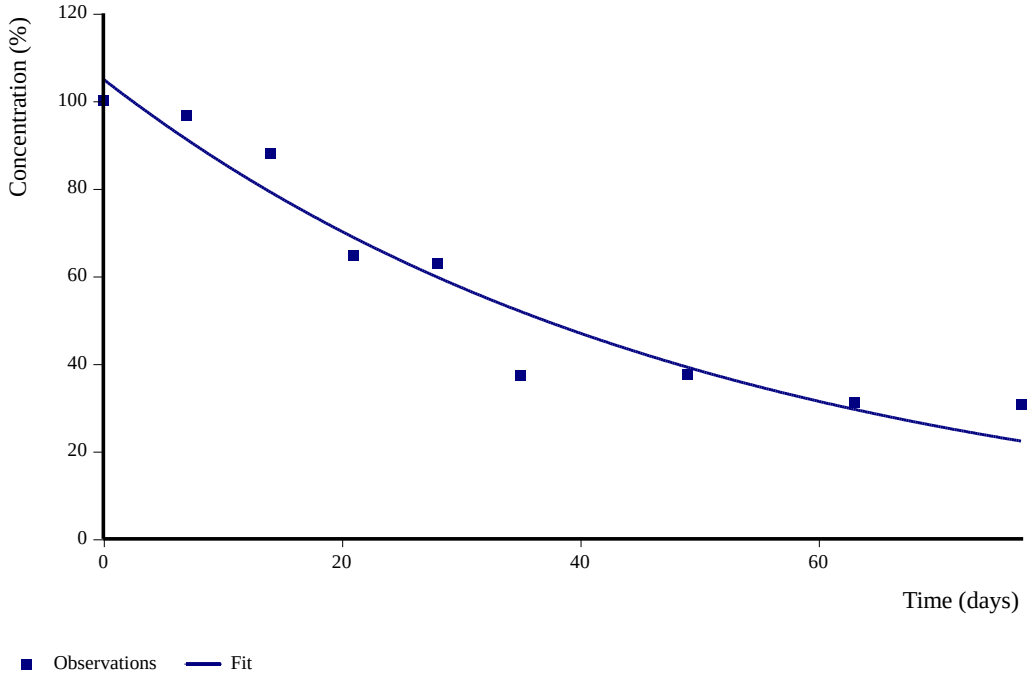
Used Extra Solver for SFO model fit: No

Reference Table:

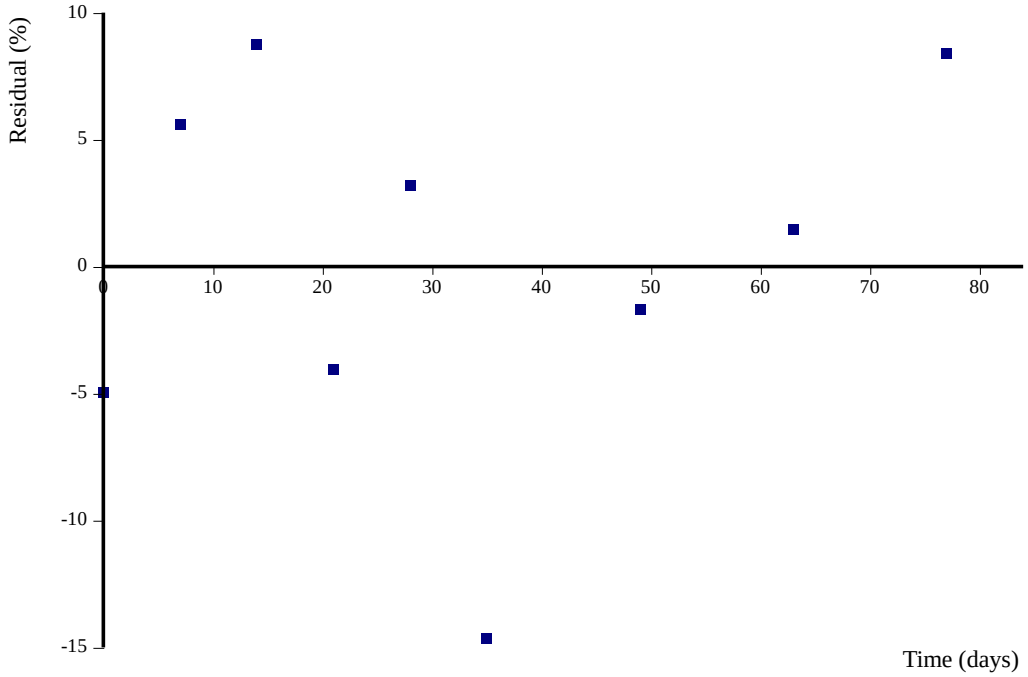
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	104.9	5.812	N/A	93.93	116	91.2	118.7
k_Parent	0.0201	0.002465	4.04E-005	0.01543	0.02477	0.01427	0.026

Sum of Squared Residuals: 449.6

χ^2

Parameter	Error %	Degrees of Freedom
All data	9.27	7
Parent	9.27	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	34.5	115

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.931	0.9306
Parent	0.931	0.9306

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.6856
k_Parent	0.6856	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	100	104.9	-4.944
7	96.78	91.17	5.605
14	87.97	79.21	8.758
21	64.76	68.81	-4.056
28	62.98	59.78	3.194
35	37.27	51.94	-14.67
49	37.52	39.2	-1.686
63	31.05	29.59	1.461
77	30.71	22.33	8.379

Sequence Creation Information:
Fit generated by CAKE version 3.4 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (DFOP)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Fit step: Final

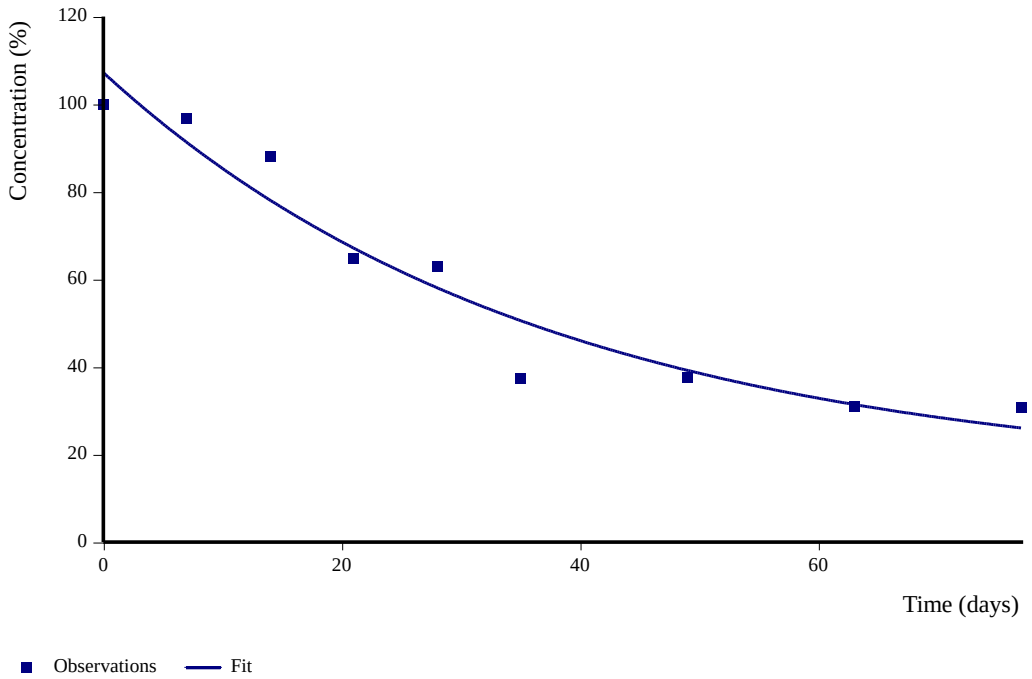
Used Extra Solver for DFOP model fit: No

Reference Table:

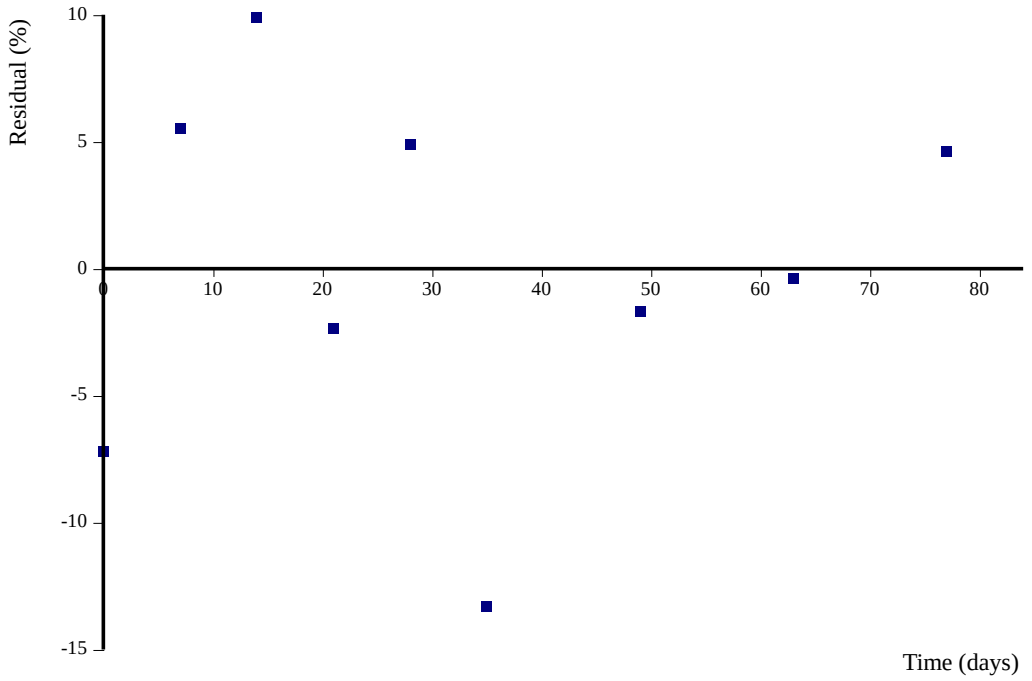
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	107.2	8.368	N/A	90.33	124.1	85.68	128.7
k1_Parent	0.02691	0.0661	0.3504	-0.1063	0.1601	-0.143	0.197
k2_Parent	8.66E-009	0.1706	0.5	-0.3437	0.3437	-0.4385	0.438
g_Parent	0.8657	2.587	N/A	-4.348	6.079	-5.785	7.516

Sum of Squared Residuals: 411.5

χ^2

Parameter	Error %	Degrees of Freedom
All data	10	5
Parent	10	5

Decay Times:

Compartment	DT50 (overall days)	DT90 (overall days)	k1 DT50 (days)	k2 DT50 (days)
Parent	32	>10,000	25.8	>10,000

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.9364	0.9364
Parent	0.9364	0.9364

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	0.4743	0.3803	-0.4017
k1_Parent	0.4743	1	0.9838	-0.9924
k2_Parent	0.3803	0.9838	1	-0.998
g_Parent	-0.4017	-0.9924	-0.998	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	100	107.2	-7.192
7	96.78	91.26	5.517
14	87.97	78.06	9.904
21	64.76	67.13	-2.374
28	62.98	58.08	4.901
35	37.27	50.58	-13.31
49	37.52	39.22	-1.703
63	31.05	31.43	-0.3765
77	30.71	26.08	4.633

Sequence Creation Information:
Fit generated by CAKE version 3.4 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (FOMC)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Fit step: Final

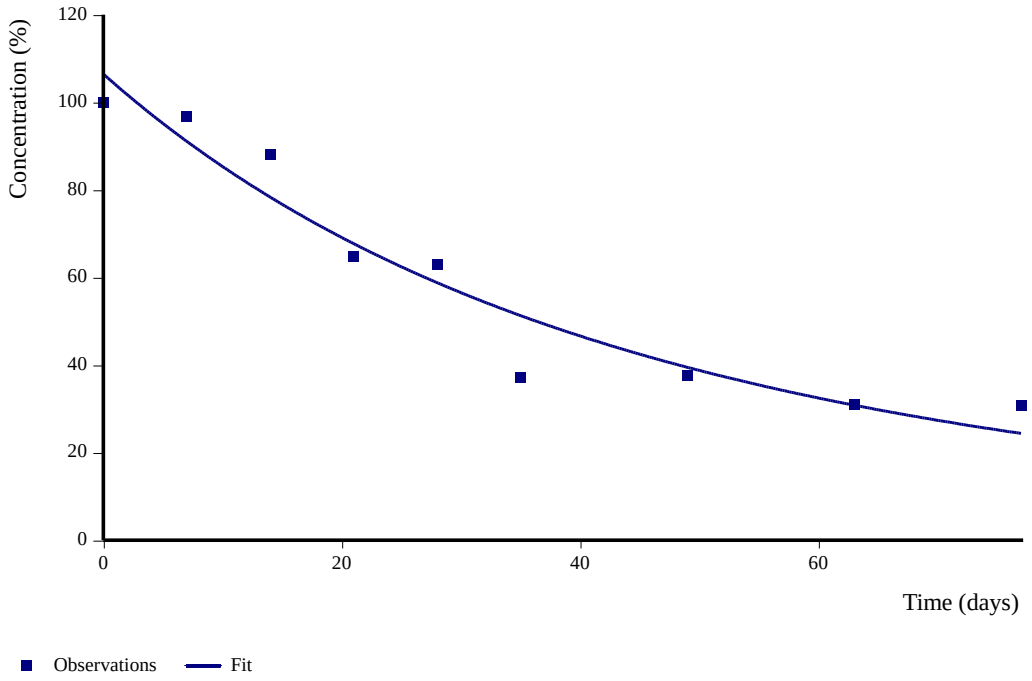
Used Extra Solver for FOMC model fit: No

Reference Table:

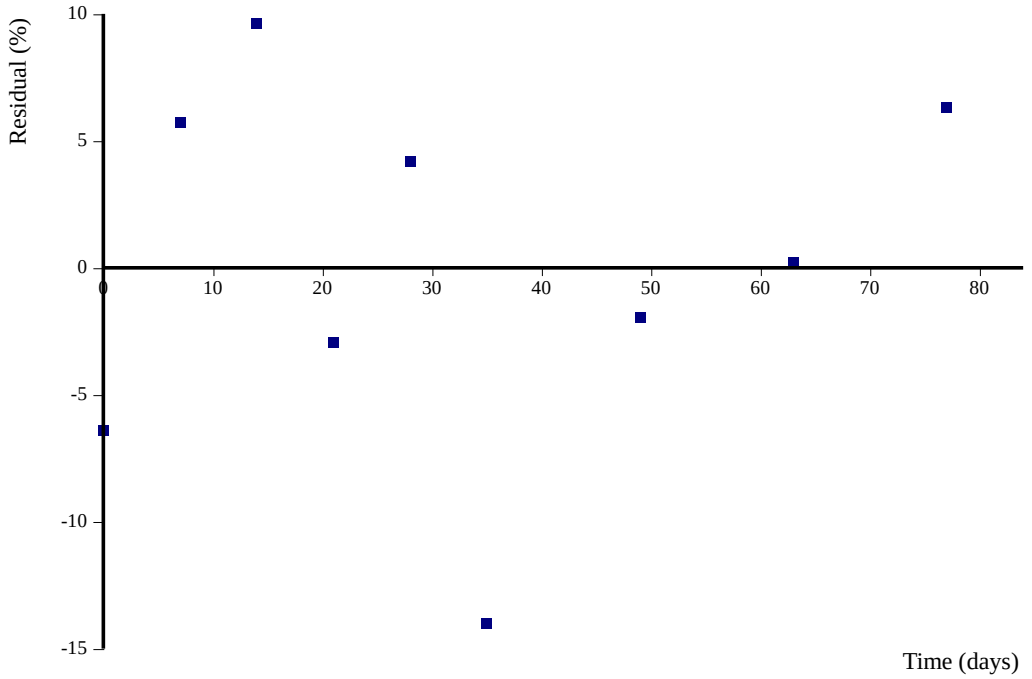
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	106.4	7.508	N/A	91.83	121	88.05	124.8
alpha	4.48	11.59	N/A	-18.04	27	-23.87	32.83
beta	197.8	574.7	N/A	-919	1.31E+003	-1208	1.60E+003

Sum of Squared Residuals: 433.1

χ^2

Parameter	Error %	Degrees of Freedom
All data	9.61	6
Parent	9.61	6

Decay Times:

Compartment	DT50 (days)	DT90 (days)	DT90 / 3.32 (days)
Parent	33.1	133	40

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.9332	0.9331
Parent	0.9332	0.9331

Parameter Correlation:

	Parent_0	alpha	beta
Parent_0	1	-0.5105	-0.5399
alpha	-0.5105	1	0.9988
beta	-0.5399	0.9988	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	100	106.4	-6.418
7	96.78	91.07	5.712
14	87.97	78.34	9.63
21	64.76	67.72	-2.96
28	62.98	58.81	4.17
35	37.27	51.29	-14.02
49	37.52	39.48	-1.968
63	31.05	30.84	0.212
77	30.71	24.4	6.314

Sequence Creation Information:
Fit generated by CAKE version 3.4 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 3.4 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK, sponsored by Syngenta
Runtime: .NET Framework 4.8.4300.0

Data set: Experiment 1 (HS)

Study date: Mittwoch, 14. April 2021
Report generated: Mittwoch, 14. April 2021

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	Automatic	0 to (unbounded)	No

Fit step: Final

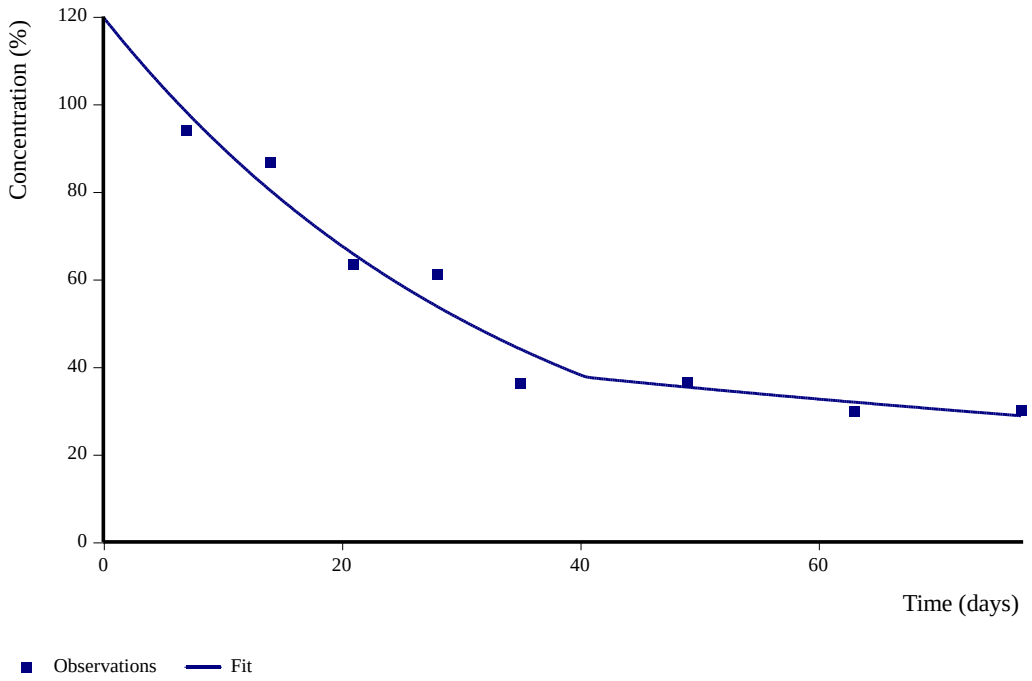
Used Extra Solver: Yes

Reference Table:

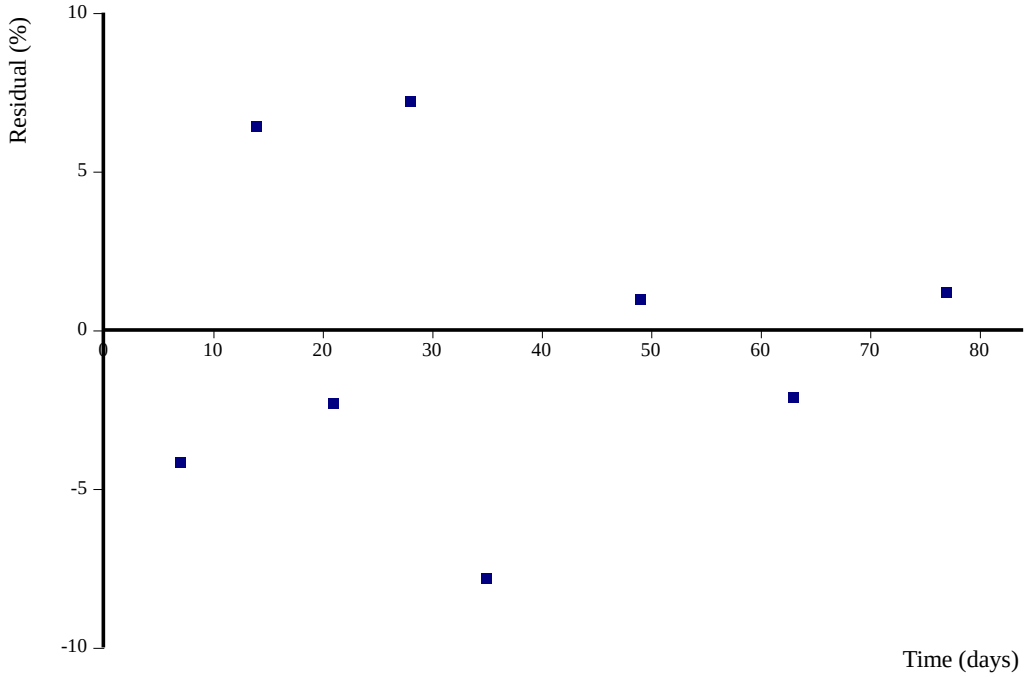
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	28	0 to (unbounded)	No

Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	119.8	9.911	N/A	98.67	140.9	92.28	147.3
k1	0.0286	0.004435	0.001488	0.01915	0.03806	0.01629	0.041
k2	0.00728	0.01003	0.2541	-0.0141	0.02866	-0.02057	0.035
tb	40.52	11.3	N/A	16.42	64.62	9.138	71.9

χ^2

Parameter	Error %	Degrees of Freedom
All data	8.06	4
Parent	8.06	4

Decay Times:

Compartment	DT50 (overall days)	DT90 (overall days)	k1 DT50 (days)	k2 DT50 (days)
Parent	24.2	198	24.2	95.2

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.9599	0.9599
Parent	0.9599	0.9599

Parameter Correlation:

	Parent_0	k1	k2	tb
Parent_0	1	0.8569	-0.1117	-0.1754
k1	0.8569	1	-0.1483	-0.2923
k2	-0.1117	-0.1483	1	-0.7477

tb	-0.1754	-0.2923	-0.7477	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
7	93.9	98.06	-4.164
14	86.7	80.27	6.43
21	63.4	65.71	-2.305
28	61	53.78	7.217
35	36.2	44.02	-7.824
49	36.3	35.35	0.9546
63	29.8	31.92	-2.121
77	30	28.83	1.172

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 3.3 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK, sponsored by Syngenta
Running on .NET version 4.0.30319.42000