Evaluating Prediction Results of Biodegradation under the OECD QSAR Assessment Framework

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OECD QSAR Assessment Framework ~ QAF ~

A systematic and harmonized framework for the regulatory assessment of (Q)SAR models, predictions, and results based on multiple predictions.

OECD (Q)SAR Model Principles

1) a defined endpoint
2) an unambiguous algorithm
3) a defined domain of applicability
4) appropriate measures of goodness-of–fit, robustness and predictivity
5) a mechanistic interpretation, if possible

→ Model Checklist

OECD (Q)SAR Prediction Principles

1) the correct input
2) the fit of the substance within the applicability domain of the model
3) the reliability of the prediction
4) the outcome's fitness for the purpose

→ Prediction & Result Checklists

We'd like to introduce examples to assess the biodegradation of two chemicals with US EPA's model by using the checklists of QAF.

Backgrounds for the assessment

- Case Study:

In this case study we aim to assess the biodegradability of target substances using QSAR under the risk assessment framework of the Chemical Substances Control Law in Japan, referred to as CSCL, by using the checklists of QAF. Two chemicals are selected, one of which does and doesn't have any multiple experiment data.

- QSAR tool : BIOWIN 5 and BIOWIN 6 version 4.11

BIOWIN is one of the prediction tools in the EPI (Estimation Programs Interface) Suite™. Specifically, BIOWIN 5 and 6 serve as predictive models to assess a compound‘s biodegradability in the Japanese MITI (Ministry of International Trade and Industry) ready biodegradation test (OECD 301C). These models are based on fragment constants developed through multiple linear and non-linear regression analyses. We frequently employ these models for the biodegradation evaluations in regulatory contexts. Based on OECD 301C pass criterion, which means 60% of maximum theoretical oxygen demand due to test substances (ThOD), the BIOWIN model interprets probabilities greater than 0.5 as predicting ready degradability and values less than 0.5 as not predicting ready degradability. For your information, the discussion of the presence and absence of degradation products should be conducted outside of the QAF scope, but still within the context of risk assessments.

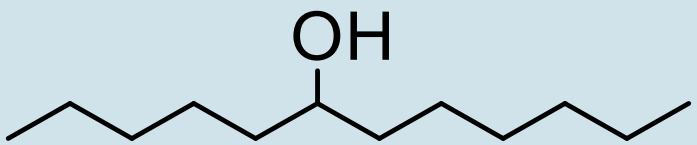
Technical Guidance Document for the Risk Assessment of Priority Assessment Chemical Substance (PACS) under CSCL (METI)
(Only Japanese)

EPI Suite™-Estimation Program Interface | US EPA
BIOWIN：Biodegradation Probability Program

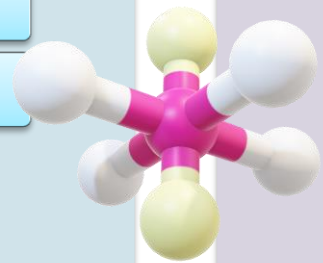
Our target substances

Case1: we can't get any appropriate experimental data

- Dodecan-6-ol
- CAS RN : 6836-38-0
- BIOWIN5 : 0.64 (RD)
- BIOWIN6 : 0.81 (RD)
- Molecular Weight : 186

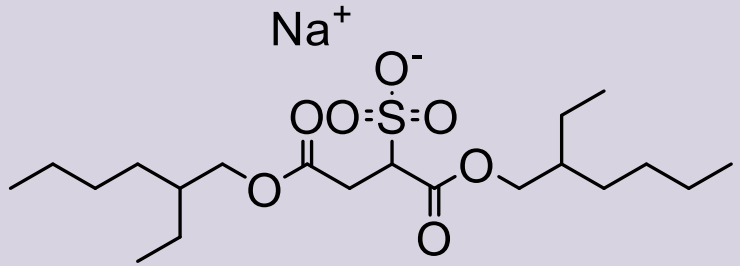


We assess the risk of chemicals with QSAR prediction results



Case2: we can get any appropriate experimental data

- Bis(2-ethylhexyl) Sulfosuccinate Sodium Salt
- CAS RN : 577-11-7
- BIOWIN5 : 0.60 (RD)
- BIOWIN6 : 0.54 (RD)
- Molecular Weight : 422



We use QSAR prediction results as one of supporting information

RD: Readily biodegradable
NRD: Not readily biodegradable

The result of the Model Checklist

Defined endpoint

1.1 Clear scientific and regulatory purpose
1.2 Transparency of the underlying experimental data
1.3 Quality of the underlying experimental data

Unambiguous algorithm

2.1 Description of the algorithm and/or software
2.2 Inputs and other options
2.3 Model accessibility

Mechanistic interpretation

5.1 Plausibility of the mechanistic interpretation

Defined domain of applicability

3.1 Clear definition of the applicability domain and limitations of the model

Appropriate measures of goodness-of-fit, robustness and predictivity

4.1 Goodness-of-fit, robustness
4.2 Predictivity

Not fulfilled
Because those models are built statistically, not mechanistically.

Overall BIOWIN5 and BIOWIN6 are acceptable for our intended purpose.

Overall results of the Prediction Checklist : 6836-38-0

Principle	Assessment element	Weight	Outcome	Uncertainty
Correct input(s) to the model				
1.1	Clear and complete description of the input and model settings	High	Fulfilled	Low
1.2	Input representative of the substance under analysis	High	Fulfilled	Low
1.3	Reliable input (parameters)	Medium	Not applicable/assessed	
Substance within the applicability domain of a valid model				
2.1	Substance within the applicability domain	High	Fulfilled	Low
2.2	Any other limitation of the model is considered	High	Fulfilled	Low
Reliable prediction				
3.1	Reproducibility	High	Fulfilled	Low
3.2	Overall performance of the model	Medium	Fulfilled	Low
3.3	Fit within the physicochemical, structural and response spaces of the training set of the model	Medium	Fulfilled	Low
3.4	Performance of the model for similar substances	High	Fulfilled	Low
3.5	Mechanistic and/or metabolic considerations	High	Not applicable/assessed	
3.6	Consistency of information	High	Not applicable/assessed	
Outcome is fit for the regulatory purpose				
4.1	Compliance with additional requirements	High	Fulfilled	Low
4.2	Correspondence between predicted property and property required by the regulation	High	Fulfilled	Low
4.3	Decidability within the specific framework	High	Fulfilled	Low

- Low
- Medium
- High

- Fulfilled
- Not fulfilled
- Not documented
- Not applicable/assessed

- Low
- Medium
- High

Prediction Checklist : 6836-38-0

Principle	Assessment element	Weight	Outcome	Uncertainty
Correct input(s) to the model				
1.1	Clear and complete description of the input and model settings	High	Fulfilled	Low
1.2	Input representative of the substance under analysis	High	Fulfilled	Low
1.3	Reliable input (parameters)	Medium	Not applicable/assessed	

1.3

- The purpose of the 1.3 assessment element is to evaluate the reliability of manually input parameters.
- This element should be assessed for models utilizing direct input beyond the chemical structure.
- Given that BIOWIN relies solely on a single input, specifically SMILES, for model execution, we consider the outcome of this element as not applicable/assessed.

Prediction Checklist : 6836-38-0

Principle	Assessment element	Weight	Outcome	Uncertainty
Substance within the applicability domain of a valid model				
2.1	Substance within the applicability domain	High	Fulfilled	Low
2.2	Any other limitation of the model is considered	High	Fulfilled	Low

- Molecular Weight Domain: 31 - 1215
- Molecular Weight: 186
- Chemical Fragment: Linear C4 terminal chain [CCC- CH3] , Aliphatic alcohol [-0H]

2.1

- The BIOWIN model utilizes a group or fragment contribution approach with molecular weight factors.
- Therefore, it can make predictions for any organic compound if the molecular weight of the target substance falls within the maximum and minimum values of the training set compounds.
- The model is based on a training set that defines 42 structural fragments, and the target substance is assessed to contain these 42 defined structural fragments.

2.2

- The BIOWIN model should not be employed to evaluate structural isomers since it utilizes a group or fragment contribution approach that assumes linear/non-linear additivity of each fragment's contributions in the molecule.

Prediction Checklist : 6836-38-0

Principle	Assessment element	Weight	Outcome	Uncertainty
Reliable prediction				
3.1	Reproducibility	High	Fulfilled	Low
3.2	Overall performance of the model	Medium	Fulfilled	Low
3.3	Fit within the physicochemical, structural and response spaces of the training set of the model	Medium	Fulfilled	Low

3.1

- BIOWIN is publicly available and can be downloaded from the website.

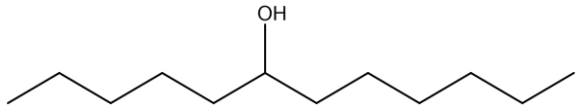
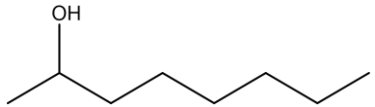
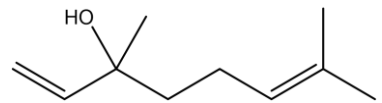
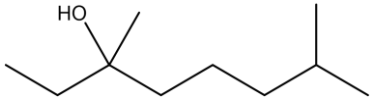
3.2

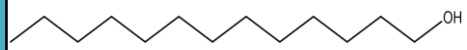
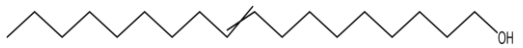
- The prediction accuracy for both the training set and validation set is generally 70% or more, demonstrating sufficient performance for its intended use.

3.3

- The molecular weight of the substance under analysis falls within the range of the maximum and minimum values of the training set compounds. Additionally, the structural fragments present in the substance are included in the 42 fragments defined based on the training set.
- In all predictions using the model, probabilities greater than or equal to 0.5 are interpreted as indicating readiness for degradation, while values less than 0.5 suggest non-readiness. For the substance under analysis, BIOWIN5 predicts 0.64, and BIOWIN6 predicts 0.81, categorizing the substance as readily degradable. These prediction values fall within the applicability domain of the models' training set.

Similar substances：6836-38-0

	target	analogue1	analogue2	analogue3
Structure				
CAS RN	6836-38-0	123-96-6	78-70-6	78-69-3
Measured value of degradability		BOD76%(301C)	BOD90%(301C)	BOD73%(301C)
BIOWIN5	0.64 (RD)	0.63 (RD)	0.34 (NRD)	0.38 (NRD)
BIOWIN6	0.81 (RD)	0.81 (RD)	0.17 (NRD)	0.29 (NRD)

	analogue4	analogue5	analogue6	analogue7
Structure				
CAS RN	112-70-9	143-28-2	506-52-5	36653-82-4
Measured value of degradability	BOD88.4%(301C)	BOD82%(301C)	BOD75%(301C)	BOD86%(301C)
BIOWIN5	0.72 (RD)	0.68 (RD)	0.76 (RD)	0.73 (RD)
BIOWIN6	0.88 (RD)	0.79 (RD)	0.86 (RD)	0.87 (RD)

RD: Readily biodegradable
NRD: Not readily biodegradable

Prediction Checklist : 6836-38-0

Principle	Assessment element	Weight	Outcome	Uncertainty
Reliable prediction				
3.4	Performance of the model for similar substances	High	Fulfilled	Low
3.5	Mechanistic and/or metabolic considerations	High	Not applicable/assessed	
3.6	Consistency of information	High	Not applicable/assessed	

3.4

- All experimental results indicate ready biodegradability.
- Within all analogues we chose, substances with tertiary alcohols are estimated as not readily biodegradable, while others are estimated as readily biodegradable.

3.5

- BIOWIN does not take into account metabolism and catabolism factors.

3.6

- There is no available data on the biodegradability of the substance under analysis.

Prediction Checklist : 6836-38-0

Principle	Assessment element	Weight	Outcome	Uncertainty
Outcome is fit for the regulatory purpose				
4.1	Compliance with additional requirements	High	Fulfilled	Low
4.2	Correspondence between predicted property and property required by the regulation	High	Fulfilled	Low
4.3	Decidability within the specific framework	High	Fulfilled	Low

4.1

- When existing chemical substances are evaluated under the CSCL in Japan and the reliable experimental results, such as in accordance with OECD301C, are not available, the predicted results from the QSAR tools are taken into consideration as one of reference materials based on the risk assessment guidance which is written in Japanese and available publicly on the Japanese government websites.

4.2

- The predicted property corresponds to the required property, because the BIOWIN5 and 6 endpoint is OECD TG 301C and the regulatory requires tests performed according to the latest version of the OECD TG 301 series.

4.3

- BIOWIN5 (Linear model prediction) estimates 0.64 as probabilities and BIOWIN6 (Non-linear model prediction) does 0.81. Both models estimate a probability greater than 0.5, which means that the substance is judged as readily biodegradable.

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Conclusion on the prediction：6836-38-0

Uncertainty

- Low
- The predictions of analogues with tertiary alcohols, which the substance under analysis doesn't include, don't match with the experiment results. Besides that, all the predictions of the analogues without tertiary alcohol match with the experiment results. Accordingly, we have identified the reason of the inconsistencies and overall uncertainty is low.

Outcome of the assessment

- Acceptable for the intended purpose

Overall results of the Prediction Checklist : 577-11-7

Principle	Assessment element	Weight	Outcome	Uncertainty
Correct input(s) to the model				
1.1	Clear and complete description of the input and model settings	High	Fulfilled	Low
1.2	Input representative of the substance under analysis	High	Fulfilled	Low
1.3	Reliable input (parameters)	Medium	Not applicable/assessed	
Substance within the applicability domain of a valid model				
2.1	Substance within the applicability domain	High	Fulfilled	Low
2.2	Any other limitation of the model is considered	High	Fulfilled	Low
Reliable prediction				
3.1	Reproducibility	High	Fulfilled	Low
3.2	Overall performance of the model	Medium	Fulfilled	Low
3.3	Fit within the physicochemical, structural and response spaces of the training set of the model	Medium	Fulfilled	Low
3.4	Performance of the model for similar substances	High	Fulfilled	Low
3.5	Mechanistic and/or metabolic considerations	High	Not applicable/assessed	
3.6	Consistency of information	High	Not fulfilled	High
Outcome is fit for the regulatory purpose				
4.1	Compliance with additional requirements	High	Fulfilled	Low
4.2	Correspondence between predicted property and property required by the regulation	High	Fulfilled	Low
4.3	Decidability within the specific framework	High	Fulfilled	High

- Low
- Medium
- High

- Fulfilled
- Not fulfilled
- Not documented
- Not applicable/assessed

- Low
- Medium
- High

Prediction Checklist : 577-11-7

Principle	Assessment element	Weight	Outcome	Uncertainty
Reliable prediction				
3.3	Fit within the physicochemical, structural and response spaces of the training set of the model	Medium	Fulfilled	Low
3.4	Performance of the model for similar substances	High	Fulfilled	Low

- Molecular Weight Domain: 31 - 1215
- Molecular Weight : 423
- Chemical Fragment: Linear C4 terminal chain [CCC-CH3],
  Ester [-C(=0)-0-C], Sulfonic acid / salt -> aliphatic attach

3.3

- The molecular weight of the substance under analysis is 423, which is in the range of the maximum value and minimum value of the training set compounds.
- All structural fragments of the substance under analysis except sulfonic acid are included in the 42 structural fragments which are defined based on the training set.
- Sulfonic acid is not included in the fragment library, but multiple substances containing sulfonic acids are included in the training set.
- In terms of the substance under analysis, BIOWIN5 predicts 0.56 and BIOWIN6 predicts 0.54, which are in the range of the training set compounds.

3.4

- The measured BOD degradability of all similar substances are 60% or more, and all are predicted as readily biodegradable.

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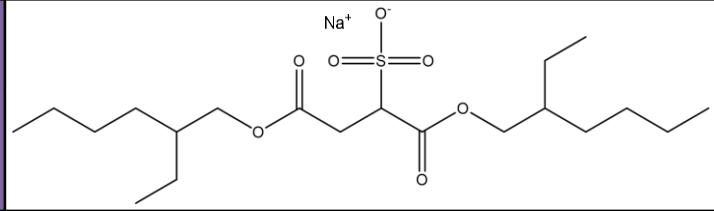
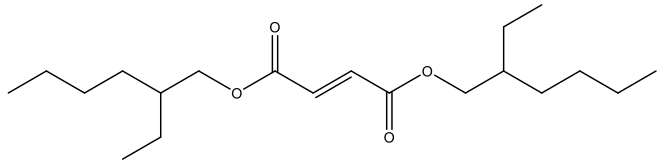
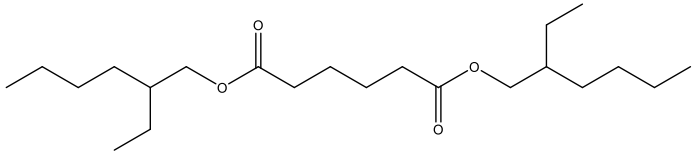
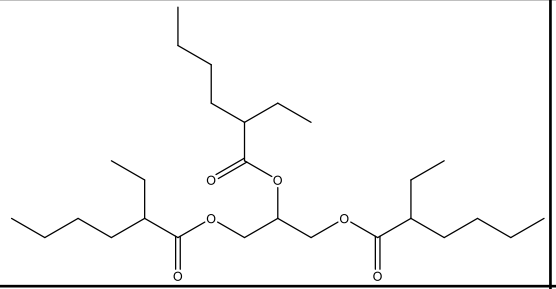
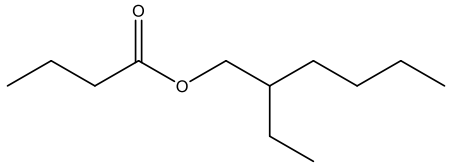
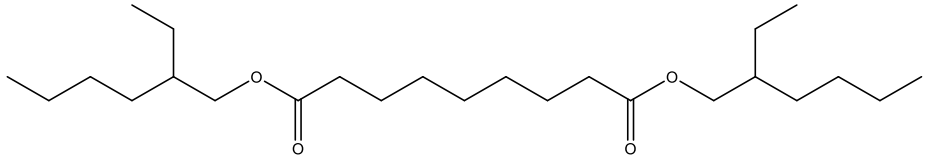
Similar substances : 577-11-7

	Target	analogue 1	Analogue2
Structure			
CAS RN	577-11-7	141-02-6	103-23-1
Measured value of degradability	3%(301C)	96%(301C)	71%(301C)
BIOWIN5	0.60 (RD)	0.75 (RD)	0.82 (RD)
BIOWIN6	0.54 (RD)	0.81 (RD)	0.89 (RD)

	Analogue3	Analogue4	Analogue5
Structure			
CAS RN	7360-38-5	25415-84-3	103-24-2
Measured value of degradability	85%(301C)	100%(301C)	95%(301C)
BIOWIN5	0.85 (RD)	0.70 (RD)	0.83 (RD)
BIOWIN6	0.85 (RD)	0.86 (RD)	0.88 (RD)

RD: Readily biodegradable, NRD: Not readily biodegradable

Prediction Checklist：577-11-7

Principle	Assessment element	Weight	Outcome	Uncertainty
Reliable prediction				
3.6	Consistency of information	High	Not fulfilled	High
Outcome is fit for the regulatory purpose				
4.3	Decidability within the specific framework	High	Fulfilled	High

3.6

- For the substance under analysis, experimental data in accordance with OECD 301C is BOD 3% and is not readily biodegradable, which is contrary to the predicted result. But there are also the following experimental data.
  - ✓301A (non-GLP) readily biodegradable (98%(DOC removal))
  - ✓301B (GLP) readily biodegradabl (94%(inorg. C analysis))
  - ✓301D (non-GLP) readily biodegradable (93% degradation (DOC removal))
  - ✓301D (non-GLP) readily biodegradable (61%(O2 consumption))
  - ✓301E (non-GLP) not readily biodegradable (25%(DOC removal))
  - ✓301F (non-GLP) readily biodegradable (BOD 76%)
  - ✓302B (non-GLP) readily biodegradable (97%(O2 consumption))
  - ✓310 (GLP) readily biodegradable (91%(inorg. C analysis))

4.3

- BIOWIN5 (Linear model prediction) estimates 0.56 as probabilities and BIOWIN6 (Non-linear model prediction) does 0.54. Both models estimate a probability greater than 0.5, which means that the substance is judged as readily biodegradable. However, the probabilities are close to 0.5, so there is not sufficient level of confidence.

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Conclusion on the prediction：577-11-7

Uncertainty

- High
- In terms of all the analogues, the prediction results match with the experiment results, while in terms of the substance under analysis, the prediction results don't match with the experiment result. Moreover, the probabilities of prediction results are close to 0.5, so there is not sufficient level of confidence.

Outcome of the assessment

- Not acceptable for the intended purpose
- There is a lack of information to judge the biodegradation of the substance under analysis from the results.

Summary

From the Model Checklist, it was confirmed that BIOWIN can be used without any issues to comply with regulations under CSCL.

In risk assessment under CSCL, it is necessary to externally demonstrate the validity of QSAR predictions. We found the Model Checklist and Prediction Checklist to be useful in this context.

In our actual risk assessment, many cases involve substances with no measured values. It was very useful to learn that the Prediction Checklist can efficiently evaluate both measured and unmeasured substances.