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Evaluating Ames mutagenicity predictions for 1,3,5-tris(2,3-dibromopropyl)-1,3,5-triazinane-2,4,6-trione under the OECD QSAR Assessment Framework

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The views expressed are those of the speaker and not an official position of NIHS or FSCJ.

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 → Model Checklist

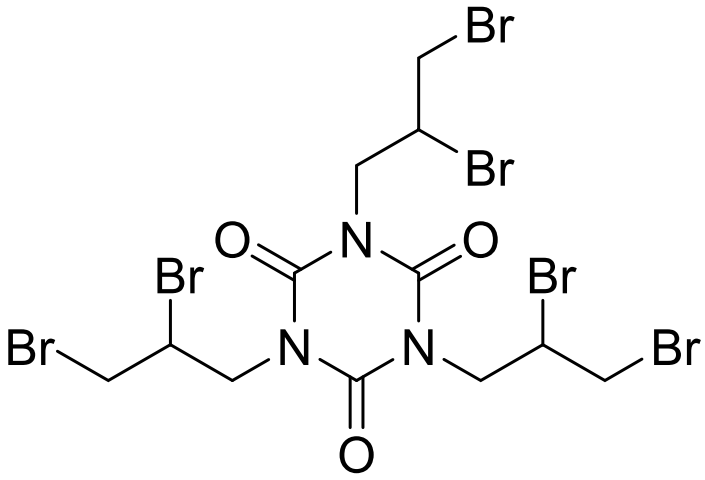
1) a defined endpoint
2) an unambiguous algorithm
3) a defined domain of applicability
4) appropriate measures of goodness-of–fit, robustness and predictivity
5) a mechanistic interpretation, if possible

OECD (Q)SAR Prediction Principles → Prediction & Result Checklists

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

A case study to assess a chemical by using the checklists

Our target substance: 1,3,5-tris(2,3-dibromopropyl)-1,3,5-triazinane-2,4,6-trione



CAS RN: 52434-90-9
MW 728.7
Log P 4.48
Ames test TA100 and TA1535 +/- S9 positive

Backgrounds for the assessment

- Case Study: We assumed to determine Ames mutagenicity of a target substance by using QSAR under an example regulation. It assumed to be evaluated by an example assessor. For assuming an example regulation (Purpose: screening, listing up Ames equivocal/positive chemicals), we referred to the draft guidance of Food Safety Commission of Japan (FSCJ) for QSAR, "Guidance for (Q)SAR to evaluate mutagenicity as health impact assessment of food". Like ICH M7 guideline, two (Q)SAR tools (statistical-based and rule-based) were used to evaluate overall mutagenicity.

  → **Multiple model's predictions. Need Result Checklist.**
- QMRF and QPRF were requested by the assessor and provided by the model user.
- QSAR tool 1: CASE Ultra Version 1.9.0.2 (MultiCASE Inc.)

  Model name : GT1_BMUT Statistical model for bacterial mutagenicity as per OECD 471
- QSAR tool 2: OASIS TIMES 2.31.2 (LMC)

  Model name : In vitro Ames Mutagenicity with S9 metabolic activation v.18.18

- To simplify the discussion, we picked up a target substance with a positive Ames test result.

Model Checklists

A 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

An unambiguous algorithm

2.1 Description of the algorithm and/or software

2.2 Inputs and other options

2.3 Model accessibility

A 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

A mechanistic interpretation if possible

5.1 Plausibility of the mechanistic interpretation

- Outcomes except for 1.2, 1.3 and 2.3 are Fulfilled
  - 1.2, 1.3 and 2.3 are Not applicable/assessed （Case Ultra）
  - 2.3 is Not applicable/assessed （OASIS TIMES）

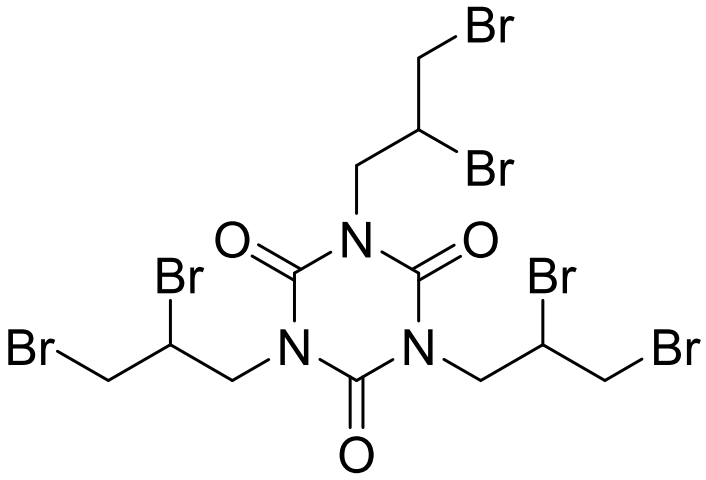
Assessment Based on Model Checklists

- The models evaluated in this study are applied under the example regulation.
- The models fill the requests for our target regulatory purposes.
- In this case study, the Model Checklists are easy to handle.
- The only issue is the transparency of models for assessors who are not able to access to the models, but not an issue for a user of the models. We might accept this limitation.

"Authorities responsible for the assessment can decide the minimum acceptable level of transparency needed for specific purposes, with the understanding that for some models the available information might be limited for e.g., commercial reasons."

"For some regulatory purposes and with a valid justification, models that do not fulfil all assessment elements (AEs) can also be accepted."

Result Checklists for 1,3,5-tris(2,3-dibromopropyl)-1,3,5-triazinane-2,4,6-trione



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OECD QSAR Prediction Principles & Result Checklist

Correct input(s) to the model

1.1 Clear and complete description of the input and model settings

1.2* Input representative of the substance under analysis

1.3 Reliable input (parameters)

The fit of the substance within the applicability domain of a valid model

2.1 Substance within the applicability domain

2.2 Any other limitation of the model is considered

Reliable prediction

3.1 Reproducibility

3.2 Overall performance of the model

3.3 Fit within the physicochemical, structural and response spaces of the training set of the model

3.4 Performance of the model for similar substances

3.5* Mechanistic and/or metabolic considerations

3.6* Consistency of information

Outcome is fit for the regulatory purpose

4.1* Compliance with additional requirements

4.2* Correspondence between predicted property and property required by the regulation

4.3* Decidability within the specific framework

Conclusion on the final result (Compile only when multiple predictions are considered)

5.1 Correct determination of the final result from individual predictions

Result Checklist

1. Outcome
   - Fulfilled
   - Not fulfilled
   - Not applicable/assessed
   - Not documented

2. Uncertainty level
   - Low
   - Medium
   - High

3. Weight
   - Low
   - Medium
   - High

* should be decided by taking into account the information from all predictions, and the same outcome should be recorded across predictions.

Accessibility of training set

Checklist (Both CASE Ultra and TIMES)

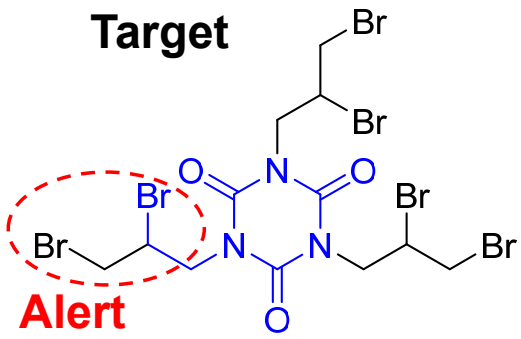
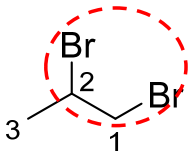
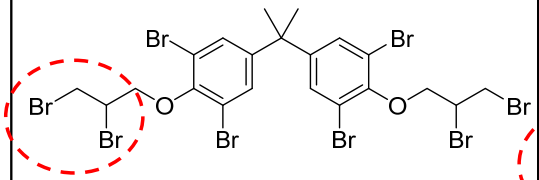
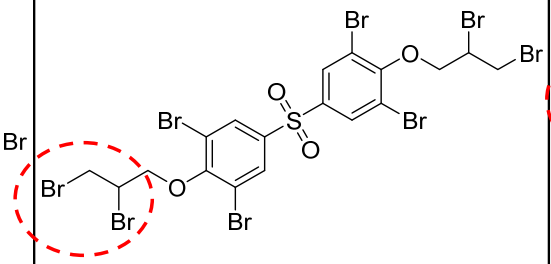
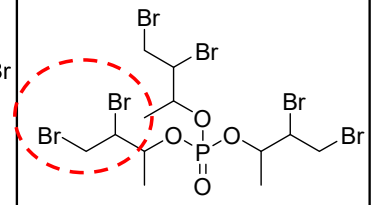
Principle	Assessment element	Weight	Outcome	Uncertainty
Substance within the applicability domain of a valid model				
2.1	Substance within the AD	High (if 3.1-3.5 not assessed) Medium (if 3.1-3.5 assessed)	Fulfilled	**Medium**

- **Most commercial models tend to treat training data as confidential and not accessible (especially) from assessors.**
  - **Now, most models include function to check if the target substance is within the Applicability Domain (AD). It is detailed in some cases, but still verifying it independently is difficult in most cases.**
  - **So, the uncertainty level of the assessment element (AD) is practically no lower than "medium" according to current definition.***

** Current definition*

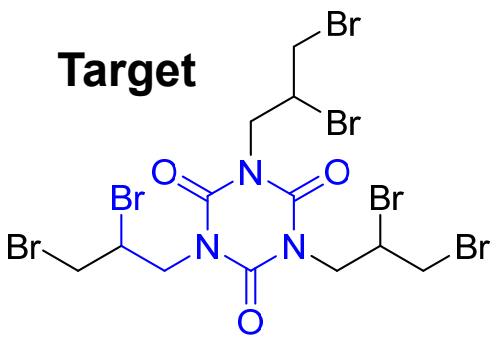
- *2.1 "AD" Uncertainty Low: the substance clearly falls within the applicability domain of the model*
- *2.1 "AD" Uncertainty Medium: the model automatically indicates that the substance is within its applicability domain,* ***but this cannot be independently verified***

Similar substances to confirm 3.4 local performance

CAS RN	**52434-90-9**	78-75-1	21850-44-2	42757-55-1	111712-45-9
MW	**728.7**	201.9	943.6	965.6	739.7
Log P	**4.48**	2.22	11.26	9.26	6.61
Case Ultra	**Positive**	Positive	Positive	Positive	Positive
Times	**Positive**	Positive	Positive	Positive	Positive
Test data*	**Positive**	Positive	Positive	Positive	Positive
					

- We'd like to emphasize the difficulties to define "similar" substances for assessing 3.4 Local performance, maybe the definition is endpoint-specific and regulation-dependent.
- Tested analogues with similar overall structures (cyanuric acid substituted with haloalkane groups) could not be found.

* Both (Either) TA100 and(or) TA 1535 positive.



Similar substances to confirm 3.4 local performance

Checklist (Both CASE Ultra and TIMES)

Principle	Assessment element	Weight	Outcome	Uncertainty
Reliable prediction				
3.4	Performance of the model for similar substances	High	Fulfilled	**Medium**

- *Uncertainty Low: similar substances with reliable experimental data are available, and the model predicts them well.*
- *Uncertainty Medium: **only moderately similar substances** (or only one similar substance) with reliable experimental data are available, and the model predicts them well.*

  - In our case, similar substances i.e. **cyanuric acid substituted with haloalkane groups** is not found and moderately similar substance's prediction is fine.

Final result from multiple predictions
Result checklist

- How to decide the overall outcome / uncertainty when they are not consistent between multiple predictions?
  - When multiple predictions show consistent results, it is basically thought supportive and expected to lower the uncertainty. So, we suppose it can be "Low".
  - AEs to conclude final result from multiple predictions:
    - ✓ Same outcome/different uncertainty : apply lower uncertainty level of them.
    - ✓ Different outcome : (basically) apply better assessment outcome.

	CASE Ultra	TIMES	Final result
Uncertainty	Medium	Medium	**Low**
Outcome of the assessment	Acceptable	Acceptable	Acceptable
Comments	The uncertainty levels of 2.1 and 3.4 were medium. Other uncertainty levels for the AEs with high weight were all low.	The uncertainty levels of 2.1 and 3.4 were medium. Other uncertainty levels for the AEs with high weight were all low.	**Two predictions are consistent.**

Next step to continue case studies

- “Each guidance/regulation may accept the limitations of models and/or predictions” is not always clear.
- Such limitations of models and predictions would be case by case and depend on endpoints (properties), but general rules will be required to fill the model, prediction and result Checklists as well as to construct QMRF and QPRF.

The optimization of QMRF and QPRF:

The acceptance criteria of model/prediction AEs in each guidance/regulation should be considered when an authority applies QAF and continuing the case studies of QAF.

Example：Ames test data quality

- Related Assessment Elements
  - Defined endpoint: the quality of training data
  - Predictivity: the quality of external validation data
  - Reliability of the predictions: the quality of similar chemical data

- How quality level of Ames data should be included in QMRF/QPRF?

Acknowledgements

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Thank you for listening