

# Framework for *In Silico* Toxicity Screening of Novel Odorants

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SOT Abstract/Poster #: 3698/P183

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# Conflict Disclosure

- No conflict to declare
- Research funded by Monell Chemical Senses Center

# Background and Objective

## Background

- This *in silico* approach was developed to support a psychophysical study of novel odorants, in which human volunteers sniffed the headspace of a solution in a vial (Lee *et al.*, 2023).
- Safety data lacking for novel odorants, restricts study.
- Develop a transparent *in silico* models to predict inhalation toxicity.
- Use this to predict allowable solution concentrations.

## Objective

- Derive toxicology-based maximum recommended solution concentrations for odorant chemicals, using chemical structure alone by an *in silico* approach.

Lee BK, Mayhew EJ, Sanchez-Lengeling B, Wei JN, Qian WW, Little KA, Andres M, Nguyen BB, Moloy T, Yasonik J, Parker JK, Gerkin RC, Mainland JD, Wiltschko AB. A principal odor map unifies diverse tasks in olfactory perception. Science. 2023 Sep; 381(6661):999-1006. doi: 10.1126/science.ade4401. Epub 2023 Aug 31. PMID: 37651511.

# Overview of *In Silico* Prediction of Inhalation Toxicity Hazard and Solution Concentration

## Hazard Prediction

Mutagen Tree

Yes

No

Gradient  
Supplemental Tree

I, II, III

No

Revised Cramer  
Tree

I, II, III

Chemical SMILES

## Exposure Limit

Assign TTC (µg/day):

Mutagen = 12\*

Class III = 90

Class II = 540

Class I = 1,800

## Exposure Prediction

Predict Vapor  
Pressure

$$\text{Headspace Mass} = \frac{VP \times MW \times V}{R \times T}$$

$$\text{Solution Concentration (\%)} = \frac{TTC \times 100\%}{\text{Headspace Mass}}$$

## Solution Concentration

Notes: SMILES = Simplified Molecular Input Line Entry System; TTC = Threshold of Toxicologic Concern.

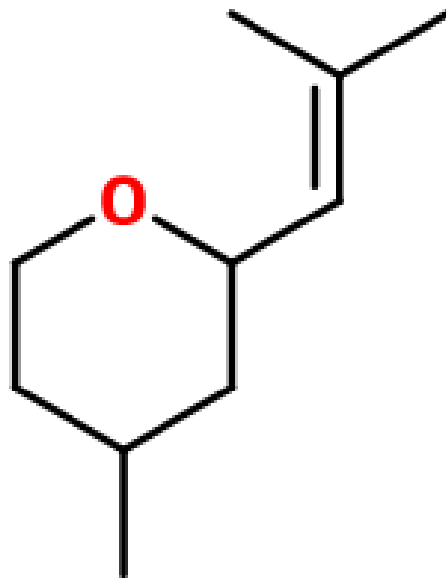
\* Based on ICH M7 TTC of 120 µg/day for ≤ 30 days exposure at 1:100,000 cancer risk level adjusted to 12 µg/day for 1:1,000,000 cancer risk level.

# Overview of *In Silico* Prediction of Inhalation Toxicity Hazard and Solution Concentration

Chemical SMILES

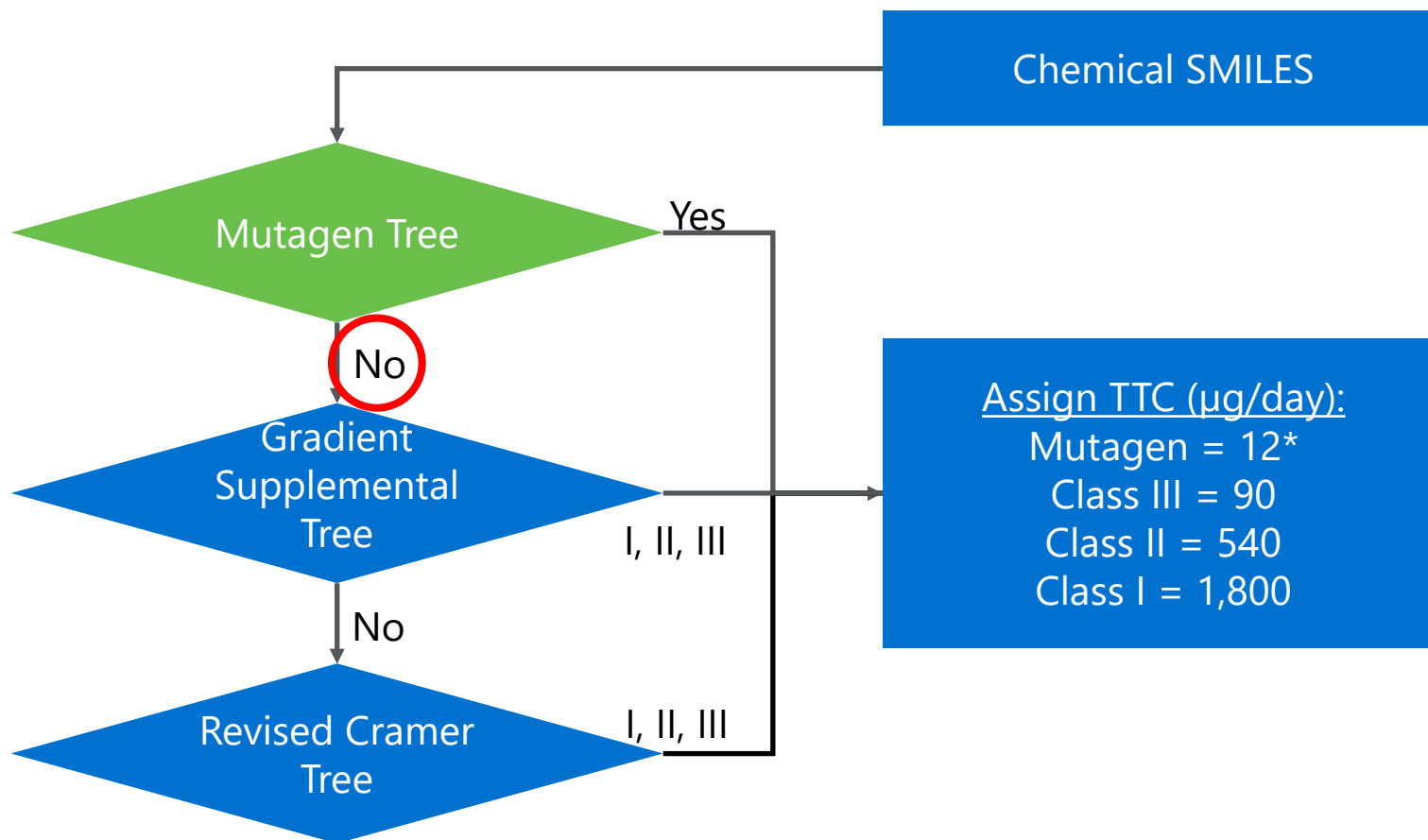
Rose oxide (CAS No. 16409-43-1)

CC1CCOC(C1)C=C(C)C



Notes: SMILES = Simplified Molecular Input Line Entry System.  
<https://pubchem.ncbi.nlm.nih.gov/compound/27866>

# Overview of *In Silico* Prediction of Inhalation Toxicity Hazard and Solution Concentration



Toxtree software (version 3.1.0)

The screenshot shows the Toxtree software interface (version 3.1.0) displaying the results of an Ames test. The window title is "In vitro mutagenicity (Ames test) alerts by ISS". The main panel shows a decision tree with the following structure:

- Alerts Ames mutagenicity
  - SA1\_Ames
    - SA2\_Ames
      - SA3\_Ames
        - SA4\_Ames
          - SA5\_Ames
            - SA6\_Ames

The "Decision node:" is QSA1\_Ames.Acyl halides. The "If 'NO' go to:" is Q.SA2\_Ames. The "Rule ID" is A1\_Ames, and the "Rule title" is Acyl halides. The "Rule explanation" is: Acyl halide  $RC(=O)[Br, Cl, F, I]$ , where R is not OH or SH. A chemical structure of an acyl halide is shown, with a nitrogen atom (N) bonded to a carbonyl group (C=O) and a chlorine atom (Cl).

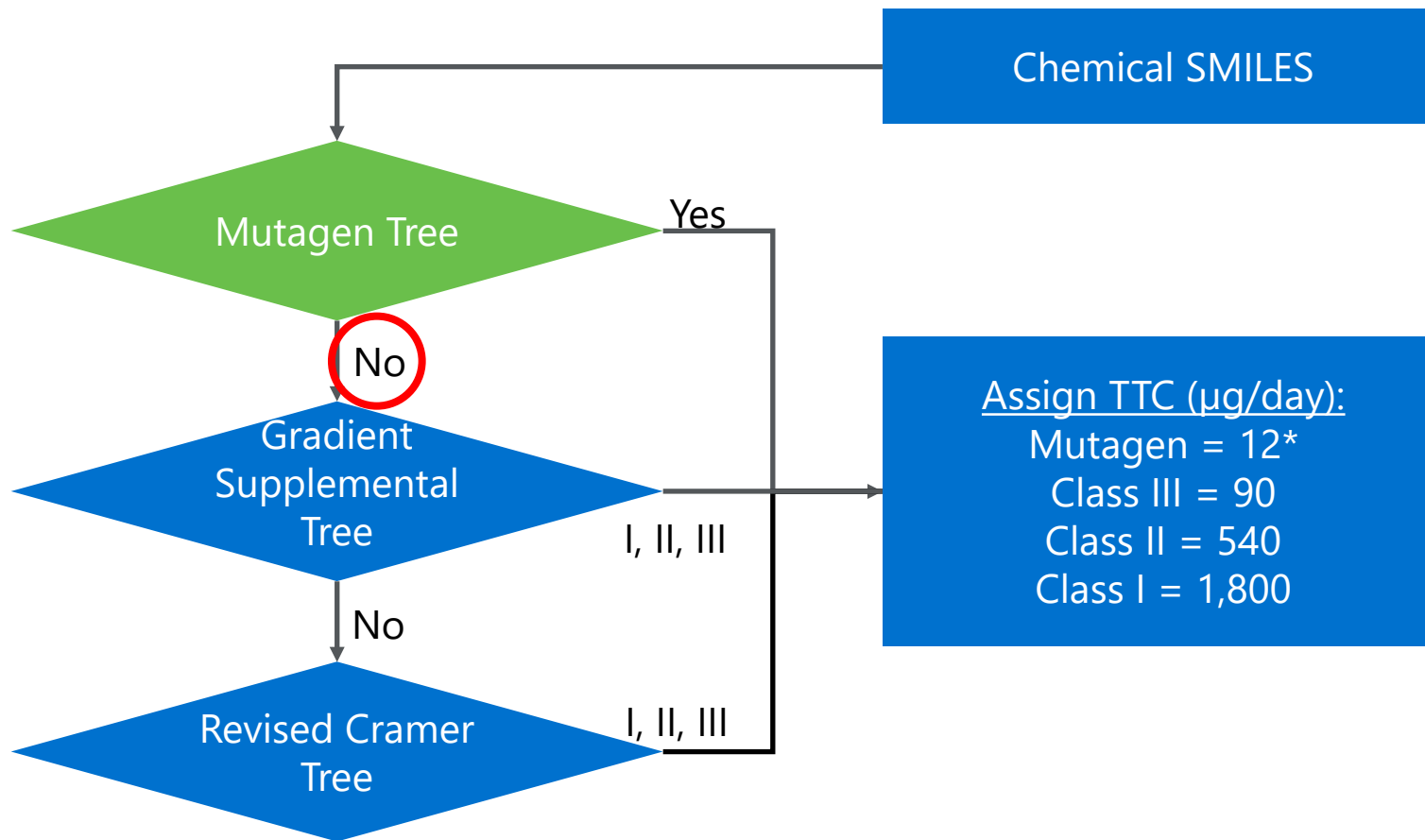
There are example molecules for each rule outcome. Select which one to display.

☒ Yes branch ☐ No branch

Ideaconsult Ltd. 2018. "Toxtree - Toxic Hazard Estimation by decision tree approach (Version 3.1.0)." Accessed at <http://toxtree.sourceforge.net>.



# Overview of *In Silico* Prediction of Inhalation Toxicity Hazard and Solution Concentration



Toxtree software (version 3.1.0)

Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v3.1.0-1851-1525...

File Edit Chemical Compounds Toxic Hazard Method Help

Chemical identifier CC1CCOC(C1)C=C(C)C Go!

Available structure attributes	
Error when applying the ...	NO
For a better assessment ...	NO
No alerts for <i>S. typhimuri</i> ...	YES
Potential <i>S. typhimuri</i> ...	NO
QSAR13 applicable?	NO
QSAR6 applicable?	NO
SA10_Ames	NO
SA11_Ames	NO
SA12_Ames	NO
SA13_Ames	NO
SA14_Ames	NO

Structure diagram

First Prev 1 / 1 Next Last

Completed.

**Toxic Hazard** by *In vitro* mutagenicity (Ames test) alerts by ISS

Estimate

**Structural Alert for *S. typhimuri* mutagenicity**

No alerts for *S. typhimuri* mutagenicity

Potential *S. typhimuri* TA100 mutagen based on QSAR

Unlikely to be a *S. typhimuri* TA100

☒ Verbose explanation

QSA65\_Ames. Halofuranones **No** CC1CCOC(C1)C=C(C)C

QSA66\_Ames. Anthrones **No** CC1CCOC(C1)C=C(C)C

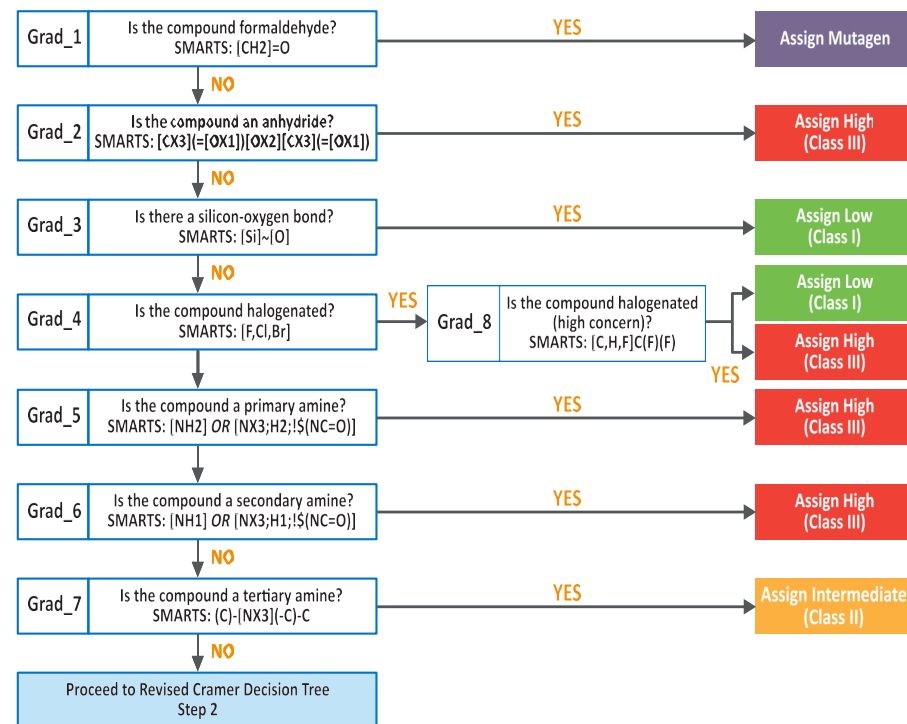
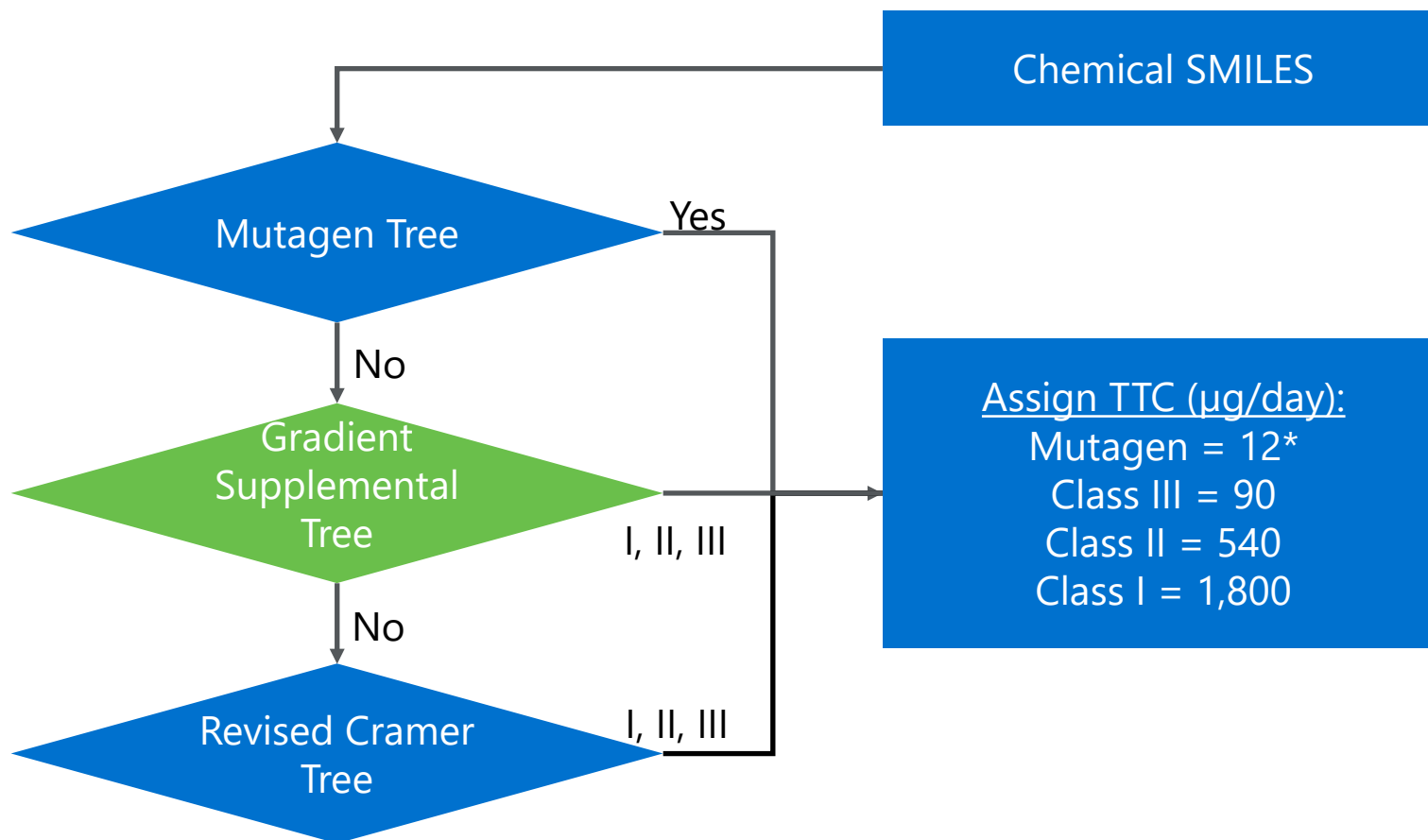
QSA67\_Ames. Triphenylimidazole and related **No** CC1CCOC(C1)C=C(C)C

QSA68\_Ames. 9,10-dihydrophenanthrenes **No** CC1CCOC(C1)C=C(C)C

QSA69\_Ames. Fluorinated quinolines **No** CC1CCOC(C1)C=C(C)C

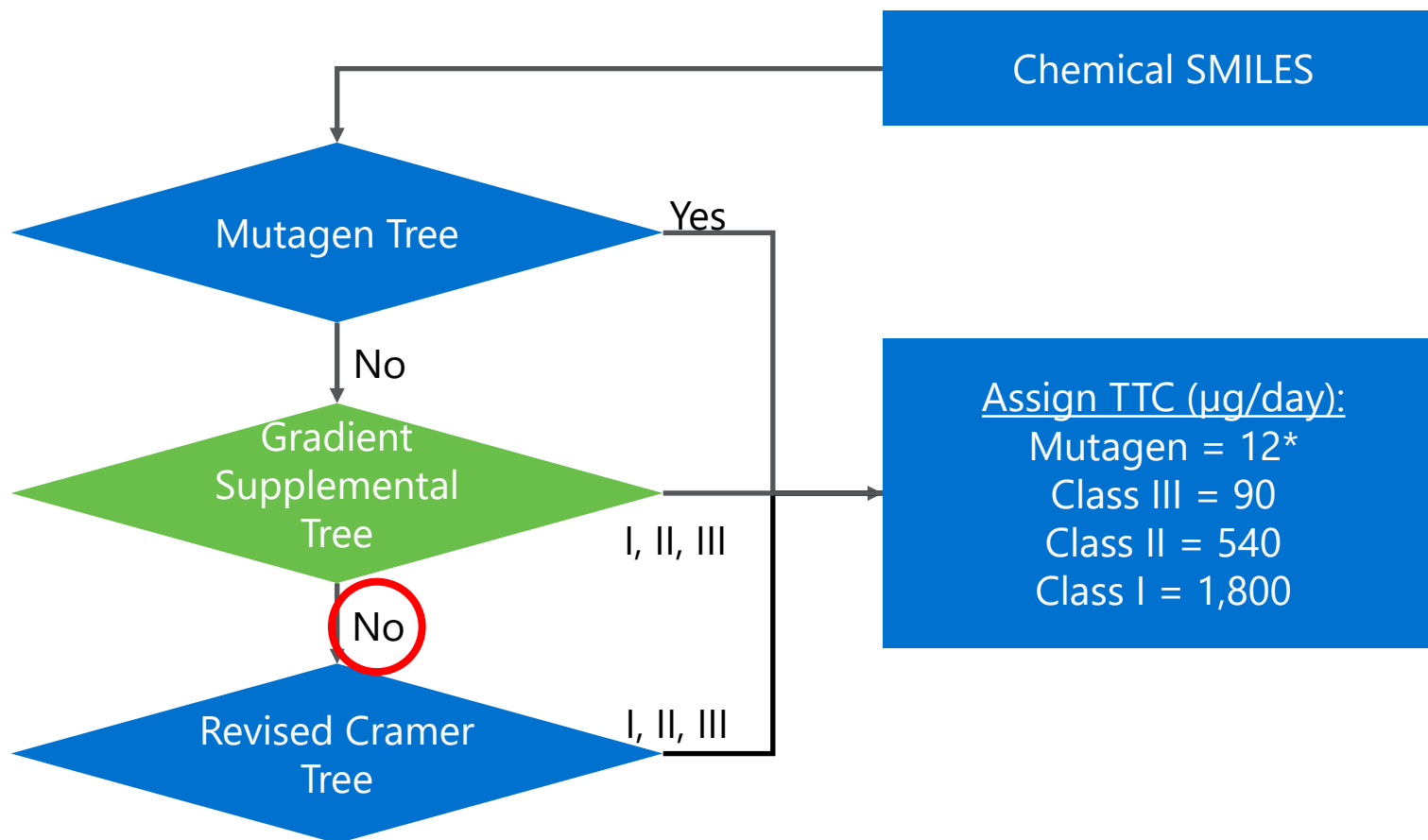
Ideaconsult Ltd. 2018. "Toxtree - Toxic Hazard Estimation by decision tree approach (Version 3.1.0)." Accessed at <http://toxtree.sourceforge.net>.

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Toxtree software (version 3.1.0)

Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v3.1.0-1851-1525...

File Edit Chemical Compounds Toxic Hazard Method Help

Chemical identifier CC1CCOC(C1)C=C(C)C Go!

Available structure attributes	
SMILES	<chem>CC1CCOC(C1)C=C(C)C</chem>
Supplementary_Rules_Gra...	No alert
Supplementary_Rules_Gra...	0N, 1N, 2N, 3N, 4N, 5N, 6N
cdk:Comment	Created from SMILES
cdk:Title	

Structure diagram

First Prev 1 / 1 Next Last

Completed.

Toxic by HazardSupplementary Rules Grad Inhalation 2

Estimate

Is secondary amine

Tertiary Amine

No alert

Low Concern Florinated

☒ Verbose explanation

Q1.Substructure by SMARTS patterns. No CC1CCOC(C1)C=C(C)C

Q2.is Siloxane No CC1CCOC(C1)C=C(C)C

Q3.Substructure by SMARTS patterns. No CC1CCOC(C1)C=C(C)C

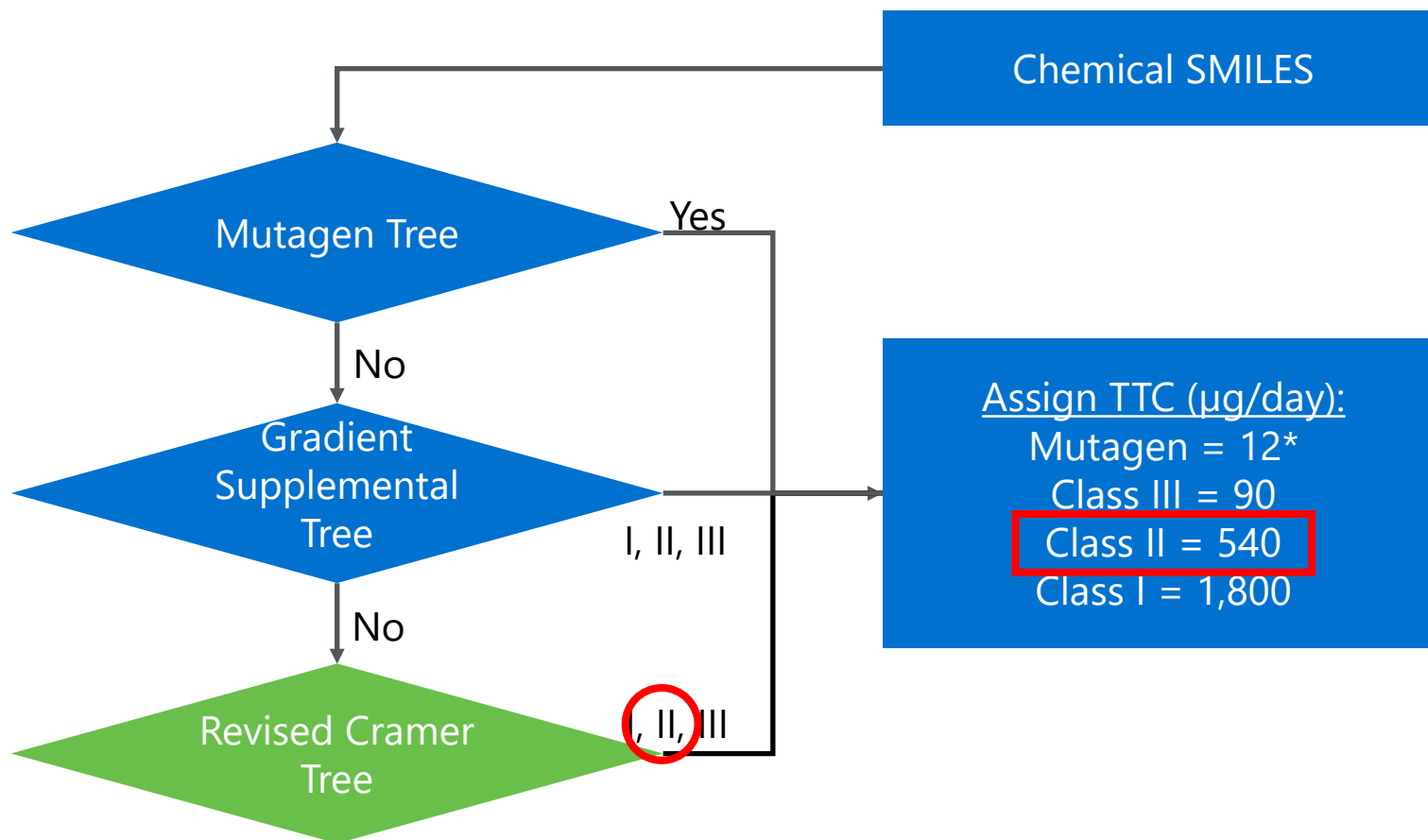
Q4.Primary Amine No CC1CCOC(C1)C=C(C)C

Q5.Secondary amine No CC1CCOC(C1)C=C(C)C

Q6.Tertiary Amine No Class No alert CC1CCOC(C1)C=C(C)C

Ideaconsult Ltd. 2018. "Toxtree - Toxic Hazard Estimation by decision tree approach (Version 3.1.0)." Accessed at <http://toxtree.sourceforge.net>.

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## Toxtree software (version 3.1.0)

Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v3.1.0-1851-1525...

File Edit Chemical Compounds Toxic Hazard Method Help

Chemical identifier CC1CCOC(C1)C=C(C)C Go!

Available structure attributes	
RevisedCDT	Intermediate (Class II)
SMILES	<chem>CC1CCOC(C1)C=C(C)C</chem>
cdk:Comment	Created from SMILES
cdk:Title	
toxtree.tree.cramer3.CDT...	1N,2N,3N,4N,6N,7Y,8N,9...

Structure diagram

First Prev 1 / 1 Next Last

Completed.

Toxic Hazard by Revised Cramer Decision Tree

Estimate

Low (Class I)

Intermediate (Class II)

High (Class III)

Substances with chemical structures that permit

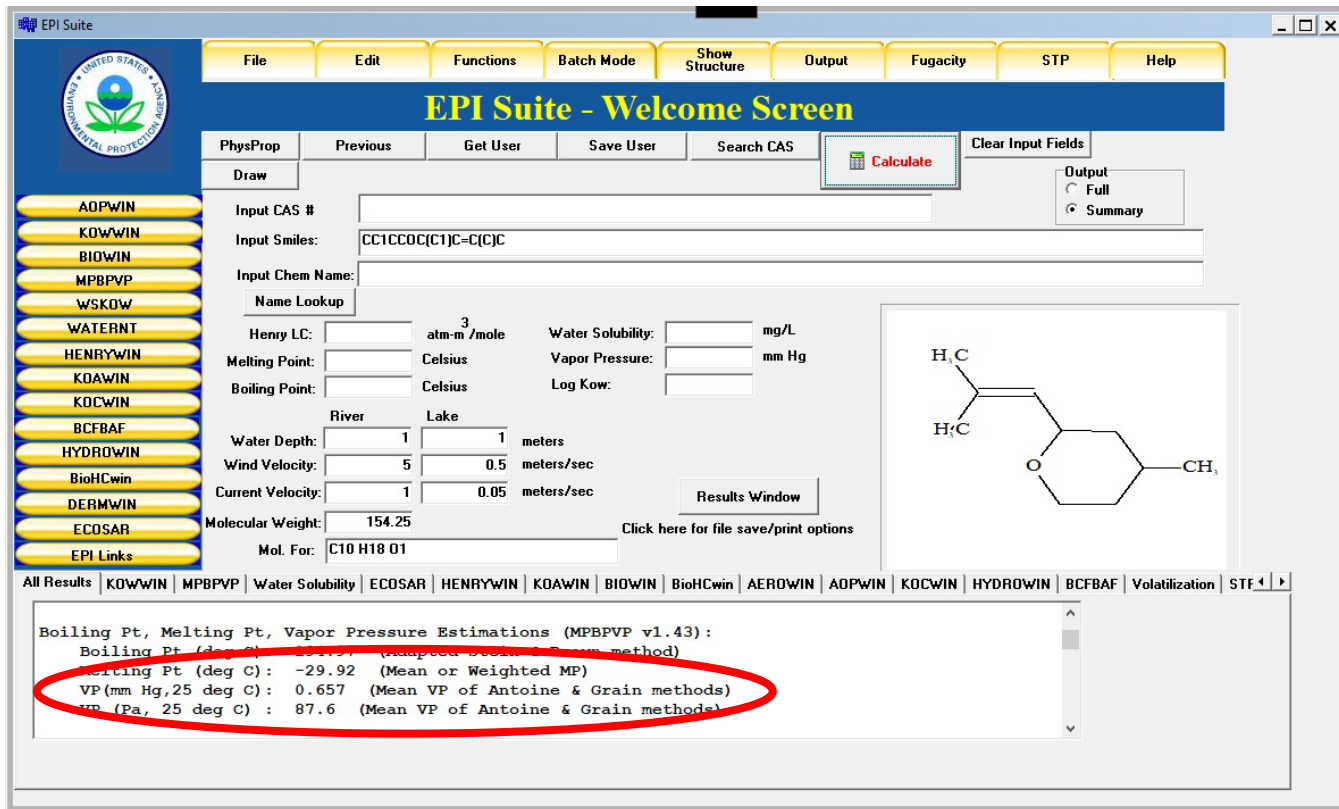
☒ Verbose explanation

other than specified? No  
CC1CCOC(C1)C=C(C)C  
Q6. Is the substance hydrocarbon, carbohydrate or terpene as specified? No  
CC1CCOC(C1)C=C(C)C  
Q7. Is the substance heterocyclic? Yes  
CC1CCOC(C1)C=C(C)C  
Q8. Is the substance heterocyclic because it contains a cyclic hemiacetal, acetal, hemiketal, ketal, or cyclic carbonate? No  
CC1CCOC(C1)C=C(C)C  
Q9A. Is the substance a cyclic diester or

Ideaconsult Ltd. 2018. "Toxtree - Toxic Hazard Estimation by decision tree approach (Version 3.1.0)." Accessed at <http://toxtree.sourceforge.net>.

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US EPA EPI Suite v 4.11 - MPBPWIN™ (v 1.43)



Chemical SMILES: CC1CCOC(C1)C=C(C)C

Results Window:

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):

Boiling Pt (deg C): 87.6 (Mean VP of Antoine & Grain methods)

Melting Pt (deg C): -29.92 (Mean or Weighted MP)

VP (mm Hg, 25 deg C): 0.657 (Mean VP of Antoine & Grain methods)

VP (Pa, 25 deg C): 87.6 (Mean VP of Antoine & Grain methods)

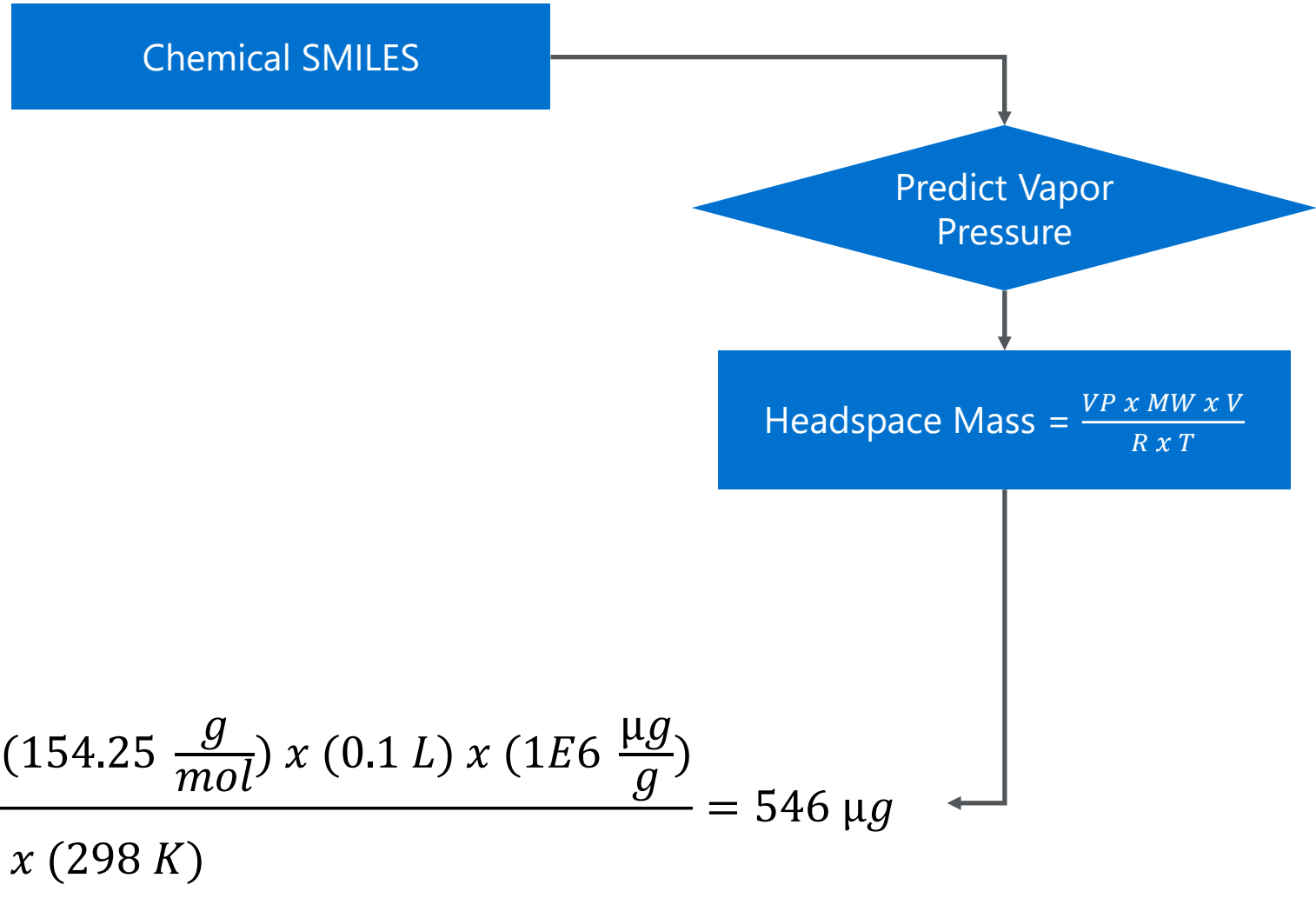
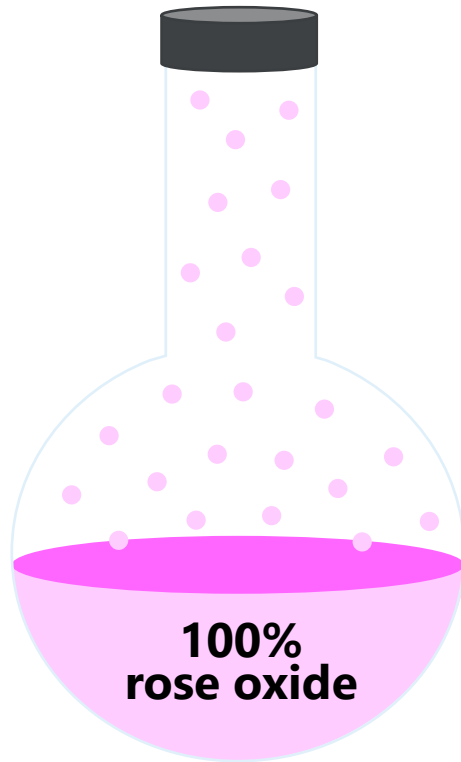
Chemical SMILES

Predict Vapor Pressure

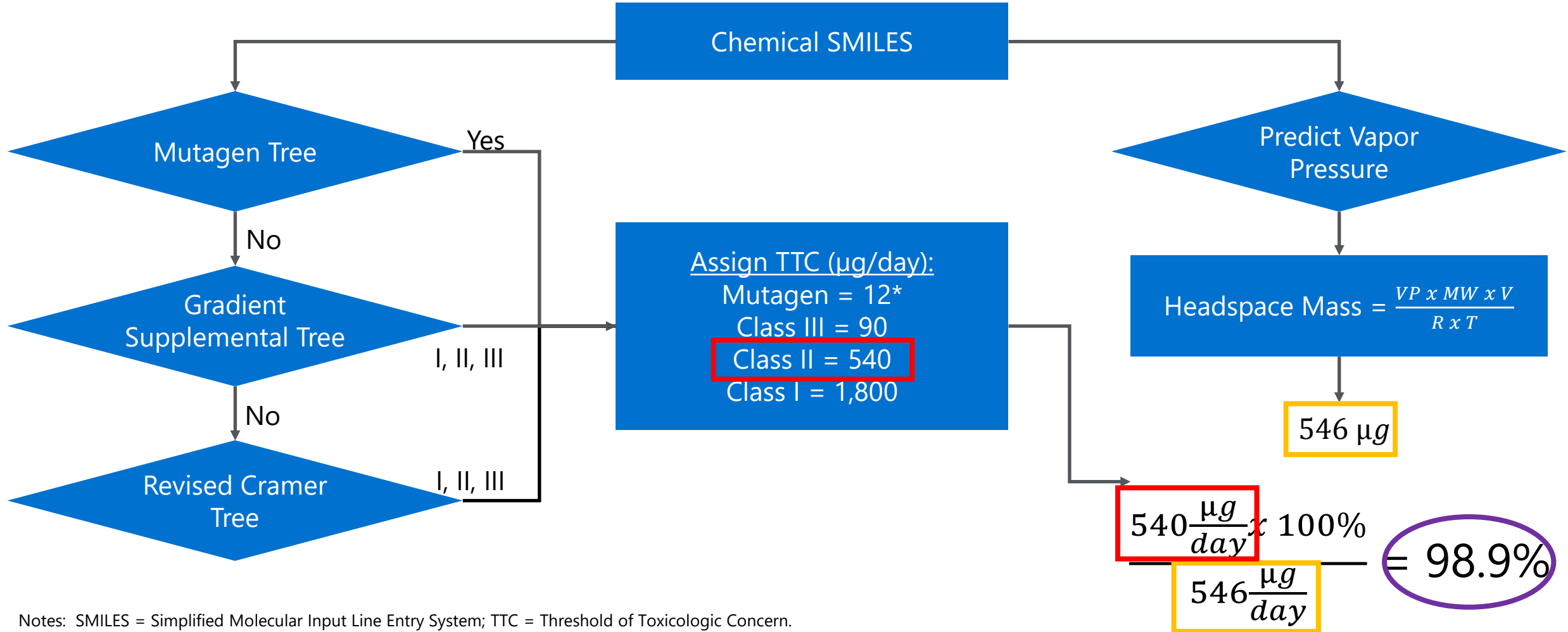
0.657 mmHg @ 25°C

US EPA. 2012. "EPI Suite v4.11." November. Accessed at <http://www.epa.gov/opptintr/exposure/pubs/episuitedi.htm>.

# Overview of *In Silico* Prediction of Inhalation Toxicity Hazard and Solution Concentration



# Overview of *In Silico* Prediction of Inhalation Toxicity Hazard and Solution Concentration

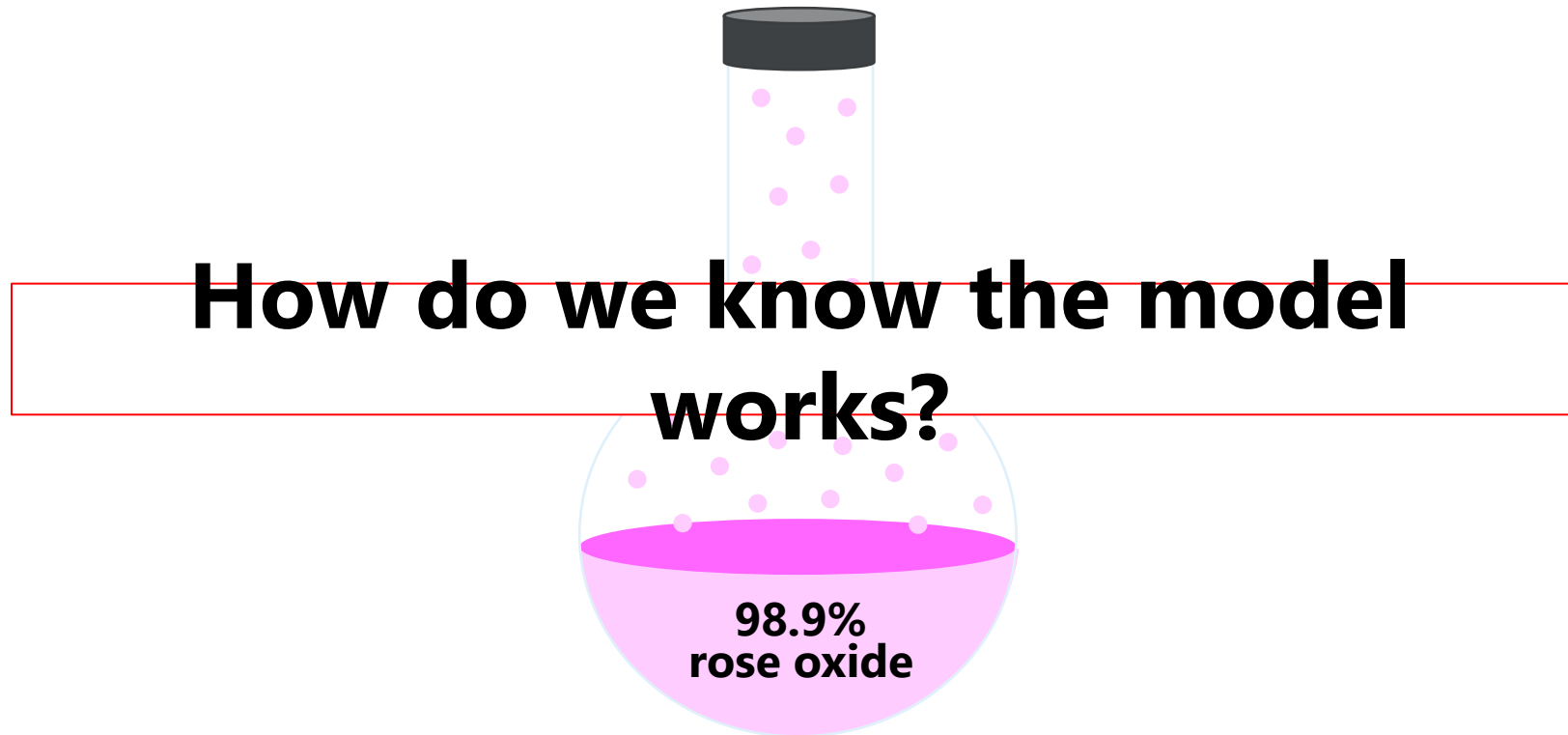


Notes: SMILES = Simplified Molecular Input Line Entry System; TTC = Threshold of Toxicologic Concern.

\* Based on ICH M7 TTC of 120  $\mu g/day$  for  $\leq 30$  days exposure at 1:100,000 cancer risk level adjusted to 12  $\mu g/day$  for 1:1,000,000 cancer risk level.

# Overview of *In Silico* Prediction of Inhalation Toxicity Hazard and Solution Concentration

- Model predicts that inhalation of a 0.1-L headspace of a 98.9% rose oxide solution would not exceed the assigned TTC.



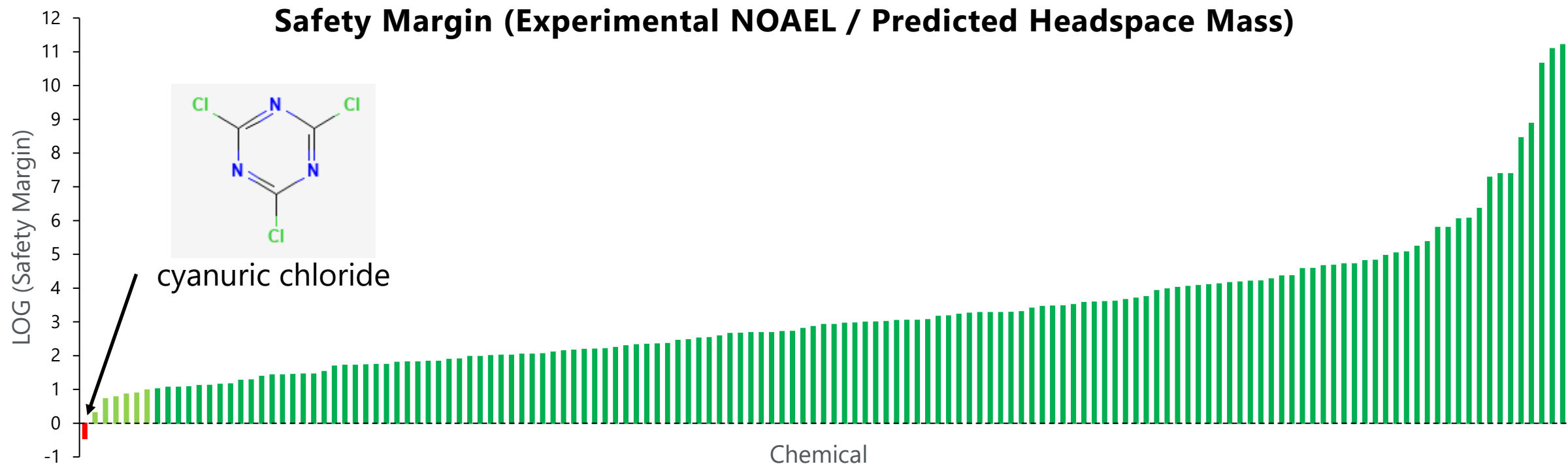


# Model Validation Against Inhalation Toxicity Dataset (Shin *et al.* 2019)

- Inhalation toxicity data for 143 compounds (90-day studies in rats) (Shin *et al.* 2019).
- Converted NOAEC (mg/L) to NOAEL (µg/day) by:
  - $NOAEL \left( \frac{\mu g}{day} \right) = NOAEC \left( \frac{mg}{L} \right) \times RMV_{rat} \left( 0.2 \frac{L}{min} \right) \times t \left( 360 \frac{min}{day} \right) \times (1000 \frac{\mu g}{mg})$
- Used our model to predicted acceptable solution concentrations based on SMILES.
- Estimated inhalation exposure (*i.e.*, headspace mass) based on predicted solution concentration (in water) assuming Raoult's Law applied.
- Compared NOAEL to exposure.  
$$Safety\ Margin = \frac{Experimental\ Inhalation\ Study\ NOAEL \left( \frac{\mu g}{day} \right)}{Acceptable\ Predicted\ Exposure\ from\ Headspace\ Mass \left( \frac{\mu g}{day} \right)}$$

Shin, JH; Lee, BH; Lee, SK. 2019. "Development of QSAR model for subchronic inhalation toxicity using random forest regression method." Bull. Korean Chem. Soc. 40(8):819-825. doi: 10.1002/bkcs.11835.

# Model Validation Against Inhalation Toxicity Dataset (Shin *et al.* 2019)



$$\text{Safety Margin} = \frac{\text{Experimental Inhalation Study NOAEL } (\frac{\mu g}{day})}{\text{Acceptable Predicted Exposure from Headspace Mass } (\frac{\mu g}{day})}$$

Safety Margin was above 1 for 99.3% of chemicals and above 10 for 95% of chemicals.

# Conclusions

- The in silico approach was developed as a transparent and health-protective tool
- Risk-based approach integrates predicted hazard and predicted inhalation exposure
- The approach showed robust performance in validation test
- Approach is conservative
  - TTCs are for repeated daily exposure while odorants will have acute / limited exposure
  - Validation used NOAEC values from 90-day repeated exposure studies
- Useful to exclude odorants where the concentration would be too low to allow reliable perceptual ratings
- Useful to flag certain (potentially toxic) chemicals for additional evaluation

# Limitations

- Cramer/Kroes TTC values derived from oral toxicity data (but for chronic daily exposure)
- Mutagenicity TTC does not apply to high potency mutagenic carcinogens (*e.g.*, N-nitroso, alkyl azoxy, aflatoxin-like)
- Does not identify potential asthmagens (but asthmatics excluded from study)
- Does not identify potential irritants (but irritation would likely be transient)
- Validation conducted with relatively small dataset that may not be representative of odorants

# Questions?

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