Workflow for collecting WoE

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Outlook

- General scheme
- Examples

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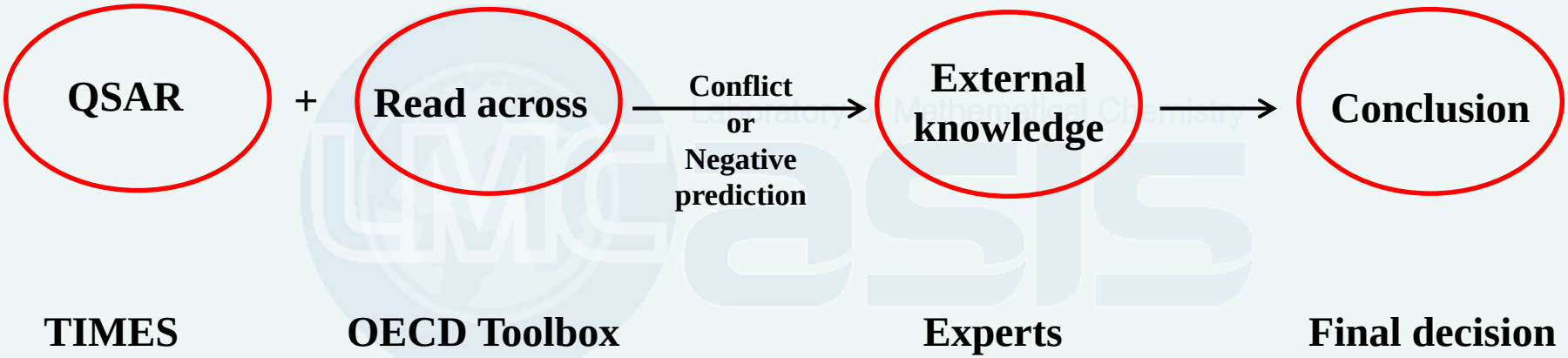
Workflow for collecting WoE

General scheme

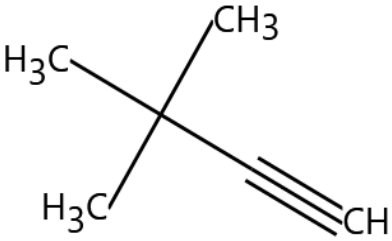


- Proposed scheme for collecting WoE is generally applicable for any QSAR study
- The application of scheme is critical in case of predictions which are:
  - ✓ Negative
  - ✓ Out of domain
  - ✓ Conflicting

Example 1

Workflow for collecting WoE

Target chemical: 3,3-Dimethylbutyne (CAS# 917-92-0)



smiles: CC(C)(C)C#C

Target endpoint: AMES Mutagenicity

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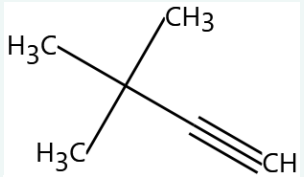
Example 1

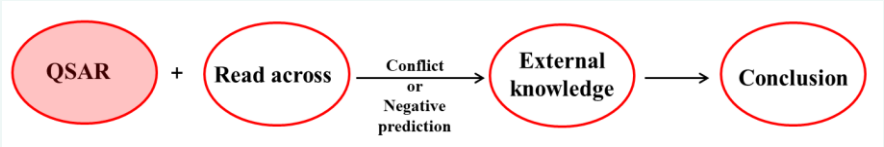
Workflow for collecting WoE



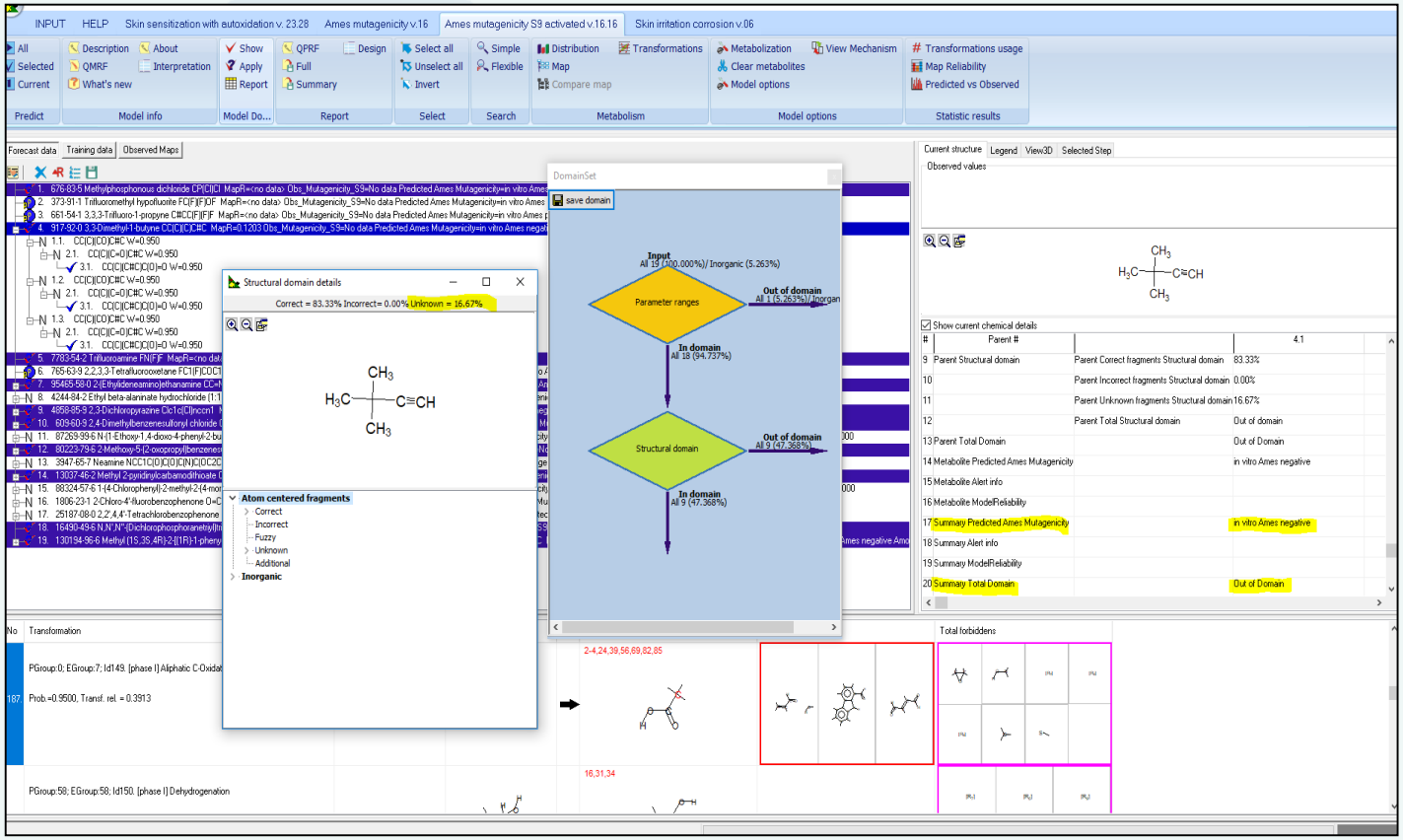
3,3-Dimethylbutyne

CAS# 917-92-0

TIMES AMES +S9 model

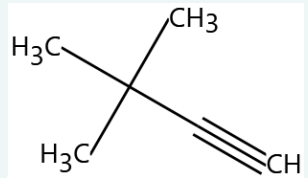


The chemical is predicted **Negative** by **TIMES AMES +S9** model. The prediction is out of the applicability domain of the model due to presence of "Unknown" atom-centered fragments extracted from the target structure



Example 1

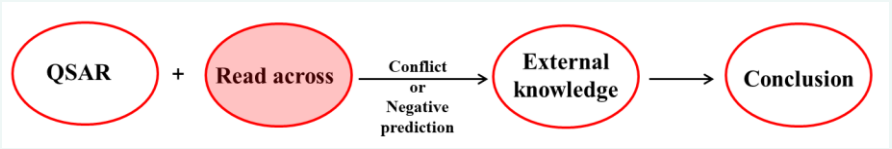
Workflow for collecting WoE



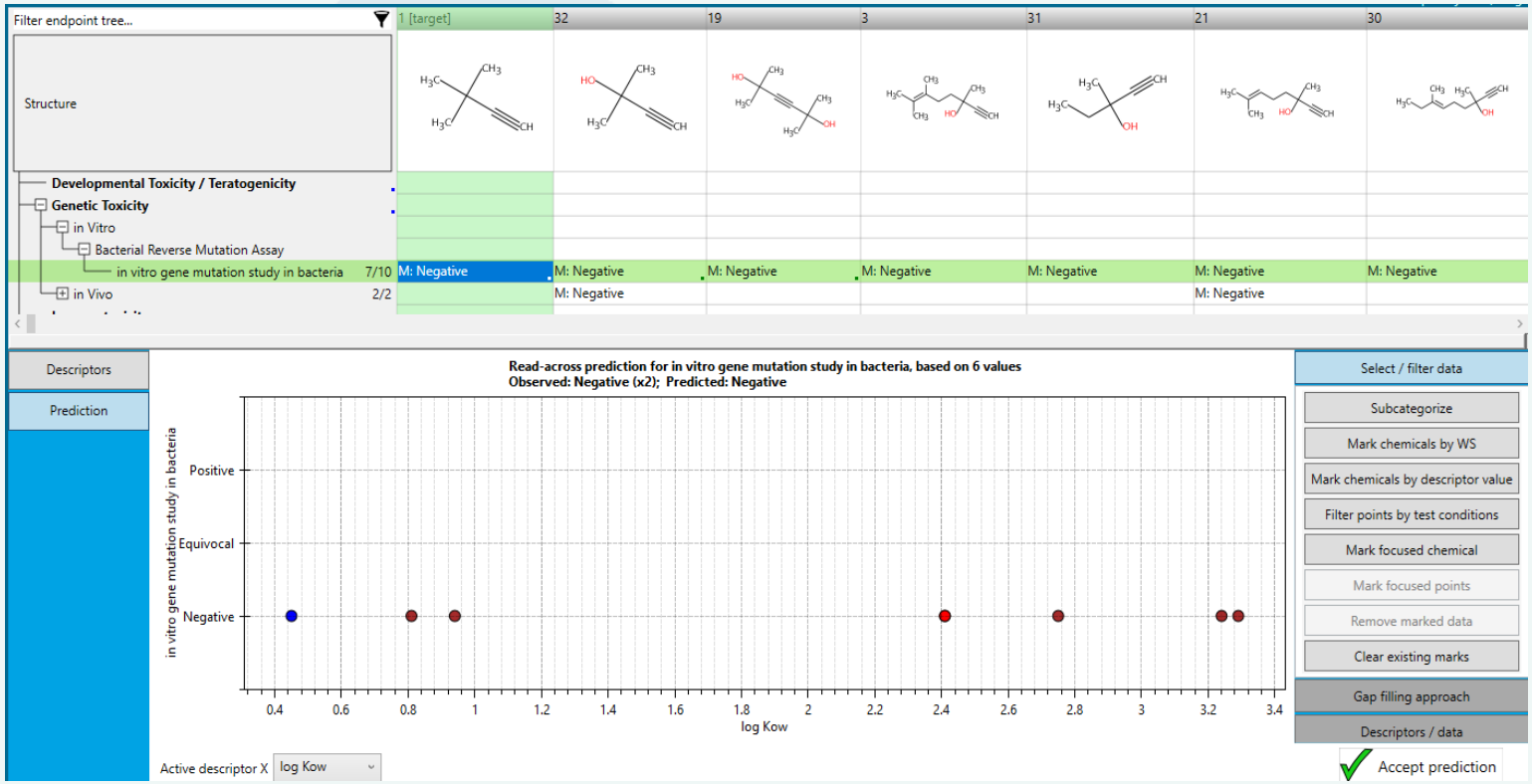
3,3-Dimethylbutyne

CAS# 917-92-0

Toolbox



By applying the category approach, four analogues of the target chemical were found all with **Negative** AMES experimental data (with and/or without S9). The read across prediction is **Negative.**



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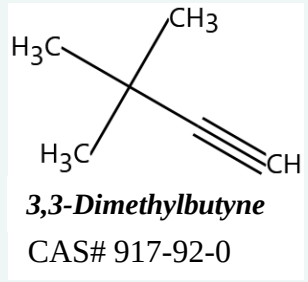
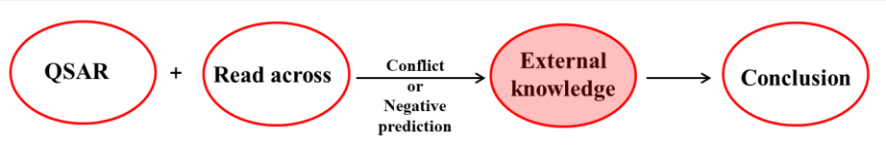
Workflow for collecting WoE



3,3-Dimethylbutyne

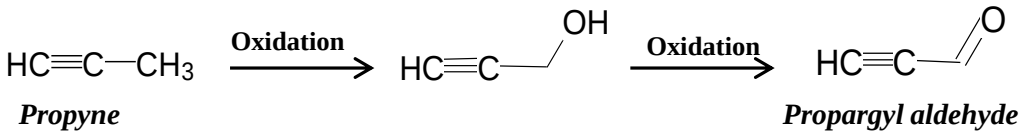
CAS# 917-92-0

Experts



Propyne could be also regarded as analogue of the target chemical. The only difference is that it does not contain the branched alkyl group. Negative experimental data were found for *Propyne* when tested under gas exposure conditions in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 both with and without metabolic activation [1]. The compound was **positive** when tested in *E.coli* strain *WP2 uvrA* both in the presence and absence of metabolic activation. These equivocal results for *propyne* obtained with different bacterial strains cannot be explained by simple DNA reactivity factors, concerning the parent chemical. After microsomal/S9 metabolic activation, however, *propyne* may be converted into *propargyl aldehyde* which is **strong bacterial mutagen** [2]:

Proposed enzymatic transformation of Propyne



Propyne ***Propargyl aldehyde***

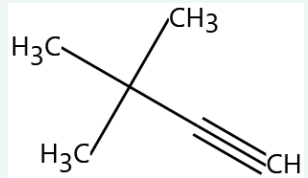
Unlike *Propyne* with compact molecular structure, the target chemical contains large, branched alkyl radical bound to the C*sp* atom. The electron-donating capacity of the alkyl radical and steric hindrance factors probably contribute to the negative *Ames* test result by making the target chemical less electrophilic and, hence, less reactive.

References:

1. Propyne (CAS No: 74-99-7) Health-Based Reassessment of Administrative Occupational Exposure Limits, Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands, No. 2000/15OSH/128, The Hague, June 8, 2004
2. Basu, A. K., L. J. Marnett, Molecular Requirements for the Mutagenicity of Malondialdehyde and Related Acroleins, Canc. Res. **44** (1984), 2848 – 2854.

Example 1

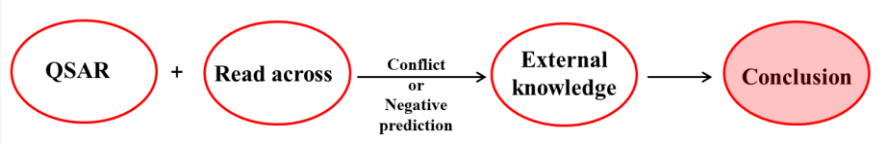
Workflow for collecting WoE



3,3-Dimethylbutyne

CAS# 917-92-0

Final decision



Expert conclusions:

- The reported experimental bacterial mutagenicity data for ***3,3-Dimethylbutyne*** are negative;
- Negative data has also been found for other branched-chain structural analogues by read-across analysis;
- *3,3-Dimethylbutyne* can be regarded as **non-mutagenic** in the Ames test.

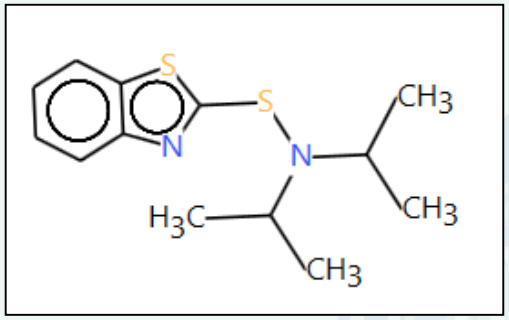
Ultimate conclusion:

3,3-Dimethylbutyne is **Non-mutagenic** in the AMES test.

Example 2

Workflow for collecting WoE

Target chemical: N,N-diisopropylbenzothiazole-2-sulphenamide (CAS# 95-29-4)



smiles: CC(C)N(Sc1nc2ccccc2s1)C(C)C

Target endpoint: Skin sensitization

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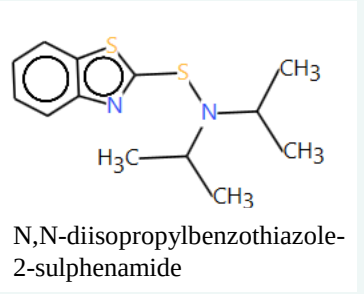
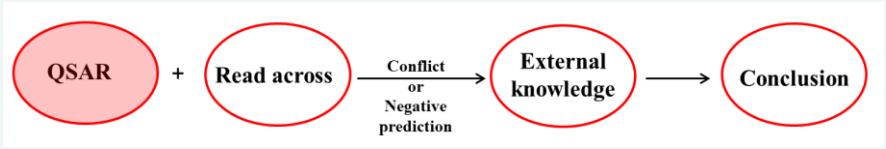
Example 2

Workflow for collecting WoE

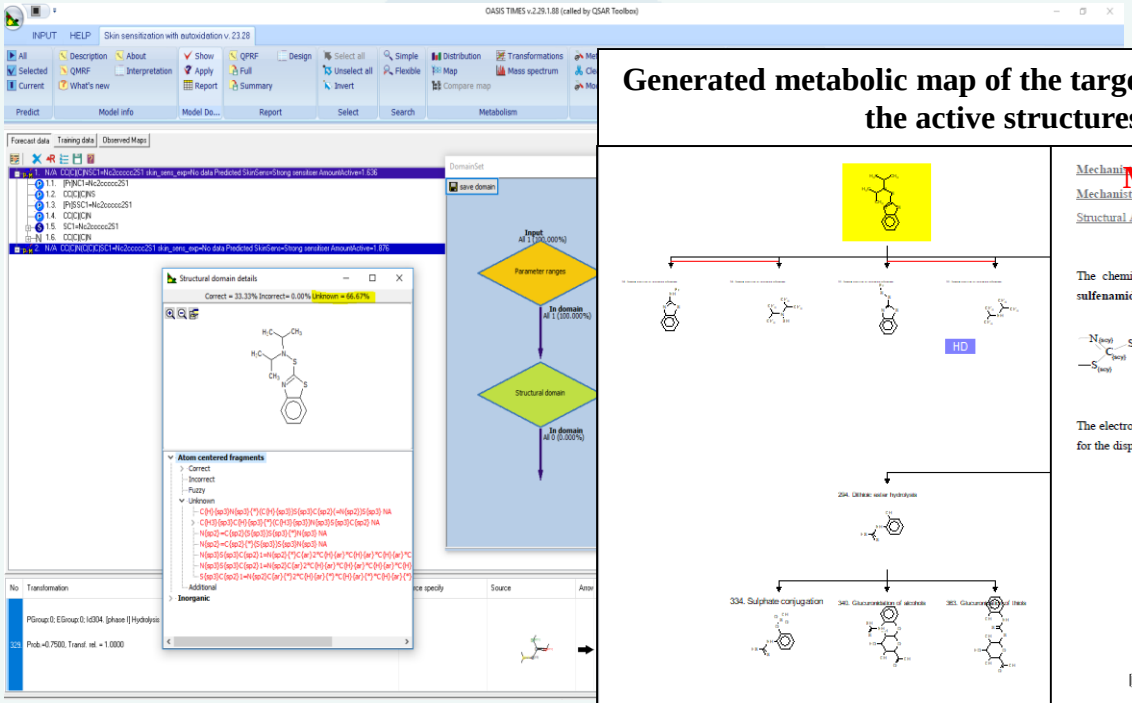


N,N-diisopropylbenzothiazole-2-sulphenamide

TIMES SS model

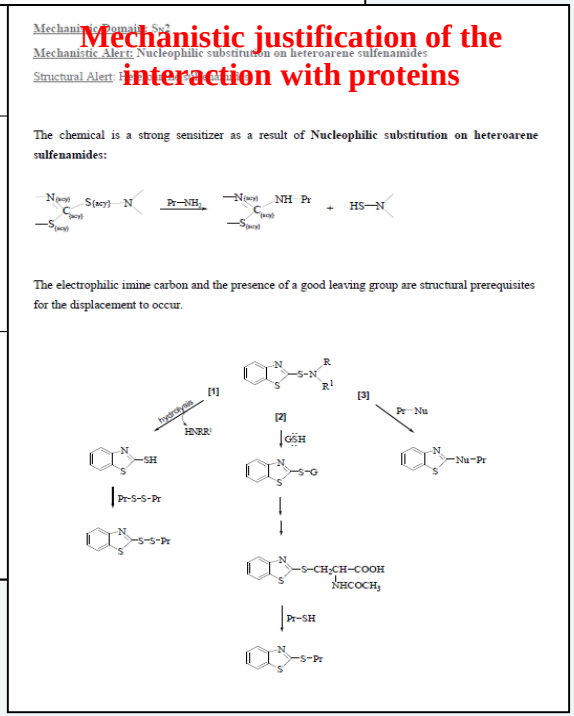


The chemical is predicted **Positive** by TIMES SS model and the prediction is out of the applicability domain of the model due to presence of "Unknown" atom centered fragments (~67%).



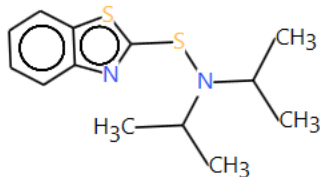
Generated metabolic map of the target with highlighted the active structures

Mechanistic justification of the interaction with proteins



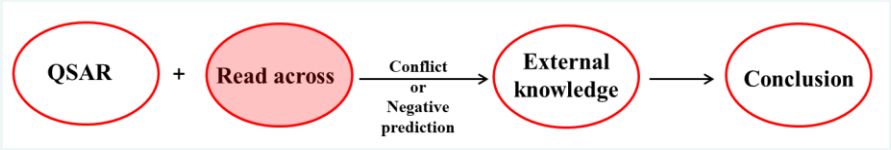
Example 2

Workflow for collecting WoE

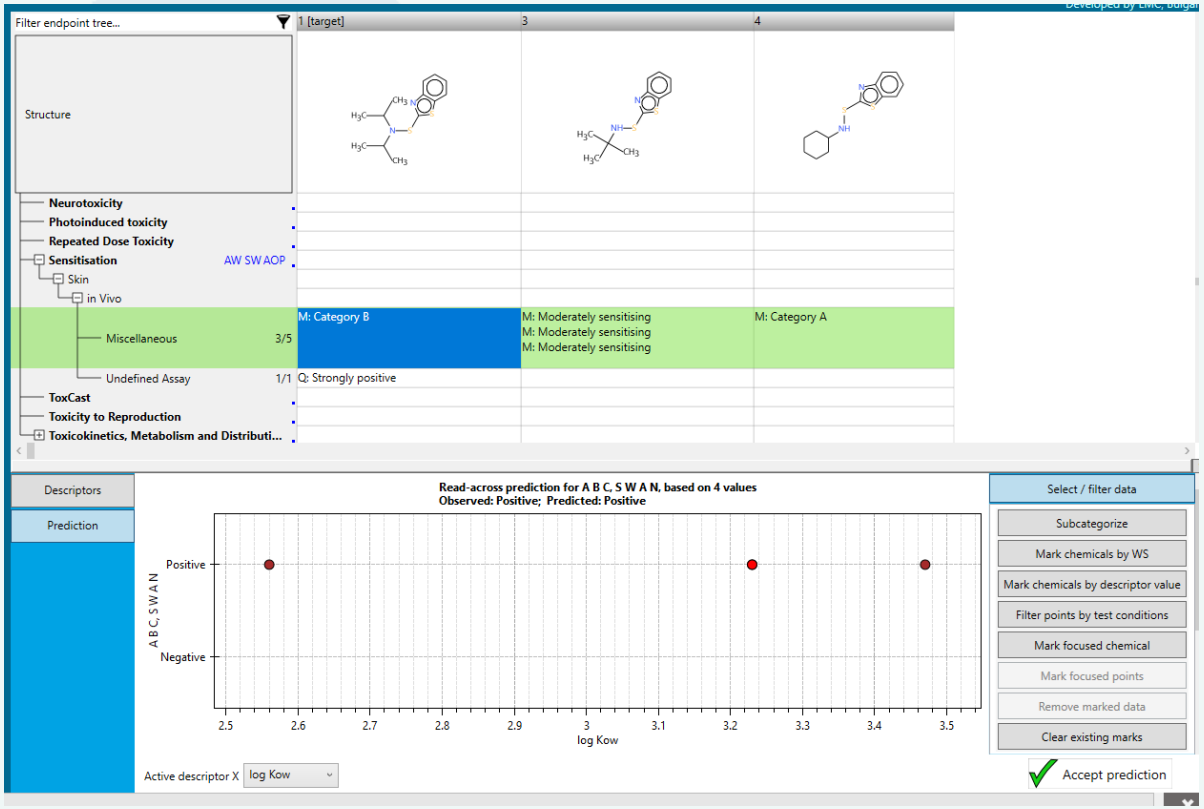


N,N-diisopropylbenzothiazole-2-sulphenamide

Toolbox

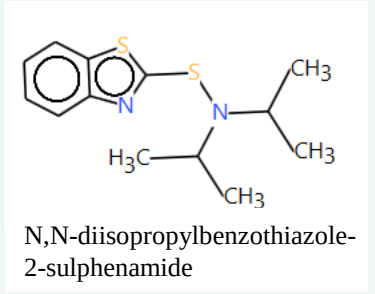


The target chemical has been predicted by applying the category approach in the Toolbox. The primary group was defined by the endpoint specific profiler "Protein binding alerts for skin sensitization by OASIS". The read across prediction is Positive based on two analogues.



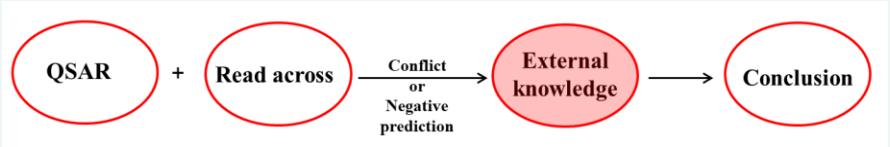
Example 2

Workflow for collecting WoE



N,N-diisopropylbenzothiazole-2-sulphenamide

Experts



- In this case, there is **no conflict** between the prediction from **TIMES SS** model and the read across from **Toolbox.**
- Expert involvement is not necessary.

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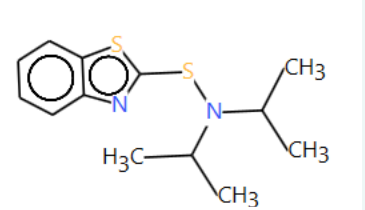
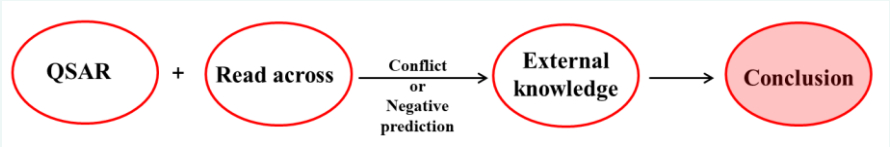
Example 2

Workflow for collecting WoE



N,N-diisopropylbenzothiazole-2-sulphenamide

Final decision



- In this case, there is **no conflict** between the prediction from **TIMES SS** model and the read across from **Toolbox.**
- Expert involvement is not necessary.

<u>Ultimate conclusion:</u>

The target chemical could be considered as **Positive skin sensitizer**.

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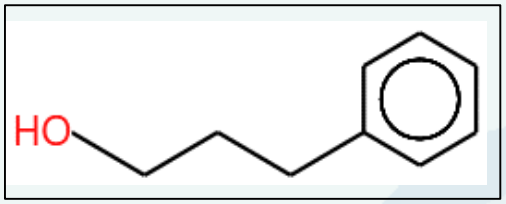
Example 3

Workflow for collecting WoE

Target chemical: 3-Phenyl-1-propanol (CAS # 122-97-4)



smiles: OCCCc1ccccc1

Target endpoint: Reproductive toxicity

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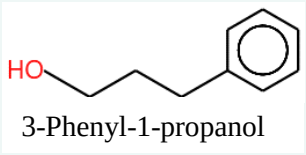
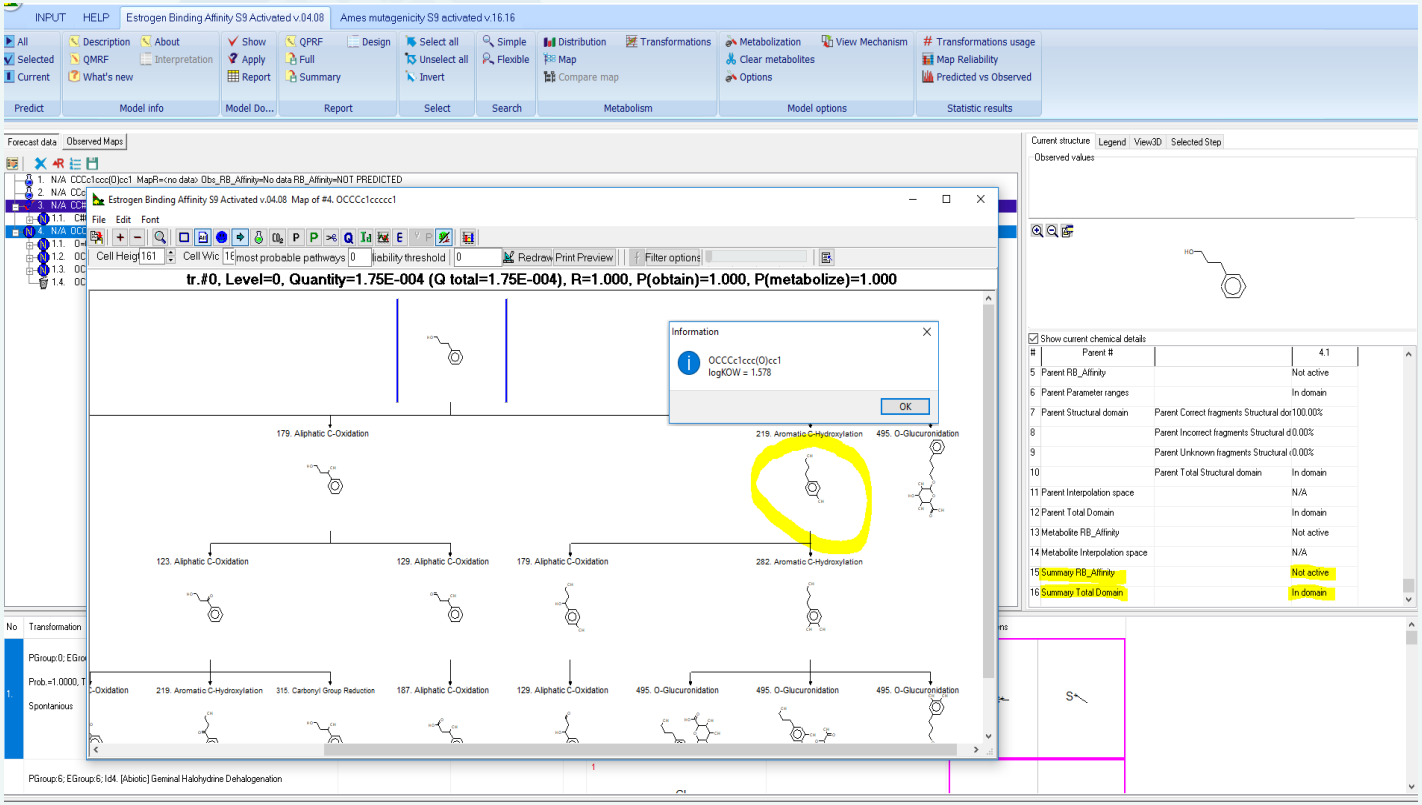
Example 3

Workflow for collecting WoE



TIMES ER binding +S9 model

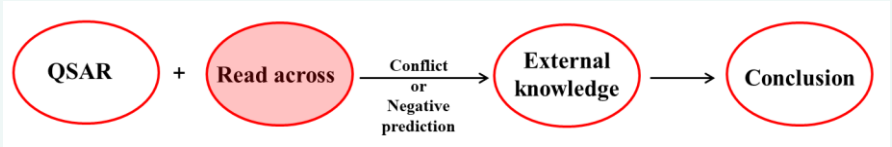
The target chemical is predicted as Non ER binder. As a result of *in vitro* S9 metabolism and the target is hydroxylated at *p*-position to alkylphenol for which alert exist in the model. However, due to parametric boundaries (not falling into the range of logKow defined for Alkylphenols) the metabolite is predicted as Non binder. The prediction is in the model domain.



Example 3

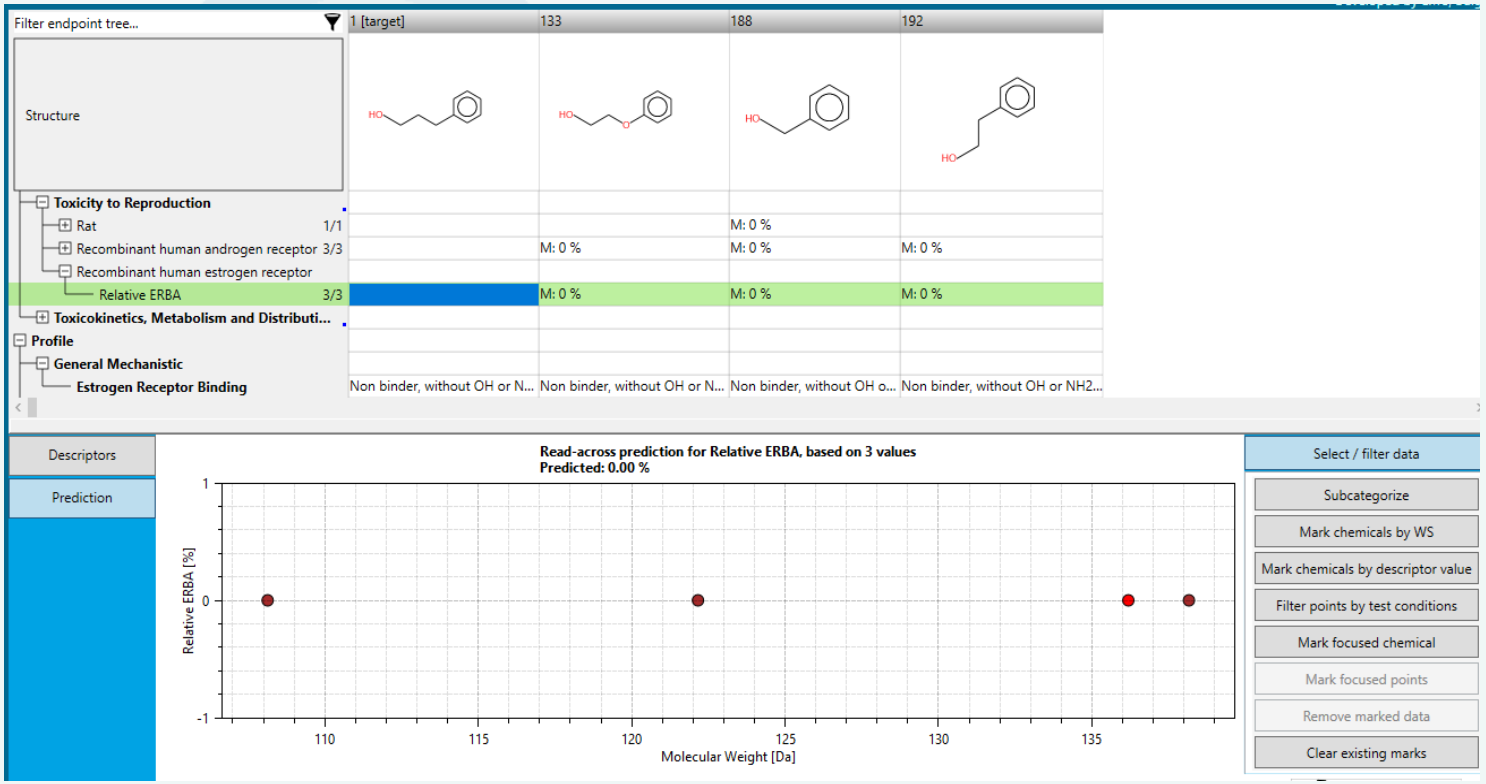
Workflow for collecting WoE

Toolbox



Read across for ERBA

By applying the category approach in Toolbox, the read across prediction for ERBA is **0%** based on three analogues.



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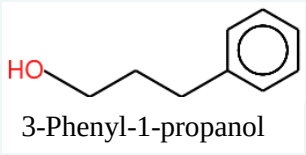
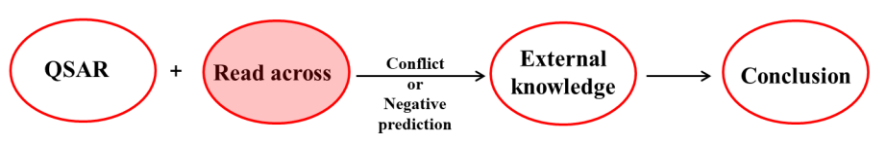
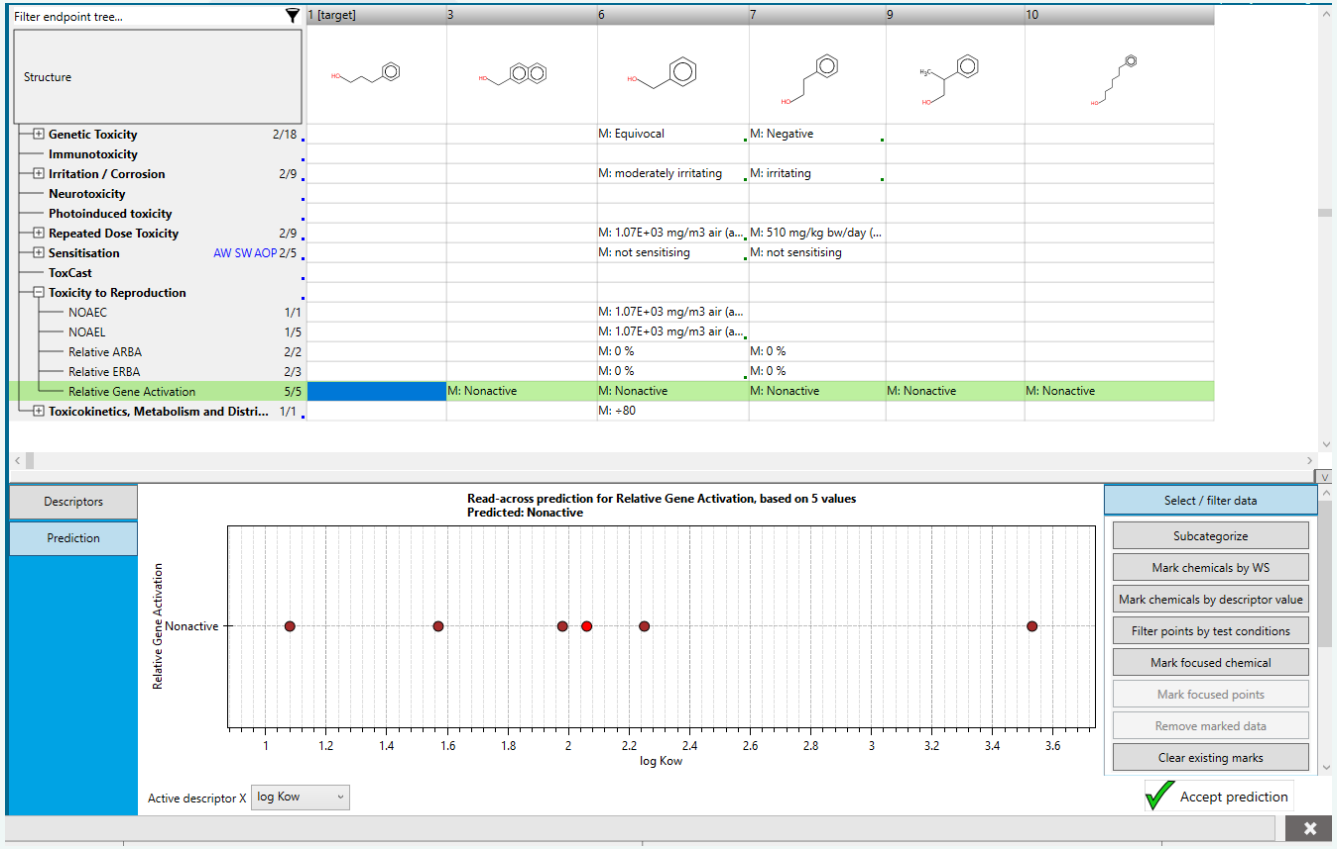


Toolbox



Read across for Relative Gene Activation

The read across prediction is **Non active** based on five analogues.



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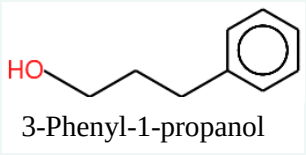
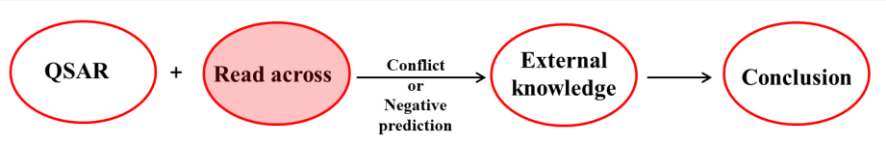
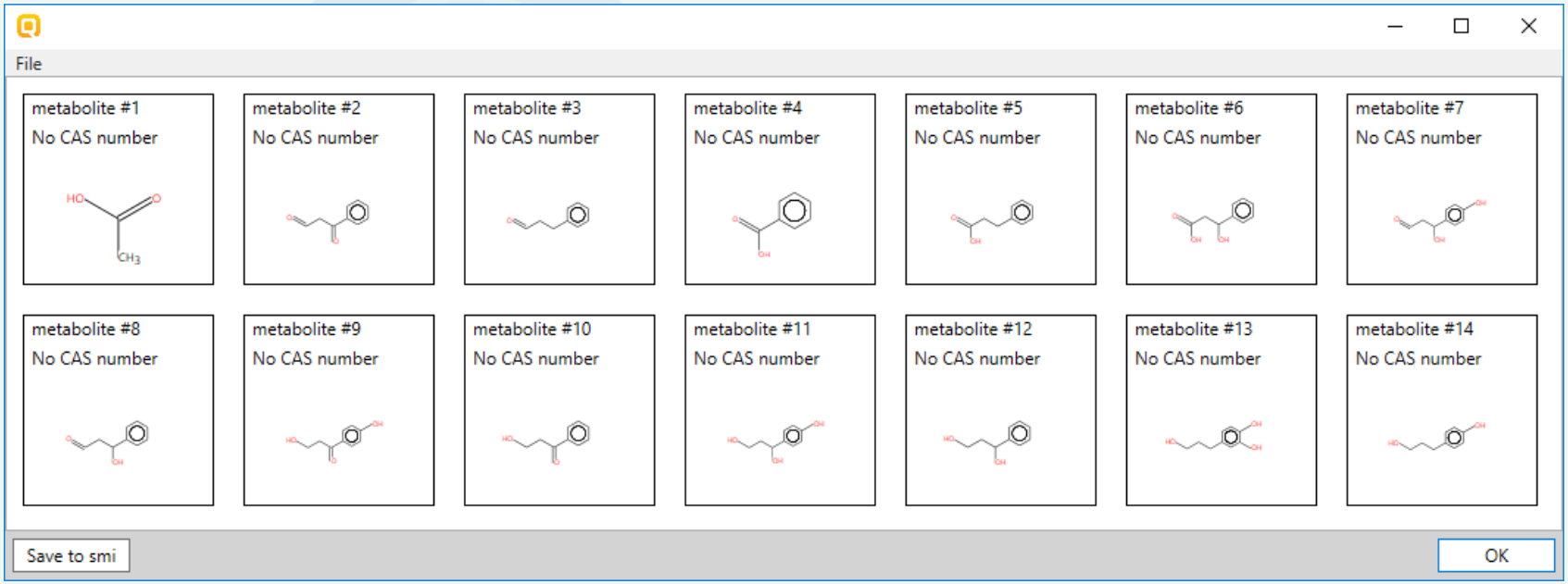


Toolbox



Analysis of metabolites

The *in vivo* rat metabolic simulator was applied on the target chemical. 14 metabolites are generated.



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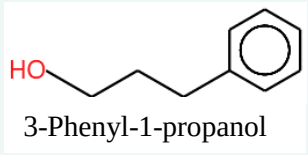
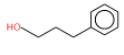
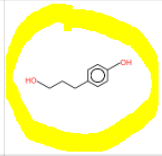


Toolbox

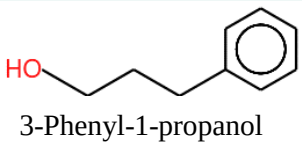
Analysis of metabolites

It was found that for one of the generated metabolites there is experimental data showing **slightly Positive effect** – **ERBA = 0.00826%.**

Filter endpoint tree...	Parent chemical [target]	metabolite #12	metabolite #1	metabolite #2	metabolite #3	metabolite #4	metabolite #5	metabolite #6
Structure								
Structure info								
Parameters								
Physical Chemical Properties 2/32			M: -0.17		M: ≥1.81÷1.88			
Environmental Fate and Transport 2/23			M: 2 d		M: >70			
Ecotoxicological Information 2/69			M: =1.12E+03 ml/ha		M: =143 mg/kg			
Human Health Hazards								
Acute Toxicity 2/11			M: >2E+03 mg/kg...		M: >0.026 mg/L/h			
Bioaccumulation								
Carcinogenicity 1/1			M: Negative					
Developmental Toxicity / Teratogenicity 1/3			M: 1.6E+03 mg/kg...					
Genetic Toxicity 2/17			M: Negative		M: Equivocal			
Immunotoxicity								
Irritation / Corrosion 2/12			M: NOT_SPECIFIED		M: Category 1 (irr...			
Neurotoxicity								
Photoinduced toxicity								
Repeated Dose Toxicity 2/11			M: =16 mg/kg bdw...		M: =2E+04 mg/kg			
Sensitisation AW SW AOP 1/5					M: not sensitising			
ToxCast								
Toxicity to Reproduction								
NOAEC 1/1			M: =0.01 mg/L					
NOAEL 1/6					M: =1.31E+03 mg...			
Relative ARBA 1/1					M: 0 %			
Relative ERBA								
Human 1/1		M: 0.00826 %						
Recombinant human estrogen rec... 2/2		M: 0.00826 %			M: 0 %			
Toxicokinetics, Metabolism and Distr... 2/49			M: 43		M: ≥0.89÷1.25			
Profile								

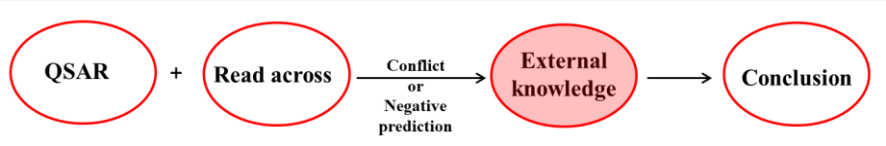
Example 3

Workflow for collecting WoE



3-Phenyl-1-propanol

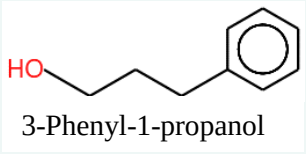
Experts



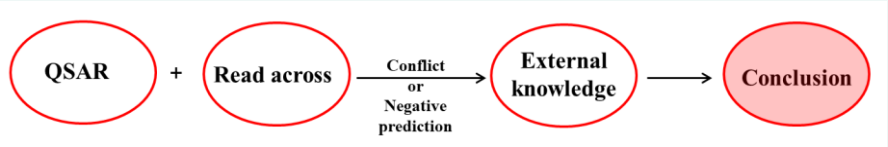
- Parent chemical has Negative reproductive toxicity.
- However, after simulating *in vivo* metabolism the chemical is hydroxylated at para position and experimental data collected for this metabolite shows that it has slightly positive result (ERBA is 0.00826%).
- The target chemical could be considered as **possible ER binder** after metabolic activation.

Example 3

Workflow for collecting WoE



Final decision



- Parent chemical has **Negative** reproductive toxicity.
- However, after simulating *in vivo* metabolism the chemical is hydroxylated at para position and experimental data collected for this metabolite shows that it has slightly positive result (ERBA is 0.00826%).
- The target chemical could be considered as **possible ER binder** after metabolic activation.

<u>Ultimate conclusion:</u>

3-Phenyl-1-propanol is **possible ER binder** after metabolic activation.