# Use of (Q)SAR and read across for assessment of genotoxicity of pesticides metabolites

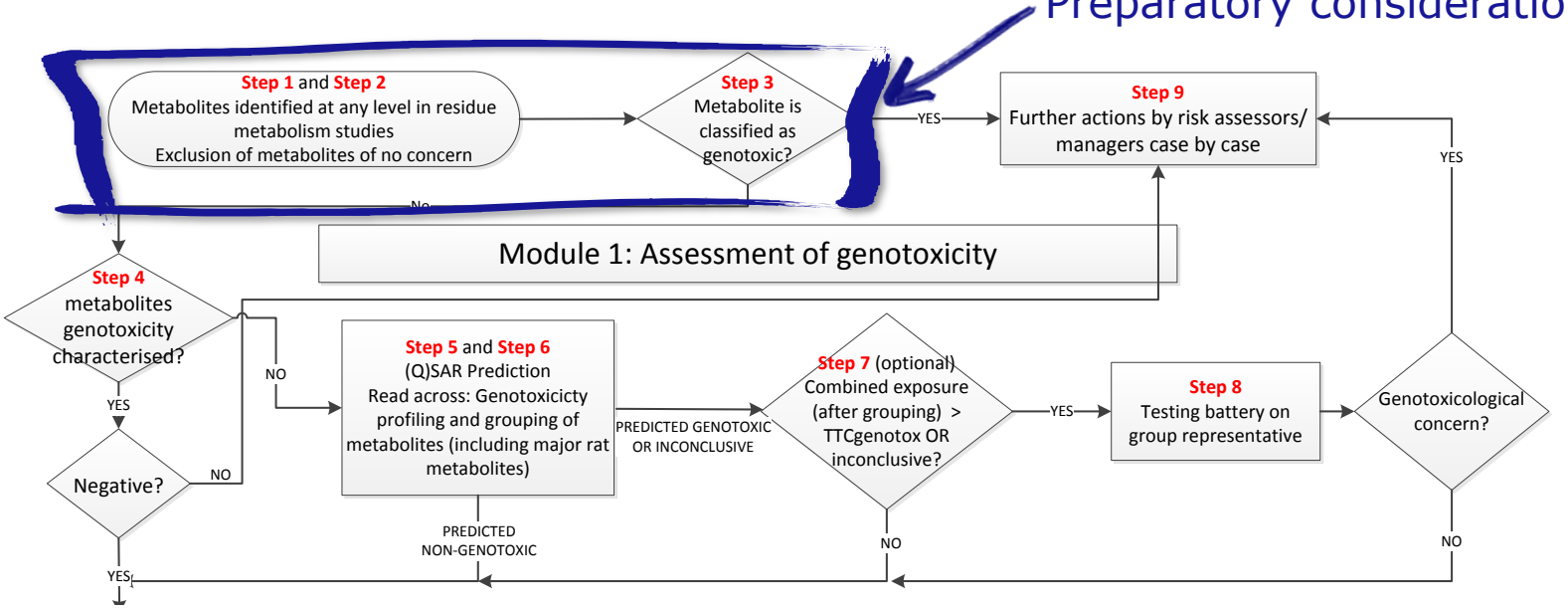
Rositsa Serafimova
Pesticides Unit, EFSA

Technical meeting on PPR Panel GD on residue definition for dietary risk assessment

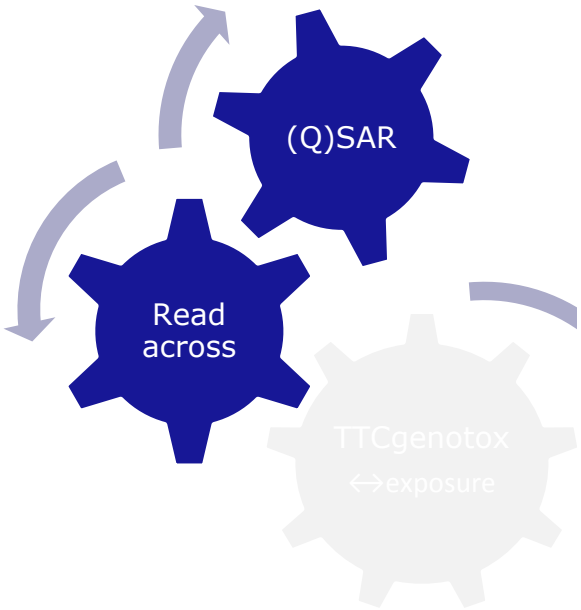
26 – 27 September, Parma

# MODULE 1: GENOTOXICITY ASSESSMENT

## Preparatory considerations

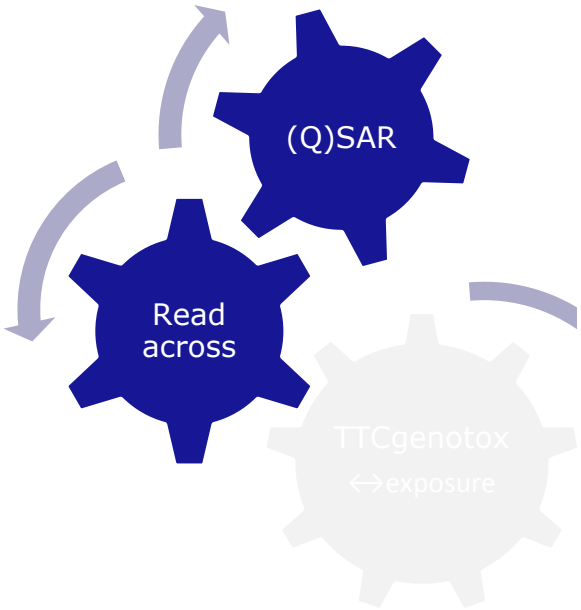


# STEPS 5 & 6: (Q)SAR PREDICTION AND READ ACROSS




- genotoxic potential of all identified metabolites is predicted by at least two independent (Q)SAR models for each endpoint
- all metabolites are subjected to read across
- weight of evidence approach for the final conclusion
- in case of different results between (Q)SAR predictions and read across, justification for the decision has to be provided

# STEPS 5 & 6: (Q)SAR PREDICTION AND READ ACROSS

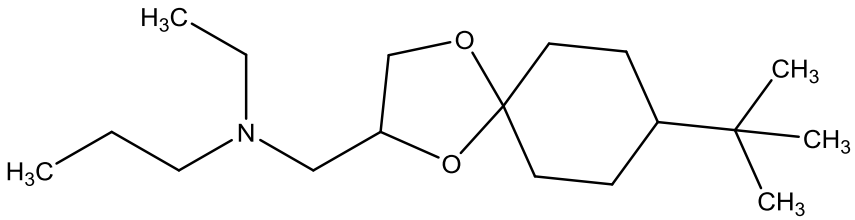



- no new methodologies were developed
- (Q)SAR models - assessment of applicability and documentation based on adapted ECHA (2008), and OECD (2007) guidance.
- Read across - performance, assessment of applicability and documentation based on adapted ECHA (2008; 2013 2015) and OECD (2014)

ECHA, 2008. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R6. ECHA, Helsinki, Finland. 134pp.
ECHA, 2013. Grouping of substances and read across approach, Part1. ECHA, Helsinki, Finland.
ECHA, 2015. Read across Assessment Framework, ECHA, Helsinki, Finland.
OECD, 2014, Series on testing & assessment No 194. Guidance on grouping of Chemicals. Second edition.
OECD, 2007. Guidance Document on the Validation of (Quantitative) Structure Activity Relationship ((Q)SAR) Models. OECD Series on Testing and Assessment No. 69.ENV/JM/MONO(2007)2.

# MODULE 1: GENOTOXICITY ASSESSMENT

## Example - Spiroxamine

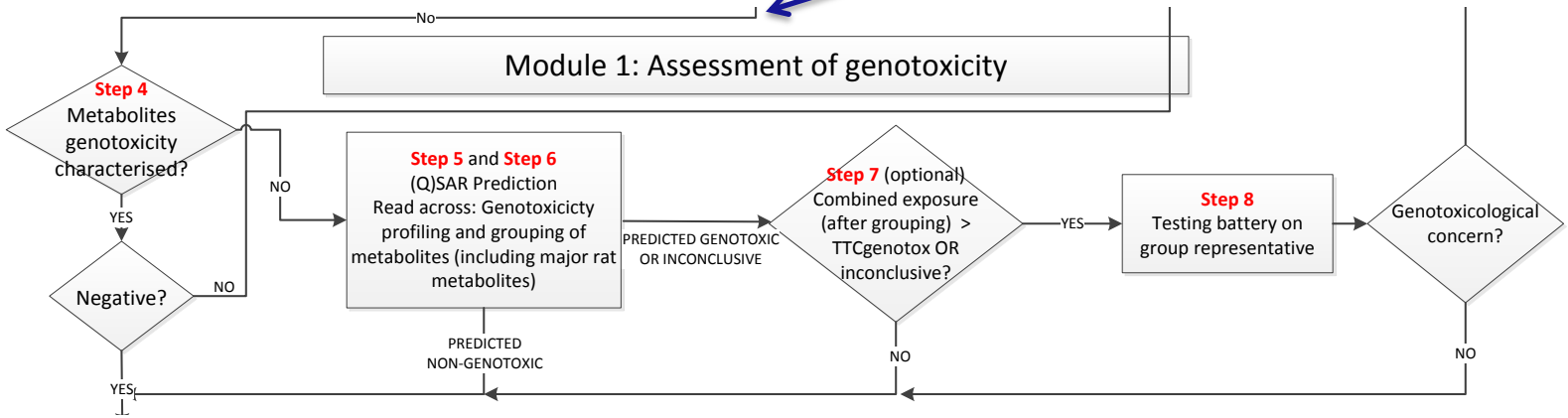


# EXAMPLE - SPIROXAMINE

1. 43 metabolites

   - 15 conjugated metabolites (glycosides, glucuronides) toxicological assessment covered by their aglycons

2. Exclusion of metabolites of no concern – None
3. Metabolite is classified as genotoxic - None



# STEP 4: METABOLITES GENOTOXICOLOGICALLY CHARACTERISED

| | | Occurrence in rat metabolism (% administered dose) | Toxicological properties covered by studies with parent compound or by specific studies |
|---|---|---|---|
| **Parent** | Spiroxamine | | Yes |
| **M01** | Desethyl | | No |
| **M02** | Despropyl | | No |
| **M03** | N-oxide | | Yes (specific studies) |
| **M04** | N-formyl-desethyl | | No |
| **M05** | Hydroxyl | | No |
| **M06** | Acid | 24.3 | Yes |
| **M07** | Hydroxy acid | | No |
| **M08** | 8-hydroxy acid | 3.6 | No |
| **M09** | Hydroxy-despropyl | | No |
| **M10** | Hydroxy-N-oxide | | No |
| **M11** | Desethyl acid | 6.1 | No |
| **M12** | Despropyl acid | 4 | No |
| **M13** | Cyclohexanol | | No |
| **M14** | Diol | | No |
| **M15** | Ketone | | No |
| **M16** | Hydroxy-ketone | | No |
| **M25** | Sulfate | 1.4 | No |
| **M26** | Desethyl-sulfate | 3.2 | No |
| **M27** | Despropyl-sulfate | 3.1 | No |
| **M28** | Aminodiol | | No |
| **M29** | Aminodiol-N-oxide | | No |
| **M30** | Desethyl-aminodiol | | No |
| **M31** | Despropyl-aminodiol | | No |
| **M35** | Docosanoic acid ester | | No |
| **M36** | Tetracosanoic acid ester | | No |
| **M37** | Cyclohexenol | 0.8 | No |
| **M38** | N-formyl-despropyl | | No |
| **M41** | Hydroxy-desethyl | | No |

# STEP 5: (Q)SAR PREDICTION OF GENOTOXICITY

- Gene mutation:

  CAESAR Mutagenicity Model (http://www.vega-qsar.eu/)

  OASIS AMES Mutagenicity model (http://oasis-lmc.org/)

- Chromosomal alterations

  Toxtree in vivo micronucleus model(http://toxtree.sourceforge.net/)

  OASIS Chromosomal Aberration model (http://oasis-lmc.org/)

# STEP 5: (Q)SAR PREDICTION OF GENOTOXICITY

| | | CAESAR prediction of gene mutation (Applicability Domain) | OASIS prediction of gene mutation (Applicability Domain) | Rule based model for prediction of in vivo CA (Toxtree) (no Applicability Domain evaluation is available) | OASIS prediction of CA (Applicability Domain) |
|---|---|---|---|---|---|
| **M01** | Desethyl | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M02** | Despropyl | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M04** | N-formyl-desethyl | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M05** | Hydroxyl | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M07** | Hydroxy acid | Negative (Out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M08** | 8-hydroxy acid | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M09** | Hydroxy-despropyl | Positive (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M10** | Hydroxy-N-oxide | Negative (Out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M11** | Desethyl acid | Negative (Out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M12** | Despropyl acid | Negative (Out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M13** | Cyclohexanol | Negative (In) | Negative (In) | Negative | Negative (out) |
| **M14** | Diol | Negative (In) | Negative (In) | Negative | Negative (In) |
| **M15** | Ketone | Negative (Could be out) | Negative (In) | Negative | Negative (out) |
| **M16** | Hydroxy-ketone | Negative (In) | Negative (In) | Negative | Negative (out) |
| **M25** | Sulfate | Negative (Out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M26** | Desethyl-sulfate | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M27** | Despropyl-sulfate | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M28** | Aminodiol | Negative (In) | Negative (In) | Positive alert for CA | Negative (In) |
| **M29** | Aminodiol-N-oxide | Negative (Out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M30** | Desethyl-aminodiol | Negative (Could be out) | Negative (In) | Positive alert for CA | Negative (out) |
| **M31** | Despropyl-aminodiol | Negative (In) | Negative (In) | Positive alert for CA | Negative (out) |
| **M35** | Docosanoic acid ester | Negative (Could be out) | Negative (In) | Negative | Negative (out) |
| **M36** | Tetracosanoic acid ester | Negative (Could be out) | Negative (In) | Negative | Negative (out) |
| **M37** | Cyclohexenol | Negative (In) | Negative (out) | Negative | Positive (In) |
| **M38** | N-formyl-despropyl | Negative (Could be out) | Negative (out) | Positive alert for CA | Negative (out) |
| **M41** | Hydroxy-desethyl | Negative (Out) | Negative (out) | Positive alert for CA | Negative (out) |

# STEP 5: (Q)SAR PREDICTION OF GENOTOXICITY

- 20 metabolites are predicted as positive (potentially genotoxic) from at least one of the models.
- 6 metabolites are predicted as negative from all models.
- All metabolites are moved to the next step – read across analysis.

# STEP 6: READ ACROSS

**Endpoint**

- Well defined endpoint
  - Gene mutation and chromosomal aberrations

**Similarity**

- Well defined and justified similarity
  - molecular initiating events – covalent binding to DNA and/or proteins
  - evaluation of the influence of the rest part of the molecule

**Data**

- High quality of the data used
  - Data for the source substance (parent and/or metabolite) - Commission Regulation (EU) No 283/2013

# STEP 6: READ ACROSS


- OECD Toolbox:
  - DNA binding by OASIS
  - DNA binding by OECD
  - Protein binding by OASIS
  - Protein binding by OECD
  - DNA alerts for AMES, MN and CA by OASIS
  - In vitro mutagenicity (AMES test) alerts by ISS
  - In vivo mutagenicity (Micronucleus) alerts by ISS
  - Protein binding alerts for Chromosomal aberrations by OASIS

  - Organic functional groups

# STEP 6: READ ACROSS

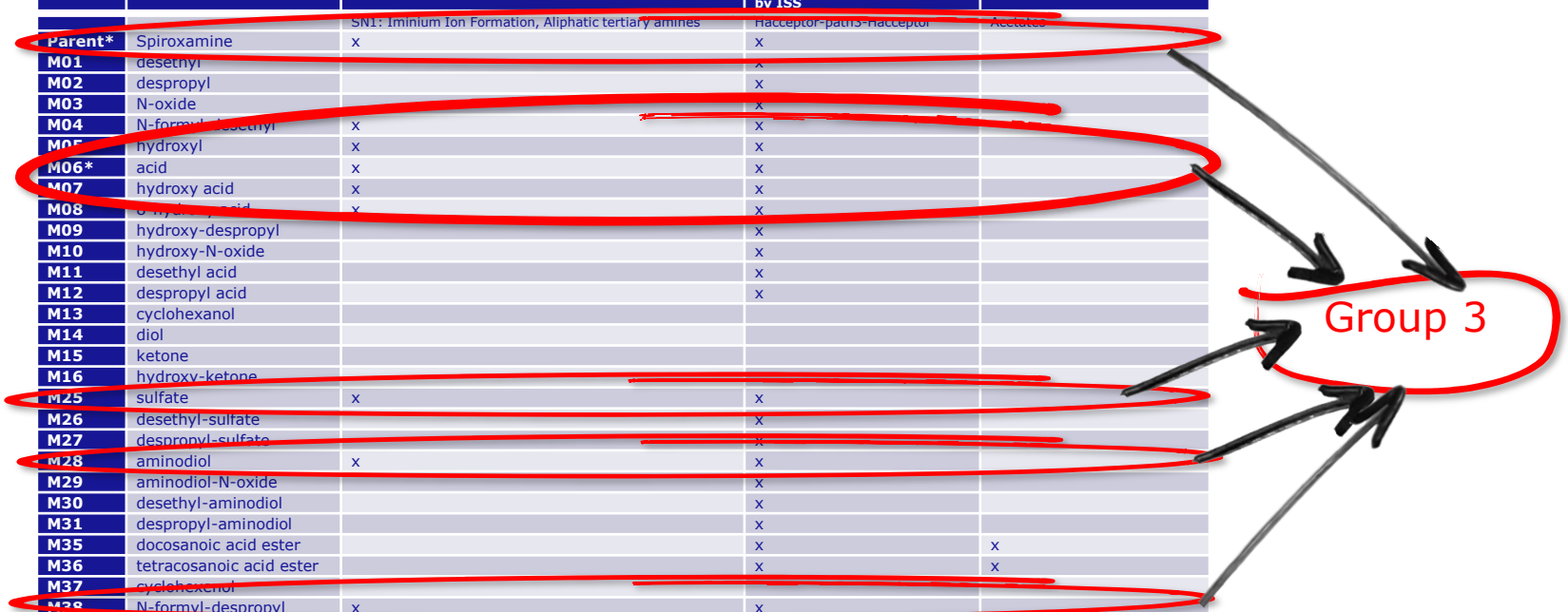
| | | DNA binding by OECD | in vivo mutagenicity (MN) by ISS | Protein binding by OECD |
|---|---|---|---|---|
| | | SN1: Iminium Ion Formation, Aliphatic tertiary amines | Hacceptor-path3-Hacceptor | Acetates |
| Parent* | Spiroxamine | x | x | |
| M01 | desethyl | | x | |
| M02 | despropyl | | x | |
| M03 | N-oxide | | x | |
| M04 | N-formyl-desethyl | x | x | |
| M05 | hydroxyl | x | x | |
| M06* | acid | x | x | |
| M07 | hydroxy acid | x | x | |
| M08 | 8-hydroxy acid | x | x | |
| M09 | hydroxy-despropyl | | x | |
| M10 | hydroxy-N-oxide | | x | |
| M11 | desethyl acid | | x | |
| M12 | despropyl acid | | | |
| M13 | cyclohexanol | | | |
| M14 | diol | | | |
| M15 | ketone | | | |
| M16 | hydroxy-ketone | | | |
| M25 | sulfate | x | x | |
| M26 | desethyl-sulfate | | x | |
| M27 | despropyl-sulfate | | x | |
| M28 | aminodiol | x | x | |
| M29 | aminodiol-N-oxide | | x | |
| M30 | desethyl-aminodiol | | x | |
| M31 | despropyl-aminodiol | | x | |
| M35 | docosanoic acid ester | | x | x |
| M36 | tetracosanoic acid ester | | x | x |
| M37 | cyclohexenol | | | |
| M38 | N-formyl-despropyl | x | x | |
| M41 | hydroxy-desethyl | | x | |

Group 2

Group 1

# STEP 6: READ ACROSS

| | | DNA binding by OECD | in vivo mutagenicity (MN) by ISS | Protein binding by OECD |
|---|---|---|---|---|
| | | SN1: Iminium Ion Formation, Aliphatic tertiary amines | Acceptor-path3-Acceptor | Acetates |
| Parent* | Spiroxamine | x | x | |
| M01 | desethyl | | x | |
| M02 | despropyl | | x | |
| M03 | N-oxide | | x | |
| M04 | N-formyl-desethyl | x | x | |
| M05 | hydroxyl | x | x | |
| M06* | acid | x | x | |
| M07 | hydroxy acid | x | x | |
| M08 | 3-hydroxy-acid | x | x | |
| M09 | hydroxy-despropyl | | x | |
| M10 | hydroxy-N-oxide | | x | |
| M11 | desethyl acid | | x | |
| M12 | despropyl acid | | x | |
| M13 | cyclohexanol | | | |
| M14 | diol | | | |
| M15 | ketone | | | |
| M16 | hydroxy-ketone | | | |
| M25 | sulfate | x | x | |
| M26 | desethyl-sulfate | | x | |
| M27 | despropyl-sulfate | | x | |
| M28 | aminodiol | x | x | |
| M29 | aminodiol-N-oxide | | x | |
| M30 | desethyl-aminodiol | | x | |
| M31 | despropyl-aminodiol | | x | |
| M35 | docosanoic acid ester | | x | x |
| M36 | tetracosanoic acid ester | | x | x |
| M37 | cyclohexenol | | | |
| M38 | N-formyl-despropyl | x | x | |
| M41 | hydroxy-desethyl | | x | |



# STEP 6: READ ACROSS

| | | DNA binding by OECD | in vivo mutagenicity (MN) by ISS | Protein binding by OECD |
|---|---|---|---|---|
| | | SN1: Iminium Ion Formation, Aliphatic tertiary amines | Hacceptor-path3-Hacceptor | Acetates |
| Parent* | Spiroxamine | x | x | |
| M01 | desethyl | | x | |
| M02 | despropyl | | x | |
| M03 | N-oxide | | x | |
| M04 | N-formyl-desethyl | x | x | |
| M05 | hydroxyl | x | x | |
| M06* | acid | x | x | |
| M07 | hydroxy acid | x | x | |
| M08 | 8-hydroxy acid | x | x | |
| M09 | hydroxy-despropyl | | x | |
| M10 | hydroxy-N-oxide | | x | |
| M11 | desethyl acid | | x | |
| M12 | despropyl acid | | x | |
| M13 | cyclohexanol | | | |
| M14 | diol | | | |
| M15 | ketone | | | |
| M16 | hydroxy-ketone | | | |
| M25 | sulfate | x | x | |
| M26 | desethyl-sulfate | | x | |
| M27 | despropyl-sulfate | | x | |
| M28 | aminodiol | x | x | |
| M29 | aminodiol-N-oxide | | x | |
| M30 | desethyl-aminodiol | | x | |
| M31 | despropyl-aminodiol | | x | |
| M35 | docosanoic acid ester | | x | x |
| M36 | tetracosanoic acid ester | | x | x |
| M37 | cyclohexenol | | | |
| M38 | N-formyl-despropyl | x | x | |
| M41 | hydroxy-desethyl | | x | |

Group 4

# STEP 6: READ ACROSS

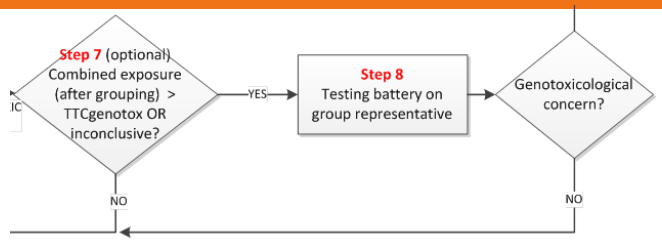
Group 1 — New alert

Group 2 — NO alerts

Group 3 — All parent's alerts

Group 4 — One parent's alert

→ Additional evaluation of the similarity



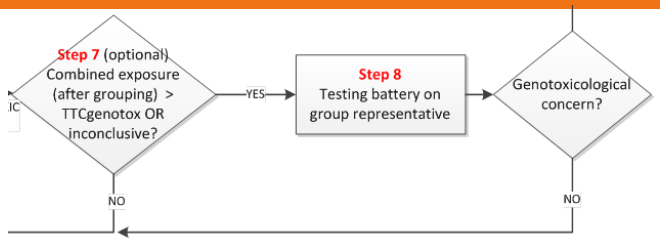
# STEP 6: READ ACROSS

Group 1 — New alert

Group 2 — NO alerts

Group 3 — All parent's alerts

Group 4 — One parent's alert




Additional evaluation of the similarity

- ✓ (non) common functional groups
- ✓ 'core structure'
- ✓ differences in reactivity, metabolism and mode of action
- ✓ steric hindrance

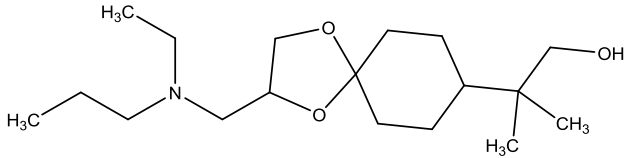
Ask the Expert!

# (Q)SAR PREDICTION AND READ ACROSS - CONCLUSION

- 4 metabolites were predicted as negative by all (Q)SAR models and no new alerts were identified by read across, hence they are not of concern for genotoxicity.
- For 6 metabolites the genotoxicity concern cannot be excluded, based on positive (Q)SAR predictions and/or read across considerations, therefore they should be subject of exposure assessment and comparison against TTC (step 8) and/or testing (step 9).
- 12 metabolites although predicted as potential genotoxicant by (Q)SAR models, as a result of read across they are not of concern for genotoxicity.
- Although no new alerts were identified for a metabolite based on the high reliable positive (Q)SAR prediction the genotoxicity concern cannot be excluded.

# (Q)SAR PREDICTION

M05




EXAMPLE

| | CAESAR prediction of gene mutation | TIMES prediction of gene mutation | Rule based model for prediction of in vivo CA (Toxtree) | TIMES prediction of CA |
|---|---|---|---|---|
| **M05** | Negative (Could be out AD) | Negative (out AD) | Positive alert for CA | Negative (out AD) |

SA34: H-acceptor-path3-H-acceptor

**Mutagenesis** vol. 25 no. 4 pp. 335–341, 2010
Advance Access Publication 1 March 2010
doi:10.1093/mutage/geq010

**Structural analysis and predictive value of the rodent *in vivo* micronucleus assay results**

**Romualdo Benigni*, Cecilia Bossa and Andrew Worth[1]**

**Table VI.** Characterization of SAs

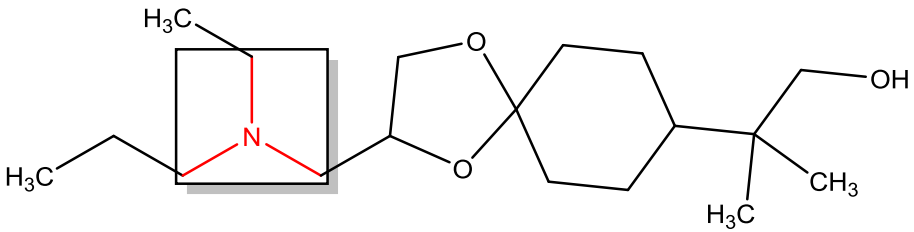
| SA | Number of substances fired | Number of positive substances | True positives (%) |
|---|---|---|---|
| SA_34: hacceptor-path3-hacceptor | 163 | 55 | 34 |

# READ ACROSS

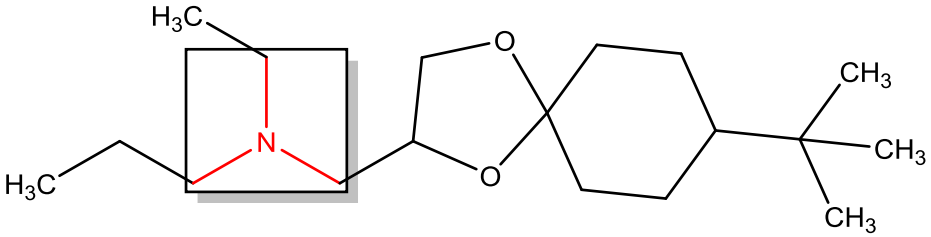
## STRUCTURAL ALERTS FOR GENOTOXICITY

EXAMPLE

SN1: Iminium Ion Formation, Aliphatic tertiary amines




**Metabolite 05**



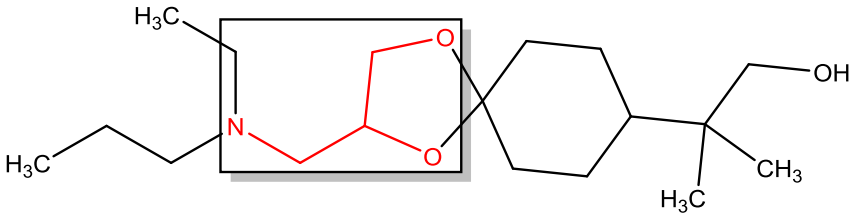

**Parent**

# READ ACROSS

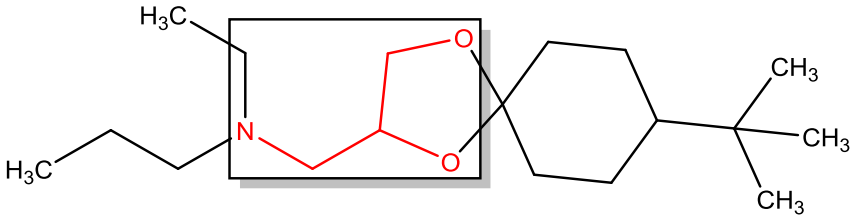
## STRUCTURAL ALERTS FOR GENOTOXICITY

EXAMPLE

Hacceptor-path3-Hacceptor




**Metabolite 05**

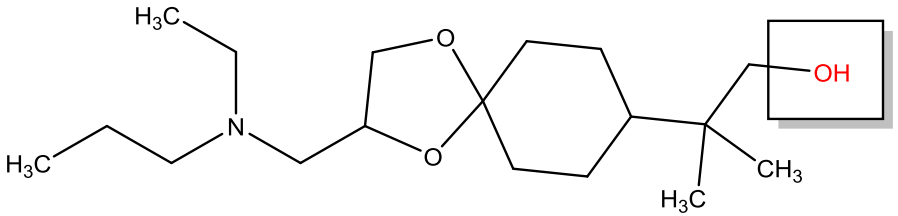



**Parent**

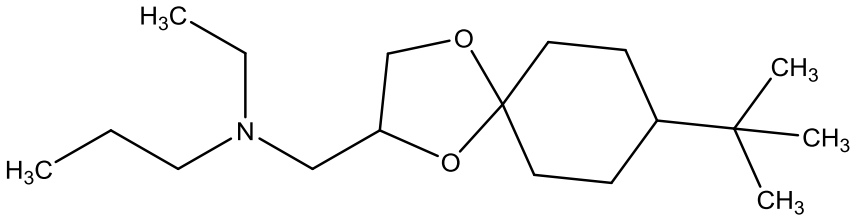
# READ ACROSS

## ORGANIC FUNCTIONAL GROUPS

EXAMPLE




**Metabolite 05**




**Parent**

# (Q)SAR PREDICTION AND READ ACROSS - CONCLUSION

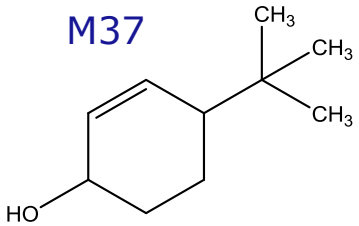
EXAMPLE

- Considerations
  - low reliability of the positive prediction for chromosomal aberrations
  - similarity with the parent substance
  - negative experimental data for genotoxicity of the parent substance

- Conclusion: NO Concern

# (Q)SAR PREDICTION

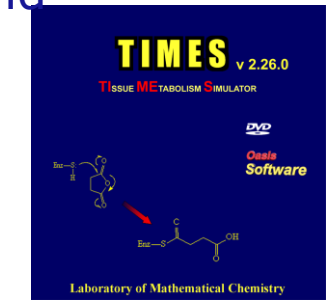
M37




another example

| | CAESAR prediction of gene mutation | TIMES prediction of gene mutation | Rule based model for prediction of in vivo CA (Toxtree) | TIMES prediction of CA |
|---|---|---|---|---|
| **M37** | Negative (In) | Negative (out) | Negative | Positive (In) |

- Prediction: **Positive** after metabolic activation
- Reliability: **High** (more or equal to 60%)
- Identified alert: Alpha/beta-unsaturated carbonyls and related compounds
- Proposed mechanism of action: Interactions with topoisomerases/proteins



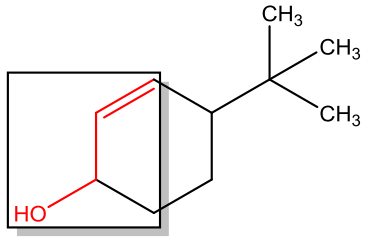
# READ ACROSS

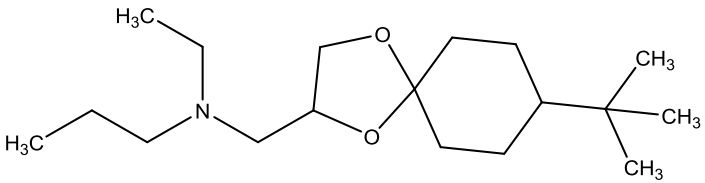
another example

## STRUCTURAL ALERTS FOR GENOTOXICITY

- **NO** alerts for genotoxicity

## ADDITIONAL EVALUATION OF THE SIMILARITY



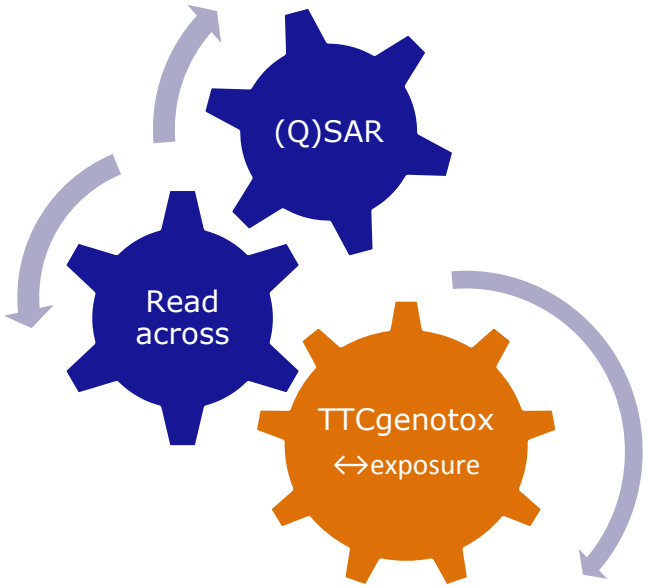

- ✓ (non) common functional groups - **YES**
- ✓ 'core structure' – **different**
- ✓ differences in reactivity, metabolism and mode of action - **YES**
- ✓ steric hindrance - NO

# (Q)SAR PREDICTION AND READ ACROSS - CONCLUSION

another example

- Considerations:
  - high reliable positive prediction for chromosomal aberrations
  - low similarity with the parent substance
- Conclusion: Concern cannot be excluded

# STEP 7: TTC ↔ EXPOSURE (OPTIONAL)




- Exposure assessment for substances **predicted to be of genotoxic concern** (following (Q)SAR prediction and read across)
- Combined exposure (i.e. exposure to all metabolites showing communality in the reaction mechanisms)

  ↔ 0.0025 µg/kg bw/day

# STEP 7: EXPOSURE GENOTOX (OPTIONAL) - EX. SPIROXAMINE

| | Wine grape | Table grape | Banana | Cereal grain | Root crops | Leafy crops |
|---|---|---|---|---|---|---|
| | STMR | HR | HR | Metabolism data | | |
| | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg |
| Metabolite M28 | 0.082 | 0.271 | 0.042 | nd | 0.005 | 0.010 |
| Metabolite M29 | 0.001 | 0.001 | nd | nd | 0.005 | 0.021 |
| Metabolite M30 | 0.002 | 0.008 | 0.001 | nd | nd | nd |
| Metabolite M31 | 0.003 | 0.009 | 0.001 | nd | 0.006 | nd |
| *Sum of metabolites* | *0.088* | *0.289* | *0.044* | - | *0.016* | *0.031* |
| Metabolite M35 | 0.053 | 0.174 | nd | nd | nd | nd |
| Metabolite M36 | 0.017 | 0.056 | nd | nd | nd | nd |
| *Sum of metabolites* | *0.070* | *0.230* | - | - | - | - |
| Metabolite M37 | 0.013 | 0.043 | nd | nd | nd | nd |
| *Sum of metabolites* | *0.013* | *0.043* | - | - | - | - |

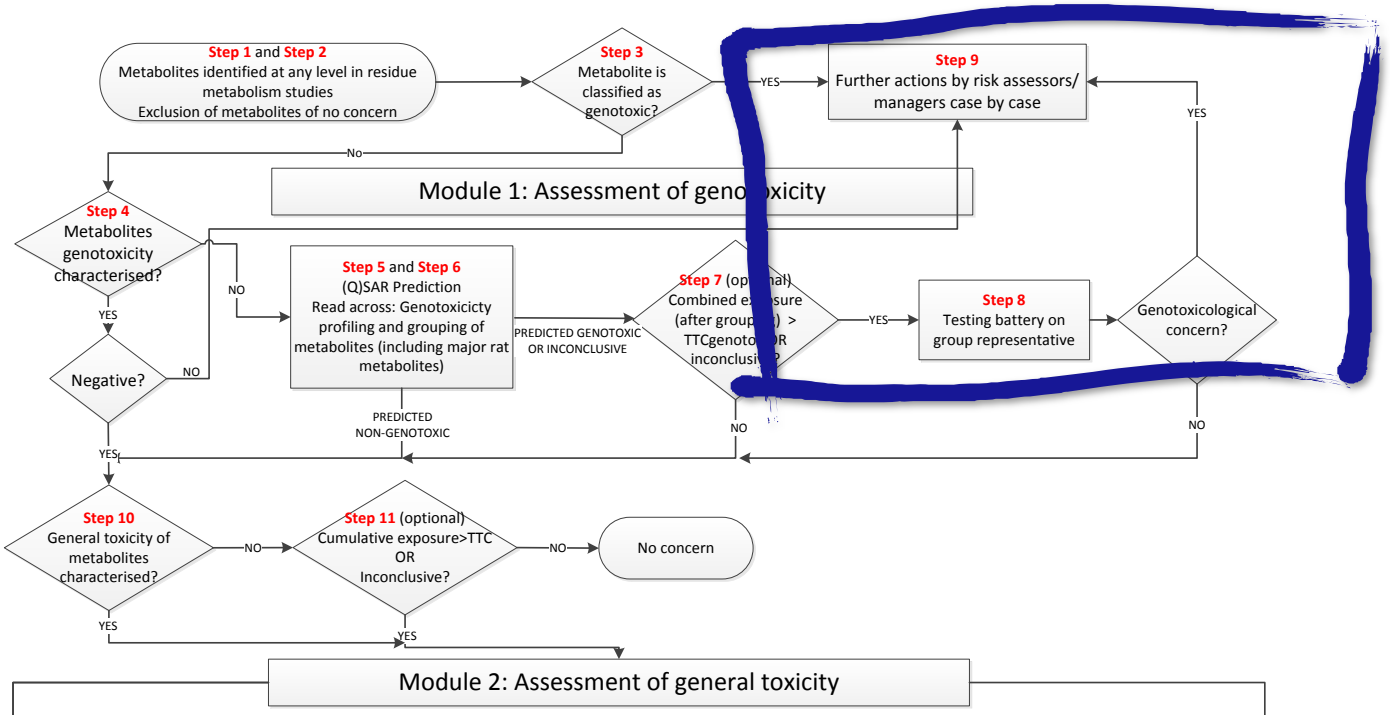
**Acute exposure (most critical; metabolite groups)**

M28-M31: 18.9 µg/kg bw/d (table grapes, children) = >10000 % $TTC_{genotoxicity}$

M35-M36: 15.1 µg/kg bw/d (table grapes, children) = >10000 % $TTC_{genotoxicity}$

M37: 2.82 µg/kg bw (table grapes, children) = >10000 % $TTC_{genotoxicity}$

# STEP 8: TESTING BATTERY FOR ASSESSMENT OF GENOTOXICITY



# TESTING BATTERY FOR ASSESSMENT OF GENOTOXICITY

- Opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011) - for selection of the most appropriate assays and results interpretation.
  - The testing battery should include as a minimum two *in vitro* tests covering gene mutations, structural and numerical chromosomal alterations.
  - The need for *in vivo* follow up testing should be considered case by case, through the evaluation of genotoxic events observed *in vitro* (if any), the data on toxicokinetics, on bioavailability and on the potential target organ.

# WRAP UP

- all identified metabolites systematically, objectively are assessed for their genotoxicity potential by (Q)SAR, Grouping & Read Across
- ‚cumulative' TTC approach is optionally applicable
- for metabolites with genotoxic concern experimental data are required
- guided strategy for testing of metabolites is provided


Thank you!