Case study from SEURAT-1

β-Olefinic alcohols: indirect acting toxicant category supported by SEURAT-1 data

Read-Across Case Study Considered in Context of the ECHA RAAF

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This assessment has been prepared to facilitate the discussion and does not represent ECHA's position or the views and policies of the European Commission.

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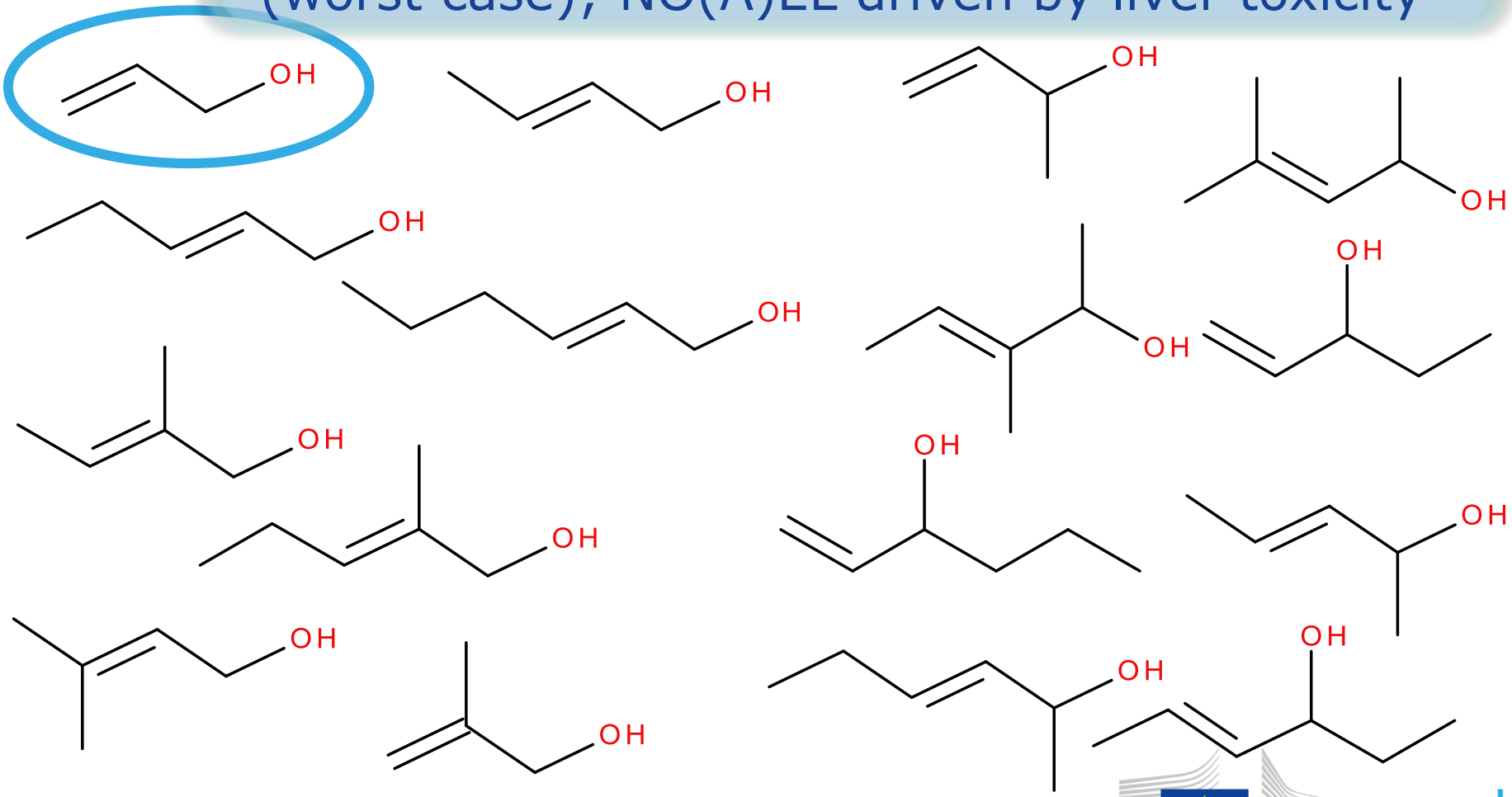
JRC Science Hub: ***ec.europa.eu/jrc***

The β-Olefinic Alcohols Case Study: Premise

- Short chain (C3 to C6) unsaturated alcohols are indirect-acting toxicants
- Same covalent mechanism of action and similar reactive potency
- Metabolism via alcohol dehydrogenase (ADH)
- Metabolites are electrophilic with *in vivo* potency related to relative thiol reactivity

β-Olefinic Alcohols: Category Members

Source substance:
90 day NOAEL read across from **2-propen-1-ol** (worst case); NO(A)EL driven by liver toxicity



The ECHA Read-Across Assessment Framework (RAAF) Approach

Scientific Assessment of Read-across argument according to scenarios defined by 3 key features:

# of substances considered:

- Analogue approach – one source and one target
- Category approach – multiple source(s) and target(s) (group)

Effect (predicted property) caused by:

- common substance for source(s) and target(s)
- different substances for source(s) and target(s)

For a Category, the predicted property:

- Follows a regular pattern (trend) across source structures
- Does not change across source structures

→ 6 Possible read-across Scenarios

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For a Category, the predicted property:

- Follows a regular pattern (trend) across source structures
- Does not follow a regular pattern (trend) across source structures

→ 6 Possible Read-Across Scenarios

Possible Scenarios:

- Analogue approach/Common toxicant
- Analogue approach/Different toxicant
- Category approach/Common toxicant/Trend in effect
- Category approach/Different toxicant/Trend in effect
- Category approach/Common toxicant/No Trend in effect
- Category approach/Different toxicant/No Trend in effect

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Scientific Assessment of Read-across argument according to scenarios defined by 3 key features:

# of source chemicals:

- Analogue approach – target and one source
- Category approach – target and multiple sources (group)

β-Olefinic Alcohols →

Scenario #4, category approach: different compounds with same type of effect, difference in effect strength

- different substances for source(s) and target

For a Category, the predicted property:

- Follows a regular pattern (trend) across source structures
- Does not change across source structures

→ 6 Possible Read-Across Scenarios

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The RAAF: Assessment Elements and Assessment Options

Set of **Assessment Elements (AE's)** per Scenario

- Describing 'crucial scientific aspects to judge validity and reliability of read-across for the Scenario'
- For each AE multiple considerations to be included in justification

Assessment Options (AO's):

- Reflect the conclusion on adequacy and scientific robustness of the information provided for the AE
- **Scores from 5 to 1**
  - **≥3** : information provided **is acceptable**
    with just (3) sufficient, (4) medium, (5) high confidence
  - **≤2** : information provided is (1) **not acceptable**,
    (2) not acceptable in its current form

The RAAF: Assessment Elements

General (Common) Assessment Elements

C.1 Substance characterisation

C.2 Structural similarity and category hypothesis

C.3 Link of structural similarities and structural differences with the proposed regular pattern

C.4 Consistency of effects in the data matrix
- order within category/clustering of strength of effects

C.5 Reliability and adequacy of the source study(ies)

C.6 Bias that influences the prediction

Scenario-Specific Assessment Elements: Scenario #4

different compounds with same effect, difference in effect strength

4.1 Compounds the test organism is exposed to

4.2 Common underlying mechanism, qualitative aspects

4.3 Common underlying mechanism, quantitative aspects

4.4 Exposure to other compounds than to those linked to the prediction

4.5 Occurrence of other effects than covered by the hypothesis

βOA Case Study Considered with the RAAF

AE		AO w/o NAM	AO with NAM	NAM added info	Remaining questions
C.1	Subst. characterisation	N/A	N/A		
C.2	Structural similarity	4	4		• Similarity and category boundaries
C.3	Link structures and regular pattern	4	5	*ex vivo* perfused liver data **link vinyl moiety and effect**	• Metabolism mechanism, exclude other than ADH
C.4	Consistency effects in data matrix; clustering	3	4	*in chemico/in silico* data show **clustering of potency**	• More details *in vivo* studies • Confirm subcategory reactivity trends
C.5	Reliability/adequacy source study(ies)	3	3		• More details on study design and quality
4.1	Compounds the organism is exposed to, transformation	4	5	*ex vivo/in silico*: **metabolites→toxicants, not tertiary alcohols**; also by hepatic organoids	• more (quant.) information: rate/speed of metabolism
4.2	Common mechanism, qualitative aspects	4	5	*in chemico/in silico/in vitro* data strengthen **mechanism evidence**, HSC activation markers the **MoA to fibrosis**	
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4.4	Exposure to other compounds	4	4		• More details (quant.) kinetics: residual parent/further metabolites & effects
4.5	Occurrence of other effects	4	4		• Rule out other effects / metabolic mechanisms
C.6	Bias influencing prediction	4	4		• Read-across valid for other structural variation members?

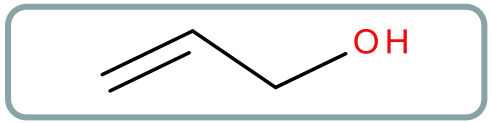
RAAF I: Category and Structural Similarity

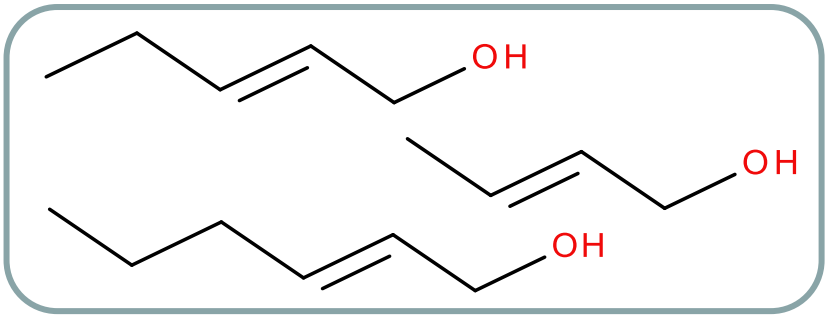
Identity (C.1)/ Structural Similarity / (Not) Allowed Differences (C.2)/ Link Structures and Effect Pattern (C.3) **/ Choice of Compounds (Bias)** (C.6)

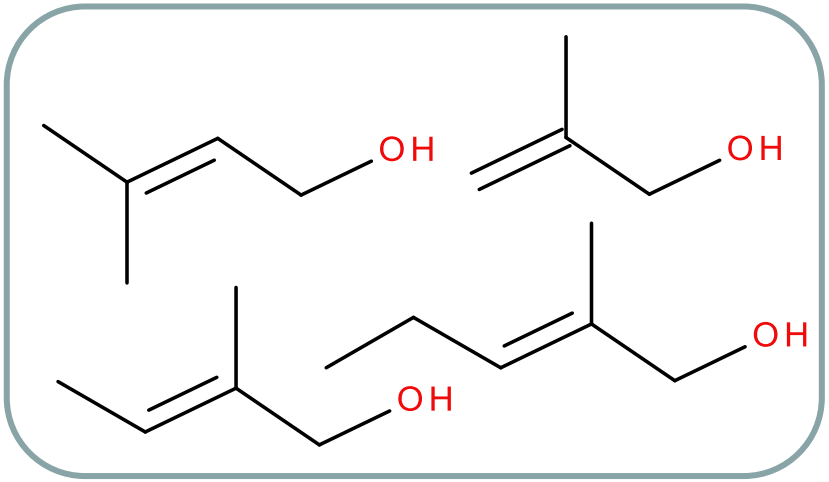
Complexity of the structural variations

- Two scaffoldings:
  primary (external –OH) / **secondary** (internal –OH)
- **External or internal vinyl group**
- Structural similarity complicated by alkyl substituents on the allylic moiety
  → **straight-chained or branched**

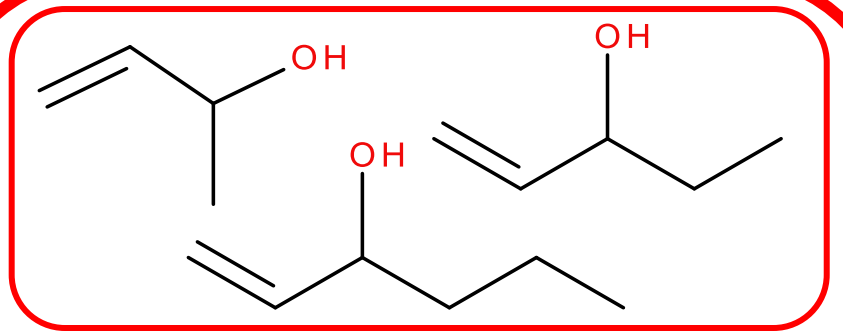
β-Olefinic Alcohols Subcategories

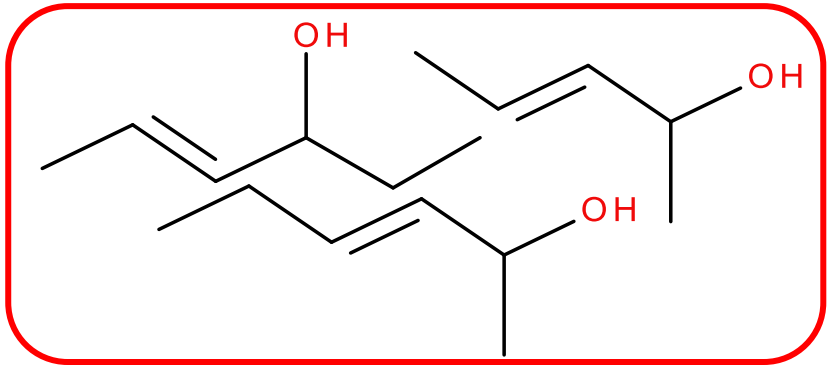


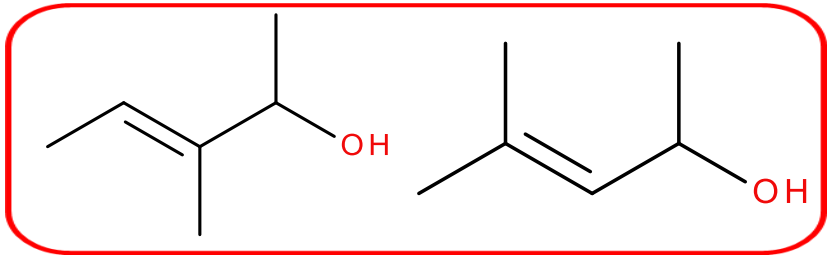




Primary Alcohols







Secondary Alcohols

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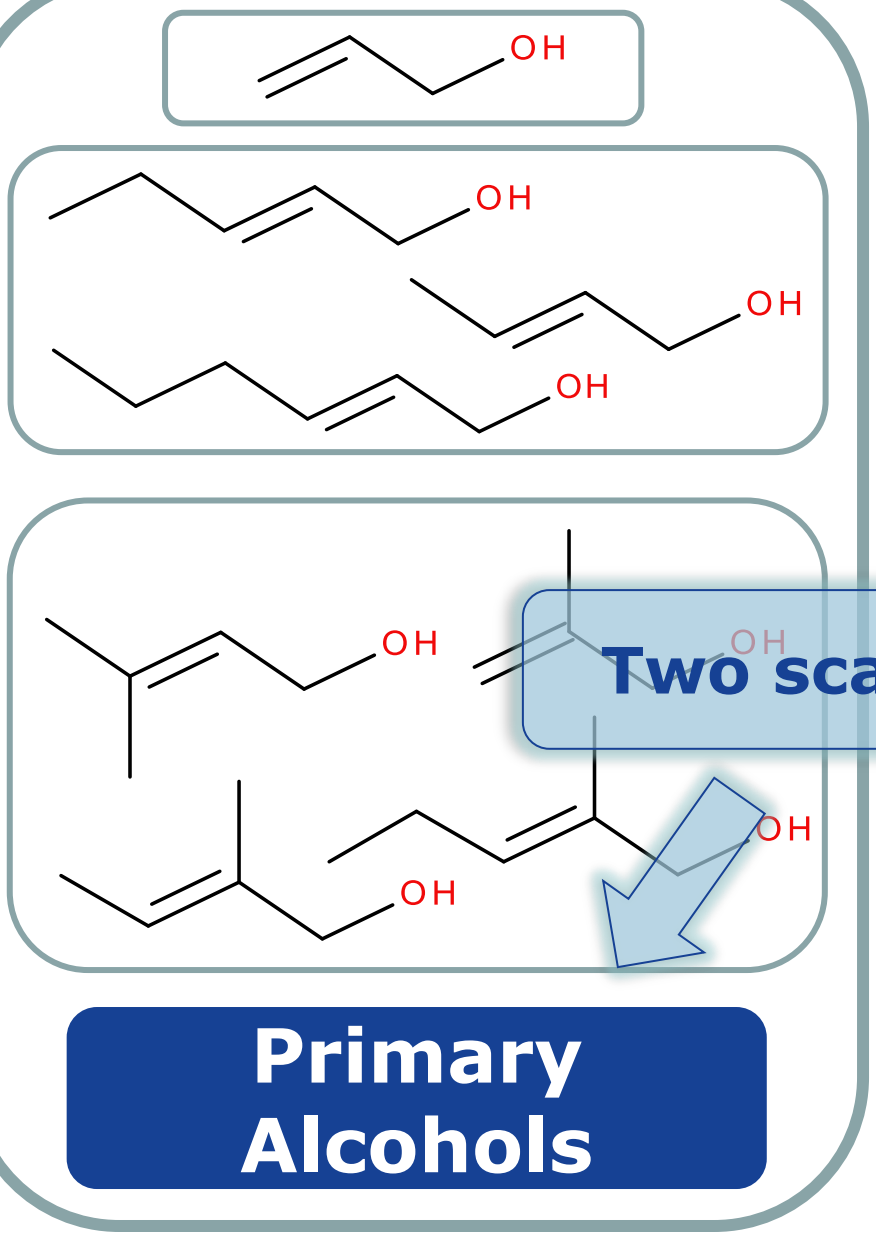
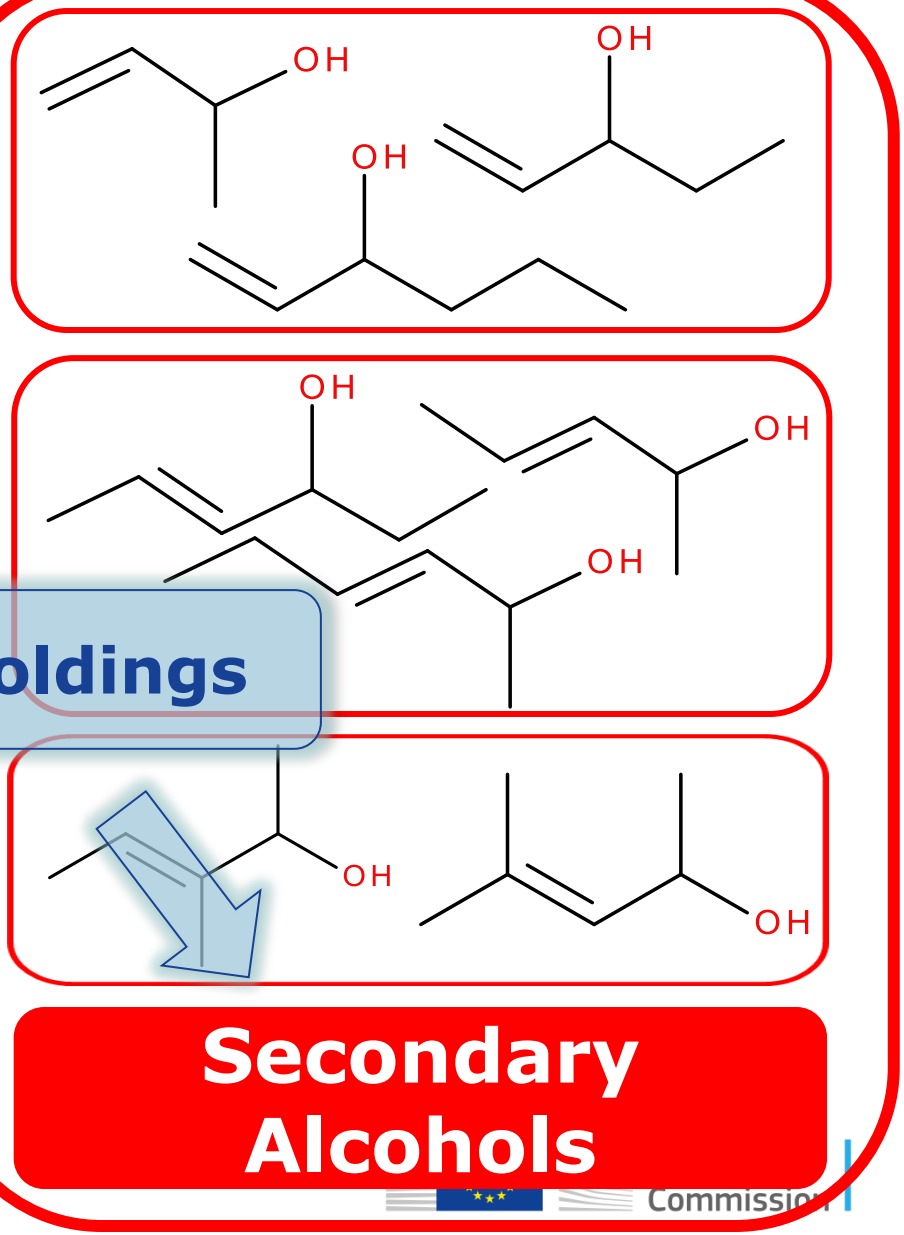
β-Olefinic Alcohols Subcategories





Two scaffoldings

Primary Alcohols

Secondary Alcohols

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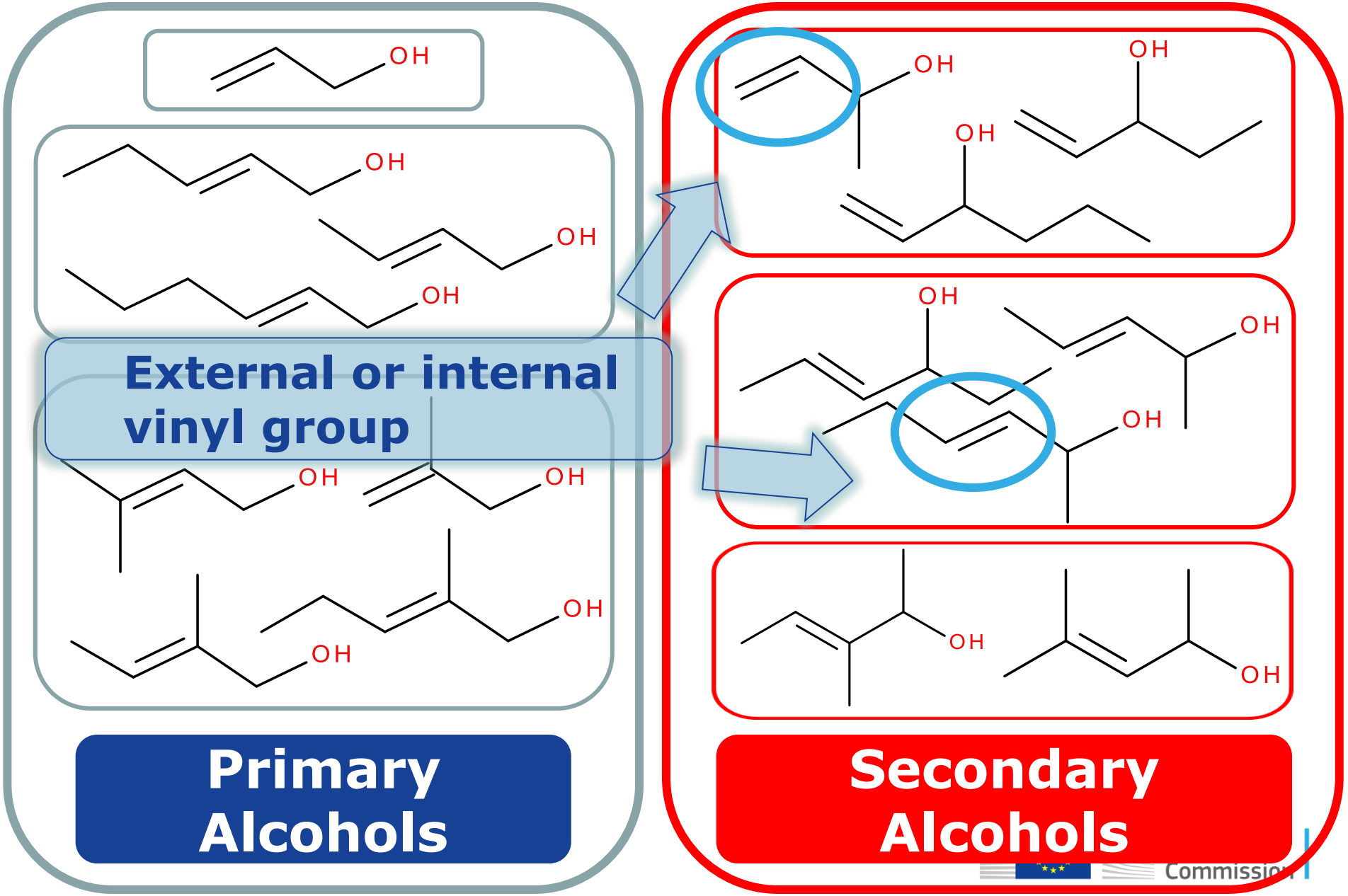
β-Olefinic Alcohols Subcategories



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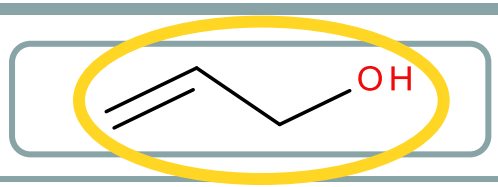
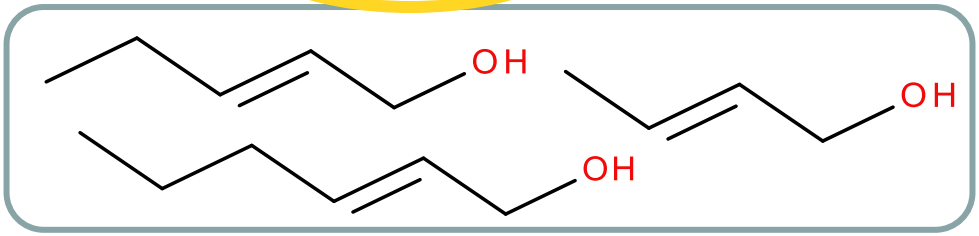
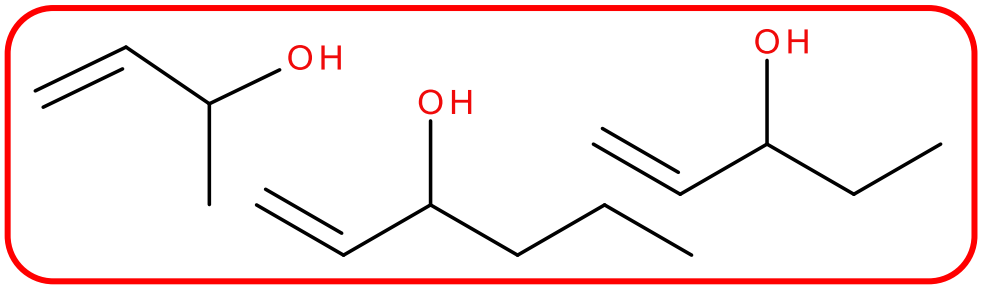
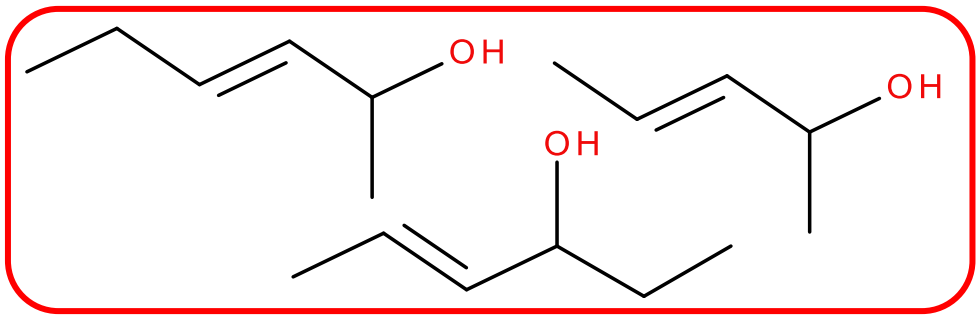
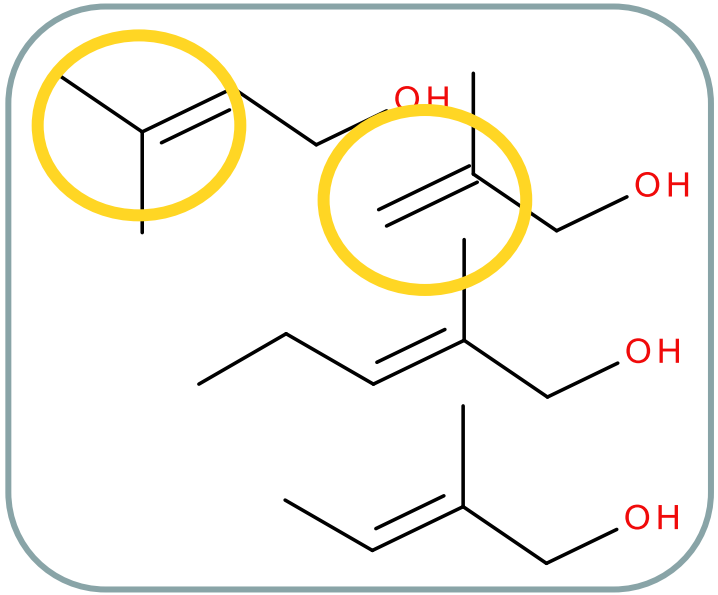


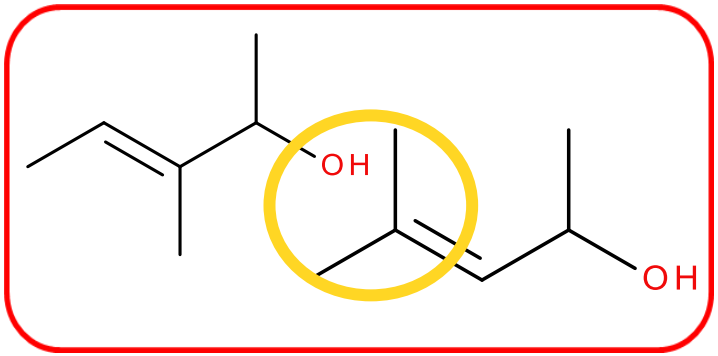






Straight-chained Alcohols





Branched Alcohols

RAAF I: Category and Structural Similarity

Identity (C.1)/ Structural Similarity / (Not) Allowed Differences (C.2)/ Link Structures and Effect Pattern (C.3) **/ Choice of Compounds (Bias)** (C.6)

Complexity of the structural variations

- → Similarity / Inclusion in category /
  How broad or narrow should a category be?

  Not included: acetylenic alcohols (metabolism difference)
- → Category valid for other structural variations?
- → Parent structure determines metabolite formed

  → Different reactivity metabolites → potency of effects

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  → Different reactivity metabolites = potency of effects

Existing NAM *ex vivo* liver perfusion:

- **Shows reactivity for different structural variants of βOAs ≠ alkanols**

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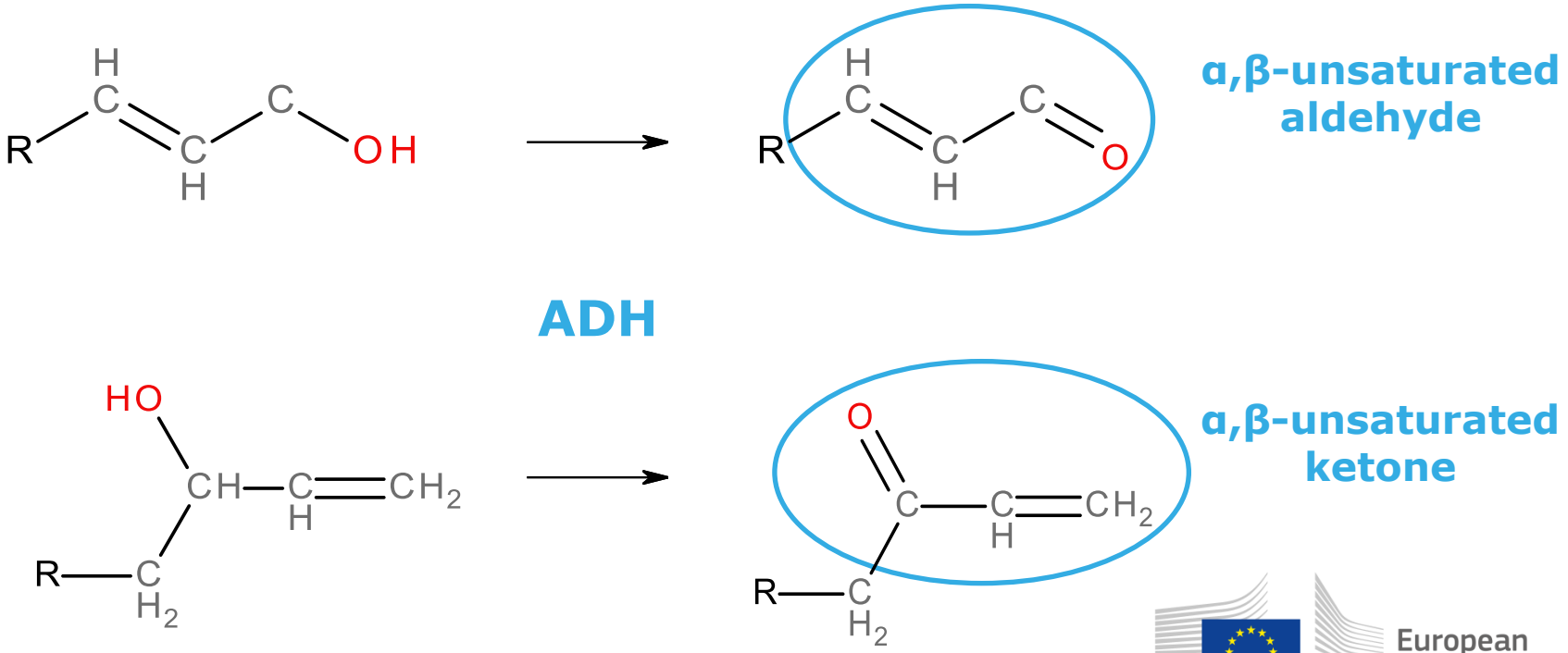
RAAF II: Metabolic Transformation to Toxicants

Link Structures and Effect Pattern (C.3) / Compounds to Which Organism Exposed (4.1) / Exposure to Other Compounds? (4.4)

Metabolites are the definitive toxicants



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RAAF II: Metabolic Transformation to Toxicants

Link Structures and Effect Pattern (C.3) **/ Compounds to Which Organism Exposed (4.1) / Exposure to Other Compounds? (4.4)**

Metabolites are the definitive toxicants

- Mechanism and kinetics of transformation
  → any other than metabolism by ADH?
- **Further metabolism pathway of aldehydes and ketones different → metabolic similarity?**
  → more quantitative kinetics information needed, relative efficiency of biotransformation
  → residual parents, further metabolites present with possible reactivity?

RAAF II: Metabolic Transformation to Toxicants

Link Structures and Effect Pattern (C.3) **/ Compounds to Which Organism Exposed (4.1) / Exposure to Other Compounds? (4.4)**

Metabolites are the definitive toxicants

Existing NAM *ex vivo* liver perfusion and *in silico*:

- **Link toxicity to metabolic transformation**
- **Electrophilic reactivity aldehydes/ketones**

Also confirmed by SEURAT-1 hepatic organoids

RAAF III: Available (*In Vivo*) Data

Consistency Data Matrix (C.4) / Reliability and Adequacy of (*In Vivo*) Data (C.5) / Choice of NOAEL to Read Across

Details and quality of *in vivo* studies

NTP study used for read-across
Rats and Mice: NOAEL 6 (m) and 25 (f) mg/kg bw/d for rats. Relating to toxicity in the liver.

RAAF III: Available (*In Vivo*) Data

Consistency Data Matrix (C.4) / Reliability and Adequacy of (*In Vivo*) Data (C.5) / Choice of NOAEL to Read Across

Details and quality of *in vivo* studies

- More information on study design and quality needed
  → selection of NAOEL to read across

- → guidance/ specific matrix needed for providing study details for RAAF?
- → also for NAM: how to evaluate new assay quality?

RAAF III: Available (*In Vivo*) Data

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Overall existing and SEURAT-1 NAM *in vivo* / *in vitro* / *in silico* data

give a consistent picture (differences in strength of effects)

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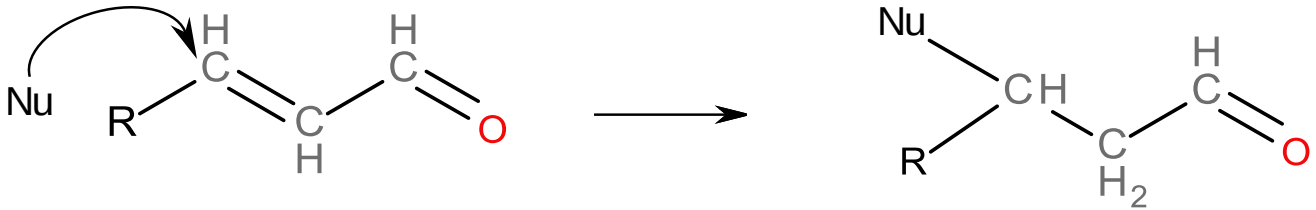
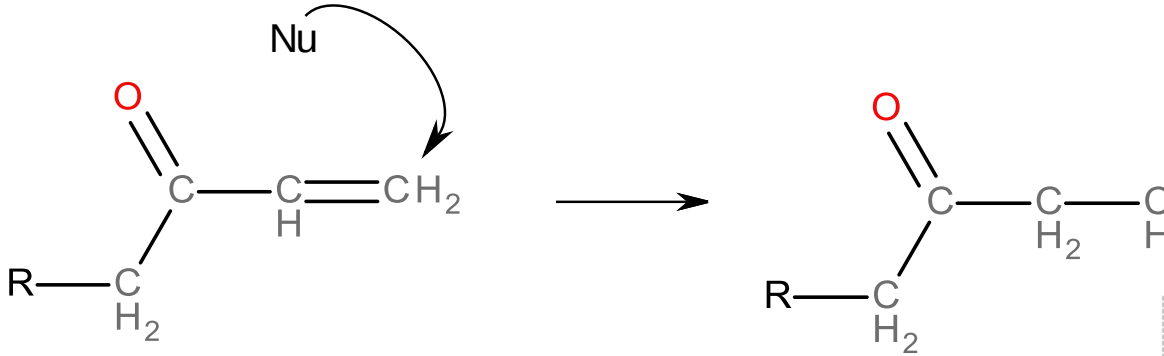
RAAF IV: Mechanism of Toxicity

Mechanism of toxicity (qualitative) (4.2) / Other effects? (4.5)

Electrophilic reactivity (Michael addition mechanism), binding to proteins → adverse effects





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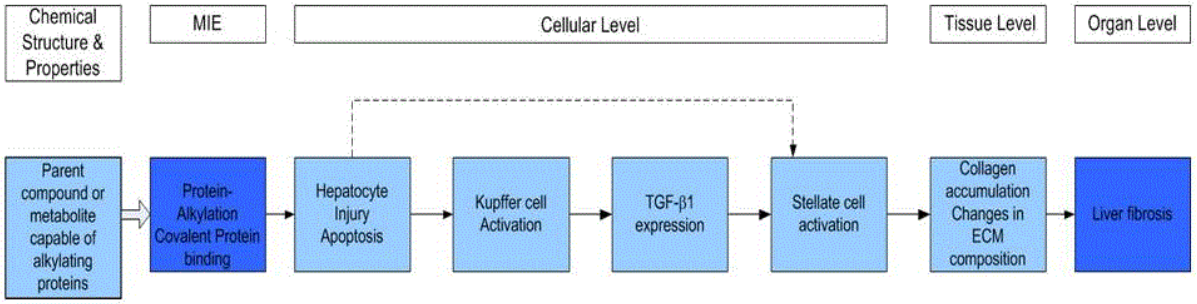
- Hypothesis of fibrosis as adverse effect



AOP for Liver Fibrosis (from Landesmann et al 2012)

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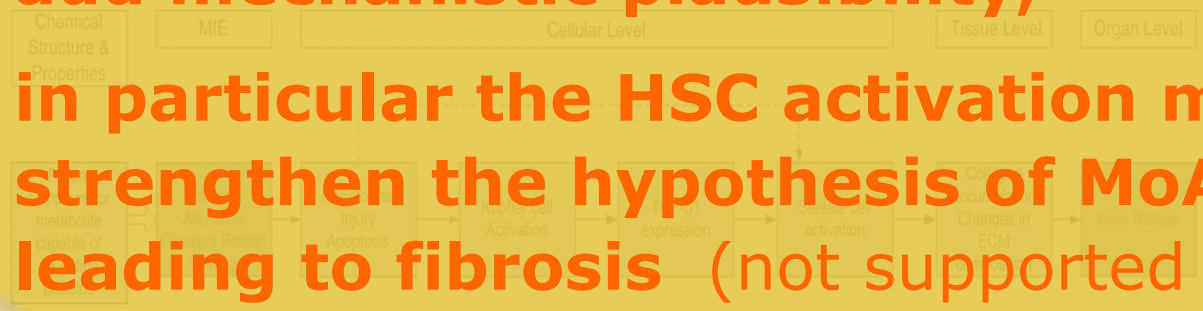
In silico/in chemico/SEURAT-1 in vitro* NAM *(hepatic organoids with HSC activation markers; stress response activation in HepG2)
add mechanistic plausibility,
in particular the HSC activation markers strengthen the hypothesis of MoA leading to fibrosis (not supported by rat data)



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RAAF V: Mechanism of Reactivity and Trends in Effect Potency

Clustering of Effects / Order of Reactivity (C.4)/ Quantitative Aspects of Mechanism and Strength of Effects (4.3) / Source is Worst Case (C.6)

Clustering of potency of effects according to chemical reactivity

- Quantitative reactivity data only from *in chemico* GSH assay supported by *in silico* predictions

Reactivity *In Chemico* and *In Silico*

	Subcategory		Compound	Metabolite	*In Chemico* reactivity GSH RC_{50} [1]	Protein binding potency [2]	*In silico* protein binding [2,3]
1	Straight chain	primary: external OH, **external =**	2-propen-1-ol	2-Propenal (acrolein)	0.085	Extremely reactive	MA, SBF
2		primary: external OH, internal =	2-buten-1-ol	2-Butenal (crotonaldehyde)	0.22	Highly reactive	MA, SBF
3			2-penten-1-ol	trans-2-Pentenal	0.35	Highly reactive	MA, SBF
4			2-hexen-1-ol	trans-2-Hexenal	0.42	Highly reactive	MA, SBF
5		secondary: internal OH, **external =**	1-buten-3-ol	Methyl vinyl ketone	0.070	Extremely reactive	MA
6			1-penten-3-ol	Ethyl vinyl ketone	0.051	Extremely reactive	MA
7			1-hexen-3-ol	Propyl vinyl ketone	0.059	Extremely reactive	MA
8		secondary: internal OH, internal =	3-penten-2-ol	3-Penten-2-one	0.15	Highly reactive	MA
9			3-hexen-2-ol	3-Hexen-2-one	not tested	Highly reactive	MA
10			4-hexen-3-ol	4-Hexen-4-one	0.34	Highly reactive	MA
11	Branched	primary: external OH, internal =	2-methyl-2-propen-1-ol **external =**	2-Methyl acrolein	not tested	Moderately reactive	MA, SBF
12			2-methyl-2-buten-1-ol	2-Methyl-2-butenal	12	Moderately reactive	MA, SBF
13			2-methyl-2-penten-1-ol	2-Methyl-2-pentenal	21	Moderately reactive	MA, SBF
14			3-methyl-2-buten-1-ol	3-Methyl-2-butenal	13	Moderately reactive	SBF no MA
15		secondary: internal OH, internal =	3-methyl-3-penten-2-ol	3-Methyl-3-penten-2-one	10	Highly reactive	MA
16			4-methyl-3-penten-2-ol	4-Methyl-3-penten-2-one	26	Highly reactive	No alert

[1] in mmol/l; [2] OECD QSAR Toolbox; [3] MA: Michael addition, SBF: Schiff base formers;

RAAF V: Mechanism of Reactivity and Trends in Effect Potency

Clustering of Effects / Order of Reactivity (C.4)/ Quantitative Aspects of Mechanism and Strength of Effects (4.3) / Source is Worst Case (C.6)

Clustering of potency of effects according to chemical reactivity

- Strengthen use of 2-propen-1-ol as source substance
- → How to prove a worst case?

- → suitability of *in chemico* assay for quantitative ranking (variability; relevance)?
- → confirm subcategory reactivity trends with testing of more (different) substances

RAAF V: Mechanism of Reactivity and Trends in Effect Potency

Clustering of Effects / Order of Reactivity (C.4)/ Quantitative Aspects of Mechanism and Strength of Effects (4.3) / Source is Worst Case (C.6)

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- → How to prove as worst case?
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Reactivity trend mostly relying on existing NAM *in chemico* data of reactivity potency clustering, supported by *in silico* and SEURAT-1 *in vitro* NAM

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βOA Case Study Considered with the RAAF

AE		AO w/o NAM	AO with NAM	NAM added info	Remaining questions
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C.2	Structural similarity	4	4		• Similarity and category boundaries
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4.1	Compounds the organism is exposed to, transformation	4	5	*ex vivo/in silico*: **metabolites→toxicants, not tertiary alcohols**; also by hepatic organoids	• more (quant.) information: rate/speed of metabolism
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4.5	Occurrence of other effects	4	4		• Rule out other effects / metabolic mechanisms
C.6	Bias influencing prediction	4	4		• Read-across valid for other structural variation members?

The beta OA Case Study: AO's and NAM

AE		AO w/o NAM	AO with NAM	NAM added info	Remaining questions
C.1	Subst. characterisation	N/A	N/A		
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C.5	Reliability/adequacy source study(ies)				• More details on study design and quality
4.1	Compounds the organism is exposed to, transformation			*ex vivo/in silico*: **metabolite variants, not tertiary alcohols**; also by hepatic cytochromes	• more (quant.) information: rate/speed [illegible] amount residual parents/other metabolites
4.2	Common mechanism, qualitative aspects	4	5	*in chemico/in silico/in vitro* data strengthen **mechanism evidence**, HSC activation markers the **MoA to fibrosis**	
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C.6	Bias influencing prediction	4	4		• Read-across valid for other structural variation members?

- **Overall AO's ≥ 3**
- **Confidence increased by existing and SEURAT-1 NAM results**

Information Added and Uncertainties Reduced by NAM

- **Metabolism of βOAs to toxicant**, reactivity of metabolites as opposed to parent compounds, link with structure (*ex vivo* perfused liver, *in silico, in chemico*)
- *in chemico* data: only quantitative data available to show **clustering of reactivity potency** (supported by *in silico*/*in vitro*)
- **Mechanism of adverse effect evidence strengthened** by *in chemico*/*in silico*/ SEURAT-1 *in vitro* data
- in particular: HSC activation markers in hepatic organoids confirm MoA **hypothesis of metabolic-mediated fibrosis**

Major Uncertainties

- Complexity of structures
  → Similarity / category boundaries and members
- Details on study design / quality of *in vivo* data, choice of NOAEL
- Potency of effects, order of reactivity
- → Proving the worst case for source compound
- Transformation mechanism (other than via ADH) / rates?
  → Reactivity potency vs kinetics
- Variation of metabolic pathways (aldehydes/ketones)
- → Possible other effects via further metabolites present (kinetics of transformations)
- Toxic reactivity mechanism? Map on AOP?

Possible further elucidation by NAM

- Complexity of structures
  → Similarity / category boundaries and members
- Details on study design / quality of *in vivo* data, choice of NOAEL
- Potency of effects, order of reactivity
- → Proving the worst case for source compound
- Transformation mechanism (other than via ADH) / rates?
  → Reactivity potency vs kinetics
- Variation of metabolic pathways (aldehydes/ketones)
- → Possible other effects via further metabolites present (kinetics of transformations)
- Toxic reactivity mechanism? Map on AOP?

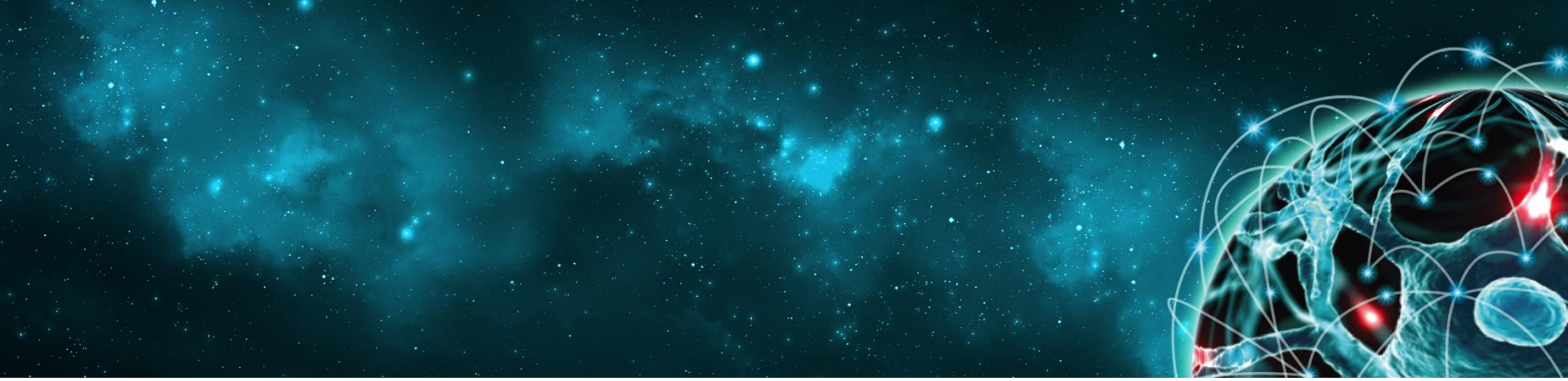
Test more substances in the GSH reactivity *in chemico* assay

Any other activation of transformation than by ADH? → *omics*?

Generally: *omics* assays for metabolism pathways?

Conclusions

- βOA case study especially features
  - High structural complexity
  - Mechanistic aspects related to structures important for considered effect
- RAAF guides systematically through checking of all important points being covered and documented in sufficient detail for regulatory assessment, highlights kinetics issues
- NAM help in reducing uncertainties in particular related to mechanism
- Uncertainties remaining
  - in particular more (quantitative) kinetics/metabolism pathway data needed as highlighted by RAAF
- → more targeted NAM testing?



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