Case Study from SEURAT-1
β-Unsaturated Alcohols: Indirect Acting Toxicant Category Supported by SEURAT-1 Data

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Grovelling Thanks to the Following Immensely Talented People:

- Liverpool John Moores University:
  - Katarzyna Przybylak, Claire Mellor, Andrea-Nicole Richarz
- University of Tennessee:
  - Terry W Schultz
- Fraunhofer ITEM:
  - Sylvia Escher
- Vrije Universiteit Brussels:
  - Sofia Leite, Leo van Grunsven
- Leiden University:
  - Bob van de Water, Steven Hiemstra

SEURAT-1

… ask not what this read-across can do for you, ask what you can do for this read-across…

Important Disclaimer!

This case study intends:

- To illustrate uncertainties in read-across
- To probe approaches for read-across
  - Compounds with a similar metabolite
- To investigate how New Approach Methodology (NAM) data may assist in increasing confidence
- To utilise the a workflow and templates
- To provide a platform for evaluation by the RAAF (…but was not designed for this purpose)

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However, it is not illustrative of a regulatory submission (and should not be seen as such)

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… What follows is my interpretation!

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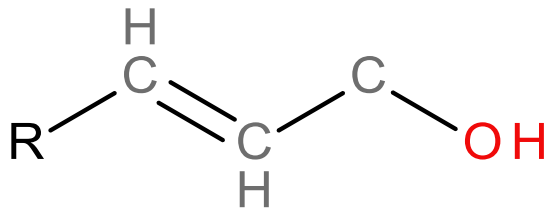
We need your opinions on NAMs….

Presumptions and Aims…

- The read-across scenario is chemical similarity as a result of metabolism to the same toxic metabolite
  - metabolite(s) acting by a common mode of toxic action are considered as the definitive toxicants
- Read-across is for olefinic β-unsaturated alcohols
  - *in vivo* data supplemented by *ex vivo*, *in vitro*, *in chemico* and SARs
- The aim was to reduce uncertainty associated with the *in vivo* prediction using the increased weight-of-evidence provided by the alternative methods data

β-Unsaturated Alcohols Include



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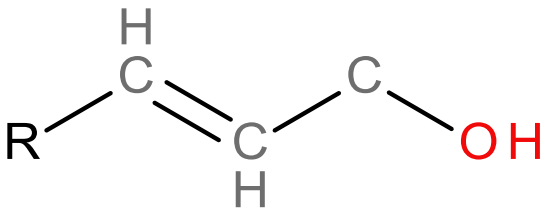

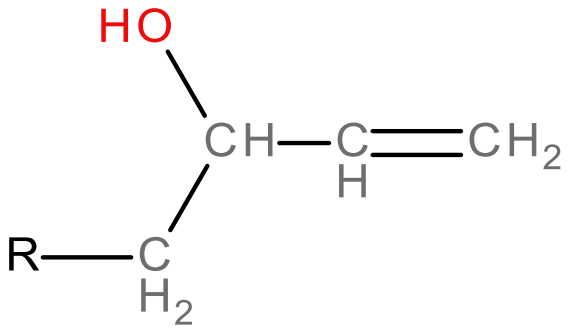

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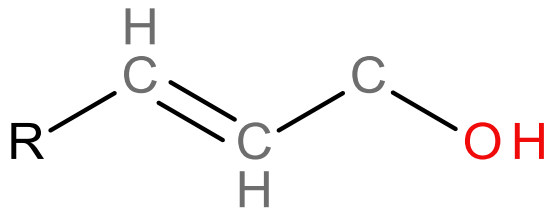
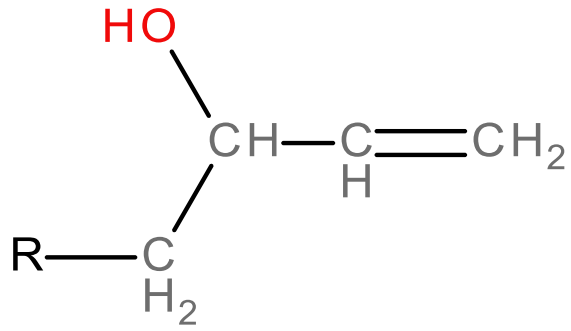
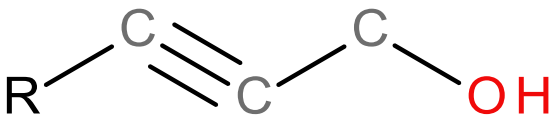
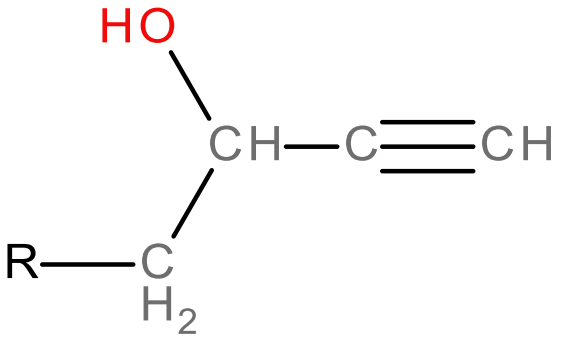


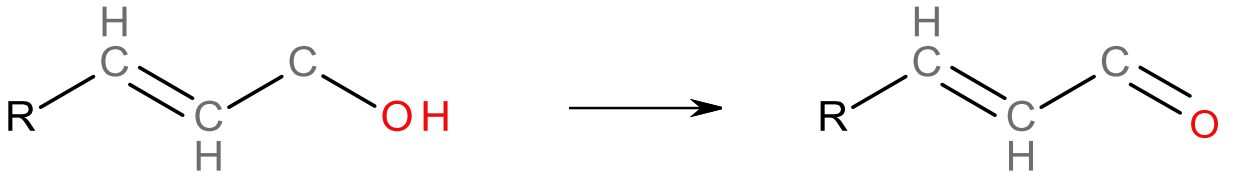


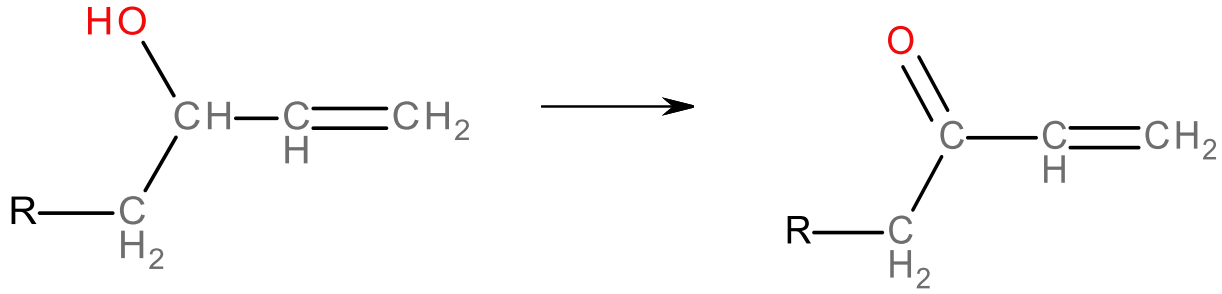




Where R is assumed to be a saturated carbon chain which may be branched or unbranched

Oxidation of Olefinic β-Unsaturated Alcohols





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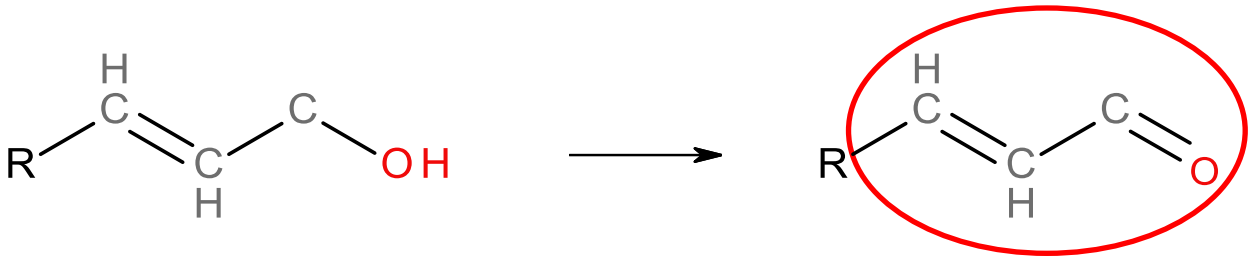
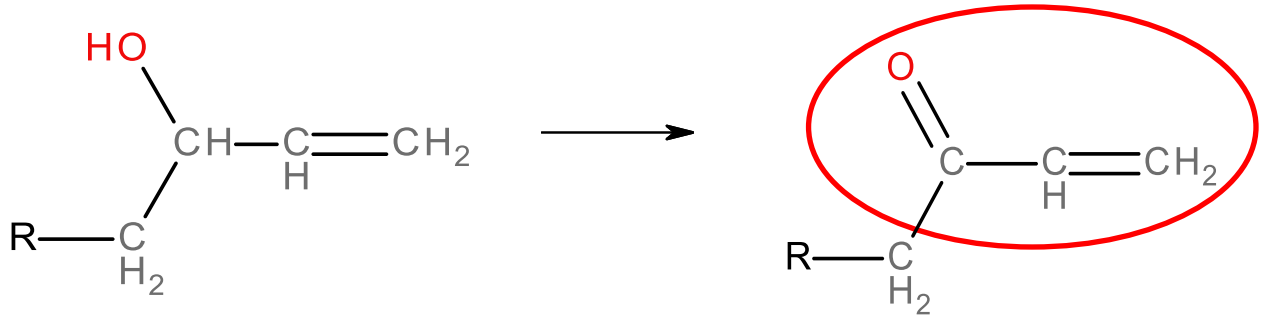
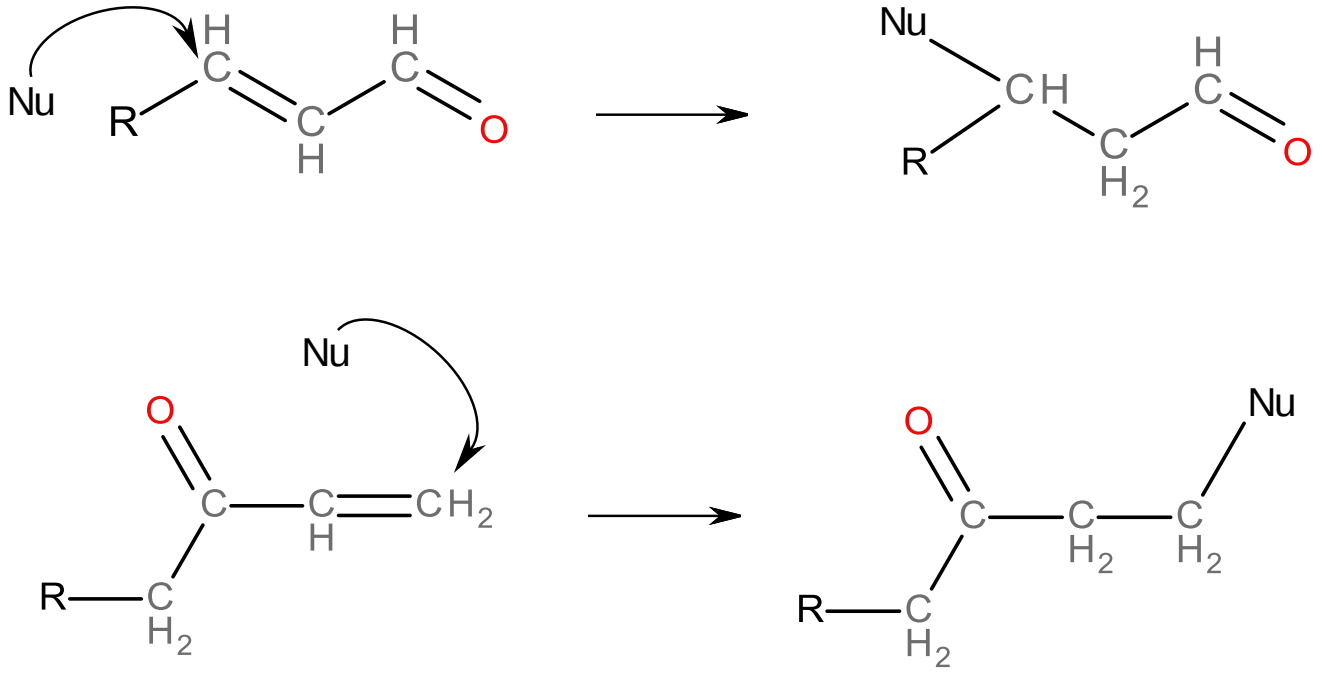


α,β-unsaturated aldehyde



α,β-unsaturated ketone

Reactivity of Metabolites Leads to Their Toxicity e.g. Liver Fibrosis



Where Nu is a nucleophilic group (e.g. –SH) on a biological macromolecule

Premise of Case Study

- Short chain (C3 to C6) unsaturated alcohols are indirect-acting toxicants
- Same covalent mechanism of action and similar reactive potency
- Little affect on in oral bioavailability
- Rapidly and extensive absorption from the gut
- Metabolism via ADH or CYP450
- Metabolites are electrophilic with *in vivo* potency related to relative thiol reactivity
- Only β-unsaturated alcohols with metabolism similar to 2-propen-1-ol and reactive potency similar to acrolein may be read across for 2-propen-1-ol with reasonable certainty
- NO(A)EL driven by liver toxicity

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- NO(A)EL driven by liver toxicity

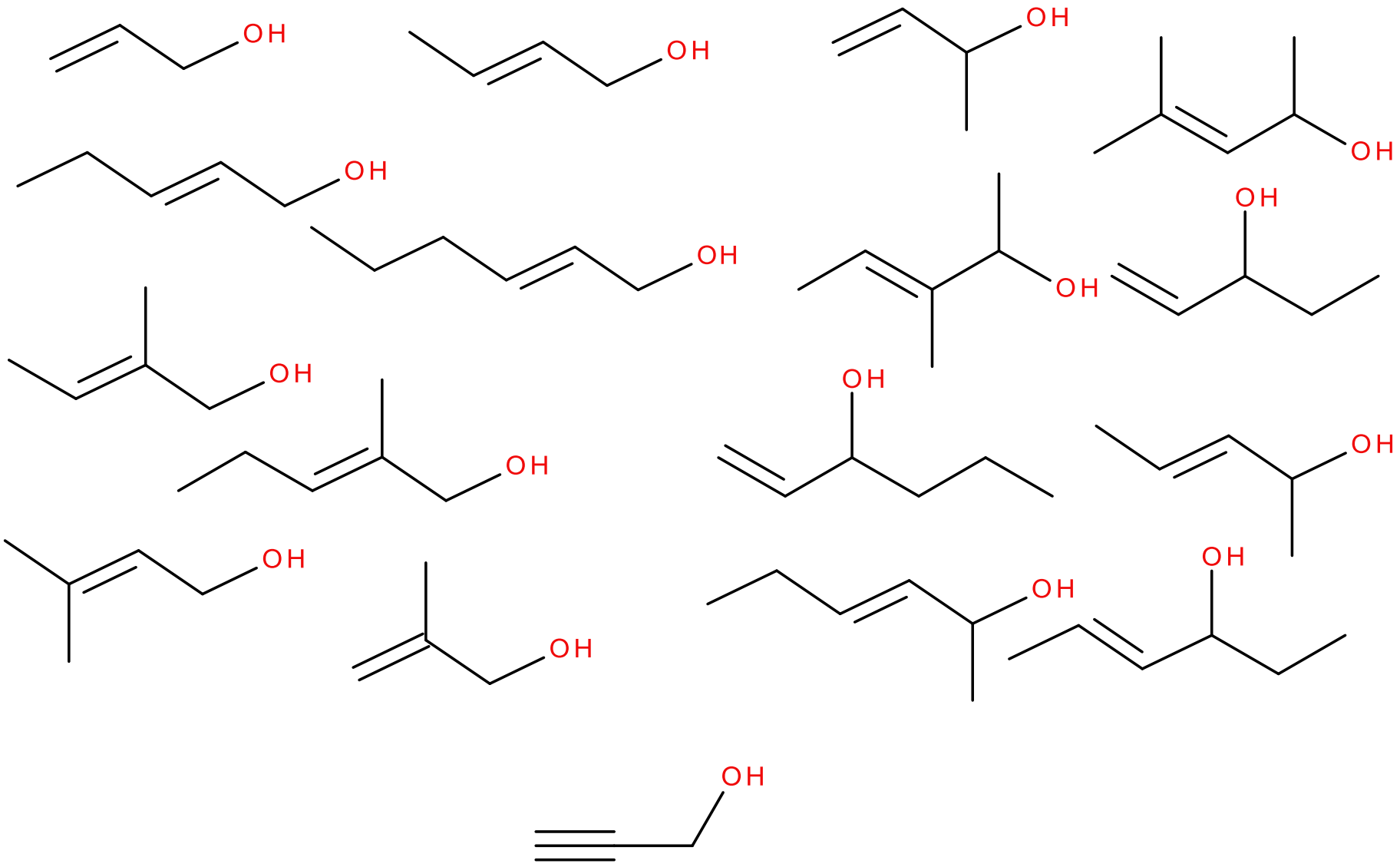
For Consideration:

How strong is the premise?

What could make it stronger?

What evidence is required?

Full Category Members: Unsaturated Alcohols



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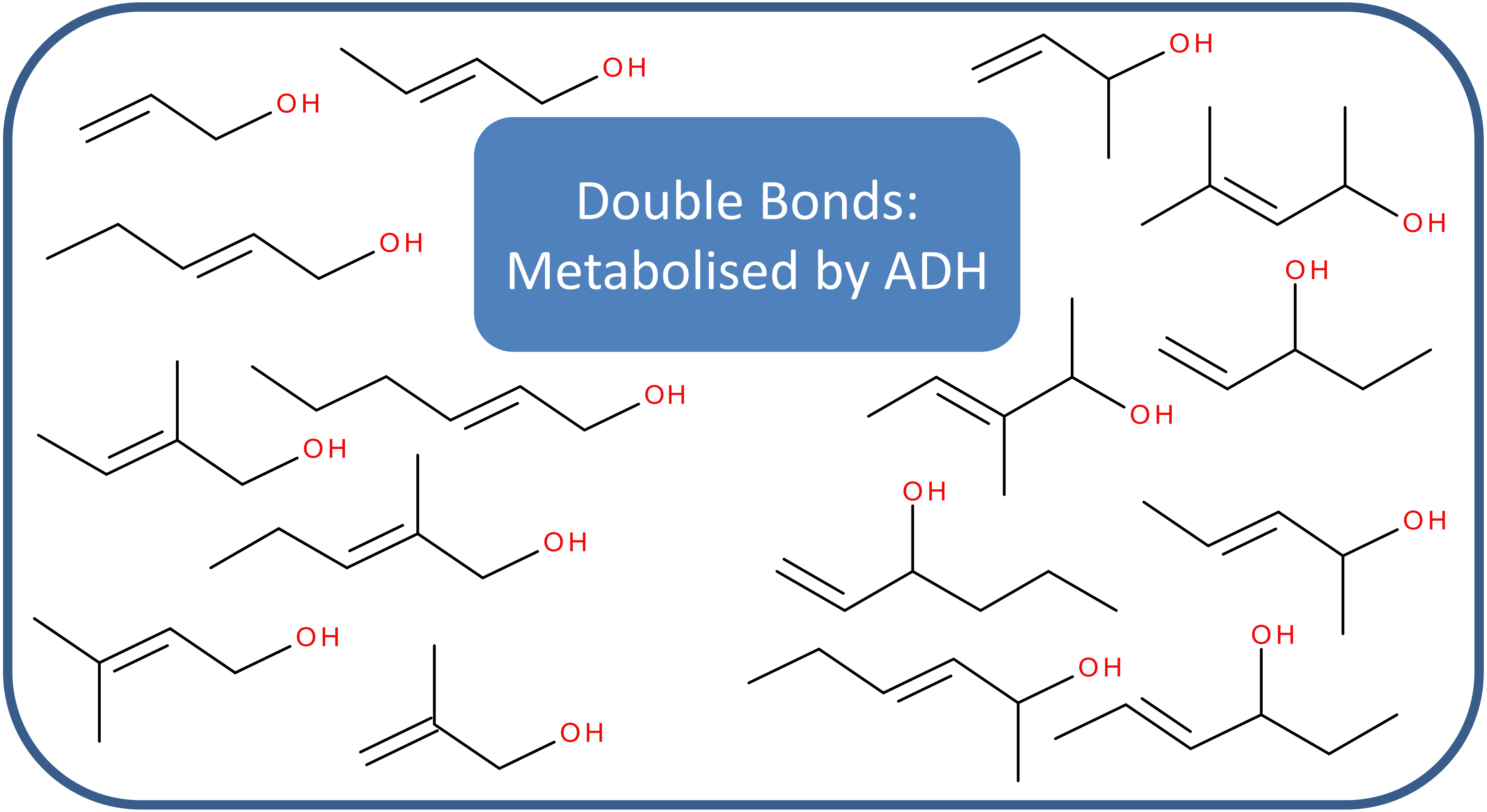

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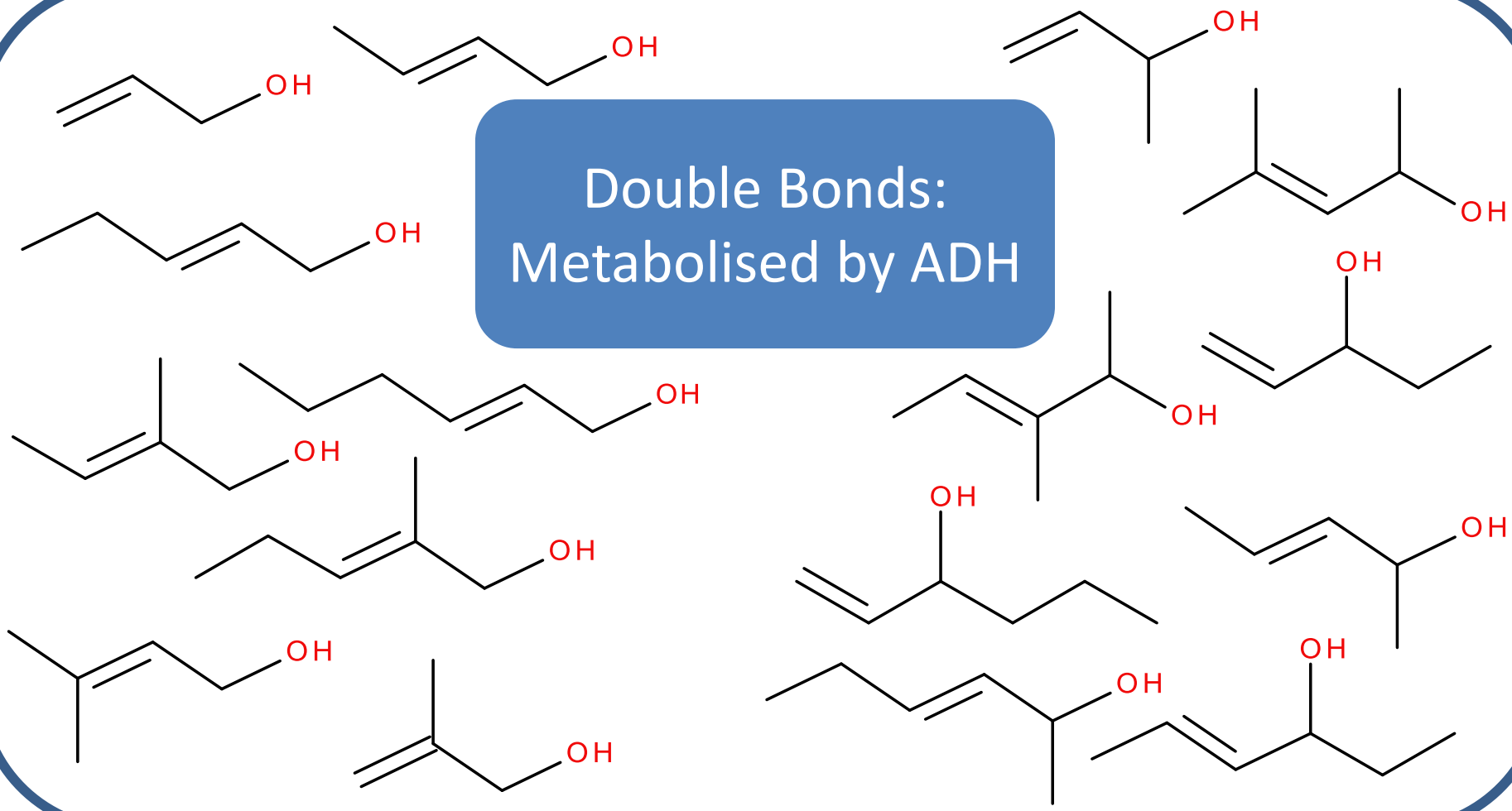
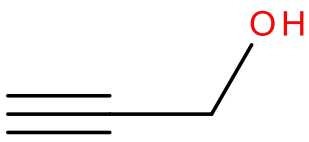
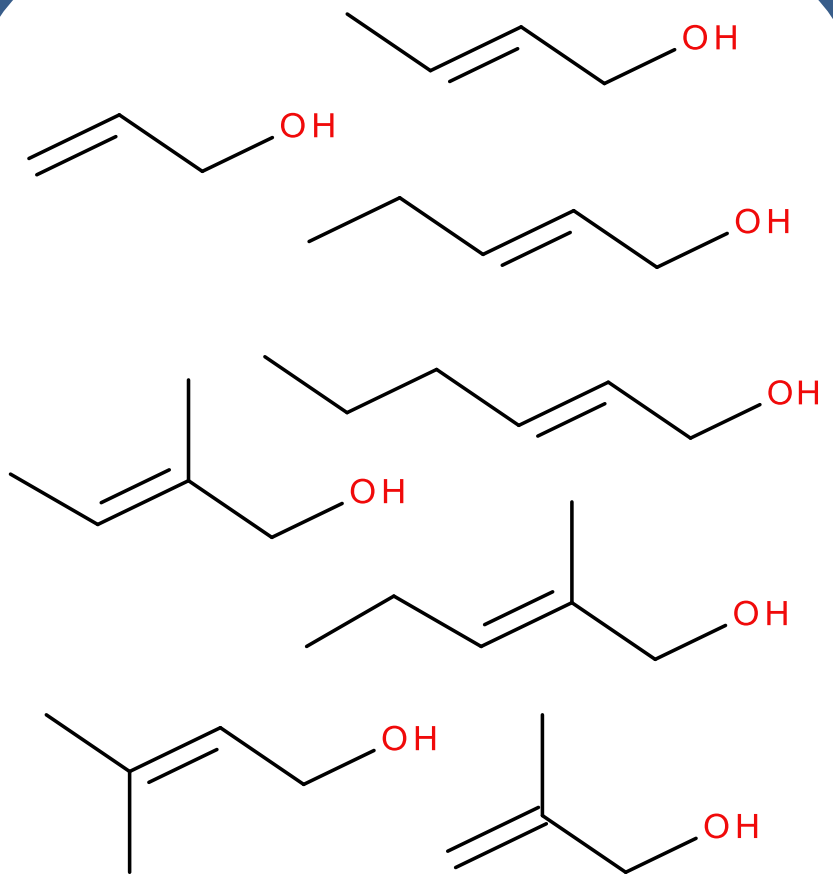




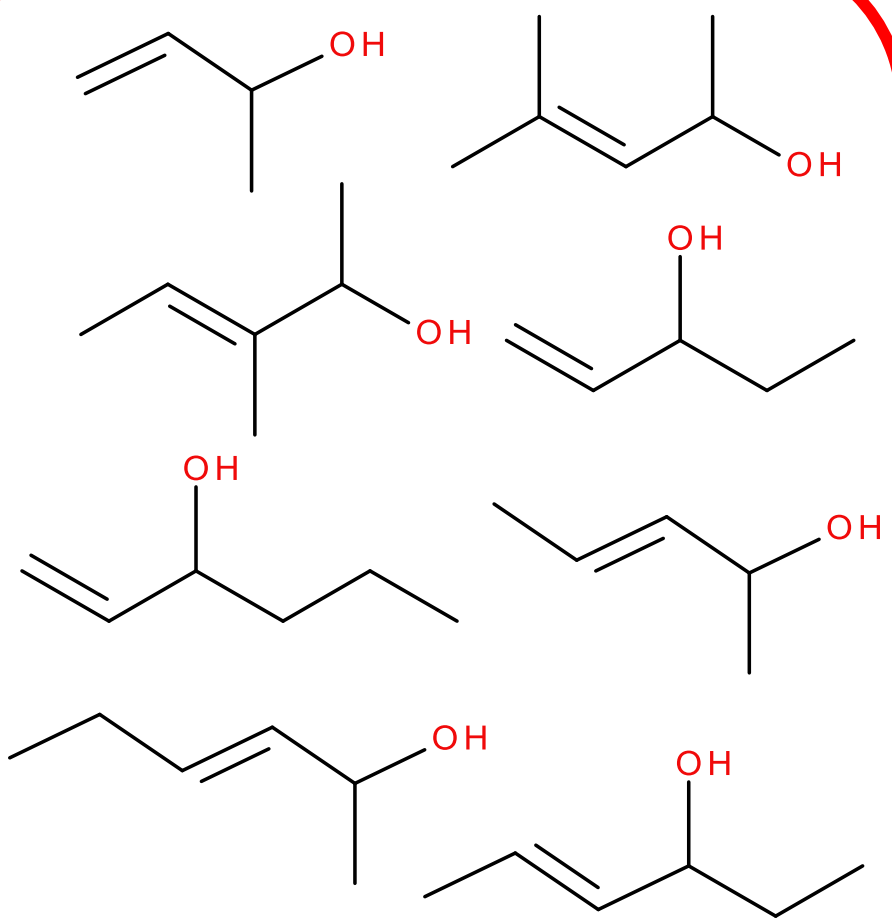
Triple Bonds:
Metabolised by CYP450

Excluded

Category Members: Unsaturated Alcohols



Primary Alcohols



Secondary Alcohols

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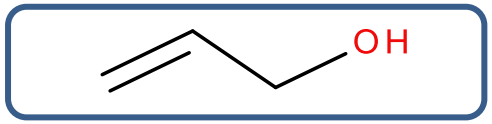

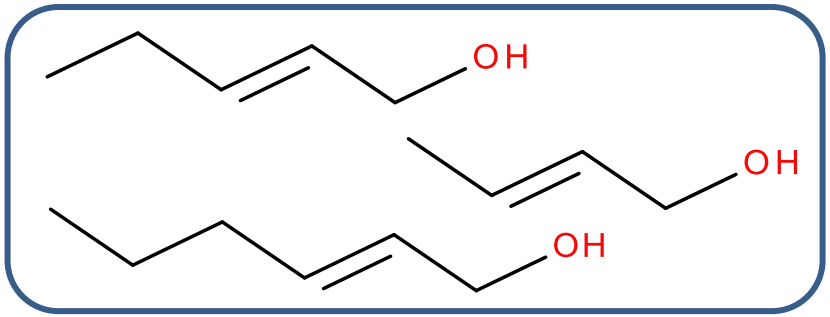

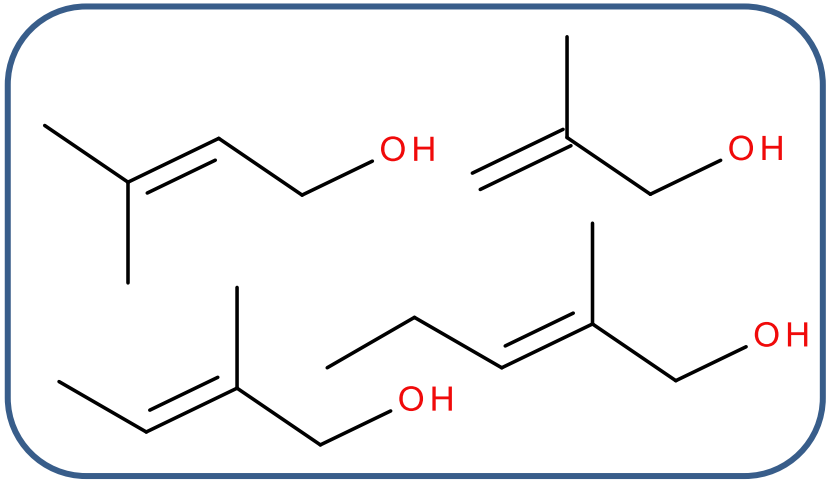

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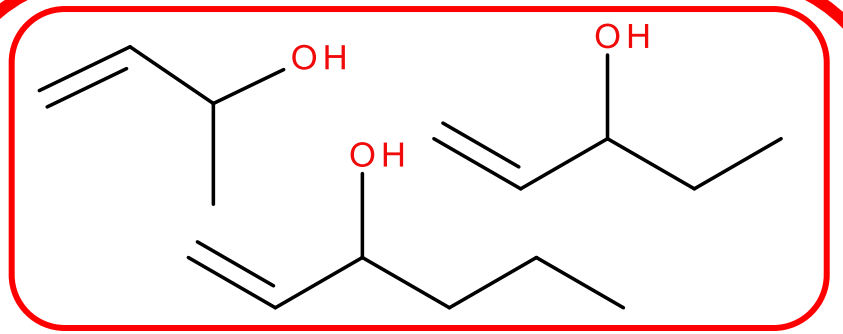

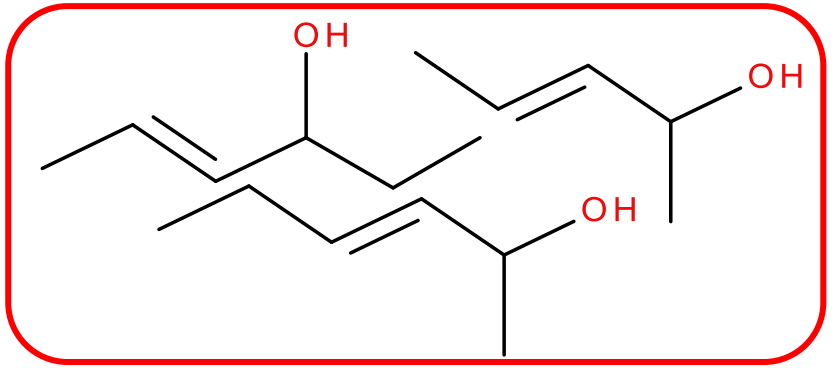

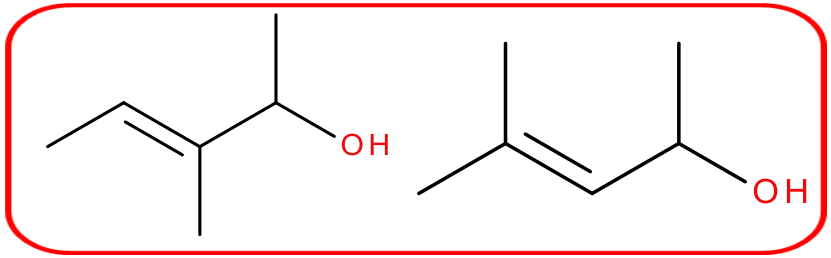

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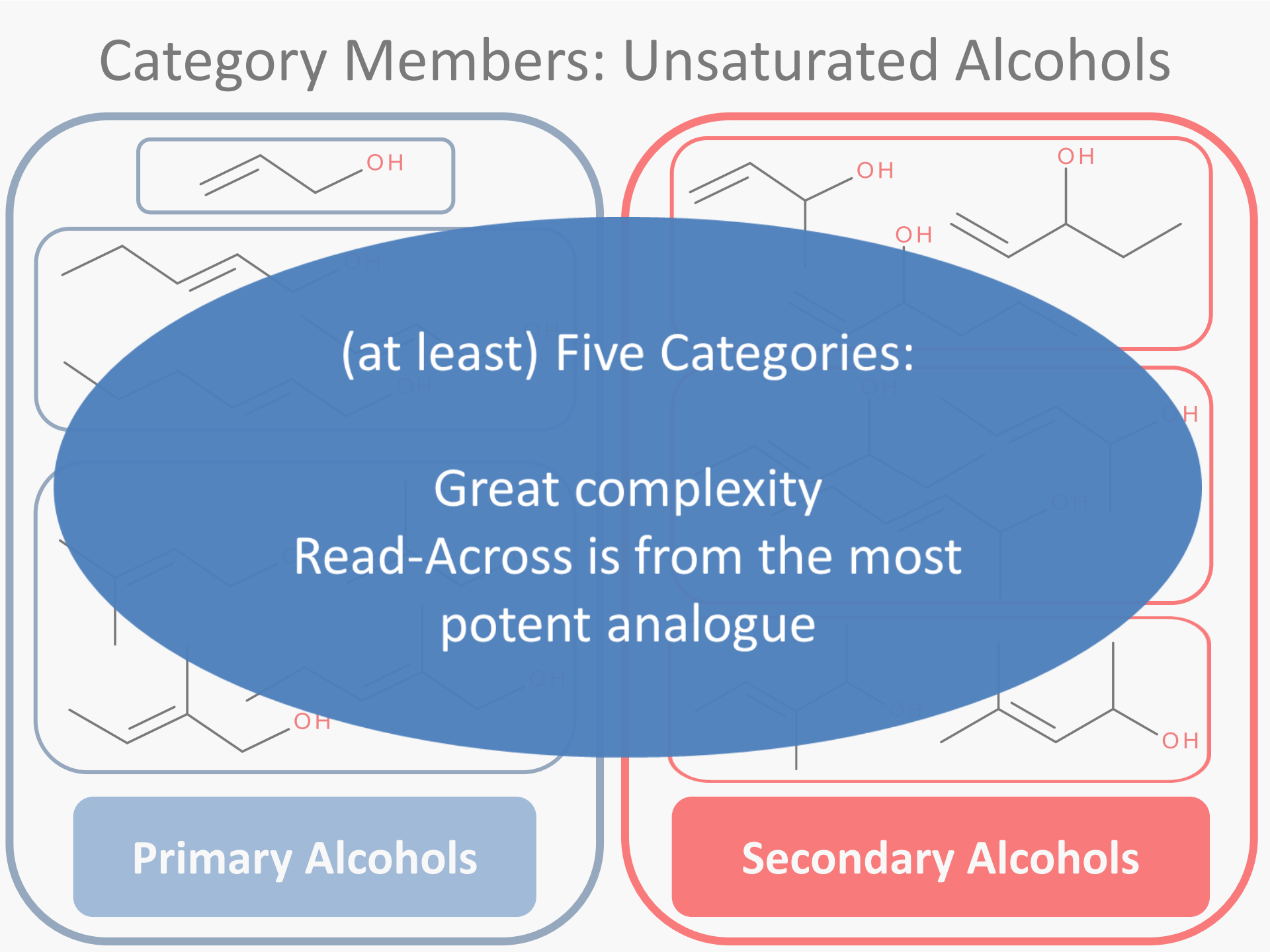

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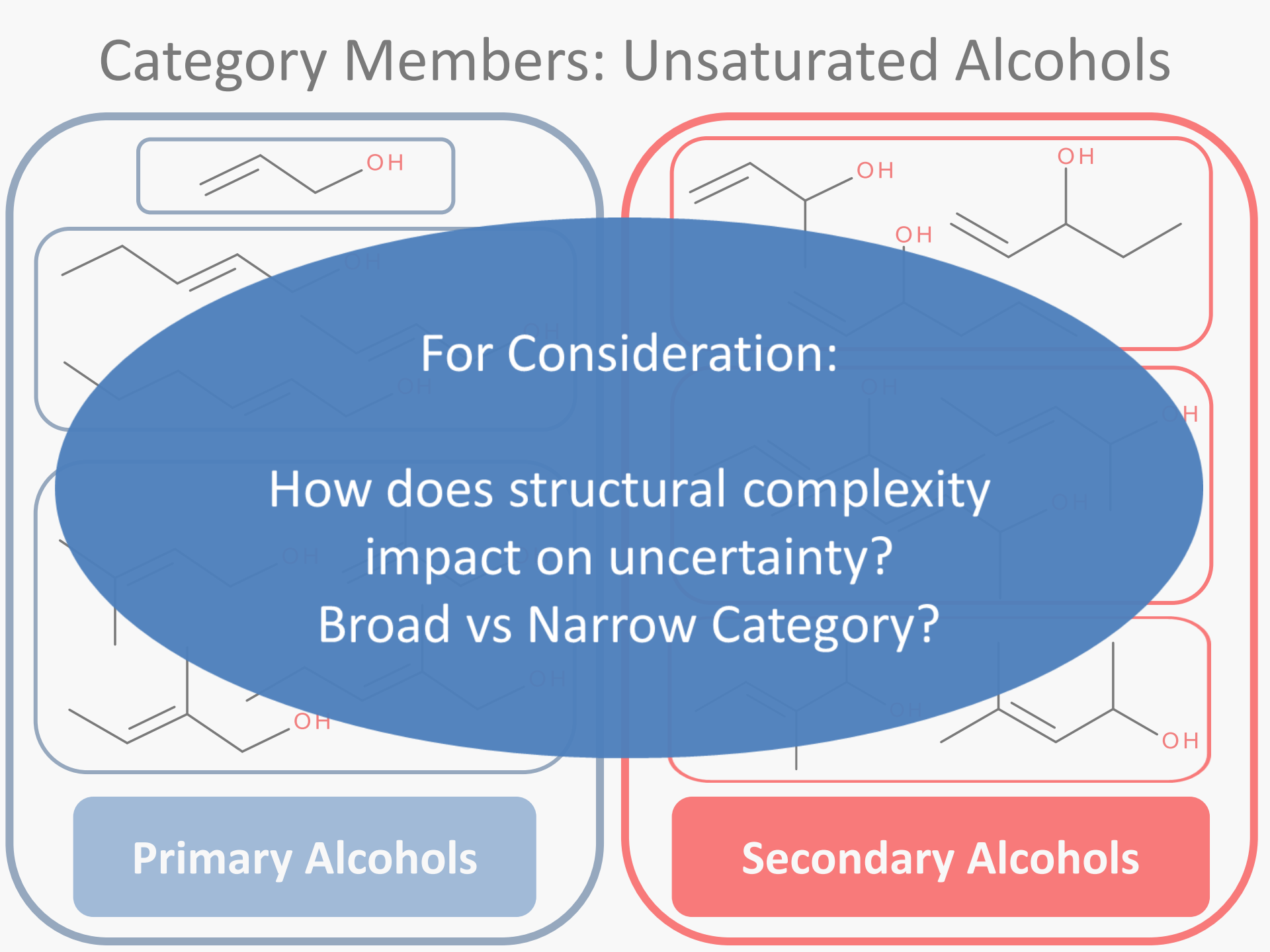

Similarity: Structural, Property, Chemical Class (Applicability Domain of Category)

- Common chemical (sub-)class: β-olefinic alcohols,
- Molecular scaffolds
  - Internal vs external hydroxyl / double bond
  - Branching
- Alkyl substituents on the C=C bond
- Very narrow value ranges of physico-chemical properties:
  - Molecular weight: 58 - 100 g/mol
  - Log P: 0.17 - 1.66
  - Density constant: 0.8 +/- 0.1 g/cm^3
  - Vapour pressure and water solubility: slight variation
  - Melting points < 0 °C
  - Boiling points > 100 °C

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From A Chemistry Point of View:

A well defined category?

Toxicokinetic Similarity

- Toxicokinetic understanding is incomplete
- Oxidation of primary olefinic alcohols to aldehydes is catalysed by ADH
  - ADH binding related to chain length and unsaturation
- Studies confirm presence of metabolites and their reactive nature
- Further oxidation of aldehyde produces an acid
  - may enter the β-oxidation pathway
  - subsequent metabolism to CO_2 or glucuronidation
  - detoxification is not relevant to repeated-dose toxicity
- Secondary alcohols may be excreted via conjugation or oxidised to ketones
  - excreted unchanged or undergo hydroxylation

Metabolic Similarity

- Predicted metabolites are α, β-unsaturated aldehydes or α, β-unsaturated ketones
  - react with GSH and protein thiols in hepatocytes
- Only primary and secondary β-olefinic alcohols can be activated by ADH
  - Limits read-across category
- Triple bonded unsaturated alcohols induce cytotoxicity via metabolic activation by CYP 2E1
  - Excluded from category

Uncertainties:

- Metabolic similarity
- Ketone vs aldehyde metabolite

How crucial are these uncertainties?

Can, if so how, NAMs reduce uncertainties?

Toxicodynamic Similarity

- *In silico* predictions indicate:
  - Most metabolites are electrophilic
  - Potency of protein binding varies between the five sub-categories

Toxicodynamic Similarity

- *In silico* predictions indicate:
  - Most metabolites are electrophilic
  - Parent and metabolites can be grouped into the five sub-categories

For Consideration:

Relevance and accuracy of *in silico* predictions?

How to support toxicodynamic similarity?


CASSIDY
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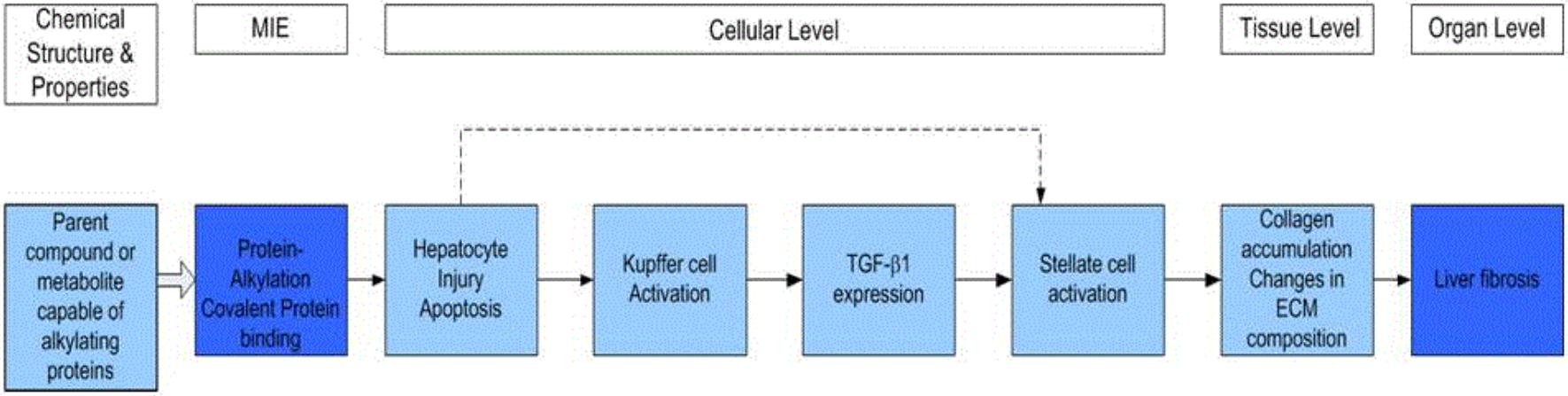
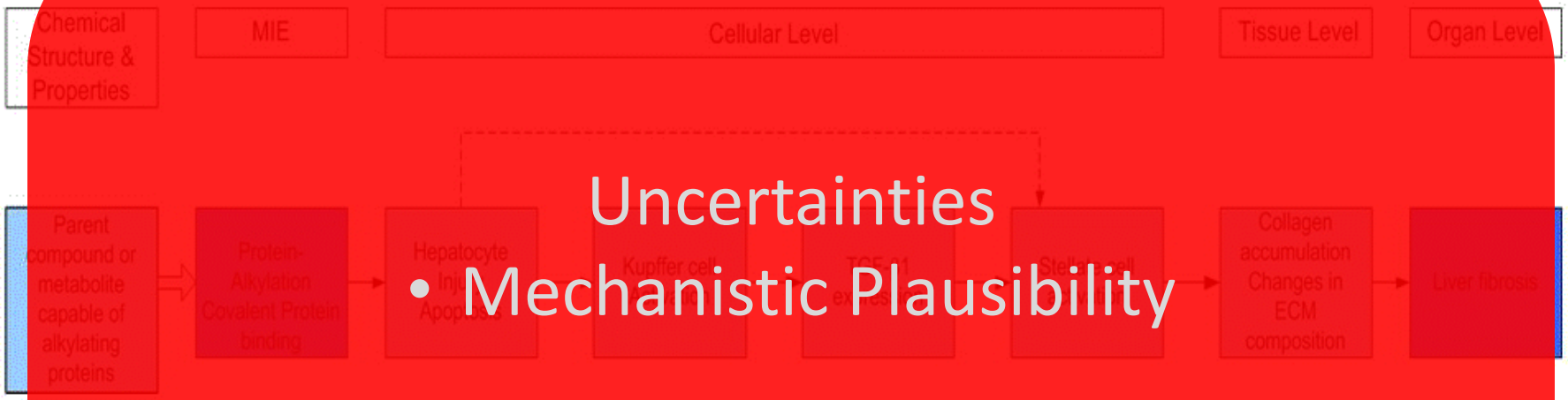
AOP for Liver Fibrosis (from Landesmann et al 2012)



- MIE is covalent binding to thiols
- Following metabolic activation in the liver, category members may bind to thiols such as GSH
- Once GSH is dissipated, react with other cellular thiols, especially in mitochondrial proteins leading to apoptosis or necrosis of hepatocytes

AOP for Liver Fibrosis



Uncertainties

- Mechanistic Plausibility

How can AOPs reduce these uncertainties?

Do NAMs relate to AOPs?

- Constructed in part from [illegible] protein.
- MIE is covalent binding to thiols.
- The non-reactive parent alcohol is converted enzymatically in the liver to the corresponding α, β-unsaturated aldehyde or α, β-unsaturated ketone.
- May bind to thiols such as GSH
- Once GSH is dissipatedreact with other cellular thiols, especially in mitochondrial proteins leading to apoptosis or necrosis of hepatocytes
- Extracted from Landesmann et al. (2012)

In Vivo Data

- 2-propen-1-ol: 90-day oral repeated-dose
  - **Rat NOAEL: 13.2 (f) and 11.6 (m) mg/kg bw/d**. Relating to increases in relative kidney (both sexes) and liver weights (males)
  - **Rat NOAEL: 4.8 (m) and 6.2 (f) mg/kg bw/d.** Relating to relative kidney weight and decrease in water intake and body weight
  - **Rats and Mice: NOAEL 6 (m) and 25 (f) mg/kg bw/d for rats.** Relating to toxicity in the liver
- 3-methyl-2-buten-1-ol: 90-day oral repeated-dose
  - **Rat NOAEL: 65.4 mg/kg bw/day (m) and 82.1 mg/kg bw/day (f).** Relating to decreased food and water consumption (m) and reduced water consumption (f) rats
  - Two further sub-acute oral studies in rats reported no other effects

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NTP study used for read-across

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  - **Rat NOAELs: 4.3 (m) and 5.2 (f) mg/kg bw/d**. Relating to relative kidney weight and decrease in water intake and body weight
  - Rats and Mice: NOAEL 6 (m) and 25 (f) mg/kg bw/day for rats. Relating to [illegible]
- 3-methyl-2-buten-1-ol: 90-day oral repeated-dose
  - **Rat NOAEL: [illegible] mg/kg bw/day (f).** Relating to increased food and water consumption (m) and reduced water consumption (f) rats
  - Two further sub-acute oral studies in rats reported no other effects

Good concordance

BUT

- Few data!
- Correct interpretation?

Existing New Approach Methodology Data 1: *In Chemico* Reactivity with Glutathione

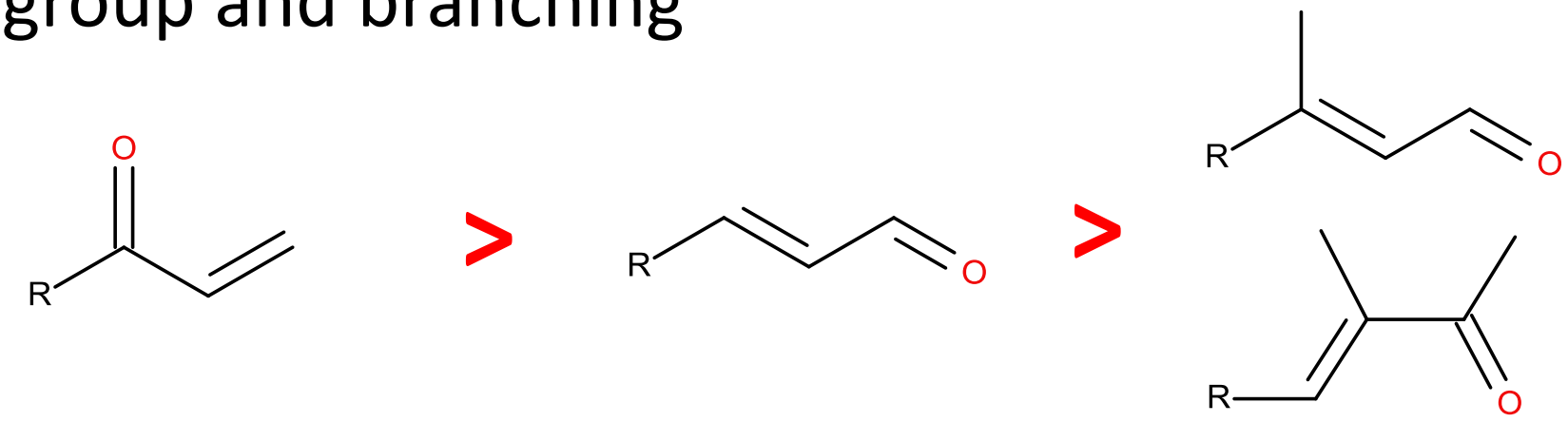
Unsaturated Alcohol + GSH → Unsaturated Alcohol–GSH

Depletion after 120 mins

k: Rate constant

Existing New Approach Methodology Data 1: *In Chemico* Reactivity with Glutathione

- Metabolites much more reactive compared to parent compound
- Reactivity of metabolites relates to functional group and branching



- GSH data confirm reactivity of metabolites and relative potency

Existing New Approach Methodology Data 2: Isolated Perfused Rat Liver

- GPT, LDH, GLDH measured after 90 mins at a single concentration
- Support concepts of
  - Metabolic activation via ADH
  - Reactivity of unsaturated alcohols
  - Greater reactivity of triple bonds
  - Lack of reactivity of saturated alcohols and tertiary alcohols

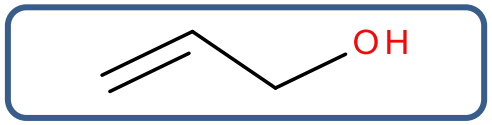
Existing New Approach Methodology Data 2: Isolated Perfused Rat Liver

- GPT, LDH, GlDH measured after 90 mins at a single concentration. Supports concepts of:
  - Reactivity of unsaturated alcohols
  - Formation of reactive metabolites
  - Lack of reactivity of saturated alcohols and tertiary alcohols

Existing NAM Data

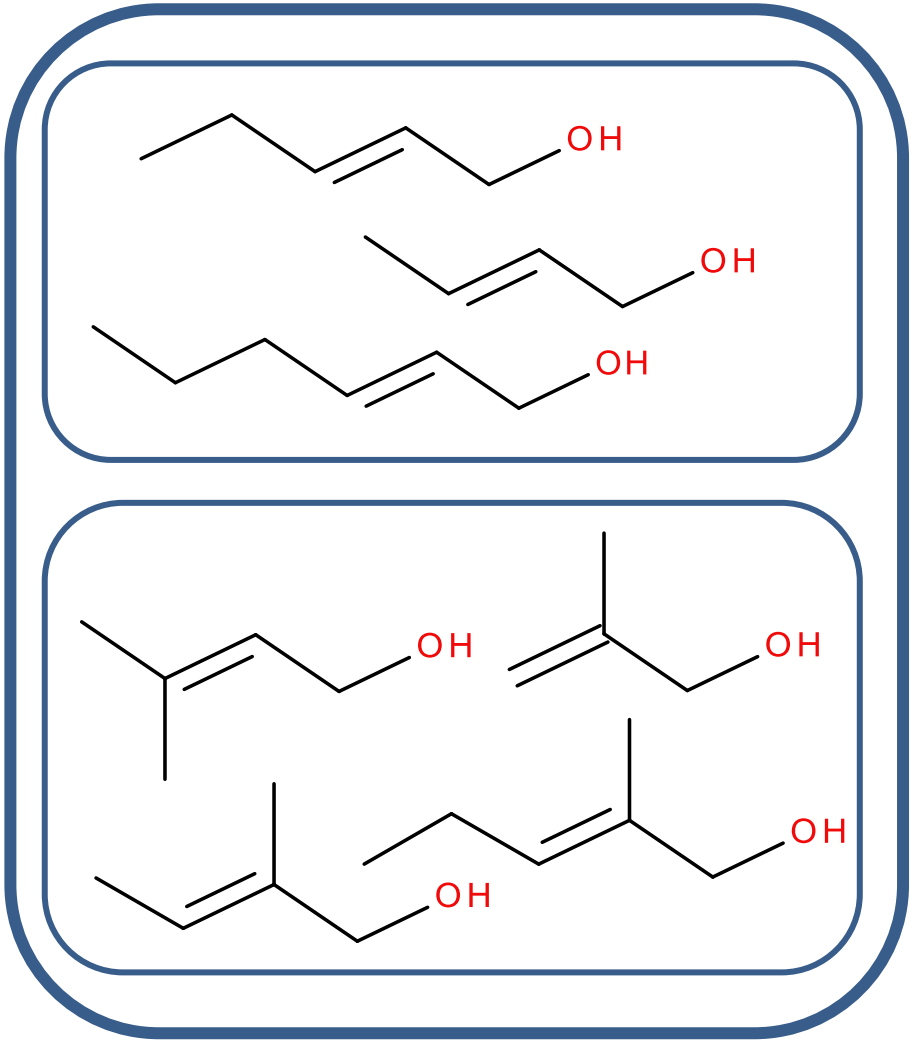
- Confirm (relative reactivity)
- How to address relevance, quality, lack of dose-response etc?

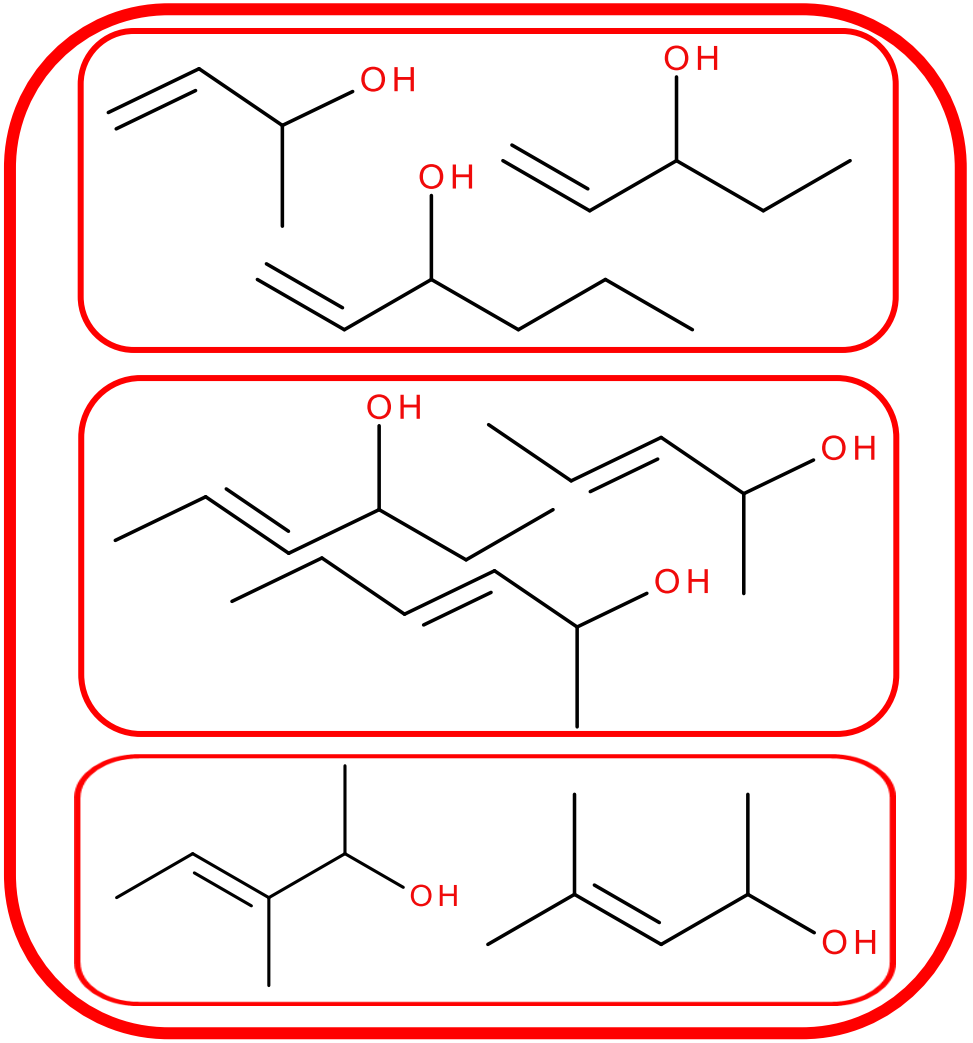
Read-Across for 2-propen-1-ol



90-day oral repeated dose NOAEL:

- 6 mg/kg (bw)/d male rat
- 25 mg/kg bw/d females rat





Uncertainties in Read-Across

Factor	Uncertainty
The problem and premise of the read-across	Low to medium
***In vivo* data read across**	
Number of analogues in the source set	Medium
Quality of the *in vivo* apical endpoint data read across	Medium
Severity of the apical *in vivo* hazard	Low
Evidence to the biological argument for RA	
Robustness of analogue data set	Low
Concordance with regard to the intermediate and apical effects and potency data	Medium
Weight of Evidence	Low to medium

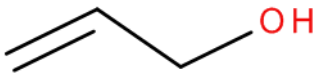
Uncertainties in Read-Across

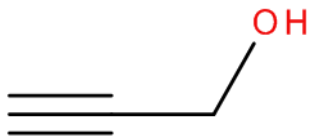
Factor	Uncertainty
The problem and premise of the read-across	Low to medium
In vivo data read across	
Number of analogues considered	Medium
Quality of the *in vivo* data for the analogues considered in the read-across	Medium
Severity of the observed *in vivo* hazard	Low
Evidence for the mechanism of action	
Robustness of analogue data set	Low
Concordance with regard to the intermediate and apical effects and potency data	Medium
Weight of Evidence	Low to medium

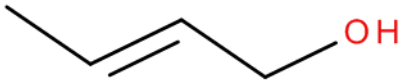
Uncertainties defined in more detail with regard to RAAF

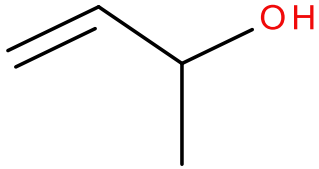
Do NAM reduce uncertainty?

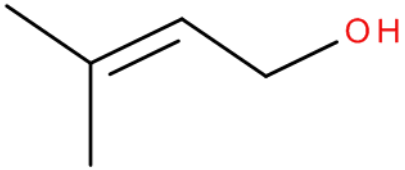
New Approach Methodology Data: Compounds Tested

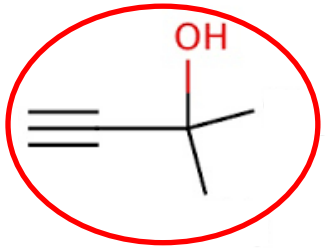






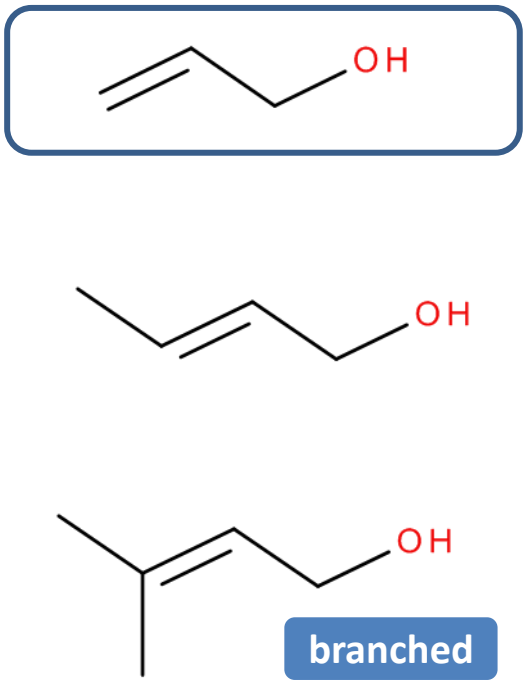




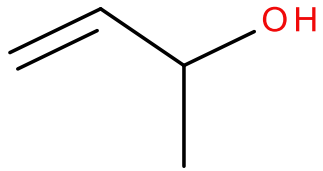


*Not in the original category

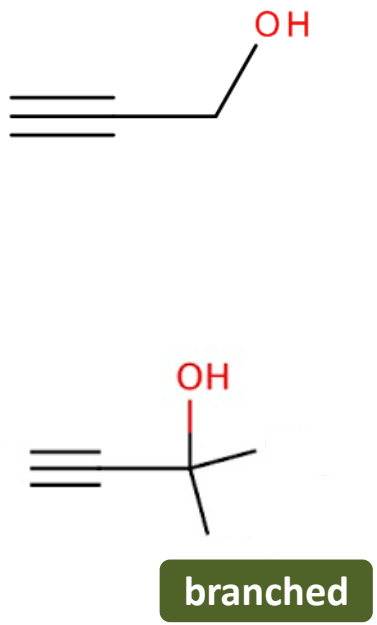
New Approach Methodology Data: Compounds Tested



1º Olefinic Alcohols



2º Olefinc Alcohol



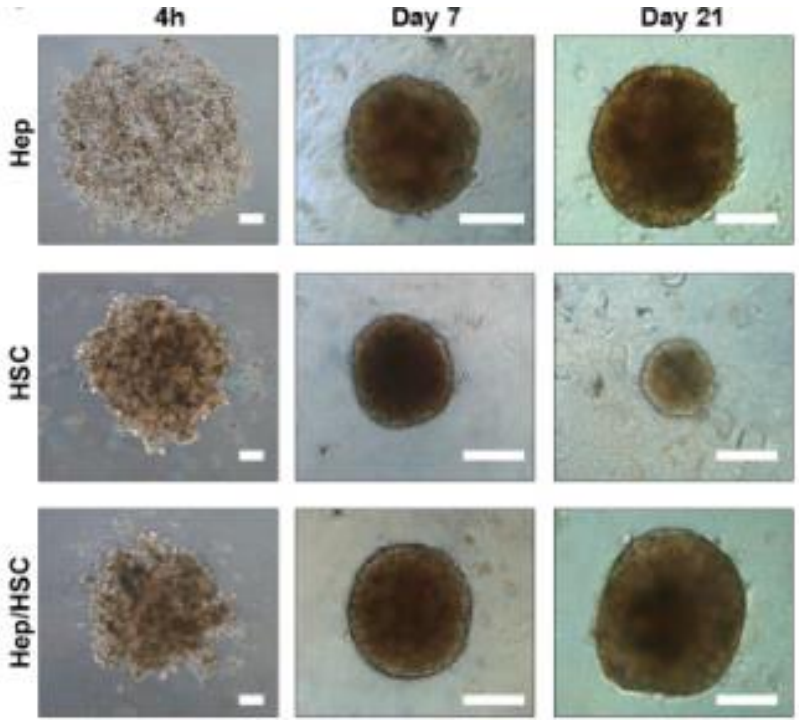
β-Acetylenic Alcohols

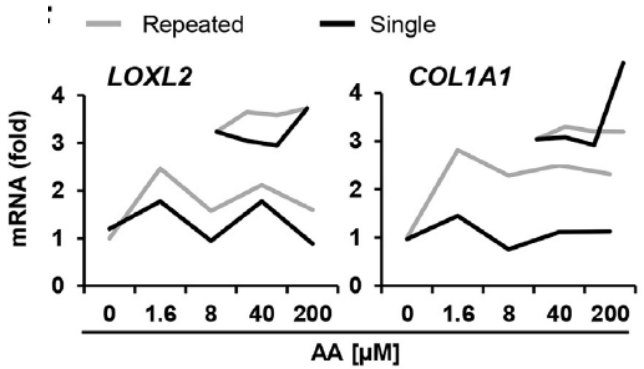
New Approach Methodology Data 1

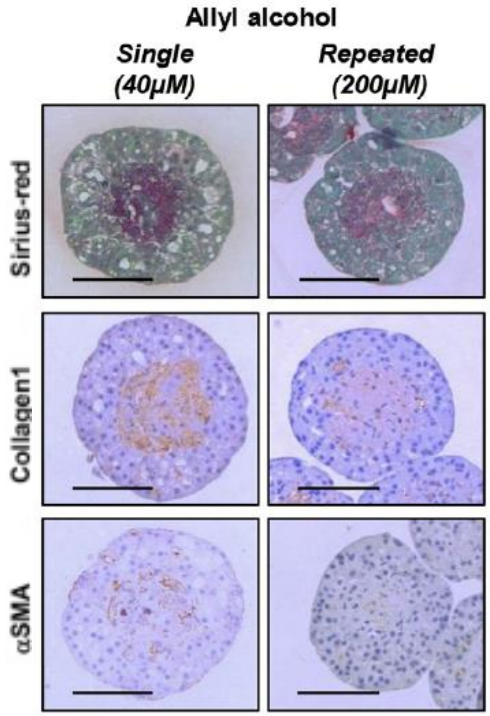
- Application of a novel human hepatic organoid model to identify fibrosis

- I'm stupid - don't ask me for details! Please refer to
  - **Leite SB et al (2016) *Biomaterials* 78: 1-10**

New Approach Methodology Data 1: Human Hepatic Organoid Model

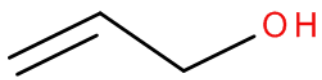


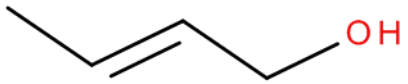


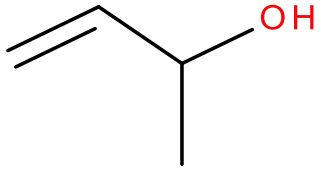


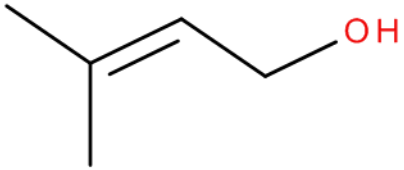
Stolen from: **Leite SB et al (2016) *Biomaterials* 78: 1-10**

New Approach Methodology Data 1: Human Hepatic Organoid Model

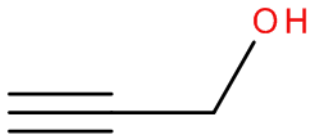


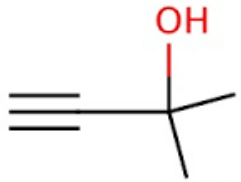






- Induce biomarkers associated with fibrosis
- Confirms fibrotic nature via ADH metabolism





- No Induction

Refer to: **Leite SB et al (2016) *Biomaterials* 78: 1-10** The new Harry Potter!

New Approach Methodology Data 2

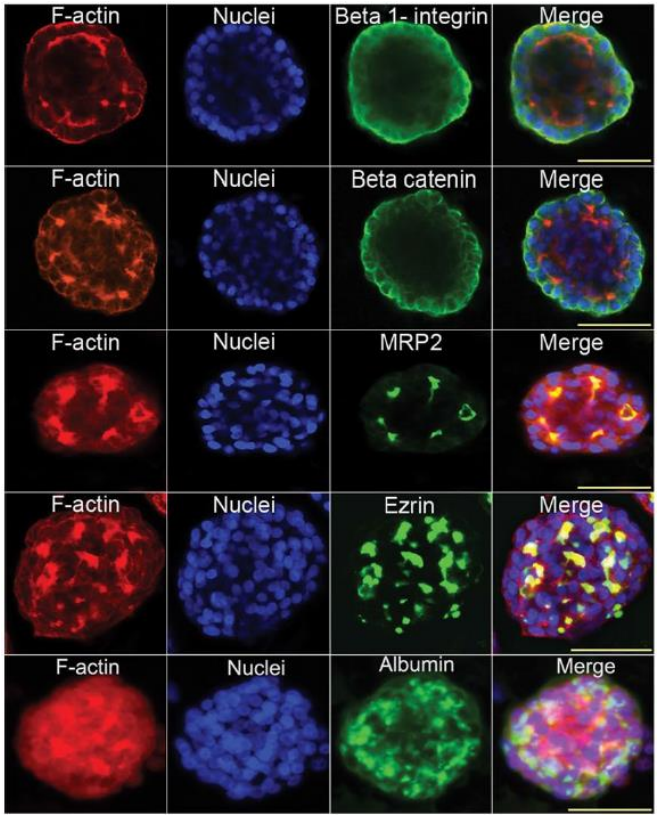
- Stress responses from 2D / 3D cultures of HepG2 following oxidative stress and DNA damage

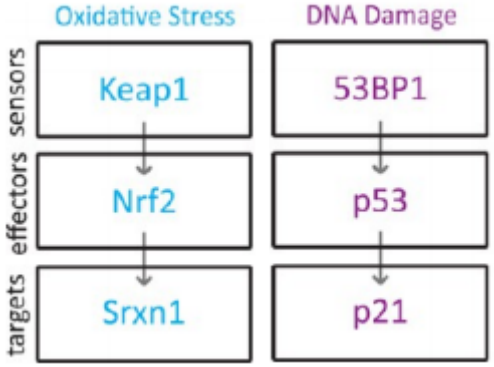
- Yet again - don't ask me for details!

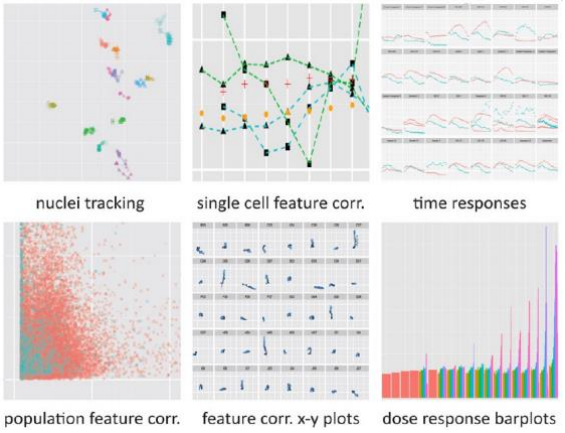
  Please refer to:

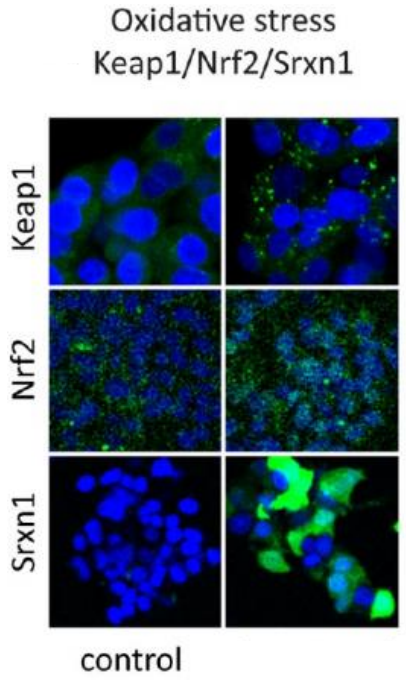
  - **Ramaiahgari SC et al (2014) *Arch Toxicol* 88: 1083-1095**
  - **Wink S et al (2014) *Chem Res Toxicol* 27: 338-355**

New Approach Methodology Data 2: Stress Response Activation in HepG2



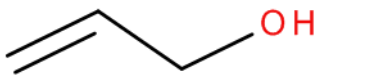


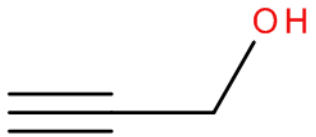


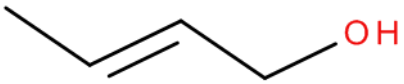


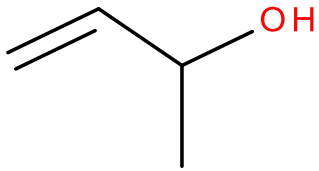
Also appearing in: **Ramaiahgari SC et al (2014) *Arch Toxicol* 88: 1083-1095**
Wink S et al (2014) *Chem Res Toxicol* 27: 338-355

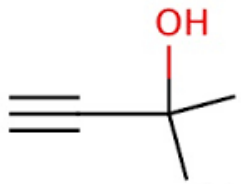
New Approach Methodology Data 2: Stress Response Activation in HepG2

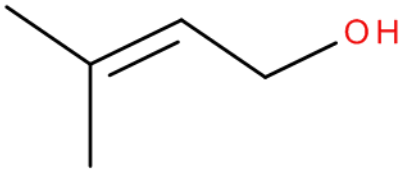












- Activate an oxidative stress response, but not DNA damage
- SRXN1-GFP activation strongest at 24 hr, possibly due to adaptation towards oxidative stress
- Consistent with ADH metabolic activation

Great to read on the beach!: **Ramaiahgari SC et al (2014) *Arch Toxicol* 88: 1083-1095**
Wink S et al (2014) *Chem Res Toxicol* 27: 338-355

New Approach Methodology Data:
Reduction of Uncertainty Related to Toxicodynamics

Does targeted testing with NAM have the potential to reduce uncertainty?

Uncertainties defined in more detail with regard to RAAF



When is Read-Across Appropriate?







Conclusions

- Complex category - many uncertainties
- The *ex vivo*, *in vitro* and *in chemico* data support the premise that 2-propen-1-ol can be read across to other primary and secondary β-alkenols
- There is less uncertainty associated with filling the data gap for the straight-chain 1-alken-3-ols and 2-alken-1-ols than for the branched-chained analogues
- NAM data reduce uncertainty for toxicodynamics