Read-Across for 90-Day Rat Oral Repeated-Dose Toxicity for Selected β-Olefinic Alcohols: A Case Study

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This read-across case study for the repeat-dose toxicity of short chain primary and secondary β-olefinic alcohols represents a scenario where metabolism is fundamental to toxicity. The pivotal issues in the applying read-across to this proposed category are whether:

i. all the β-olefinic alcohols considered are transformed to metabolites having the same mechanism of electrophilic reactivity;
ii. the metabolic pathway is the same for all compounds in the category;
iii. the rates of transformation are sufficient so that the reactive metabolites are the definitive toxicant for the endpoint being read across;
iv. the metabolites have similar in reactive potency.

The applicability domain of the category was limited to small (i.e., carbon (C) atom chain lengths from C3 to C6) primary and secondary β-olefinic alcohols. The category considered in this case study was confined to five subclasses of β-olefinic alcohols. Mechanistically, these β-unsaturated alcohols are considered to be readily metabolised by alcohol dehydrogenase (ADH) to polarised α,β-unsaturated aldehydes and ketones. These metabolites are able to react with thiol groups in proteins via the Michael addition mechanism resulting in cellular apoptosis and/or necrosis. The main route of exposure for β-olefinic alcohols is oral with direct absorption from the upper gastrointestinal tract. They are distributed unbound in the blood and are subsequently readily enzymatically oxidised, especially in the liver, to reactive metabolites. There are 90 day repeated-dose toxicity test results for only 2-propen-1-ol and 3-methyl-2-buten-1-ol.

Compounds representing each of five sub-structural groups were tested in an *ex vivo* model; the results from a 2-hr rat isolated, perfused liver assay, are consistent with metabolic activation to soft electrophiles. *In chemico* reactivity data with glutathione also support this chemical category. In order to support category membership, a human cell-based hepatic organoid *in vitro* model was used to assess fibrosis of 4 β-unsaturated alcohols, as well as 2 β-acetylenic alcohols (the data measured within SEURAT-1). The *in vivo*, *ex vivo*, *in vitro* and *in chemico* data support the read-across premise. Specifically, all the category members are highly likely to be

i. transformed to metabolites having the same mechanism of electrophilic reactivity (i.e., Michael acceptors;
ii. metabolised via the same pathway (i.e., ADH-mediated);
iii. to have rates of transformation sufficient so the reactive metabolites are the definitive toxicant for repeated-dose.

However, the category members have metabolites with different reactive potencies (i.e., GSH RC_{50} values). In order to reduce the uncertainty associated with reactivity, consideration was given to sub-categorising the category into straight-chained and branched derivatives.