Case study from SEURAT-1: β-Unsaturated alcohols – indirect acting toxicant category supported by SEURAT-1 data

SEURAT-1 β-OLEFINIC ALCOHOLS READ-ACROSS CASE STUDY CONSIDERED IN CONTEXT OF THE ECHA RAAF

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Case Study: **Read-across of 90-Day Oral Repeated-Dose Toxicity for β-Olefinic Alcohols: A Case Study of Compounds with Similar Metabolism**
KR Przybylak, TW Schultz et al

β-Olefinic Alcohols Case Study Read-Across Hypothesis:

Read-across is proposed to fill data gaps for 90-day oral repeated dose toxicity of selected (C3-C6) primary and secondary β-olefinic alcohols (βOAs). The read-across hypothesis is that as a result of their chemical structural similarity, the toxicological properties of the category members are likely to be similar. More specifically that biotransformation of these β-unsaturated alcohols by alcohol dehydrogenase results in formation of reactive unsaturated aldehyde or ketone metabolites that have electrophilic reactivity and are indirect-acting toxicants with the same mode of action.

Quantitative variations in effects (potency) observed across the category follow a trend related to the chemical reactivity via Michael addition of the metabolites; a worst-case approach is taken based on the proposed most reactive category member. The read-across hypothesis is that category member (straight-chained) 2-propen-1-ol is biotransformed to the most reactive metabolite of all category members; therefore the 90-day oral repeated dose toxicity study for 2-propen-1-ol is proposed for use in read-across to fill the data gaps for all other category members which lack a 90-day oral repeated dose toxicity study.

Read-Across Scenario according to the ECHA RAAF:

This read-across is consistent with ECHA Read-Across Assessment Framework (RAAF) Scenario # 4 – i.e. category approach for which the hypothesis is based on different compounds (in this case formed by biotransformation via a common pathway from different parent compounds) which have the same type of effect(s), and where differences in strength of the effect(s) exist that may form a regular pattern. In this scenario, the predicted property is either based on the established regular pattern or a worst-case approach.

For the βOA Case Study, the prediction (and read-across) is based on a worst-case approach. Taken together the available *in vivo* and *in vitro* data support identification of the critical properties of category members that result in toxicity and from that support identification of 2-propen-1-ol as the worst-case category member and source for the read-across for other category members lacking a 90-day oral repeated dose toxicity study.

Evaluation of the βOA Case Study according to the ECHA RAAF:

All relevant read-across 'Assessment Elements' (i.e. crucial scientific aspects of the read-across justification) for RAAF Scenario #4 are evaluated and an 'Assessment Option' (AO), i.e. score based on strength of the information/evidence provided, is proposed for each Element. This is done first based on consideration of the traditional *in vivo* study data and then including consideration of New Approach Methodology (NAM) data, defined as including any *in silico*, *in chemico* or *in vitro* technique supporting the substance evaluation, in order to elucidate the utility of the NAM data to strengthen the read-across.

The table below represents consideration of the βOA case study in the context of the ECHA RAAF.

Contributions of New Approach Methodology (NAM) data and how the additional information improves the AOs are highlighted in orange;
in particular, NAM data from the SEURAT-1 initiative in dark orange.

EVALUATION OF THE βOA CASE STUDY ACCORDING TO THE ECHA RAAF

RAAF SCENARIO #4		βOA Case Study		RAAF Assessment Option	
AE#	**Assessment Element /** Details	**Page# Lines**	**Relevant Text and Tables in Case Study Report**	**AO#**	**Rationale / Comments**
C.1	**Identify/characterise substances which are members of the category, including impurity profile**			N/A	No impurity profiles for category members was available. To address the RAAF: Provide impurity profile of category members. However, it is appreciated that this is a case study "on paper" and no production information is available; this would be easily done for a real-case registration.
		p.9 248-251	β-unsaturated aliphatic alcohols with carbon chain lengths from C3 to C6. Specifically, primary (external hydroxyl group) and secondary (internal hydroxyl group) with a β-positioned vinylic moiety.		
		p.9-10 263-288; Annex I, Table 1	Lists of category members including structures and CAS-numbers: Table 1: Potential category analogues for β-olefinic alcohols. Annex I Table 1: Substance identification and structure		
		p.10 293-296	The purity/impurity profile for the analogues listed in 2.4 is unknown. The most likely impurities are other isomers (e.g. cis vs. trans conformations). *NOTE: The substances used for the in vitro assays within SEURAT-1 were obtained from Sigma-Aldrich with listed purities of 96-99% (no impurities specified in the catalogue).*		
C.2	**Describe the structural similarity and allowable differences for the category**			4	Detailed information on structural similarity and allowable differences is available in the case study.
			β-olefinic aliphatic alcohols of C3-C6, primary and secondary, straight-chained or branched		
		p.10 302-303	All category members 1) belong to a common chemical class, β-unsaturated alcohols, 2) the subclass β-olefinic alcohols.		
		p.11 319-321	All the β-olefinic alcohols included in the category have common constituents in the form of: 1) a single polar substituent, -OH, 2) a β-positioned olefin (C=C) moiety. Other structural fragments are limited to -H, -CH3 and -CH2- groups.		
		Annex I, Tables 1 and 3	See also Annex I Tables 1 and 3.		

			Allowable differences: Difference in chain length: number of C-atoms from C3 to C6.		To increase AO score: More description of the structural variations of e.g. 2-propen-1-ol, 3-methyl-2-buten-1-ol and 4-Methyl-3-penten-2-ol could be added to make clear why the differences do not impact overall their inclusion in this category.
		p.19 594-595	The narrow range of carbon atoms was chosen for the applicability domain to limit the impact of bioavailability.		
		p.10 303-306	The category members possess one of two molecular scaffoldings, primary with an external hydroxyl or secondary with an internal hydroxyl configuration. Structural similarity is complicated by the presence or absence of alkyl substituents (i.e., methyl groups) on the allylic moiety. The β-olefinic alcohol category includes five sub-groups (external or internal hydroxyl group, external or internal vinylic moiety). The sub-groups can be clustered into two sub-categories – straight-chained and branched β-olefinic alcohols: 1) primary alcohol, straight-chain, external hydroxyl, internal vinyl 2) secondary alcohol, straight-chain, internal hydroxyl, external (terminal) vinyl 3) secondary alcohol, straight-chain, internal hydroxyl, internal vinyl 4) primary alcohol, branched, external hydroxyl, internal vinyl 5) secondary alcohol, branched, internal hydroxyl, internal vinyl		
		p.10 306-307	In addition to these subcategories, the potential source substance, 2-propen-1-ol, is a unique β-olefinic alcohol, one with both a terminal hydroxyl and terminal vinyl group.		
		p.10 308-309	3-methyl-2-buten-1-ol, is dissimilar as it has an alkyl substituent on the olefinic carbon that can inhibit the protein binding site of the vinyl group; this is also the case for 4-Methyl-3-penten-2-ol.		
			These two analogues (i.e. their metabolites) show different behaviour than other category members in the prediction of protein and DNA binding (Annex I Table 6B): no alerts for Michael addition, which is the discussed mechanism of toxicity. This fact supports a dissimilarity of the substances. The *in chemico* results (Table 3, p. 17) however show reactivity similar to the other (sub-category) members. Inclusion in the overall category is therefore confirmed.		
			Non allowable differences:		
		p.4 114-116 p.9 251-252	Tertiary alcohols are excluded since not compatible with the common mechanism linked to toxicity, metabolisation to aldehyde/ketone not possible, which drives the toxicity (see C.3): Only primary and secondary β -olefinic alcohols can undergo first step oxidation to α, β-unsaturated aldehydes or α, β-unsaturated ketones, respectively, because a key structural feature is the presence of a free H-atom on the hydroxyl-containing C-atom.		

C.3	**Explain the link between the structural similarities/differences and the proposed (regular) pattern; does it apply to all category members**			4 5	The *ex vivo* perfused liver data support the link between structures and effect for category members by confirming metabolisation of primary/secondary and not tertiary unsaturated alcohols to potent metabolites.
		p.6-7 179-182	Oxidative metabolism of primary and secondary β-olefinic alcohols results in the corresponding α, β-unsaturated aldehyde or α, β-unsaturated ketone. These α,β-unsaturated aldehydes or α,β-unsaturated ketones are the definitive electrophilic toxicants. – This applies to all members in the category.		
		p.11 324-325	The oxidation of primary alkanols and primary olefinic alcohols to the corresponding aldehydes is catalysed by NAD+/NADH-dependent alcohol dehydrogenase.		
		p.12 367-370	From a structural standpoint, only primary and secondary (not tertiary) β-olefinic alcohols are able to be activated by ADH to form polarised α,β-unsaturated electrophiles. The availability of H-atoms on the C-atom with hydroxyl group is crucial to the metabolic activations and subsequent expression of relative toxic potency.		
		p.4-5 117-118	The metabolites formed have the capability to be reactive with biological macromolecules as Michael acceptors.		
		p.4 108-114	Olefinic β-unsaturated alcohols vary in molecular structure (see C.2). The overall structure of the parent alcohol determines the metabolic pathway and the specific metabolite formed. The metabolites exhibit different levels of reactivity and toxicity, depending on their structure.		
		p.19 590-592	The structural differences within the β-olefinic alcohols lead to 1) different likely metabolite (e.g., aldehyde or ketone), 2) different *ex vitro* metabolism (i.e., free GSH levels) and 3) different rates of *in chemico* reactivity (i.e., GSH reactivity).		
		p.16 479-484	The results of the Strubelt study (1999) support the premise that 1-alken-3-ols, 2-alken-1-ols, and 2-methyl-2-alken-1-ols are metabolised and give rise to a metabolite of similar potency to 2-propen-1-ol. The Strubelt data also support the structural selectivity as tertiary β-unsaturated alcohols, as well as alkanols, do not reduce GSH i.e., are not metabolised to reactive electrophiles.		

C.4	**Demonstrate/discuss consistency of effects in data matrix and any clustering in strengths of effects**			3	To more directly address the RAAF: increase details in *in vivo* data matrix, in particular separate NOAEL by effects, species, male/female etc. More directly state the choice of the NOAEL value to be read across for 2-propen-1-ol as worst case.
	Documentation includes discussion of consistency of data for predicted property, and any inconsistencies are explained (strength of effects vary)	Annex I, Table 8 p.7-8 190-215 p.8-9 240-246 p.2 40-44 p.8 220-221 p.2 44-46	There are 90 day repeated dose toxicity data only for 2-propen-1-ol and 3-methyl 2-buten-1-ol as shown in Annex I Table 8. Several 90-day oral repeated-dose toxicity evaluations of 2-propen-1-ol have been conducted. NOTE: Consistent targets of kidney and/or liver in all studies, along with local gastrointestinal irritation observed in gavage study. While protocols vary, three studies have experimentally evaluated 2-propen-1-ol and one study evaluated 3-methyl-2-buten-1-ol in 90-day, oral repeated-dose testing schemes. Repeated-dose toxicity data on 2-propen-1-ol indicate liver and kidney are the target organs. For the 3-methyl-2-buten-1-ol, only the reduction in food and water consumption was observed. The 90-day NOAEL values for oral administration are between 3 and 15 mg/kg bw/d for 2-propen-1-ol and 60 -85 mg/kg bw/d for 3-methyl-2-buten-1-ol. These ranges of NOAEL values are 10-100 times smaller than those reported for saturated derivatives. The 90-day oral repeated dose toxicity No Observed Adverse Effect Levels (NOAELs) in rats, chosen as worst case for 2-propen-1-ol, were 6 mg/kg body weight (bw)/d in males based on increase in relative weight of liver and 25 mg/kg bw/d in females based on bile duct hyperplasia and periportal hepatocyte hypertrophy in the liver. To our knowledge, only one 90-day repeated dose toxicity evaluation of 3-methyl-2-buten-1-ol has been conducted. The 90-day oral repeated dose NOAELs for 3-methyl 2-buten-1-ol in rat were reported based on the decreased food and water consumption: 65.4 mg/kg bw/day in males and 82.1 mg/kg bw/day in females. NOTE: These *in vivo* data demonstrate a potency difference between these structures (i.e. straight-chained terminal allyl alcohol and the branched internal allyl structure). This is supported by *in vitro* data, see below.	4	The NAM data increases the WOE and demonstration of consistency. In particular, *in chemico* GSH assay results show clustering of potency corresponding to structural subcategories, supported by *in silico* predictions of reaction potency. Testing a few more substances would allow to confirm the reactivity trend within subcategories, i.e. prevalent influence of branching, branching with external vinyl group (e.g. 2-methyl-2-propen-1-ol/ 2-methyl-acrolein) etc.
	Documentation includes discussion of occurrence of any other relevant effects (than predicted property)	p.7 187-189 p.8 234-239	In general, toxicological data on 2-propen-1-ol demonstrate significant toxicity. The oral LD50 for rat is 37 mg/kg for 2-propen-1-ol, while the rat oral LD50 for the saturated 1-propanol is 1870 mg/kg. Two more sub-acute oral studies in rats do not show any other effects of 3-methyl-2-buten-1-ol. Specifically, a 14-days drinking water study with rats (3/sex/dose) exposed to 250, 500, 750 and 1500 mg/kg bw/d reported acute toxic effects at 1500 mg/kg bw/d; reduced food and water intake was observed at 250 mg/kg bw/d. So there is good concordance with 90-day test results. In a 14-day gavage test with rats exposed to 250, 500 and 750 mg/kg bw/d no treatment related effects were observed.		

	Order within the category is described	p.17-18 539-547 p.18 548-551 p.7-9 190-246 p. 22-23 667-687 p.26 756-757	It has been reported that for α,β unsaturated carbonyl compounds, such as those derived from hepatic oxidative metabolism of β-olefinic alcohols: 1) terminal vinyl-substituted derivatives (H2C=C-) were more reactive (with GSH) than the internal vinylene-substituted ones (-CH=CH-) (e.g. 2-propen-1-ol, 1-buten-3-ol, 1-penten-3-ol, 1-hexen-3-ol vs 3-penten-2-ol, 4-hexen-3-ol in Table 3, p.17) 2) methyl-substitution on the vinyl carbon atoms diminishes reactivity (see all branched compounds in Table 3, p.17) 3) methyl-substitution on the carbon atom farthest from the carbonyl group (C(=O)C=C(C) causes a larger reduction than methyl-substitution on the carbon atom nearest to the carbonyl group (C(=O)C(C)=C), and 4) derivatives with carbon-carbon double bond on the end of the molecule (i.e., vinyl ketones) were more reactive than ones with the carbon-oxygen double bond at the end of the molecule (i.e., aldehydes). The results from the thiol reactivity experiments suggest that the ability of α,β-unsaturated carbonyl compounds other than acrolein (and thus, β-olefinic alcohol other than 2-propen-1-ol) to elicit kidney and liver targeted toxicity may be reduced, especially for branched alcohols with alkyl substitutions on the vinyl carbon atoms. This is in line with the *in vivo* study results regarding different strength of effects (NOAELs) for branched vs unbranched substance (see above). The fact that methyl-substituted β-olefinic alcohols are less reactive is also supported by results from the *in vitro* studies in hepatic organoids (3D co-culture), where the up-regulation of HSC activation markers in the hepatic organoids (3D co-culture) is weaker for 3-methyl-2-buten-1-ol.		
	Any clustering of strength of effects across structural features of category (or subcategories) is characterised	Table 3, p.17; Annex I, Table 6B	*In chemico* GSH assay results show clustering of potency corresponding to structural subcategories, supported by *in silico* predictions of the potential reaction mechanism and reaction potency, as summarised in the following table. The metabolites of 2-propen-1-ol and the straight-chain β-olefinic alcohols, i.e. metabolites with an external vinyl group, are the most reactive. The metabolites of the straight-chain β-olefinic alcohols are more reactive than the branched alcohols. The reactivity clustering by sub-categories is shown in the following table. ([1] OECD QSAR Toolbox; [2] MA: Michael addition, SBF: Schiff base formers; [3] in mmol/l; [4] no structural alerts coded for di-beta substituted chemicals, *in chemico* data show reactivity however)		

	Subcategory		Compound	Metabolite	*In chemico* reactivity GSH RC_{50} [3]	Protein binding potency [1, 2]	*In silico* protein binding [1]
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: **alkyl substituent on olefinic C that can inhibit protein binding site of vinyl group**	3-Methyl-2-butenal	13	Moderately reactive	SBF **no MA** [4]
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: **alkyl substituent on olefinic C that can inhibit protein binding site of vinyl group**	4-Methyl-3-penten-2-one	26	Highly reactive	**No alert** [4]

		p.16 492-493	The basic structure-activity relationships for chemical reactivity via Michael additions to thiols are pivotal for understanding hepatotoxic potency both *in vitro* and *in vivo*. NOTE: Availability of test results for 2-methyl-2-propen-1-ol/ 2-methyl-acrolein would be useful to confirm the trend regarding reactivity of external vinyl group reactivity vs substituent on olefinic C.		
C.5	**Demonstrate reliability and adequacy of the source data to meet the info requirements**			3	Insufficient detail on source study design and test material. The study design should be evaluated in more detail regarding matching default REACH requirements, e.g. it should be stated whether robust study summaries are available. To more directly address the RAAF: Clearly and directly cite read-across source study/studies and include detail on study design relative to published test guidelines and test material used.
	Read-across source study is of adequate and reliable design	p.8 222 Annex II Table 2, p.36	The 90-day oral repeated-dose toxicity evaluation of 3-methyl-2-buten-1-ol has been conducted following OECD test guideline 408. The following assessment of the quality of the data to be read across is given in Annex II Table 2, assigning a medium uncertainty: High quality empirical data for the stated regulatory endpoint exists from multiple studies for 2-propen-1-ol and a single study 3-methyl-2-buten-1-ol. These data are inconsistent in regards to qualitative and quantitative descriptions of effects. NOTE: It is not clear whether the values to be read-across for male/female rats are from two different studies. An explicit statement could be added for clarification.		
	Test material used represents source in terms of purity/impurities		*No information in case study text / not applicable.* NOTE: Test material for SEURAT-1 *in vitro* tests: from Sigma Aldrich, 96-99% purity.		
	Results of read-across source study are sufficient for C&L and/or risk assessment purposes	p.28-29 824-829	The evaluation of overall uncertainty concludes that the oral 90-day repeated-dose NOAEL of 6 and 25 mg/kg bw/d, in male and female rats, respectively, reported for 2-propen-1-ol can be read across to untested straight-chained β-olefinic alcohols (i.e., 1-alken-3-ols and 2-alken-1-ols) with acceptable uncertainty for all regulatory decisions including risk assessment. Read-across from 2-propen-1-ol to untested methyl-substituted β-olefinic alcohols is a conservative prediction which may estimate lower than likely repeated-dose potencies.		

4.1	**Identify the compounds to which the test organism is exposed**			4	Mechanism of transformation to compounds to which organism is exposed is clearly presented and confirmed by NAM data. Some more (quantitative) information could be given to show the rate/speed of the metabolic transformation, amount of residual parent compounds or further metabolites.
	Substances to which the organism is exposed (for target and all sources) are identified as well as how they are formed, here: biotransformation products of source and target substances	p.6-7 179-182	Oxidative metabolism of primary and secondary β-olefinic alcohols results in the corresponding α, β-unsaturated aldehyde or α, β-unsaturated ketone. These α, β-unsaturated aldehydes or α,β-unsaturated ketones are the definitive electrophilic toxicants.		
		p.14 422-423	Upon reaching the liver, the non-reactive parent alcohol is converted enzymatically to the corresponding α,β-unsaturated aldehyde or α, β-unsaturated ketone.		
		p.11 325-327	The oxidation of primary alkanols and primary olefinic alcohols to the corresponding aldehydes is catalysed by NAD+/NADH-dependent alcohol dehydrogenase (ADH).		
		p.5 129-130	For example, the parent alcohol 2-propen-1-ol is relatively non-toxic, however its metabolite acrolein, an α,β-unsaturated aldehyde, is a Michael-type soft electrophile.		
	Supporting evidence, if possible qualitative or quantitative kinetics information, is provided	p.14-15 427-432	The short-term isolated perfused liver represents an *ex vivo* model which is close to the *in vivo* condition. The major advantages are that the three-dimensional architecture of the liver and the metabolic capabilities of the hepatocytes are preserved. Strubelt et al. (1999) studied acute toxicity and metabolism in a series of short-chain alcohols, i.e. the effects at a single concentration (65.1 mmol/l) in isolated rat livers perfused at 60 ml/hr for two hours were examined. The results for the compounds representing each of five sub-structural groups of the case study β-olefinic alcohols are consistent with metabolic activation to soft electrophiles. Specifically, all tested primary and secondary β-olefinic alcohols exhibit a dramatic reduction (90-99%) in glutathione (GSH) as compared to controls; whereas saturated alcohols elicited no change in GSH levels.		
		p.2 47-50			
		p.16 476-478			
		p.16 479-482	The major weakness of the Strubelt study is the lack of dose-response data. However, the results of the Strubelt study support the premise that, similarly as 2-propen-1-ol, the β-olefinic alcohols are metabolised and give rise to a reactive metabolite, and thus are very likely to cause similar repeated-dose toxicity. Alkanols do not reduce GSH (i.e., are not metabolised to reactive electrophiles) and, as shown *in vivo* studies, do not elicit the same repeated-dose effects.		
		p.16 483-490			
		p.13 393-395	In silico predictions support the premise that the metabolites are the toxicants: only the metabolites of β-olefinic alcohols and not the parent compounds triggered the OECD protein and DNA binding profilers within the OECD QSAR Toolbox v3.3.5.		
		p.12 341-347	In kinetic studies it was found that 2-buten-1-ol was readily oxidised by equine liver ADH, with electrospray-mass spectrometry confirming that 2-butanal was the main metabolite formed. It was also reported that in mouse hepatocytes, 2-buten-1-ol produced marked time-and concentration-dependent cell killing as well as pronounced		

			glutathione depletion. Most importantly, as also confirmed by other studies in the literature, both cytotoxicity and glutathione loss were eliminated with the addition of the ADH inhibitor 4-methylpyrazole, indicating the ADH-mediated pathway is responsible for producing these effects.		
		p.18 557-560	The role of ADH activity in metabolic activation and enhanced toxicity was confirmed in fish. Specifically, the ADH in the gill epithelial cells metabolises the appropriate alcohol to the corresponding aldehyde (or ketone), which are the reactive toxicants.		
		p. 18 564-568	This mechanism of formation of the metabolite toxicants from β-olefinic alcohols is also supported by data from acute toxicity studies with the fathead minnow (Pimephales promelas), showing that primary and secondary allylic alcohols exhibit potency in excess of that predicted by saturated alcohols and baseline narcosis QSAR models, but on the contrary, tertiary olefinic alcohols exhibit fish toxic potency consistent with baseline narcosis models.		
		p.23 687-692	Moreover, in the SEURAT-1 *in vitro* assay with hepatic organoids (3D co-culture) of human hepatocyte-like cells, the triple-bond containing β-acetylenic alcohols, one tertiary, do not exhibit 2-propen-1-ol-like up-regulation of hepatic stellate cells activation markers (see 4.2). This also supports the hypothesis, that tertiary alcohols are not metabolised to the reactive metabolites implicated in liver toxicity. 2-propyn-1-ol which is activated via CYP 2E1 activity is toxicokinetically dissimilar.		
		Annex II, Table 5	The *in silico* metabolisation prediction tools (Annex II, Table 5) also generally predict metabolisation for example by oxidation for all of the β-olefinic alcohols included in the category.		
			Available kinetic information on the metabolisation:		
		p.11 327-329	Comparison of the alcohol structure with the enzyme binding affinity of alcohol dehydrogenase indicates that increased binding (lower Km) occurs with increasing chain length (C3-C6) of the alcohols and the presence of unsaturation.		
		p.11 335-338	The maximum rates of oxidation were essentially constant, regardless of the alcohol structure, suggesting that alcohol-enzyme binding is not the rate-limiting step for oxidation. The activity of the enzyme appears to be dependent on the lipophilic character of the alcohol.		
		p.5 142-144 p.12 351-352 p.12-13 370-377	Primary alcohols have one alkyl-group; thus, two H-atoms are available for metabolism. Secondary alcohols have two alkyl-groups and one H-atom available for ADH attack. Tertiary alcohols are substituted with three alkyl-groups on the α-carbon. Since at least one H-atom must be freely available for cleavage by ADH, which is not the case for tertiary alcohols, the latter are not metabolised to Michael acceptor electrophilic derivatives by ADH. It also follows that primary β-olefinic alcohols are likely to be more readily converted to reactive metabolites than secondary ones.		

4.2	**Identify the common mechanism underlying the category hypothesis, qualitative aspects**			4	
	The common underlying mechanism that links the structures of the compounds to which the organism is exposed with the predicted effect (but with different strength of effect)		**Target organ:**		
		p.2 30-33	The β-olefinic alcohols taken up orally are directly absorbed from the upper gastrointestinal tract. They are distributed unbound in the blood and are subsequently enzymatically oxidised, especially in the liver, to form the reactive metabolites. With a view to the location of metabolisation, the liver can be considered as one of the main target organs.	5	The NAM data strengthen the evidence for the mechanism, in particular the hypothesis of the mode-of-action leading to fibrosis
		p.5 124-128	Overall, currently available data suggest that the kidney, liver and lung are potential targets for 2-propen-1-ol, following repeated oral or inhalation exposure. In oral repeated-dose toxicity testing, exposure to 2-propen-1-ol may lead to liver fibrosis.		
		p.5 145-149	*In vivo* oral exposure to 2-propen-1-ol leads to periportal necrosis and subsequent connective tissue development. Histopathological studies of 2-propen-1-ol exposed to repeatedly dosed rat livers showed signs of necrosis around the portal triad, with relatively little damage around the central vein. In addition, ductular proliferation, connective tissue accumulation and cirrhosis were evident.		
			Mechanism underlying the effect of the toxicants:		
		p.5 130-134 p.16-17 498-500	The polarised α,β-unsaturated aldehydes and ketones (the metabolites of the β-unsaturated alcohols) are Michael-type soft electrophiles preferentially covalently interact with thiol groups in proteins, in the form of Michael addition, leading to necrotic or apoptotic cell death. During the *in vivo* response to cell death, stellate cells in the liver are activated, for example by transforming growth factor beta (TGF-β) and connective tissue is formed.		
		p.5 135-144	Several studies in the literature, e.g. with radiolabelled ^{14}C 2-propen-1-ol and its deuterated derivative, point out the mechanism of covalent binding of acrolein to essential sulfhydryl groups in cellular macromolecules such as proteins in periportal hepatocytes, leading to cellular damage.		
		p.12 347-351	For example, in keeping with expectations that Michael addition adducts would feature prominently during protein modification, it was noted in a study that exposure to 2-buten-1-ol resulted in marked carbonylation of a range of cell proteins. Damage to a subset of small proteins (e.g., 29, 32, 33 kDa) is closely correlated with the severity of cell death.		
		p.14 409-410	The cellular damage and effects leading from the molecular initiating event of covalent protein binding to the adverse effect of liver fibrosis are described in the preliminary adverse outcome pathway (AOP) reported by Landesmann et al. (2012).		

		p.14 411-419	They noted a number of key intermediate events including: Hepatocyte injury and death, activation of Kupffer cells (liver macrophages), inflammation, oxidative stress, activation of TGF- β, activation of stellate cells (mesenchymal stem cells), collagen synthesis and accumulation, alteration in connective tissue extracellular matrix.		
		p.14 421-426	The molecular initiating event of this pathway is covalent binding to thiols. More specifically, upon reaching the liver, the non-reactive parent alcohol is converted enzymatically to the corresponding α, β-unsaturated aldehyde or α,β-unsaturated ketone. These reactive species, in turn, bind to thiols such as GSH. Once GSH is dissipated, the α,β-unsaturated substrates react with other cellular thiols, especially in mitochondrial proteins. This denaturing of proteins leads to apoptosis or necrosis of hepatocytes and subsequent events along the AOP.		
		p.4 117-119	While all of these oxidative metabolites have the capability to be reactive with biological macromolecules as Michael acceptors, they exhibit different levels of reactivity and toxicity (see C.4, 4.3).		
	Supporting qualitative evidence from *in vivo*, *in vitro* or *in silico* studies	p.20 612-615 p.2 50-59	High quality *in chemico* reactivity data – in the form of the concentration eliciting a 50% reduction in free GSH after 2 hours exposure of the α,β-unsaturated carbonyl compounds – exist for 14 of the 16 category members based on the proposed α,β-unsaturated metabolites of the β-olefinic alcohols, i.e. such as those derived from hepatic metabolism, and their reactivity with GSH. These 14 derivatives include more than one representative of four of the five structural sub-groups (the other group has only a single analogue, i.e. 2-propen-1-ol). All 14 analogues exhibit GSH reactivity, showing some potency differences (see 4.3).		
		p.13 393-397	In silico predictions with the OECD protein and DNA binding profilers within the OECD QSAR Toolbox v3.3.5 showed that all metabolites of β-olefinic alcohols are associated with Michael addition (MA) and/or Schiff base formation mechanisms. Two exceptions are noted (3-methyl-2-buten1-ol and 4-methyl-3-penten-2-ol) not being predicted to react via MA. Both have an alkyl substituent on the olefinic carbon that can inhibit the protein binding site of the vinyl group. This sterically hindrance of the MA was assumed for the coding of the structural alerts. However, in the *in chemico* assay both substances proved to be reactive and thus are part of the overall category.		
		p.18 552-563	In fish, this mode of toxic action involves metabolism of the parent alcohol to the corresponding α,β-unsaturated aldehyde or ketone via ADH (in the gill epithelial cells) and while the parent aliphatic alcohols elicit baseline narcosis toxic action, the metabolites are electrophilic toxicants, which react with cellular proteins. The end result is death of the gill epithelia cells, which results in the loss of the ability to extract oxygen causing subsequent hypoxia and fish mortality. This mechanism was described for model electrophiles by respiratory and cardiovascular responses in trout.		

		p.18 564-568	Acute toxicity studies with the fathead minnow (Pimephales promelas) found that primary and secondary allylic alcohols exhibit potency in excess of that predicted by saturated alcohols and baseline narcosis QSAR models. However, tertiary olefinic alcohols exhibit fish toxic potency consistent with baseline narcosis models.		
			Within the SEURAT-1 cluster, the following analogues of the case study, representing different subcategories, were tested with new *in vitro* assays: 2-propen-1-ol, 2-buten-1-ol, 1-buten-3-ol, 3-methyl-2-buten-1-ol, as well as the β-acetylenic alcohols 2-methyl-3-butyn-2-ol and 2-propyn-1-ol.		
		p.21-22 643-662	The following *in vitro* model was used to assess fibrosis: hepatic organoids (3D co-culture) of human hepatocyte-like cells (HepaRG and primary human hepatic stellate cells (HSC)). This culture model has shown to maintain good hepatocyte functionalities and maintain HSCs in a quiescent-like state for 3 weeks. – Cells were lysed either for ATP measurements (as a toxicity assessment) or mRNA analysis.		
		p.22 663-666	Both single and repeated-dose assays revealed 2-propen-1-ol is toxic (i.e., reduction in % control ATP) at the high concentrations (i.e., 40 and 200 µM) with potency increasing for all tested concentrations upon repeated exposure. However, none of the other tested alcohols showed toxicity for the same concentrations.		
		p.22 667-673	The analysis of fibrosis-related gene expression was adopted as an easy and accurate way to screen HSC activation. *In vivo*, upon liver injury HSCs respond by activation which is accompanied by an increased transcription and production/secretion of extracellular matrix; once the injury is repeated the described phenotype will lead to the development of fibrosis. The up-regulation of HSC activation markers such as COL1A1, COL3A1 and LOXL2 at the mRNA level has been established as a way to detect HSC activation in the current 3D model.		
		p.22-23 681-692	2-propen-1-ol strongly induced (i.e., >3-4 fold up-regulation) the expression of all three tested markers, but only upon repeated exposure. This pattern (strong induction of 3 out of 3 tested markers after repeated exposure), is also observed for 1-buten-3-ol. The up-regulation of the three genes upon repeated exposure, although to a lesser extent, was also observed for 3-methyl-2-buten-1-ol, however this compound also produces an effect in single exposure. 2-buten-1-ol up-regulates two out of the three tested genes (i.e., COL1A1 and LOXL2) in repeated exposure. The only alcohols that do not strongly induce (i.e., >3-4 fold up-regulation) of at least two out of three markers are 2-methyl-3-butyn-2-ol and 2-propyn-1-ol.		
		p.23 697-701	In another SEURAT-1 new method study, stress response activation of SRXN1, a target of the transcription factor NRF2, which is activated upon oxidative stress, and stress response activation of p21 and BTG2, both targets of the transcription factor p53, which is activated upon DNA damage, were evaluated. The HepG2 BAC- GFP reporter system was used.		

		p.24 713-724	Since 2-propen-1-ol causes oxidative stress in primary human and rat hepatocytes as well as under *in vivo* circumstances initial work focused on the SRXN1 reporter. After 24-hrs, all tested β-unsaturated alcohols showed a significant and dose dependent activation of SRXN1-GFP 716 expression in both the 2D and 3D assays. 2D cultures were more sensitive to pick of the GFP-SRXN1 reporter activity. A clear dose dependent effect was observed. More than 50% of the cells showed a response to all tested alcohols. The response for p21-GFP induction was also evaluated. In general, a limited percentage of cells responded to the tested analogues, all treatments showed maximal up to 20 % of the cells reaching the threshold of two times the negative control (i.e., DMSO) GFP intensity. In contrast, almost all cells responded to Aflatoxin B1. The response for the BTG2-GFP reporter, which reflects a DNA damage response through activation of p53 was also examined. No significant induction was observed in 2D after 24-hrs of treatment. Also in 3D spheroids, while exposure to some unsaturated alcohols resulted in a significant induction of BTG2-GFP; induction was not observed at all concentrations. After 48-hrs exposure, the average GFP intensity in 2D cultures showed some significant up-regulation. However, no significant up-regulation is observed in 3D cultures. For all unsaturated alcohols, between 25% and 45% of cells were observed to express GFP intensity higher than two times DMSO GFP intensity. In summary, the SEURAT-1 new methods data show that: • Straight chain β-olefinic alcohols induce the main HSC activation markers (i.e., COL1A1, COL3A1 and LOXL); the exception COL3A1 for 2-buten-1-ol is noted. • Vinylic methyl-substituted β -olefinic alcohols weakly induce the main HSC activation markers tested. • β-acetylenic alcohols typically do not induce the main HSC activation markers. • β-unsaturated alcohols primarily activate an oxidative stress response, but not a DNA damage response. • β-unsaturated alcohols all strongly activate the KEAP1/Nrf2 pathway reporter SRXN1-GFP. • β-unsaturated alcohols do not effectively activate the p21-GFP and BTG2-GFP reporter and responses are only generally observed >100 µM. Overall, these new methods data support the hypothesised mode-of-action for β-olefinic alcohols, in particular the premise of metabolic-mediated fibrosis.		
		p.24-25 725-738			
		p.25 739-749			
		p.26-27 754-775			

4.3	**Describe the quantitative aspects of the common underlying mechanism and differences in strength of effects**			3 4	Insufficient explanation of the source substance being worst-case. The NAM *in chemico* data contribute to the only quantitative information to address the differences in potency, but leave some uncertainty. More data or evidence is needed to demonstrate that – for the category members that have the external vinyl group – 2-propen-1-ol is truly worst case. To reduce the uncertainty in addressing that question, discuss in more detail particularly whether the GSH RC50 data are sufficient to make quantitative comparisons or relative ranking of category members with regard to reactivity (e.g. variance and reproducibility). Testing of more substances to clarify nuances in potency for different structural variations.
	Describe quantitative variations in the effect; explanation of how differences in exposure or potency determine the quantitative variations in the effects observed, based on the differences in chemical structures.		<u>The differences in strengths of effects are not due to exposure to the toxicant, i.e. kinetics differences, but to differences in potency.</u> **Available information on kinetics/biavailability differences possibly leading to different exposure:**		
		p.6 176-177 p.6 174-175	All short-chain β-olefinic alcohols are rapidly and nearly completely absorbed from the gut; within the C3 to C6 derivatives, C-atom chain length or branching does not significantly affect oral bioavailability.		
		p. 12-13 370-377	Primary alcohols have one alkyl-group and thus two H-atoms available for metabolism via alcohol dehydrogenase (ADH). Secondary alcohols have two alkyl-groups and one H-atom available for ADH attack. It follows that primary β-olefinic alcohols are likely to be more readily converted to reactive metabolites than secondary ones.		
		p.11 327-338	A comparison of the alcohol structure with the enzyme binding affinity of alcohol dehydrogenase indicates that increased binding (lower Km) occurs with increasing chain length (C3-C6) of the alcohols and the presence of unsaturation. However, the maximum rates of oxidation were essentially constant, regardless of the alcohol structure, suggesting that alcohol-enzyme binding is not the rate-limiting step for oxidation. Overall, it is considered that not the bioavailability of the parent or formation of the metabolites, and thus exposure, is the deciding step, but the structure-related potency.		
			Potency-related differences in strength of effects:		
		p.7 181-182	The key feature for the differences of strength of effects across the category is <u>different potency based on differences in chemical structure</u>: α,β-unsaturated aldehydes or α,β-unsaturated ketones are the definitive electrophilic toxicants and their <u>*in vivo* potency is related to relative thiol reactivity</u>, as further detailed in the following.		
		p.2 54-59 p.3 72-73	*In chemico* reactivity assay data, in the form of the concentration eliciting a 50% reduction in free GSH after 2 hours, show that α,β-unsaturated aldehydes and ketones, such as those derived from hepatic metabolism of primary and secondary olefinic β-unsaturated alcohols, readily react with GSH. However, the category members have metabolites with different reactive potencies (i.e., GSH RC50 values). Specifically, α,β-unsaturated carbonyl derivatives of straight-chain alcohols: 1-alken-3-ols, 2-alken-1-ols exhibit 2-hour RC50 values between 0.05 and 0.40 mmol/l, while those of branched alcohols: 2-methyl-2-alken-1-ols, 3-methyl-2-alken-1-ols, 3-methyl-3-alken-2-ols and 4-methyl-3-alken-2-ols exhibit RC50 values between 12-26 mmol/l.		

		p.17-18 505-547	The differences in potency were described as follows based on the results from the *in chemico* reactivity assay for the potential reactive metabolites of β-olefinic alcohols (see also C.4): 1) terminal vinyl-substituted derivatives (H2C=C-) were more reactive (with GSH) than the internal vinylene-substituted ones (-CH=CH-) (e.g. metabolites of 2-propen-1-ol, 1-buten-3-ol, 1-penten-3-ol, 1-hexen-3-ol vs 3-penten-2-ol, 4-hexen-3-ol) 2) methyl-substitution on the vinyl carbon atoms diminishes reactivity (see all branched compounds in Table 3, p.17) 3) methyl-substitution on the carbon atom farthest from the carbonyl group (C(=O)C=C(C) causes a larger reduction than methyl-substitution on the carbon atom nearest to the carbonyl group (C(=O)C(C)=C), and 4) derivatives with carbon-carbon double bond on the end of the molecule (i.e., vinyl ketones) were more reactive than ones with the carbon-oxygen double bond at the end of the molecule (i.e., aldehydes).		
		p.20 615-618	Overall, there is consistent potency between the overarching sub-categories of straight-chained and branched β-olefinic alcohols. Specifically, the results showed that β-olefinic alcohols with a methyl group substituted on a vinyl C-atom are 100 times less reactive than the non-methyl-substituted β-olefinic alcohol.		
		p.7-9 190-246	The *in chemico* results are in line with the *in vivo* study results regarding different strength of effects (NOAELs) for branched vs unbranched substance (see above).		
		p. 22-23 667-687 p.26 756-757	The fact that methyl-substituted β-olefinic alcohols are less reactive is also supported by results from the *in vitro* studies in hepatic organoids (3D co-culture), where the up-regulation of HSC activation markers in the hepatic organoids (3D co-culture) is weaker for 3-methyl-2-buten-1-ol.		
		p.16 492-493	The basic structure-activity relationships for chemical reactivity via Michael additions to thiols are pivotal for understanding hepatotoxic potency both *in vitro* and *in vivo*.		
		p.16 496-502	Structural features associated with relative reactivity of polarised α,β-unsaturated molecules, especially where an olefinic moiety conjugated to a carbonyl group, toward the model nucleophile glutathione, has been examined. This α,β-unsaturated structure conveys the capacity to undergo a covalent interaction with the thiol group of cysteine in the form of Michael addition. Quantitatively, reactivity of the α,β-unsaturated carbonyl compounds with glutathione is reliant upon the specific molecular structure, with several trends being observed and reported in the literature.		
		Annex II, Table 6B	The results above are supported by *in silico* predictions (OECD QSAR Toolbox) of the potential reaction mechanism and reaction potency (see summarising table in C.4).		

		p.10 308-309	In the *in silico* predictions for protein binding and DNA binding, all metabolites of β-olefinic alcohols, but 4-methyl-3-penten-2-one are associated with Michael addition (MA) and/or Schiff base formation mechanisms. The compounds not being predicted to react via MA are 3-methyl-2-buten1-ol and 4-methyl-3-penten-2-ol, both have an alkyl substituent on the olefinic carbon that may inhibit the protein binding site of the vinyl group, i.e. MA would be sterically hindered. In coding the profilers used in the OECD QSAR Toolbox, substances di-substituted at the β-carbon were not included in these structural alerts. The hypothesis of steric hindrance is generally in line with the weaker effects shown in *in chemico* reactivity assays, which demonstrate on the other hand, that the compounds do react, but with less potency than the straight-chained compounds.		
	Prediction based on regular pattern or worst-case approach? Justification of the chosen source substance being worst case.	p.7-9 190-246 p. 22-23 667-687 p.26 756-757	There is not a linear trend for prediction of the effects, but the differences in strength of effects are based on differences in chemical reactivity, related to relative thiol reactivity and based on differences in chemical structure. Therefore the prediction model is based on a worst case approach. 90-day study NOAELs, based on liver effects, for 2-propen-1-ol are proposed for read-cross to the other category members. Not many quantitative data are available to demonstrate 2-propen-1-ol as the most potent, i.e. worst case category member. Apart from the *in vivo* study data (NOAELs) showing that 3-methyl-2-buten-1-ol is less potent than 2-propen-1-ol, and the up-regulation of HSC activation markers in the hepatic organoids (3D co-culture) also showing a weaker response for 3-methyl-2-buten-1-ol, i.e. a methyl-substituted β-olefinic alcohol, quantitative differences between category members are demonstrated only by the *in chemico* data from the GSH assay. They are supported *by in silico* prediction of the potency of protein binding with the OECD QSAR Toolbox. The data are summarised in the table in section C.4 of this document (p. 7). The results clearly show differences in potency of the reactivity of the metabolite toxicants considered between subgroups in the category (see clustering of the effects in C.4). The “extremely reactive” metabolites are 2-propenal/acrolein (metabolite of 2-propen-1-ol) and the vinyl ketones (metabolites of the secondary non-branched 1-alken-3-ols), which all feature an external vinyl group, which determines the reactivity. However, considering the numeric values of the *in chemico* reactivity, the vinyl ketones seem to be even more reactive than acrolein. It should be determined whether the GSH RC50 data are sufficient and the variability in the *in vitro* assay allows a quantitative comparison and statement on relative ranking of category members with regard to reactivity. Thus the demonstration of the worst case of the chosen source 2-propen-1-ol for read-across is left with some uncertainty.		

4.4	**Exposure to other compounds than those linked to the prediction: characterise (or demonstrate no) influence on the prediction**			4	A more detailed statement on quantitative metabolisation rates/presence of residual parent or further transformation products and on potential effects of the further metabolisation products should be provided.
	Does the documentation indicate that other compounds are present (e.g. parent compounds or impurities) or formed (e.g. via other biotransformation pathways or as intermediates)?	p.10 293-296 p.12 354-361	The purity/impurity profile for the analogues listed in 2.4 is unknown. The most likely impurities are other isomers (e.g. cis vs. trans conformations). Further oxidation of the aldehyde produces the corresponding acid. The corresponding carboxylic acid may enter the β-oxidation pathway and be subsequently metabolised to CO_2 via the tricarboxylic acid pathway or be glucuronidated prior to excretion in the urine. Secondary alcohols are expected to be excreted via conjugation or oxidised to ketones, which cannot be further oxidised. Additionally, they can be excreted unchanged or undergo hydroxylation of the carbon chain, which in turn may give rise to a metabolite that can be more readily excreted. More information is needed on possibly formed further metabolisation products and their reactivity.		
	Are there indications that such compounds may have an influence on the prediction of the property under consideration?	p. 10 293-295 Annex I, Tables 6A/6B	Since the category is structurally limited, the potential impact of any impurities on the endpoint being considered is very limited. The parent compounds are considered as relatively non-toxic, which is supported by the *in silico* profiler predictions, where only the metabolites of the β-olefinic alcohols and not the parent compounds triggered for example the protein binding profiler. More information is needed on possible reactivity of possibly formed further metabolisation products.		
4.5	**Characterise (or demonstrate no) occurrence of other effects than those covered by the read-across hypothesis and justification**			4	The possible occurrence of other effects should be explained in more detail; more information necessary to be able to rule other metabolic mechanisms out completely or dismiss other chemical reactions.
	Other mechanisms than the common one claimed to drive the toxicity? Is qualitative and quantitative evaluation of data matrix indicative of any?	p.19 595-600 p.15-16, Table 2 p.27 771-773 Annex I Tables 5, 6B	The most likely metabolic pathway of all analogues is considered to be metabolisation via ADH oxidation to similar reactive derivatives eliciting the same mechanism of chemical reactivity. This metabolic activation is supported indirectly by the results of the liver profusion studies by Strubelt et al. (1999). The *in vitro* assay data from SEURAT-1 are also consistent with the ADH pathway of metabolic activation. However, other metabolic mechanisms, such as ROS formation or P450 activation, cannot be completely ruled out. The *in silico* prediction for the mechanism of protein (GSH) binding includes either Michael addition only or Michael addition and Schiff base formation (Annex I Table 6B);		

			other metabolic transformation products, in addition to oxidation, are possible according to *in silico* predictions (Annex I Table 5). The possible differences in or effects via an additional mechanism of metabolisation or reactivity other than Michael addition are not further discussed. Overall, however, the GSH binding *in chemico* (Table 3, p. 17) shows consistent electrophilic reactivity via the mechanism considered for the different (sub-category) members' metabolites considered.		
C.6	**Demonstrate there is no bias influencing the prediction**			4	The choice of the individual category members could be further detailed. Would the read-across be valid for members not explicitly mentioned but within this structural applicability domain?
	Criteria used in selection of category members are described and no otherwise suitable members have been excluded (or if so a justification is provided)		The inclusion criteria for category members have been described, based on structure and related to the mechanism of toxicity, e.g. exclusion of tertiary alcohols because of non-reactivity regarding the considered mechanism of toxicity, i.e. metabolisation to toxic metabolite not possible (see C.2). Borders of the category regarding chain length selected: Within the C3 to C6 derivatives, C-atom chain length or branching does not significantly affect oral bioavailability. The choice of the individual category members has not been justified in detail, but is seen to cover exemplarily all possible structural variations for primary and secondary β-olefinic alcohols, i.e. non-branched or branched, type of branching.		
		p.13 378-379	β-acetylenic alcohols have been considered as category members, but based on metabolic similarity, the read-across category was limited to primary and secondary β-olefinic alcohols.		
		p.13 379-391 p.21 641-642	See Moridani et al. (2001): primary β-acetylenic alcohol, 2-propyn-1-ol, induces cytotoxicity via metabolic activation to form 2-propynal which in turn causes hepatocyte lysis as a result of GSH depletion and lipid peroxidation. However, the metabolic activation is due to Cytochrome P450 2E1 and not ADH. Thus 2-propyn-1-ol is toxicokinetically dissimilar.		
		p.23 690-692	Consistent with this consideration, in the SEURAT-1 *in vitro* assay, the triple-bond containing β-acetylenic alcohol did not exhibit 2-propen-1-ol-like up-regulation of HSC activation markers.		
	Conservative 'worst case' (highest concern) studies available on source(s) are used in RA or if not a justification is provided	p.8 214-215 Annex II, Table 2, p.36	Considering and based on the effects in the liver, i.e. bile duct aplasia and periportal hepatocyte hypertrophy in the liver, the NOAEL values of 6 and 25 mg/kg bw/day in male and female rats, respectively, have been established from the available NOAELs as the values to be read across.		The study details and reliability could be described in more detail to follow the justification of the chosen NOAELs.