Read-Across for 90-Day Oral Repeated-Dose Toxicity for Low or No Toxicity Substances: The Importance of Toxicokinetic Similarity

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DATA GAP FILLING BY READ-ACROSS

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Introduction & Aim

One of the key advantages of read-across is its potential to predict a large number of substances with low potency for eliciting chronic endpoints and thereby avoid use of standard *in vivo* testing regimes, while maintaining high confidence in risk assessments.

Establishing similarity based on structure and chemical properties is often not enough sufficient to accept a toxicological read across, especially for chronic health endpoints. The principal philosophy of a toxicological read-across is that chemicals with similar molecular structure will exhibit similar chemical properties and in so doing exhibit similar toxic potency. An underlying assumption is that similarity of experimentally-derived toxicity data, which can be used to fill data gaps for an untested compounds, is associated with similar toxicokinetic and toxicodynamic properties from one substance, the source chemical, to the other compounds in the applicability domain of the read across. Toxicodynamic and toxicokinetic similarity can be established with relevant *in vivo* and *in vitro* data. Analysis of chemical metabolism can restrict the applicability domain for toxicokinetic similarity and thus the overall applicability domain for the read across.

The aim of this study was to demonstrate the need for evaluating toxicokinetic similarity by using a set of alkanols (saturated primary aliphatic alcohols). Based on *in vivo* and *in vitro* assays and mechanistic data alkanols are considered to have low or no toxicity at concentrations up to solubility in the exposure media.

The results of this read-across exercise illustrate how toxicokinetic analyses inform the certainty of similarity assumptions.

Methods

In this example, uncertainty was initially reduced by limiting the applicability domain to intermediate size (i.e., carbon atom (C) chain length of C5 to C13) alkanols.

20 analogues (9 n-alkanol and 11 2-alkyl-1-alkanols) were included in the evaluation.

Assessments of similarity (chemistry, toxicokinetics and toxicodynamics), systematic characterisation of uncertainty (i.e., similarity rationale, the read across data, and overall approach and conclusion) as well as application of high throughput toxicological data to further inform the read across predictions were conducted.

Assessments were supported by a data matrix of chemical properties, biotic modification properties, including a summary of metabolic pathways and metabolites, as well as toxicodynamic properties.

Rat *In vivo* Data

Alkanol	Oral LD50 (mg/kg)	90-d Oral NOEL (mg/kg w/d)	Alkanol	Oral LD50 (mg/kg)	90-d Oral NOEL (mg/kg bw/d)
1-Pentanol	2200	1000	2-Methyl-1-butanol	4010	ND
	≈3600		2-Methyl-1-pentanol	ND	ND
1-Hexanol	4590	1127 M	2-Ethyl-1-butanol	1850	ND
	4870	1243 F	2-Ethyl-1-hexanol	>3730	125
1-Heptanol	3250	ND		≈2000	
	6200 M	>1000	2-Propyl-1-pentanol	ND	ND
	5500 F		2-Methyl-1-octanol	ND	ND
1-Octanol	>5000	ND	2-Ethyl-1-octanol	ND	ND
1-Nonanol	3560	ND	2-Propyl-1-heptanol	5400	150
1-Decanol	4720	ND	2-Methyl-1-undecanol	ND	ND
1-Undecanol	3000	2000	2-Ethyl-1-decanol	ND	ND
1-Dodecanol	>2000	2000	2-Propyl-1-decanol	ND	ND
1-Tridecanol	ND	ND			ND = not determined

Non-Mammalian *In vivo* and *In vitro* Data

Because this is a well-tested and well-understood group of compounds, confidence in the read across for these chemicals is high. Uncertainty associated with mechanistic relevance and completeness of the read-across is low.

Fish and tadpole alkanol toxicity studies show symptoms consistent with general anaesthesia. Fish, rodent (inhalation exposure) and cellular toxicity data support the hypothesis that alkanols act as narcotics (see Mechanistic Plausibility).

Perfused rat liver toxicity data for the C5 primary alkanol exposure of 65.1 mmol/l for 2 hours suggest that 2-alkyl-1-alkanols may not be in the same read-across category as other primary alkanols (Table 1). These data support the premise that *in vitro* toxicity (e.g., O_2 consumption and ATP production) of 2-alkyl-1-alkanols is due, in large part, to loss of membrane integrity as indicated by cytosolic enzyme (LDH) leakage). While it is likely that enzyme leakage is the result of alteration in membrane fluidity due to partitioning into the cell membrane, loss of membrane integrity as a result of soft electrophilic reactivity is indicated by a 50% reduction in free glutathione (GSH).

Table 1. *In vitro* toxicity profiles for selected alkanols.

Name	log Kow	O_2 (µmol/g x min)	ATP (µmol/g)	LDH (U/l)	GSH (µmol/g)
Control		1.54 ± 0.07	1.25 ± 0.20	1109 ± 265	2.52 ± 0.29
2-Methyl-1-butanol	1.30	0.30 ± 0.03	0.10 ± 0.01	20521 ± 1087	1.33 ± 0.29
3-Methyl-1-butanol	1.16	0.22 ± 0.07	0.27 ± 0.05	8680 ± 1216	2.27 ± 0.37
1-Pentanol	1.40	0.06 ± 0.01	0.20 ± 0.03	28959 ± 4142	2.82 ± 0.36

ToxCast Data

ToxCast results reveal primary alkanols are among the least promiscuous chemical classes examined. Specifically only 104 of 4412 (2.4%) ToxCast test results from up to 700 assays showed any activity. None of the active assays were associated with a particular pathway or specific bioactivity. While these alkanols were not tested up to solubility, the lack of specific responses is not inconsistent with an assumption of baseline narcosis.

Mechanistic Plausibility

Narcosis in the broadest sense is the non-covalent disruption of hydrophobic interactions within membranes with a particular volume fraction, rather than molar fraction, causing narcosis. It is the accumulation of alcohols in cell membranes which disturbs their function, however, the exact mechanism is not known yet. There are three competing theories of general anaesthetic action; 1) the lipid solubility-anaesthetic potency correlation (i.e., the Meyer-Overton correlation), 2) the modern lipid hypothesis, and 3) the membrane protein hypothesis. Chemicals eliciting baseline narcosis often have low or no toxicity for chronic health effects at expected levels of exposure. This group of alkanols are considered nonpolar narcotics, which act via unspecific interaction with biological membrane in a manner similar to depressant anaesthetics.

Toxicokinetic Data

Primary alkanols are readily absorbed by the gastrointestinal tract and are distributed in the blood. In the case of n-alkanols, metabolism leads to two-step oxidation in the liver with the corresponding carboxylic acid undergoing mitochondrial β-oxidation to CO_2 with minor amounts of glucuronidation and subsequent elimination in the urine. In the case of 2-alkyl-1-alkanols, metabolism, while highly efficient, involves metabolic steps that are more complex than with n-alkanols. Experimental data reveal the major pathways of metabolism and fate of 2-alkyl-1-alkanols include: 1) conjugation of the alcohol group with glucuronic acid; 2) oxidation of the alcohol group, and 3) side-chain oxidation yielding additional polar metabolites, which may be subsequently conjugated and be excreted or further oxidised.

Conclusions

To assure high confidence in alkanol similarity assignments, toxicodynamic and toxicokinetic analyses indicate primary alkanols should be subcategorised based on structure prior to read-across.

With reasonable certainty, a 90-day oral repeated-dose toxicity NOEL value of 1000 mg/kg bw/d can be read across to fill data gaps of untested n-alkanols and a NOEL value of 125 mg/kg bw/d can be read across to fill data gaps of untested 2-alkyl-1-alkanols.