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

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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.

**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 \(\beta\)-oxidation to \(\mathrm{CO}_2\) 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.

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

<table>
<thead>
<tr>
<th>Name</th>
<th>log Kow ((\mu\text{mol/g} \times \text{min}))</th>
<th>(\text{O}<em>2) Consumption ((\mu\text{mol/O}</em>{2}))</th>
<th>ATP ((\mu\text{mol/g}))</th>
<th>LDH (U/l)</th>
<th>GSH ((\mu\text{mol/g}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00 ± 0.07</td>
<td>1.25 ± 0.20</td>
<td>1109 ± 265</td>
<td>2.52 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>2-Methyl-1-butanol</td>
<td>1.30 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>20521 ± 1087</td>
<td>1.33 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>3-Methyl-1-butanol</td>
<td>1.16 ± 0.02</td>
<td>0.27 ± 0.05</td>
<td>8680 ± 1216</td>
<td>2.27 ± 0.37</td>
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</tr>
<tr>
<td>1-Pentanol</td>
<td>1.40 ± 0.06</td>
<td>0.20 ± 0.03</td>
<td>28959 ± 4142</td>
<td>2.82 ± 0.36</td>
<td></td>
</tr>
</tbody>
</table>

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**Rat In vivo Data**

Table 1. In vivo toxicity profiles for selected alkanols.