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Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected 2-alkyl-1-alkanols

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ABSTRACT

2-Alkyl-1-alkanols offer an example whereby the category approach to read-across can be used to predict repeated-dose toxicity for a variety of derivatives. Specifically, the NOAELs of 125 mg/kg bw/d for 2-ethyl-1-hexanol and 2-propyl-1-heptanol, the source substances, can be read across with confidence to untested 2-alkyl-1-alkanols in the C5 to C13 category based on a LOAEL of low systemic toxicity. These branched alcohols, while non-reactive and exhibiting unspecific, reversible simple anaesthesia or nonpolar narcosis mode of toxic action, have metabolic pathways that have significance to repeated-dose toxic potency. In this case study, the chemical category is limited to the readily bioavailable analogues. The read-across premise includes rapid absorption via the gastrointestinal tract, distribution in the circulatory system and first-pass metabolism in the liver via Phase 2 glucuronidation prior to urinary elimination. 2-Ethyl-1-hexanol and 2-propyl-1-heptanol, the source substances, have high quality 90-day oral repeated-dose toxicity studies (OECD TG 408) that exhibit qualitative and quantitative consistency. Findings include only mild changes consistent with low-grade effects including decreased body weight and slightly increased liver weight, which in some cases is accompanied by clinical chemical and haematological changes but generally without concurrent histopathological effects at the LOAEL. These findings are supported by results from the TG 408 assessment of a semi-defined mixture of isotridecanols. Chemical similarity between the analogues is readily defined and data uncertainty associated with toxicokinetic and toxicodynamics similarities are low. Uncertainty associated with mechanistic relevance and completeness of the read-across is reduced by the concordance of *in vivo* and *in vitro* results, as well as high throughput and *in silico* methods data. As shown in detail, the 90-day rat oral repeated-dose NOAEL values for the two source substances can be read across to fill the data gaps of the untested analogues in this category with uncertainty deemed equivalent to results from a TG 408 assessment.

Introduction

Read-across

In a toxicity based read-across, it is imperative to demonstrate that all target substances exhibit similar chemical, toxicokinetic and toxicodynamic properties so experimentally-derived information and data from the source substances may be read across to fill the data gap for the target substances [1,2]. This type of data gap filling is particularly useful for cosmetic ingredients where *in vivo* testing in Europe is prohibited by legislation [3].

While read-across has been used by industry and regulators for decades, recent advances, especially in non-animal test methods, has resulted in read-across today being held to a higher standard [4,5].

The read-across strategy employed here focuses on assessing the similarity between target(s) and source substance(s) and the uncertainties in the read-across process and ultimate prediction, two fundamentals of a read-across estimation [6]. Briefly, the justification of read-across prediction needs to be robust, reliable and easily explicable. The crucial principles of similarity are clearly documented and supported by scientific literature and data. Sources of uncertainty, the uncertainty associated with the justification of similarity, and the uncertainty associated with the particular application are identified and accommodated.

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As such, the current study describes a case that illustrates a number of issues associated with a category approach for the scenario in which metabolism, while straight forward, is important in determining molecular similarity. Thus, establishing toxicodynamic, as well as toxicokinetic similarity, is critical to reducing uncertainties associated with the repeated-dose toxicity predictions.

The present study builds on an early finding [2]. Specifically, an initial evaluation of a wide variety of saturated alcohols revealed that, based on consideration of a common metabolic pathway the saturated alcohols need to be sub-categorised prior to making read-across predictions.

C5-C13 2-alkyl-1-alkanols: Overview of existing knowledge

As previously noted [2], intermediate chain-length primary alkanols are considered non-polar narcotics which act mechanistically in a manner similar to depressant anaesthetics. Perfused rat liver toxicity data from Strubelt et al. [7] for the C5 primary alkanol exposure of 65.1 mmol/l for 2 h suggests 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 in the cell membrane, loss of membrane integrity as a result of soft electrophilic reactivity and indicated by a 50% reduction in free glutathione (GSH) is not likely.

Due to bioavailability, and distribution and mechanistic considerations, the applicability domain for this case study is limited to 2-alkyl-1-alkanols with a carbon atom (C) chain length range of C5 to C13. Since long-chain derivatives are typically transported via carrier molecules, alcohols of C14 and greater are not included in this category. Since shorter-chain derivatives (e.g., isopropyl alcohol) have the potential to volatilise, they also are not included in this category.

Among the 2-alkyl-1-alkanols, 2-ethyl-1-hexanol is the most widely studied [8–12].

Dermal penetration of intermediate size alkanols does not readily occur and absorption from inhalation is extremely limited [13]. Thus, the primary route of exposure, which is toxicologically relevant, is oral. Two-alkyl-1-alkanols within the range C5-C13 are expected to be readily absorbed by the gastrointestinal tract and distributed in the blood in solution [14].

Metabolism of 2-alkyl-1-alkanols, while highly efficient, involves processes that are more complex than n-alkanol metabolism. Experimental data reveals 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; 3) side-chain oxidation yielding additional polar metabolites, which may be subsequently conjugated and be excreted or further oxidised, and 4) excretion of the unchanged parent compound. For example, in rabbits, the glucuronide of 2-ethyl-1-hexanoic acid was identified as the main metabolite (87%) after oral application of 2-ethyl-1-hexanol [15,16]. In contrast, in the same species, only about 9% of the administered dose of 2-methyl-1-butanol was found in the form of the glucuronides [15,16].

Belsito et al. [14] reviewed the toxicity of branched chain saturated alcohols, including secondary ones. Patocka and Kuca [17] summarized the toxicity of C1 to C6 alkanols. The efficacy of alkanols to induce ataxia [18] and enzyme release from liver cells [19] has been interpreted as being due to the hydrophobic property of the alkanols. Based on rat and fish studies, 2-alkyl-1-alkanols, like other alkanols, act in a manner similar to depressant anaesthetics [20,21]. Koleva et al. [22] reported multiple-regression type quantitative structure-toxicity relationships (QSARs) for oral log $LD50^{-1}$ data for rodents and the 1-octanol/water partition coefficient (log Kow). Comparison of measured toxicity data with predictions from baseline QSARs reveals that straight-chain and branched, saturated monohydric alcohols consistently behave as classic nonpolar narcotics.

A cursory summary of the rodent oral acute and oral repeated-dose toxicity of intermediate size 2-alkyl-1-akanols are presented in Table 2. In general, 2-alkyl-1-akanols acute oral toxicity (LD50) is very low ranging from ≈2000 to <5000 mg/kg bw with an average value of ≈3500 mg/kg bw.

2-Alkyl-1-akanols are slightly toxic in oral repeated-dose testing; typically, the rodent, oral, 90-day, repeated-dose No Observed Adverse Effect Level (NOAEL) in mg/kg bw/d is ≥125 mg/kg bw/d (see Table 2). This value is characteristically based on clinical symptoms, haematological values outside the normal range, or whole body effects different from normal. However, if ingested in large enough quantities, alkanols may cause systemic damage to the liver, heart, kidneys, and/or nervous system.

Method and materials

This evaluation of selected 2-alkyl-1-akanols follows the workflow of Schultz et al. [2]. It is in accord with the guidance proposed by Organization for Economic Co-Operation and Development (OECD) [30] and Schultz and co-workers [6]. *In vivo* data used in the assessment were taken from the literature, including ECHA REACH Registered Substances database [31]. Mechanistic relevance, as well as toxicokinetic and toxicodynamic similarity of the category analogues, was established using relevant non-animal data.

Target and source substances

In this case study, the analogues (listed in Table 3) include ten target and two source chemicals; the latter, those with repeated-dose data derived from a 90-day OECD TG 408 assay, are noted in bold print. This list is not meant to be all inclusive, rather it represents existing industrial organic materials that are likely to be found in a governmental or industrial inventory (e.g., OECD High Production Volume Chemicals). Additional substance identifier information, such as chemical structures and molecular formulas are available in Table 1 of the supplemental information.

Table 1
In vitro toxicity profiles for selected alkanols.

Name	log Kow	O_2 Consumption (μmol/g × 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	20,521 ± 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	28,959 ± 4142	2.82 ± 0.36

LDH – lactate dehydrogenase; ATP – adenosine triphosphate; GSH – reduced glutathione.