Read-Across of 90-day Rat Oral Repeated-Dose Toxicity: A Case Study for Selected n-Alkanols

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Highlights

- A category of saturated alcohols was created
- Data compilation was undertaken for the category of n-alkanaols
- Repeat dose NOELs were read across for low toxicity compounds
- *In vitro* data reduce uncertainty in read-across

Abstract: n-Alkanols provide an excellent example where a category-approach to read-across may be used to estimate the repeated-dose endpoint for a number of untested derivatives (target chemicals) using experimental data for tested derivatives (source chemicals). n-Alkanols are non-reactive and exhibit the unspecific, reversible simple anaesthesia or non-polar narcosis mode of toxic action in that the metabolic products of the parent alcohols do not contribute to the toxic endpoint evaluated. In this case study, the chemical category is limited to the readily bioavailable (C5 to C13) analogues. The toxicokinetic premise includes rapid absorption via the gastrointestinal tract, distribution in the circulatory system, and first-pass metabolism in the liver resulting in metabolism via oxidation to CO_2 and with minor elimination of oxidative intermediate as glucuronides. Two analogues have experimental 90-day oral repeated-dose toxicity data which exhibit qualitative and quantitative consistency. Typical findings include decreased body weight, slightly increased liver weight which, in some cases, is accompanied by clinical chemical and haematological changes but generally without concurrent histopathological effects at the Lowest Observed Effect Level (LOEL). Chemical similarity between the analogues is readily defined by a variety of structure-related properties; data uncertainty associated with toxicokinetic and toxicodynamic similarities is 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 oral repeated-dose toxicity No Observed Effect Level (NOEL) value of 1000 mg/kg bw/d for 1-pentanol and 1-hexanol based on LOEL of very low systemic toxicity can be read across to fill the data gaps of the untested analogues in this category with acceptable uncertainty.

Keywords: read-across, n-alkanols, repeated-dose toxicity, No Observed Effect Level (NOEL), Lowest Observed Effect Level (LOEL), weight-of-evidence (WoE), uncertainty

1 Introduction

1.1 Read-across

The principal philosophy of a toxicological read-across is chemicals that are similar in molecular structure will exhibit similar chemical properties, and as such, they will exhibit similar toxicokinetic and toxicodynamic properties. Thus, experimentally-derived toxicokinetic and toxicodynamic information and data from one chemical, the source substance, can be read across to fill the data gap for a second chemical, the target substance which is similar. This type of data gap filling is particularly useful for cosmetic ingredients where *in vivo* testing in Europe is prohibited by legislation [1].

As a predictive tool, read-across has been used by industry and regulators for decades [2]. With advances in non-animal test methods, read-across today is held to a different standard than at the turn of the century. Specifically, there is greater expectation in terms of the identifying similarities and addressing uncertainties within the read-across argument [3].

In order to facilitate the development of better practical guidance on how to formulate high quality read-across justifications, a series of case studies have been conducted by the authors. This case study illustrates specific considerations where metabolism of all the analogues in the chemical category is highly similar and plays no role in determining toxicological similarity [4]. The case study is also intended to illustrate how non-animal data, in the form of high throughput screening (HTS) data and *in silico* molecular screening, may be used to reduce uncertainties, as well as, add to mechanistic plausibility and weights-of-evidence (WoE) to any read-across argument.

While it is easy to establish similarity based on structure and chemical properties, this similarity alone is often not enough to accept a toxicological read-across prediction for sub-chronic and chronic health endpoints. To justify the applicability domain of the category it is often necessary to establish toxicodynamic, and to a greater extent toxicokinetic, similarity within the category.

The purpose of this research was to demonstrate the how read-across predictions of the repeated-dose toxicity no observed effect level (NOEL) value based on a consistent set of lowest observed effect level (LOEL) symptoms could be performed and substantiated for a category of n-alkanol analogues. Specifically, the category based data providing information to reduce uncertainties, and add to the WoE associated with read-across predictions of specified *in vivo* data. Thus, the estimations from the read-across are quantitative and with sufficiently low uncertainty that they may be used in risk assessments. As such, the predicted 90-day repeated-dose NOEL values are accompanied by sufficient relevant *in vivo* and non-animal test data to make the uncertainties equal to what would be expected from running a test using a protocol similar to Organization for Economic Co-Operation and Development (OECD) TG 408. In the present study, a previously reported ‘strategy’ was employed to assess similarities and overall completeness of the read-across [5].

1.2 C5 – C13 n-alkanols: overview of existing knowledge

Historically, intermediate chain-length n-alkanols are considered nonpolar narcotics which act mechanistically in a manner similar to depressant anaesthetics. Fang, McKim, Koleva and their co-workers [6-8] 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 saturated monohydric alcohols consistently behave as classic nonpolar narcotics [9].

The efficacy of n-alkanols to induce ataxia [10] and enzyme release from liver cells [11] has been interpreted as being due to the hydrophobic property of the alkanols. Perfused rat liver toxicity data from Strubelt et al. [12] for 1-pentanol (exposure 65.1 mmol/l for 2 hours) are reported in Table 1. These data support the premise that mammalian ex *vivo* toxicity (e.g., O_2 consumption and ATP production) of n-alkanols is due to membrane partitioning resulting in loss of membrane integrity (i.e., cytosolic enzyme leakage (LDH) but not glutathione (GSH) binding).

Table 1. *In vitro* toxicity profiles for 1-pentanol.
LDH – lactate dehydrogenase; ATP - adenosine triphosphate; GSH – reduced glutathione

Name	**log Kow**	**O_2 consumption (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
1-Pentanol	1.40	0.06 ± 0.01	0.20 ± 0.03	28959 ± 4142	2.82 ± 0.36

Due to bioavailability, and distribution and mechanistic considerations, the applicability domain for this case study is limited to n-alkanols with a carbon atom (C) chain length range of C5 to C13. For example, since longer-chain derivatives are typically transported via carrier molecules, they are not included in this chemical category. Also, shorter-chain derivatives are not included in this chemical category, as they have the potential to volatilise.

The general anaesthetic potency of several members of this homologous series of saturated aliphatic alcohols was determined in tadpoles, using the loss of righting reflex as the criterion of anaesthesia [13]. In this series, anaesthetic potency increased with chain length and was maximal for 1-dodecanol. The cut-off in potency was between C12 and C14, such that 1-tridecanol was a partial anaesthetic.

n-Alkanols within the range C5-C13 are expected to be readily absorbed by the gastrointestinal tract and distributed in the blood in solution. n-Alkanols are metabolised mainly in the liver via alcohol dehydrogenase to corresponding aldehydes and, subsequently, by aldehyde dehydrogenase to the corresponding carboxylic acids [14]. The fatty acid derivatives of intermediate size n-alkanols are readily taken up by mitochondria, where they are degraded by β-oxidation, especially in hepatocytes and myocytes [14]. However, generally <10% of the dose of these primary alcohols form glucuronic acid conjugates which are excreted in the urine [15].

Voskoboinikova [16] and Opdyke [17] have summarised the historical literature on aliphatic alcohol toxicity. More recently, the toxicity of alkanols containing from one to six C-atoms has been reviewed [18]. A cursory summary of the rat oral acute and oral repeated-dose toxicity of intermediate size n-alkanol are presented in Table 2. In general, n-alkanols acute oral toxicity (i.e., LC50) is very low, ranging from 1500 to 5000 mg/kg bw with an average value of ≈3000 mg/kg bw. n-Alkanols are only slightly toxic in oral repeated-dose testing; typically, the rodent, oral, 90-day, repeated-dose NOEL in mg/kg bw/d is in the range of 1/2 - 1/3 the LC50 value. 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 (i.e., near lethal doses), n-alkanols have the potential to cause systemic damage to the liver, heart, kidneys, and/or nervous system (see citations in Table 2 for details).

Table 2. Rat oral acute and repeated-dose toxicity of selected n-alkanols.

Alcohol	Oral LD50 (mg/kg)	Reference	90-d Oral NOAEL (mg/kg bw/d)	Reference
1-Pentanol	2200	[19]	1000	[20]
	3645	[21]	1000	[21]
1-Hexanol	4590	[22]	1127 M	[23]
	4870	[24]	1243 F	[23]
1-Heptanol	3250	[24]	> 1000	[26]
	6200 M	[25, 26]		
	5500 F	[25, 26]		

1-Octanol	>5000	[27]	Not determined	
Nonyl alcohol (assumed 1-nonanol)	3560	[17]	Not determined	
1-Decanol	4720	[28]	Not determined	
Undecyl alcohol (assumed 1-undecanol)	3000	[29]	2000[a]	[30]
Lauryl alcohol (assumed 1-dodecanol)	> 2000	[31]	2000	[31, 32]
1-Tridecanol	17200	[33]	Not determined	

[a] NOAEL value is recorded as experimental result, but the details in the report indicate that it is read across from 1-dodecanol (CAS 112-53-8).
M- male, F- female

2. Method and Materials

This evaluation of selected n-alkanols follows the workflow of Przybylak et al. [5]. It is in accord with the guidelines proposed by OECD [34] and Schultz and co-workers [35]. *In vivo* data used in the assessment were taken from the literature, including ECHA REACH Registered Substances database [36]. Mechanistic relevance, as well as toxicokinetic and toxicodynamic similarity of the category analogues was established using relevant non-animal data.

2.1 Target and Source Substances

In this case study, the analogues (listed in Table 3) include seven 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 inclusive, as defined by the limitations of the applicability domain. The analogues represents n-alkanols which are found in governmental or industrial inventories (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 3. n-Alkanols considered as part of the chemical category for read-across. Compounds in bold indicate the source substances.

ID	Name	CAS No.	SMILES
1	**1-Pentanol**	**71-41-0**	**CCCCCO**
2	**1-Hexanol**	**111-27-3**	**CCCCCCO**
3	1-Heptanol	111-70-6	CCCCCCCO
4	1-Octanol	111-87-5	CCCCCCCCO
5	1-Nonanol	143-08-8	CCCCCCCCCO
6	1-Decanol	112-30-1	CCCCCCCCCCO
,	1-Undecanol	112-42-5	CCCCCCCCCCCO
8	1-Dodecanol	112-53-8	CCCCCCCCCCCCO
9	1-Tridecanol	112-70-9	CCCCCCCCCCCCCO

2.2 Endpoint

The NOEL for the 90-day rat oral repeated-dose is the single endpoint for which this category approach is applied. The 90-day oral repeated-dose data for 1-pentanol and 1-hexanol are particularly well-suited for read-across; the NOELs are based on experimental results from a 4-dose exposure scenario (0, <100, between 100 and 500, and ≥ 1000 mg/kg bw/d) following a standard test guideline (OECD TG 408) where the LOEL symptoms are reported. Moreover, there are supporting repeated-dose results for 1-heptanol, 1-undecanol and 1-dodecanol from OECD TG 422 studies, with the exposure durations for males being 28 days and for females 54 days.

2.3 Hypothesis of the category

The premise for this read-across case study is:

- n-Alkanols of intermediate chain length (i.e., C5 to C13) are direct acting toxicants (i.e., metabolic activation and detoxification is not a factor in toxicity) with a similar reversible mode of action (i.e., non-polar narcosis or simple anaesthesia).

- Within C5 to C13 derivatives, C-atom chain length affects most physico-chemical properties (e.g., Log Kow values increase with increasing chain length). However, this trend, while toxicologically relevant in fish toxicity and *in vitro* assays, is not observed in mammalian acute and sub-chronic toxicity via oral exposure.
- These primary alkanols are rapidly and nearly completely absorbed from the gut and distributed in the blood in solution; first pass metabolism leads to two-step oxidative metabolism in the liver resulting in corresponding carboxylic acid, which subsequently undergoes mitochondrial β-oxidation to CO_2 with minor amounts of glucuronidation with subsequent elimination of the phase II metabolite in the urine.
- Toxicodynamically, these primary alkanols are highly similar. Briefly, *in vivo* they exhibit very low systemic toxicity; *in vitro* and *in silico* they exhibit no chemical reactivity or receptor-mediated interactions.
- 90-day oral rat repeated-dose NOAEL data for 1-pentanol and 1-hexanol can be read across to other category members listed in Table 3 with acceptable uncertainty.

3 Results

3.1 Read-across justification

In order to conduct a read-across, there is the requirement for high quality *in vivo* data for the endpoint under consideration [5, 34, 35]. In this case, is 90-day oral repeated dose-toxicity for rats in the form of a NOEL value and LOEL symptoms from a study similar to OECD TG 408.

From a repeated-dose perspective, test results of n-alkanols are extensive. 1-Pentanol was orally administered to rats following OECD TG 408 at dose levels of 0, 50, 150 or 1000 mg/kg bw/d for 13 weeks [20, 21]. The “no-outward-effect level” (assumed to be the NOEL) was 1000 mg/kg/day.

In a non-standard rat oral repeated-dose assay similar to an OECD TG 408 assay, animals were exposed to 0.25% (based on nominal concentrations in the diet) and 0.50% for 13 weeks; 1.0% for 10 weeks, then 2.0% (week 11), 4.0% (week 12) and 6.0% 13 weeks of 1-hexanol [23]. The NOAEL for 1-hexanol was determined to be ≈1100 mg/kg bw/d (1127 mg/kg bw/d for male and 1243 mg/kg bw/d for female rats).

While the endpoint read across in this exercise is the 90-day oral repeated-dose NOEL, there is also high quality repeated-dose toxicity NOEL/LOEL data for shorter duration studies (e.g., OECD TG 422). Since these data are both qualitatively and quantitatively similar to the 90-day data, they may be used as WoE and to confirm that all category members are within the endpoint domain.

1-Heptanol was administered orally to rats under OECD TG 422 and 0, 100, 300 and 1000 mg/kg bw/d [26]. No treatment related changes were noted for all parameters (e.g., biochemical, haematological and clinical parameters, as well as body weight, food consumption and neurobehavioral effects).

Following OECD TG 422, oral repeated-dose toxicity of 1-undecanol in rats was evaluated at doses of ≈ 0, 100, 500, 2000 mg/kg bw/d [30]. A NOEL for systemic toxicity of 2000 mg/kg bw/d was determined in male rats, in the absence of toxicologically significant effects at any dose level.

Following OECD TG 422, rats were exposed to 1-dodecanol in the diet in concentrations of ≈ 0, 100, 500 and 2000 mg/kg/ bw/d [31]. A NOEL for systemic toxicity of 2000 mg/kg bw/d was determined in male rats, in the absence of toxicologically significant effects at any dose level.

In summary, while protocols vary, results for repeated-dose toxicity test results exhibit qualitative and quantitative consistency. Phenotypic results from repeated exposure to n-alkanols reflect mild changes consistent with low-grade effects and include decreased body weight, accompanied by clinical chemical and haematological changes, but generally without concurrent histopathological effects.

3.2 Applicability domain

As previously noted, the applicability domain for this case study is confined to straight-chain primary alkanols of intermediate size, C5 to C13.

3.3 Purity/impurities

Read-across is based on the structural similarity of the main constituents of the source and target substances. Toxicity may actually be determined by an impurity, therefore it is important to provide a purity/impurity profile for all analogues. However, in this case it was not possible to take into account impurities based on production. Since the category is structurally limited, the impurities are expected to be similar if not the same across the members and are not expected to significantly impact the toxicity profile of any analogue. However, it is acknowledged for regulatory decisions such information may be required.

3.4 Data matrices for assessing similarity

In order for a read-across prediction to be accepted, there is the requirement to establish similarity between the source and target substance [5, 34, 35]. While structural similarity is a minimum, toxicokinetic similarity, especially for metabolism, and toxicodynamic similarity, especially in regard to mechanistic plausibility, is required for sub-chronic endpoints such as 90-day oral repeated dose-toxicity [5].

3.4.1 Structural similarity

As demonstrated in Tables 1 and 3 of the supplemental information, all the n-alkanols included in the category are structurally highly similar. Specifically, they: 1) belong to a common chemical class, aliphatic alcohols and subclass, n-alkanols, and 2) possess common molecular scaffolding, a C-atom backbone with a straight-chain configuration. Structurally, the only variable is the length of the hydrocarbon backbone, C5-C13.

3.4.2 Chemical property similarity

As demonstrated in Table 2 of the supplemental information, all the n-alkanols included in the category have many of their physico-chemical properties determined experimentally. Thus, when required calculated values, which are based on these measured values can be accepted with high confidence. Properties, with the exception of density and pKa, trend in value related to C-atom number within the scaffold. Specifically, all category members exhibit molecular weights from 88 to 200 g/mol. Hydrophobicity (as modelled by log Kow) increases with number of C-atoms from >1.0 to <6.0, vapour pressure and water solubility decrease with molecular size, melting point and boiling point increase with molecular size, and density is constant at $0.8 \pm 0.1 g/cm^3$. Since there is no readily ionisable substituent the pKa is consistent at ≈ 15.2.

3.4.3 Chemical constituent similarity

As shown in Table 3 of the supplemental information, all the n-alkanols included in the category have common constituents in the form of: 1) a single key substituent, -OH, and 2) structural fragments, $-CH_3$ and $-CH_2-$.

3.4.4 Toxicokinetic similarity

Limiting the range of C-atoms for the applicability domain reduced the impact of size on adsorption, distribution, metabolism and elimination (ADME). From a bioavailability standpoint, the analogues exhibit in *in silico* models linear trend with molecular weight. Such modelling reflects hydrophobic-dependent uptake.

The toxicokinetic understanding of alkanols is reasonably complete despite the fact that the experimental data, as summarised in Table 4 of the supplemental information, are limited. Absorption, distribution and elimination are not considered factors in these predictions. For example, 1-octanol is rapidly absorbed after oral administration (i.e., bioavailability >80%). 1-Octanol is excreted mainly as CO_2, and to a lesser extent as n-octyl glucuronide [17, 27, 37]. Other n-alkanols exhibit similar toxicokinetics, with n-alcohols generally forming <10% of the dose as glucuronic acid conjugates and are excreted in the urine [15].

It is generally accepted that, regardless of species, metabolism of n-alkanols is highly efficient and proceeds in a similar fashion [38]. Basically, there only degradative or detoxification pathways involved in the metabolism of n-alkanols. It is universality accepted that in the first step of the biotransformation, the alcohols undergo stepwise intracellular oxidation to the corresponding carboxylic acids, followed by a stepwise C2 unit elimination via mitochondrial β-oxidation [38].

3.4.5 Metabolic similarity

As demonstrated in Table 5 of the supplemental information, all of the category members undergo oxidation and hydroxylation in metabolic simulations. Briefly, mammalian catabolism of fatty acids, which most often takes place in the mitochondria, leads to the formation of acetyl-coenzyme A (CoA), enters the TCA cycle and reduces nicotinamide adenine dinucleotide (NADH) and flavin adenine nucleotide (FADH2) which are used by the electron transport chain to produce ATP [14].

While other processes, including ω-oxidation and α-oxidation, are known to take place, β-oxidation is the most common catabolic process in n-alkanol metabolism. It is highly likely that the n-alkanols included in the category will be nearly completely metabolised (i.e., >90%) via the tricarboxcylic acid (TCA) cycle. It is generally agreed that cytosolic fatty acids are activated for degradation by conjugation with CoA. β-Oxidation of saturated fatty acids consists of a recurring cycle of four reactions [14]. In acids with an even number of C-atoms, this cycling continues until two molecules of Acetyl-CoA are produced in the final reaction. Acetyl-CoA is available to be further metabolised in the TCA cycle. In acids with an odd number of C-atoms, the end product is propionyl-CoA, which must be converted to succinyl-CoA to enter the TCA cycle.

3.4.6 Toxicophore similarity

The severe limitation of the structural domains sharply reduces the likelihood of differences in toxicophores between the target and source analogues. As demonstrated in Table 6 of the supplemental information, none of the n-alkanols included in the category are associated with any toxicophore based on *in silico* profilers within the OECD QSAR Toolbox V3.4.

3.4.7 Mechanistic plausibility similarity

While there is no mammalian adverse outcome pathway for the hypothesized mode of action, it is generally accepted that the acute toxicity of intermediate chain n-alcohols is the result of narcosis [5-9]. There are both theoretical and biochemical evidence for the cell membrane being the site of action for anaesthetic-like chemicals [10-11]. Narcosis, in the broadest sense, is the reversible, non-covalent disruption of hydrophobic interactions within membranes with a particular volume fraction, rather than molar fraction [39]. It is the accumulation of alcohols in cell membranes which disturbs their function; however, the exact mechanism is not yet known. 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 [c.f., 40-41].

As stated in Table 7 of the supplemental information, the n-alkanols included in the category are associated with the simple narcosis mechanism of toxicity that is equivalent to depressant anaesthetics [6]. Additivity of primary alkanols in joint effect studies was demonstrated in injection anaesthesia studies in rats [6],. This observation of additivity is consistent with the premise that n-alkanols exhibit a common mechanism of action. More importantly, Fang et al. [6] demonstrated additivity or slight deviations from additivity for alkanols with the conventional inhaled anaesthetic desflurane (1,2,2,2-tetrafluoroethyl difluoromethyl ether). The latter support the contention that the mechanisms of action of n-alkanols is depressant anaesthesia.

The effect of various primary alkanols on the CNS was studied by using rat brain synaptosomal membranes as an *in vitro* model [41]. The activity of (Ca_2^+/Mg_2^+) ATPase and the membrane fluidity were determined. Specifically, the n-alkanols exhibited an increased molar inhibition of the ATPase activity, with an increase in the carbon chain length up to 1-octanol. 1-Octanol and 1-decanol caused a biphasic effect on the ATPase activity, depending on the n-alkanol concentration, whereas 1-dodecanol caused a stimulation of the ATPase activity. All alkanols studied caused an increased fluidity of the membrane; however, changes in the membrane fluidity do not seem to be a pre-requisite of the ATPase inhibition [41].

The Fish Acute Toxicity Syndrome (FATS) approach put forth by McKim et al. [7] has furthered our mechanistic understanding and the effects of intermediate chain saturated alcohols in fish more than anything else. The FATS approach is based on physiological response sets from spinally transected rainbow trout (*Oncorhynchus mykiss*) exposed to model chemicals. Briefly, *in vivo* biochemical and respiratory-cardiovascular responses were measured during lethal aqueous exposures; the responses and their interdependence formed a complex data matrix, with the best response variables for mechanisms of action being determined with multivariate statistics. The FATS for 1-octanol is characterised by a striking slow-down in all respiratory and cardiovascular functions [7] that makes it distinct from other modes of actions. The action of 1-octanol is consistent with depressant anaesthesia.

The contributions of functional groups in acute rat oral toxicity have been calculated using alkanes as the baseline [40]. The toxic contribution of the OH group is -0.108. This situation (negative contribution to toxicity as compared to corresponding alkane) has not been observed in acute fish toxicity because the threshold of excess toxicity is too high to distinguish differences in toxicity. Critical body residues (CBRs) calculated from percentage of absorption and bioconcentration factors indicate that most of aliphatic alcohols share the same modes of toxic action between fish and rat. Specifically, fish and rat log (1/CBR) and number of alcohols are 1.65; 18 and 1.58; 348, respectively [40].

In summary, there are several lines of evidence that support the contention that all the analogues within the domain act in a similar fashion and that fashion is not different from simple anaesthesia or non-polar narcosis.

3.4.8 Other *in vivo* endpoint similarity

In mammals, alkanols are considered baseline inhalation toxicants which model as simple narcotics [9]. Based on acute oral toxicity, n-alkanols belong to Category 4 which do not require a hazard label for acute oral toxicity. Their LD50 values are very low, typically ranging from 1000 to >5000 mg/kg bw with an average value of ≈3000 mg/kg bw (see Table 2). In mammals, mild to moderate sub-lethal toxicity from a single oral dose of intermediate size alkanols include general gastrointestinal symptoms (e.g., nausea, vomiting, abdominal cramps and diarrhoea) associated with irritation. High ingested doses (i.e., near acute lethal levels) can cause gastrointestinal haemorrhage and liver injury. For example, in the rat, the LD50 for 1-octanol is >5000 mg/kg [17]; the only symptoms of intoxication observed were moderately to severely ruffled fur and mild sedation. The symptoms had disappeared completely 24 hours later. The growth of the exposed animals was similar to that of the controls.

In fish, alkanols are considered to act via the nonpolar narcosis mode of action [42, 43]. Within the USEPA DSSTox Fathead Minnow Acute Toxicity (EPAFHM) database, alkanols are represented. They exhibit toxic potencies not statistically different from baseline predictions. Because of concerns for aquatic toxicity, a large number of alcohols, especially saturated ones, have been tested *in vitro* for cell population growth inhibition [44]. Structure-activity results from *in vivo* and *in vitro* tests are highly consistent [45]. Briefly, from a structural standpoint, the aquatic toxicity of alkanols is partition-dependent, regardless of endpoint being assessed.

Generally, for alkanol exposures in *in vitro* assays, results are attributed to unspecific interactions with biological membranes [11]; such effects are typically directly correlated with 1-octanol/water partition coefficients (c.f. [46]).

3.4.9 Relevant *in vitro* and *in silico* data

In an effort to further support the mechanistic argument for this read-across information form two new methods were examined. Specifically, relevant HTS data in the form of ToxCast data [47, 48] and of *in silico* nuclear receptor binding predictions [49] were evaluated. Within the USEPA toxicity forecaster program (ToxCast) [50], data are available for the majority of the n-alkanol derivatives (see Table 8 of the supplemental information). Of the 711 possible assays that form the ToxCast scheme, 1-octanol, 1-undecanol, 1-dodecanol and 1-tridecanol have been evaluated in 602 of them. Additionally, 1-hexanol, 1-heptanol and 1-decanol have been assessed in about 250 assays. Lastly, 1-nonanol has been tested in 150 ToxCast assays. The number of active assays varies from none for 1-octanol to 25 for 1-undecanol and 30 for 1-tridecanol. Within ToxCast, the n-alkanols are among the “least promiscuous chemical classes”; < 2.74% of the ToxCast assays showing any activity up to highest concentration tested and none of the active assay are associated with specific bioactivity.

Only four non-specific cell viability qHTS assays within the Toxcast suite were positive for four of the tested n-alkanol analogues; no assay exhibits activity for five or more of the category analogues. Specifically, the Tox21_ELG1_LUC_Agonist_viability, Tox21_TR_LUC_GH3_Antagonist_viability, Tox21_AhR_viability and Tox21_Aromatase_Inhibition_viability show a positive response with four n-alkanols but there is no consistency among which analogues are positive.

Alkanols were screened with a variety of *in silico* profilers [49]. Specifically, profilers for nuclear receptor binding were run to identify potential binding to the following nuclear receptors; PPARs (peroxisome proliferator-activated receptors), AR (androgen receptor), AHR (aryl hydrocarbon receptor), ER (oestrogen receptor), GR (glucocorticoid receptor), PR (progesterone receptor), FXR (farnesoid X receptor), LXR (liver X receptor), PXR (pregnane X receptor), THR (thyroid hormone receptor), VDR (vitamin D receptor), as well as RAR/RXR (retinoic acid receptor/ retinoid X receptor). The evaluation of potential binding to the receptors is based on structural fragments and physico-chemical features that have been identified as essential to bind to these nuclear receptors and induce a response. As noted in Table 6 of the supplemental information, no potential receptor binding was predicted. It is worth noting that ToxCast also tested for all of these receptors, and all corresponding assays were negative.

Taken collectively, the HTS and *in silico* findings are not inconsistent with the cited *in vivo* data. The premise that, oral repeated-dose toxicity of n-alkanols are considered to be nonpolar narcotics and act in a manner similar to depressant anesthetics is consistent with the ToxCast data and receptor binding simulations results which indicate no activity associated with a specific mode of action.

4. Statement of uncertainty

The categorical assessments of uncertainties along with summary comments are presented in Tables 4 and 5. Briefly, chemical similarity is limited by chain length but has no impact on repeated-dose toxicity. Data uncertainty with the fundamental aspects of toxicokinetics is low. Regardless of the species of mammals, all such category members are judged to be readily absorbed orally and to have similar distributions; metabolised via oxidation to the acid derivative, subsequently degraded to CO_2 via mitochondrial oxidation, and/or eliminated as a glucuronide. Data uncertainty with the fundamental aspects of toxicodynamics is low, in that category members exhibit a very low-toxic profile with respect to *in vivo* effects (i.e., NOEL and LOEL), as well as with respect to *in vitro* and new-methods effects. n-Alkanols are experimentally associated with the nonpolar narcosis mechanisms of toxicity. The simple narcosis (i.e., reversible anaesthesia) mode of toxic action is driven by partitioning into the biophase. While well-studied, this molecular mechanism is not well-understood and no adverse outcome pathway (AOP) is currently available. Moreover, it is unclear if oral repeated-dose toxicity is related to this mechanism; however, there is no evidence to suggest it is not. Uncertainty associated with mechanistic relevance and completeness of the read-across (i.e., uncertainty in the predictions) while initially low-to-moderate is reduced to low with the addition of ToxCast and *in silico* screening data. The major source of uncertainty for this group of alcohols is associated with what is essentially a "low-toxic" prediction.

Table 4. Assessment of data uncertainty and strength-of-evidence associated with the fundamentals of chemical, transformation/toxicokinetic and toxicodynamic similarity**.**

Similarity Parameter	Data Uncertainty[a]	Strength-of-Evidence[b]	Comment
Substance identification, structure and chemical classifications	low	high	All category members are discrete organic substance of simple structure. They all have CAS numbers, similar 2D structure and belong to the same chemical class (primary aliphatic alcohols) and same subclass (straight-chain alcohols).
Physio-chem & molecular properties	Empirical: low Modelled: low	high	All category members are appropriately similar with respect to key physico-chemical and molecular properties. Where appropriate (e.g., log Kow) changes in values are linked to changes in C-atom chain length. There is a high degree of consistency between measured and model estimated values.
Substituents, functional groups, & extended structural fragments	low	high	Substituents and functional groups are consistent across all category members. There are no extended structural fragments.
Transformation/tox icokinetics and	Empirical: *in vivo*: low	medium	While *in vivo* absorption data are reported for only one category member, there is evidence for similar

Similarity Parameter	Data Uncertainty[a]	Strength-of-Evidence[b]	Comment
metabolic similarity	*in vitro*: none Simulated: low		toxicokinetics and metabolic pathways. Comparison of results from empirical studies and model predictions indicate similar metabolism among category members. It is universally accepted that n-alkanols are typically degraded to CO_2. Absorption and distribution are not considered factors in these predictions.
Potential metabolic products	Simulated: low	high	Based on *in silico* metabolic simulations, metabolites from oxidation and hydroxylation are predicted to be produced by all the category members.
Toxicophores /mechanistic alerts	medium	high	Based on *in silico* profilers, no category member contains any established toxicophores.
Mechanistic plausibility and AOP-related events	medium	high	Although no AOP is currently available for the hypothesized mode of action, many category members have been tested for what is generally accepted as mechanistically-relevant events (i.e., anaesthesia and narcosis).
Other relevant, *in vivo*, *in vitro* and *ex vivo* endpoints	low	high	Although not directly related to the repeated-dose endpoint, many category members have been tested for *in vivo* acute effects in rodents and fish. In addition, many category members have been tested *in vitro* for cellular effects. There is general agreement in the trend of the reported EC50 values. The primary alkanols are among the "least promiscuous chemical classes" within ToxCast with no positive assay being associated with specific bioactivity. Primary alkanols reveal no propensity for nuclear receptor binding within the COSMOS suite of *in silico* profilers.

[a] Uncertainty associated with underlying information/data used in the exercise (empirical, modelled; low, medium, high)

[b] Consistency within the information/data used to support the similarity rational and prediction (low, medium, high)

Table 5. Assessment of uncertainty associated with mechanistic relevance and completeness of the read-across.

Factor	Mechanistic Uncertainty[a]	Comment
The problem and premise of the read-across	Low	The endpoint to be read across, oral 90-day repeated-dose toxicity, for n-alkanols is well-studied and fairly well-understood mechanistically. The scenario of the read-across hinges on metabolism not affecting toxicity and the mode of toxic action being reversible narcosis. Thus, n-alkanols have no obvious chemical reactivity, do not bind to any known receptor and exhibit no specific mode of toxic action.
***In vivo* data read-across**		
Number of analogues in the source set	Low; 5of 9 analogues	While there are five tested category members, two 1-pentanol and 1-hexanol, have high quality *in vivo* 90-day, oral repeated-dose data usable for read-across.
Quality of the *in vivo* apical	Low; consistent lowest observed	Generally, the *in vivo* data are consistent with regard to qualitative description of repeated-dose effects. LOEL affects are typically haematological or whole body parameters and not

endpoint data read across	effect concentration (LOEC) symptoms	organ-specific effects. The high quality empirical data (e.g., OECD TG 408) for the 90-day repeated-dose endpoint exists for 1-pentanol and 1-hexanol are supported by lower quality (i.e., OECD TG 422) oral repeated-dose toxicity data for 1-heptanol, 1-unidecanol and 1-dodecanol.
Severity of the apical *in vivo* hazard	Low; strong evidence that the 90-day NOAEL value is 1/20 to 1/10 of the LD50 values.	The consensus is that n-alkanols have no obvious chemical reactivity, do not bind to any known receptor and exhibit no specific mode of toxic action. Potency data for the *in vivo* 90-d oral repeated-dose NOAEL are ≈1000 mg/kg bw/d based on general whole body effects for both sexes.
Evidence to the biological argument for read-across		
Robustness of analogue data set	Low; numerous endpoints reveal the same structure-activity relationships.	The available data from acute *in vivo* and *in vitro* studies for the category members are extensive with several assays being used to assess most if not all the analogues, especially the source analogues. The tests were judged to be reliable and conducted under the appropriate conditions.
Concordance with regard to the intermediate and apical effects and potency data	Low to medium; limited by indirect rationale (e.g., acute to chronic) of mechanistic plausibility.	Since there is no toxicity pathway for repeated-dose effects for this chemical category, there are no true intermediate events. However, there is concordance between anaesthesia and slow-down in all respiratory and cardiovascular functions. There is agreement among the dose-response relationships of the tested category members for other relevant endpoints.
Weight-of-Evidence	High; experimental and predicted information among and between the category member is consistent with stated premise	Overall the available information is generally consistent with the stated premise. The structural limitations (i.e., n-alkanols) on the category strengthen the weight-of –evidence (WoE). While the toxicokinetics data are limited, the consistency in metabolism and simplicity of the metabolic pathway adds to the WoE. The fact the source substances *in vivo* data is supported by similar data for other analogues adds to the WoE. The fact that there is consistent relevant *in vitro* data for most category members strengthens the WoE. The consistency in results as related to simple membrane partitioning strengthens the WoE. The consistent negative results with ToxCast assays and screening with *in silico* receptor-binding profilers add to the WoE.

[a] Uncertainty: low, medium, high

5. Statement of the conclusions

This is the second in a series of read-across case studies; this particular study examines a category of similar compounds that do not require (or do not undergo) metabolism to exert a potential adverse human health effect [51]. *In vivo* oral repeated-dose exposure to n-alkanols gives rise to a set of nonspecific symptoms, including clinical symptoms, haematological values outside the normal range, or whole body effects different from normal. Limiting the category to C5 to C13 analogues assures that the impact of bioavailability on the toxicokinetic and toxicodynamic profiles is very limited. Primary alkanols are direct-acting toxicants with a reversible mode of toxic action described as nonpolar narcosis (i.e., unspecific interaction with biological membrane in a manner similar to simple anaesthetics). The main route of exposure for alkanols is oral via rapid gastrointestinal absorption. The majority of an oral dose of any n-alkanol is promptly degraded via simple cellular oxidation; the remainder is eliminated as the glucuronide conjugate.

Repeated-dose toxicity test results exhibit qualitative consistency in results between and within species. While protocols vary, results of oral repeated-dose testing exhibit qualitative consistency between and within mammals. Typical findings are only mild changes including decreased body weight, slightly increased liver weight, as well as clinical chemical and haematological changes, but typically without concurrent histopathological effects.

Within ToxCast, the n-alkanols are among the “least promiscuous chemical classes”; < 2.74% of the ToxCast assays showing any activity and none of the active assay being associated with specific bioactivity. Screening with *in silico* profilers reveals that n-alkanols have no predicted potential of nuclear receptor binding.

This is a category read-across (i.e., many-to-one several times). While several analogues have been evaluated experimentally in oral repeated-dose testing schemes, the 90-day oral repeated-dose toxicity data and the NOAELs of 1000 mg/kg bw/d for 1-pentanol and1-hexanol is the conservative prediction. A no systemic toxic conclusion with a NOAEL of 1000 mg/kg bw/d can be read across with confidence to untested n-alkanols in the C5 to C13 category listed in Table 3.

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Supplementary material

Read-Across of 90-day Rat Oral Repeated-Dose Toxicity: A Case Study for Selected n-Alkanols

Tables for Assessing Similarity of Analogues and Category Members for Read-Across

Table 1: Comparison of Substance Identification, Structure and Chemical Classifications

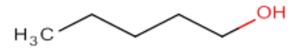
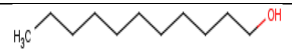
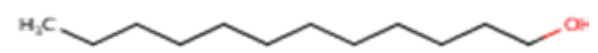
ID	Name	CAS No	SMILES	2D Structure	Molecular Formula
1	1-Pentanol	71-41-0	CCCCCO		C5H12O
2	1-Hexanol	111-27-3	CCCCCCO		C6H14O
3	1-Heptanol	111-70-6	CCCCCCCO		C7H16O
4	1-Octanol	111-87-5	CCCCCCCCO		C8H18O
5	1-Nonanol	143-08-8	CCCCCCCCCO		C9H20O
6	1-Decanol	112-30-1	CCCCCCCCCCO		C10H22O
7	1-Undecanol	112-42-5	CCCCCCCCCCCO		C11H24O
8	1-Dodecanol	112-53-8	CCCCCCCCCCCCO		C12H26O
9	1-Tridecanol	112-70-9	CCCCCCCCCCCCCO		C13H28O

Table 2: Comparison of Physico-Chemical and Molecular Properties[1]

ID	Name	Molecular Weight [1]	Log Kow[1a]	Vapour Pressure (Pa, 25 deg C) [1b]	Density [2] (g/cm^3)	Melting Point (deg C) [1b]	Water Solubility (mg/L, 25 deg C) [1c]	Boiling Point (deg C) [1b]	pKa [3]
1	1-Pentanol	88.15	1.33 1.51 (M)	353 293 (M)	0.8±0.1	-49.96 -78.9 (M)	20890 22000 (M)	136.95 137.9 (M)	15.24
2	1-Hexanol	102.18	1.82 2.03 (M)	117 124 (M)	0.8±0.1	-37.86 -44.6 (M)	6885 5900/6260 (M)	159.09 157.6 (M)	15.38
3	1-Heptanol	116.21	2.31 2.62 (M)	39.8 31.2 (M)	0.8±0.1	-26.03 -34 (M)	1940 1670/1800 (M)	180.33 176.4 (M)	15.38
4	1-Octanol	130.23	2.81 3.00 (M)	13.2 10.6	0.8±0.1	-14.46 -15.5 (M)	814 540 (M)	200.67 195.1 (M)	15.27
5	1-Nonanol	144.26	3.30 3.77 (M)	4.38 3.03 (M)	0.8±0.1	-3.15 -5 (M)	156.8 140 (M)	220.09 213.3 (M)	15.22
6	1-Decanol	158.29	3.79 4.57 (M)	1.45 1.13 (M)	0.8±0.1	7.89 6.9 (M)	28.21 37 (M)	238.62 231.1 (M)	15.21
7	1-Undecanol	172.31	4.28	0.68 0.396 (M)	0.8±0.1	18.67 19 (M)	43.04	256.24 243 (M)	15.2
8	1-Dodecanol	186.34	4.77 5.13 (M)	0.242 0.113 (M)	0.8±0.1	29.19 24 (M)	6.898 4 (M)	272.96 259 (M)	15.2
9	1-Tridecanol	200.37	5.26	0.0316 0.0581(M)	0.8±0.1	0.0316	4.533	288.77 152 (M)	15.2

M = measured value

[1]Values typically derived from EPISuite v4.1, [a] KOWWIN Program (v1.68), [b] MPBPWIN v1.43, [c] at 25 deg C; (mg/L) Kow (WSKOW v1.42); [2] ACD/Lab Percepta Platform - PhysChem Module (from ChemSpider); [3] Predicted by PERCEPTA; predicted by ACD (Advanced Chemistry Development Inc., Toronto, Canada)

Table 3: Comparison of Substituents, Functional Groups, and Extended Structural Fragments

ID	Name	Key Substituent(s)	Functional Group(s)	Extended Fragment(s)	Chemical Class:	Chemical Sub-Class:
1	1-Pentanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
2	1-Hexanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
3	1-Heptanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
4	1-Octanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
5	1-Nonanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
6	1-Decanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
7	1-Undecanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
8	1-Dodecanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
9	1-Tridecanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain

Table 4: Comparison of Abiotic Transformation and Toxicokinetics

ID	Name	Abiotic Transformation	Toxicokinetics			
			Absorption	Bioavailability	half-life	Elimination
1	1-Pentanol					
2	1-Hexanol	Phototransformation in air - half-life: 30.8 hrs[c]				Rabbit:10.3% as hexyl glucuronide[e]
3	1-Heptanol	Phototransformation in air - half-life: 28.1[c]				Rabbit: 5.3% as heptyl glucuronide[e]
4	1-Octanol	Phototransformation in air - half-life: 26.7 hrs[d]	orally rapidly absorbed[a,b]	>80%[a,b]		mainly as CO_2, small amount as n-octyl glucuronide[a,b]; Rabbit: 9.5% as octyl glucuronide[e]
5	1-Nonanol					Rabbit: 4.1% as nonyl glucuronide[e]
6	1-Decanol					Rabbit: 3.5% as decyl glucuronide[e]
7	1-Undecanol					
8	1-Dodecanol					
9	1-Tridecanol					

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Table 5: Comparison of Potential Metabolic Products

ID	Name	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
		Rat liver S9	Skin metabolism			
1	1-Pentanol	Hydroxylation (1) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1) beta-Oxidation of Carboxylic Acids (1)
2	1-Hexanol	Hydroxylation (1) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1) beta-Oxidation of Carboxylic Acids (1)
3	1-Heptanol	Hydroxylation (1) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)
4	1-Octanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)

ID	Name	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
		Rat liver S9	Skin metabolism			
				Dealkylation Dehydration Demethylation		
5	1-Nonanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)
6	1-Decanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)
7	1-Undecanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)

ID	Name	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
		Rat liver S9	**Skin metabolism**			
8	1-Dodecanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)
9	1-Tridecanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)

() - The number of metabolites for specific transformation.

Table 6: Comparison of Toxicophores

ID	Name	Toxicophores[1]	DNA binding by OECD[1]	Protein binding by OECD[1]	Nuclear receptor binding[2]	Liver& Mitochondria toxicity[2]
1	1-Pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
2	1-Hexanol	Cramer Class I	No alert	No alert	Inactive	No alert
3	1-Heptanol	Cramer Class I	No alert	No alert	Inactive	No alert
4	1-Octanol	Cramer Class I	No alert	No alert	Inactive	No alert
5	1-Nonanol	Cramer Class I	No alert	No alert	Inactive	No alert
6	1-Decanol	Cramer Class I	No alert	No alert	Inactive	No alert
7	1-Undecanol	Cramer Class I	No alert	No alert	Inactive	No alert
8	1-Dodecanol	Cramer Class I	No alert	No alert	Inactive	No alert
9	1-Tridecanol	Cramer Class I	No alert	No alert	Inactive	No alert

[1] OECD QSAR Toolbox 3.3. [2] COSMOS profilers available via COSMOS space: http://cosmosspace.cosmostox.eu

Table 7: Comparison of Mechanistic Plausibility and Adverse Outcome Pathway-Related Event Data

ID	Name	Mechanistic Plausibility	Adverse Outcome Pathway or Mode of Toxic Action:	Molecular Initiating Event:	Key Event 1 etc.:	Key Event Relationship 1 etc.:	Other Mechanistically-Relevant Events
1	1-Pentanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2	1-Hexanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			CNS depression
3	1-Heptanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
4	1-Octanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			CNS depression biphasic effect on the ATPase activity
5	1-Nonanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
6	1-Decanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			biphasic effect on the ATPase activity
7	1-Undecanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			

ID	Name	Mechanistic Plausibility	Adverse Outcome Pathway or Mode of Toxic Action:	Molecular Initiating Event:	Key Event 1 etc.:	Key Event Relationship 1 etc.:	Other Mechanistically-Relevant Events
8	1-Dodecanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			stimulation of the ATPase activity
9	1-Tridecanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			

Table 8: Comparison of Toxicologically Relevant *In Vivo*, *In Vitro* and *Ex Vivo* Data

Name	1-Pentanol	1-Hexanol	1-Heptanol	1-Octanol	1-Nonanol	1-Decanol	1-Undecanol	1-Dodecanol	1-Tridecanol
Endpoint: NOAEL (Repeat dose toxicity)	1000 (mg/kg bw/d) [1]	1127 mg/kg bw/d for male and 1243 mg/kg bw/d for female [3]	1000 (mg/kg bw/d) [39]				2000 (mg/kg bw/d) [9]	2000 (mg/kg bw/d) [11]	
Endpoint: NOEL (Repeat dose toxicity)	≥ 6400 (mg/m^3) [2]			1300 (mg/kg bw/d) [4,58]			<100 (mg/kg bw/d) [9]	100 (mg/kg bw/d) [10]	
Endpoint: LOAEL (Repeat dose toxicity)		1000 (mg/kg bw/d) [3]							
Endpoint: HNEL (Repeat dose toxicity)	882 (mg/kg bw/d) [4]		50 (mg/kg bw/d) [6]	130 (mg/kg bw/d) [7]					
Endpoint: LEL (Repeat dose toxicity)	5080 (mg/kg bw/d) [5]			650-2564 (mg/kg bw/d) [7,8]				3324 (mg/kg bw/d) [12]	

Name	1-Pentanol	1-Hexanol	1-Heptanol	1-Octanol	1-Nonanol	1-Decanol	1-Undecanol	1-Dodecanol	1-Tridecanol
Endpoint: LOEL (Repeat dose toxicity)								100-2000 (mg/kg/d) [13]	
Endpoint: NOAEL (Reproductive toxicity)	1000 (mg/kg/d [1]								
Endpoint: NOAEL (Teratogenicity)		370-1240 (mg/kg/d) [3]		1300 (mg/kg/d) [16]					
Endpoint: NOAEC (Teratogenicity)	14 (mg/L air) [15]	3.5 (mg/L air) [3]				>100(mg/L air) [61]			
Endpoint: LOAEL (Maternal toxicity)				130 (mg/kg/d) [17]		130 (mg/kg/d) [61]			
Endpoint: NOAEC (Maternal toxicity)				>0.4 (mg/L) [16]					

Name	1-Pentanol	1-Hexanol	1-Heptanol	1-Octanol	1-Nonanol	1-Decanol	1-Undecanol	1-Dodecanol	1-Tridecanol
Endpoint: Carcinogenic/ Genotoxicity	1 X Negative [66]	5 x Negative [3]		2 X Negative [16]			1 X Negative [9]	7X Negative 1x Positive [19-25]	
Endpoint: LC50 (Acute toxicity)		>21 (mg/L air) >21 (mg/ L/hour) >5030 (mg/L air) [3, 35]					>700 (mg/m^3) [9]		
Endpoint: LD50 (Acute toxicity) **From different routes of exposure**	140-4585 (mg/kg/) 2.83-5.66 (mL/kg) [14, 27-31, 34,54-55, 66]	103-4870 (mg/kg) [3, 36-38]	500-6200 (mg/kg) [37, 39-41, 67]	1790 - ≥5000 (mg/kg) [16,42,43]	800-6400 (mg/kg) 44 (mmol/kg) 5660 (uL/kg) [44-46,55, 59,60]	1000-5000 mg/kg [18, 61, 68]	3000-> 15800 (mg/kg) [9, 62, 69]	1500- >26530 (mg/kg/d) >12.8 - > 36 (ml/kg) [11,47,63,6 4]	5600- 17200 (mg/kg) [48]
Endpoint: LDLo (Acute toxicity)	122- 2000(mg/k g) [32,33,57]								

Name	1-Pentanol	1-Hexanol	1-Heptanol	1-Octanol	1-Nonanol	1-Decanol	1-Undecanol	1-Dodecanol	1-Tridecanol
Endpoint: Genotoxicity (AMES, Chromosomal abrration, gene mutation)	2 x Negative [52,57]	1 x Negative [3]	1 x Negative [39]	1 x Negative [50]		2 x Negative [26, 51]			
Toxcast overview [53]	-	250 (1 active)	250 (10 active)	602 (0 active)	150 (4 active)	257(15 active)	602 (25 active)	602 (3 active)	602 (30 active)

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