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

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16 **Highlights**

- 17 • A category of saturated alcohols was created
- 18 • Data compilation was undertaken for the category of n-alkanaols
- 19 • Repeat dose NOELs were read across for low toxicity compounds
- 20 • *In vitro* data reduce uncertainty in read-across
- 21

22 **Abstract:** n-Alkanols provide an excellent example where a category-approach to read-across
23 may be used to estimate the repeated-dose endpoint for a number of untested derivatives (target
24 chemicals) using experimental data for tested derivatives (source chemicals). n-Alkanols are
25 non-reactive and exhibit the unspecific, reversible simple anaesthesia or non-polar narcosis mode
26 of toxic action in that the metabolic products of the parent alcohols do not contribute to the toxic
27 endpoint evaluated. In this case study, the chemical category is limited to the readily bioavailable
28 (C5 to C13) analogues. The toxicokinetic premise includes rapid absorption via the
29 gastrointestinal tract, distribution in the circulatory system, and first-pass metabolism in the liver
30 resulting in metabolism via oxidation to CO₂ and with minor elimination of oxidative
31 intermediate as glucuronides. Two analogues have experimental 90-day oral repeated-dose
32 toxicity data which exhibit qualitative and quantitative consistency. Typical findings include
33 decreased body weight, slightly increased liver weight which, in some cases, is accompanied by
34 clinical chemical and haematological changes but generally without concurrent histopathological
35 effects at the Lowest Observed Effect Level (LOEL). Chemical similarity between the analogues
36 is readily defined by a variety of structure-related properties; data uncertainty associated with
37 toxicokinetic and toxicodynamic similarities is low. Uncertainty associated with mechanistic
38 relevance and completeness of the read-across is reduced by the concordance of *in vivo* and *in*
39 *vitro* results, as well as high throughput and *in silico* methods data. As shown in detail, the 90-
40 day oral repeated-dose toxicity No Observed Effect Level (NOEL) value of 1000 mg/kg bw/d for
41 1-pentanol and 1-hexanol based on LOEL of very low systemic toxicity can be read across to fill
42 the data gaps of the untested analogues in this category with acceptable uncertainty.

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

46 **1 Introduction**

47 1.1 Read-across

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

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

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

67 While it is easy to establish similarity based on structure and chemical properties, this similarity
68 alone is often not enough to accept a toxicological read-across prediction for sub-chronic and

69 chronic health endpoints. To justify the applicability domain of the category it is often necessary
70 to establish toxicodynamic, and to a greater extent toxicokinetic, similarity within the category.
71 The purpose of this research was to demonstrate the how read-across predictions of the repeated-
72 dose toxicity no observed effect level (NOEL) value based on a consistent set of lowest observed
73 effect level (LOEL) symptoms could be performed and substantiated for a category of n-alkanol
74 analogues. Specifically, the category based data providing information to reduce uncertainties,
75 and add to the WoE associated with read-across predictions of specified *in vivo* data. Thus, the
76 estimations from the read-across are quantitative and with sufficiently low uncertainty that they
77 may be used in risk assessments. As such, the predicted 90-day repeated-dose NOEL values are
78 accompanied by sufficient relevant *in vivo* and non-animal test data to make the uncertainties
79 equal to what would be expected from running a test using a protocol similar to Organization for
80 Economic Co-Operation and Development (OECD) TG 408. In the present study, a previously
81 reported ‘strategy’ was employed to assess similarities and overall completeness of the read-
82 across [5].

83 1.2 C5 – C13 n-alkanols: overview of existing knowledge

84 Historically, intermediate chain-length n-alkanols are considered nonpolar narcotics which act
85 mechanistically in a manner similar to depressant anaesthetics. Fang, McKim, Koleva and their
86 co-workers [6-8] reported multiple-regression type quantitative structure-toxicity relationships
87 (QSARs) for oral log LD₅₀⁻¹ data for rodents and the 1-octanol/water partition coefficient (log
88 Kow). Comparison of measured toxicity data with predictions from baseline QSARs reveals that
89 saturated monohydric alcohols consistently behave as classic nonpolar narcotics [9].

90 The efficacy of n-alkanols to induce ataxia [10] and enzyme release from liver cells [11] has
91 been interpreted as being due to the hydrophobic property of the alkanols. Perfused rat liver
92 toxicity data from Strubelt et al. [12] for 1-pentanol (exposure 65.1 mmol/l for 2 hours) are
93 reported in Table 1. These data support the premise that mammalian *ex vivo* toxicity (e.g., O₂
94 consumption and ATP production) of n-alkanols is due to membrane partitioning resulting in
95 loss of membrane integrity (i.e., cytosolic enzyme leakage (LDH) but not glutathione (GSH)
96 binding).

97 **Table 1.** *In vitro* toxicity profiles for 1-pentanol.

98 LDH – lactate dehydrogenase; ATP - adenosine triphosphate; GSH – reduced glutathione

Name	log K _{ow}	O ₂ consumption ($\mu\text{mol/g x min}$)	ATP ($\mu\text{mol/g}$)	LDH (U/l)	GSH ($\mu\text{mol/g}$)
Control		1.54 \pm 0.07	1.25 \pm 0.20	1109 \pm 265	2.52 \pm 0.29
1-Pentanol	1.40	0.06 \pm 0.01	0.20 \pm 0.03	28959 \pm 4142	2.82 \pm 0.36

99

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

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

110 n-Alkanols within the range C5-C13 are expected to be readily absorbed by the gastrointestinal
111 tract and distributed in the blood in solution. n-Alkanols are metabolised mainly in the liver via

112 alcohol dehydrogenase to corresponding aldehydes and, subsequently, by aldehyde
 113 dehydrogenase to the corresponding carboxylic acids [14]. The fatty acid derivatives of
 114 intermediate size n-alkanols are readily taken up by mitochondria, where they are degraded by β -
 115 oxidation, especially in hepatocytes and myocytes [14]. However, generally <10% of the dose of
 116 these primary alcohols form glucuronic acid conjugates which are excreted in the urine [15].

117 Voskoboinikova [16] and Opdyke [17] have summarised the historical literature on aliphatic
 118 alcohol toxicity. More recently, the toxicity of alkanols containing from one to six C-atoms has
 119 been reviewed [18]. A cursory summary of the rat oral acute and oral repeated-dose toxicity of
 120 intermediate size n-alkanols are presented in Table 2. In general, n-alkanols acute oral toxicity
 121 (i.e., LC50) is very low, ranging from 1500 to 5000 mg/kg bw with an average value of \approx 3000
 122 mg/kg bw. n-Alkanols are only slightly toxic in oral repeated-dose testing; typically, the rodent,
 123 oral, 90-day, repeated-dose NOEL in mg/kg bw/d is in the range of 1/2 - 1/3 the LC50 value.
 124 This value is characteristically based on clinical symptoms, haematological values outside the
 125 normal range, or whole body effects different from normal. However, if ingested in large enough
 126 quantities (i.e., near lethal doses), n-alkanols have the potential to cause systemic damage to the
 127 liver, heart, kidneys, and/or nervous system (see citations in Table 2 for details).

128 **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	

129 ^a NOAEL value is recorded as experimental result, but the details in the report indicate that it is read across from 1-dodecanol
130 (CAS 112-53-8).
131 M- male, F- female
132

133 2. Method and Materials

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

139 2.1 Target and Source Substances

140 In this case study, the analogues (listed in Table 3) include seven target and two source
141 chemicals; the latter, those with repeated-dose data derived from a 90-day OECD TG 408 assay,
142 are noted in bold print. This list is inclusive, as defined by the limitations of the applicability
143 domain. The analogues represents n-alkanols which are found in governmental or industrial
144 inventories (e.g., OECD High Production Volume Chemicals). Additional substance identifier
145 information, such as chemical structures and molecular formulas, are available in Table 1 of the
146 supplemental information.

147 **Table 3.** n-Alkanols considered as part of the chemical category for read-across. Compounds in
148 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	CCCCCCCCCO
,	1-Undecanol	112-42-5	CCCCCCCCCO
8	1-Dodecanol	112-53-8	CCCCCCCCCO
9	1-Tridecanol	112-70-9	CCCCCCCCCO

149

150 2.2 Endpoint

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

159 2.3 Hypothesis of the category

160 The premise for this read-across case study is:

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

- 164 • Within C5 to C13 derivatives, C-atom chain length affects most physico-chemical
165 properties (e.g., Log Kow values increase with increasing chain length). However, this
166 trend, while toxicologically relevant in fish toxicity and *in vitro* assays, is not observed in
167 mammalian acute and sub-chronic toxicity via oral exposure.
- 168 • These primary alkanols are rapidly and nearly completely absorbed from the gut and
169 distributed in the blood in solution; first pass metabolism leads to two-step oxidative
170 metabolism in the liver resulting in corresponding carboxylic acid, which subsequently
171 undergoes mitochondrial β -oxidation to CO₂ with minor amounts of glucuronidation with
172 subsequent elimination of the phase II metabolite in the urine.
- 173 • Toxicodynamically, these primary alkanols are highly similar. Briefly, *in vivo* they
174 exhibit very low systemic toxicity; *in vitro* and *in silico* they exhibit no chemical
175 reactivity or receptor-mediated interactions.
- 176 • 90-day oral rat repeated-dose NOAEL data for 1-pentanol and 1-hexanol can be read
177 across to other category members listed in Table 3 with acceptable uncertainty.

178 **3 Results**

179 3.1 Read-across justification

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

183 From a repeated-dose perspective, test results of n-alkanols are extensive. 1-Pentanol was orally
184 administered to rats following OECD TG 408 at dose levels of 0, 50, 150 or 1000 mg/kg bw/d

185 for 13 weeks [20, 21]. The “no-outward-effect level” (assumed to be the NOEL) was 1000
186 mg/kg/day.

187 In a non-standard rat oral repeated-dose assay similar to an OECD TG 408 assay, animals were
188 exposed to 0.25% (based on nominal concentrations in the diet) and 0.50% for 13 weeks; 1.0%
189 for 10 weeks, then 2.0% (week 11), 4.0% (week 12) and 6.0% 13 weeks of 1-hexanol [23]. The
190 NOAEL for 1-hexanol was determined to be \approx 1100 mg/kg bw/d (1127 mg/kg bw/d for male and
191 1243 mg/kg bw/d for female rats).

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

197 1-Heptanol was administered orally to rats under OECD TG 422 and 0, 100, 300 and 1000
198 mg/kg bw/d [26]. No treatment related changes were noted for all parameters (e.g., biochemical,
199 haematological and clinical parameters, as well as body weight, food consumption and
200 neurobehavioral effects).

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

205 Following OECD TG 422, rats were exposed to 1-dodecanol in the diet in concentrations of ≈ 0 ,
206 100, 500 and 2000 mg/kg/ bw/d [31]. A NOEL for systemic toxicity of 2000 mg/kg bw/d was
207 determined in male rats, in the absence of toxicologically significant effects at any dose level.

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

213 3.2 Applicability domain

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

216 3.3 Purity/impurities

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

224 3.4 Data matrices for assessing similarity

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

230 3.4.1 Structural similarity

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

236 3.4.2 Chemical property similarity

237 As demonstrated in Table 2 of the supplemental information, all the n-alkanols included in the
238 category have many of their physico-chemical properties determined experimentally. Thus, when
239 required calculated values, which are based on these measured values can be accepted with high
240 confidence. Properties, with the exception of density and pKa, trend in value related to C-atom
241 number within the scaffold. Specifically, all category members exhibit molecular weights from
242 88 to 200 g/mol. Hydrophobicity (as modelled by log Kow) increases with number of C-atoms
243 from >1.0 to <6.0, vapour pressure and water solubility decrease with molecular size, melting
244 point and boiling point increase with molecular size, and density is constant at $0.8\pm 0.1\text{g/cm}^3$.
245 Since there is no readily ionisable substituent the pKa is consistent at ≈ 15.2 .

246 3.4.3 Chemical constituent similarity

247 As shown in Table 3 of the supplemental information, all the n-alkanols included in the category
248 have common constituents in the form of: 1) a single key substituent, -OH, and 2) structural
249 fragments, -CH₃ and -CH₂-.

250 3.4.4 Toxicokinetic similarity

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

255 The toxicokinetic understanding of alkanols is reasonably complete despite the fact that the
256 experimental data, as summarised in Table 4 of the supplemental information, are limited.

257 Absorption, distribution and elimination are not considered factors in these predictions. For
258 example, 1-octanol is rapidly absorbed after oral administration (i.e., bioavailability >80%). 1-
259 Octanol is excreted mainly as CO₂, and to a lesser extent as n-octyl glucuronide [17, 27, 37].
260 Other n-alkanols exhibit similar toxicokinetics, with n-alcohols generally forming <10% of the
261 dose as glucuronic acid conjugates and are excreted in the urine [15].

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

268 3.4.5 Metabolic similarity

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

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

285 3.4.6 Toxicophore similarity

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

290 3.4.7 Mechanistic plausibility similarity

291 While there is no mammalian adverse outcome pathway for the hypothesized mode of action, it
292 is generally accepted that the acute toxicity of intermediate chain n-alcohols is the result of
293 narcosis [5-9]. There are both theoretical and biochemical evidence for the cell membrane being
294 the site of action for anaesthetic-like chemicals [10-11]. Narcosis, in the broadest sense, is the
295 reversible, non-covalent disruption of hydrophobic interactions within membranes with a
296 particular volume fraction, rather than molar fraction [39]. It is the accumulation of alcohols in
297 cell membranes which disturbs their function; however, the exact mechanism is not yet known.
298 There are three competing theories of general anaesthetic action: 1) the lipid solubility-
299 anaesthetic potency correlation (i.e., the Meyer-Overton correlation), 2) the modern lipid
300 hypothesis, and 3) the membrane protein hypothesis [c.f., 40-41].

301 As stated in Table 7 of the supplemental information, the n-alkanols included in the category are
302 associated with the simple narcosis mechanism of toxicity that is equivalent to depressant
303 anaesthetics [6]. Additivity of primary alkanols in joint effect studies was demonstrated in
304 injection anaesthesia studies in rats [6]. This observation of additivity is consistent with the
305 premise that n-alkanols exhibit a common mechanism of action. More importantly, Fang et al.
306 [6] demonstrated additivity or slight deviations from additivity for alkanols with the conventional
307 inhaled anaesthetic desflurane (1,2,2,2-tetrafluoroethyl difluoromethyl ether). The latter support
308 the contention that the mechanisms of action of n-alkanols is depressant anaesthesia.

309 The effect of various primary alkanols on the CNS was studied by using rat brain synaptosomal
310 membranes as an *in vitro* model [41]. The activity of (Ca²⁺/Mg²⁺) ATPase and the membrane
311 fluidity were determined. Specifically, the n-alkanols exhibited an increased molar inhibition of
312 the ATPase activity, with an increase in the carbon chain length up to 1-octanol. 1-Octanol and
313 1-decanol caused a biphasic effect on the ATPase activity, depending on the n-alkanol

314 concentration, whereas 1-dodecanol caused a stimulation of the ATPase activity. All alkanols
315 studied caused an increased fluidity of the membrane; however, changes in the membrane
316 fluidity do not seem to be a pre-requisite of the ATPase inhibition [41].

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

327 The contributions of functional groups in acute rat oral toxicity have been calculated using
328 alkanes as the baseline [40]. The toxic contribution of the OH group is -0.108. This situation
329 (negative contribution to toxicity as compared to corresponding alkane) has not been observed in
330 acute fish toxicity because the threshold of excess toxicity is too high to distinguish differences
331 in toxicity. Critical body residues (CBRs) calculated from percentage of absorption and
332 bioconcentration factors indicate that most of aliphatic alcohols share the same modes of toxic
333 action between fish and rat. Specifically, fish and rat log (1/CBR) and number of alcohols are
334 1.65; 18 and 1.58; 348, respectively [40].

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

338 3.4.8 Other *in vivo* endpoint similarity

339 In mammals, alkanols are considered baseline inhalation toxicants which model as simple
340 narcotics [9]. Based on acute oral toxicity, n-alkanols belong to Category 4 which do not require
341 a hazard label for acute oral toxicity. Their LD50 values are very low, typically ranging from
342 1000 to >5000 mg/kg bw with an average value of ≈ 3000 mg/kg bw (see Table 2). In mammals,
343 mild to moderate sub-lethal toxicity from a single oral dose of intermediate size alkanols include
344 general gastrointestinal symptoms (e.g., nausea, vomiting, abdominal cramps and diarrhoea)
345 associated with irritation. High ingested doses (i.e., near acute lethal levels) can cause
346 gastrointestinal haemorrhage and liver injury. For example, in the rat, the LD50 for 1-octanol is
347 >5000 mg/kg [17]; the only symptoms of intoxication observed were moderately to severely
348 ruffled fur and mild sedation. The symptoms had disappeared completely 24 hours later. The
349 growth of the exposed animals was similar to that of the controls.

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

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

360 3.4.9 Relevant *in vitro* and *in silico* data

361 In an effort to further support the mechanistic argument for this read-across information form
362 two new methods were examined. Specifically, relevant HTS data in the form of ToxCast data
363 [47, 48] and of *in silico* nuclear receptor binding predictions [49] were evaluated. Within the
364 USEPA toxicity forecaster program (ToxCast) [50], data are available for the majority of the n-
365 alkanol derivatives (see Table 8 of the supplemental information). Of the 711 possible assays
366 that form the ToxCast scheme, 1-octanol, 1-undecanol, 1-dodecanol and 1-tridecanol have been
367 evaluated in 602 of them. Additionally, 1-hexanol, 1-heptanol and 1-decanol have been assessed
368 in about 250 assays. Lastly, 1-nonanol has been tested in 150 ToxCast assays. The number of
369 active assays varies from none for 1-octanol to 25 for 1-undecanol and 30 for 1-tridecanol.
370 Within ToxCast, the n-alkanols are among the “least promiscuous chemical classes”; < 2.74% of
371 the ToxCast assays showing any activity up to highest concentration tested and none of the
372 active assay are associated with specific bioactivity.

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

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

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

395

396 **4. Statement of uncertainty**

397 The categorical assessments of uncertainties along with summary comments are presented in
398 Tables 4 and 5. Briefly, chemical similarity is limited by chain length but has no impact on
399 repeated-dose toxicity. Data uncertainty with the fundamental aspects of toxicokinetics is low.
400 Regardless of the species of mammals, all such category members are judged to be readily
401 absorbed orally and to have similar distributions; metabolised via oxidation to the acid

402 derivative, subsequently degraded to CO₂ via mitochondrial oxidation, and/or eliminated as a
 403 glucuronide. Data uncertainty with the fundamental aspects of toxicodynamics is low, in that
 404 category members exhibit a very low-toxic profile with respect to *in vivo* effects (i.e., NOEL and
 405 LOEL), as well as with respect to *in vitro* and new-methods effects. n-Alkanols are
 406 experimentally associated with the nonpolar narcosis mechanisms of toxicity. The simple
 407 narcosis (i.e., reversible anaesthesia) mode of toxic action is driven by partitioning into the
 408 biophase. While well-studied, this molecular mechanism is not well-understood and no adverse
 409 outcome pathway (AOP) is currently available. Moreover, it is unclear if oral repeated-dose
 410 toxicity is related to this mechanism; however, there is no evidence to suggest it is not.
 411 Uncertainty associated with mechanistic relevance and completeness of the read-across (i.e.,
 412 uncertainty in the predictions) while initially low-to-moderate is reduced to low with the addition
 413 of ToxCast and *in silico* screening data. The major source of uncertainty for this group of
 414 alcohols is associated with what is essentially a “low-toxic” prediction.

415 **Table 4.** Assessment of data uncertainty and strength-of-evidence associated with the
 416 fundamentals of chemical, transformation/toxicokinetic and toxicodynamic similarity.
 417

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/toxicokinetics and	Empirical: <i>in vivo</i> : low	medium	While <i>in vivo</i> 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	<i>in vitro</i> : 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 <i>in silico</i> metabolic simulations, metabolites from oxidation and hydroxylation are predicted to be produced by all the category members.
Toxicophores /mechanistic alerts	medium	high	Based on <i>in silico</i> 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, <i>in vivo</i> , <i>in vitro</i> and <i>ex vivo</i> endpoints	low	high	Although not directly related to the repeated-dose endpoint, many category members have been tested for <i>in vivo</i> acute effects in rodents and fish. In addition, many category members have been tested <i>in vitro</i> 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 <i>in silico</i> profilers.

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

419

420 ^b Consistency within the information/data used to support the similarity rationale and prediction (low, medium, high)

421

422

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

424

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.
<i>In vivo</i> data read-across		
Number of analogues in the source set	Low; 5 of 9 analogues	While there are five tested category members, two 1-pentanol and 1-hexanol, have high quality <i>in vivo</i> 90-day, oral repeated-dose data usable for read-across.
Quality of the <i>in vivo</i> apical	Low; consistent lowest observed	Generally, the <i>in vivo</i> data are consistent with regard to qualitative description of repeated-dose effects. LOEL effects 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 <i>in vivo</i> 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 <i>in vivo</i> 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 <i>in vivo</i> and <i>in vitro</i> 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 <i>in vivo</i> data is supported by similar data for other analogues adds to the WoE. The fact that there is consistent relevant <i>in vitro</i> 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 <i>in silico</i> receptor-binding profilers add to the WoE.

425 ^a Uncertainty: low, medium, high

426 5. Statement of the conclusions

427 This is the second in a series of read-across case studies; this particular study examines a
428 category of similar compounds that do not require (or do not undergo) metabolism to exert a
429 potential adverse human health effect [51]. *In vivo* oral repeated-dose exposure to n-alkanols
430 gives rise to a set of nonspecific symptoms, including clinical symptoms, haematological values
431 outside the normal range, or whole body effects different from normal. Limiting the category to
432 C5 to C13 analogues assures that the impact of bioavailability on the toxicokinetic and

433 toxicodynamic profiles is very limited. Primary alkanols are direct-acting toxicants with a
434 reversible mode of toxic action described as nonpolar narcosis (i.e., unspecific interaction with
435 biological membrane in a manner similar to simple anaesthetics). The main route of exposure for
436 alkanols is oral via rapid gastrointestinal absorption. The majority of an oral dose of any n-
437 alkanol is promptly degraded via simple cellular oxidation; the remainder is eliminated as the
438 glucuronide conjugate.

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

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

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

453

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461

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601

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	CCCCCCCCCO		C10H22O
7	1-Undecanol	112-42-5	CCCCCCCCCO		C11H24O
8	1-Dodecanol	112-53-8	CCCCCCCCCO		C12H26O
9	1-Tridecanol	112-70-9	CCCCCCCCCO		C13H28O

Table 2: Comparison of Physico-Chemical and Molecular Properties¹

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

¹Values 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					

^aWilliams, R.T. 1959. The metabolism of some aliphatic aldehydes, ketones and acids. In: Detoxication mechanisms. The metabolism and detoxication of drugs, toxic substances and other organic compounds, 2nd Ed., London: Chapman & Hall, Ltd., chapter four, pp. 88-113;

^bOpdyke, D.L. 1973. Monographs on fragrance raw materials. Food Cosmet. Toxicol. 11: 95-115; ^cKwok, E.S.C. and Atkinson, R., 1994. Gas-phase atmospheric chemistry of dibenzo-pdioxin and dibenzofuran. Environ.Sci.Technol. 28:528-533; ^dAtkinson, R. 1994. Gas-phase tropospheric chemistry of organic compounds. J. Phys. Chem. Ref. Data, Monograph 2:1-216. ^eKamil, I.A., Smith, J.N. and Williams, R.T. 1953. Studies in detoxication. 46. The metabolism of aliphatic alcohols. The glucuronic acid conjugation of acyclic aliphatic alcohols. Biochem. J. 53: 129-136.

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	Toxicophores¹	DNA binding by OECD¹	Protein binding by OECD¹	Nuclear receptor binding²	Liver & Mitochondria toxicity²
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) [11,47,63,64]	5600-17200 (mg/kg) [48]
Endpoint: LDLo (Acute toxicity)	122-2000(mg/kg) [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 aberration, 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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