

Article

Read-across of 90-Day Rat Oral Repeated-Dose Toxicity: A Case Study for Selected 2-Alkyl-1-alkanols

Schultz, T.W, Przybylak, P.R, Richardz, A-N, Mellor, Claire, Bradbury, S.P and Cronin, M.T.D

Available at <http://clock.uclan.ac.uk/17640/>

Schultz, T.W, Przybylak, P.R, Richardz, A-N, Mellor, Claire ORCID: 0000-0002-7647-2085, Bradbury, S.P and Cronin, M.T.D (2017) Read-across of 90-Day Rat Oral Repeated-Dose Toxicity: A Case Study for Selected 2-Alkyl-1-alkanols. Computational Toxicology .

It is advisable to refer to the publisher's version if you intend to cite from the work.

<http://dx.doi.org/10.1016/j.comtox.2017.02.005>

For more information about UCLan's research in this area go to <http://www.uclan.ac.uk/researchgroups/> and search for <name of research Group>.

For information about Research generally at UCLan please go to <http://www.uclan.ac.uk/research/>

All outputs in CLoK are protected by Intellectual Property Rights law, including Copyright law. Copyright, IPR and Moral Rights for the works on this site are retained by the individual authors and/or other copyright owners. Terms and conditions for use of this material are defined in the <http://clock.uclan.ac.uk/policies/>

1 **Read-across of 90-Day Rat Oral Repeated-Dose Toxicity:**

2 **A Case Study for Selected 2-Alkyl-1-alkanols**

3
4 Terry W. Schultz^{a*}, Katarzyna R. Przybylak^b, Andrea-Nicole Richarz^b, Claire L. Mellor^b,
5 Steven P Bradbury^c and Mark T. D. Cronin^b
6

7 ^aThe University of Tennessee, College of Veterinary Medicine, 2407 River Drive, Knoxville,
8 TN 37996-4543 USA; ^bLiverpool John Moores University, Byrom Street, L33AF Liverpool
9 United Kingdom; ^cDepartment of Natural Resource Ecology and Management Department of
10 Entomology Toxicology Graduate Program Iowa State University Ames, Iowa 50011, USA
11

12 *Corresponding author: Terry W. Schultz, email: tschultz@utk.edu
13

14 **Abstract:** 2-Alkyl-1-alkanols offer an example whereby the category approach to read-across
15 can be used to predict repeated-dose toxicity for a variety of derivatives. Specifically, the
16 NOAELs of 125 mg/kg bw/d for 2-ethyl-1-hexanol and 2-propyl-1-heptanol, the source
17 substances, can be read across with confidence to untested 2-alkyl-1-alkanols in the C5 to C13
18 category based on a LOAEL of low systemic toxicity. These branched alcohols, while non-
19 reactive and exhibiting unspecific, reversible simple anaesthesia or nonpolar narcosis mode of
20 toxic action, have metabolic pathways that have significance to repeated-dose toxic potency. In
21 this case study, the chemical category is limited to the readily bioavailable analogues. The
22 read-across premise includes rapid absorption via the gastrointestinal tract, distribution in the
23 circulatory system and first-pass metabolism in the liver via Phase 2 glucuronidation prior to

24 urinary elimination. 2-Ethyl-1-hexanol and 2-propyl-1-heptanol, the source substances, have
25 high quality 90-day oral repeated-dose toxicity studies (OECD TG 408) that exhibit qualitative
26 and quantitative consistency. Findings include only mild changes consistent with low-grade
27 effects including decreased body weight and slightly increased liver weight, which in some
28 cases is accompanied by clinical chemical and haematological changes but generally without
29 concurrent histopathological effects at the LOAEL. These findings are supported by results
30 from the TG 408 assessment of a semi-defined mixture of isotridecanols. Chemical similarity
31 between the analogues is readily defined and data uncertainty associated with toxicokinetic and
32 toxicodynamics similarities are low. Uncertainty associated with mechanistic relevance and
33 completeness of the read-across is reduced by the concordance of *in vivo* and *in vitro* results, as
34 well as high throughput and *in silico* methods data. As shown in detail, the 90-day rat oral
35 repeated-dose NOAEL values for the two source substances can be read across to fill the data
36 gaps of the untested analogues in this category with uncertainty deemed equivalent to results
37 from a TG 408 assessment.

38 **Keywords:** read-across, n-alkanols, repeated-dose toxicity, No Observed Adverse Effect Level
39 (NOAEL), Lowest Observed Adverse Effect Level (LOAEL), weight-of-evidence (WoE),
40 uncertainty

41

42

43 **Highlights:**

- 44 • The category is limited to readily bioavailable 2-alkyl-1-alkanols of intermediate size
45 (C5 to C13)
- 46 • 2-Alkyl-1-alkanols are toxicants acting via a simple narcosis mechanism
- 47 • Toxicokinetically and toxicodynamically, the 2-alkyl-1-alkanols are highly similar
- 48 • 2-ethyl-hexanol and 2-propyl-1-heptanol can be read across to other analogues with
49 acceptable uncertainty

50

51 **1 Introduction**

52 1.1 Read-across

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

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

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

68 As such, the current study describes a case that illustrates a number of issues associated with a
69 category approach for the scenario in which metabolism, while straight forward, is important in
70 determining molecular similarity. Thus, establishing toxicodynamic, as well as toxicokinetic

71 similarity, is critical to reducing uncertainties associated with the repeated-dose toxicity
72 predictions.

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

77 1.2 C5-C13 2-alkyl-1-alkanols: Overview of Existing Knowledge

78 As previously noted [2], intermediate chain-length primary alkanols are considered non-polar
79 narcotics which act mechanistically in a manner similar to depressant anaesthetics. Perfused rat
80 liver toxicity data from Strubelt et al. [7] for the C5 primary alkanol exposure of 65.1 mmol/l
81 for 2 hours suggests that 2-alkyl-1-alkanols may not be in the same read-across category as
82 other primary alkanols (Table 1). These data support the premise that *in vitro* toxicity (e.g., O₂
83 consumption and ATP production) of 2-alkyl-1-alkanols is due, in large part, to loss of
84 membrane integrity, as indicated by cytosolic enzyme (LDH) leakage. While it is likely that
85 enzyme leakage is the result of alteration in membrane fluidity due to partitioning in the cell
86 membrane, loss of membrane integrity as a result of soft electrophilic reactivity and indicated
87 by a 50% reduction in free glutathione (GSH) is not likely.

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

Name	log Kow	O ₂ Consumption ($\mu\text{mol/g} \times \text{min}$)	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
2-Methyl-1-butanol	1.30	0.30 \pm 0.03	0.10 \pm 0.01	20521 \pm 1087	1.33 \pm 0.29
3-Methyl-1-butanol	1.16	0.22 \pm 0.07	0.27 \pm 0.05	8680 \pm 1216	2.27 \pm 0.37
1-Pentanol	1.40	0.06 \pm 0.01	0.20 \pm 0.03	28959 \pm 4142	2.82 \pm 0.36

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

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

96 Among the 2-alkyl-1-alkanols, 2-ethyl-1-hexanol is the most widely studied [8, 9, 10, 11, 12].

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

101 Metabolism of 2-alkyl-1-alkanols, while highly efficient, involves processes that are more
102 complex than n-alkanol metabolism. Experimental data reveals the major pathways of
103 metabolism and fate of 2-alkyl-1-alkanols include: 1) conjugation of the alcohol group with
104 glucuronic acid; 2) oxidation of the alcohol group; 3) side-chain oxidation yielding additional
105 polar metabolites, which may be subsequently conjugated and be excreted or further oxidised,
106 and 4) excretion of the unchanged parent compound. For example, in rabbits, the glucuronide
107 of 2-ethyl-1-hexanoic acid was identified as the main metabolite (87%) after oral application of
108 2-ethyl-1-hexanol [15, 16]. In contrast, in the same species, only about 9% of the administered
109 dose of 2-methyl-1-butanol was found in the form of the glucuronides [15, 16].

110 Belsito et al. [14] reviewed the toxicity of branched chain saturated alcohols, including
111 secondary ones. Patocka and Kuca [17] summarized the toxicity of C1 to C6 alkanols. The

112 efficacy of alkanols to induce ataxia [18] and enzyme release from liver cells [19] has been
 113 interpreted as being due to the hydrophobic property of the alkanols. Based on rat and fish
 114 studies, 2-alkyl-1-alkanols, like other alkanols, act in a manner similar to depressant
 115 anaesthetics [20, 21]. Koleva et al. [22] reported multiple-regression type quantitative
 116 structure-toxicity relationships (QSARs) for oral log LD50⁻¹ data for rodents and the 1-
 117 octanol/water partition coefficient (log Kow). Comparison of measured toxicity data with
 118 predictions from baseline QSARs reveals that straight-chain and branched, saturated
 119 monohydric alcohols consistently behave as classic nonpolar narcotics.

120 A cursory summary of the rodent oral acute and oral repeated-dose toxicity of intermediate size
 121 2-alkyl-1-alkanols are presented in Table 2. In general, 2-alkyl-1-alkanols acute oral toxicity
 122 (LD50) is very low ranging from ≈2000 to < 5000 mg/kg bw with an average value of ≈3500
 123 mg/kg bw.

124 **Table 2.** Acute and repeated-dose oral toxicity of selected 2-alkyl-1-alkanols

Alcohol	Species	Oral LD50 (mg/kg)	Reference	90-d Oral NOAEL (mg/kgbw/d)	Reference
2-Methyl-1-butanol	Rat	4010	[23]	Not determined	
2-Methyl-1-pentanol		Not determined		Not determined	
2-Ethyl-1-butanol	Rat	1850	[24]	Not determined	
2-Ethyl-1-pentanol	Rat	Not determined		Not determined	
2-Ethyl-1-hexanol	Rat	>3730	[25]	125	[26, 27]
	Rat	≈2000	[27]	Not determined	
	Mouse	2500	[28]	125	[26]
2-Propyl-1-pentanol		Not determined		Not determined	
2-Methyl-1-octanol		Not determined		Not determined	
2-Ethyl-1-octanol		Not determined		Not determined	
2-Propyl-1-heptanol	Rat	5400	[29]	150	[29]
2-Methyl-1-undecanol		Not determined		Not determined	
2-Ethyl-1-decanol		Not determined		Not determined	
2-Propyl-1-decanol		Not determined		Not determined	

126 2-Alkyl-1-alkanols are slightly toxic in oral repeated-dose testing; typically, the rodent, oral,
127 90-day, repeated-dose No Observed Adverse Effect Level (NOAEL) in mg/kg bw/d is ≥ 125
128 mg/kg bw/d (see Table 2). This value is characteristically based on clinical symptoms,
129 haematological values outside the normal range, or whole body effects different from normal.
130 However, if ingested in large enough quantities, alkanols may cause systemic damage to the
131 liver, heart, kidneys, and/or nervous system.

132 **2 Method and Materials**

133 This evaluation of selected 2-alkyl-1-alkanols follows the workflow of Schultz et al. [2]. It is in
134 accord with the guidance proposed by Organization for Economic Co-Operation and
135 Development (OECD) [30] and Schultz and co-workers [6]. *In vivo* data used in the assessment
136 were taken from the literature, including ECHA REACH Registered Substances database [31].
137 Mechanistic relevance, as well as toxicokinetic and toxicodynamic similarity of the category
138 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 ten target and two source
141 chemicals; the latter, those with repeated-dose data derived from a 90-day OECD TG 408
142 assay, are noted in bold print. This list is not meant to be all inclusive, rather it represents
143 existing industrial organic materials that are likely to be found in a governmental or industrial
144 inventory (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.** 2-Alkyl-1-alkanols considered part of the chemical category. The source chemicals
148 are in bold.

ID	Name	CAS	Molecular formula
1	2-Methyl-1-butanol	137-32-6	C ₅ H ₁₂ O
2	2-Methyl-1-pentanol	105-30-6	C ₆ H ₁₄ O
3	2-Ethyl-1-butanol	97-95-0	C ₆ H ₁₄ O
4	2-Ethyl-1-pentanol	27522-11-8	C ₇ H ₁₆ O
5	2-Ethyl-1-hexanol	104-76-7	C₈H₁₈O
6	2-Propyl-1-pentanol	58175-57-8	C ₈ H ₁₈ O
7	2-Methyl-1-octanol	818-81-5	C ₉ H ₂₀ O
8	2-Ethyl-1-octanol	20592-10-3	C ₁₀ H ₂₂ O
9	2-Propyl-1-heptanol	10042-59-8	C₁₀H₂₂O
10	2-Methyl-1-undecanol	10522-26-6	C ₁₂ H ₂₆ O
11	2-Ethyl-1-decanol	21078-65-9	C ₁₂ H ₂₆ O
12	2-Propyl-1-decanol	60671-35-4	C ₁₃ H ₂₈ O

149

150 2.2 Endpoint

151 The NOAEL for the 90-day rat oral repeated-dose is the single endpoint for which this
152 category approach is applied. The 90-day oral repeated-dose data for 2-ethyl-hexanol and 2-
153 propyl-1-heptanol are particularly well-suited for read-across; the NOAELs are based on
154 experimental results from a 4-dose exposure scenario (0, <100, between 100 and 200 and >
155 500 mg/kg bw/d) following a standard test guideline (OECD TG 408) where the LOAEL
156 symptoms are reported.

157 2.3 Hypothesis of the category

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

- 159 • 2-Alkyl-1-alkanols of intermediate chain length (i.e., C₅ to C₁₃) are direct-acting
160 toxicants (i.e., metabolic activation and detoxification is not a major factor in toxicity)
161 with a similar reversible mode of action (i.e., non-polar narcosis or simple anaesthesia).
- 162 • The chemical category is based on simple structure similarities- C-atom chain length
163 and 2-alkan-1-ol hydrocarbon scaffolding.

- 164 • With C5 to C13 2-alkanol-1-ol derivatives, C-atom chain length affects most physico-
165 chemical properties with property values increasing with increasing chain length.
166 However, this trend is not toxicologically significant and does not significantly affect
167 bioavailability in sub-chronic oral exposure.
- 168 • These 2-alkyl-1-alkanols are rapidly and nearly completely absorbed from the gut and
169 distributed in the blood in solution; first past metabolism leads to glucuronidation with
170 subsequent elimination in the urine and/or oxidative metabolism in the liver resulting in
171 a carboxylic acid, which subsequently undergoes mitochondrial β -oxidation, and/or
172 resulting in additional polar metabolites which are glucuronidated prior to excretion in
173 the urine.
- 174 • Toxicodynamically, these 2-alkyl-1-alkanols are highly similar. Briefly, *in vivo* they
175 exhibit low systemic toxicity and *in vitro* they exhibit no chemical reactivity or
176 receptor-mediated interactions.
- 177 • Repeated-dose tested NOAEL data for 2-ethyl-hexanol and 2-propyl-1-heptanol can be
178 read across to other category members listed in Table 3 with acceptable uncertainty.

179 **3. Results**

180 3.1. Read-across Justification

181 3.1.1 Rodent repeated-dose toxicity for 2-ethyl-1-hexanol

182 From a repeated-dose perspective, 2-ethyl-1-hexanol is well-studied. More specifically, in a
183 90-day study similar in design to an OECD TG408, Fischer F344 rats were administered doses
184 of 0, 25, 125, 250 or 500 mg 2-ethyl-1-hexanol/kg bw/d by gavage [32]. A NOAEL of 125

185 mg/kg bw/d based on reduced body weight and body weight gain, changes in blood chemistry
186 were reported.

187 A second sub-chronic gavage study is reported by the same authors [33] in which Fischer rats
188 were exposed to doses of 0, 25, 250 and 500 mg/kg bw/d. Relative weight changes are reported
189 for kidney and liver, as well as a decrease of alanine aminotransferase at 250 mg/kg bw/d.
190 Further weight changes occurred in brain, testes and stomach at highest dose, together with a
191 slight decrease in body weight. Changes in clinical chemistry parameters were reported,
192 including an increased activity of the enzyme palmitoyl coenzyme A activity (pCoA), decrease
193 of cholesterol, total protein and albumin, as well as an increase in reticulocytes. Since no doses
194 between 25 and 250 were tested, the NOAEL of this study is 25 mg/kg bw/d.

195 In a chronic Fischer F344 rat study, 2-ethyl-1-hexanol was administered by gavage at doses of
196 0, 50, 150 or 500 mg/kg bw/d, 5 days per week for 2 years [34]. Food consumption, body
197 weights, and haematological parameters were examined at specific intervals during the study.
198 At the end of the study, gross and histopathological examinations were conducted. No
199 treatment-related adverse effects were observed at the 50 mg/kg bw/d dose level. At the 150
200 mg/kg bw/d dose level, rats exhibited a body weight gain reduction of approximately 16% in
201 males and 12% in females. An increase of brain and liver weight also is reported. However, no
202 histopathological changes were observed at same or higher doses. In addition, the rats also
203 displayed a slightly increased incidence of clinical signs, such as poor general condition and
204 laboured breathing. We conclude that the NOAEL for this study is 150 mg/kg bw/d.

205 Shorter-term repeated dose studies are also available for 2-ethyl-1-hexanol. In an 11-day study,
206 Fischer 344 rats were exposed by gavage at doses of 0, 100, 330, 1000 and 1500 mg/kg bw/d
207 [35]. From 330 mg/kg bw/d on, atrophy of the thymus was reported being most pronounced at

208 1500 mg/kg bw/d. At 1000 mg/kg bw/d a decrease in reticulocytes and clinical chemistry
209 parameters such as cholesterol, glucose and ALAT was reported, as well as a marked
210 inflammation of the forestomach. At highest tested dose, additional adverse effects were
211 reported, including focal hepatocellular necrosis, hepatocellular hypertrophy and several organ
212 weight changes. Transient clinical signs were reported at 1000 and 1500 mg/kg bw/d, namely
213 ataxia, lethargia and lateral and abdominal posturing. A NOAEL of 100 mg/kg bw/d was
214 determined.

215 A second short-term gavage study was done with Fischer rats exposed to doses of 0, 100, 320
216 and 950 mg/kg bw/d for 28 days [36]. At the highest dose of 950 mg/kg bw/d body weight gain
217 was reduced and kidney and liver weight and triglycerides were increased. At 320 mg/kg bw/d
218 an induction of peroxisome proliferation was observed, as well as hepatic cyanide-insensitive
219 palmitoyl coenzyme A activity (pCoA). At 100 mg/kg bw/d a reduction of neutral lipids in
220 liver is reported; however, we do not consider this toxicologically relevant and, thus, we
221 conclude the NOAEL for this study to be 100 mg/kg bw/d.

222 In a 90-day study, B6C3F1 mice received doses of 0, 25, 125, 250 or 500 mg 2-ethyl-1-
223 hexanol/kg bw/d [26] and the 90-day oral NOEL was noted as 125 mg/kg bw/d.

224 In another B6C3F1 mouse study, 2-ethyl-1-hexanol mice were administered by gavage at doses
225 of 0, 50, 200 or 750 mg/kg bw/d, five days per week for 18 months [32]. Food consumption,
226 body weights and haematological parameters were examined at specific intervals during the
227 study. At the end of the study, gross and histopathological examinations were conducted.

228 While no treatment-related adverse effects were observed in the mice receiving 50 or 200 mg
229 2-ethyl-1-hexanol/kg bw/d, at the 750 mg/kg bw/d dose level, body weight gain reductions of
230 approximately 26 and 24% in males and females, respectively. Further high dose effects

231 consist of changes in haematology (lymphocytes, neutrophil increase after 12 months), weight
232 changes of different organs (kidney, liver), and hyperplasia in the forestomach. We conclude
233 the NOAEL for this study to be 200 mg/kg bw/d.

234 3.1.2 Rodent repeated-dose toxicity for 2-propyl-1-heptanol

235 In an OECD TG 408 test, oral 90-day repeated-dose assay, male and female Fischer 344 rats
236 were exposed via gavage to 0, 30, 150 and 600 mg/kg bw/d of 2-propyl-1-heptanol [29].
237 Histopathological findings at 600 mg/kg bw/d include diffuse liver hypertrophy, likely the
238 result of peroxisome proliferation, diffuse hypertrophy of follicular cells in the thyroid gland,
239 and vacuolation of basophilic (thyrotropic) cells in the glandular part of the pituitary gland.
240 Additionally, alterations based on clinical signs were observed at 600 mg/kg bw/d.
241 Disregarding peroxisomal proliferation, the NOAEL for this study was 150 mg/kg bw/d.

242 3.1.3 Other related rodent repeated-dose studies

243 Isotridecanol (i.e., C13-rich mixture of iso-alcohols of C11-14, CAS No. 68526-86-3) was
244 tested by gavage to Sprague–Dawley rats [14]. In a 90-day study, according to OECD TG 408
245 with doses of 0, 100, 500, or 1000 mg/kg bw/d, the NOAEL of 100 mg/kg bw/d was reported
246 [14].

247 While ECHA CHEM notes a reliable read-across from 3-methyl-1-butanol to 2-methyl-1-
248 butanol, the current study disregarded these data. This decision was based on the finding of
249 Strubelt and co-workers. [7]. Data (see Table 1) for the C5 primary alkanols exposure 65.1
250 mmol/l for 2 hours suggest that 2-methyl-1-butanol may not be in the same read-across
251 category as 3-methyl-1-butanol or n-pentanol.

252 In summary, two 2-alkyl-1-alkanols (i.e., 2-ethyl-hexanol and, 2-propyl-1-heptanol) have high
253 quality quantitative (e.g., OECD TG 408) 90-day oral exposure repeated-dose test data. These
254 data exhibit qualitative and quantitative consistency between and within rodent species.
255 Specifically, results of oral repeated-dose testing for these two source substances suggest mild
256 changes consistent with low-grade effects, including decreased body weight, accompanied by
257 clinical chemical and haematological changes but generally without concurrent
258 histopathological effects. While it can be argued that these effects are not adverse, we still
259 considered them in determining the NOAEL. The 90-day oral exposure repeated-dose NOAEL
260 values ≥ 125 mg/kg bw/d are based on experimental results from a four dose exposure
261 scenario, typically 0, <100, between 100 and 200, >200 and ≥ 500 . While there is not repeated-
262 dose toxicity data for 2-methyl derivatives, they are included in the category.

263 3.2. Applicability domain

264 As previously noted, the applicability domain for this case study is confined to branched
265 primary alkanols of intermediate size, C5 to C13. Straight-chain derivatives, which exhibit a
266 different toxicokinetic profile, are excluded from this chemical category. Briefly, metabolism
267 of straight-chain saturated alcohols resulting in the corresponding carboxylic acid, which
268 subsequently undergoes mitochondrial β -oxidation to CO₂ with only minor amounts of Phase 2
269 glucuronidation [2].

270 3.3. Purity/impurities

271 A purity/impurity profile for the analogues listed in Table 3 is not reported. No effort was
272 made to take into account impurities based on production. Since the category is structurally

273 limited, the impurities are expected to be similar if not the same across the members and are
274 not expected to significantly impact the toxicity profile of any analogue.

275 3.4 Data matrices for assessing similarity

276 As earlier noted, in order for a read-across prediction to be accepted, there is the requirement to
277 establish similarity between the source and target substance; toxicokinetic similarity, especially
278 for metabolism, and toxicodynamic similarity, especially in regard to mechanistic plausibility
279 is required for repeated dose-toxicity endpoints [1, 2].

280 3.4.1 Structural similarity

281 As demonstrated in Tables 1 and 3 of the supplemental information, all the branched alkanols
282 included in the category are structurally highly similar. Specifically, they: 1) belong to a
283 common chemical class, aliphatic alcohols and the subclasses primary alkanols and 2-alkyl-1-
284 alkanols, and 2) possess a similar molecular scaffolding, a C-atom backbone with alkyl
285 branching in the 2-position. Structurally, the main variations are the length of the backbone,
286 C5-C11 and the length of the alkyl-substituent, C1-C3.

287 3.4.2 Chemical property similarity

288 As demonstrated in Table 2 of the supplemental information, all the primary alkanols included
289 in the category have a large portion of their physio-chemical properties determined
290 experimentally. Properties, with the exception of density and pKa, trend in values related to C-
291 atom number within a scaffold. Specifically, all category members exhibit molecular weights
292 from 88 to 200 g/mol. While hydrophobicity (log Kow) increases with number of C-atoms
293 from >1.0 to <6.0, density and pKa are constant at 0.8 g/cm³ and 15. While vapour pressure

294 and water solubility decrease with molecular size, melting point and boiling point increase with
295 molecular size.

296 3.4.3 Chemical constituent similarity

297 As demonstrated in Table 3 of the supplemental information, all the branched primary alkanols
298 included in the category have common constituents in the form of: 1) a single key substituent,
299 OH, and 2) structural fragments, CH₃, CH₂ and CH.

300 3.4.4 Toxicokinetic similarity

301 As demonstrated in Table 4 of the supplemental information, while the analogues tested are
302 limited, the toxicokinetic understanding of 2-position branched primary alkanol is fairly
303 complete. Two-alkyl-1-alkanols are rapidly absorbed following oral administration [13] and
304 are rapidly excreted [37]. Data for 2-ethyl-1-hexanol and to a lesser extent 2-methyl-1-butanol
305 and 2-ethyl-1-butanol demonstrate that branched primary alcohols exhibit common metabolic
306 pathways. These metabolic pathways include oxidation of the alcohol group and oxidation of
307 the side chain at various positions, glucuronidation of the oxidation products and
308 decarboxylation [37]. Glucuronidation increases with increased chain length of the alkanols
309 [38].

310 Two adult male CD-strain rats (300 g) were gavaged with radiolabeled 2-ethyl-1-¹⁴C-hexanol
311 (¹⁴C- labeled 2-ethyl-1-hexanol; 1 μCi; 8.8 μg) in cotton seed oil. Two others were given the
312 same amount of ¹⁴C-EH and cotton seed oil but also were given 0.1 ml (0.64 mmol) of
313 unlabeled 2-ethyl-1-hexanol. Following administration, rats were housed in metabolism cages
314 and expired CO₂, urine, and faeces were collected every hour for 28 hrs. Most (99.8%) of the

315 orally administered radioactivity was accounted for by radioactivity in expired CO₂, urine,
316 faeces, an ethanol wash of the metabolism cage at the end of the experiment, heart, brain, liver,
317 kidneys, and "residual carcass". Two-ethyl-1-hexanol was efficiently absorbed following oral
318 administration and rapidly excreted in respired CO₂ (6-7%), urine (80-82%), and faeces (8-
319 9%); elimination was essentially complete by 28 hrs [10, 27, 37].

320 Deisinger et al. [39, 40] examined the elimination of ¹⁴C-labeled 2-ethyl-1-hexanol in rats.
321 After oral administration to rats, 69-75% of a dose of 500 mg ¹⁴C-labeled 2-ethyl-1-hexanol/kg
322 bw was excreted in the urine within 96 hours; about 13 to 15% of the dose was excreted in the
323 faeces and about the same amount was exhaled as ¹⁴C-labeled CO₂. After intravenous
324 administration to rats, about 74% of a dose of 1 mg ¹⁴C-labeled 2-ethyl-1-hexanol/kg bw was
325 excreted in the urine within 96 hours. About 4% of the dose was excreted in the faeces and
326 23% was exhaled. More than 50% of the dose was excreted within 8 hours and the terminal
327 half-life was estimated to be 60 hours [39, 40].

328 Haggard et al. [41] examined the metabolic fate of 2-methyl-1-butanol in rats. Specifically,
329 intraperitoneal injection in four equal doses of 250mg/kg bw at 15-min intervals resulted in a
330 maximum blood concentration of 550 mg/l. Blood concentration decreased over the next nine
331 hours. Of the total dose of 1000mg/kg bw, only 5.6% was excreted in air and 2% in the urine.
332 The remainder was metabolised, first to the corresponding aldehyde and then to the acid [41].
333 After a single oral dose of 25 mmoles of 2-methyl-1-butanol to rabbits [15], 9.6% of the dose
334 was excreted in the urine as glucuronides. Glucuronide excretion occurred within 24 hours, the
335 urine did not contain aldehydes or ketones. Iwersen and Schmoldt [42] studied the alcohol
336 dehydrogenase-independent metabolism of aliphatic alcohols (oxidation and glucuronidation).
337 Briefly, male Sprague-Dawley rats were pre-treated with 10% ethanol in the drinking water for

338 two weeks. Rats were sacrificed and microsomes were prepared for glucuronidation
339 experiments and trials, as well as oxidation experiments with aliphatic alcohols. *In vitro*
340 experiments have demonstrated additional oxidation of 2-methyl-1-butanol by rat liver
341 microsomes via CYP P450 enzymes and glucuronidation. At very low ethanol concentrations
342 (5-10 mmol/L) competitive inhibiting effect of ethanol on oxidation of 2-methyl-1-butanol was
343 observed [42].

344 A rabbit was given 2.55g of 2-ethyl-1-butanol and the 24-hr urine was collected [16]. 2-Ethyl-
345 1-butanol was excreted mainly as glucuronides, along with a minor amount of methyl n-propyl
346 ketone.

347 3.4.5 Metabolic similarity

348 As demonstrated in Table 5 of Annex I with data from *in silico* predictions, it is highly likely
349 that all of the category members undergo successive oxidation to their corresponding aldehyde
350 and carboxylic acid [43, 44].

351 Kamil et al. [15, 16] examined the metabolic fate of 2-methyl-1-hexanol in rats. Via acid
352 extraction of urine, the major urinary metabolite of 2-ethyl-1-hexanol was revealed to be 2-
353 ethyl hexanoic acid. This metabolite may undertake partial β -oxidation and decarboxylation to
354 produce $^{14}\text{CO}_2$ and 2- and 4-heptanone (in the urine). Other urinary metabolites identified in
355 this study were 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-5-ketohexanoic acid, and 2-ethyl-1,6-
356 hexanedioic acid. Approximately 3% of the parent compound was excreted unchanged.
357 Metabolic saturation was seen with 500 mg/kg body weight applied [15, 16].

358 Typically, the presence of a side chain does not terminate the oxidation process of alkanols.
359 However, in most cases, it alters it. The position and size of the alkyl substituent plays a role in

360 metabolism with degradation to CO₂ decreasing and glucuronidation increasing with branching
361 and increasing chain length.

362 Alkyl acids formed during metabolic transformation of branched alkanols have their own set of
363 metabolic pathways. Acids with a methyl substituent located at an even-numbered carbon (e.g.,
364 2-methyl pentanoic acid or 4-methyl decanoic acid) are extensively metabolised to CO₂ via β -
365 oxidative cleavage in the fatty acid pathway. If the methyl group is located at the 3-position, β -
366 oxidation is inhibited and omega (ω -) oxidation predominates, primarily leading to polar,
367 acidic metabolites capable of being further oxidised or conjugated and excreted in the urine
368 [44]. As chain length and lipophilicity increase, ω -oxidation competes with β -oxidative
369 cleavage. Methyl substituted acids (e.g., 3-methylnonanoic acid) are, to some extent, ω -
370 oxidized in animals to form diacids which can be detected in the urine [45].

371 Oxidation of these branched fatty acids is accomplished by alpha (α -) oxidation. α -Oxidation is
372 a complex catabolic process. It initially involves hydroxylation of the α -C atom. Subsequently,
373 the terminal carboxyl group is removed, and there is a concomitant conversion of the α -
374 hydroxyl group to a new terminal carboxyl group. Lastly, there is a linking of CoA to the
375 terminal carboxyl group. This new branched, fatty acyl-CoA functions in the β -oxidation. In
376 humans, α -oxidation is used in peroxisomes to break down dietary branched acids which
377 cannot undergo β -oxidation due to β -methyl branching.

378 Metabolism of methyl-substituted alcohols is determined primarily by the position of the
379 methyl group(s) on the hydrocarbon-chain. Following successive oxidation to the
380 corresponding carboxylic acids, the branched-chain acids are metabolised via β -oxidation.
381 With longer branched-chain derivatives, this is followed by cleavage to yield linear acid
382 fragments which are typically completely metabolised to CO₂. At high-dose levels, the longer

383 branched-chain acids may go through omega-oxidation to yield diacids, which subsequently
384 may undergo further oxidation and cleavage.

385 The presence of an ethyl- or propyl-substitution at the α -position, such as in 2-ethyl-1-hexanol,
386 inhibits β -oxidation [46]. Detoxication pathways of ω - and ω -1 oxidation compete with β -
387 oxidation of these sterically hindered substances; the parent alcohol or corresponding
388 carboxylic acid undergoes a combination of reactions (e.g., ω - or ω -1 oxidation and functional
389 group oxidation) leading to polar, acidic metabolites capable of being excreted in the urine [40,
390 45]. When the principal pathway is saturated, the corresponding carboxylic acid conjugates
391 with glucuronic acid and is excreted in the urine [37, 40, 45].

392 One of the best studied 2-position branched carboxylic acid is 2-propyl pentanoic acid (valproic
393 acid). The toxicokinetic aspects of 2-propyl pentanoic acid have been reviewed [47, 48]. 2-
394 Propyl pentanoic acid is almost entirely metabolised by the liver, so it is not surprising that the
395 liver is also the dominant target organ of toxicity. The multiple metabolic pathways involved in
396 2-propyl pentanoic acid biotransformation give rise to more than 50 known metabolites [47].
397 Ghodke-Puranik and co-workers [48] estimate that, while 30 - 50% of 2-propyl pentanoic acid
398 is excreted in the urine as a glucuronide conjugate, 40% goes through mitochondrial β -
399 oxidation and about 10% undergoes cytochrome P450-mediated oxidation. It has been
400 postulated that the hepatotoxicity of 2-propyl-pentanoic acid results from the mitochondrial β -
401 oxidation of its cytochrome P450 metabolite, 2-propyl-4-pentenoic acid to 2-propyl-(E)-2,4-
402 pentadienoic acid which, in the CoA thioester form, either depletes GSH or produces a putative
403 inhibitor of β -oxidation enzymes. Pent-4-enoate, 2-propyl-4-pentenoic acid and 2-propyl-(E)-
404 2,4-pentadienoic acid are potent inducers of microvesicular steatosis in rats [49]. However,
405 since 2-propyl-pentanoic acid failed to induce discernible liver lesions in young rats, even at

406 near lethal doses of 700 mg/kg/day, Kesterson et al. [49] suggested that β -oxidation inhibition
407 observed in both valproic acid and unsaturated metabolite-treated rats occurred by different
408 mechanisms. Specifically, 2-propyl pentanoic acid inhibits transient sequestering of CoA,
409 while the CoA esters of some metabolites, particularly 2-propyl-4-pentenoic acid, inhibit
410 specific enzyme(s) in the β -oxidation sequences [49].

411 Ghodke-Puranik et al. [48] rationalised the involvement of 2-propyl-4-pentenoic acid.
412 Specifically, 2-propyl-4-pentenoic acid enters the mitochondria, forms a complex with CoA
413 ester and subsequent β -oxidation forms the reactive 2-propyl-(E)-2,4-pentadienoic acid-CoA
414 ester. The latter is the putative cytotoxic metabolite that binds with glutathione to form thiol
415 conjugates. The reactive metabolite, 2-propyl-(E)-2,4-pentadienoic acid-CoA ester, has the
416 potential to deplete mitochondrial glutathione pools and form conjugates with CoA, which in
417 turn inhibits enzymes in the β -oxidation pathway [48].

418 In summary, the experimental toxicokinetic data for 2-alkyl-1-alkanols show consistency in
419 absorption, distribution and metabolic pathways. In contrast, there is less consistency in
420 excretion. In particular, derivatives with 2-position ethyl and propyl groups are more likely to
421 be excreted as a glucuronidated metabolite, while 2-position-methylated analogues are more
422 likely to be oxidized to CO₂. The latter are metabolically similar to the less toxic n-alkanols
423 [2]. The metabolic evidence supporting the idea that some 2-position branched carboxylic acids
424 are metabolised to thiol reactive metabolites is not considered toxicologically relevant to this
425 read-across, as repeated-dose toxicity through a reactive mechanism is considered unlikely as
426 long as the reactive half-life is shorter than the dosing interval (e.g., <8-hr vs. 24-hr) and the
427 Phase 2 conjugation mechanism is not saturated.

428 3.4.6 Toxicophore similarity

429 As shown in Table 6 of the supplemental information, 2-alkyl-1-alkanols themselves do not
430 contain a known toxicophore. However, the carboxylic acid metabolites of the same 2-position
431 branched isomers (e.g., 2-ethyl-1-hexanol and 2-propyl-1-heptanol) are linked to
432 developmental toxicity and chronic oral toxicity via the short-chain carboxylic acid pathway
433 [50].

434 3.4.7 Mechanistic plausibility similarity

435 It is generally accepted that the toxicity of intermediate size 2-alkyl-1-alkanols, like other
436 saturated alcohols, is the result of narcosis. While there is theoretical evidence for the
437 membrane as the site of action for anaesthetic-like 2-alkyl-1-alkanols, biochemical, cellular
438 and physiological evidence is largely restricted to 1-alkanol derivatives [20, 21]. Narcosis, in
439 the broadest sense, is the non-covalent disruption of hydrophobic interactions within
440 membranes with a particular volume fraction rather than molar fraction [51]. It is the
441 accumulation of alcohols in cell membranes which disturbs their function; however, the exact
442 mechanism is not known yet. There are three competing theories of general anaesthetic action:
443 1) the lipid solubility-anaesthetic potency correlation (i.e., the Meyer-Overton correlation); 2)
444 the modern lipid hypothesis and 3) the membrane protein hypothesis.

445 As shown in Table 7 of Annex I, the alkanols included in the category are associated with the
446 simple narcosis mechanism of toxicity that is equivalent to depressant anaesthetics. Measured
447 acute toxicity for 2-alkyl-1-alkanols is consistent with predictions from QSAR models [52, 53]
448 for the nonpolar narcosis mode of action [54].

449 The contributions of functional groups in acute rat oral toxicity have been calculated using
450 alkanes as the baseline [55]. The toxic contribution of alcohols is -0.108. This situation has not
451 been observed in acute fish toxicity because the threshold of excess toxicity is too high to
452 distinguish differences in toxicity. Critical body residues (CBRs) calculated from percentage of
453 absorption and bioconcentration factors indicate that most of aliphatic alcohols share the same
454 modes of toxic action between fish and rat. Specifically, fish and rat log (1/CBR) and number
455 of alcohols are 1.65; 18 and 1.58; 348, respectively [55].

456 It should be noted that some 2-alkyl-1-alkanols are associated with development toxicity via
457 their conversion to the corresponding 2-alkyl-carboxylic acids. The experimental evidence is
458 largely confined to 2-ethyl-1-hexanol and the results are mixed.

459 In rats administered 1600 mg/kg bw 2-ethyl-1-hexanol by gavage (but not 800 mg/kg bw) on
460 day 12 of gestation, Ritter et al. [56] reported a statistically significant increase in the number
461 of teratogenic live fetuses; malformations included hydronephrosis, tail and limb defects.
462 Maternal toxicity was not reported in this study.

463 In another study, Ritter et al. [57] proposed that the teratogen di(2-ethylhexyl) phthalate acts by
464 *in vivo* hydrolysis to 2-ethyl-1-hexanol, which in turn is metabolised to the definitive teratogen
465 2-ethyl-1-hexanoic acid. They conducted teratological studies with Wistar rats administering
466 one of the three agents on day 12 of gestation. Briefly, it was revealed that, on an equimolar
467 basis, the phthalate derivative was least potent, the alcohol derivative was intermediate, and the
468 acid derivative was most potent. Similarity in the types of malformation induced by each
469 derivative suggests a common mechanism of action. *In toto*, these findings are consistent with
470 the hypothesis [57].

471 Two-ethyl-1-hexanol was evaluated for developmental toxicity in mice [58]. There were no
472 effects on any gestational parameters upon exposure to dietary 2-ethyl-1-hexanol. Specifically,
473 the number of corpora lutea, uterine implantation sites (live, dead, resorbed), pre- and post-
474 implantation loss, sex ratio (% males), and live fetal body weight per litter (all foetuses or
475 separately by sex) were all equivalent across all groups. Moreover, there were no maternal
476 toxic effects observed at any of the concentrations tested [58].

477 Tyl et al. [59] examined the developmental toxicity of 2-ethyl-1-hexanol administered
478 dermally. In range-finding (8 females / treatment) and definitive investigations (25 females /
479 treatment), 2-ethyl-1-hexanol was administered by occluded dermal application for 6-hours per
480 day on gestation days 6 through 15 to pregnant Fischer 344 rats. Treatment levels for range-
481 finding were equivalent to 0, 420, 840, 1680, and 2520 mg/kg bw/d; treatment levels for
482 definitive experiments were equivalent to 0, 252, 840, and 2520 mg/kg bw/d. Controls included
483 negative- deionised water, dermal-positive- 2-methoxyethanol and oral reference - valproic
484 acid.

485 For 2-ethyl-1-hexanol, the findings are: 1) maternal weight gain was reduced at the two highest
486 dose levels, 2) maternal liver, kidney, thymus, spleen, adrenal and uterine weights, as well as
487 gestational and foetal parameters were unaffected by any treatment, and 3) there were no
488 treatment-related increases in the incidence of individual or pooled external, visceral, and
489 skeletal malformations or variations. The dermal NOAELs for the maternal toxicity of 2-ethyl-
490 1-hexanol were 252 mg/kg/d based on skin irritation and 840 mg/kg/d based on systemic
491 toxicity. The developmental toxicity NOAEL was at least 2520 mg/kg/d, with no
492 teratogenicity. While the Fischer 344 rat is susceptible to known rodent teratogens, such as 2-
493 methoxyethanol by the dermal route and valproic acid by the oral route, in the Fischer 344 rat,

494 2-ethyl-1-hexanol is not a developmental toxicant by the dermal route at and below treatment
495 levels which produce maternal toxicity.

496 Narotsky et al. [60] studied the developmental toxicity and structure-activity relationships of
497 aliphatic acids in rats. 14 acids were administered by gavage to Sprague-Dawley rats once
498 daily during organogenesis. Only 2-ethyl hexanoic and 2-propyl hexanoic acid caused effects
499 similar to valproic acid (i.e., mortality, extra pre-sacral vertebrae, fused ribs, and delayed
500 parturition) on rat development. Developmental toxicity of α -branched acids is, in part, due to
501 maternal toxicity resulting in alterations in zinc (Zn) metabolism that affects the developing
502 conceptus [61]. Developmentally toxic doses of 2-ethyl hexanoic acid, 2-ethyl-1-hexanol and
503 valproic acid on Zn metabolism were investigated in the pregnant rat. At the higher dose levels
504 of 2-ethyl-1-hexanoic acid, 2-ethyl-1-hexanol, and at all dosages of valproic acid, the
505 percentage of ^{65}Zn retained in maternal liver was higher than controls, while that in the
506 embryos was lower than controls. Two-ethyl-1-hexanoic acid exposed dams fed Zn-containing
507 diets during gestation exhibited a dose-dependent reduction in teratogenic effects.

508 Toxicokinetic parameters are important determinants of teratogenic outcome of α -alkyl-
509 substituted carboxylic acids, which helps explain differing potencies of structurally similar
510 chemicals [62]. Valproic acid (2-propyl-1-pentanoic acid), 2-ethyl-1-hexanoic acid, and 1-
511 octanoic acid are isomeric analogues with markedly different teratogenic potencies. Valproic
512 acid induces moderate to severe malformations after a single oral administration of 6.25
513 mmoles/kg on day 12 of rat pregnancy. Twice as much 2-ethyl-1-hexanoic acid (12.5
514 mmoles/kg) induces a less severe response and 1-octanoic acid is non-teratogenic, even at the
515 higher dose of 18.75 mmoles/kg [62]. While 1-octanoic acid exhibits poor intestinal
516 absorption, the peak concentration and duration of exposure to valproic acid and 2-ethyl-1-

517 hexanoic acid were very similar. A fourth agent, 2-methyl-1-hexanoic acid, which is non-
518 teratogenic when administered orally at 14.1 mmol/kg, exhibits peak concentration and
519 duration of exposure intermediate to 2-ethyl-1-hexanoic acid and 1-octanoic acid. The
520 differences in the severity of developmental malformations for the α -alkyl-substituted
521 derivatives indicated higher intrinsic activity for analogues with C2 and especially, C3 α -alkyl-
522 substituents.

523 In summary, there is reasonable evidence that some 2-alkyl-1-alkanols via oxidation to their
524 corresponding acid are probable development toxicants. However, there is no evidence that this
525 mechanism is related to repeated-dose toxicity.

526 3.4.8 Other endpoint similarity

527 In mammals, alkanols, in general, are considered baseline inhalation toxicants which model as
528 simple narcotics [53].

529 In fish, alkanols are considered to act via the nonpolar narcosis mode of action, as first reported
530 by Veith et al. [52]. Alkanols are also represented within the USEPA DSSTox Fathead
531 Minnow Acute Toxicity (EPAFHM) database. They exhibit toxic potencies not statistically
532 different from baseline predictions. Because of concerns for aquatic toxicity, a large number of
533 alcohols, especially saturated ones, have been tested *in vitro* for cell population growth
534 inhibition [63]. Structure-activity results from *in vivo* and *in vitro* tests are highly consistent
535 [64]. Briefly, from a structural standpoint, the aquatic toxicity of alkanols is partition-
536 dependent, regardless of endpoint being assessed.

537 Generally, *in vitro*, alkanols ascribed to unspecific interactions with biological membranes;
538 such effects are directly correlated with 1-octanol/water partition coefficients [65]. The 2-

539 alkyl-1-alkanols were screened with a variety of *in silico* nuclear receptor binding predictions
540 [66]. Specifically, profilers for nuclear receptor binding were run to identify potential binding
541 to the following nuclear receptors: PPARs (peroxisome proliferator-activated receptors), AR
542 (androgen receptor), AHR (aryl hydrocarbon receptor), ER (oestrogen receptor), GR
543 (glucocorticoid receptor), PR (progesterone receptor), FXR (farnesoid X receptor), LXR (liver
544 X receptor), PXR (pregnane X receptor), THR (thyroid hormone receptor), VDR (vitamin D
545 receptor), as well as RAR/RXR (retinoic acid receptor/ retinoid X receptor). The evaluation of
546 potential binding to the receptors is based on structural fragments and physico-chemical
547 features that have been identified as essential to bind to these nuclear receptors and induce a
548 response. No potential receptor binding was predicted. It is worth noting that ToxCast also
549 tested for all of these receptors, and all assays were negative.

550 HTS data from US EPA's ToxCast [67, 68] are available for a variety of saturated alcohols
551 [69]. Of the 711 assays available in ToxCast ToxCast, 2-ethyl-1-hexanol has been evaluated in
552 602 of them and 2-propyl-1-heptanol has been assessed in about 250 assays. The number of
553 active assays varies, six for 2-ethyl-1-hexanol and four for 2-propyl-1-heptanol. No other
554 category members have been screened by ToxCast. However, alkanols, in general, are one of
555 the least promiscuous chemical classes with < 3% of the ToxCast assays show any activity up
556 to highest concentration tested. None of the active assay are associated with specific bioactivity
557 [2].

558 Taken collectively, the findings for other endpoints are not inconsistent with the previously
559 cited *in vivo* data and the premise that in oral repeated-dose toxicity, 2-alkyl-1-alkanols act in a
560 manner similar to depressant anaesthetics.

561 **4. Statement of uncertainty**

562 The categorical assessments of uncertainties along with summary comments are presented in
563 Tables 4 and 5. 2-Alkyl-1-alkanols are a category with acceptable data uncertainty and robust
564 strengths-of-evidence for repeated-dose toxicity. Briefly, chemical dissimilarity has no impact
565 on repeated-dose toxicity. Data uncertainty with the fundamental aspects of toxicokinetics is
566 low. Regardless of the species of mammal, all such category members are judged to be readily
567 absorbed orally and to have similar distributions metabolism elimination as glucuronides. Data
568 uncertainty with the fundamental aspects of toxicodynamics is low, in that category members
569 exhibit a low-toxic profile with respect to *in vivo* repeated-dose NOAEL and LOAEL values.

570 The uncertainty associated with mechanistic relevance and completeness of the read-across is
571 acceptable. While relevant non-animal data are minimal, the *in vivo* WoE is high. 2-Alkyl-1-
572 alkanols are thought to be associated with the nonpolar narcosis mechanisms of toxicity. While
573 well-studied, this molecular mechanism is not well-understood and no adverse outcome
574 pathway (AOP) is currently available. Moreover, it is unclear if oral repeated-dose toxicity is
575 related to this mechanism; however, there is no evidence to suggest it is not.

576 **Table 4.** Assessment of data uncertainty and strengths-of-evidence associated with the
577 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 and subclass.
Physio-chem & molecular properties	Empirical: Low	High	All category members are appropriately similar with respect to key physicochemical and molecular properties. Where appropriate (e.g., log Kow) changes in values are linked to

Similarity Parameter	Data Uncertainty ^a	Strength-of-Evidence ^b	Comment
	Modelled: Low		changes in C-atom number. 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/t oxicokinetics and metabolic similarity	Empirical: <i>In vivo</i> : Medium <i>In vitro</i> : none Simulated: Medium	Medium	Based on <i>in vivo</i> data for multiple category members, there is evidence for similar toxicokinetics and metabolic pathways. It is extremely likely that absorption and distribution are consistent within the category. It is likely that the metabolic pathways are consistent with the category. Comparison of results from empirical studies and model predictions indicate similar metabolism among category members. Experimental data support the idea that 2-alkyl-alkanols often undergo oxidation of the alcohol group to an acid with degradation to CO ₂ , as well as oxidation or hydroxylation of the alkyl chains at various positions, and subsequent glucuronidation prior to excretion. There is evidence the % of glucuronidation varies within the category; higher % of glucuronidation is associated with 2-position branching > C1. There is also evidence supporting the idea that some 2-position branched carboxylic acids are metabolised to thiol reactive metabolites which exhibit enhanced cellular toxicity. Bioavailability while affected by size is not considered a factor in these predictions.
Potential metabolic products	Simulated: Low	High	Based on <i>in silico</i> metabolic simulations, metabolites from hydroxylation and oxidation are predicted to be produced by any of the category members.
Toxicophores /mechanistic alerts	Medium	High	Based on <i>in silico</i> profilers, no category member contains any established toxicophores related to repeated-dose toxicity.
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 LD50, LC50 and EC50 values. The primary alkanols (both straight-chain and branched) are among the “least promiscuous chemical classes” (i.e., only 104 of 4412 assay are positive) within ToxCast with no positive assay being associated with specific bioactivity. None of the 2-alkyl-1-

Similarity Parameter	Data Uncertainty ^a	Strength-of-Evidence ^b	Comment
			alkanols reveal any propensity for receptor binding within the SEURAT-1 suite of <i>in silico</i> profilers.

578 ^a Uncertainty associated with underlying information/data used in the exercise (empirical, modelled; low, medium,
579 high)
580 ^b Consistency within the information/data used to support the similarity rational and prediction (low, medium,
581 high)

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

Factor	Uncertainty or WoE ^a	Comment
The problem and premise of the read-across	Low	The endpoint to be read across, oral 90-day repeated-dose toxicity, for 2-alkyl-1-alkanols is well-studied and fairly well-understood mechanistically. The scenario of the read-across hinges on metabolism affecting toxic potency but not the mode of toxic action (i.e., reversible narcosis). 2-alkyl-1-alkanols, themselves, have no obvious chemical reactivity, do not bind to any known receptor and exhibit no specific receptor interactions.
<i>In vivo</i> data read across		
Number of analogues in the source set	Low; 2 of 12 analogues	There are two suitable category members (i.e., 2-ethyl-1-hexanol, 2-propyl-1-heptanol) with high quality <i>in vivo</i> 90-day, oral repeated-dose data usable for read-across.
Quality of the <i>in vivo</i> apical endpoint data read across	Low	Generally, the <i>in vivo</i> data are consistent in regards to qualitative description of repeated-dose effects. Lowest observed effects are typically haematological or whole body parameters and not organ-specific effects. High quality empirical data from accepted guidelines for the 90-day repeated-dose endpoint exist for 2-ethyl-1-hexanol and 2-propyl-1-heptanol and are supported by 90-day oral repeated-dose toxicity data for the isotridecano1 mixture.
Severity of the apical <i>in vivo</i> hazard	Low	The consensus is 2-alkyl-1-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 is ≈ 125 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 is 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	Low to medium; limited by indirect rationale (e.g., acute to chronic) of	Since there is no toxicity pathway for repeated-dose effects for this chemical category, there are no true intermediate events. There is agreement among the dose-response relationships of the

apical effects and potency data	mechanistic plausibility.	tested category members for relevant <i>in vitro</i> events.
Weight of Evidence	High/ medium for 2-methyl-1-alkanols	Overall the available information is mainly consistent with the stated premise. The structural limitations (i.e., 2-alkyl-1-alkanols) of the category strengthen the WoE. While the toxicokinetics data is limited, the consistency of the metabolic pathway adds to the WoE. Having two well-studied source substances with highly similar <i>in vivo</i> 90-day repeated-dose data that are supported by similar data for a mixture of C11 to C14 branched alkanols adds to the <i>in vivo</i> WoE. Having both 28-day repeated-dose and chronic (18-month and 2-year) studies for 2-ethyl-1-hexanol with qualitative and quantitative data similar to the 90-day repeated-dose data adds to the <i>in vivo</i> WoE. Having repeated-dose studies for 2-ethyl-1-hexanol with qualitative and quantitative similar data in both rat and mouse data adds to the <i>in vivo</i> WoE. The lack of <i>in vivo</i> repeated-dose data for 2-methyl derivatives reduced the WoE for including these analogues in the category.

584 ^a Uncertainty: low, medium, high

585

586 One observed uncertainty is associated with the fact that, while 2-methyl-substituted

587 derivatives are considered with the domain of the category, there is no *in vivo* experimental

588 data supporting their inclusion. However, there is high quality repeated-dose data for 3-methyl-

589 1-butanol (CAS 123-51-3). In a 90-day study with rats, according to OECD Test Guideline

590 408, 3-methyl-1-butanol was administered in the drinking water in concentrations of 0, ≈80,

591 ≈340 and ≈1250 mg/kg bw/d [70]. A NOAEL of 340 mg/kg bw/d for males and 1250 mg/kg

592 bw/d for females was reported. 3-Methyl-1-butanol was also tested in a 17-week toxicity study

593 with Ash/CSE rats [71]. The test substance was administered by gavage to group of 15 rats/sex

594 at dose levels of 0, 150, 500, or 1000 mg/kg bw/d in corn oil. While a variety of whole body

595 clinical pathological and histopathological endpoints were examined, the only observed effects

596 were a statistically significant reduced body weight in males and a non-statistical reduction in

597 food intake at the highest dose level. A NOAEL of 500 mg/kg bw/d for males and 1000 mg/kg

598 bw/d for females was reported. In addition, 3-methyl-1-butanol was administered to male and

599 female Wistar rats (≈ 2000 mg/kg bw/d) in drinking water for 56 weeks. No treatment-related
600 effects were observed for whole body, clinical pathology or histopathological endpoints [72].
601 In rats, oral administration of 2000 mg 3-methyl-1-butanol /kg bw led to a peak concentration
602 of 170 mg/l blood at 1 hour [13, 73]; more than 50% of the dose was excreted within 24 hours.
603 In another study [41], rats were intraperitoneally administered of 250 mg/kg bw four times in
604 15 minute-intervals. Complete absorption of the substance was observed within 1 hr after final
605 administration. No test substance was detectable after 4 hrs. Excretion was 2% in urine and 5.6
606 in expired air. Kamil et al. [15] reported after gavage administration of a dose of 25 mmol per
607 rabbit (corresponding to ≈ 735 mg/kg bw) of 1-pentanol, 3-methyl-1-butanol, and 2-methyl-1-
608 butanol, approximately 7%, 9%, and 10% of the dose was excreted by the rabbits into urine as
609 glucuronides, respectively. Furthermore, the urine did not contain aldehydes or ketones. It is
610 assumed the remaining 90+% of the tested derivative was excreted as CO₂.

611 The collective results for 3-methyl-1-butanol show it is toxicodynamically more similar to
612 tested n-alkanols (i.e., NOAEL = 1000 mg/kg bw/d) than it is to tested 2-alkyl-1-alkanols (i.e.,
613 NOAEL = 125 mg/kg bw/d). Toxicokinetically, 3-methyl-1-butanol and 2-methyl-1-butanol
614 are highly similar to n-alkanols, especially 1-pentanol.

615 **5. Conclusions**

616 This is the third in a series of read-across case studies. This specific study is a result of findings
617 which came to light during evaluations of n-alkanols [2]. *In vivo* oral repeated-dose exposure to
618 2-alkyl-1-alkanols gives rise to a set of non-specific symptoms, including clinical symptoms,
619 haematological values outside the normal range, or whole body effects different from normal.
620 The category limitation to C5 to C13 analogues assures that the impact of bioavailability on the

621 toxicokinetic and toxicodynamic profiles is limited. 2-Alkyl-1-alkanols are toxicants which act
622 via a reversible mode of toxic action. The main route of exposure is oral with rapid
623 gastrointestinal absorption, distribution via the blood, prompt Phase 2 metabolism and
624 eliminated in the urine.

625 Repeated-dose toxicity test results exhibit qualitative consistency between and within species.
626 While protocols vary, results of oral repeated-dose testing exhibit qualitative consistency
627 between and within mammals. Typical findings are only mild changes, including decreased
628 body weight, slightly increased liver weight, as well as clinical chemical and haematological
629 changes, but typically without concurrent histopathological effects. The 90-day rat oral
630 repeated-dose NOAEL values for 2-ethyl-1-hexanol and 2-propyl-1-heptanol are particularly
631 well suited for read-across. Moreover, the predictions are supported by highly similar results
632 for an isotridecanol mixture.

633 A NOAEL value of 125 mg/kg bw/d can be read across to fill the data gaps among the
634 analogues in this category for the purpose of risk assessment. Specifically, the data gaps for 2-
635 propyl-1-pentanol and 2-ethyl-1-octanol are filled with very low uncertainty (very high
636 confidence) by interpolation from 2-ethyl-1-hexanol and 2-propyl-1-heptanol. The data gaps
637 for 2-ethyl-1-butanol, 2-ethyl-1-pentanol, 2-ethyl-1-decanol and 2-propyl-1-decanol are filled
638 with low uncertainty (high confidence) by extrapolation from 2-ethyl-1-hexanol and 2-propyl-
639 1-heptanol. The data gaps for 2-methyl-1-butanol, 2-methyl-1-pentanol, 2-methyl-1-octanol
640 and 2-methyl-1-undecanol are filled with acceptable uncertainty as worst-case scenarios. The
641 latter uncertainty results from incomplete knowledge of how a methyl group, rather than an
642 ethyl or propyl moiety, affects the ratio of excretion in respired CO₂, in urine as a conjugate
643 and in faeces, as well as repeated-dose toxic potency.

644 **6. Acknowledgements**

645 This work was funded in part by the Physicians Committee for Responsible Medicine. TWS
646 acknowledges funding by Cosmetics Europe, the personal care association. KRP, ANR, CLM
647 and MTDC acknowledge funding from the COSMOS Project, which was funded by the
648 European Community's Seventh Framework Programme (FP7/2007-2013) under grant
649 agreement number 266835 and Cosmetics Europe.

650

651 **7. References**

652 [1] Przybylak, K.R., Schultz, T.W., Richarz, A.-N., Mellor, C.L., Escher, S.E., and Cronin,
653 M.T.D. 2016. Read-across of 90-day rat oral repeated-dose toxicity: A case study for
654 selected β -olefinic alcohols. *Computational Toxicology*.

655 <http://dx.doi.org/10.1016/j.comtox.2016.11.001>

656 [2] Schultz, T.W., Przybylak, K.R., Richarz, A.-N., Mellor, C.L., Escher, S.E., Bradbury, S.P.
657 and Cronin, M.T.D. 2017. Read-across of 90-day rat oral repeated-dose toxicity: A Case
658 Study for selected n-alkanols. *Computational Toxicology*.

659 [3] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30

660 November 2009 on cosmetic products, replacing Directive 76/768/EC. *Off. J. Eur. Union*.

661 L 342: 59-209.

662 [4] Cronin, M.T.D., Madden, J.C., Enoch S.J., Roberts, D.W., (Eds.), 2013. *Chemical Toxicity*
663 *Prediction: Category Formation and Read-Across Applications*. The Royal Society of
664 Chemistry, Cambridge UK.

- 665 [5] Teubner W. and Landsiedel, R., 2015. Read-across for hazard assessment: The ugly
666 duckling in growing up. *ATLA* 43: 67-71.
- 667 [6] Schultz, T.W., Amcoff, P. Berggren, E., Gautier, F. Klaric, M., Knight, D. J. Mahony, C.
668 Schwarz, M., White, A. and Cronin, M.T.D. 2015. A strategy for structuring and reporting
669 a read-across prediction of toxicity. *Reg. Toxicol. Pharmacol.* 72: 586-601.
- 670 [7] Strubelt, O., Deters, M., Pentz, R., Siegers, C.-P. and Younes, M. 1999. The toxic and
671 metabolic effects of 23 aliphatic alcohols in the isolated perfused rat liver. *Toxicol. Sci.*
672 49: 133-142.
- 673 [8] Opdyke, D.L. 1973. Monographs on fragrance raw materials. *Food Cosmet. Toxicol.* 11:
674 95-115.
- 675 [9] Organization for Economic Co-Operation and Development (OECD), 1995. SIDS Initial
676 Assessment Profile (SIAP) on 2-Ethylhexanol. Available at
677 <http://webnet.oecd.org/Hpv/UI/handler.axd?id=ffab6db1-9916-48a0-833e-7de4fe550dc7>
- 678 [10] Joint FAO/WHO expert Committee on Food Additives (JECFA), 1993. Evaluation of
679 certain food additives and contaminants. 2-ethyl-1-hexanol. 41st report of the Joint
680 FAO/WHO Expert Committee on Food Additives. WHO Geneva, WHO Technical
681 Report Series No. 837.
- 682 [11] Joint FAO/WHO expert Committee on Food Additives (JECFA), 1999. Evaluation of
683 certain food additives and contaminants. Saturated aliphatic acyclic branched-chain
684 primary alcohols, aldehydes and acids. 49th report of the Joint FAO/WHO Expert
685 Committee on Food Additives, WHO Geneva, WHO Technical Report Series No. 884.

- 686 [12] MAK 2012. 2-Ethylhexanol [MAK Value Documentation, 2003]. The MAK Collection
687 for Occupational Health and Safety. 136–178.
- 688 [13] Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.M., Rogers, A.E.,
689 Saurat, J.H., Sipes, I.G. and Tagami, H. 2010. A safety assessment of branched chain
690 saturated alcohols when used as fragrance ingredients. *Food Chem. Toxicol.* 48(S4): S1-
691 S46.
- 692 [14] Gaillard, D. and Derache, R. 1965. Metabolisation de different alcools, present dans les
693 buissons alcooliques, chez le rat. *Trav. Soc. Pharm. Montp.* 25: 51-62.
- 694 [15] Kamil, I.A., Smith, J.N. and Williams, R.T. 1953a. Studies in detoxication. 46. The
695 metabolism of aliphatic alcohols. The glucuronic acid conjugation of acyclic aliphatic
696 alcohols. *Biochem. J.* 53: 129-136.
- 697 [16] Kamil, I.A., Smith, J.N. and Williams, R.T. 1953b. Studies in detoxication. 47. The
698 formation of ester glucuronides of aliphatic acids during the metabolism of 2-
699 ethylbutanol and 2-ethylhexanol. *Biochem. J.* 53: 137-140.
- 700 [17] Patocka, J. and Kuca, K. 2012. Toxic alcohols: Aliphatic saturated alcohols. *Mil. Med.*
701 *Sci. Lett. (Voj. Zdrav. Listy)* 81: 142-163.
- 702 [18] McCreery, M.J. and Hunt, W.A. 1978. Physico-chemical correlates of alcohol
703 intoxication. *Neuropharmacology* 17: 451-461.
- 704 [19] McKarns, S.C., Hansch, C., Caldwell, W.S., Morgan, W.T., Moore, S.K. and Doolittle,
705 D.J. 1997. Correlations between hydrophobicity of short-chain aliphatic alcohols and
706 their ability to alter plasma membrane integrity. *Fundam. Applied Toxicol.* 36: 62-70.

- 707 [20] Fang, Z., Ionescu, P., Chortkoff, B.S., Kandel, L., Sonner, J., Laster, M.J. and Eger, E.I.
708 1997. Anesthetic potencies of n-alkanols: Results of additivity and solubility studies
709 suggest a mechanism of action similar to that for conventional inhaled anesthetics.
710 *Anesth. Analgesia* 84: 1042-1048.
- 711 [21] McKim, J.M., Bradbury, S.P. and Niemi, G.J. 1987. Fish acute toxicity syndromes and
712 their use in the QSAR approach to hazard assessment. *Environ. Health Perspect.* 71: 171-
713 186.
- 714 [22] Koleva, Y.K., Cronin, M.T., Madden, J.C. and Schwöbel, J.A. 2011. Modelling acute oral
715 mammalian toxicity. 1. Definition of a quantifiable baseline effect. *Toxicol. In Vitro* 25:
716 1281-1293.
- 717 [23] Rowe, V.K. and McCollister, S.B. 1982. Alcohols. In: Clayton, G.D. & Clayton, F.E., eds,
718 Patty's Industrial Hygiene and Toxicology, 3rd Revised Ed., Vol. 2C, New York: John
719 Wiley & Sons, chapter 35, pp. 4527-4708.
- 720 [24] Smyth, H.F.J., Carpenter, C.P., Weil, C.S. and Pozzani, U.C. 1954. Range-finding toxicity
721 data. List V. *Arch. Indust. Hyg. Occup. Med.* 10: 61-68.
- 722 [25] Scala, R.A., Burtis, E.G. 1973. Acute toxicity of a homologous series of branched-chain
723 primary alcohols. *Am. Ind. Hyg. Assoc. J.*, 34, 493-499.
- 724 [26] Astill, B.D., Gingell, R., Guest, D., Hodgson, J.R., Murphy, S.R. and Tyler, T.R. 1993.
725 Subacute and subchronic oral toxicity of 2-ethylhexanol to Fischer 344 rats and B6C3F1
726 mice. *Toxicologist* 13: 70.
- 727 [27] ECHA CHEM A for 2-Ethyl-1-hexanol: [http://echa.europa.eu/registration-dossier/-](http://echa.europa.eu/registration-dossier/-/registered-dossier/15194)
728 [/registered-dossier/15194](http://echa.europa.eu/registration-dossier/-/registered-dossier/15194) (accessed 28.06.2016).

- 729 [28] Chvapil, M., Zahradnik, R. and Cmuchalová, B. 1962. Influence of alcohols and
730 potassium salts of xanthogenic acids on various biological objects. Arch. Int.
731 Pharmacodyn. Ther. 135: 330-343.
- 732 [29] ECHA CHEM B for 2-Propyl-1-heptanol: [http://echa.europa.eu/registration-dossier/-](http://echa.europa.eu/registration-dossier/-/registered-dossier/13788)
733 [/registered-dossier/13788](http://echa.europa.eu/registration-dossier/-/registered-dossier/13788) (accessed 28.06.2016).
- 734 [30] Organization for Economic Co-Operation and Development (OECD) 2015. Guidance
735 Document on the Reporting of Integrated Approaches to Testing and Assessment
736 (IATA). ENV/JM/HA(2015)7.
- 737 [31] European Chemicals Agency (ECHA) Registered substances. Available from:
738 <https://echa.europa.eu/information-on-chemicals/registered-substances>.
- 739 [32] Astill, B.D., Deckardt, K., Gembardt, Chr., Gingell, R., Guest, D., Hodgson, J.R., Mellert,
740 W., Murphy, S.R. and Tyler, T.R. 1996a. Prechronic toxicity studies on 2-ethylhexanol in
741 F334 rats and B6C3F1 mice. Fundam. Appl. Toxicol., 29: 31-39.
- 742 [33] Astill, B.D., Gingell, R., Guest, D., Hellwig, J., Hodgson, J.R., Kuettler, K., Mellert, W.,
743 Murphy, S.R., Sielken, R.L. and Tyler, T.R. 1996b. Oncogenicity testing of 2-
744 ethylhexanol in Fischer 344 rats and B6C3F1 mice. Fundam. Appl. Toxicol., 31: 29-41.
- 745 [34] BASF AG, Department of Toxicology. 1992. Report on the study of the oncogenic
746 potential of 2-ethylhexanol in rats after administration by gavage (aqueous emulsion), 24
747 months study duration, satellite study. Unpublished report, project No. 99C0622/88045.
748 The Chemical Manufacturers Association, Washington, USA.
- 749 [35] BASF AG, Department of Toxicology. 1991. Report on the study of the oral toxicity of 2-
750 ethylhexanol in rats after administration by gavage for 11 days (9 applications; solution

751 in corn oil). Unpublished report, project No. 11C0631/87104. The Chemical
752 Manufacturers Association, Washington, USA.

753 [36] Hodgson JR. 1987. Results of peroxisome induction studies on tri(2-
754 ethylhexyl)trimellitate and 2-ethylhexanol. *Toxicol Ind Health*. 3(2):49-61.

755 [37] Albro, P.W. 1975. The metabolism of 2-ethylhexanol in rats. *Xenobiotica* 5: 625-636.

756 [38] Jurowich, S., Sticht, G. and Käferstein, H. 2004. Glucuronidation of aliphatic alcohols in
757 human liver microsomes in vitro. *Alcohol* 32: 187-194.

758 [39] Deisinger, P.J., Boatman, R.J. and Guest, D. 1993. Pharmacokinetic studies with 2-
759 ethylhexanol in the female Fischer 344 rat. *Toxicologist* 13: 179.

760 [40] Deisinger, P.J., Boatman, R.J. and Guest, D. 1994. Metabolism of 2-ethylhexanol
761 administered orally and dermally to the female Fischer 344 rat. *Xenobiotica* 24: 429-440.

762 [41] Haggard, H.W., Miller, D.P. and Greenberg, L.A. 1945. The amyl alcohols and their
763 ketones: their metabolic fates and comparative toxicities. *J. Ind. Hyg. Toxicol.* 27: 1-14.

764 [42] Iwersen, S. and Schmoldt, A. 1995. ADH independent metabolism of aliphatic alcohols:
765 Comparisons of oxidation and glucuronidation. *Advan. Forensic Sci.* 4: 19-22.

766 [43] Bosron, W.F. and Ting-Kai, L. 1980. Alcohol dehydrogenase. In: Jacoby, W.B. ed.
767 *Enzymatic Basis of Detoxification*. Academic Press, New York, Vol. I, pp. 231-248.

768 [44] Levi, E. and Hodgson, E. 1989. Metabolites resulting from oxidative and reductive
769 processes. In: Hutson, D.H., Caldwell, J., and Paulson, G.D. ed. *Intermediary Xenobiotic*
770 *Metabolism in Animals*. Taylor and Francis, London, pp. 119-138.

- 771 [45] Williams, R.T. 1959. The metabolism of some aliphatic aldehydes, ketones and acids. In:
772 Detoxication mechanisms. The metabolism and detoxication of drugs, toxic substances
773 and other organic compounds, 2nd Ed., London: Chapman & Hall, Ltd., chapter four, pp.
774 88-113.
- 775 [46] Deuel, H.J. 1957. The Lipids, Their Chemistry and Biochemistry - Volume III. Wiley
776 Interscience, New York.
- 777 [47] Silva, M.F.B., Aires, C.C.P., Luis, P.B.M., Ruiter, J.P.N. IJlst, L., Duran, M., Wanders,
778 R.J.A. and Tavares de Almeida, I. 2008. Valproic acid metabolism and its effects on
779 mitochondrial fatty acid oxidation: A review. *J. Inherit. Metab. Dis.* 31: 205-216.
- 780 [48] Ghodke-Puranik, Y., Thorn, C.F., Lamba, J.K., Leeder J.S., Song, W. Birnbaum, A.K.,
781 Altman, R.B. and Klein, T.E. 2013. Valproic acid pathway: pharmacokinetics and
782 pharmacodynamics, *Pharmacogenet. Genomics* 23: 236–241.
- 783 [49] Kesterson, J.W., Granneman, G.R. and Machinist, J.M. 1984. The hepatotoxicity of
784 valproic acid and its metabolites in rats. I. Toxicologic, biochemical and histopathologic
785 studies. *Hepatotoxicology* 4: 1143-1152.
- 786 [50] Przybylak, K.R. and Schultz, T.W. 2013. Informing chemical categories through
787 development of adverse outcome pathway. In: Cronin, M., Madden, J., Enoch, S. and
788 Roberts, D. (eds), *Chemical Toxicity Prediction Category Formation and Read-Across*.
789 The Royal Society of Chemistry pp. 44-71.
- 790 [51] Alifimoff, J., Firestone, L. and Miller, K. 1989. Anaesthetic potencies of primary alkanols:
791 implications for the molecular dimensions of the anaesthetic site. *Br. J. Pharmacol.* 96: 9-
792 16.

- 793 [52] Veith, G.D., Call, D.J. and Brooke, L.T. 1983. Structure-toxicity relationships for the
794 fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. Can. J. Fish.
795 Aquat. Sci. 40: 743-748.
- 796 [53] Veith, G.D., Petkova, E.P. and Wallace, K.B. 2009. A baseline inhalation toxicity model
797 for narcosis in mammals. SAR QSAR Environ. Res. 20: 567-578.
- 798 [54] Raevsky, O.A., Grigorev, V.Y., Weber, E.E. and Dearden, J.C. 2008. Classification and
799 quantification of the toxicity of chemicals to guppy, fathead minnow and rainbow trout:
800 Part 1. Nonpolar narcosis mode of action. QSAR Comb. Sci. 27: 1274–1281.
- 801 [55] He, J., Fu, L., Wang, Y., Li, J.J., Wang, X.H., Su, L.M., Sheng, L.X. and Zhao, Y.H.
802 2014. Investigation on baseline toxicity to rats based on aliphatic compounds and
803 comparison with toxicity to fish: Effect of exposure routes on toxicity. Reg. Toxicol.
804 Pharmacol. 70: 98-106.
- 805 [56] Ritter, E.J., Scott, W.J., Fradkin, R. and Ritter, J.M. 1986. Computer analysis of rat
806 teratology data following administration of phthalates and their metabolites. Teratology
807 33(3), 93C.
- 808 [57] Ritter, E.J., Scott, W.J. Jr., Randell, J.L and Ritter, J.M. 1987. Teratogenicity of di(2-
809 ethylhexyl) phthalate, 2-ethylhexanol, 2-ethylhexanoic acid, and valproic acid, and
810 potentiation by caffeine. Teratology 35: 41-46.
- 811 [58] National Toxicology Program (NTP) 1991. Final report on the developmental toxicity of 2
812 ethylhexanol in CD-1-Swiss mice. National Toxicology Program, Research Triangle
813 Park, NC, USA (PB91-185900).

- 814 [59] Tyl, R.W., Fisher, L.C., Kubena, M.F., Vrbanic, M.A., Gingell, R., Guest, D., Hodgson,
815 J.R., Murphy, S.R., Tyler, T.R. and Astill, B.D. 1992. The developmental toxicity of 2-
816 ethylhexanol applied dermally to pregnant Fischer 344 rats. *Fundam. Appl. Toxicol.*
817 19:176-185.
- 818 [60] Narotsky, M.G., Francis, E.Z., and Kavlock, R.T. 1994. Developmental toxicity and
819 structure-activity relationships of aliphatic acids, including dose-response assessment of
820 valproic acid in mice and rats. *Fundam. Appl. Toxicol.* 22: 251-265.
- 821 [61] Bui, L.M., Taubeneck, M.W., Commisso, J.F., Faber, W.D. and Keen, C.L. 1998. Altered
822 zinc metabolism contributes to the developmental toxicity of 2-ethylhexanoic acid, 2-
823 ethylhexanol and valproic acid. *Toxicology* 126: 9-21.
- 824 [62] Scott, W.J. Jr., Collins, M.D. And Nau, H. 1994. Pharmacokinetic determinants of
825 embryotoxicity in rats associated with organic acids. *Environ Health Perspect.* 102(S11):
826 97-101.
- 827 [63] Schultz, T.W., Seward-Nagel, J., Foster, K.A. and Tucker, V.A. 2004. Structure-activity
828 relationships for aliphatic alcohols and aquatic toxicity to *Tetrahymena*. *Environ.*
829 *Toxicol.* 19: 1-10.
- 830 [64] Schultz, T.W., Sinks, G.D. and Bearden, A.P. 1998. QSARs in aquatic toxicology: A
831 mechanism of action approach comparing toxic potency to *Pimephales promelas*,
832 *Tetrahymena pyriformis*, and *Vibrio fischeri*. In: Devillers, J. (ed.) *Comparative QSAR*.
833 Taylor and Francis, London, pp. 52-109.

- 834 [65] Benane, S.G., Richard, A.M., Blackman, C.F. and Lytle, C.D. 1993. Quantitative
835 structure-toxicity relationships for a series of primary alcohols in a mammalian viral host
836 cell reactivation assay. *In Vitro Toxicol.* 6: 267-277.
- 837 [66] Mellor, C.L, Steinmetz, F.P, Cronin, M.T.D. 2016. Using molecular initiating events to
838 develop a structural alert based screening workflow for nuclear receptor ligands
839 associated with hepatic steatosis. *Chem. Res. Toxicol.* 29: 203-212.
- 840 [67] US EPA ToxCast 2014. US EPA website with access to the ToxCast data.
841 <https://www.epa.gov/chemical-research/toxicity-forecasting>. (Accessed 16/06/2016)
- 842 [68] Richard, A.M., Judson, R.S., Houck, K.A., Grulke, C.M., Volarath, P., Thillainadarajah,
843 I., Chihae Yang, C., Rathman, J., Martin, M.T., Wambaugh, J.T., Knudsen, T.B.,
844 Kancherla, J., Mansouri, K. Patlewicz, G., Williams, A.J., Little, S.B., Crofton, K.M. and
845 Thomas, R.S. 2016. ToxCast chemical landscape: Paving the road to 21st century
846 toxicology. *Chem. Res. Toxicol.* 29: 1225–1251
- 847 [69] Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen,
848 H.M., Reif, D.M., Richard, A.M., Rotroff, D.M., Shah, I. and Dix, D.J. 2010. Predictive
849 *in vitro* screening of environmental chemicals – The ToxCast project. *Environ. Health*
850 *Perspect.* 118:485-492.
- 851 [70] Schilling, K., Kayser, M., Deckardt, K., Küttler, K. and Klimisch, H.J. 1997. Subchronic
852 toxicity studies of 3-methyl-1-butanol and 2-methyl-1-propanol in rats. *Human Exper.*
853 *Toxicol.* 16: 722-726.
- 854 [71] Carpanini, F.M. and Gaunt, I.F. 1973. Short-term toxicity of isoamyl alcohol in rats. *Food*
855 *Cosmet. Toxicol.* 11: 713-724.

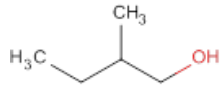
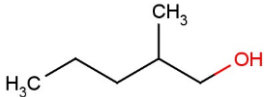
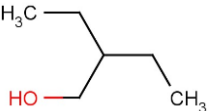
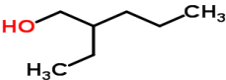
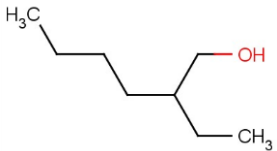
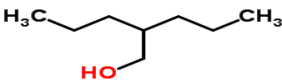
- 856 [72] Johannsen, E. and Purchase, I.F.H. 1969. Kaffir corn malting and brewing studies. XXI:
857 The effect of the fusel oils of bantu beer on rat liver. South African Med. J. 43: 326-328.
- 858 [73] ECHA CHEM C for 3-methyl-1-butanol: [http://echa.europa.eu/registration-dossier/-](http://echa.europa.eu/registration-dossier/-/registered-dossier/13936)
859 /[registered-dossier/13936](http://echa.europa.eu/registration-dossier/-/registered-dossier/13936) (accessed 28.06.2016).

Supplementary material

Read-across of 90-Day Rat Oral Repeated-Dose Toxicity: A Case Study for Selected 2-Alkyl-1-alkanols

Annex I 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	2-Methyl-1-butanol	137-32-6	<chem>CCC(C)CO</chem>		C ₅ H ₁₂ O
2	2-Methyl-1-pentanol	105-30-6	<chem>CCCC(C)CO</chem>		C ₆ H ₁₄ O
3	2-Ethyl-1-butanol	97-95-0	<chem>CCC(CC)CO</chem>		C ₆ H ₁₄ O
4	2-Ethyl-1-pentanol	27522-11-8	<chem>CCCC(CC)CO</chem>		C ₇ H ₁₆ O
5	2-Ethyl-1-hexanol	104-76-7	<chem>CCCCCC(CC)CO</chem>		C ₈ H ₁₈ O
6	2-Propyl-1-pentanol	58175-57-8	<chem>CCCC(CCC)CO</chem>		C ₈ H ₁₈ O

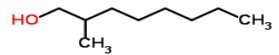
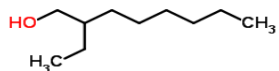
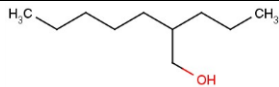
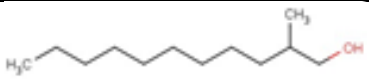
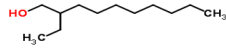
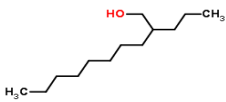
ID	Name	CAS No	SMILES	2D Structure	Molecular Formula
7	2-Methyl-1-octanol	818-81-5	<chem>CCCCCCC(C)CO</chem>		C ₉ H ₂₀ O
8	2-Ethyl-1-octanol	20592-10-3	<chem>CCCCCCC(CC)CO</chem>		C ₁₀ H ₂₂ O
9	2-Propyl-1-heptanol	10042-59-8	<chem>CCCCCC(CCC)CO</chem>		C ₁₀ H ₂₂ O
10	2-Methyl-1-undecanol	10522-26-6	<chem>CCCCCCCCC(C)CO</chem>		C ₁₂ H ₂₆ O
11	2-Ethyl-1-decanol	21078-65-9	<chem>CCCCCCCCC(CC)CO</chem>		C ₁₂ H ₂₆ O
12	2-Propyl-1-decanol	60671-35-4	<chem>CCCCCCCCC(CCC)CO</chem>		C ₁₃ H ₂₈ O

Table 2: Comparison of Physico-Chemical and Molecular Properties¹

ID	Name	Molecular Weight ¹	Log Kow ^{1a}	Vapor Pressure (Pa, 25 degC) ^{1b}	Density ² (g/cm ³)	Melting Point (deg C) ^{1b}	Water Solubility (mg/L, 25 degC) ^{1c}	Boiling Point (deg C) ^{1b}	pKa ³
1	2-Methyl-1-butanol	88.15	1.26 1.29 (M)	606 416 (M)	0.8±0.1	-61.49	32200 29700 (M)	123.17 128 (M)	15.24
2	2-Methyl-1-pentanol	102.18	1.75	191 256 (M)	0.8±0.1	-49.23	11950 6000 (M)	145.86 149 (M)	15.05
3	2-Ethyl-1-butanol	102.18	1.75	213 204 (M)	0.8±0.1	-49.23 <-15 (M)	11950 4000 (M)	145.86 147 (M)	15.05
4	2-Ethyl-1-pentanol	116.21	2.24	66.2	0.8±0.1	-37.23	4089	167.64	15.05
5	2-Ethyl-1-hexanol	130.23	2.73	24.6 18.1 (M)	0.8±0.1	-25.50 -70 (M)	1379 880 (M)	188.52 184.6 (M)	15.05
6	2-Propyl-1-pentanol	130.23	2.73	19.5	0.8±0.1	-25.50	1379	188.52	15.05
7	2-Methyl-1-octanol	144.26	3.22	5.88	0.8±0.1	-14.04	459.7	208.49	15.09
8	2-Ethyl-1-octanol	158.29	3.71	1.81	0.8±0.1	-2.83	151.8	227.56	15.09
9	2-Propylheptan-1-ol	158.29	3.71	3.38	0.8±0.1	-2.83	151.8	227.56 217.5 (M)	15.09

ID	Name	Molecular Weight ¹	Log Kow ^{1a}	Vapor Pressure (Pa, 25 degC) ^{1b}	Density ² (g/cm ³)	Melting Point (deg C) ^{1b}	Water Solubility (mg/L, 25 degC) ^{1c}	Boiling Point (deg C) ^{1b}	pKa ³
10	2-Methyl-1-undecanol	186.34	4.70	0.186	0.8±0.1	18.78	16.18	262.99	15.04
11	2-Ethyl-1-decanol	186.34	4.70	0.186	0.8±0.1	18.78	16.18	262.99	15.04
12	2-Propyl-1-decanol	200.37	5.19	0.0615	0.8±0.1	29.19	5.237	279.35	15.06

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 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	2-Methyl-1-butanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2	2-Methyl-1-pentanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
3	2-Ethyl-1-butanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
4	2-Ethyl-1-pentanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
5	2-Ethyl-1-hexanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
6	2-Propyl-1-pentanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
7	2-Methyl-1-octanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
8	2-Ethyl-1-octanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
9	2-Propylheptan-1-ol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
10	2-Methyl-1-undecanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol

ID	Name	Key Substituent(s)	Functional Group(s)	Extended Fragment(s)	Chemical Class	Chemical Sub-Class
11	2-Ethyl-1-decanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
12	2-Propyl-1-decanol	-OH	-CH ₃ , -CH ₂ -, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol

Table 4: Comparison of Abiotic Transformation and Toxicokinetics

ID	Name	Abiotic Transformation	Toxicokinetics		
			Absorption	Half-life	Elimination
1	2-Methyl-1-butanol		Efficiently following oral administration ^a	< 24 hrs	9.6% excreted in the urine as a glucuronides within 24 hrs ^b 5.6% excreted in air and 2% in urine, remainder metabolized, first to the corresponding aldehyde, then to the acid ^c Additional oxidation of 2-methyl-1-butanol by rat liver microsomes via CYP P450 enzymes, and glucuronidation ^d
2	2-Methyl-1-pentanol		Efficiently following oral administration ^a		
3	2-Ethyl-1-butanol		Efficiently following oral administration ^a		Excreted mainly as a glucuronides ^e
4	2-Ethyl-1-pentanol		Efficiently following oral administration ^a		

5	2-Ethyl-1-hexanol	atmospheric lifetime of 24.6 hrs	Efficiently following oral administration ^a	< 24 hrs terminal half-life 60 hours	Rapidly excreted in respired CO ₂ (6-7%), urine mainly as glucuronides (80-82%), and faeces (8-9%); elimination was essentially complete by 28 hrs ^f After oral administration to rats, within 96 hrs; 69-75% excreted in urine, about 13-15% in faeces, about the same amount exhaled. After intravenous administration to rats, within 96 hours about 74% excreted in urine, about 4% in faeces and 23% exhaled. More than 50% excreted within 8 hrs. ^g Glucuronide main metabolite (87%) in rabbits ^{b,e}
6	2-Propyl-1-pentanol		Efficiently following oral administration ^a		
7	2-Methyl-1-octanol		Efficiently following oral administration ^a		
8	2-Ethyl-1-octanol		Efficiently following oral administration ^a		
9	2-Propyl-1heptanol		Efficiently following oral administration ^a		
10	2-Methyl-1-undecanol		Efficiently following oral administration ^a		

11	2-Ethyl-1-decanol		Efficiently following oral administration ^a		
12	2-Propyl-1-decanol		Efficiently following oral administration ^a		

^a Gaillard, D. and Derache, R. 1965. Metabolisation de different alcools, present dans les buissons alcooliques, chez le rat. *Trav. Soc. Pharm. Montp.*, 25: 51-62; ^bKamil, I.A., Smith, J.N. and Williams, R.T. 1953a. Studies in detoxication. 46. The metabolism of aliphatic alcohols. The glucuronic acid conjugation of acyclic aliphatic alcohols. *Biochem. J.* 53: 129-136; ^c Haggard, H.W., Miller, D.P. and Greenberg, L.A. 1945. The amyl alcohols and their ketones: their metabolic fates and comparative toxicities. *J. Ind. Hyg. Toxicol.* 27: 1-14; ^dIwersen, S. and Schmoldt, A. 1995. ADH independent metabolism of aliphatic alcohols: Comparisons of oxidation and glucuronidation. *Advan. Forensic Sci.* 4: 19-22; ^eKamil, I.A., Smith, J.N. and Williams, R.T. 1953b. Studies in detoxication. 47. The formation of ester glucuronides of aliphatic acids during the metabolism of 2-ethylbutanol and 2-ethylhexanol. *Biochem. J.* 53: 137-140; ^fAlbro, P.W. 1975. The metabolism of 2-ethylhexanol in rats. *Xenobiotica* 5: 625-636, ECHA CHEM A for 2-Ethyl-1-hexanol: <http://echa.europa.eu/registration-dossier/-/registered-dossier/15194>, Joint FAO/WHO expert Committee on Food Additives (JECFA), 1993. Evaluation of certain food additives and contaminants. 2-ethyl-1-hexanol. 41st report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Geneva, WHO Technical Report Series No. 837; ^g Deisinger, P.J., Boatman, R.J. and Guest, D. 1993. Pharmacokinetic studies with 2-ethylhexanol in the female Fischer 344 rat. *Toxicologist* 13: 179, Deisinger, P.J., Boatman, R.J. and Guest, D. 1994. Metabolism of 2-ethylhexanol administered orally and dermally to the female Fischer 344 rat. *Xenobiotica* 24: 429-440.

Table 5: Comparison of Potential Metabolic Products as Predicted *in silico*

ID	Name	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
		Rat liver S9	Skin metabolism			
1	2-Methyl-1-butanol	Hydroxylation (3) Oxidation (1)	Hydroxylation (3)	Hydroxylation Oxidation Acylation	Possible sites of metabolism have been identified	Hydroxylation (4) Oxidation (1)
2	2-Methyl-1-pentanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (1)	Hydroxylation Oxidation Acylation Methylation Dealkylation	Possible sites of metabolism have been identified	Hydroxylation (2) Oxidation (1)
3	2-Ethyl-1-butanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dealkylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)
4	2-Ethyl-1-pentanol	Hydroxylation (4) Oxidation (1)	Hydroxylation (3)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation	Possible sites of metabolism have been identified	Hydroxylation (4) Oxidation (1)
5	2-Ethyl-1-hexanol	Hydroxylation (4) Oxidation (1)	Hydroxylation (4)	Hydroxylation Oxidation Acylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (6) 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			
6	2-Propyl-1-pentanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (4)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (2) Oxidation (1) beta-Oxidation of Carboxylic Acids (1)
7	2-Methyl-1-octanol	Hydroxylation (3) Oxidation (1)	Hydroxylation (3)	Hydroxylation Oxidation Methylation Dealkylation Demethylation Alkylation Acylation	Possible sites of metabolism have been identified	Hydroxylation (5) Oxidation (1)
8	2-Ethyl-1-octanol	Hydroxylation (4) Oxidation (1)	Hydroxylation (4)	Hydroxylation Oxidation Methylation Dealkylation Dehydration Demethylation Alkylation Acylation	Possible sites of metabolism have been identified	Hydroxylation (6) Oxidation (1)
9	2-Propyl-1-heptanol	Hydroxylation (4) Oxidation (1)	Hydroxylation (4)	Hydroxylation Oxidation Acylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (7) Oxidation (1)
10	2-Methyl-1-undecanol	Hydroxylation	Hydroxylation (3)	Hydroxylation	Possible sites of	Hydroxylation (5)

ID	Name	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
		Rat liver S9	Skin metabolism			
		(3) Oxidation (1)		Oxidation Acylation Methylation Alkylation Dealkylation Demethylation	metabolism have been identified	Oxidation (1)
11	2-Ethyl-1-decanol	Hydroxylation (4) Oxidation (1)	Hydroxylation (3)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (4) Oxidation (1)
12	2-Propyl-1-decanol	Hydroxylation (3) Oxidation (1)	Hydroxylation (1)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Dehydration	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)

Table 6: Comparison of Toxicophores

ID	Name	Toxicophores¹	DNA binding by OECD¹	Protein binding by OECD¹	Nuclear receptor binding²	Liver & Mitochondria toxicity²
1	2-Methyl-1-butanol	Cramer Class I	No alert	No alert	Inactive	No alert
2	2-Methyl-1-pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
3	2-Ethyl-1-butanol	Cramer Class I	No alert	No alert	Inactive	No alert
4	2-Ethyl-1-pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
5	2-Ethyl-1-hexanol	Cramer Class I	No alert	No alert	Inactive	No alert
6	2-Propyl-1-pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
7	2-Methyl-1-octanol	Cramer Class I	No alert	No alert	Inactive	No alert
8	2-Ethyl-1-octanol	Cramer Class I	No alert	No alert	Inactive	No alert
9	2-Propyl-1-heptanol	Cramer Class I	No alert	No alert	Inactive	No alert
10	2-Methyl-1-undecanol	Cramer Class I	No alert	No alert	Inactive	No alert
11	2-Ethyl-1-decanol	Cramer Class I	No alert	No alert	Inactive	No alert
12	2-Propyl-1-decanol	Cramer Class I	No alert	No alert	Inactive	No alert

¹ OECD QSAR Toolbox 3.3; ² COSMOS profilers available via COSMOS space: <http://cosmospace.cosmostox.eu>

Table 7: Comparison of Mechanistic Plausibility and AOP-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	2-Methyl-1-butanol		Narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
2	2-Methyl-1-pentanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
3	2-Ethyl-1-butanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
4	2-Ethyl-1-pentanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
5	2-Ethyl-1-hexanol		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
6	2-Propyl-1-pentanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
7	2-Methyl-1-octanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
8	2-Ethyl-1-octanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
9	2-Propyl-1-heptanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
10	2-Methyl-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
11	2-Ethyl-1-decanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			
12	2-Propyl-1-decanol		narcosis - depressant anesthesia	Unspecific interactions with biological membranes			

Table 8: Comparison of Toxicologically Relevant *in vivo*, *in vitro* and *ex vivo* Data

Name	2-Methyl-1-butanol	2-Methyl-1-pentanol	2-Ethyl-1-butanol	2-Ethyl-1-pentanol	2-Ethyl-1-hexanol	2-Propyl-1-pentanol	2-Methyl-1-octanol	2-Ethyl-1-octanol	2-Propyl-1-heptanol	2-Methyl-1-undecanol	2-Ethyl-1-decanol	2-Propyl-1-decanol
Endpoint: NOAEL (Repeat dose toxicity)					25-1000 (mg/kg/d) [2, 3, 5, 22]				30-150 (mg/kg/d) [4, 21]			
Endpoint: NOEL (Repeat dose toxicity)	≥6400 (mg/m ³) [1]											
Endpoint: NOAEL (short-term repeated dose study)					100-200 (mg/kg bw/d) [5, 23-26]							
Endpoint: LOAEL (Repeat dose toxicity)					1525 (mg/kg/d) [5]				150-600 (mg/kg/d) [4]			
Endpoint: NOAEC (Repeat dose toxicity)					120-638.4 (mg/kg/d) [6]							
Endpoint: NOAEL (Reproductive toxicity)					130-2520 (mg/kg/d) [6]				50 (mg/kg/d) [7]			
Endpoint: NOAEL (Teratogenicity)					191-650 (mg/kg/d) [6]				158-600 (mg/kg/d) [7]			
Endpoint: HNEL (Carcinogenic/ Genotoxicity)					50-200 (mg/kg/d) [8]							
Endpoint: LEL					150-750 (mg/kg/d)							

(Carcinogenic/ Genotoxicity)					[8]							
Endpoint: LC50 (Acute toxicity)					0.89- 5.3 (mg/Lair) [6]				>0.13(mg/L air) [7]			
Endpoint: LD50 (Acute toxicity)		1900-5000 (mg/kg) 12.53- 16.6 (mg/Lair) 3.54 (mL/kg) [1, 9]			3730 (mg/kg) [6, 10, 11]				5100-5400 (mg/kg) [7]			
Endpoint: oral LD50 (mg/kg) (Acute toxicity)		4010 mg/kg bw [17]		1850 mg/kg bw [18]	2000-3730 mg/kg bw [5, 19-20]				5400 mg/kg bw [21]			
Endpoint: LDLo (Acute toxicity)		1900- 2448 (mg/kg) [1, 12]										
Endpoint: Genotoxicity (AMES, Chromosomal abrration, gene mutation)		2 x Negative [13-16]			9 x Negative [5]				5 x Negative [4]			
Toxcast [27]	ATG_ERa_TRA NS				11.9							
	ATG_ERa_TRA NS_perc				5.77							
	ATG_PXRE_CI S				31.1							
	ATG_PXRE_CI S_perc				31.1							
	OT_ERa_EREL UC_AG_1440								3.14			
	Tox21_AR_BL A_Agonist_ch1								0.00219			

Tox21_ELG1_L UC_Agonist_viability										54.9			
--------------------------------------	--	--	--	--	--	--	--	--	--	------	--	--	--

References for Table 8

- [1] ECHA CHEM A for 2-Methyl-1-butanol. <http://echa.europa.eu/registration-dossier/-/registered-dossier/12040/7/6/3> (accessed 28.06.2016).
- [2] Toxicity Testing Reports of Environmental (Ministry of Health and Welfare, Japan) 1997, Rep Dose Tox Fraunhofer.
- [3] Schilling, K., Kayser, M., Deckardt, K., Küttler, K. and Klimisch, H.J. 1997. Subchronic toxicity studies of 3-methyl-1-butanol and 2-methyl-1-propanol in rats. *Human Exper. Toxicol.* 16: 722-726.
- [4] ECHA CHEM B for 2-Propyl-1-heptanol: <https://echa.europa.eu/registration-dossier/-/registered-dossier/13788/1> (accessed 28.06.2016).
- [5] ECHA CHEM C for 2-Ethylhexanol: <https://echa.europa.eu/registration-dossier/-/registered-dossier/15194> (accessed 28.06.2016).
- [6] Toxicity Testing Reports of Environmental (Ministry of Health and Welfare, Japan) 1997, Rep Dose Tox Fraunhofer
- [7] ChemIDplus, <http://chem.sis.nlm.nih.gov/chemidplus/rn/100-51-6>. Original reference given; *Cancer Research* 33, 3069-3085, 1973
- [8] Astill, B.D., Gingell, R., Guest, D., Hellwig, J., Hodgson, J.R., Kuettler, K., Mellert, W., Murphy, S.R., Sielken, R.L. and Tyler, T.R. 1996. Oncogenicity testing of 2-ethylhexanol in Fischer 344 rats and B6C3F1 mice. *Fundam. Appl. Toxicol.*, 31: 29-41.
- [9] ChemIDplus search, <http://chem.sis.nlm.nih.gov/chemidplus/rn/71-36-3>. Original reference quoted; *South African Med. J.* Vol. 43, Pg. 795, 1969
- [10] EPA Document No. 86-870001383, Neste Oxo AB Stenungsund (71), Microfische No OTS515545 (1940). (<http://www.epa.govt.nz/search-databases/Pages/ccid-details.aspx?SubstanceID=1845>)
- [11] Nihs search, http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- [12] European Chemicals Bureau; IUCLID Dataset, 1-Hexanol (111-27-3) (2000 CD-ROM edition). Available from: <http://esis.jrc.ec.europa.eu/>
- [13] Nakajima, D., Ishii, R., Kageyama, S., Onji, Y., Mineki, S., Morooka, N., Takatori, K and Goto, S (2006). Genotoxicity of microbial volatile organic compounds. *J. Health Sci.* 52: 148–153.
- [14] Seidel, H. and Plappert, U. 1999. Zur Toxikologie zweier häufig nachgewiesener MVOC: 1 Octen-3-ol und 3-Methyl-1-butanol. *Umweltmed. Forsch. Prax.* 4: 285-288, 1999
- [15] Edelfors, S. and Ravn-Jonsen, A. 1990. The effects of alcohols in vitro on the nervous cell membrane measured by changes in the (Ca²⁺/Mg²⁺) ATPase activity and fluidity of the synaptosomal membrane. *Pharmacol. Toxicol.* 67: 56-60.

- [16] Bacterial mutagenicity ISSSTY DATABASE; <http://www.iss.it/meca/index.php?lang=1&anno=2013&tipo=25>
- [17] Rowe, V.K. and McCollister, S.B. 1982. Alcohols. In: Clayton, G.D. & Clayton, F.E., eds, Patty's Industrial Hygiene and Toxicology, 3rd Revised Ed., Vol. 2C, New York: John Wiley & Sons, chapter 35, pp. 4527-4708.
- [18] Smyth, H.F.J., Carpenter, C.P., Weil, C.S. and Pozzani, U.C. 1954. Range-finding toxicity data. List V. Arch. Indust. Hyg. Occup. Med. 10: 61-68.
- [19] Scala, R.A. and Burtis, E.G. 1973. Acute toxicity of a homologous series of branched-chain primary alcohols. Am. Ind. Hyg. Assoc. J., 34: 493-499.
- [20] Chvapil, M., Zahradnik, R. and Cmuchalová, B. 1962. Influence of alcohols and potassium salts of xanthogenic acids on various biological objects. Arch. Int. Pharmacodyn. Ther. 135: 330-343.
- [21] ECH CHEM for 2-Propyl-1-heptanol: <https://echa.europa.eu/registration-dossier/-/registered-dossier/13788/1>
- [22] Astill, B.D., Gingell, R., Guest, D., Hodgson, J.R., Murphy, S.R. and Tyler, T.R. 1993. Subacute and subchronic oral toxicity of 2-ethylhexanol to fischer 344 rats and B6C3F1 mice. Toxicologist 13: 70.
- [23] RIFM (Research Institute for Fragrance Materials, Inc.), 1992. Oral Toxicity of 2- Ethylhexanol in Mice after Administration by Gavage for 11 days. Unpublished Report from BASF. RIFM Report Number 18665. RIFM, Woodcliff Lake, NJ, USA.
- [24] Astill, B.D., Gingell, R., Guest, D., Hellwig, J., Hodgson, J.R., Kuettler, K., Mellert, W., Murphy, S.R., Sielken, R.L. and Tyler, T.R. 1996b. Oncogenicity testing of 2-ethylhexanol in Fischer 344 rats and B6C3F1 mice. Fundam. Appl. Toxicol. 31: 29-41.
- [25] Hodgson JR. 1987. Results of peroxisome induction studies on tri(2-ethylhexyl)trimellitate and 2-ethylhexanol. Toxicol. Ind. Health 3: 49-61.
- [26] Astill, B.D., Deckardt, K., Gemhardt, Chr., Gingell, R., Guest, D., Hodgson, J.R., Mellert, W., Murphy, S.R. and Tyler, T.R. 1996a. Prechronic toxicity studies on 2-ethylhexanol in F334 rats and B6C3F1 mice. Fundam. Appl. Toxicol. 29: 31-39.
- [27] Toxcast™ data. Provider the USEPA: <http://www.epa.gov/ncct/toxcast/data.html>