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

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**Article**

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

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

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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.

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**Highlights:**

- The category is limited to readily bioavailable 2-alkyl-1-akanols of intermediate size (C5 to C13)

- 2-Alkyl-1-akanols are toxicants acting via a simple narcosis mechanism

- Toxicokinetically and toxicodynamically, the 2-alkyl-1-akanols are highly similar

- 2-ethyl-hexanol and 2-propyl-1-heptanol can be read across to other analogues with acceptable uncertainty

## 1 Introduction

1.1 Read-across

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

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

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

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

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., $O_2$

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 ($\mu$mol/g x min) | ATP ($\mu$mol/g) | LDH (U/l) | GSH ($\mu$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-akanols are presented in Table 2. In general, 2-alkyl-1-akanols 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 | |

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126     2-Alkyl-1-akanols 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 $\geq$ 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-akanols 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 | C5H12O |
| 2 | 2-Methyl-1-pentanol | 105-30-6 | C6H14O |
| 3 | 2-Ethyl-1-butanol | 97-95-0 | C6H14O |
| 4 | 2-Ethyl-1-pentanol | 27522-11-8 | C7H16O |
| 5 | **2-Ethyl-1-hexanol** | **104-76-7** | **C8H18O** |
| 6 | 2-Propyl-1-pentanol | 58175-57-8 | C8H18O |
| 7 | 2-Methyl-1-octanol | 818-81-5 | C9H20O |
| 8 | 2-Ethyl-1-octanol | 20592-10-3 | C10H22O |
| 9 | **2-Propyl-1-heptanol** | **10042-59-8** | **C10H22O** |
| 10 | 2-Methyl-1-undecanol | 10522-26-6 | C12H26O |
| 11 | 2-Ethyl-1-decanol | 21078-65-9 | C12H26O |
| 12 | 2-Propyl-1-decanol | 60671-35-4 | C13H28O |

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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-akanols 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-akanols 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-akanols 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-akanols (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 $\geq$ 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 $\geq$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 $\beta$-oxidation to $CO_2$ 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, tread 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 $g/cm^3$ 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, $CH_3$, $CH_2$ 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-$^{14}$C-hexanol

311 ($^{14}$C- labeled 2-ethyl-1-hexanol; 1 µCi; 8.8 µg) in cotton seed oil. Two others were given the

312 same amount of $^{14}$C-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 $CO_2$, 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 $CO_2$, 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 $CO_2$ (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 ^{14}C-labeled 2-ethyl-1-hexanol in rats.

321   After oral administration to rats, 69-75% of a dose of 500 mg ^{14}C-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 ^{14}C-labeled $CO_2$. After intravenous

324   administration to rats, about 74% of a dose of 1 mg ^{14}C-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 mmo/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 ß-oxidation and decarboxylation to

354     produce $^{14}CO_2$ 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 $CO_2$ 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 $CO_2$ via ß-

365     oxidative cleavage in the fatty acid pathway. If the methyl group is located at the 3-position, ß-

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 ß-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 ß-oxidation.

381     With longer branched-chain derivatives, this is followed by cleavage to yield linear acid

382     fragments which are typically completely metabolised to $CO_2$. 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 ß-oxidation [46]. Detoxication pathways of ω- and ω-1 oxidation compete with ß-

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-postion 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 $CO_2$. 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-akanols 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-carboxcylic acids. The experimental evidence is

458 largely confined to 2-ethyl-1-hexanol and the results are mixed.

459 In rats administrated 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 mmoles/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/toxicokinetics and metabolic similarity | Empirical: *In vivo*: Medium<br><br>*In vitro*: none<br><br>Simulated: Medium | Medium | Based on *in vivo* 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 *in silico* 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 *in silico* 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, *in vivo*, *in vitro* and *ex vivo* endpoints | Low | High | Although not directly related to the repeated-dose endpoint, many category members have been tested for *in vivo* acute effects in rodents and fish. In addition, many category members have been tested *in vitro* for cellular effects. There is general agreement in the trend of the reported 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 *in silico* profilers. |

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

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

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

| Factor | 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 know receptor and exhibit no specific receptor interactions. |
| *In vivo* **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 *in vivo* 90-day, oral repeated-dose data usable for read-across. |
| Quality of the *in vivo* apical endpoint data read across | Low | Generally, the *in vivo* 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 *in vivo* 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 *in vivo* 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 *in vivo* and *in vitro* 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 *in vitro* 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 *in vivo* 90-day repeated-dose data that are supported by similar data for a mixture of C11 to C14 branched alkanols adds to the *in vivo* 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 *in vivo* 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 *in vivo* WoE. The lack of *in vivo* 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 $CO_2$.

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 $CO_2$, in urine as a conjugate

643    and in faeces, a as well as repeated-dose toxic potency.

## 6. Acknowledgements

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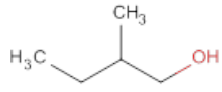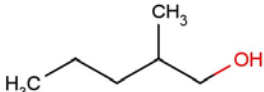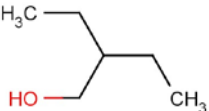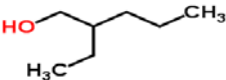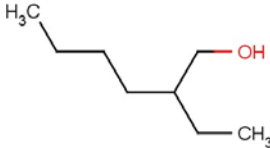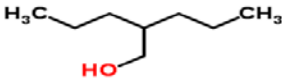
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# Supplementary material


**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 | CCC(C)CO |  | C5H12O |
| 2 | 2-Methyl-1-pentanol | 105-30-6 | CCCC(C)CO |  | C6H14O |
| 3 | 2-Ethyl-1-butanol | 97-95-0 | CCC(CC)CO |  | C6H14O |
| 4 | 2-Ethyl-1-pentanol | 27522-11-8 | CCCC(CC)CO |  | C7H16O |
| 5 | 2-Ethyl-1-hexanol | 104-76-7 | CCCCC(CC)CO |  | C8H18O |
| 6 | 2-Propyl-1-pentanol | 58175-57-8 | CCCC(CCC)CO |  | C8H18O |

| ID | Name | CAS No | SMILES | 2D Structure | Molecular Formula |
|---|---|---|---|---|---|
| 7 | 2-Methyl-1-octanol | 818-81-5 | CCCCCCC(C)CO |  | C9H20O |
| 8 | 2-Ethyl-1-octanol | 20592-10-3 | CCCCCCC(CC)CO |  | C10H22O |
| 9 | 2-Propyl-1-heptanol | 10042-59-8 | CCCCCC(CCC)CO |  | C10H22O |
| 10 | 2-Methyl-1-undecanol | 10522-26-6 | CCCCCCCCC(C)CO |  | C12H26O |
| 11 | 2-Ethyl-1-decanol | 21078-65-9 | CCCCCCCC(CC)CO |  | C12H26O |
| 12 | 2-Propyl-1-decanol | 60671-35-4 | CCCCCCCC(CCC)CO |  | C13H28O |
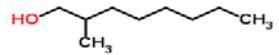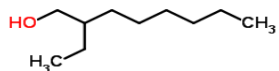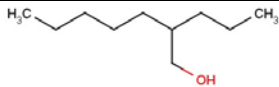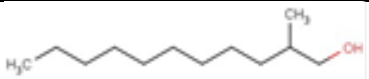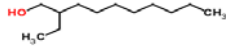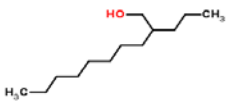
**Table 2: Comparison of Physico-Chemical and Molecular Properties[1]**

| ID | Name | Molecular Weight[1] | Log Kow[1a] | Vapor Pressure (Pa, 25 degC)[1b] | Density[2] (g/cm³) | 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 <br> 1.29 (M) | 606 <br> 416 (M) | 0.8±0.1 | -61.49 | 32200 <br> 29700 (M) | 123.17 <br> 128 (M) | 15.24 |
| 2 | 2-Methyl-1-pentanol | 102.18 | 1.75 | 191 <br> 256 (M) | 0.8±0.1 | -49.23 | 11950 <br> 6000 (M) | 145.86 <br> 149 (M) | 15.05 |
| 3 | 2-Ethyl-1-butanol | 102.18 | 1.75 | 213 <br> 204 (M) | 0.8±0.1 | -49.23 <br> <-15 (M) | 11950 <br> 4000 (M) | 145.86 <br> 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 <br> 18.1 (M) | 0.8±0.1 | -25.50 <br> -70 (M) | 1379 <br> 880 (M) | 188.52 <br> 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 <br> 217.5 (M) | 15.09 |

| ID | Name | Molecular Weight[1] | Log Kow[1a] | Vapor Pressure (Pa, 25 degC)[1b] | Density[2] (g/cm³) | 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

[1]Values typically derived from EPISuite v4.1, [a] KOWWIN Program (v1.68), [b] MPBPWIN v1.43, [c] at 25 deg C; (mg/L) Kow (WSKOW v1.42); [2] ACD/Lab Percepta Platform - PhysChem Module (from ChemSpider); [3] Predicted by 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] <br> 5.6% excreted in air and 2% in urine, remainder metabolized, first to the corresponding aldehyde, then to the acid [c] <br> 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<br><br>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]<br>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]<br>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] | | |

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**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)<br>Oxidation (1) | Hydroxylation (3) | Hydroxylation<br>Oxidation<br>Acylation | Possible sites of metabolism have been identified | Hydroxylation (4)<br>Oxidation (1) |
| 2 | 2-Methyl-1-pentanol | Hydroxylation (2)<br>Oxidation (1) | Hydroxylation (1) | Hydroxylation<br>Oxidation<br>Acylation<br>Methylation<br>Dealkylation | Possible sites of metabolism have been identified | Hydroxylation (2)<br>Oxidation (1) |
| 3 | 2-Ethyl-1-butanol | Hydroxylation (2)<br>Oxidation (1) | Hydroxylation (2) | Hydroxylation<br>Oxidation<br>Acylation<br>Dealkylation | Possible sites of metabolism have been identified | Hydroxylation (3)<br>Oxidation (1) |
| 4 | 2-Ethyl-1-pentanol | Hydroxylation (4)<br>Oxidation (1) | Hydroxylation (3) | Hydroxylation<br>Oxidation<br>Acylation<br>Dehydroxylation<br>Methylation<br>Alkylation<br>Dealkylation | Possible sites of metabolism have been identified | Hydroxylation (4)<br>Oxidation (1) |
| 5 | 2-Ethyl-1-hexanol | Hydroxylation (4)<br>Oxidation (1) | Hydroxylation (4) | Hydroxylation<br>Oxidation<br>Acylation<br>Methylation<br>Alkylation<br>Dealkylation<br>Dehydration<br>Demethylation | Possible sites of metabolism have been identified | Hydroxylation (6)<br>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)<br>Oxidation (1) | | Oxidation<br>Acylation<br>Methylation<br>Alkylation<br>Dealkylation<br>Demethylation | metabolism have been identified | Oxidation (1) |
| 11 | 2-Ethyl-1-decanol | Hydroxylation (4)<br>Oxidation (1) | Hydroxylation (3) | Hydroxylation<br>Oxidation<br>Acylation<br>Dehydroxylation<br>Methylation<br>Dealkylation<br>Dehydration<br>Demethylation | Possible sites of metabolism have been identified | Hydroxylation (4)<br>Oxidation (1) |
| 12 | 2-Propyl-1-decanol | Hydroxylation (3)<br>Oxidation (1) | Hydroxylation (1) | Hydroxylation<br>Oxidation<br>Acylation<br>Dehydroxylation<br>Methylation<br>Dehydration | Possible sites of metabolism have been identified | Hydroxylation (3)<br>Oxidation (1) |

**Table 6: Comparison of Toxicophores**

| ID | Name | Toxicophores[1] | DNA binding by OECD[1] | Protein binding by OECD[1] | Nuclear receptor binding[2] | Liver& Mitochondria toxicity[2] |
|---|---|---|---|---|---|---|
| 1 | 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://cosmosspace.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)** | $\geq$6400 (mg/m$^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) | | | | | | | |

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| Endpoint | Assay | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **(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_TRANS | | | | | 11.9 | | | | | | | |
| | ATG_ERa_TRANS_perc | | | | | 5.77 | | | | | | | |
| | ATG_PXRE_CIS | | | | | 31.1 | | | | | | | |
| | ATG_PXRE_CIS_perc | | | | | 31.1 | | | | | | | |
| | OT_ERa_ERELUC_AG_1440 | | | | | | | | 3.14 | | | | |
| | Tox21_AR_BLA_Agonist_ch1 | | | | | | | | 0.00219 | | | | |

| | | | | | | | | | 54.9 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tox21_ELG1_LUC_Agonist_viability | | | | | | | | | | | | |

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