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

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16   **Highlights**

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

Abstract: n-Alkanols provide an excellent example where a category-approach to read-across

may be used to estimate the repeated-dose endpoint for a number of untested derivatives (target

chemicals) using experimental data for tested derivatives (source chemicals). n-Alkanols are

non-reactive and exhibit the unspecific, reversible simple anaesthesia or non-polar narcosis mode

of toxic action in that the metabolic products of the parent alcohols do not contribute to the toxic

endpoint evaluated. In this case study, the chemical category is limited to the readily bioavailable

(C5 to C13) analogues. The toxicokinetic premise includes rapid absorption via the

gastrointestinal tract, distribution in the circulatory system, and first-pass metabolism in the liver

resulting in metabolism via oxidation to CO_2 and with minor elimination of oxidative

intermediate as glucuronides. Two analogues have experimental 90-day oral repeated-dose

toxicity data which exhibit qualitative and quantitative consistency. Typical findings include

decreased body weight, slightly increased liver weight which, in some cases, is accompanied by

clinical chemical and haematological changes but generally without concurrent histopathological

effects at the Lowest Observed Effect Level (LOEL). Chemical similarity between the analogues

is readily defined by a variety of structure-related properties; data uncertainty associated with

toxicokinetic and toxicodynamic similarities is low. Uncertainty associated with mechanistic

relevance and completeness of the read-across is reduced by the concordance of *in vivo* and *in*

vitro results, as well as high throughput and *in silico* methods data. As shown in detail, the 90-

day oral repeated-dose toxicity No Observed Effect Level (NOEL) value of 1000 mg/kg bw/d for

1-pentanol and 1-hexanol based on LOEL of very low systemic toxicity can be read across to fill

the data gaps of the untested analogues in this category with acceptable uncertainty.

1 Introduction

1.1 Read-across

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

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

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

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

69 chronic health endpoints. To justify the applicability domain of the category it is often necessary

70 to establish toxicodynamic, and to a greater extent toxicokinetic, similarity within the category.

71 The purpose of this research was to demonstrate the how read-across predictions of the repeated-

72 dose toxicity no observed effect level (NOEL) value based on a consistent set of lowest observed

73 effect level (LOEL) symptoms could be performed and substantiated for a category of n-alkanol

74 analogues. Specifically, the category based data providing information to reduce uncertainties,

75 and add to the WoE associated with read-across predictions of specified *in vivo* data. Thus, the

76 estimations from the read-across are quantitative and with sufficiently low uncertainty that they

77 may be used in risk assessments. As such, the predicted 90-day repeated-dose NOEL values are

78 accompanied by sufficient relevant *in vivo* and non-animal test data to make the uncertainties

79 equal to what would be expected from running a test using a protocol similar to Organization for

80 Economic Co-Operation and Development (OECD) TG 408. In the present study, a previously

81 reported 'strategy' was employed to assess similarities and overall completeness of the read-

82 across [5].

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

84 Historically, intermediate chain-length n-alkanols are considered nonpolar narcotics which act

85 mechanistically in a manner similar to depressant anaesthetics. Fang, McKim, Koleva and their

86 co-workers [6-8] reported multiple-regression type quantitative structure-toxicity relationships

87 (QSARs) for oral log $LD50^{-1}$ data for rodents and the 1-octanol/water partition coefficient (log

88 Kow). Comparison of measured toxicity data with predictions from baseline QSARs reveals that

89 saturated monohydric alcohols consistently behave as classic nonpolar narcotics [9].

90   The efficacy of n-alkanols to induce ataxia [10] and enzyme release from liver cells [11] has

91   been interpreted as being due to the hydrophobic property of the alkanols. Perfused rat liver

92   toxicity data from Strubelt et al. [12] for 1-pentanol (exposure 65.1 mmol/l for 2 hours) are

93   reported in Table 1. These data support the premise that mammalian ex *vivo* toxicity (e.g., O_2

94   consumption and ATP production) of n-alkanols is due to membrane partitioning resulting in

95   loss of membrane integrity (i.e., cytosolic enzyme leakage (LDH) but not glutathione (GSH)

96   binding).

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

Name	log Kow	O_2 consumption (μmol/g x min)	ATP (μmol/g)	LDH (U/l)	GSH (μmol/g)
Control		1.54 ± 0.07	1.25 ± 0.20	1109 ± 265	2.52 ± 0.29
1-Pentanol	1.40	0.06 ± 0.01	0.20 ± 0.03	28959 ± 4142	2.82 ± 0.36

99

100   Due to bioavailability, and distribution and mechanistic considerations, the applicability domain

101   for this case study is limited to n-alkanols with a carbon atom (C) chain length range of C5 to

102   C13. For example, since longer-chain derivatives are typically transported via carrier molecules,

103   they are not included in this chemical category. Also, shorter-chain derivatives are not included

104   in this chemical category, as they have the potential to volatilise.

105   The general anaesthetic potency of several members of this homologous series of saturated

106   aliphatic alcohols was determined in tadpoles, using the loss of righting reflex as the criterion of

107   anaesthesia [13]. In this series, anaesthetic potency increased with chain length and was maximal

108   for 1-dodecanol. The cut-off in potency was between C12 and C14, such that 1-tridecanol was a

109   partial anaesthetic.

110   n-Alkanols within the range C5-C13 are expected to be readily absorbed by the gastrointestinal

111   tract and distributed in the blood in solution. n-Alkanols are metabolised mainly in the liver via

112   alcohol dehydrogenase to corresponding aldehydes and, subsequently, by aldehyde

113   dehydrogenase to the corresponding carboxylic acids [14]. The fatty acid derivatives of

114   intermediate size n-alkanols are readily taken up by mitochondria, where they are degraded by β-

115   oxidation, especially in hepatocytes and myocytes [14]. However, generally <10% of the dose of

116   these primary alcohols form glucuronic acid conjugates which are excreted in the urine [15].

117   Voskoboinikova [16] and Opdyke [17] have summarised the historical literature on aliphatic

118   alcohol toxicity. More recently, the toxicity of alkanols containing from one to six C-atoms has

119   been reviewed [18]. A cursory summary of the rat oral acute and oral repeated-dose toxicity of

120   intermediate size n-alkanol are presented in Table 2. In general, n-alkanols acute oral toxicity

121   (i.e., LC50) is very low, ranging from 1500 to 5000 mg/kg bw with an average value of ≈3000

122   mg/kg bw. n-Alkanols are only slightly toxic in oral repeated-dose testing; typically, the rodent,

123   oral, 90-day, repeated-dose NOEL in mg/kg bw/d is in the range of 1/2 - 1/3 the LC50 value.

124   This value is characteristically based on clinical symptoms, haematological values outside the

125   normal range, or whole body effects different from normal. However, if ingested in large enough

126   quantities (i.e., near lethal doses), n-alkanols have the potential to cause systemic damage to the

127   liver, heart, kidneys, and/or nervous system (see citations in Table 2 for details).

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

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

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

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

M- male, F- female

2. Method and Materials

This evaluation of selected n-alkanols follows the workflow of Przybylak et al. [5]. It is in accord

with the guidelines proposed by OECD [34] and Schultz and co-workers [35]. *In vivo* data used

in the assessment were taken from the literature, including ECHA REACH Registered

Substances database [36]. Mechanistic relevance, as well as toxicokinetic and toxicodynamic

similarity of the category analogues was established using relevant non-animal data.

2.1 Target and Source Substances

In this case study, the analogues (listed in Table 3) include seven target and two source

chemicals; the latter, those with repeated-dose data derived from a 90-day OECD TG 408 assay,

are noted in bold print. This list is inclusive, as defined by the limitations of the applicability

domain. The analogues represents n-alkanols which are found in governmental or industrial

inventories (e.g., OECD High Production Volume Chemicals). Additional substance identifier

information, such as chemical structures and molecular formulas, are available in Table 1 of the

supplemental information.

Table 3. n-Alkanols considered as part of the chemical category for read-across. Compounds in bold indicate the source substances.

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

149

150 2.2 Endpoint

151 The NOEL for the 90-day rat oral repeated-dose is the single endpoint for which this category

152 approach is applied. The 90-day oral repeated-dose data for 1-pentanol and 1-hexanol are

153 particularly well-suited for read-across; the NOELs are based on experimental results from a 4-

154 dose exposure scenario (0, <100, between 100 and 500, and ≥ 1000 mg/kg bw/d) following a

155 standard test guideline (OECD TG 408) where the LOEL symptoms are reported. Moreover,

156 there are supporting repeated-dose results for 1-heptanol, 1-undecanol and 1-dodecanol from

157 OECD TG 422 studies, with the exposure durations for males being 28 days and for females 54

158 days.

159 2.3 Hypothesis of the category

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

161 • n-Alkanols of intermediate chain length (i.e., C5 to C13) are direct acting toxicants (i.e.,

162 metabolic activation and detoxification is not a factor in toxicity) with a similar reversible

163 mode of action (i.e., non-polar narcosis or simple anaesthesia).

164   • Within C5 to C13 derivatives, C-atom chain length affects most physico-chemical

165   properties (e.g., Log Kow values increase with increasing chain length). However, this

166   trend, while toxicologically relevant in fish toxicity and *in vitro* assays, is not observed in

167   mammalian acute and sub-chronic toxicity via oral exposure.

168   • These primary alkanols are rapidly and nearly completely absorbed from the gut and

169   distributed in the blood in solution; first pass metabolism leads to two-step oxidative

170   metabolism in the liver resulting in corresponding carboxylic acid, which subsequently

171   undergoes mitochondrial β-oxidation to CO_2 with minor amounts of glucuronidation with

172   subsequent elimination of the phase II metabolite in the urine.

173   • Toxicodynamically, these primary alkanols are highly similar. Briefly, *in vivo* they

174   exhibit very low systemic toxicity; *in vitro* and *in silico* they exhibit no chemical

175   reactivity or receptor-mediated interactions.

176   • 90-day oral rat repeated-dose NOAEL data for 1-pentanol and 1-hexanol can be read

177   across to other category members listed in Table 3 with acceptable uncertainty.

178   **3 Results**

179   3.1 Read-across justification

180   In order to conduct a read-across, there is the requirement for high quality *in vivo* data for the

181   endpoint under consideration [5, 34, 35]. In this case, is 90-day oral repeated dose-toxicity for

182   rats in the form of a NOEL value and LOEL symptoms from a study similar to OECD TG 408.

183   From a repeated-dose perspective, test results of n-alkanols are extensive. 1-Pentanol was orally

184   administered to rats following OECD TG 408 at dose levels of 0, 50, 150 or 1000 mg/kg bw/d

185   for 13 weeks [20, 21]. The "no-outward-effect level" (assumed to be the NOEL) was 1000

186   mg/kg/day.

187   In a non-standard rat oral repeated-dose assay similar to an OECD TG 408 assay, animals were

188   exposed to 0.25% (based on nominal concentrations in the diet) and 0.50% for 13 weeks; 1.0%

189   for 10 weeks, then 2.0% (week 11), 4.0% (week 12) and 6.0% 13 weeks of 1-hexanol [23]. The

190   NOAEL for 1-hexanol was determined to be ≈1100 mg/kg bw/d (1127 mg/kg bw/d for male and

191   1243 mg/kg bw/d for female rats).

192   While the endpoint read across in this exercise is the 90-day oral repeated-dose NOEL, there is

193   also high quality repeated-dose toxicity NOEL/LOEL data for shorter duration studies (e.g.,

194   OECD TG 422). Since these data are both qualitatively and quantitatively similar to the 90-day

195   data, they may be used as WoE and to confirm that all category members are within the endpoint

196   domain.

197   1-Heptanol was administered orally to rats under OECD TG 422 and 0, 100, 300 and 1000

198   mg/kg bw/d [26]. No treatment related changes were noted for all parameters (e.g., biochemical,

199   haematological and clinical parameters, as well as body weight, food consumption and

200   neurobehavioral effects).

201   Following OECD TG 422, oral repeated-dose toxicity of 1-undecanol in rats was evaluated at

202   doses of ≈ 0, 100, 500, 2000 mg/kg bw/d [30]. A NOEL for systemic toxicity of 2000 mg/kg

203   bw/d was determined in male rats, in the absence of toxicologically significant effects at any

204   dose level.

205 Following OECD TG 422, rats were exposed to 1-dodecanol in the diet in concentrations of ≈ 0,

206 100, 500 and 2000 mg/kg/ bw/d [31]. A NOEL for systemic toxicity of 2000 mg/kg bw/d was

207 determined in male rats, in the absence of toxicologically significant effects at any dose level.

208 In summary, while protocols vary, results for repeated-dose toxicity test results exhibit

209 qualitative and quantitative consistency. Phenotypic results from repeated exposure to n-alkanols

210 reflect mild changes consistent with low-grade effects and include decreased body weight,

211 accompanied by clinical chemical and haematological changes, but generally without concurrent

212 histopathological effects.

213 3.2 Applicability domain

214 As previously noted, the applicability domain for this case study is confined to straight-chain

215 primary alkanols of intermediate size, C5 to C13.

216 3.3 Purity/impurities

217 Read-across is based on the structural similarity of the main constituents of the source and target

218 substances. Toxicity may actually be determined by an impurity, therefore it is important to

219 provide a purity/impurity profile for all analogues. However, in this case it was not possible to

220 take into account impurities based on production. Since the category is structurally limited, the

221 impurities are expected to be similar if not the same across the members and are not expected to

222 significantly impact the toxicity profile of any analogue. However, it is acknowledged for

223 regulatory decisions such information may be required.

224 3.4 Data matrices for assessing similarity

225 In order for a read-across prediction to be accepted, there is the requirement to establish

226 similarity between the source and target substance [5, 34, 35]. While structural similarity is a

227 minimum, toxicokinetic similarity, especially for metabolism, and toxicodynamic similarity,

228 especially in regard to mechanistic plausibility, is required for sub-chronic endpoints such as 90-

229 day oral repeated dose-toxicity [5].

230 3.4.1 Structural similarity

231 As demonstrated in Tables 1 and 3 of the supplemental information, all the n-alkanols included

232 in the category are structurally highly similar. Specifically, they: 1) belong to a common

233 chemical class, aliphatic alcohols and subclass, n-alkanols, and 2) possess common molecular

234 scaffolding, a C-atom backbone with a straight-chain configuration. Structurally, the only

235 variable is the length of the hydrocarbon backbone, C5-C13.

236 3.4.2 Chemical property similarity

237 As demonstrated in Table 2 of the supplemental information, all the n-alkanols included in the

238 category have many of their physico-chemical properties determined experimentally. Thus, when

239 required calculated values, which are based on these measured values can be accepted with high

240 confidence. Properties, with the exception of density and pKa, trend in value related to C-atom

241 number within the scaffold. Specifically, all category members exhibit molecular weights from

242 88 to 200 g/mol. Hydrophobicity (as modelled by log Kow) increases with number of C-atoms

243 from >1.0 to <6.0, vapour pressure and water solubility decrease with molecular size, melting

244 point and boiling point increase with molecular size, and density is constant at 0.8 ± 0.1 g/cm^3.

245 Since there is no readily ionisable substituent the pKa is consistent at ≈ 15.2.

246 3.4.3 Chemical constituent similarity

247   As shown in Table 3 of the supplemental information, all the n-alkanols included in the category

248   have common constituents in the form of: 1) a single key substituent, -OH, and 2) structural

249   fragments, $-CH_3$ and $-CH_2-$.

250   3.4.4 Toxicokinetic similarity

251   Limiting the range of C-atoms for the applicability domain reduced the impact of size on

252   adsorption, distribution, metabolism and elimination (ADME). From a bioavailability standpoint,

253   the analogues exhibit in *in silico* models linear trend with molecular weight. Such modelling

254   reflects hydrophobic-dependent uptake.

255   The toxicokinetic understanding of alkanols is reasonably complete despite the fact that the

256   experimental data, as summarised in Table 4 of the supplemental information, are limited.

257   Absorption, distribution and elimination are not considered factors in these predictions. For

258   example, 1-octanol is rapidly absorbed after oral administration (i.e., bioavailability >80%). 1-

259   Octanol is excreted mainly as CO_2, and to a lesser extent as n-octyl glucuronide [17, 27, 37].

260   Other n-alkanols exhibit similar toxicokinetics, with n-alcohols generally forming <10% of the

261   dose as glucuronic acid conjugates and are excreted in the urine [15].

262   It is generally accepted that, regardless of species, metabolism of n-alkanols is highly efficient

263   and proceeds in a similar fashion [38]. Basically, there only degradative or detoxification

264   pathways involved in the metabolism of n-alkanols. It is universality accepted that in the first

265   step of the biotransformation, the alcohols undergo stepwise intracellular oxidation to the

266   corresponding carboxylic acids, followed by a stepwise C2 unit elimination via mitochondrial β-

267   oxidation [38].

268   3.4.5 Metabolic similarity

269   As demonstrated in Table 5 of the supplemental information, all of the category members

270   undergo oxidation and hydroxylation in metabolic simulations. Briefly, mammalian catabolism

271   of fatty acids, which most often takes place in the mitochondria, leads to the formation of acetyl-

272   coenzyme A (CoA), enters the TCA cycle and reduces nicotinamide adenine dinucleotide

273   (NADH) and flavin adenine nucleotide (FADH2) which are used by the electron transport chain

274   to produce ATP [14].

275   While other processes, including ω-oxidation and α-oxidation, are known to take place, β-

276   oxidation is the most common catabolic process in n-alkanol metabolism. It is highly likely that

277   the n-alkanols included in the category will be nearly completely metabolised (i.e., >90%) via

278   the tricarboxcylic acid (TCA) cycle. It is generally agreed that cytosolic fatty acids are activated

279   for degradation by conjugation with CoA. β-Oxidation of saturated fatty acids consists of a

280   recurring cycle of four reactions [14]. In acids with an even number of C-atoms, this cycling

281   continues until two molecules of Acetyl-CoA are produced in the final reaction. Acetyl-CoA is

282   available to be further metabolised in the TCA cycle. In acids with an odd number of C-atoms,

283   the end product is propionyl-CoA, which must be converted to succinyl-CoA to enter the TCA

284   cycle.

285   3.4.6 Toxicophore similarity

286   The severe limitation of the structural domains sharply reduces the likelihood of differences in

287   toxicophores between the target and source analogues. As demonstrated in Table 6 of the

288   supplemental information, none of the n-alkanols included in the category are associated with

289   any toxicophore based on *in silico* profilers within the OECD QSAR Toolbox V3.4.

290   3.4.7 Mechanistic plausibility similarity

291 While there is no mammalian adverse outcome pathway for the hypothesized mode of action, it

292 is generally accepted that the acute toxicity of intermediate chain n-alcohols is the result of

293 narcosis [5-9]. There are both theoretical and biochemical evidence for the cell membrane being

294 the site of action for anaesthetic-like chemicals [10-11]. Narcosis, in the broadest sense, is the

295 reversible, non-covalent disruption of hydrophobic interactions within membranes with a

296 particular volume fraction, rather than molar fraction [39]. It is the accumulation of alcohols in

297 cell membranes which disturbs their function; however, the exact mechanism is not yet known.

298 There are three competing theories of general anaesthetic action: 1) the lipid solubility-

299 anaesthetic potency correlation (i.e., the Meyer-Overton correlation), 2) the modern lipid

300 hypothesis, and 3) the membrane protein hypothesis [c.f., 40-41].

301 As stated in Table 7 of the supplemental information, the n-alkanols included in the category are

302 associated with the simple narcosis mechanism of toxicity that is equivalent to depressant

303 anaesthetics [6]. Additivity of primary alkanols in joint effect studies was demonstrated in

304 injection anaesthesia studies in rats [6],. This observation of additivity is consistent with the

305 premise that n-alkanols exhibit a common mechanism of action. More importantly, Fang et al.

306 [6] demonstrated additivity or slight deviations from additivity for alkanols with the conventional

307 inhaled anaesthetic desflurane (1,2,2,2-tetrafluoroethyl difluoromethyl ether). The latter support

308 the contention that the mechanisms of action of n-alkanols is depressant anaesthesia.

309 The effect of various primary alkanols on the CNS was studied by using rat brain synaptosomal

310 membranes as an *in vitro* model [41]. The activity of (Ca_2^+/Mg_2^+) ATPase and the membrane

311 fluidity were determined. Specifically, the n-alkanols exhibited an increased molar inhibition of

312 the ATPase activity, with an increase in the carbon chain length up to 1-octanol. 1-Octanol and

313 1-decanol caused a biphasic effect on the ATPase activity, depending on the n-alkanol

314 concentration, whereas 1-dodecanol caused a stimulation of the ATPase activity. All alkanols

315 studied caused an increased fluidity of the membrane; however, changes in the membrane

316 fluidity do not seem to be a pre-requisite of the ATPase inhibition [41].

317 The Fish Acute Toxicity Syndrome (FATS) approach put forth by McKim et al. [7] has furthered

318 our mechanistic understanding and the effects of intermediate chain saturated alcohols in fish

319 more than anything else. The FATS approach is based on physiological response sets from

320 spinally transected rainbow trout (*Oncorhynchus mykiss*) exposed to model chemicals. Briefly, *in*

321 *vivo* biochemical and respiratory-cardiovascular responses were measured during lethal aqueous

322 exposures; the responses and their interdependence formed a complex data matrix, with the best

323 response variables for mechanisms of action being determined with multivariate statistics. The

324 FATS for 1-octanol is characterised by a striking slow-down in all respiratory and cardiovascular

325 functions [7] that makes it distinct from other modes of actions. The action of 1-octanol is

326 consistent with depressant anaesthesia.

327 The contributions of functional groups in acute rat oral toxicity have been calculated using

328 alkanes as the baseline [40]. The toxic contribution of the OH group is -0.108. This situation

329 (negative contribution to toxicity as compared to corresponding alkane) has not been observed in

330 acute fish toxicity because the threshold of excess toxicity is too high to distinguish differences

331 in toxicity. Critical body residues (CBRs) calculated from percentage of absorption and

332 bioconcentration factors indicate that most of aliphatic alcohols share the same modes of toxic

333 action between fish and rat. Specifically, fish and rat log (1/CBR) and number of alcohols are

334 1.65; 18 and 1.58; 348, respectively [40].

335   In summary, there are several lines of evidence that support the contention that all the analogues

336   within the domain act in a similar fashion and that fashion is not different from simple

337   anaesthesia or non-polar narcosis.

338   3.4.8 Other *in vivo* endpoint similarity

339   In mammals, alkanols are considered baseline inhalation toxicants which model as simple

340   narcotics [9]. Based on acute oral toxicity, n-alkanols belong to Category 4 which do not require

341   a hazard label for acute oral toxicity. Their LD50 values are very low, typically ranging from

342   1000 to >5000 mg/kg bw with an average value of ≈3000 mg/kg bw (see Table 2). In mammals,

343   mild to moderate sub-lethal toxicity from a single oral dose of intermediate size alkanols include

344   general gastrointestinal symptoms (e.g., nausea, vomiting, abdominal cramps and diarrhoea)

345   associated with irritation. High ingested doses (i.e., near acute lethal levels) can cause

346   gastrointestinal haemorrhage and liver injury. For example, in the rat, the LD50 for 1-octanol is

347   >5000 mg/kg [17]; the only symptoms of intoxication observed were moderately to severely

348   ruffled fur and mild sedation. The symptoms had disappeared completely 24 hours later. The

349   growth of the exposed animals was similar to that of the controls.

350   In fish, alkanols are considered to act via the nonpolar narcosis mode of action [42, 43]. Within

351   the USEPA DSSTox Fathead Minnow Acute Toxicity (EPAFHM) database, alkanols are

352   represented. They exhibit toxic potencies not statistically different from baseline predictions.

353   Because of concerns for aquatic toxicity, a large number of alcohols, especially saturated ones,

354   have been tested *in vitro* for cell population growth inhibition [44]. Structure-activity results

355   from *in vivo* and *in vitro* tests are highly consistent [45]. Briefly, from a structural standpoint, the

356   aquatic toxicity of alkanols is partition-dependent, regardless of endpoint being assessed.

357 Generally, for alkanol exposures in *in vitro* assays, results are attributed to unspecific

358 interactions with biological membranes [11]; such effects are typically directly correlated with 1-

359 octanol/water partition coefficients (c.f. [46]).

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

361 In an effort to further support the mechanistic argument for this read-across information form

362 two new methods were examined. Specifically, relevant HTS data in the form of ToxCast data

363 [47, 48] and of *in silico* nuclear receptor binding predictions [49] were evaluated. Within the

364 USEPA toxicity forecaster program (ToxCast) [50], data are available for the majority of the n-

365 alkanol derivatives (see Table 8 of the supplemental information). Of the 711 possible assays

366 that form the ToxCast scheme, 1-octanol, 1-undecanol, 1-dodecanol and 1-tridecanol have been

367 evaluated in 602 of them. Additionally, 1-hexanol, 1-heptanol and 1-decanol have been assessed

368 in about 250 assays. Lastly, 1-nonanol has been tested in 150 ToxCast assays. The number of

369 active assays varies from none for 1-octanol to 25 for 1-undecanol and 30 for 1-tridecanol.

370 Within ToxCast, the n-alkanols are among the "least promiscuous chemical classes"; $< 2.74\%$ of

371 the ToxCast assays showing any activity up to highest concentration tested and none of the

372 active assay are associated with specific bioactivity.

373 Only four non-specific cell viability qHTS assays within the Toxcast suite were positive for four

374 of the tested n-alkanol analogues; no assay exhibits activity for five or more of the category

375 analogues. Specifically, the Tox21_ELG1_LUC_Agonist_viability,

376 Tox21_TR_LUC_GH3_Antagonist_viability, Tox21_AhR_viability and

377 Tox21_Aromatase_Inhibition_viability show a positive response with four n-alkanols but there is

378 no consistency among which analogues are positive.

379 Alkanols were screened with a variety of *in silico* profilers [49]. Specifically, profilers for

380 nuclear receptor binding were run to identify potential binding to the following nuclear

381 receptors; PPARs (peroxisome proliferator-activated receptors), AR (androgen receptor), AHR

382 (aryl hydrocarbon receptor), ER (oestrogen receptor), GR (glucocorticoid receptor), PR

383 (progesterone receptor), FXR (farnesoid X receptor), LXR (liver X receptor), PXR (pregnane X

384 receptor), THR (thyroid hormone receptor), VDR (vitamin D receptor), as well as RAR/RXR

385 (retinoic acid receptor/ retinoid X receptor). The evaluation of potential binding to the receptors

386 is based on structural fragments and physico-chemical features that have been identified as

387 essential to bind to these nuclear receptors and induce a response. As noted in Table 6 of the

388 supplemental information, no potential receptor binding was predicted. It is worth noting that

389 ToxCast also tested for all of these receptors, and all corresponding assays were negative.

390 Taken collectively, the HTS and *in silico* findings are not inconsistent with the cited *in vivo* data.

391 The premise that, oral repeated-dose toxicity of n-alkanols are considered to be nonpolar

392 narcotics and act in a manner similar to depressant anesthetics is consistent with the ToxCast

393 data and receptor binding simulations results which indicate no activity associated with a specific

394 mode of action.

395

396 **4. Statement of uncertainty**

397 The categorical assessments of uncertainties along with summary comments are presented in

398 Tables 4 and 5. Briefly, chemical similarity is limited by chain length but has no impact on

399 repeated-dose toxicity. Data uncertainty with the fundamental aspects of toxicokinetics is low.

400 Regardless of the species of mammals, all such category members are judged to be readily

401 absorbed orally and to have similar distributions; metabolised via oxidation to the acid

402 derivative, subsequently degraded to CO_2 via mitochondrial oxidation, and/or eliminated as a

403 glucuronide. Data uncertainty with the fundamental aspects of toxicodynamics is low, in that

404 category members exhibit a very low-toxic profile with respect to *in vivo* effects (i.e., NOEL and

405 LOEL), as well as with respect to *in vitro* and new-methods effects. n-Alkanols are

406 experimentally associated with the nonpolar narcosis mechanisms of toxicity. The simple

407 narcosis (i.e., reversible anaesthesia) mode of toxic action is driven by partitioning into the

408 biophase. While well-studied, this molecular mechanism is not well-understood and no adverse

409 outcome pathway (AOP) is currently available. Moreover, it is unclear if oral repeated-dose

410 toxicity is related to this mechanism; however, there is no evidence to suggest it is not.

411 Uncertainty associated with mechanistic relevance and completeness of the read-across (i.e.,

412 uncertainty in the predictions) while initially low-to-moderate is reduced to low with the addition

413 of ToxCast and *in silico* screening data. The major source of uncertainty for this group of

414 alcohols is associated with what is essentially a "low-toxic" prediction.

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

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

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

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

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

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

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

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

425 [a] Uncertainty: low, medium, high

426 **5. Statement of the conclusions**

427 This is the second in a series of read-across case studies; this particular study examines a

428 category of similar compounds that do not require (or do not undergo) metabolism to exert a

429 potential adverse human health effect [51]. *In vivo* oral repeated-dose exposure to n-alkanols

430 gives rise to a set of nonspecific symptoms, including clinical symptoms, haematological values

431 outside the normal range, or whole body effects different from normal. Limiting the category to

432 C5 to C13 analogues assures that the impact of bioavailability on the toxicokinetic and

433   toxicodynamic profiles is very limited. Primary alkanols are direct-acting toxicants with a

434   reversible mode of toxic action described as nonpolar narcosis (i.e., unspecific interaction with

435   biological membrane in a manner similar to simple anaesthetics). The main route of exposure for

436   alkanols is oral via rapid gastrointestinal absorption. The majority of an oral dose of any n-

437   alkanol is promptly degraded via simple cellular oxidation; the remainder is eliminated as the

438   glucuronide conjugate.

439   Repeated-dose toxicity test results exhibit qualitative consistency in results between and within

440   species. While protocols vary, results of oral repeated-dose testing exhibit qualitative consistency

441   between and within mammals. Typical findings are only mild changes including decreased body

442   weight, slightly increased liver weight, as well as clinical chemical and haematological changes,

443   but typically without concurrent histopathological effects.

444   Within ToxCast, the n-alkanols are among the "least promiscuous chemical classes"; < 2.74% of

445   the ToxCast assays showing any activity and none of the active assay being associated with

446   specific bioactivity. Screening with *in silico* profilers reveals that n-alkanols have no predicted

447   potential of nuclear receptor binding.

448   This is a category read-across (i.e., many-to-one several times). While several analogues have

449   been evaluated experimentally in oral repeated-dose testing schemes, the 90-day oral repeated-

450   dose toxicity data and the NOAELs of 1000 mg/kg bw/d for 1-pentanol and1-hexanol is the

451   conservative prediction. A no systemic toxic conclusion with a NOAEL of 1000 mg/kg bw/d can

452   be read across with confidence to untested n-alkanols in the C5 to C13 category listed in Table 3.

453

454   **6. Acknowledgements**

461

7. References

463 [1] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30

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

465 L 342: 59-209.

466 [2] Cronin, M.T.D., Madden, J.C., Enoch S.J., Roberts, D.W., (Eds.), 2013. Chemical Toxicity

467 Prediction: Category Formation and Read-Across Applications. The Royal Society of

468 Chemistry, Cambridge UK.

469 [3] Teubner, W. and Landsiedel, R., 2015. Read-across for hazard assessment: The ugly

470 duckling in growing up. ATLA 43: 67-71.

471 [4] European Chemicals Agency (ECHA). 2012. Practical Guide 6 — How to report read

472 across and categories [ECHA-10-B-11.1-EN]. 32pp. European Chemicals Agency,

473 Helsinki, Finland.

474 [5] Przybylak, K.R., Schultz, T.W., Richarz, A.-N., Mellor, C.L., Escher, S.E., and Cronin,

475 M.T.D. 2016. Read-across of 90-day rat oral repeated-dose toxicity: A case study for

476 selected β-olefinic alcohols. Computational Toxicology (in press).

477   [6] Fang, Z., Ionescu, P., Chortkoff, B.S., Kandel, L., Sonner, J., Laster, M.J. and Eger, E.I.

478   1997. Anesthetic potencies of n-alkanols: Results of additivity and solubility studies

479   suggest a mechanism of action similar to that for conventional inhaled anesthetics. Anesth.

480   Analgesia 84: 1042-1048.

481   [7] McKim, J.M., Bradbury, S.P. and Niemi, G.J. 1987. Fish acute toxicity syndromes and their

482   use in the QSAR approach to hazard assessment. Environ. Health Perspect. 71: 171-186.

483   [8] Koleva, Y.K., Cronin, M.T., Madden, J.C. and Schwöbel, J.A. 2011. Modelling acute oral

484   mammalian toxicity. 1. Definition of a quantifiable baseline effect. Toxicol. In Vitro 25:

485   1281-1293.

486   [9] Veith, G.D., Petkova, E.P. and Wallace, K.B. 2009. A baseline inhalation toxicity model for

487   narcosis in mammals. SAR QSAR Environ. Res. 20: 567-578.

488   [10] McCreery, M.J. and Hunt, W.A. 1978. Physico-chemical correlates of alcohol intoxication.

489   Neuropharmacology 17: 451-461.

490   [11] McKarns, S.C., Hansch, C., Caldwell, W.S., Morgan, W.T., Moore, S.K. and Doolittle,

491   D.J. 1997. Correlations between hydrophobicity of short-chain aliphatic alcohols and their

492   ability to alter plasma membrane integrity. Fundam. Applied Toxicol. 36: 62-70.

493   [12] Strubelt, O., Deters, M., Pentz, R., Siegers, C.-P. and Younes, M. 1999. The toxic and

494   metabolic effects of 23 aliphatic alcohols in the isolated perfused rat liver. Toxicol. Sci. 49:

495   133-142.

496   [13] Pringle, M.J., Brown, K.B. and Miller, K.W. 1981. Can the lipid theories of anesthesia

497   account for the cutoff in anesthetic potency in homologous series of alcohols? Mol.

498   Pharmacol. 19: 49-55.

499   [14] Voet, D. and Voet, J.G. 1990. *Biochemistry*. John Wiley & Sons, New York.

500   [15] Kamil, I.A., Smith, J.N. and Williams, R.T. 1953. Studies in detoxication. 46. The

501   metabolism of aliphatic alcohols. The glucuronic acid conjugation of acyclic aliphatic

502   alcohols. Biochem. J. 53: 129-136.

503   [16] Voskoboinikova, V.B. 1966. Substantiation of the maximum permissible concentration of

504   the flotation reagent IM-68 and its component alcohols (hexyl-, heptyl- and octyl) in water

505   reservoirs. Hyg. Sanit. (GUS) 31: 310-315.

506   [17] Opdyke, D.L. 1973. Monographs on fragrance raw materials. Food Cosmet. Toxicol. 11:

507   95-115.

508   [18] Patocka, J. and Kuca, K. 2012. Toxic alcohols: Aliphatic saturated alcohols. Mil. Med. Sci.

509   Lett. (Voj. Zdrav. Listy) 81: 142-163.

510   [19] ECB (European Chemicals Bureau) 2000. IUCLID Dataset, Pentan-1-ol (71-41-0) (2000

511   CD-ROM edition).

512   [20] Butterworth, K.R., Gaunt, I.F., Heading, C.E., Grasso, P. and Gangolli, S.D. 1976. Short-

513   term toxicity of n-amylalcohol in rats. Food Cosmet. Toxicol. 16: 203-207.

514   [21] ECHA CHEM A 1-pentanol: http://www.echa.europa.eu/web/guest/registration-dossier/-

515   /registered-dossier/2115 (accessed 27.01.2016).

516   [22] ITII (International Technical Information Institute) 1988. Toxic and Hazardous Industrial

517   Chemicals Safety Manual. Tokyo, Japan.

518   [23] ECHA CHEM B 1-hexanol: http://www.echa.europa.eu/web/guest/registration-dossier/-

519   /registered-dossier/13265 (accessed 27.01.2016).

520   [24] Bingham, E., Cohrssen, B., and Powell, C.H. 2001. Patty's Toxicology Volumes 1-9 5th

521   ed. John Wiley & Sons. New York, N.Y. p. 6:440

522   [25] Truhaut, R. 1974. Contribution à l'étude toxicologique de l'alcool heptylique. Archives des

523   maladies professionelles de médecine du travail, 35: 501-509.

524   [26] ECH CHEM C 1-heptanol: http://www.echa.europa.eu/web/guest/registration-dossier/-

525   /registered-dossier/5921/7/1 (accessed 27.01.2016).

526   [27] ECHA CHEM D 1-octanol: http://www.echa.europa.eu/web/guest/registration-dossier/-

527   /registered-dossier/15210 (accessed 27.01.2016).

528   [28] Lewis, R.J. Sr. (ed) 2004a. Sax's Dangerous Properties of Industrial Materials. 11th

529   Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. p. 1077.

530   [29] Verschueren, K. 2001. Handbook of Environmental Data on Organic Chemicals. Volumes

531   1-2. 4th ed. John Wiley & Sons. New York, NY. p. 2164.

532   [30] ECHA CHEM E 1-undecanol; http://www.echa.europa.eu/web/guest/registration-dossier/-

533   /registered-dossier/2096 (accessed 27.01.2016).

534   [31] ECHA CHEM F1-dodecanol: I http://www.echa.europa.eu/web/guest/registration-dossier/-

535   /registered-dossier/15424 (accessed 27.01.2016).

536   [32] Organization for Economic Co-Operation and Development (OECD) and Screening

537   Information Datasets (SIDS) 2006. High Production Volume Chemicals 1-Dodecanol (Cas

538   No.: 112-53-83). Processed by United Nations Environmental Program (UNEP). Available

539   from http://www.inchem.org/documents/sids/sids/112538.pdf.

540   [33] Raw Material Data Handbook. 1974. Organic Solvents.1: 114.

541   [34] Organization for Economic Co-Operation and Development (OECD) 2015. Guidance

542   Document on the Reporting of Integrated Approaches to Testing and Assessment (IATA).

543   ENV/JM/HA(2015)7.

544   [35] Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D. J., Mahony,

545   C., Schwarz, M., White, A. and Cronin, M.T.D. 2015. A strategy for structuring and

546   reporting a read-across prediction of toxicity. Reg. Toxicol. Pharmacol. 72: 586-601.

547   [36] European Chemicals Agency (ECHA) Registered substances. Available from:

548   https://echa.europa.eu/information-on-chemicals/registered-substances.

549   [37] Williams, R.T. 1959. The metabolism of some aliphatic aldehydes, ketones and acids. In:

550   Detoxication mechanisms. The metabolism and detoxication of drugs, toxic substances and

551   other organic compounds, 2nd Ed., London: Chapman & Hall, Ltd., chapter four, pp. 88-

552   113.

553   [38] Moyes, C.D. and Schulte, P.M. 2006. *Principles of Animal Physiology*. Pearson Benjamin

554   Cummings, San Francisco. p734.

555   [39] Alifimoff, J., Firestone, L. and Miller, K. 1989. Anaesthetic potencies of primary alkanols:

556   implications for the molecular dimensions of the anaesthetic site. Br. J. Pharmacol. 96: 9-

557   16.

558   [40] Edelfors, S. and Ravn-Jonsen, A. 1990. The effects of alcohols in vitro on the nervous cell

559   membrane measured by changes in the (Ca_2^+/Mg_2^+) ATPase activity and fluidity of the

560   synaptosomal membrane. Pharmacol. Toxicol. 67: 56-60.

561   [41] He, J., Fu, L., Wang, Y., Li, J.J., Wang, X.H., Su, L.M., Sheng, L.X. and Zhao, Y.H.

562   2014. Investigation on baseline toxicity to rats based on aliphatic compounds and

563   comparison with toxicity to fish: Effect of exposure routes on toxicity. Reg. Toxicol.

564   Pharmacol. 70: 98-106.

565   [42] Veith, G.D., Call, D.J. and Brooke, L.T. 1983. Structure-toxicity relationships for the

566   fathead minnow, *Pimpehales promelas*: narcotic industrial chemicals. Can. J. Fish. Aquat.

567   Sci. 40: 743-748.

568   [43] Raevsky, O.A., Grigorev, V.Y., Weber, E.E. and Dearden, J.C. 2008. Classification and

569   quantification of the toxicity of chemicals to guppy, fathead minnow and rainbow trout:

570   Part 1. Nonpolar narcosis mode of action. QSAR Comb. Sci. 27: 1274–1281.

571   [44] Schultz, T.W., Seward-Nagel, J., Foster, K.A. and Tucker, V.A. 2004. Structure-activity

572   relationships for aliphatic alcohols and aquatic toxicity to *Tetrahymena*. Environ. Toxicol.

573   19: 1-10.

574   [45] Schultz, T.W., Sinks, G.D. and Bearden, A.P. 1998. QSARs in aquatic toxicology: A

575   mechanism of action approach comparing toxic potency to Pimephales promelas,

576   *Tetrahymena pyriformis*, and *Vibrio fischeri*. In: Devillers, J. (ed.) Comparative QSAR.

577   Taylor and Francis, London, pp. 52-109.

578   [46] Benane, S.G., Richard, A.M., Blackman, C.F. and Lytle, C.D. 1993. Quantitative

579   structure-toxicity relationships for a series of primary alcohols in a mammalian viral host

580   cell reactivation assay. In Vitro Toxicol. 6: 267-277.

581   [47] US EPA ToxCast 2014. US EPA website with access to the ToxCast data.

582   https://www.epa.gov/chemical-research/toxicity-forecasting (accessed 16.06.2016).

583   [48] Richard, A.M., Judson, R.S., Houck, K.A., Grulke, C.M., Volarath, P., Thillainadarajah, I.,

584   Chihae Yang, C., Rathman, J., Martin, M.T., Wambaugh, J.T., Knudsen, T.B., Kancherla,

585   J., Mansouri, K. Patlewicz, G., Williams, A.J., Little, S.B., Crofton, K.M. and Thomas,

586   R.S. 2016. ToxCast chemical landscape: Paving the road to 21st century toxicology. Chem.

587   Res. Toxicol. 29: 1225–1251.

588   [49] Mellor, C.L, Steinmetz, F.P, Cronin, M.T.D. 2016. Using molecular initiating events to

589   develop a structural alert based screening workflow for nuclear receptor ligands associated

590   with hepatic steatosis. Chem. Res. Toxicol. 29: 203-212.

591   [50] Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M.,

592   Reif, D.M., Richard, A.M., Rotroff, D.M., Shah, I. and Dix, D.J. 2010. Predictive *in vitro*

593   screening of environmental chemicals – The ToxCast project. Environ. Health

594   Perspect.118:485-492.

595   [51] Berggren, E., Amcoff, P., Benigni, R., Blackburn, K. Carney, E. Cronin, M., Deluyker, H.,

596   Gautier, F., Judson, R.S., Kass, G.E.N., Keller, D., Knight, D., Lilienblum, W., Mahony,

597   C., Rusyn, I., Schultz, T., Schwarz, M., Schüürman, G., White, A., Burton, J., Lostia, A.,

598   Munn, S., and Worth, A. 2015. Chemical safety assessment using read-across: How can

599   novel testing methods strengthen evidence base for decision-making? Environ. Health

600   Perspect. 123: 1232–1240.

601

Supplementary material

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

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

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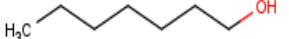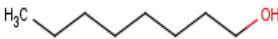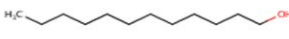
ID	Name	CAS No	SMILES	2D Structure	Molecular Formula
1	1-Pentanol	71-41-0	CCCCCO		C5H12O
2	1-Hexanol	111-27-3	CCCCCCO		C6H14O
3	1-Heptanol	111-70-6	CCCCCCCO		C7H16O
4	1-Octanol	111-87-5	CCCCCCCCO		C8H18O
5	1-Nonanol	143-08-8	CCCCCCCCCO		C9H20O
6	1-Decanol	112-30-1	CCCCCCCCCCO		C10H22O
7	1-Undecanol	112-42-5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

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

ID	Name	Molecular Weight [1]	Log Kow[1a]	Vapour Pressure (Pa, 25 deg C) [1b]	Density [2] (g/cm³)	Melting Point (deg C) [1b]	Water Solubility (mg/L, 25 deg C) [1c]	Boiling Point (deg C) [1b]	pKa [3]
1	1-Pentanol	88.15	1.33 1.51 (M)	353 293 (M)	0.8±0.1	-49.96 -78.9 (M)	20890 22000 (M)	136.95 137.9 (M)	15.24
2	1-Hexanol	102.18	1.82 2.03 (M)	117 124 (M)	0.8±0.1	-37.86 -44.6 (M)	6885 5900/6260 (M)	159.09 157.6 (M)	15.38
3	1-Heptanol	116.21	2.31 2.62 (M)	39.8 31.2 (M)	0.8±0.1	-26.03 -34 (M)	1940 1670/1800 (M)	180.33 176.4 (M)	15.38
4	1-Octanol	130.23	2.81 3.00 (M)	13.2 10.6	0.8±0.1	-14.46 -15.5 (M)	814 540 (M)	200.67 195.1 (M)	15.27
5	1-Nonanol	144.26	3.30 3.77 (M)	4.38 3.03 (M)	0.8±0.1	-3.15 -5 (M)	156.8 140 (M)	220.09 213.3 (M)	15.22
6	1-Decanol	158.29	3.79 4.57 (M)	1.45 1.13 (M)	0.8±0.1	7.89 6.9 (M)	28.21 37 (M)	238.62 231.1 (M)	15.21
7	1-Undecanol	172.31	4.28	0.68 0.396 (M)	0.8±0.1	18.67 19 (M)	43.04	256.24 243 (M)	15.2
8	1-Dodecanol	186.34	4.77 5.13 (M)	0.242 0.113 (M)	0.8±0.1	29.19 24 (M)	6.898 4 (M)	272.96 259 (M)	15.2
9	1-Tridecanol	200.37	5.26	0.0316 0.0581(M)	0.8±0.1	0.0316	4.533	288.77 152 (M)	15.2

M = measured value

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

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

ID	Name	Key Substituent(s)	Functional Group(s)	Extended Fragment(s)	Chemical Class:	Chemical Sub-Class:
1	1-Pentanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
2	1-Hexanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
3	1-Heptanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
4	1-Octanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
5	1-Nonanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
6	1-Decanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
7	1-Undecanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
8	1-Dodecanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain
9	1-Tridecanol	-OH	-CH$_3$, -CH$_2$-	–	saturated aliphatic alcohols	straight-chain

Table 4: Comparison of Abiotic Transformation and Toxicokinetics

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

[a]Williams, R.T. 1959. The metabolism of some aliphatic aldehydes, ketones and acids. In: Detoxication mechanisms. The metabolism and detoxication of drugs, toxic substances and other organic compounds, 2nd Ed., London: Chapman & Hall, Ltd., chapter four, pp. 88-113; [b]Opdyke, D.L. 1973. Monographs on fragrance raw materials. Food Cosmet. Toxicol. 11: 95-115; [c]Kwok, E.S.C. and Atkinson, R.,1994. Gas-phase atmospheric chemistry of dibenzo-pdioxin and dibenzofuran. Environ.Sci.Technol. 28:528-533; [d]Atkinson, R. 1994. Gas-phase tropospheric chemistry of organic compounds. J. Phys. Chem. Ref. Data, Monograph 2:1-216. [e]Kamil, I.A., Smith, J.N. and Williams, R.T. 1953. Studies in detoxication. 46. The metabolism of aliphatic alcohols. The glucuronic acid conjugation of acyclic aliphatic alcohols. Biochem. J. 53: 129-136.

Table 5: Comparison of Potential Metabolic Products

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

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

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

() - The number of metabolites for specific transformation.

Table 6: Comparison of Toxicophores

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

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

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

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

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

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

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

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

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

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

References for Table 8

[1]   Butterworth, K.R., Gaunt, F., Heading, C.E., Grassot, P. and Gangolli, S.D. 1978. Short-term toxicity of n-amyl alcohol in rats. Fd. Cosmet. Toxicol. 16: 203-207.

[2]   ECHA CHEM for 1-pentanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/2115

[3]   ECHA CHEM for 1-hexanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/13265

[4]   Ray S.D, Mehendale H.D 1990. Potentiation of CCl4 and CHCl3 hepatotoxicity and lethality by various alcohols Fundam. Appl. Toxicol. 15(3): 429-440.

[5]   Vaquera, J., Vaquera, A. and Girbes, T. 2002. Effects of chronic administration of either ethanol or pentanol on rat duodenum morphology. Histol. Histopathol. 17:199-203.

[6]   US FDA CFSAN PAFA 1; Uch Zap Mosk Nauchn Issled Inst Gigieny 9: 40-46.

[7]   US FDA CFSAN PAFA 27; Food. Chem. Toxicol. 35:489-500.

[8]   US FDA CFSAN PAFA 18; Acta Pathol. Jpn. 41: 405-413.

[9]   ECHA CHEM for 1-undecanol; http://echa.europa.eu/registration-dossier/-/registered-dossier/2096

[10]   Hansen, E. 1992. Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test on 1-Dodecanol in Rats. Institute of Toxicology, Danish National Food Agency, IT 921105.

[11]   ECHA CHEM for 1-dodecanol; http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15424;

[12] Wakabayashi, T., Adachi, K. and Popinigis, J. 1991. Effects of Alkyl Alcohols and Related Chemicals on Rat Liver Structure and Function. Acta. Pathol. Jpn. 41: 405-413.

[13] Danish National Food Agency 1992. Combined repeated dose and reproductive/developmental toxicity screening test in rats. Danish National Food Agency, IT 9211105

[14] Toxicity Testing Reports of Environmental (Ministry of Health and Welfare, Japan) 1997. Rep Dose Tox Fraunhofer

[15] Nelson B.K, Brightwell S.W, Khan A, Krieg E.F and Hoberman A.M 1989. Developmental Toxicology Evaluation of 1-Pentanol, 1-Hexanol, and 2-Ethyl-1-Hexanol Administered by Inhalation to Rats. J Am Col Toxicol 8(2): 405-410.

[16] ECHA CHEM search for 1-octanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15210

[17] Nelson, B.K, Brightwell, W.W., Khan, A., Krieg, E.F. and Hoberman, A.M. 1990. Developmental toxicology assessment of 1-Octanol 1-Nonanol and 1-Decanol administered by inhalation to rats. J. Amer. College Toxicol 9(l): 93-97.

[18] Klimisch, H.-J. and Hellwig, J. 1995. Studies on the Prenatal Toxicity of 3-Methyl-1-butanol and 2-Methyl-1-propanol in Rats and Rabbits Following Inhalation Exposure. Fund. Appl. Toxicol. 27(1): 77-89.

[19] Banduhn, N. 1992. Henkel KGaA, Toxikologie, 1-dodecanol (Lorol C12-99), Micronucleus Test in vivo in Bone Marrow Cells of the Mouse, Report, May 1992.

[20] Barilyak, I.R. and Kozachuk, S.Y. 1988. Studies in cytogenetic action of some monohydric alcohols on the rat bone marrow cells (Russian). Tsitol. Genet. 22: 49-52.

[21] Stoner, G.D., Shimkin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K. and Gori, G.B. 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by pulmonary tumor response in strain A mice. Cancer Research, 33: 3069-3085.

[22] van Duuren, B.L and Goldschmidt, B.M. 1976. Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. J. Nat. Cancer Inst. 56: 1237-1242.

[23] Sice, J. 1966. Tumor-promoting activity of n-alkanes and 1-alkanols. 1966. Toxicol. Applied Pharmacol. 9: 70-73.

[24] Bingham, E.L. and Falk, H.L. 1969. Environmental carcinogens: the modifying effect of cocarcinogens on the threshold response. Arch. Environ. Health 19: 779-783.

[25] Ando, K., Kodama, K., Kato, A., Tamura, G. and Arima, K. 1972. Antitumor activity of glyceryl ethers. Cancer Research 32: 125-129.

[26] Ccris database, http://toxnet.nlm.nih.gov/newtoxnet/ccris.htm

[27] Jenner, P.M., Hagen, E.C., Taylor, J.M., Cook, E.L. and Fitzhugh, O.G. 1964. Food flavourings and compounds of related structure. Acute oral toxicity. Fd. Cosmet. Toxicol. 2: 327-343.

[28] ChemIDplus search results, http://chem.sis.nlm.nih.gov/chemidplus/rn/64-17-5. Original reference given; EHP, Environmental Health Perspectives.1985. Vol. 61, Pg. 321.

[29] National Technical Information Service. Vol. OTS0534716, http://www.ntis.gov/about/

[30] ECHA CHEM registered substances, http://echa.europa.eu/information-on-chemicals/registered-substances,Study report 1973

[31]   ECHA CHEM registered substances, http://echa.europa.eu/information-on-chemicals/registered-substances,Study report 1978

[32]   ChemIDplus search, http://chem.sis.nlm.nih.gov/chemidplus/rn/100-51-6. Original reference cited; Journal of Pharmacology and Experimental Therapeutics. Vol. 16, Pg. 1, 1920.

[33]   ChemIDplus search, http://chem.sis.nlm.nih.gov/chemidplus/rn/71-41-0. Original reference cited: Comptes Rendus Hebdomadaires des Seances, Academie des Sciences. 1975. Vol. 81, Pg. 192.

[34]   Chvapil, M., Zahradnik, R. and Cmuchalova, B. 1962. Influence of alcohols and potassium salts of xanthogenic acids on vari. Arch Int Pharmacodyn Ther. 1962 Feb 1ous biological objects; 135: 330.

[35]   European Chemicals Bureau; IUCLID Dataset, 1-Hexanol (111-27-3) (2000 CD-ROM edition). Available from, as of April 20, 2006: http://esis.jrc.ec.europa.eu/

[36]   Lewis, R.J. Sr. 1955. Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004.

[37]   Bingham, E., Cohrssen, B. and Powell, C.H. 2001. Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001) p. 6-440.

[38]   ITII (1988). Toxic and Hazardous Industrial Chemicals Safety Manual. Tokyo, Japan: The International Technical Information Institute, p. 267.

[39]   ECHA CHEM for 1-heptanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/5921/7/1

[40]   US FDA CFSAN PAFA 2; Gig Sanit 31: 310-316.

[41]   US FDA CFSAN PAFA 18; Chemosphere 36: 1475-1483.

[42]   US FDA CFSAN PAFA 1; Gig Sanit 31:310-316.

[43]   US FDA CFSAN PAFA 9; Fd. Cosmet. Toxicol. 11: 101.

[44]   Opdyke, D.L.J. 1973. Monographs on fragrance raw materials: Acetate C-9. Fd. Cosmet. Toxicol. 11: 95.

[45]   Izmerov, N.F., Sanotsky, I.V. and Sidorov, K.K. 1982.Toxicometric Parameters of Industrial Toxic Chemicals Under Single Exposure. Moscow, Centre of International Projects, GKNT, Pg. 94.

[46]   Hau, K.M., Connell, D.W. and Richardson, B.J. 1999. Mechanism of acute inhalation toxicity of alkanes and aliphatic alcohols. Environ. Toxicol. Pharmacol. 7: 159-167.

[47]   Lewis, R.J. 1967. Food Additives Handbook. Medizin und Ernaehrung. Vol. 8, p244.

[48]   Raw Material Data Handbook 1974. Vol.1: Organic Solvents. p114.

[49]   ChemIDplus search, http://chem.sis.nlm.nih.gov/chemidplus/rn/137-32-6. Original reference quoted; Journal of Industrial Hygiene and Toxicology. 1945. Vol. 27, Pg. 1

[50]   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 membrane fluidity. Pharmacol. Toxicol. 67: 56-60.

[51]   Kreja, L. and Seidel, H.-J. 2002. Evaluation of the genotoxic potential of some microbial volatile organic compounds (MVOC) with the comet assay, the micronucleus assay and the HPRT gene mutation assay. Mut. Res. 513: 143-150.

[52]   Nakajima, D., Rhii, R., Kageyama, S., Onji, Y., Mineki, S., Morooka, N., Takatori, K. and Goto, S. 2006. Genotoxicity of microbial volatile organic compounds. J. Health Sci. 5: 148-153.

[53]   Toxcast^{TM} data, Provider the USEPA; http://www.epa.gov/ncct/toxcast/data.html

[54]   ChemIDPlus search. Original reference quoted: Gigiena i Sanitariya. For English translation, see HYSAAV. 1970 Vol.35, p 88.

[55]   ChemIDPlus search. Original reference quoted: Gigiena i Sanitariya. For English translation, see HYSAAV. 1986. Vol.51, p61.

[56]   ChemIDPlus search. Original reference quoted: Archives Internationales de Pharmacodynamie et de Therapie.1935. Vol. 50, p296.

[57]   ECHA. Study report 1986. http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d8a5727-6a82-2e4a-e044-00144f67d249/AGGR-0517495b-7701-4b59-bb0c-32d003c0f680_DISS-9d8a5727-6a82-2e4a-e044-00144f67d249.html#AGGR-0517495b-7701-4b59-bb0c-32d003c0f680

[58]   Hellwig, J., and Jäckh, R. 1997. Differential prenatal toxicity of one straight-chain and five branched-chain primary alcohols in rats. Food Chem. Toxicol. 35: 489-500.

[59]   Opdyke, D.L.J. 1973. Monographs on fragrance raw materials: Alcohol C-8. Fd. Cosmet. Toxicol. 11:103-104.

[60]   Patty's Industrial Hygiene and Toxicology 3rd rev. 1982. ed., Clayton, G.D., and F.E. Clayton, eds., New York, John Wiley & Sons, Inc., 1978-82. Vol. 3 originally pub. in 1979; pub. as 2n rev. ed. in 1985.Vol. 2C, p4628.

[61]   ECHA CHEM for 1-decanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15951/1

[62]   US FDA CFSAN PAFA 1; Fd. Cosmet. Toxicol. 16: 641.

[63]   Patty's Industrial Hygiene and Toxicology. Vol. II, part D. Clayton, G. D. and Clayton, F. E. (eds.). John Wiley & Sons Inc. New York. Chapter 55.

[64]   "Alcohols," Patty's Industrial Hygiene and Toxicology, 3rd. Ed., Vol. 2C, 1982. Clayton, G., et. al., ed., John Wiley & Sons, New York, p. 4632-4633.

[65]   Stoner, G. D., Shimkin, M. B., Kniazeff, A.J., Weisburger, J.H, Weisburger, E. K. and Gori, G.B. 1973.Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res. 33: 3069-3085.

[66]   European Chemicals Bureau; IUCLID Dataset, Pentan-1-ol (71-41-0) (2000 CD-ROM edition.

[67]   Truhaut, R. 1974. Contribution à l'étude toxicologique de l'alcool heptylique. Archives des maladies professionelles de médecine du travail, 35: 501-509.

[68]   Lewis, R.J. Sr. (ed) 2004. Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. p. 1077.

[69]   Verschueren, K. 2001. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. p. 2164.