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CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED 2-ALKYL-1-ALKANOLS: READ-ACROSS

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Paris 2017

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FOREWORD

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by International Council for Animal Protection in OECD Programmes (ICAPO) for illustrating practical use of IATA and submitted to the 2016 review cycle of the IATA Case Studies project. This case study was reviewed by the project team. The document was endorsed at the1st meeting of the Working Party on Hazard Assessment in June 2017.

The following four case studies were also reviewed in the project in 2016 and are published with this case study:

1. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR THE REPEATED-DOSE TOXICITY OF PHENOLIC BENZOTRIAZOLES, ENV/JM/MONO(2017)23, Series on Testing & Assessment No. 271.

2. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR PESTICIDE CUMULATIVE RISK ASSESSMENT & ASSESSMENT OF LIFESTAGE SUSCEPTIBILITY, ENV/JM/MONO(2017)24, Series on Testing & Assessment No. 272.

3. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED N-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)25, Series on Testing & Assessment No. 273.

4. CHEMICAL SAFETY ASSESSMENT WORKFLOW BASED ON EXPOSURE CONSIDERATIONS AND NON-ANIMAL METHODS, ENV/JM/MONO(2017)27, Series on Testing & Assessment No. 275.

In addition, a considerations document summarizing the learnings and lessons of the review experience of the case studies is published with the case studies:

> REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) -Second Review Cycle (2016) - ENV/JM/MONO(2017)22, Series on Testing & Assessment No. 270.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

TABLE OF CONTENTS

Please Note: *This case study has been designed to illustrate specific issues associated with Integrated Approaches for Testing and Assessment (IATA), in particular read-across and to stimulate discussion on the topic. It is not intended to be related to any currently ongoing regulatory discussions on this group of compounds.*

SUMMARY

2-Alkyl-1-alkanols (2-position branched saturated primary aliphatic alcohols) provide an example where category approaches to read-across can be used to estimate the No Observed Adverse Effect Level (NOAEL) for the 90-day rat oral repeated-dose endpoint for a number of derivatives. The intended use of these data is in substance-specific quantitative risk assessments. In this case study, the chemical category represents derivatives which are non-reactive and exhibit nonpolar narcosis, and metabolic products of the parent alcohols have no toxicological significance (i.e., these alkanols are direct-acting toxicants). Briefly, nonpolar narcotics act via unspecific, reversible interactions with biological membrane which are non-reactive and exhibit no specific mode of toxic action, and metabolism, while complex, is straight forward and well-documented. In an effort to reduce uncertainty associated with bioavailability, the applicability domain for this case study is limited to intermediate size (i.e., carbon (C) atom number of 5 to 13) 2-alkyl-1-alkanols. Like their n-alkanol counter-parts, 2-alkyl-1-alkanols are considered to act in a manner similar to depressant anaesthetics. The read-across approach assumes 2-alkyl-1-alkanols are rapidly absorbed via the gastrointestinal tract and are partially degraded via simple cellular oxidation to CO_2 but typically eliminated as glucuronides. Two analogues, namely 2-ethyl-1-hexanol and 2-propyl-1-heptanol, have been experimentally evaluated in high quality 90-day oral repeated-dose toxicity studies. Repeated-dose toxicity test results exhibit qualitative consistency. Results of oral repeated-dose testing report only mild changes consistent with low-grade effects; 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. For this case study, these effects are considered ‘adverse’.

Chemical similarity between the analogues is readily defined, and data uncertainty associated with the similarities in chemical transformation/toxicokinetic, as well as toxicodynamics, are low. Uncertainty associated with mechanistic relevance and completeness of the read-across is low to moderate, largely because of the difficulty of proving the narcosis mode of action. Because this is a well-tested and well-understood group of chemicals, the lines-of-evidence associated with the fundamentals of chemical transformation/toxicokinetic and toxicodynamic are high. Uncertainty associated with mechanistic relevance and completeness of the read-across is reduced by the concordance of *in vivo*, *in vitro*, ToxCast results, and other new-methods data. For example, while not tested to saturation, primary alkanols are among the least promiscuous chemical classes examined with 700 ToxCast assays with only 88 of 3315 (2.7%) results showing any activity up to highest tested concentration. Moreover, none of the active assays were associated with a particular pathway or specific bioactivity. In addition, primary alkanols reveal no propensity for receptor binding within a suite of predictive *in silico* models addressing the binding to thirteen nuclear receptors.

As will be shown in detail, the 90-day rat oral repeated-dose NOAEL values for 2-ethyl-1-hexanol and 2-propyl-1-heptanol are particularly well-suited for read-across. Moreover, the predictions are supported by results for isotridecanol. With acceptable uncertainty, a NOAEL value of 125 mg/kg bw/d can be read across to fill the data gaps among the analogues in this category.

INTRODUCTION TO READ-ACROSS

The principal philosophy of a toxicological read-across is chemicals that are similar in molecular structure will exhibit similar chemical properties and in so doing, exhibit similar toxicokinetic and toxicodynamic properties. As a consequence, experimentally-derived toxicokinetic and toxicodynamic properties from one substance, the source chemical, can be read across to fill the data gap for a second substance, the target chemical that is similar.

While it is easy to establish similarity based on structure and chemical properties, this similarity is often not enough to accept a toxicological read-across for chronic health endpoints. Establishing toxicodynamic and to a greater extent toxicokinetic similarity often requires limiting the applicability domain.

Read-across can be fitted to different purposes. The format or style of the read-across differs with purpose. While there are a variety of circumstances in which a read-across prediction may be of value, there are a limited number of read-across formats. First, there is the narrow-domain format. Narrow-domain read-across exercises include ones associated with the development of a substance-specific assessment such as with a REACH dossier. Such a read-across is a one-to-one or many-to-one estimation (i.e., analogue- and category-based, respectively). They have a single target chemical and often one, but generally three or less, source substances.

At the other extreme is the wide-domain format. Wide-domain exercises are associated with screening and priority setting. Wide-domain applications are basically a one-to-many or a many-to-many estimation. They have multiple target chemicals and often one, but generally three or less, source substances.

In between these extremes is the intermediate-domain format; these exercises are one-to-one or many-to-one repeated several times within the same assessment. For example, C8 is the source substance and is more similar to C9 (the read-across is accepted with high confidence) than to C10 (the read-across is accepted with lower confidence). The C5 and C11 analogues are sufficiently dissimilar to be considered outside the domain of the read-across. Thus, the read-across for C5 and/or C11 may not be accepted without further information. The C5 and C11 analogues break the series with respect to the toxic property being predicted and thus, are points-of-departure. In some cases, it may be reasoned that the data gaps for the C5 and/or C11 analogues may be filled as worst-possible-scenarios.

A similar line of reasoning can be made for a many-to-one or category-based read-across. In the latter example, C8 and C10 are both source substances, so the read-across to C9 is predicted with high confidence by interpolation, while the read-across to C11 is predicted with lower confidence via extrapolation. C5 is again dissimilar and thus, outside the domain of the read-across. The C5 analogue breaks the series with respect to the toxic property being predicted and thus is a point-of-departure. However, it may be argued that the C5 data gap may be filled as a worst-possible-scenario.

Two-alkyl-1-alkanols provide an example where the category approach of many-to-one read-across is repeated several times in an endeavour to clear a number of derivatives for a specific endpoint (i.e., the oral 90-day repeated-dose NOAEL). As a case study, this series of assessments is designed to illustrate specific issues associated with this type of IATA, especially as related to chronic health effects and stimulate discussion on how to develop guidance to address these issues. It is not meant to be related to any regulatory discussions by OECD member countries or other agencies of this group of substances.

Two-alkyl-1-alkanols: Existing Knowledge

Perfused rat liver toxicity data from Strubelt et al. (1999) for the C5 primary alkanol exposure 65.1 mmol/l for 2 hours suggests that 2-alkyl-1-alkanols may not be in the same read-across category as other primary alkanols (Table 1). These data support the premise that *in vitro* toxicity (e.g., O_2 consumption and ATP production) of 2-alkyl-1-alkanols is due, in large part, to loss of membrane integrity as indicated by cytosolic enzyme (LDH) leakage. While it is likely that enzyme leakage is the result of alteration in membrane fluidity due to partitioning into the cell membrane, loss of membrane integrity as a result of soft electrophilic reactivity and indicated by a 50% reduction in free glutathione (GSH) is demonstrated.

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

Name	**log Kow**	**O_2 (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
2-Methyl-1-butanol	1.30	0.30 ± 0.03	0.10 ± 0.01	20521 ± 1087	1.33 ± 0.29
3-Methyl-1-butanol	1.16	0.22 ± 0.07	0.27 ± 0.05	8680 ± 1216	2.27 ± 0.37
1-Pentanol	1.40	0.06 ± 0.01	0.20 ± 0.03	28959 ± 4142	2.82 ± 0.36

The applicability domain for this case study is limited to 2-alkyl-1-akanols with the number of carbon (C) atoms range of C5 to C13. Since long-chain derivatives are typically transported via carrier molecules, alcohols of C14 and greater are not included in this category. Since shorter-chain derivatives have the potential to volatilize, they are not initially included in this category.

Among the 2-alkyl-1-alkanols, 2-ethyl-1-hexanol is the best studied (see Opdyke, 1973; OECD, 1995; Joint FAO/WHO Expert Committee on Food Additives, **1993, 1999;** MAK, 2012, NICNAS - IMAP).

2-Alkyl-1-alkanols within the range C5-C13 are expected to be readily absorbed by the gastrointestinal tract (Gaillard and Derache, 1965). Dermal penetration of these intermediate size alkanols does not readily occur and absorption from inhalation is extremely limited (Belsito et al., 2010). Thus, the primary route of exposure, which is toxicologically relevant is oral.

Metabolism of 2-alkyl-1-alkanols, while highly efficient, involves processes that are more complex than n-alkanol metabolism. Experimental data reveals the major pathways of metabolism and fate of 2-alkyl-1-alkanols include: 1) conjugation of the alcohol group with glucuronic acid, 2) oxidation of the alcohol group, 3) side-chain oxidation yielding additional polar metabolites, which may be subsequently conjugated and be excreted or further oxidized, and 4) excretion of the unchanged parent compound. For example, in rabbits, the glucuronide of 2-ethyl-1-hexanoic acid was identified as the main metabolite (87%) after oral application of 2-ethyl-1-hexanol (Kamil et al., 1953a, 1953b). In contrast, in the same species, only about 9% of the administered dose of 2-methyl-1-butanol was found in the form of the glucuronides (Kamil et al., 1953a, 1953b).

Belsito et al. (2010) reviewed the toxicity of branched chain saturated alcohols, including secondary ones. Patocka and Kuca (2012) summarized the toxicity of C1 to C6 alkanols. The efficacy of alkanols to induce ataxia (McCreery and Hunt, 1978) and enzyme release from liver cells (McKarns et al., 1997) has been interpreted as being due to the hydrophobic property of the alkanols. Based on rat and fish studies, 2-alkyl-1-alkanols, like other alkanols, act in a manner similar to depressant anaesthetics (Fang et al., 1997; McKim et al., 1987). Koleva et al. (2011) reported multiple-regression type quantitative structure-toxicity relationships (QSARs) for oral log $LD50^{-1}$ data for rodents and the 1-octanol/water partition coefficient (log Kow). Comparison of measured toxicity data, with predictions from baseline QSARs, reveal straight-chain and branched, saturated monohydric alcohols consistently behave as classic nonpolar narcotics.

In general, 2-alkyl-1-akanols acute oral toxicity (LD50) is very low ranging from >1800 to < 5500 mg/kg bw with an average value of ≈3500 mg/kg bw (see Table 2).

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

Alcohol	**Species**	**Oral LD50 (mg/kg)**	**Citation**	**90-d Oral NOAEL (mg/kg bw/d)**	**Citation**
2-Methyl-1-butanol	rat	4010	Rowe and McCollister, 1982	Not determined	
2-Methyl-1-pentanol		Not determined		Not determined	
2-Ethyl-1-butanol	rat	1850	Smyth et al., 1954	Not determined	
2-Ethyl-1-pentanol	rat			Not determined	Not determined
2-Ethyl-1-hexanol	rat	>3730	Scala and Burtis, 1973	125	Astill et al., 1993; ECHA CHEM A
	Rat	≈2000	ECHA CHEM A		
	mouse	2500	Chvapil et al., 1962	125	Astill et al., 1993
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	ECHA CHEM B	150	ECHA CHEM B
2-Methyl-1-undecanol		Not determined		Not determined	
2-Ethyl-1-decanol		Not determined		Not determined	
2-Propyl-1-decanol		Not determined		Not determined	

Astill, B.D., Gingell, R., Guest, D., Hodgson, J.R., Murphy, S.R. and Tyler, T.R. 1993. Subacute and subchronic oral toxicity of 2-ethylhexanol to fischer 344 rats and B6C3F1 mice. Toxicologist 13: 70.

Chvapil, M., Zahradnik, R. and Cmuchalová, B. 1962. Influence of alcohols and potassium salts of xanthogenic acids on various biological objects. Arch. Int. Pharmacodyn. Ther. 135: 330-343.

ECH CHEM A for 2-Ethyl-1-hexanol: http://echa.europa.eu/registration-dossier/-/registered-dossier/15194

ECH CHEM B for 2-Propyl-1-heptanol: http://echa.europa.eu/registration-dossier/-/registered-dossier/13788

Rowe, V.K. and McCollister, S.B. 1982. Alcohols. In: Clayton, G.D. & Clayton, F.E., eds, *Patty's Industrial Hygiene and Toxicology*, 3rd Revised Ed., Vol. 2C, New York: John Wiley & Sons, chapter 35, pp. 4527-4708.

Scala, R.A., Burtis, 1973. E.G. Acute toxicity of a homologous series of branched-chain primary alcohols. Am. Ind. Hyg. Assoc. J., 34, 493–499.

Smyth, H.F.J., Carpenter, C.P., Weil, C.S. and Pozzani, U.C. 1954. Range-finding toxicity data. List V. Arch. Indust.Hyg. Occup. Med.10: 61-68.

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

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 (Schultz et al., 2015). Briefly, the justification of a 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.

EVALUATION OF SELECTED 2-ALKYL-1-ALKANOL FOLLOWING A READ-ACROSS WORKFLOW

1. PURPOSE

1.1. Purpose of use

The proposed use of the data estimations resulting from this IATA is risk assessment. As such, the predicted NOAEL values from the IATA must be accompanied by low uncertainty. In this case study, acceptable uncertainty is one that is equal to testing using the protocol similar to OECD TG 408.

1.2. Target and Source Substances

The analogues listed in Table 3 include the ten target and two source chemicals (noted in bold) of this case study. This list is not meant to be all inclusive, rather it represents existing industrial organic materials that are likely to be found in a governmental or industrial inventory (e.g., OECD High Production Volume Chemicals).

Table 3. 2-alkyl-1-alkanols considered part of the chemical category

Name CAS No.	Structure	
2-Methyl-1-butanol	137-32-6	C5H12O
2-Methyl-1-pentanol	105-30-6	C6H14O
2-Ethyl-1-butanol	97-95-0	C6H14O
2-Ethyl-1-pentanol	27522-11-8	C7H16O
2-Ethyl-1-hexanol	**104-76-7**	**C8H18O**
2-Propyl-1-pentanol	58175-57-8	C8H18O
2-Methyl-1-octanol	818-81-5	C9H20O
2-Ethyl-1-octanol	20592-10-3	C10H22O
2-Propyl-1-heptanol	**10042-59-8**	**C10H22O**
2-Methyl-1-undecanol	10522-26-6	C12H26O
2-Ethyl-1-decanol	21078-65-9	C12H26O
2-Propyl-1-decanol	60671-35-4	C13H28O

1.3 Endpoint

The NOAEL for the 90-day rat oral repeated-dose is the single endpoint for which this category approach is applied. The 90-day oral repeated-dose data for 2-ethyl-hexanol and 2-propyl-1-heptanol are particularly well-suited for read-across; the NOAELs are based on experimental results from a 3-dose exposure scenario (<100, between 100 and 200 and > 500 mg/kg bw/d) following a standard test guideline (OECD TG 408).

2. HYPOTHESIS OF THE CATEGORY

The premise for this read-across case study is:

- 2-alkyl-1-akanols of intermediate chain length (i.e., C5 to C13) are direct-acting toxicants (i.e., metabolic activation is not a factor in toxicity) with a similar reversible mode of action (i.e. non-polar narcosis simple anaesthesia).

- The chemical category is based on simple structure similarities- C-atom chain length and 2-alkan-1-ol hydrocarbon scaffolding.

- With C5 to C13 2-alkanol-1-ol derivatives, C-atom chain length affects most physico-chemical properties with property values increasing with increasing chain length. However, this trend is not toxicologically significant and does not significantly affect bioavailability in chronic oral exposure.

- These 2-alkyl-1-akanols are rapidly and nearly completely absorbed from the gut; first past metabolism leads to glucuronidation and subsequent elimination in the urine and/or oxidative metabolism in the liver resulting in a carboxylic acid, which subsequently undergoes mitochondrial β-oxidation, and/or a polar metabolite which is excreted in the urine.

- Toxicodynamically, these 2-alkyl-1-akanols are highly similar. In vivo they exhibit no systemic toxicity and in vitro they exhibit no chemical reactivity or receptor-mediated interactions.

- Repeated-dose tested NOAEL data for 2-ethyl-hexanol and 2-propyl-1-heptanol can be read across to other category members listed in Table 3.

2.1. Justification

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

From a repeated-dose perspective, 2-ethyl-1-hexanol is well-studied. More specifically, in a 90-day study similar in design to an OECD TG408, Fischer F344 rats were administered doses of 0, 25, 125, 250 or 500 mg 2-ethyl-1-hexanol/kg bw/d by gavage (Astill et al., 1993). A NOAEL of 125 mg/kg bw/d based on reduced body weight and body weight gain, changes in blood chemistry was reported.

A second sub-chronic gavage study is reported by the same authors (Astill et al., 1996a) in which Fischer rats were exposed to doses of 25, 250 and 500 mg/kg bw/d. Relative weight changes are reported for kidney and liver, as well as a decrease of Alanine aminotransferase at 250 mg/kg bw/d. Further weight changes occurred in brain, testes and stomach at highest dose, together with a slight decrease in body weight. Changes in clinical chemistry parameters were reported, including an increased activity of the enzyme palmitoyl coenzyme A activity (pCoA), decrease of cholesterol, total protein and albumin, as well as an increase in reticulocytes. Since no doses between 25 and 250 were tested, the NOAEL of this study is 25 mg/kg bw/d.

In a chronic Fischer F344 rat study, 2-ethyl-1-hexanol were administered by gavage at doses of 0, 50, 150 or 500 mg/kg bw/d, 5 days per week for 2 years (Astill et al., 1996b; ECHA CHEM A). Food consumption, body weights, and haematological parameters were examined at specific intervals during the study. At the end of the study, gross and histopathological examinations were conducted. No treatment-related adverse effects were observed at the 50 mg/kg bw/d dose level. At the 150 mg/kg bw/d dose level, rats exhibited a body weight gain reduction of approximately 16% in males and 12% in females. An increase of brain and liver weight also is reported. However, no histopathological changes were observed at same or higher doses. In addition, the rats also displayed a slightly increased incidence of clinical signs, such as poor general condition and laboured breathing. We conclude that the NOAEL for this study is 150 mg/kg bw/d.

Shorter-term repeated dose studies are also available for 2-ethyl-1-hexanol. In an 11-day study, Fischer 344 rats were exposed by gavage at doses of 100, 330, 1000 and 1500 mg/kg bw/d (RIFM, 1992). From 330 mg/kg bw/d on atrophy of the thymus was reported being most pronounced at 1500 mg/kg bw/d. At 1000 mg/kg bw/d a decrease in reticulocytes and clinical chemistry parameters such as cholesterol, glucose and ALAT was reported, as well as a marked inflammation of the forestomach. At highest tested dose, additional adverse effects were reported including focal hepatocellular necrosis, hepatocellular hypertrophy and several organ weight changes. Transient clinical signs were reported at 1000 and 1500 mg/kg bw/d, namely ataxia, lethargia and lateral and abdominal posturing. A NOAEL of 100 mg/kg bw/d was determined.

A second gavage short-term gavage study was done with Fischer rats exposed to doses of 100, 320 and 950 mg/kg bw/d for 28 days (Hodgeson, 1987). At the highest dose of 950 mg/kg bw/d body weight gain was reduced and kidney and liver weight and trigylcerides were increased. At 320 mg/kg bw/d an induction of peroxisome proliferation was observed, as well as hepatic cyanide-insensitive palmitoyl coenzyme A activity (pCoA). At 100 mg/kg bw/d a reduction of neutral lipids in liver is reported; however, we do not consider this toxicologically relevant and, thus, we conclude the NOAEL for this study to be 100 mg/kg bw/d.

In a 90-day study, B6C3F1 mice received doses of 0, 25, 125, 250 or 500 mg 2-ethyl-1-hexanol/kg bw/d (Astill et al., 1993) and the 90-day oral NOEL was noted as 125 mg/kg bw/d.

In another B6C3F1 mouse study, 2-ethyl-1-hexanol mice were administered by gavage at doses of 0, 50, 200 or 750 mg/kg bw/d, five days per week for 18 months (Astill et al., 1996a). Food consumption, body weights and haematological parameters were examined at specific intervals during the study. At the end of the study, gross and histopathological examinations were conducted. While no treatment-related adverse effects were observed in the mice receiving 50 or 200 mg 2-ethyl-1-hexanol/kg bw/d, at the 750 mg/kg bw/d dose level, body weight gain reductions of approximately 26 and 24% in males and females, respectively. Further high dose effects consist of changes in haematology (lymphocytes, neutrophil increase after 12 months), weight changes of different organs (kidney, liver), and hyperplasia in the forestomach. We conclude the NOAEL for this study to be 200 mg/kg bw/d.

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

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

Other related rodent repeated-dose studies

Isotridecanol (i.e., C13-rich mixture of iso-alcohols of C11-14, CAS No. 68526-86-3) was tested by gavage to Sprague–Dawley rats. In a 90-day study, according to OECD TG 408 with doses of 0, 100, 500, or 1000 mg/kg body weight/day the NOAEL of 100 mg/kg bw/d was reported (see Belsito et al., 2010).

While ECHA CHEM notes a reliable read-across from 3-methyl-1-butanol to 2-methyl-1-butanol, the current study disregarded these data. This decision was based on the finding of Strubelt et al. (1999). Data (see Table 1) for the C5 primary alkanols exposure 65.1 mmol/l for 2 hours suggest that 2-methyl-1-butanol may not be in the same read-across category as 3-methyl-1-butanol or n-pentanol.

In summary, two 2-alkyl-1-akanols (i.e., 2-ethyl-hexanol and, 2-propyl-1-heptanol) have high quality quantitative (e.g., OECD TG 408) 90-day oral exposure repeated-dose test data. These data exhibit qualitative and quantitative consistency between and within rodent species. Specifically, results of oral repeated-dose testing for these two source substances suggest mild changes consistent with low-grade effects including decreased body weight, accompanied by clinical chemical and haematological changes but generally without concurrent histopathological effects. While it can be argued that these effects are not adverse, we still considered them in determining the NOAEL. The 90-day oral exposure repeated-dose NOAEL values ≥ 125 mg/kg bw/d are based on experimental results from a 3 or 4 dose exposure scenario, typically <100, between 100 and 200, >200 and ≥500. While there is not repeated-dose toxicity data for 2-methyl derivatives, there are included in the category.

2.2. Applicability domain

The applicability domain for this read-across is confined to branched primary alkanols of intermediate size, C5 to C13. Longer-chain derivatives, which are typically transported via carrier molecules, and shorter-chain derivatives, having the potential to volatilize, are not initially included in this chemical category.

2.3. Purity/impurities

A purity/impurity profile for the analogues listed in Table 3 is not reported. No effort was made to take into account impurities based on production. However, since the category is structurally limited, the potential impact of any impurities on the endpoint being considered is considered very limited. The most likely impurities are other saturated derivatives or stereoisomers.

3. DATA MATRICES FOR ASSESSING SIMILARITY

The data supporting the similarity argument for the analogues listed in Table 3 are reported in Annex I.

Structural similarity

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

Chemical property similarity

As demonstrated in Table 2 of Annex I, all the primary alkanols included in the category have a large portion of their physio-chemical properties determined experimentally. Thus, calculated values can be taken with high confidence. Properties, with the exception of density and pKa, tread in values related to C-atom number within a scaffold. Specifically, all category members exhibit molecular weights from 88 to 200 g/mol. While hydrophobicity (log Kow) increases with number of C-atoms from >1.0 to <6.0, density and pKa are constant at 0.8 g/cm^3 and 15. While vapour pressure and water solubility decrease with molecular size, melting point and boiling point increase with molecular size.

Chemical constituent similarity

As demonstrated in Table 3 of Annex I, all the branched primary alkanols included in the category have common constituents in the form of: 1) a single key substituent, OH, and 2) structural fragments, CH_3, CH_2 and CH.

Toxicokinetic similarity

As demonstrated in Table 4 of Annex I, while the analogues tested are limited, the toxicokinetic understanding of 2-position branched primary alkanol is fairly complete. Two-alkyl-1-alkanols are rapidly absorbed following oral administration (Gaillard and Derache, 1965) and are rapidly excreted (Albro 1975). Data for 2-ethyl-1-hexanol and to a lesser extent 2-methyl-1-butanol and 2-ethyl-1-butanol demonstrate that branched primary alcohols exhibit common metabolic pathways. These metabolic pathways include oxidation of the alcohol group and oxidation of the side chain at various positions, glucuronidation of the oxidation products, and decarboxylation (Albro, 1975). Glucuronidation increases with increased chain length of the alkanols (Jurowich et al., 2004).

Two adult male CD-strain rats (300 g) were gavaged with radiolabeled 2-ethyl-1-^{14}C-hexanol (^{14}C- labeled 2-ethyl-1-hexanol; 1 µCi; 8.8 µg) in cottonseed oil. Two others were given the same amount of ^{14}C-EH and cottonseed oil but also were given 0.1 ml (0.64 mmol) of unlabeled 2-ethyl-1-hexanol. Following administration, rats were housed in metabolism cages; expired CO_2, urine, and faeces were collected every hour for 28 hrs. Most (99.8%) of the orally administered radioactivity was accounted for by radioactivity in expired CO_2, urine, faeces, an ethanol wash of the metabolism cage at the end of the experiment, heart, brain, liver, kidneys, and "residual carcass". Two-ethyl-1-hexanol was efficiently absorbed following oral administration and rapidly excreted in respired CO_2 (6-7%), urine (80-82%), and faeces (8-9%); elimination was essentially complete by 28 hrs (Albro, 1975; ECHA CHEM A; JECFA, 1993**)**.

Deisinger et al. (1993, 1994) examined the elimination of ^{14}C-labeled 2-ethyl-1-hexanol in rats. After oral administration to rats, 69-75% of a dose of 500 mg ^{14}C-labeled 2-ethyl-1-hexanol/kg bw was excreted in the urine within 96 hours; about 13 to 15% of the dose was excreted in the faeces and about the same amount was exhaled as ^{14}C-labeled CO_2. After intravenous administration to rats, about 74% of a dose of 1 mg ^{14}C-labeled 2-ethyl-1-hexanol/kg bw was excreted in the urine within 96 hours. About 4% of the dose was excreted in the faeces and 23% was exhaled. More than 50% of the dose was excreted within 8 hours and the terminal half-life was estimated to be 60 hours (Deisinger et al., 1993, 1994).

Haggard et al. (1945) examined the metabolic fate of 2-methyl-1-butanol in rats. Specifically, intraperitoneal injection in four equal doses of 250mg/kg bw at 15-min intervals resulted in a maximum blood concentration of 550 mg/l. Blood concentrations decreased over the next nine hours. Of the total dose of 1000mg/kg bw, only 5.6% was excreted in air and 2% in the urine. The remainder was metabolized, first to the corresponding aldehyde and then to the acid (Haggard et al., 1945). After a single oral dose of 25 mmoles 2-methyl-1-butanol to rabbits (Kamil et al., 1953a), 9.6% of the dose was excreted in the urine as glucuronides. Glucuronide excretion occurred within 24 hours, the urine did not contain aldehydes or ketones. Iwersen and Schmoldt (1995) studied the alcohol dehydrogenase-independent metabolism of aliphatic alcohols (oxidation and glucuronidation). Briefly, male Sprague-Dawley rats were pretreated with 10% ethanol in the drinking water for two weeks. Rats were sacrificed and microsomes were prepared for glucuronidation experiments and trials, as well as oxidation experiments with aliphatic alcohols. *In vitro* experiments have demonstrated additional oxidation of 2-methyl-1-butanol by rat liver microsomes via CYP P450 enzymes and glucuronidation. At very low ethanol concentrations (5-10 mmo/L) competitive inhibiting effect of ethanol on oxidation of 2-methyl-1-butanol was observed (Iwersen and Schmoldt, 1995).

A rabbit was given 2.55g of 2-ethyl-1-butanol and the 24-hr urine was collected (Kamil et al., 1953b). 2-Ethyl-1-butanol was excreted mainly as glucuronides, along with a minor amount of methyl n-propyl ketone.

Metabolic similarity

As demonstrated in Table 5 of Annex I with data from *in silico* predictions, it is highly likely that all of the category members undergo successive oxidation to their corresponding aldehyde and carboxylic acid (Bosron and Ting-Kai, 1980; Levi and Hodgson, 1989).

Kamil et al. (1953a, 1953b) examined the metabolic fate of 2-methyl-1-hexanol in rats. Via acid extraction of urine, the major urinary metabolite of 2-ethyl-1-hexanol was revealed to be 2-ethyl hexanoic acid. This metabolite may undertake partial ß-oxidation and decarboxylation to produce $^{14}CO_2$ and 2- and 4-heptanone (in the urine). Other urinary metabolites identified in this study were 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-5-ketohexanoic acid, and 2-ethyl-1,6-hexanedioic acid. Approximately 3% of the parent compound was excreted unchanged. Metabolic saturation was seen with 500 mg/kg body weight applied (Kamil et al., 1953a, 1953b).

Typically, the presence of a side chain does not terminate the oxidation process of alkanols. However, in most cases, it alters it. The position and size of the alkyl substituent plays a role in metabolism with degradation to CO_2 decreasing and glucuronidation increasing with branching and increasing chain length.

Alkyl acids formed during metabolic transformation of branched alkanols have their own set of metabolic pathways. Acids with a methyl substituent located at an even-numbered carbon (e.g., 2-methyl pentanoic acid or 4-methyl decanoic acid) are extensively metabolized to CO_2 via ß-oxidative cleavage in the fatty acid pathway. If the methyl group is located at the 3-position, ß-oxidation is inhibited and omega- (ω-) oxidation predominates, primarily leading to polar, acidic metabolites capable of being further oxidized or conjugated and excreted in the urine (Williams, 1959). As chain length and lipophilicity increase, ω-oxidation competes with ß-oxidative cleavage. Methyl substituted acids (e.g., 3-methylnonanoic acid) are, to some extent, ω-oxidized in animals to form diacids, which can be detected in the urine (Williams, 1959).

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

Metabolism of methyl-substituted alcohols is determined primarily by the position of the methyl group(s) on the hydrocarbon-chain. Following successive oxidation to the corresponding carboxylic acids, the branched-chain acids are metabolized via ß-oxidation. With longer branched-chain derivatives, this is followed by cleavage to yield linear acid fragments, which are typically completely metabolized to CO_2. At high-dose levels, the longer branched-chain acids may go through omega-oxidation to yield diacids, which subsequently may undergo further oxidation and cleavage.

The presence of an ethyl- or propyl-substitution at the α-position, such as in 2-ethyl-1-hexanol, inhibits ß-oxidation (Deuel, 1957). Detoxication pathways of omega- and omega-1 oxidation compete with ß-oxidation of these sterically-hindered substances; the parent alcohol or corresponding carboxylic acid undergoes a combination of reactions (e.g., omega- or omega-1 oxidation and functional group oxidation) leading to polar, acidic metabolites capable of being excreted in the urine (Williams, 1959; Deisinger *et al.*, 1994). When the principal pathway is saturated, the corresponding carboxylic acid conjugates with glucuronic acid and is excreted in the urine (Williams, 1959; Albro, 1975; Deisinger et al., 1994).

One of the best studied 2-postion branched carboxylic acid is 2-propyl pentanoic acid (valproic acid). The toxicokinetic aspects of 2-propyl pentanoic acid have been reviewed (Silva et al., 2008; Ghodke-Puranik et al., 2013). 2-Propyl pentanoic acid is almost entirely metabolized by the liver, so it is not surprising that the liver is also the dominant target organ of toxicity. The multiple metabolic pathways involved in 2-propyl pentanoic acid biotransformation give rise to more than 50 known metabolites (Silvia et al., 2008). Ghodke-Puranik et al. (2013) estimate that, while 30 - 50% of 2-propyl pentanoic acid is excreted in the urine as a glucuronide conjugate, 40% goes through mitochondrial β-oxidation and about 10% undergoes cytochrome P450-mediated oxidation. It has been postulated that the hepatotoxicity of 2-propyl-pentanoic acid results from the mitochondrial β-oxidation of its cytochrome P450 metabolite, 2-propyl-4-pentenoic acid, to 2-propyl-(E)-2,4-pentadienoic acid which, in the CoA thioester form, either depletes GSH or produces a putative inhibitor of β-oxidation enzymes. Pent-4-enoate, 2-propyl-4-pentenoic acid and 2-propyl-(E)-2,4-pentadienoic acid are potent inducers of microvesicular steatosis in rats (Kesterson et al., 1984). However, since 2-propyl-pentanoic acid failed to induce discernible liver lesions in young rats, even at near lethal doses of 700 mg per kg per day, Kesterson et al. (1984) suggested that β-oxidation inhibition observed in both valproic acid and unsaturated-metabolite-treated rats occurred by different mechanisms. Specifically, 2-propyl pentanoic acid inhibits transient sequestering of CoA, while the CoA esters of some metabolites, particularly 2-propyl-4-pentenoic acid, inhibit specific enzyme(s) in the β-oxidation sequences (Kesterson et al., 1984).

Ghodke-Puranik et al. (2013) rationalized the involvement of 2-propyl-4-pentenoic acid. Specifically, 2-propyl-4-pentenoic acid enters the mitochondria, forms a complex with CoA ester and subsequent β-oxidation forms the reactive 2-propyl-(E)-2,4-pentadienoic acid-CoA ester. The latter is the putative cytotoxic metabolite that binds with glutathione to form thiol conjugates. The reactive metabolite, 2-propyl-(E)-2,4-pentadienoic acid-CoA ester, has the potential to deplete mitochondrial glutathione pools and form conjugates with CoA, which in turn inhibits enzymes in the β-oxidation pathway (Ghodke-Puranik et al., 2013).

In summary, the experimental toxicokinetic data for 2-alkyl-1-alkanols shows consistency in absorption, distribution and metabolic pathways. In contrast, there is less consistency in excretion. In particular, derivatives with 2-position ethyl and propyl groups are more likely to be excreted as a glucuronidated metabolite, while 2-position-methylated analogues are more likely to be oxidized to CO_2. The latter are metabolically similar to the less toxic n-alkanols (Schultz et al., 2016). The metabolic evidence supporting the idea that some 2-position branched carboxylic acids are metabolized to thiol reactive metabolites is not considered toxicologically relevant to this read-across as repeated-dose toxicity through a reactive mechanism is considered unlikely as long as the reactive half-life is shorter than the dosing interval (e.g., <8-hr vs. 24-hr) and the phase 2 conjugation mechanism is not saturated.

Toxicophore similarity

As shown in Table 6 of Annex I, 2-alkyl-1-akanols themselves do not contain a known toxicophore. However, the carboxylic acid metabolites of the same 2-position branched isomers (e.g., 2-ethyl-1-hexanol and 2-propyl-1-heptanol) are linked to developmental toxicity and chronic oral toxicity via the short-chain carboxylic acid pathway (Przybylak and Schultz, 2013).

Mechanistic plausibility similarity

It is generally accepted that the toxicity of intermediate size 2-alkyl-1-alkanols, like other saturated alcohols, is the result of narcosis. While there is theoretical evidence for the membrane as the site of action for anaesthetic-like 2-alkyl-1-alkanols, biochemical, cellular and physiological evidence is largely restricted to 1-alkanol derivatives (e.g., Fang et al., 1997; McKim et al., (1987)). Narcosis, in the broadest sense, is the non-covalent disruption of hydrophobic interactions within membranes with a particular volume fraction rather than molar fraction (Alifimoff et al., 1989). It is the accumulation of alcohols in cell membranes which disturbs their function, however, the exact mechanism is not known yet. There are three competing theories of general anaesthetic action: 1) the lipid solubility-anaesthetic potency correlation (i.e., the Meyer-Overton correlation), 2) the modern lipid hypothesis, and 3) the membrane protein hypothesis.

As shown in Table 7 of Annex I, the alkanols included in the category are associated with the simple narcosis mechanism of toxicity that is equivalent to depressant anaesthetics. Measured acute toxicity for 2-alkyl-1-alkanols is consistent with predictions from QSAR models (Veith et al., 1983; 2009) for the nonpolar narcosis mode of action (Raevsky et al., 2008).

The contributions of functional groups in acute rat oral toxicity have been calculated using alkanes as the baseline (He et al., 2014). The toxic contribution of alcohols is -0.108. This situation has not been observed in acute fish toxicity because the threshold of excess toxicity is too high to distinguish differences in toxicity. Critical body residues (CBRs) calculated from percentage of absorption and bioconcentration factors indicate that most of aliphatic alcohols share the same modes of toxic action between fish and rat. Specifically, fish and rat log (1/CBR) and number of alcohols are 1.65; 18 and 1.58; 348, respectively (He et al., 2014).

It would be remise to not note that some 2-alkyl-1-alkanols are associated with development toxicity via their conversion to the corresponding 2-alkyl-carboxcylic acids. The experimental evidence is largely confined to 2-ethyl-1-hexanol and the results are mixed.

In rats administrated 1600 mg/kg bw 2-ethyl-1-hexanol by gavage (but not 800 mg/kg bw) on day 12 of gestation, Ritter et al. (1986) reported a statistically significant increase in the number of teratogenic live fetuses; malformations included hydronephrosis, tail and limb defects). Maternal toxicity was not reported in this study.

In another study, Ritter et al. (1987) proposed that the teratogen di(2-ethylhexyl) phthalate acts by *in vivo* hydrolysis to 2-ethyl-1-hexanol, which in turn is metabolized to the definitive teratogen 2-ethyl-1-hexanoic acid. They conducted teratological studies with Wistar rats administering one of the three agents on day 12 of gestation. Briefly it was revealed that on an equimolar basis the phthalate derivative was least potent, the alcohol derivative was intermediate, and the acid derivative was most potent. Similarity in the types of malformation induced by each derivative suggests a common mechanism of action. *In toto,* these findings are consistent with the hypothesis (Ritter et al. (1987).

Two-ethyl-1-hexanol was evaluated for developmental toxicity in mice (NTP, 1991). There were no effects on any gestational parameters upon exposure to dietary 2-ethyl-1-hexanol. Specifically, the number of corpora lutea, uterine implantation sites (live, dead, resorbed), pre- and post-implantation loss, sex ratio (% males) and live fetal body weight per litter (all fetuses or separately by sex) were all equivalent across all groups. Moreover, there were no maternal toxic effects observed at any of the concentrations tested (NTP, 1991).

Tyl et al. (1992) examined the developmental toxicity of 2-ethyl-1-hexanol administered dermally. In range-finding (8 females/ treatment) and definitive investigations (25 females per treatment), 2-ethyl-1-hexanol was administered by occluded dermal application for 6-hours per day on gestation days 6 through 15 to pregnant Fischer 344 rats. Treatment levels for range-finding were equivalent to 0, 420, 840, 1680, and 2520 mg/kg bw/d; treatment levels for definitive experiments were equivalent to 0, 252, 840, and 2520 mg/kg bw/d. Controls included negative- deionized water, dermal-positive- 2-methoxyethanol and oral reference- valproic acid.

For 2-ethyl-1-hexanol: 1) maternal weight gain was reduced at the two highest dose levels, 2) maternal liver, kidney, thymus, spleen, adrenal, and uterine weights, and gestational and fetal parameters were unaffected by any treatment, and 3) there were no treatment-related increases in the incidence of individual or pooled external, visceral, and skeletal malformations or variations. The dermal NOAELs for the maternal toxicity of 2-ethyl-1-hexanol were 252 mg/kg/day based on skin irritation and 840 mg/kg/day based on systemic toxicity. The developmental toxicity NOAEL was at least 2520 mg/kg/day, with no teratogenicity. While the Fischer 344 rat is susceptible to known rodent teratogens- 2-methoxyethanol by the dermal route and valproic acid by the oral route, in the Fischer 344 rat, 2-ethyl-1-hexanol is not a developmental toxicant by the dermal route at and below treatment levels which produce maternal toxicity.

Narotsky et al. (1994) studied the developmental toxicity and structure-activity relationships of aliphatic acids in rats. Fourteen acids were administered by gavage to Sprague-Dawley rats once daily during organogenesis. Only 2-ethyl hexanoic and 2-propyl hexanoic acid caused valproic acid-like effects (i.e., mortality, extra pre-sacral vertebrae, fused ribs, and delayed parturition) on rat development. Developmental toxicity of α-branched acids is, in part, due to maternal toxicity resulting in alterations in zinc (Zn) metabolism that affects the developing conceptus (Bui et al., 1998). Developmentally toxic doses of 2-ethyl hexanoic acid, 2-ethyl-1-hexanol and valproic acid on Zn metabolism were investigated in the pregnant rat. At the higher dose levels of 2-ethyl-1-hexanoic acid, 2-ethyl-1-hexanol, and at all dosages of valproic acid, the percentage of ^{65}Zn retained in maternal liver was higher than controls, while that in the embryos was lower, than controls. Two-ethyl-1- hexanoic acid exposed dams fed Zn-containing diets during gestation exhibited a dose dependent reduction in teratogenic effects.

Toxicokinetic parameters are important determinants of teratogenic outcome of α-alkyl-substituted carboxylic acids, which helps explain differing potencies of structurally similar chemicals (Scott et al., 1994). Valproic acid (2-propyl-1- pentanoic acid), 2-ethyl-1-hexanoic acid, and 1-octanoic acid are isomeric analogues with markedly different teratogenic potencies. Valproic acid induces moderate to severe malformations after a single oral administration of 6.25 mmoles/kg on day 12 of rat pregnancy. Twice as much 2-ethyl-1-hexanoic acid (12.5 mmoles/kg) induces a less severe response and 1-octanoic acid is non-teratogenic, even at the higher dose of 18.75 mmoles/kg (Scott et al., 1994). While 1-octanoic acid exhibits poor intestinal absorption, the peak concentration and duration of exposure to valproic acid and 2-ethyl-1-hexanoic acid were very similar. A fourth agent, 2-methyl-1-hexanoic acid, which is non-teratogenic when administered orally at 14.1 mmoles/kg, exhibits peak concentration and duration of exposure intermediate to 2-ethyl-1-hexanoic acid, and 1-octanoic acid. The differences in the severity of developmental malformations for the α-alkyl-substituted derivatives indicated higher intrinsic activity for analogues with C2 and especially C3 α-alkyl-substituents.

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

Other endpoint similarity

In mammals, alkanols are considered baseline inhalation toxicants which model as simple narcotics (Veith et al., 2009).

Acute oral toxicity studies are performed mainly for classification and labelling in order to assign substances their potential hazard categories and estimate the dose required to cause chronic toxicity. Four toxicity categories based on their acute oral toxicity properties are typically used. Specifically, the categories are as follows: Category 1 (LD50 < 5 mg/kg bw), Category 2 (5 < LD50< 50 mg/kg bw), Category 3 (50 < LD50 < 300 mg/kg bw), and Category 4 (300 < LD50 < 2000 mg/kg bw). In general, 2-alkyl-1-alkanol acute oral toxicity (LD50) is Category 3 (see Table 2) and thus, are not required to have a hazard label for acute oral toxicity.

In mammals, mild to moderate sub-lethal toxicity from a single oral dose of intermediate size alkanols include general gastrointestinal symptoms (e.g., nausea, vomiting, abdominal cramps and diarrhoea) associated with irritation. Severe ingestions (i.e., near acute lethal levels) can cause gastrointestinal haemorrhage and liver injury. For example, in the rat, the LD50 for 1-octanol is >5000 mg/kg (Opdyke, 1973). The only symptoms of intoxication observed here were moderately to severely ruffled fur and mild sedation. The symptoms had regressed completely 24 hours later. The growth of the exposed animals was like that of the controls.

In fish, alkanols are considered to act via the nonpolar narcosis mode of action (as first reported by Veith et al, 1983; Raevsky et al., 2008). Within the USEPA DSSTox Fathead Minnow Acute Toxicity (EPAFHM) database, alkanols are represented. They exhibit toxic potencies not statistically different from baseline predictions. Because of concerns for aquatic toxicity, a large number of alcohols, especially saturated ones, have been tested *in vitro* for cell population growth inhibition (Schultz et al., 2004). Structure-activity results from *in vivo* and *in vitro* tests are highly consistent (Schultz et al., 1998). Briefly, from a structural standpoint, the aquatic toxicity of alkanols is partition-dependent, regardless of endpoint being assessed.

Generally, *in vitro*, alkanols ascribed to unspecific interactions with biological membranes; such effects are directly correlated with 1-octanol/water partition coefficients (c.f. Benane et al., 1993).

New-methods similarity

ToxCast data are available for a large number of alkanols (Judson et al., 2010). Of the 711 assays that form the ToxCast scheme, 2-ethyl-1-hexanol has been evaluated in 602 of them and 2-propyl-1-heptanol has been assessed in about 250 assays. The number of active assays varies, six for 2-ethyl-1-hexanol and four for 2-propyl-1-heptanol. Within ToxCast, the alkanols, in general, are one of the least promiscuous chemical classes; for alkanols < 3% of the ToxCast assays show any activity up to highest concentration tested and none of the active assay are associated with specific bioactivity (Schultz et al., 2016).

The 2-alkyl-1-alkanols were screened with a variety of *in silico* profilers within the COSMOS Project of SEURAT-1 (see Table 6 of Annex I). Specifically, profilers for nuclear receptor binding were run to identify potential binding to the following nuclear receptors; PPARs (peroxisome proliferator-activated receptors), AR (androgen receptor), AHR (aryl hydrocarbon receptor), ER (estrogen receptor), GR (glucocorticoid receptor), PR (progesterone receptor), FXR (farnesoid X receptor), LXR (liver X receptor), PXR (pregnane X receptor), THR (thyroid hormone receptor), VDR (vitamin D receptor), as well as RXR (retinoic acid receptor). The evaluation of potential binding to the receptors is based on structural fragments and physico-chemical features that have been identified as essential to bind to these nuclear receptors and induce a response. No potential receptor binding was predicted. It is notable that ToxCast also tested for all of these receptors, and all assays were negative.

Taken collectively, the findings with new-method data is not inconsistent with the previously cited data. The premise that in oral repeated-dose toxicity, 2-alkyl-1-alkanols are considered to be nonpolar narcotics and thereby act in a manner similar to depressant anaesthetics is consistent with the ToxCast data and receptor binding simulations results, which do indicate any activity associated with a specific mode of action.

4. STATEMENT OF UNCERTAINTY

In Tables 4 and 5, are presented the assessments of uncertainties. 2-Alkyl-1-alkanols are a well-studied category with a robust strength-of-evidence. Data uncertainty associated with the fundamentals of chemistry, transformation/toxicokinetic and toxicological similarity (i.e., chemical, toxicokinetic and toxicodynamic similarity of category members) is low.

In terms of chemistry, the narrowly defined applicability domain of this category leads to all analogues or category members being chemically highly similar. Specifically, the key feature, being a primary alkanol, is common within the category and relevant to a “no/low toxicity” read-across. While there are differences among the category members with respect to physicochemical properties, these differences are not considered toxicologically relevant outside of their impact on bioavailability.

From a toxicokinetic standpoint, there are differences among the 2-alkyl-1-alkanols in the composition of the final metabolites. The position and size of the alkyl substituent plays a role in metabolism, with degradation to expired CO_2 decreasing and glucuronidation increasing with increasing chain lengths. Absorption, distribution, metabolic pathways and half-lives of all analogues is considered to be highly similar. There is uncertainty whether the branched analogues, especially ones with an alkyl group larger than a methyl group in the 2-position, are metabolized to reactive intermediates. However, reading across from 2-ethyl-1-hexanol is considered to be toxicokinetically conservative. In other words, methyl-substituted derivatives may be less toxic; they will not be more toxic.

Alkanols, including 2-alkyl-1-alkanols, are experimentally associated with the nonpolar narcosis mechanisms of toxicity. The simple narcosis (i.e., reversible anaesthesia) mode of toxic action is driven by partition into the biophase. While well-studied, this molecular mechanism is not well understood and no adverse outcome pathway is currently available. Moreover, it is unclear if oral repeated-dose toxicity is limited to this mechanism; however, there is evidence (Strubelt et al., 1999; see Table 1) to suggest it is not.

All analogues or category members are considered, from a toxicodynamic standpoint, to be similar. Specifically, from a qualitative and quantitative standpoint, tested analogues exhibit highly similar toxicological profiles for *in vivo* repeated-dose effects. The new-methods data increases confidence in the mechanistic relevance and completeness of the read-across (i.e., reduces the uncertainty in the predictions).

One observed uncertainty is associated with the fact that, while 2-methyl-substituted derivatives are considered with the domain of the category, there is no *in vivo* experimental data supporting their inclusion. However, there is high quality repeated-dose data for 3-methyl-1-butanol [CAS 123-51-3].

In a 90-day study with rats, according to OECD Test Guideline 408, 3-methyl-1-butanol was administered in the drinking water in concentrations of 0, ≈80, ≈340 and ≈1250 mg/kg bw/d (Schilling et al., 1997). A NOAEL of 340 mg/kg bw/d for males and 1250 mg/kg bw/d for females was reported. 3-Methyl-1-butanol was also tested in a 17-week toxicity study with Ash/CSE rats (Carpanini and Gaunt, 1973). The test substance was administered by gavage to 15 /sex/group at dose levels of 0, 150, 500, or 1000 mg/kg bw/d in corn oil. While a variety of whole body clinical pathological and histopathological endpoints were examined, the only observed effects were a statistically significant reduced body weight in males and a non-statistical reduction in food intake at the highest dose level. A NOAEL of 500 mg/kg bw/d for males and 1000 mg/kg bw/d for females was reported. In addition, 3-methyl-1-butanol was administered to male and female Wistar rats (≈2000 mg/kg bw/d) in drinking water for 56 weeks. No treatment-related effects were observed for whole body, clinical pathology or histopathological endpoints (Johnansen and Purchase 1969).

In rats, oral administration of 2000 mg 3-methyl-1-butanol /kg bw led to a peak concentration of 170 mg/l blood at 1 hour (Gaillard and Derache, 1965; ECHA CHEM c); more than 50% of the dose was excreted within 24 hours. In another study (Haggard et al., 1945), rats were intraperitoneally administered 4 treatments of 250 mg/kg bw in 15 minute-intervals. Complete absorption of the substance was observed within 1 hr after final administration. No test substance was detectable after 4 hrs. Excretion was 2% in urine and 5.6 expired air. Kamil et al. (1953a) reported after gavage administration of a dose of 25 mmol per rabbit (corresponding to ≈ 735 mg/kg bw) of 1-pentanol, 3-methyl-1-butanol, and 2-methyl-1-butanol ≈ 7%, 9%, and 10% of the dose was excreted by the rabbits into urine as glucuronides, respectively. Furthermore, the urine did not contain aldehydes or ketones. It is assumed the remaining 90+% of the tested derivative was excreted as CO_2.

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

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

Similarity Parameter	**Data Uncertainty** [a] **(empirical, modelled)** **(low, medium, high)**	**Strength-of-Evidence** [b] **(low, medium, high)**	**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 Modelled: 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 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.

Similarity Parameter	**Data Uncertainty** [a] **(empirical, modelled)** **(low, medium, high)**	**Strength-of-Evidence** [b] **(low, medium, high)**	**Comment**
Transformation/ Toxicokinetics and Metabolic Similarity	Empirical: In vivo: moderate In vitro: none 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 metabolized 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-alkanols reveal any propensity for receptor binding within the SEURAT-1 suite of *in silico* profilers.
Overall uncertainty in similarity of category members: Chemical similarity is limited but has limited impact other than possibly on bioavailability. While weight-of evidence is high, uncertainty associated with the fundamentals of chemical, transformation/toxicokinetic is low to moderate. The weight-of evidence is high and uncertainty associated with the toxicodynamics is low. Uncertainty associated with mechanistic relevance and completeness of the read-across (i.e., uncertainty in the predictions) is reduced to low with the addition of new-methods data. Summary: Key features of chemistry are similar within the category. Key features of transformation toxicokinetics and metabolism are common within the category. Category members are considered mechanistically similar. Category members exhibit a narcosis toxic profile with respect to *in vivo* and *in vitro* toxicity. Category members exhibit a non-toxic profile with respect to new-methods effects.			

[a] Uncertainty associated with underlying information/data used in the exercise

[b] Consistency within the information/data used to support the similarity rational and prediction

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

Factor	**Uncertainty** **(low, medium, high)**	**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 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. There is agreement among the dose-response relationships of the tested category members for relevant *in vitro* events.

Factor	**Uncertainty** **(low, medium, high)**	**Comment**
Weight of Evidence	Low	Overall the available information is generally 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. The fact there are two well studied source substances with highly similar *in vivo* data that are supported by similar data for other analogues adds to the WoE. The fact that there is consistent relevant *in vitro* data for many 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 and other profilers add to the WoE.
While it is difficult to prove a negative, the overall uncertainty associated with mechanistic relevance and completeness of the read-across is judged to be low.		

5. STATEMENT OF THE CONCLUSIONS

In vivo oral repeated-dose exposure to 2-alkyl-1-alkanols gives rise to a set of non-specific symptoms, including clinical symptoms, haematological values outside the normal range, or whole body effects different from normal. The category limitation to C5 to C13 analogues assures that the impact of bioavailability on the toxicokinetic and toxicodynamic profiles is limited. These primary alkanols are direct-acting toxicants with a reversible mode of toxic action described as nonpolar narcotics (i.e., unspecific interaction with biological membrane in a manner similar to simple anaesthetics). The main route of exposure is oral with rapid gastrointestinal absorption; the 2-alkyl-1-alkanols are promptly degraded via simple cellular oxidation and/or eliminated as glucuronide conjugates.

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

A NOAEL value of 125 mg/kg bw/d can be read across to fill the data gaps among the analogues in this category for the purpose of risk assessment. Specifically, the data gaps for 2-propyl-1-pentanol and 2-ethyl-1-octanol are filled with low uncertainty (high confidence) by interpolation from 2-ethyl-1-hexanol and 2-propyl-1-heptanol. The data gaps for 2-ethyl-1-butanol, 2-ethyl-1-pentanol, 2-ethyl-1-decanol and 2-propyl-1-decanol are filled with low uncertainty (high confidence) by extrapolation from 2-ethyl-1-hexanol and 2-propyl-1-heptanol. The data gaps for 2-methyl-1-butanol, 2-methyl-1-pentanol, 2-methyl-1-octanol and 2-methyl-1-undecanol are filled with acceptable uncertainty as worst-case scenarios. The latter uncertainty results from incomplete knowledge of how a methyl group rather than an ethyl or propyl moiety, affects the ratio of excretion in respired CO_2, urine as a conjugate and faeces and its impact on repeated-dose potency.

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ECHA CHEM B for 2-Propyl-1-heptanol: http://echa.europa.eu/registration-dossier/-/registered-dossier/13788

ECHA CHEM C for 3-Methyl-1-butanol: http://echa.europa.eu/registration-dossier/-/registered-dossier/13936

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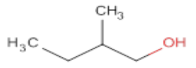
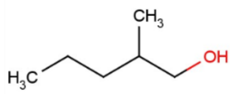
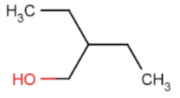
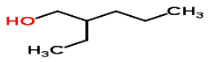
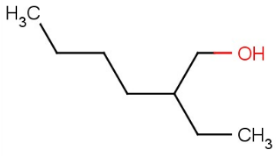
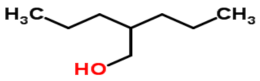
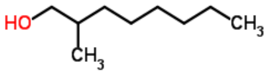
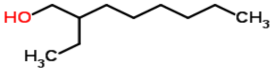
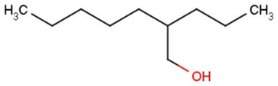
ANNEX I TABLES FOR ASSESSING SIMILARITY OF ANALOGUES AND CATEGORY MEMBERS FOR READ-ACROSS

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

	Name	CAS No	SMILES	2D Structure	Molecular Formula
2-Methyl-1-butanol	2-Methybutanol	137-32-6	CCC(C)CO		C5H12O
2-Methyl-1-pentanol	2-Methylpentanol	105-30-6	CCCC(C)CO		C6H14O
2-Ethyl-1-butanol	2-Ethylbutanol	97-95-0	CCC(CC)CO		C6H14O
2-Ethyl-1-pentanol	2-Ethylpentanol	27522-11-8	CCCC(CC)CO		C7H16O
2-Ethyl-1-hexanol	2-Ethylhexanol	104-76-7	CCCCC(CC)CO		C8H18O

	Name	CAS No	SMILES	2D Structure	Molecular Formula
2-Propyl-1-pentanol	2-Propylpentanol	58175-57-8	CCCC(CCC)CO		C8H18O
2-Methyl-1-octanol	2-methyloctanol	818-81-5	CCCCCCC(C)CO		C9H20O
2-Ethyl-1-octanol	2-ethyloctanol	20592-10-3	CCCCCCC(CC)CO		C10H22O
2-Propyl-1-heptanol	2-Propylheptanol	10042-59-8	CCCCCC(CCC)CO		C10H22O
2-Methyl-1-undecanol	2-Methylundecanol	10522-26-6	CCCCCCCCCC(C)CO		C12H26O

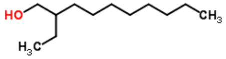
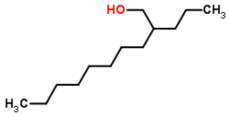
	Name	CAS No	SMILES	2D Structure	Molecular Formula
2-Ethyl-1-decanol	2-Ethyldecanol	21078-65-9	CCCCCCCCC(CC)CO		C12H26O
2-propyl-1-decanol	2-Propyldecanol	60671-35-4	CCCCCCCCC(CCC)CO		C13H28O

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

	Molecular Weight [1]	**Log Kow**[1a]	**Vapor Pressure (Pa, 25 degC)** [1b]	**Density** [2] **(g/cm^3)**	**Melting Point (deg C)** [1b]	**Water Solubility (mg/L, 25 degC)** [1c]	**Boiling Point (deg C)** [1b]	**pKa** [3]
2-Methyl-1-butanol	88.15	1.26 1.29 (M)	606 416 (M)	0.8±0.1	-61.49	32200 29700 (M)	123.17 128 (M)	15.24
2-Methyl-1-pentanol	102.18	1.75	191 256 (M)	0.8±0.1	-49.23	11950 6000 (M)	145.86 149 (M)	15.05
2-Ethyl-1-butanol	102.18	1.75	213 204 (M)	0.8±0.1	-49.23 <-15 (M)	11950 4000 (M)	145.86 147 (M)	15.05
2-Ethyl-1-pentanol	116.21	2.24	66.2	0.8±0.1	-37.23	4089	167.64	15.05
2-Ethyl-1-hexanol	130.23	2.73	24.6 18.1 (M)	0.8±0.1	-25.50 -70 (M)	1379 880 (M)	188.52 184.6 (M)	15.05
2-Propyl-1-pentanol	130.23	2.73	19.5	0.8±0.1	-25.50	1379	188.52	15.05
2-Methyl-1-octanol	144.26	3.22	5.88	0.8±0.1	-14.04	459.7	208.49	15.09
2-Ethyl-1-octanol	158.29	3.71	1.81	0.8±0.1	-2.83	151.8	227.56	15.09
2-Propylheptan-1-ol	158.29	3.71	3.38	0.8±0.1	-2.83	151.8	227.56 217.5 (M)	15.09
2-Methyl-1-undecanol	186.34	4.70	0.186	0.8±0.1	18.78	16.18	262.99	15.04

	Molecular Weight [1]	**Log Kow[1a]**	**Vapor Pressure (Pa, 25 degC) [1b]**	**Density [2] (g/cm^3)**	**Melting Point (deg C) [1b]**	**Water Solubility (mg/L, 25 degC) [1c]**	**Boiling Point (deg C) [1b]**	**pKa [3]**
2-Ethyl-1-decanol	186.34	4.70	0.186	0.8±0.1	18.78	16.18	262.99	15.04
2-propyl-1-decanol	200.37	5.19	0.0615	0.8±0.1	29.19	5.237	279.35	15.06

[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

	Key Substituent(s)	Functional Group(s)	Extended Fragment(s)	Chemical Class:	Chemical Sub-Class:
2-Methyl-1-butanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Methyl-1-pentanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Ethyl-1-butanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Ethyl-1-pentanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Ethyl-1-hexanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Propyl-1-pentanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Methyl-1-octanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Ethyl-1-octanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Propylheptan-1-ol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Methyl-1-undecanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
2-Ethyl-1-decanol	-OH	$-CH_3$, $-CH_2-$, -CH-	–	saturated aliphatic alcohols	2-alkyl-1-alkanol
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

Name	*Abiotic Transformation*	*Toxicokinetics*			
		Absorption	**Bioavailability**	**half-life**	**Elimination**
2-Methyl-1-butanol		efficiently following oral administration [d]		< 24 hrs	9.6% excreted in the urine as a glucuronides within 24 hrs[a] 5.6% excreted in air and 2% in urine, remainder metabolized, first to the corresponding aldehyde, then to the acid [f] additional oxidation of 2-methyl-1-butanol by rat liver microsomes via CYP P450 enzymes, and glucuronidation [g]
2-Methyl-1-pentanol		efficiently following oral administration [d]			
2-Ethyl-1-butanol		efficiently following oral administration [d]			excreted mainly as a glucuronides [b]
2-Ethyl-1-pentanol		efficiently following oral administration [d]			

2-Ethyl-1-hexanol	atmospheric lifetime of 24.6 hrs	efficiently following oral administration [d]		< 24 hrs terminal half-life 60 hours	rapidly excreted in respired CO_2 (6-7%), urine mainly as glucuronides (80-82%), and faeces (8-9%); elimination was essentially complete by 28 hrs [c] 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. [e] Glucuronide main metabolite (87%) in rabbits [a,b]
2-Propyl-1-pentanol		efficiently following oral administration [d]			
2-Methyl-1-octanol		efficiently following oral administration [d]			
2-Ethyl-1-octanol		efficiently following oral administration [d]			
2-Propyl-1heptanol		efficiently following oral administration [d]			
2-Methyl-1-undecanol		efficiently following oral administration [d]			
2-Ethyl-1-decanol		efficiently following oral administration [d]			
2-propyl-1-decanol		efficiently following oral administration [d]			

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

	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
	Rat liver S9	**Skin metabolism**			
2-Methyl-1-butanol	Hydroxylation (3) Oxidation (1)	Hydroxylation (3)	Hydroxylation Oxidation Acylation	Possible sites of metabolism have been identified	Hydroxylation (4) Oxidation (1)
2-Methyl-1-pentanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (1)	Hydroxylation Oxidation Acylation Methylation Dealkylation	Possible sites of metabolism have been identified	Hydroxylation (2) Oxidation (1)
2-Ethyl-1-butanol	Hydroxylation (2) Oxidation (1)	Hydroxylation (2)	Hydroxylation Oxidation Acylation Dealkylation	Possible sites of metabolism have been identified	Hydroxylation (3) Oxidation (1)
2-Ethyl-1-pentanol	Hydroxylation (4) Oxidation (1)	Hydroxylation (3)	Hydroxylation Oxidation Acylation Dehydroxylation Methylation Alkylation Dealkylation	Possible sites of metabolism have been identified	Hydroxylation (4) Oxidation (1)

	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
	Rat liver S9	**Skin metabolism**			
2-Ethyl-1-hexanol	Hydroxylation (4) Oxidation (1)	Hydroxylation (4)	Hydroxylation Oxidation Acylation Methylation Alkylation Dealkylation Dehydration Demethylation	Possible sites of metabolism have been identified	Hydroxylation (6) Oxidation (1)
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)
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)

	Liver metabolism simulator Toolbox v3.3		**MetaPrint2D-React software**	**SMARTCyp version 2.4.2**	**Meteor Nexus**
	Rat liver S9	**Skin metabolism**			
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)
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)
2-Methyl-1-undecanol	Hydroxylation (3) Oxidation (1)	Hydroxylation (3)	Hydroxylation Oxidation Acylation Methylation Alkylation Dealkylation Demethylation	Possible sites of metabolism have been identified	Hydroxylation (5) Oxidation (1)

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

Table 6: Comparison of Toxicophores

	Toxicophores[1]	DNA binding by OECD[1]	Protein binding by OECD[1]	Nuclear receptor binding[2]	Liver& Mitochondria toxicity[2]
2-Methyl-1-butanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Methyl-1-pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Ethyl-1-butanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Ethyl-1-pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Ethyl-1-hexanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Propyl-1-pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Methyl-1-octanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Ethyl-1-octanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Propyl-1-heptanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Methyl-1-undecanol	Cramer Class I	No alert	No alert	Inactive	No alert
2-Ethyl-1-decanol	Cramer Class I	No alert	No alert	Inactive	No alert
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

	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
2-Methyl-1-butanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Methyl-1-pentanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Ethyl-1-butanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Ethyl-1-pentanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Ethyl-1-hexanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Propyl-1-pentanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			

	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**
2-Methyl-1-octanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Ethyl-1-octanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Propyl-1-heptanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Methyl-1-undecanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
2-Ethyl-1-decanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
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)					125-1000 (mg/kg/d) [2, 3] 125 (mg/kg bw/d) (rat 90d oral) [24, 27] 125 (mg/kg bw/d) (mouse 90d oral) [27] 25 (mg/kg bw/d) (subchronic gavage) [27]				30-150 (mg/kg/d) [4] 150 (mg/kg bw/d) (rat 90d oral) [26]			
Endpoint: NOEL (Repeat dose toxicity)	≥6400 (mg/m^3) [1]											

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 (short-term repeated dose study)					100 (mg/kg bw/d) (rat 11d) [28] 100 (mg/kg bw/d) (rat 28d) [30]							
Endpoint: NOAEL (chronic)					150 (mg/kg bw/d) (rat 2years) [24, 29] 200 (mg/kg bw/d) (mouse 18months) [31]							
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]							

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 (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 (Carcinogenic/ Genotoxicity)					150-750 (mg/kg/d) [8]							
Endpoint: LC50 (Acute toxicity)					0.89-5.3(mg/La ir) [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) [9-12]				3730(mg/kg) [6, 13, 14]				5100-5400(mg/kg) [7]			

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: oral LD50 (mg/kg) (Acute toxicity)		4010 (rat) [21]		1850 (rat) [22]		>3730 (rat) [23] ≈2000 (rat) [24] 2500 (mouse) [25]				5400 (rat) [26]			
Endpoint: LDLo (Acute toxicity)		1900-2448 (mg/kg) [15, 9]											
Endpoint: Genotoxicity (AMES, , Chromosomal abrration, gene mutation)		2 x Negative [16-19]				9 x Negative [20]				5 x Negative [20]			
Toxcast [32]	ATG_ERa_TRANS					11.9							
	ATG_ERa_TRANS_perc					5.77							
	ATG_PXRE_CIS					31.1							
	ATG_PXRE_CIS_perc					31.1							

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
	OT_ERa_ERELUC_AG_1440									3.14			
	Tox21_AR_BLA_Agonist_ch1									0.00219			
	Tox21_ELG1_LUC_Agonist_viability									54.9			

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