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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 N-ALKANOLS: READ-ACROSS

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Environment Directorate
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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 2-ALKYL-1-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)26, Series on Testing & Assessment No. 274.

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.

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Please Note: This case study has been designed to illustrate specific issues associated with 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

n-Alkanols (unbranched saturated primary aliphatic alcohols) provide an excellent example where a category-approach to read-across can be used to estimate the repeated-dose endpoint for a number of derivatives. The intended use of these data is in substance-specific quantitative risk assessments. In this case the chemical category represents analogues 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 membranes in a manner similar to depressant anaesthetics. The read-across premise includes absorption via the gastrointestinal tract, distribution in the circulatory system, and first-pass metabolism in the liver, with metabolism via oxidation to CO_2 and with minor elimination of oxidative intermediate as glucuronides. Five analogues have experimental oral repeated-dose toxicity data. Repeated-dose toxicity test results exhibit qualitative consistency in symptoms. The 90-day findings are quantitatively consistent with NOAELs $\geq$ 1000 mg/kg bw/d. 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.

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 a negative. 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,* USEPA toxicity forecaster (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. The 90-day oral repeated-dose toxicity NOAEL value of 1000 mg/kg bw/d for 1-pentanol and 1-hexanol can be read across to fill the data gaps of the untested analogues in this category with acceptable uncertainty.

INTRODUCTION TO THE 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 is often not enough to establish similarity with sufficient uncertainty to accept a toxicological read-across for chronic health endpoints. To establish toxicodynamic and to a greater extent toxicokinetic similarity one must limit 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. There 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 red-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.

n-Alkanols provide excellent examples where the category approach to IATA may provide fill data gaps for the oral 90-day repeated-dose endpoint. In this scenario, the chemical category represents analogues which are non-reactive and exhibit no specific mode of toxic action, and metabolism has no toxicological relevance. 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 to stimulate discussion on how to develop guidance on how to address these issues. It is not intended to be related to any regulatory discussions on this chemical group.

n-Alkanols: Existing Knowledge

Based on rat and fish studies, n-alkanols are considered nonpolar narcotics, which 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, reveals saturated monohydric alcohols consistently behave as classic nonpolar narcotics (Veith et al., 2009).

The efficacy of n-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. Perfused rat liver toxicity data from Strubelt et al. (1999) for 1-pentanol (exposure 65.1 mmol/l for 2 hours) are reported in Table 1. These data support the premise that *in vitro* toxicity (e.g., O_2 consumption and ATP production) of n-alkanols is due to membrane partitioning (which is assumed to correlate with log Kow) resulting in loss of membrane integrity (i.e., cytosolic enzyme leakage (LDH) but not glutathione (GSH) binding).

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

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
1-Pentanol	1.40	0.06 ± 0.01	0.20 ± 0.03	28959 ± 4142	2.82 ± 0.36

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

The general anaesthetic potency of several members of this homologous series of saturated aliphatic alcohols was determined in tadpoles, using the loss of righting reflex as the criterion of anaesthesia (Pringle et al., 1981). In this series, anaesthetic potency increased with chain length and was maximal for 1-dodecanol. The cut-off in potency was between C12 and C14, such that 1-tridecanol was a partial anaesthetic.

n-Alkanols within the range C5-C13 are expected to be readily absorbed by the gastrointestinal tract. Dermal penetration of these intermediate size alkanols does not readily occur and absorption from inhalation is extremely limited. Thus, the primary route of exposure of repeated-dose toxicological interest is oral.

n-Alkanols are metabolized mainly in the liver via alcohol dehydrogenase to corresponding aldehydes and, subsequently, by aldehyde dehydrogenase to the corresponding carboxylic acids (Voet and Voet, 1990). The fatty acid derivatives of intermediate size n-alkanols are readily taken up by mitochondria, where they are degraded by β-oxidation, especially in hepatocytes and myocytes (Voet and Voet, 1990). However, generally <10% of the dose of these primary alcohols form glucuronic acid conjugates, which are excreted in the urine (Kamil et al., 1953).

Voskoboinikova (1966) and Opdyke (1973) have summarized the historical literature on aliphatic alcohol toxicity. More recently, the toxicity of alkanols containing from C1 to C6 has been reviewed (Patocka and Kuca, 2012). In general, n-alkanols acute oral toxicity (i.e., LC50) is very low, ranging from 1500 to 5000 mg/kg bw with an average value of ≈3000 mg/kg bw (see Table 2). n-Alkanols are only slightly toxic or nontoxic 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 in the range of 1/2 - 1/3 the LC50 value (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 (i.e., near lethal doses), n-alkanols may cause systemic damage to the liver, heart, kidneys, and/or nervous system.

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

Alcohol	Oral LD50 (mg/kg)	Citation	90-d Oral NOAEL (mg/kg bw/d)	Citation
1-Pentanol	2200	ECB, 2000	1000	Butterworth et al., 1978
	3645	ECHA CHEM a	1000	ECHA CHEM a
1-Hexanol	4590	IITI, 1988	1127 M	ECHA CHEM b
	4870	Bingham et al., 2001	1243 F	ECHA CHEM b
1-Heptanol	3250	Bingham et al., 2001	Not determined	
	6200 M	Truhaut, 1974; ECHA CHEM c	> 1000	ECHA CHEM c
	5500 F	Truhaut, 1974; ECHA CHEM c		
1-Octanol	>5000	ECHA CHEM D	Not determined	
Nonyl alcohol (assumed 1-nonanol)	3560	Opdyke, 1973	Not determined	
1-Decanol	4720	Lewis, 2004a	Not determined	
Undecyl alcohol (assumed 1-undecanol)	3000	Verschueren, 2001	a	
Lauryl alcohol (assumed 1-dodecanol)	> 2000	ECHA CHEM F	2000	ECHA CHEM f; OECD SIDS, 2006
1-Tridecanol 1	7200	Raw Material Data Handbook	Not determined	

[a] NOAEL of 2000 mg/kg bw/d is recorded on ECHA CHEM website (http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/2096/1). Although this NOAEL value is recorded as experimental result, the details in the report indicate that it is read across from 1-dodecanol (CAS 112-53-8) (ECHA CHEM e)

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

Butterworth, K.R., Gaunt, I.F., Heading, C.E., Grasso, P. and Gangolli, S.D. 1978. Short-term toxicity of n-amylalcohol in rats. Food Cosmet. Toxicol. 16: 203-207.

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

ECHA CHEM a 1-pentanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/2115; Accessed 27.01.2016

ECHA CHEM b 1-hexanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/13265; Accessed 27.01.2016

ECH CHEM c 1-heptanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/5921/7/1; Accessed 27.01.2016

ECHA CHEM d 1-octanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15210; Accessed 27.01.2016

ECHA CHEM e 1-undecanol; http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/2096; Accessed 27.01.2016

ECHA CHEM f 1-dodecanol: I http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15424; Accessed 27.01.2016

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

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

Opdyke, D.L.J. 1973. Monographs on fragrance raw materials. Food and Cosmetics Toxicology. 11(1): 95-116.

Organization for Economic Co-Operation and Development (OECD) and Screening Information Datasets (SIDS) 2006. High Production Volume Chemicals 1-Dodecanol (Cas No.: 112-53-83). Processed by United Nations Environmental Program (UNEP). Available Online:OECD; Screening Information Data Set for. 2006. http://www.inchem.org/documents/sids/sids/112538.pdf.

Raw Material Data Handbook, Vol.1: Organic Solvents, 1974. Vol. 1, Pg. 114, 1974.

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

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

EVALUATION OF THE N-ALKANOL CASE STUDY FOLLOWING THE 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 associated with the IATA must be accompanied by low uncertainty. In this case study, acceptable uncertainty is one that is equal to doing a test using a protocol similar to the OECD TG 408.

1.2. Target and Source Substances

The analogues listed in Table 3 include the seven target and two source chemicals (noted in bold) of this case study. This list is inclusive as defined by the limitations of the applicability domain; it represents n-alcohols which are found in governmental or industrial inventories (e.g., OECD High Production Volume Chemicals).

Table 3. n-Alkanols considered as part of the chemical category for read-across.

	Name	CAS No.	Structure
1)	**1-Pentanol**	**71-41-0**	**C5H12O**
2)	**1-Hexanol**	**111-27-3**	**C6H14O**
3)	1-Heptanol	111-70-6	C7H16O
4)	1-Octanol	111-87-5	C8H18O
5)	1-Nonanol	143-08-8	C9H20O
6)	1-Decanol	112-30-1	C10H22O
7)	1-Undecanol	112-42-5	C11H24O
8)	1-Dodecanol	112-53-8	C12H26O
9)	1-Tridecanol	112-70-9	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 1-pentanol and 1-hexanol are particularly well-suited for read-across; the NOAELs are based on experimental results from a 3-dose exposure scenario (<100, between 100 and 500 and ≥ 1000 mg/kg bw/d) following a standard test guideline (OECD TG 408). Moreover, there are supporting repeated-dose results for 1-heptanol, 1-undecanol and 1-dodecanol from OECD TG 422.

2. HYPOTHESIS OF THE CATEGORY

The premise for this read-across case study is:

- n-Alkanols 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 or simple anaesthesia).

- The chemical category is based on simple structure similarities - C-atom chain length and straight-chain hydrocarbon scaffolding.

- With C5 to C13 derivatives, C-atom chain length affects most physico-chemical properties (e.g., Low Kow values increase with increasing chain length). However, this trend, while toxicologically relevant in fish toxicity and in vitro assays, is not observed in mammalian acute and chronic toxicity via oral exposure.

- These primary alkanols are rapidly and nearly completely absorbed from the gut; first pass metabolism leads to two-step oxidative metabolism in the liver resulting in the corresponding carboxylic acid, which subsequently undergoes mitochondrial β-oxidation to CO_2 with minor amounts of glucuronidation with subsequent elimination of the phase II metabolite in the urine.

- Toxicodynamically, these primary alkanols are highly similar. In vivo they exhibit no systemic toxicity; in vitro they exhibit no chemical reactivity or receptor-mediated interactions.

- Repeated-dose testing data for 1-pentanol and 1-hexanol can be read across to other category members listed in Table 3.

2.1. Justification

From a repeated-dose perspective, test results of n-alkanols are extensive. 1-pentanol was orally administered to rats following OECD TG 408 at dose levels of 0, 50, 150 or 1000 mg/kg body weight/day for 13 wk (Butterworth et al. 1978; ECHA CHEM A). The no-outward-effect level was 1000 mg/kg/day.

In a non-standard rat oral repeated-dose assay similar to an OECD TG 408 assay, animals were exposed to 0.25% (alkanol in vehicle) and 0.50% for 13 weeks; 1.0% for 10 weeks then 2.0% (week 11), 4.0% (week 12) and 6.0% 13 weeks of 1-hexanol (ECHA CHEM b). The NOAEL for 1-hexanol was determined to be ≈1100 mg/kg bw/d (1127 mg/kg bw/d for male and 1243 mg/kg bw/d for female rats).

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

Following OECD TG 422, oral repeated-dose toxicity of 1-undecanol in rats was evaluated at doses of ≈ 0, 100, 500, 2000 mg/kg bw/d (ECHA CHEM e). A NOAEL for systemic toxicity of 2000 mg/kg bw/d was determined in male rats, in the absence of toxicologically significant effects at any dose level.

Following OECD TG 422, rats were exposed to 1-dodecanol in the diet in concentrations of ≈ 0, 100, 500 and 2000 mg/kg/ bw/d (ECHA CHEM f). A NOAEL for systemic toxicity of 2000 mg/kg bw/d was determined in male rats, in the absence of toxicologically significant effects at any dose level.

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

2.2. Applicability domain

The applicability domain for this read-across is confined to straight-chain 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 included in this chemical category. Branched derivatives, which exhibit a different toxicokinetic profile, are also excluded from this chemical category. Briefly, the presence of alkyl side chains does not terminate the oxidation process; however, in most cases, it alters it. Moreover, the position and size of the alkyl substituent plays a role in metabolism, with glucuronidation increasing with increased branching.

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. 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.

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 n-alkanols included in the category are structurally highly similar. Specifically, they: 1) belong to a common chemical class, aliphatic alcohols and subclass, n-alkanols, and 2) possess common molecular scaffolding, a C-atom backbone with a straight-chain configuration. Structurally, the main variable is the length of the backbone, C5-C13.

Chemical property similarity

As demonstrated in Table 2 of Annex I, all the n-alkanols included in the category have many of their physico-chemical properties determined experimentally. Thus, calculated values based on these measured values can be used with high confidence. Properties, with the exception of density and pKa, trend in value related to C-atom number within the scaffold. Specifically, all category members exhibit molecular weights from 88 to 200 g/mol. Hydrophobicity (log Kow) increases with number of C-atoms from >1.0 to <6.0, vapour pressure and water solubility decrease with molecular size, melting point and boiling point increase with molecular size, density is constant at 0.8±0.1g/cm^3 and pKa at around 15.2.

Chemical constituent similarity

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

Toxicokinetic similarity

The narrow range of C-atoms for the applicability domain limits the impact on adsorption, distribution, metabolism and elimination (ADME). From a bioavailability standpoint, the analogues exhibit a linear trend with molecular weight. This curve reflects hydrophobic-dependent uptake.

The toxicokinetic understanding of alkanols is reasonably complete despite the fact that the experimental data, as summarised in Table 4 of Annex I, is limited. Absorption, distribution and elimination are not considered factors in these predictions. For example, 1-octanol is rapidly absorbed after oral administration (i.e., bioavailability >80%). 1-Octanol is excreted mainly as CO_2, and to a lesser extent as n-octyl glucuronide (Williams, 1959; Opdyke, 1973). Other n-alkanols exhibit similar toxicokinetics, with n-alcohols generally forming <10% of the dose as glucuronic acid conjugates and are excreted in the urine (Kamil et al., 1953).

It is universally accepted that, regardless of species, metabolism of n-alkanols is highly efficient and proceeds in a similar fashion (Moyes and Schulte, 2006). In the first step of the biotransformation, the alcohols undergo stepwise intracellular oxidation to the corresponding carboxylic acids, followed by a stepwise C2 unit elimination via mitochondrial β-oxidation.

Metabolic similarity

As demonstrated in Table 5 of Annex I, all of the category members can undergo oxidation and hydroxylation. It is highly likely the n-alkanols included in the category will be nearly completely metabolized (i.e., >90%) via the tricarboxcylic acid (TCA) cycle. Briefly, mammalian catabolism of fatty acids, which most often takes place in the mitochondria, leads to the formation of acetyl- coenzyme A (CoA), enters the TCA cycle and reduces nicotinamide adenine dinucleotide (NADH) and flavin adenine nucleotide (FADH2) which are used by the electron transport chain to produce ATP (Voet and Voet, 1990). While β-oxidation is the most common catabolic process, other processes, including ω-oxidation and α-oxidation, are known to take place.

Cytosolic fatty acids are activated for degradation by conjugation with CoA. β-Oxidation of saturated fatty acids consists of a recurring cycle of four reactions (Voet and Voet, 1990). In acids with an even number of C-atoms, this cycling continues until two molecules of acetyl-CoA are produced in the final reaction. Acetyl-CoA is available to be further metabolized in the TCA cycle. In acids with an odd number of C-atoms, the end product is propionyl-CoA, which must be converted to succinyl-CoA to enter the TCA cycle.

Toxicophore similarity

As demonstrated in Table 6 of Annex I, none of the n-alkanols included in the category are associated with any toxicophore based on *in silico* modelling.

Mechanistic plausibility similarity

It is generally accepted that the toxicity of intermediate chain n-alcohols is the result of narcosis. There are both theoretical and biochemical evidence for the cell membrane being the site of action for anaesthetic-like chemicals. Narcosis, in the broadest sense, is the reversible, 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 yet known. 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 stated 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 (Fang et al., 1997). While Central Nervous System (CNS) depression is reported after repeated bolus doses of 1-hexanol and 1-octanol, members of this category with higher C-chain lengths are not CNS depressants; the latter is most likely due to reduced bioavailability related to low water solubility.

The Fish Acute Toxicity Syndrome (FATS) approach put forth by McKim et al., (1987) has furthered our understanding of the effects of intermediate chain saturated alcohols in fish more than anything else. The FATS approach is based on physiological response sets from spinally transected rainbow trout (*Oncorhynchus mykiss*) exposed to model chemicals. Briefly, *in vivo* biochemical and respiratory-cardiovascular responses were measured during lethal aqueous exposures; the responses and their interdependence formed a complex data matrix, with the best response variables for mechanisms of action being determined with multivariate statistics. The FATS for 1-octanol is characterized by a striking slow-down in all respiratory and cardiovascular functions (McKim et al., 1987) that makes it distinct from other modes of actions. The action of 1-octanol is consistent with depressant anaesthesia.

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 the OH group is -0.108. This situation (negative contribution to toxicity as compared to corresponding alkane) 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).

In injection anaesthesia studies in rats (Fang et al., 1997), additivity of primary alkanols in joint effect studies was demonstrated. This observation of additivity is consistent with the premise that n-alkanols exhibit a common mechanism of action. More importantly, Fang et al. (1997) demonstrated additivity or slight deviations from additivity for alkanols with the conventional inhaled anaesthetic desflurane (1,2,2,2-tetrafluoroethyl difluoromethyl ether). The latter support the contention that the mechanisms of action of n-alkanols is depressant anaesthesia.

The effect of various primary alkanols on the CNS was studied by using rat brain synaptosomal membranes as an *in vitro* model (Edelfors and Ravn-Jonsen, 1990). The activity of (Ca_2^+/Mg_2^+) ATPase and the membrane fluidity were determined. Specifically, the n-alkanols exhibited an increased molar inhibition of the ATPase activity, with an increase in the carbon chain length up to 1-octanol. 1-Octanol and 1-decanol caused a biphasic effect on the ATPase activity, depending on the n-alkanol concentration, whereas 1-dodecanol caused a stimulation of the ATPase activity. All alkanols studied caused an increased fluidity of the membrane; however, changes in the membrane fluidity do not seem to be a pre-requisite of the ATPase inhibition (Edelfors and Ravn-Jonsen, 1990).

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' 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). n-Alkanols belong to Category 4 and do not require a hazard label for acute oral toxicity. Their LD50 values are very low, typically ranging from 1000 to >5000 mg/kg bw with an average value of ≈3000 mg/kg bw (see Table 2).

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. High ingested doses (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 were moderately to severely ruffled fur and mild sedation. The symptoms had disappeared completely 24 hours later. The growth of the exposed animals was similar to that of the controls.

In fish, alkanols are considered to act via the nonpolar narcosis mode of action (as 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, for alkanol exposures in *in vitro* assays, results are attributed to unspecific interactions with biological membranes; such effects are typically directly correlated with 1-octanol/water partition coefficients (c.f. Benane et al., 1993).

New-methods similarity

Within the USEPA toxicity forecaster program (ToxCast), data are available for the majority of the analogues (Judson et al., 2010). Of the 711 assays that form the ToxCast scheme, 1-octanol, 1-undecanol, 1-dodecanol and 1-tridecanol have been evaluated in 602 of them. Additionally, 1-hexanol, 1-heptanol and 1-decanol have been assessed in about 250 assays. Lastly, 1-nonanol has been tested in 150 ToxCast assays. The number of active assays varies from none for 1-octanol to 30 for 1-tridecanol and 25 for 1-undecanol. Within ToxCast, the n-alkanols are among the "least promiscuous chemical classes"; < 2.74% of the ToxCast assays showing any activity up to highest concentration tested and none of the active assay are associated with specific bioactivity. No assay exhibits activity for five or more of the category analogues. Only four assays, Tox21_ELG1_LUC_Agonist_viability, Tox21_TR_LUC_GH3_Antagonist_viability, Tox21_AhR_viability and Tox21_Aromatase_Inhibition_viability show activity with four alkanols and with these assays, there is no consistency among which analogues are active. Moreover, each of the four active assays is a non-specific cell viability qHTS assay.

Alkanols were screened with a variety of *in silico* profilers within the COSMOS Project of SEURAT-1. These results are reported in 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. Note 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 n-alkanols are considered to be nonpolar narcotics and act in a manner similar to depressant anaesthetics is consistent with the ToxCast data and receptor binding simulations results which indicate no activity associated with a specific mode of action.

4. STATEMENT OF UNCERTAINTY

The assessments of uncertainties are presented in Tables 4 and 5. Chemical similarity is high, and data uncertainty associated with the similarities in transformation/toxicokinetic, as well as toxicodynamics, is low. Uncertainty associated with mechanistic relevance and completeness of the read-across is low to moderate, largely because of the difficulty of proving a negative. Strengths-of-evidence associated with the fundamentals of chemistry, transformation/toxicokinetic, and toxicodynamic is high (i.e., this is a well-tested and well-understood group of chemicals).

In terms of chemistry, the narrowly defined applicability domain of this category leads to all analogues or category members being highly similar. While there are differences among the category members with respect to physico-chemical properties, these differences are not considered toxicologically relevant outside of their impact on bioavailability.

From a toxicokinetic standpoint, all straight-chain analogues are considered to be highly similar. Regardless of the species of mammals, all such category members are judged to be readily absorbed orally, metabolized via oxidation to the acid derivative, subsequently degraded to CO_2 via mitochondrial oxidation, and/or eliminated as a glucuronide. All analogues are judged to have similar distributions.

From a toxicodynamic standpoint, all category members are considered to be highly similar. n-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 related to this mechanism; however, there is no evidence to suggest it is not. From both qualitative and quantitative standpoints, all tested analogues exhibit highly similar toxicological profiles for *in vivo* oral acute and repeated-dose effects. Traditional *in vitro* data are from aqueous-based assays which quantify acute effects. Results for n-alkanols are typically dependent on hydrophobicity and are not relevant to repeated-dose mammalian toxicity.

The major source of uncertainty for this group of alcohols is associated with what is essentially a "non-toxic" prediction.

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 (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 Metabolic Similarity	Empirical: In vivo: low In vitro: none Simulated: low	medium	While *in vivo* absorption data are reported for only one category member, there is evidence for similar 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).

Similarity Parameter	Data Uncertainty [a] (empirical, modelled) (low, medium, high)	Strength-of-Evidence [b] (low, medium, high)	Comment
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.
Overall uncertainty in similarity of category members: Chemical similarity is limited by chain length but has no impact other than possibly bioavailability. Data uncertainty with the fundamentals of transformation/toxicokinetic and toxicodynamic similarity of category members is low. Uncertainty associated with mechanistic relevance and completeness of the read-across (i.e., uncertainty in the predictions) while initially low-to-moderate 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 non-toxic profile with respect to *in vivo* effects (i.e. LD50 and NOAEL). Category members exhibit a non-toxic profile with respect to *in vitro* and 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 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 endpoint data read across	Low; consistent LOEC symptoms	Generally, the *in vivo* data are consistent with regard to qualitative description of repeated-dose effects. LOEC are typically haematological or whole body parameters and not 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.

Factor	Uncertainty (low, medium, high)	Comment
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 ≈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.
Strenght-of-Evidence	Low; consistent relevant acute and *in vitro* data	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.
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 n-alkanols gives rise to a set of nonspecific symptoms, including clinical symptoms, haematological values outside the normal range, or whole body effects different from normal. Limiting the category to C5 to C13 analogues assures that the impact of bioavailability on the toxicokinetic and toxicodynamic profiles is very limited. Primary alkanols are direct-acting toxicants with a reversible mode of toxic action described as nonpolar narcosis (i.e., unspecific

interaction with biological membrane in a manner similar to simple anaesthetics). The main route of exposure for alkanols is oral via rapid gastrointestinal absorption. The majority of an oral dose of any n-alkanol is promptly degraded via simple cellular oxidation; the remainder is eliminated as the glucuronide conjugate.

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.

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

This is a category read-across (i.e., many-to-one several times). While several analogues have been evaluated experimentally in oral repeated-dose testing schemes, the 90-day oral repeated-dose toxicity data and the NOAELs of 1000 mg/kg bw/d for 1-pentanol and1-hexanol is the conservative prediction. A no systemic toxic conclusion with a NOAEL of 1000 mg/kg bw/d can be read across with confidence to untested n-alkanols in the C5 to C13 category listed in Table 3.

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ECHA CHEM B 1-hexanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/13265; Accessed 27.01.2016

ECH CHEM C 1-heptanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/5921/7/1; Accessed 27.01.2016

ECHA CHEM D 1-octanol: http://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15210; Accessed 27.01.2016

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

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

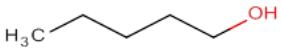
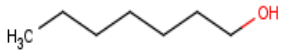
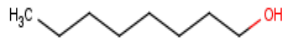
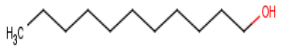
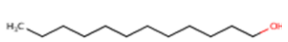
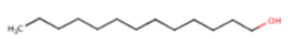
	Name	CAS No	SMILES	2D Structure	Molecular Formula
1-Pentanol	1-Pentanol	71-41-0	CCCCCO		C5H12O
1-Hexanol	1-Hexanol	111-27-3	CCCCCCO		C6H14O
1-Heptanol	1-Heptanol	111-70-6	CCCCCCCO		C7H16O
1-Octanol	1-Octanol	111-87-5	CCCCCCCCO		C8H18O
1-Nonanol	1-Nonanol	143-08-8	CCCCCCCCCO		C9H20O
1-Decanol	1-Decanol	112-30-1	C(O)CCCCCCCCC		C10H22O
1-Undecanol	1-Undecanol	112-42-5	CCCCCCCCCCCO		C11H24O
1-Dodecanol	1-Dodecanol	112-53-8	CCCCCCCCCCCCO		C12H26O
1-Tridecanol	1-Tridecanol	112-70-9	CCCCCCCCCCCCCO		C13H28O

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

	Molecular Weight [1]	Log Kow[1a]	Vapour Pressure (Pa, 25 deg C) [1b]	Density [2] (g/cm^3)	Melting Point (deg C) [1b]	Water Solubility (mg/L, 25 deg C) [1c]	Boiling Point (deg C) [1b]	pKa [3]
1-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
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
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
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
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
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
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
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
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

	Key Substituent(s)	Functional Group(s)	Extended Fragment(s)	Chemical Class:	Chemical Sub-Class:
1-Pentanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Hexanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Heptanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Octanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Nonanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Decanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Undecanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Dodecanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain
1-Tridecanol	-OH	$-CH_3$, $-CH_2-$	–	saturated aliphatic alcohols	straight-chain

Table 4: Comparison of Abiotic Transformation and Toxicokinetics

Name	Abiotic Transformation	Toxicokinetics			
		Absorption	Bioavailability	half-life	Elimination
1-Pentanol					
1-Hexanol	Phototransformation in air - half-life: 30.8 hrs[c]				Rabbit:10.3% as hexyl glucuronide[e]
1-Heptanol	Phototransformation in air - half-life: 28.1[c]				Rabbit: 5.3% as heptyl glucuronide[e]
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]
1-Nonanol					Rabbit: 4.1% as nonyl glucuronide[e]
1-Decanol					Rabbit: 3.5% as decyl glucuronide[e]
1-Undecanol					
1-Dodecanol					
1-Tridecanol					

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Table 5: Comparison of Potential Metabolic Products

	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
	Rat liver S9	**Skin metabolism**			
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)
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)
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)

	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
	Rat liver S9	**Skin metabolism**			
1-Octanol	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)
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)
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)

	Liver metabolism simulator Toolbox v3.3		MetaPrint2D-React software	SMARTCyp version 2.4.2	Meteor Nexus
	Rat liver S9	**Skin metabolism**			
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)
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)
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

	Toxicophores[1]	DNA binding by OECD[1]	Protein binding by OECD[1]	Nuclear receptor binding[2]	Liver& Mitochondria toxicity[2]
1-Pentanol	Cramer Class I	No alert	No alert	Inactive	No alert
1-Hexanol	Cramer Class I	No alert	No alert	Inactive	No alert
1-Heptanol	Cramer Class I	No alert	No alert	Inactive	No alert
1-Octanol	Cramer Class I	No alert	No alert	Inactive	No alert
1-Nonanol	Cramer Class I	No alert	No alert	Inactive	No alert
1-Decanol	Cramer Class I	No alert	No alert	Inactive	No alert
1-Undecanol	Cramer Class I	No alert	No alert	Inactive	No alert
1-Dodecanol	Cramer Class I	No alert	No alert	Inactive	No alert
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

	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-Pentanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
1-Hexanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			CNS depression
1-Heptanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
1-Octanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			CNS depression biphasic effect on the ATPase activity
1-Nonanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
1-Decanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			biphasic effect on the ATPase activity

	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-Undecanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			
1-Dodecanol		narcosis - depressant anesthesia	unspecific interactions with biological membranes			stimulation of the ATPase activity
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) [40]				2000 (mg/kg bw/d) [9]	2000 (mg/kg bw/d) [11]	
Endpoint: NOEL (Repeat dose toxicity)	$\geq$ 6400 (mg/m^3) [2]			1300 (mg/kg bw/d) [4,60]			<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]	
Endpoint: LOEL (Repeat dose toxicity)								100-2000 (mg/kg/d) [13]	

Name	1-Pentanol	1-Hexanol	1-Heptanol	1-Octanol	1-Nonanol	1-Decanol	1-Undecanol	1-Dodecanol	1-Tridecanol
Endpoint: NOAEL (Reproductive toxicity)	1000 (mg/kg/d [1]								
Endpoint: NOAEL (Teratogenicity)		370-1240 (mg/kg/d) [3]		1300 (mg/kg/d) [17]					
Endpoint: NOAEC (Teratogenicity)	14 (mg/L air) [16]	3.5 (mg/L air) [3]				>100 (mg/L air) [63]			
Endpoint: LOAEL (Maternal toxicity)				130 (mg/kg/d) [18]		130 (mg/kg/d) [63]			
Endpoint: NOAEC (Maternal toxicity)				>0.4 (mg/L) [17]					
Endpoint: Carcinogenic/ Genotoxicity	1 X Negative [67]	5 x Negative [3]		2 X Negative [17]			1 X Negative [9]	7X Negative 1x Positive [20-26]	

Name	1-Pentanol	1-Hexanol	1-Heptanol	1-Octanol	1-Nonanol	1-Decanol	1-Undecanol	1-Dodecanol	1-Tridecanol
Endpoint: LC50 (Acute toxicity)		>21 (mg/L air) >21 (mg/ L/hour) >5030 (mg/L air) [3, 36]					>700 (mg/m^3) [9]		
Endpoint: LD50 (Acute toxicity) From different routes of exposure	140-4585 (mg/kg/) 2.83-5.66 (mL/kg) [28, 29-32, 35,55-57, 68]	103-4870 (mg/kg) [3, 37-39]	500-6200 (mg/kg) [38, 40-42, 69]	1790 - ≥5000 (mg/kg) [17,43,44]	800-6400 (mg/kg) 44 (mmol/kg) 5660 (uL/kg) [45,47,56, 61,62]	1000-5000 mg/kg [63, 70]	3000-> 15800 (mg/kg) [9, 64, 71]	1500->26530 (mg/kg/d) >12.8 - > 36 (ml/kg) [11,48,65,66]	5600-17200 (mg/kg) [49]
Endpoint: LDLo (Acute toxicity)	122-2000 (mg/kg) [33,34,59]								
Endpoint: Genotoxicity (AMES, Chromosomal abrration, gene mutation)	2 x Negative [53,59]	1 x Negative [3]	1 x Negative [40]	1 x Negative [51]		2 x Negative [27]			
Toxcast overview [54]	-	250 (1 active)	250 (10 active)	602 (0 active)	150 (4 active)	257 (15 active)	602 (25 active)	602 (3 active)	602 (30 active)

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