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A CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR SUB-CHRONIC REPEATED-DOSE TOXICITY OF SIMPLE ARYL ALCOHOL ALKYL CARBOXYLIC ESTERS: READ-ACROSS

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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 2017 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team. The document was endorsed at the 2nd meeting of the Working Party on Hazard Assessment in June 2018.

The following three case studies were also reviewed in the project in 2017 and are published with this case study:

1. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR ESTROGENICITY OF THE SUBSTITUTED, ENV/JM/MONO(2018)26, Series on Testing & Assessment No. 290.
2. PRIORITISATION OF CHEMICALS USING THE INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA)-BASED ECOLOGICAL RISK CLASSIFICATION, ENV/JM/MONO(2018)27, Series on Testing & Assessment No. 291.
3. CASE STUDY ON GROUPING AND READ-ACROSS FOR NANOMATERIALS GENOTOXICITY OF NANO-TiO_2, ENV/JM/MONO(2018)28, Series on Testing & Assessment No. 293.

These case studies are illustrative examples, and their publication as OECD monographs does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries.

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) -Third Review Cycle (2017)- ENV/JM/MONO(2018)25, Series on Testing & Assessment No. 289.

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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INTRODUCTION

Integrated Approaches for Testing and Assessment (IATA) have the potential to clear untested chemicals of regulatory concern without the need for additional *in vivo* testing (Organisation for Economic Co-operation and Development; OECD, 2015). Rat sub-chronic repeated-dose toxicity is a health-related endpoint that is typically data poor and thereby a target for IATA approaches to data gap filling. Cosmetic ingredients include a large number of carboxylic acid esters (Richarz et al., 2015; see COSIng: https://ec.europa.eu/growth/sectors/cosmetics/cosing_en). Since in Europe, cosmetic ingredients are legislatively prohibited by the European Commission (EC) from *in vivo* testing (EC, 2009), alternative methods are needed to conduct safety assessments of these esters. One such assessment tool is category formation and read-across (Cronin et al., 2013). The issue of ester hydrolysis was addressed as part of the allyl ester case study presented and discussed as part of the first OECD workshop on IATA (OECD, 2016a). In this study, we follow up on this toxicokinetic-driven exercise and demonstrate how other, but not all, esters may be grouped and cleared from further testing.

Cosmetic-related carboxylic acid esters vary markedly in structure. They include: 1) simple aliphatic esters such as alkyl acetates and other alkyl alkanoates, either straight-chain or branched, 2) cyclic esters such as cyclohexane ethyl acetates and, 3) alkyl aryl esters. While the alcohol moiety of cosmetics-related esters varies in its general structure, the acid group is typically a saturated C5 of less moiety or a larger linear analogue. Cosmetically-related primary aryl alcohols include benzyl-, 2-phenethyl- and 3-phenpropyl-derivatives. The phenyl ring may be unsubstituted or substituted with small alkyl groups, typically at the para-position or the ortho- and para-positions.

Since the turn of the century, the International Joint FAO/WHO Expert Committee on Food Additives (JECFA) have published a series of safety evaluations of selected esters (e.g., JECFA, 1998, 1999, 2001, 2008a, 2008b); specifically, several benzyl- and 2-phenethyl-derivatives have been evaluated as food additives (JECFA, 2001, 2008a, respectively). More recently, Belsito et al. (2012) assessed the toxicity of aryl alkyl esters. It is well-accepted that the initial step in *in vivo* detoxification of carboxylic acid esters is the rapid and nearly complete hydrolysis to their corresponding alcohol and carboxylic acid (Heymann, 1982). This hydrolysis is very efficiently catalysed by carboxylic ester hydrolases (EC 3.1.1). These ubiquitous enzymes exhibit broad and overlapping substrate specificity toward esters and amides, and the same substrate is often hydrolysed by more than one enzyme (Heymann, 1980). It is well known that esterase activity varies considerably between species (Satoh et al., 1998). For aliphatic esters, the rate of hydrolysis usually decreases in the species order rat > rabbit > dog > human.

Linear and short-chain branched carboxylic acids, in particular saturated ones, feed into common physiological pathways like the citric acid cycle, sugar synthesis and lipid synthesis, and exhibit very low toxicity in sub-chronic repeated-dose toxicity experiments. For example, in a 90-day dietary study conducted on groups of 20 Sprague Dawley rats per sex per dose group treated with 0 or 0.62%, 1.25%, 2.5%, or 5% propionic acid (i.e., equal to or approximately 0, 312, 625, 1,250 or 2,500 mg/kg bw/d),

the NOAEL for local and systemic effects was 1,250 and 2,500 mg/kg bw/d for males and females, respectively. In contrast, the repeated-dose toxicity of alcohols varies markedly with structure (Schultz et al., 2017a, 2017b). The net result is the repeated-dose toxicity of esters is typically determined by the alcohol moiety.

Since read-across exercises of repeated-dose toxicity of n-alkanols, 2-alkyl-1-alkanols have recently been conducted (Schultz et al., 2017a, 2017b), respectively, these predictions can likely be extended to simple carboxylic acid esters of n-alkanols and 2-alkyl-1-alkanols. However, similar exercises for simple aryl alkyl alcohols and esters have yet to be conducted.

Simple aryl alkyl alcohols and their corresponding carboxylic acid esters are chemicals of importance to the cosmetics industry; for example, several benzyl alkanoates and 2-phenethyl alkanoates are fragrance materials (Belsito et al., 2012). While there is extensive mammalian acute toxicity data for short-chain (C5 or less) benzyl alkanoates and 2-phenethyl alkanoates, repeated-dose toxicity data is more limited (Belsito et al., 2012).

Simple aryl alkyl carboxylic acid esters (aryl alkanoates) exhibit a common route of primary metabolism– hydrolysis by carboxylesterases and the subsequent formation of the simple acid and the corresponding aryl alkyl alcohol (Heymann, 1980). This fact, combined with availability of sub-chronic repeated-dose toxicity data for benzyl alcohol and 2-phenethyl alcohol, as well as most carboxylic acid derivatives, makes them excellent candidates for filling data-gaps of simple aryl alkyl carboxylic acid esters by read-across.

The principle of a toxicological read-across is that substances that are similar in molecular structure will exhibit similar chemical properties, and thereby, they will exhibit similar toxicokinetic and toxicodynamic properties. Thus, experimentally-derived toxicokinetic and toxicodynamic information and data from a source substance, can be read across to fill the data gap of a target substance which is similar. This read-across exercise is consistent with Scenario # 3– a category approach of the European Chemical Agency (ECHA) read-across assessment framework or RAAF (ECHA, 2016). Specifically, the read-across hypothesis is based on compounds with different structures where biotransformation leads to common metabolites. This case study follows the template used for the 2016 case studies (OECD, 2017).

While this study focuses on selected aryl alkyl carboxylic acid esters, it is designed to be an example for how data gaps for other esters (e.g., methyl alkanoates) can be filled by read-across. Key to this illustration is how considerations of simple and well-documented metabolism of category members leads to the same metabolite (i.e., an aryl alcohol) which is the determinate of repeated-dose toxicity and highly similar metabolites (i.e., carboxylic acids) which play little or no role in determining repeated-dose toxicity.

The aim of this investigation is to examine the sub-chronic repeated-dose toxicity of three subcategories of similar aryl alkanoates by the use of read-across. Benzyl alkanoates from C2 (benzyl acetate) to C12 (benzyl dodecanoate) were selected as the initial category members. While *in vivo* toxicokinetic and repeated-dose toxicity data exists for benzyl acetate, a literature search reveals such data is lacking for the remaining category members. However, toxicokinetic and repeated-dose toxicity data is available for the common metabolite benzyl alcohol, as well as many carboxylic acid derivatives. Subsequently, the study was expanded to include the 2-phenethyl alkanoate

subcategory– C2 (2-phenethyl acetate) to C12 (2-phenethyl dodecanoate). In the latter subcategory, *in vivo* toxicodynamic data exists for 2-phenethyl acetate and *in vivo* toxicokinetic and repeated-dose toxicity data for 2-phenethyl alcohol. Lastly, the study was further expanded to include the 3-phenpropyl alkanoate subcategory– C2 to C12 where *in vivo* toxicokinetic and repeated-dose toxicity data are neither available for any ester nor 3-phenpropyl alcohol. As revealed in Table 1, the experimental weight-of evidence (WoE) varies between the three subcategories. Therefore, the uncertainty associated with the read-across predictions between the three sub-categories varies– benzyl alkanoates < 2-phenethyl alkanoates < 3-phenpropyl alkanoates.

Table 1. Summary of definitive toxicants, similarities and data.

Subcategory	Definitive Toxicant	Similarities Toxicokinetic	Toxicodynamic	
Benzyl alkanoates			experimental	experimental
	Benzyl alcohol	experimental	experimental	
2-Phenethyl alkanoates			read across	experimental
	2-Phenethyl alcohol		experimental	experimental
3-Phenpropyl alkanoates			read across	read across
	3-Phenpropyl alcohol		read across	read across

The following read-across abides by the guidance provided by OECD on the reporting of defined approached used within IATA (OECD, 2016b, 2017).

1. PURPOSE

1.1. Purpose of use

The purpose of this IATA is to fill data gaps with sufficient confidence in the predictions that may be used by member countries in risk assessment or other regulatory processes. In addition, the read-across strategy presented in this study has been developed with the expectation of trying to pass the ECHA RAAF, save for the assessment element of impurities (ECHA, 2016). Impurity information is often proprietary and provided by the manufacture.

1.2. Target chemical(s)/category definition

The category for this read-across exercise is defined as the C2 to C12 benzyl alkanoates (Table 1a of the Annex), C2 to C12 2-phenethyl alkanoates (Table 1b of the Annex) and C2 to C12 3-phenpropyl alkanoates (Table 1c of the Annex). In this category approach, the common features of the category members are common hydrolysis products, either benzyl alcohol (Table 1a of the Annex), 2-phenethyl alcohol (Table 1b of the Annex) or 3-phenpropyl alcohol (Table 1c of the Annex). The common boundaries are the C2 to C12 range of the saturated carboxylic acids hydrolysis products (Table 1d of the Annex).

1.3. Endpoint

This category approach read-across is applied to sub-chronic repeated-dose toxicity. It is based on experimental data availability from protocols equivalent to or similar to OECD TG 408 (Subchronic Oral Toxicity: 90-Day Study), OECD TG 411 (Subchronic Dermal Toxicity: 90-Day Study) or an appropriate chronic test.

2. HYPOTHESIS FOR THE CATEGORY APPROACH

It is hypothesised that read-across can be performed, within the category, ester to ester, as well as the common alcohol metabolite. Specifically, hydrolysis by carboxylesterases and the subsequent formation of the simple saturated C2 to C12 carboxylic acids and either benzyl alcohol, 2-phenethyl alcohol or 3-phenpropyl alcohol.

It is further hypothesised that benzyl alcohol, 2-phenethyl alcohol or 3-phenpropyl alcohol, respectively, are the primary determinates of repeated-dose toxicity of each sub-category.

It is further hypothesised that these alcohols have highly similar toxicokinetic (i.e., absorption, distribution, metabolism and excretion), toxicodynamic (e.g., mode/mechanism of action or adverse outcome pathways) and chemical/biological interaction information.

It is also further hypothesised that the simple saturated carboxylic acids have highly similar toxicokinetic and toxicodynamic profiles with low local toxicity and no systemic toxicity of any consequence.

While the aryl alkanoate category members have varied physical-chemical properties and other molecular properties, it is hypothesised these properties are not toxicologically relevant.

The route of expected exposure is either dermal or oral. The duration is 90-days or more. The mode of toxic action of the source substances (primary aryl alcohols) is nonpolar narcosis where the alcohols act via unspecific, reversible interactions with biological membranes in a manner similar to depressant anaesthetics.

Thus, data gaps for repeated-dose toxicity of benzyl alkanoates may be filled with acceptable uncertainty for risk assessment by read-across from benzyl acetate and benzyl alcohol with increased WoE from 2-phenethyl alcohol.

Thus, data gaps for repeated-dose toxicity of 2-phenethyl alkanoates may be filled with acceptable uncertainty for risk assessment by read-across from 2-phenethyl alcohol with increased WoE from benzyl acetate and benzyl alcohol.

Additionally, data gaps for repeated-dose toxicity of 3-phenpropyl alkanoates may be filled with acceptable uncertainty for prioritisation and screening but not risk assessment by read-across from benzyl acetate, benzyl alcohol and 2-phenethyl alcohol.

This research demonstrates how read-across predictions of the repeated-dose toxicity no observed adverse effect level (NOAEL) value based on a consistent set of lowest observed adverse effect level (LOAEL) symptoms could be performed and substantiated for a category of selected aryl alkanoate analogues. Specifically, metabolism gives rise to one of three common definitive toxicants. It is shown that the category-based experimental data provides information which reduce uncertainties, and collectively adds *in vivo* WoE associated with read-across predictions of the specified endpoint. In the case of the of benzyl alkanoates and the 2-phenethyl alkanoates, the estimations from the read-across are quantitative and with sufficiently low uncertainty that they may be used in risk assessments. As such, the predicted 90-day repeated-dose NOAEL values are accompanied by sufficient relevant *in vivo* test data to make the uncertainties equal

to what would be expected from running a test using a protocol similar to OECD TG 408 or TG 409.

There are several lines of evidence that supports the contention that all the analogues within the domain act in a similar manner, that is, simple anaesthesia or non-polar narcosis. Specifically, while there is no mammalian adverse outcome pathway for the hypothesised mode of action, it is generally accepted that the toxicity of saturated esters is based on their saturated alcohol metabolite and thereby is the result of narcosis (Schultz et al., 2017a). There are both theoretical and biochemical evidence for the cell membrane being the site of action for anaesthetic-like chemicals such as saturated alcohols (McCreery and Hunt, 1978; Fang et al., 1997; McKarns et al., 1997). These findings are supported by animal data (Munch, 1972; McKim et al., 1987; Veith et al., 2009; Koleva, et al., 2011). 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). While the exact mechanism is yet to be elucidated, it is the accumulation of alcohols in cell membranes which disturbs cellular functions.

McCloskey et al. (1986) reported an attempt to alter benzyl alcohol's toxicity by using pyrazole and disulfiram to inhibit the activities of alcohol dehydrogenase and aldehyde dehydrogenase, respectively. Treatment with pyrazole, before benzyl alcohol exposure, resulted in an increase in benzyl alcohol levels to 203% of controls concomitant with a marked increase in toxicity. Although pre-treatment with disulfiram led to benzaldehyde levels which were 368% of controls, toxicity was unchanged. These data imply that the acute toxicity of benzyl alcohol, which includes sedation, dyspnoea and loss of motor function is due to the alcohol itself and not to its metabolite, benzaldehyde (McCloskey et al., 1986).

While and AOP for narcosis is under development (http://www.oecd.org/chemicalsafety/testing/projects-adverse-outcome-pathways.htm) no pathway has been evaluated. However, the basic premise of such a pathway (i.e., partitioning of nonpolar narcotic compounds into cellular membranes leading to decreased physiological performance) is consistent with the mode of action proposed in case studies on read-across data gap filling of saturated alcohols discussed in review cycle 2016. ToxCast data shows the aryl alkanoates and corresponding alcohols are a very innocuous group of chemicals.

3. SOURCE CHEMICALS/CATEGORY MEMBERS

3.1. Identification and selection of source chemicals/category members

Based on the hypothesis described in Section 2, the source chemicals identified are, benzyl acetate, benzyl alcohol and 2-phenethyl alcohol. Based on the well accepted metabolic pathway of simple hydrolysis leading to a common toxicant, the category members are either benzyl alkanoates, 2-phenethyl alkanoates or 3-phenpropyl alkanoates with saturated carboxylic acid moieties of C2 to C12. The category members are compounds, which are commonly found in a governmental or industrial inventory (e.g., OECD High Production Volume Chemicals). Since *in vivo* testing of such compound is banned in some geographic regions, read-across was proposed for data gap filling of the sub-chronic repeated-dose endpoint for quantitative risk assessment. As there were only limited possibilities, there was no bias in selecting the source substances.

3.2. List of source chemicals/ category members

The source chemicals and category members along with common chemical identifiers (i.e., name, CAS number, SMILES notation and chemical structure) are presented for each subcategory in Tables 1a - 1c of the Annex. In addition, in Table 1d of the Annex, similar information is presented for the relevant saturated carboxylic acid metabolites.

4. JUSTIFICATION OF DATA GAP FILLING

4.1. Data gathering

In vivo data for target and source chemicals used in the assessment were taken from the literature, including ECHA REACH Registered Substances database1 and Research Institute for Fragrance Materials (RIFM) safety assessment monographs and group summaries.

[1]European Chemicals Agency (ECHA) Registered substances. Available from: https://echa.europa.eu/information-on-chemicals/registered-substances.

4.2. Data matrix

The data, on which this investigation is based, are summarised in Tables 4 - 5 of the Annex.

4.3. Justification

Based on the data matrices in Section 4, the hypothesis described in Section 2 is supported as follows.

4.3.1. Similarities and Differences in Chemistry

As demonstrated in Tables 1a - 1c, respectively, of the Annex, all the benzyl alkanoates, 2-phenethyl alkanoates and 3-phenpropyl alkanoates included in the category are structurally similar. Specifically, they: 1) belong to a common chemical class, esters, and a common subclass, aryl alkane esters, and 2) possess similar molecular scaffoldings of benzyl, 2-phenethyl or 3-phenpropyl esters of a carboxylic acid. Outlined in Tables 3a – 3d, all the parent category esters have common structural constituents in the form of: 1) a key polar substituent, -OC(=O)-, and 2) structural fragments, C_6H_5-, -CH_3, -CH_2- and -CH-. Structurally, the main variable is the length of the carboxylic acid backbone, C2 to C12.

The physico-chemical properties of the benzyl alkanoates, 2-phenethyl alkanoates and 3-phenpropyl alkanoates are reported in Tables 2a - 2c, respectively, of the Annex. Many of these property values are determined experimentally and calculated values based on these measured values can be used with high confidence. Properties, with the exception of density, trend in value related to C-atom number and branching within the scaffold.

As demonstrated in Tables 1d and 3d of the Annex, all the aliphatic carboxylic acids, which are metabolites of the alkanoates included in the category are structurally similar. Specifically, they: 1) belong to a common chemical class, carboxylic acids, and a common subclass, alkane acids, and 2) possess similar molecular scaffoldings of common structural constituents in the form of: 1) a key polar substituent, HOC(=O)-, and 2) structural fragments, C_6H_5-, -CH_3, -CH_2- and -CH-. Structurally, the main variable is the length of the hydrocarbon backbone, C1 to C11.

The physico-chemical properties of the aliphatic carboxylic acid metabolites are reported in Table 2d of the Annex. Many of these property values are determined

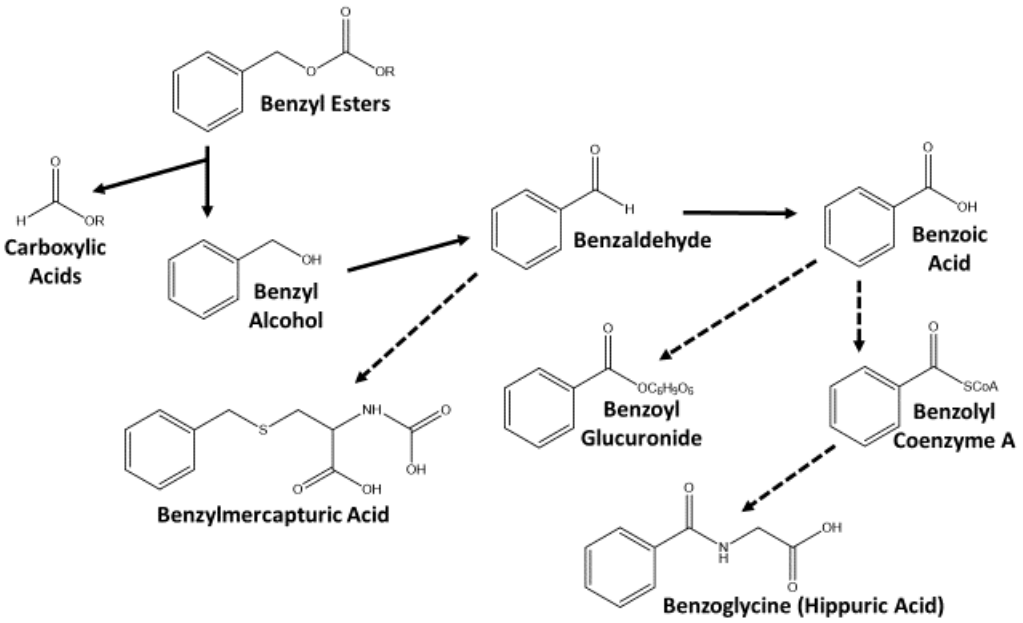
experimentally and calculated values based on these measured values can be used with high confidence. Properties, with the exception of density, trend in value related to C-atom number and branching within the scaffold.

4.3.2. In vitro and in vivo toxicokinetic similarity

Available experimental toxicokinetic data is summarised in Table 4 of the Annex. Briefly, the toxicokinetics of aryl alkanoates are highly similar. Such esters are rapidly absorbed, distributed via the blood, metabolised and excreted. As compared to dermal exposure, esters are more extensively absorbed from the gut. In the latter case toxicokinetic potency is great via gavage than from dosed feed administration. The results of studies of ester hydrolysis *in vitro* indicate that hydrolysis is a universal metabolic step (Leegwater and van Straten, 1974; Longland et al., 1977). The rate of hydrolysis of straight-chain esters is approximately 100 times greater than that of branched-chain esters (Drake et al., 1975). Select isoenzymes exhibit an increase in enzyme binding (lower K_m) and maximum velocity (V_{max}) as the carbon chain length of either the alcohol or carboxylic acid component of the substrate increases (Heymann, 1980).

Benzyl esters are hydrolysed *in vivo* to benzyl alcohol and corresponding carboxylic acids (Williams, 1959; JCFA, 2001). For example, benzyl acetate is hydrolysed rapidly to benzyl alcohol and acetic acid (Heymann, 1980; Yuan et al., 1995). Benzyl alcohol is rapidly oxidised to benzaldehyde and subsequently to benzoic acid (Williams, 1959). Benzoic acid is readily conjugated with glucuronic acid or glycine, primarily in the liver, and excreted in the urine as conjugates (Bridges et al., 1970; JCFA, 2001). At high doses, where glycine is depleted, free benzoic acid may sequester acetyl coenzyme A or be excreted as the glucuronic acid conjugate or unchanged (Bray et al., 1951; Abdo et al., 1998). The metabolic pathway for benzyl esters is shown in (Figure 1).

Figure 1 Metabolism of benzyl esters.



Solid arrows – Phase 1 oxidisation; Dashed arrows – Phase 2 conjugation.

Phenethyl esters are rapidly hydrolysed *in vivo* to yield phenethyl alcohol and corresponding carboxylic acids (Williams, 1959; JCFA, 2008a). Phenethyl alcohol is oxidised to phenylacetic acid, which is conjugated and excreted primarily in the urine (Williams, 1959; James et al., 1972). Specifically, phenethyl alcohol is readily oxidised to phenylacetaldehyde by an assortment of NAD^+-dependent alcohol and aldehyde dehydrogenases (Bosron and Li, 1980). The greatest activity of mammalian alcohol dehydrogenases (ALDH) occurs in the liver, where they show broad substrate specificity for the oxidation of primary aliphatic and aromatic alcohols. Human liver ALDH showed a decreased Michaelis-Menten constant (K_m, the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity with increasing lipophilicity); however, the maximum rate or velocity of an enzymatic reaction which is indicative of all the enzyme active site(s) complexed with substrate, V_{max}, remained essentially constant, suggesting that the rate-limiting step does not involve the binding or release of the alcohol or aldehyde intermediate (Pietruszko et al., 1973). Longland et al. (1977) studied the *in vitro* potential for hydrolysis of 16 esters including 2-phenethyl acetate. Ester hydrolysis by artificial gastric and pancreatic juices followed first-order kinetics. The ester hydrolysis rate constant and time to effect 50% hydrolysis showed a relatively slow rate of hydrolysis by artificial gastric juice and a faster reaction in artificial pancreatic juice. Hydrolysis in rat liver and small intestinal tissue preparations also followed first order rate kinetics. The tissue rates (liver tissue > intestinal mucosal tissue) showed esters are hydrolysed more readily in tissues than artificial pancreatic juices. The overall results show relatively rapid hydrolysis of phenethyl acetate by artificial gastro-intestinal juices and by liver and intestinal tissues and confirms the indications of rapid metabolic detoxification in the liver following

repeat exposure and also confirms the lack of bioaccumulation potential. Thus, the metabolic pathway for phenethyl esters is similar to benzyl esters (see Figure 1).

4.3.2.1. Benzyl acetate

Chidgey and Caldwell, (1986) report plasma pharmacokinetics and metabolism of [methylene-^{14}C]benzyl acetate in the rat. This study was conducted in two phases-kinetics and metabolite analysis. In the kinetics phase, benzyl acetate the animals were treated for 24 hours. In the excretion phase, the animals were analysed only after three days of exposure.

Briefly, a minimum of three animals/treatment group received [methylene-^{14}C]benzyl acetate by gavage at doses of 5, 250 or 500 mg/kg bw, as neat substance, in corn oil or in propylene glycol. Urine and faeces were collected and urinary metabolites were assayed by radio-TLC and HPLC. Other animals were killed at various times and exsanguinated, plasma levels of ^{14}C in plasma occurred earliest and were highest when benzyl acetate was given neat. Peak levels were lowered and absorption was delayed with the propylene glycol vehicle. The use of corn oil as the dose vehicle at the higher doses (250 and 500 mg/kg) led to the maintenance of plateau plasma levels, at about ½ of the peak levels seen with the neat compound, for up to 8 hours after administration. At 5 mg/kg bw the plasma levels of ^{14}C were essentially the same whether the dose was given in corn oil or propylene glycol. At 250 and 500 mg/kg bw, at all time points, the major metabolite in plasma was benzoic acid with smaller amounts of hippuric acid. Benzyl alcohol was also detected in some plasma samples. At 5 mg/kg bw the major plasma metabolite was hippuric acid together with a smaller amount of benzoic acid. When propylene glycol was used as the vehicle at this dose level, benzylmercapturic acid was also present in the plasma. The major urinary metabolite was hippuric acid (≈ 66% of the dose), with benzoic acid (2%) and benzylmercapturic acid (1%) also present. The elimination of benzoyl glucuronide increased with increasing dose, from ≈ 3 to 11% of the dose.

Chidgey et al. (1987) and Caldwell et al. (1987) report studies of the percutaneous absorption, distribution and metabolism of benzyl acetate. Briefly, [methylene-^{14}C]benzyl acetate was applied over an area of 6.25, 12 or 18 cm^2 to the shaved backs of three male Fischer 344 rats/treatment group under an occlusive dressing at dose levels of 100, 250 and 500 mg/kg. The compound was administered either as the neat substance or as a 50% (v/v) solution in ethanol. After six-hours the dressing was removed, the shaven area was washed with ethanol and the dressing and washings were counted for ^{14}C. Urine and faeces were collected for 72 hours from the start of treatment and urinary metabolites were assayed by radio-TLC and HPLC.

Following administration of the neat compound, 28-48% of the dose was recovered from the application site. Similarly, 28-46% was absorbed and excreted in the urine during the first 24 hours. Excretion of ^{14}C in the urine over 0-24 hours accounted for ≈ 95% of absorbed ^{14}C with <4% of the dose present in the carcass at the end of the experiments. The total recovery of radioactivity was 79-84% at the end of 72 hours.

The extent of absorption of benzyl acetate per unit area of skin, as assessed by the recovery of its metabolites in urine, rose with increasing concentration (mg/cm^2) of the test compound on the skin. The absorption of topically applied benzyl acetate was essentially the same when the dose was administered in a 50% ethanol solution. In all cases, the major urinary metabolite (≈95%) was hippuric acid; other metabolites

included much smaller amounts of benzoyl glucuronide, benzoic acid and benzylmercapturic acid.

McMahon et al. (1989) examined the *in vivo* metabolism and excretion of benzyl acetate in male rats and C57BL/6N mice. Briefly, rats aged 3-4, 9 and 25 months received a single oral dose of either 5 or 500 mg/kg bw [^{14}C]benzyl acetate, while mice aged 2, 13 and 25 months received single oral dose of 10 mg/kg bw ^{14}C-benzyl acetate. Urine and faeces were collected for 96 hours. Biliary excretion and plasma elimination were examined in rats after iv admin of 5 mg/kg [^{14}C]benzyl acetate. In both young and old rats and mice, hippuric acid was the major urinary metabolite after oral dosing. No significant age-related difference was observed in rats in urinary elimination of benzyl acetate-derived radioactivity or % of total dose excreted as hippuric acid (≈95%). Twenty-five-month-old rats excreted a significantly higher % of total dose as benzyl mercapturic acid (2%) than 3-to-4-month-old rats (1%) at 5 mg dose. Benzyl mercapturic acid excretion in 3-to-4-month-old rats also increased significantly at 500 versus 5 mg/kg bw benzyl acetate. Faecal excretion of benzyl acetate-derived radioactivity declined significantly in 25-month-old rats at both doses. This decrease was reflected by an age-related decline in biliary excretion and higher plasma levels of radioactivity. Exam of plasma metabolites revealed a significantly higher level of hippuric acid and benzoyl glucuronide in 25-month rats. In 25-month-old mice, a significant decrease in 24-hour urinary excretion of benzyl acetate-derived radioactivity was observed relative to 2- and 13-month-old mice, whereas faecal excretion was highest in this age group. Results indicate changes in minor routes of metabolism and excretion of benzyl acetate occur with age, but formation of hippuric acid from benzyl acetate is not affected by aging.

Yuan et al. (1995) examined the effects of gavage versus dosed feed administration on the toxicokinetics of benzyl acetate. Studied were conducted in male F344 rats and B6C3F1 mice with plasma concentrations of benzoic acid, benzyl alcohol and hippuric acid being determined. Briefly, 6 rats and 12 mice/dose, and 10 rats and mice/dose were treated by oral gavage oral and feed, respectively. For the oral gavage treated group samples were taken at 5, 10, 20 and 40 minutes and 1, 1.5, 2, 3, 3.7, 6, 9 and 24 hours after administration with one animal per time point. For the oral feed treated group blood samples were taken on day 7 at 18:00, 23:00, 05:30 (next day), 07:30 (next day) and 09:00 (next day) with two animals/species being bled at each time point.

Benzyl acetate was rapidly hydrolysed to benzyl alcohol and then oxidised to benzoic acid. After gavage administration of benzyl acetate in corn oil at 500 mg/kg bw (rats) and 1,000 mg/kg bw (mice), high benzoic acid plasma concentrations were observed. In contrast, much lower benzoic acid plasma concentrations were found after dosed feed administration at (10,800 ppm for rats and 2,700 ppm for mice (≈ 615 mg/kg bw/d for rats and ≈ 850 mg/kg bw/d for mice). Results show that although the daily doses of benzyl acetate are comparable, bolus gavage administration effectively saturated the benzoic acid elimination pathway whereas, dosed feed administration did not. In contrast, hippuric acid plasma concentrations were similar after both gavage and dosed feed administration due to the depletion of the glycine supply pool.

Regardless of route of exposure, the metabolism of benzyl acetate proceeds by hydrolysis to benzyl alcohol, the bulk of which is oxidised to benzoic acid before undergoing conjugation to yield hippuric acid or glucuronide. The various metabolic pathways appear to be solely involved in the detoxification of benzyl acetate.

4.3.2.2. Benzyl alcohol

Toxicokinetic of benzyl alcohol is well-studied https://echa.europa.eu/registration-dossier/-/registered-dossier/14748/7/2/2/?documentUUID=33018239-1a4c-4b40-8cb7-c8076364e2db. Benzyl esters, benzyl alcohol, and benzoic acid considered as a single category regarding human health, as they are all rapidly metabolised and excreted via a common pathway within 24 hours. A comprehensive document providing discussion and justification for the category read-across is attached to the cited study record in the IUCLID database. In a Cosmetic Ingredient Review (CRI, 2001), it was reported that benzyl alcohol is metabolised to benzoic acid, which reacts with glycine and excreted as hippuric acid in the human body.

Humans, rabbits and rats readily oxidise benzyl alcohol to benzoic acid, which subsequently conjugated with glycine prior to being rapidly eliminated as hippuric acid in the urine (Williams, 1959). Within six hours after the oral administration of 0.40 g benzyl alcohol/kg bw, rabbits eliminated 65.7% of the dose as hippuric acid in the urine (Diack and Lewis, 1928). Metabolites identified in the urine of rabbits given an oral dose of 0.25 g/kg benzyl alcohol are chiefly glycine conjugate (74%) and glucosiduronic acid (14%) (Bray et al., 1958).

The metabolism of benzyl alcohol is oxidised to benzoic acid before undergoing conjugation to yield hippuric acid or glucuronide. This metabolic pathway appears to be solely involved in the detoxification of benzyl alcohol.

4.3.2.3. 2-Phenethyl acetate

While there is *in vitro* evidence for 2-phenethyl acetate hydrolysis, there is no *in vivo* evidence.

4.3.2.4. 2-Phenethyl alcohol

Toxicokinetic of 2-phenethyl alcohol is well-studied (https://echa.europa.eu/registration-dossier/-/registered-dossier/13615/7/2/2). The ECHA dossier reports a number of toxicokinetic studies. One study described a comparison of the kinetics of 2-phenylacetic acid and 2-phenethyl alcohol in the CD-1 rat following single dose administration via dermal application, oral gavage or in the diet. Peak plasma concentration of unchanged 2-phenethyl alcohol was reached within 0.5 hour, on the average, following dermal application and oral administration (gavage) of 0.43 ml/kg 2-phenethyl alcohol. Plasma concentrations of 2-phenethyl alcohol were higher after the dermal route compared to oral administration. 2-Phenethyl alcohol is eliminated by oxidative metabolism to the correspondent acid (2-phenylacetic acid), which is subsequently eliminated by the following parallel processes; renal excretion of unchanged phenylacetic acid, conjugation with glycine and formation of a glucuronic acid conjugate and subsequent renal elimination.

Following oral administration (gavage or ad libitum ingestion) of phenethyl alcohol, approximately 70% of the administered dose is eliminated via the renal route as phenylacetic acid or its conjugates within 24 hours. The lower proportion eliminated in the urine following dermal application is most probably related to a lower extent of percutaneous absorption relative to the oral route. After a single dermal application of 0.43 ml 2-phenethyl alcohol/kg, the mean concentration of 2-phenylacetic acid in 0-24-hour urine was 2.77 mg/ml and the mean amount of 2-phenylacetic acid excreted was 28.5 mg. After a single oral dose of 0.43 ml 2-phenethyl alcohol/kg, the mean

concentration of 2-phenylacetic acid in 0-24-hour urine was 73.5 mg. After ad libitum oral administration of 0.43 ml 2-phenethyl alcohol/kg, the mean concentration of 2-phenylacetic acid in 0-24 hour urine was 4.67 mg/ml and the mean amount of 2-phenylacetic acid excreted was 42.0 mg.

Following a single oral dose of 0.43 ml/kg 2-phenethyl alcohol by gavage, plasma concentrations area under the curve (AUC) of 2-phenylacetic acid were markedly higher than those observed after a similar oral dose of phenethyl alcohol ingested over 24 hours in the diet. It appears that metabolic clearance of 2-phenylacetic acid is reduced at higher plasma concentration due to capacity- limited metabolism in an analogues manner to that described for salicylic acid; that is the elimination of 2-phenylacetic following oral and dermal doses appears to be characterised by both zero-order and first-order kinetics. The differences in AUC values of 2-phenylacetic acid following equal dose of 2-phenethyl alcohol as oral gavage or in the diet is most probably due to a slower input of 2-phenethyl alcohol following the diet; this results in a lower 2-phenylacetic plasma concentration profile and higher average clearance.

Following increasing dermal doses of 0.43, 0.7 and 1.4 ml/kg 2-phenethyl alcohol, AUCs of both unchanged drug and 2-phenylacetic acid tended to increase proportionally with dose and the terminal half-life of 2-phenethyl alcohol remained at about 5 hours, irrespective of the applied dose. It was concluded that 2-phenethyl alcohol is rapidly absorbed after oral and ad libitum route administration. The absorption rate by dermal administration is much lower due to loss in dressing and probably to evaporation.

In another study, after a single dermal dose application of 2-phenethyl alcohol at dosage level of 0.14 and 0.7 ml/kg the peak plasma concentration was 520 µg/ml after 4 hours and declined rapidly to 0.29 µg/ml at 24 hours after dosing. The rates of 2-phenylacetic acid concentration declined and appeared similar after application of 2-phenethyl alcohol at each dosage level. Therefore, the origin of the non-linearity may be dose-dependent dermal absorption of 2-phenethyl alcohol or dose-dependent formation of 2-phenylacetic acid from absorbed precursor.

In the latter study, 76 female rats were treated with a single dermal dose of 2-phenethyl alcohol at dosage level of 0.14 and 0.7 ml/kg. Gas chromatography/mass spectrometry techniques were used to determine the concentrations of 2-phenylacetic acid (i.e., the major metabolite of the alcohol), which is eliminated in urine as glycine and glucuronic acid conjugates. The peak plasma concentration of 520 µg/ml was reached after 4 hours and declined rapidly to 0.29 µg/ml at 24 hours after dosing. At concentration level of 1.4 ml 2-phenethyl alcohol, the peak plasma concentration was 671 µg/ml at 4 hours and declined rapidly to 0.48 µg/ml at 24 hours after dosing. After application of 2-phenethyl alcohol at levels of 1.4 ml/kg and 0.7 ml/kg, in a ratio of 2.0, the mean peak plasma 2-phenylacetic acid concentration ratio was 1.3, the man AUC ratio was 1.6 and the mean 24-hour post-dose plasma concentration ratio was 1.7. These ratios appear to be lower than those required for linearity of response with dosage level of parent compound. The rates of 2-phenylacetic acid concentration decline appeared similar after application of 2-phenethyl alcohol at each dosage level. Therefore, the origin of the non-linearity may be dose-dependent dermal absorption of 2-phenethyl alcohol or dose-dependent formation of 2-phenylacetic acid from absorbed precursor.

In a third study, the results obtained indicated that 2-phenethyl alcohol was rapidity absorbed and excreted by the rat after dermal application. The oxidative biotransformation primarily led to 2-phenylacetic acid, most of which was conjugated as 2-phenaceturic acid.

The absorption and disposition of 2-phenethyl alcohol was studied in female CD rats after single and repeated dermal application of the ^{14}C-compound at dose levels of 0.14 and 0.7 ml/kg. The tissue distribution of radioactivity was studied in male Long-Evans rats after single and repeated dermal application of the ^{14}C-compound at dose level of 0.14 ml/kg. After single dermal doses of [^{14}C]2-phenethyl alcohol to female CD rats at dose level of 0.14 ml/kg means of 80.7 and 1.3% dose were excreted in urine and faeces, respectively. After a single dermal dose of [^{14}C]2-phenethyl alcohol to female CD rats at dose level of 0.7 ml/kg, means of 39.0 and 0.4% dose were excreted in urine and faeces during 120 hours. A mean total of 44.4% was recovered from excreta, tissues, skin and dressings. The results indicated that at dose level of 0.7 ml/kg, the absorption of 2-phenethyl alcohol is not so rapid as for 0.14 ml/kg. After five repeated dermal doses of [^{14}C]2-phenethyl alcohol to female CD rats at dose level of 0.14 ml/kg, means of 68.6 and 1.1% of the cumulative dose were recovered from urine and faeces, respectively, during 216 hours after the first dose. Results indicated that the compound is rapidly absorbed and eliminated during 0-24 hours. After five repeated dermal doses of [^{14}C[2-phenethyl alcohol to female CD rats at dose level of 0.7 ml/kg, means of 40.2 and 0.6% of the cumulative dose were recovered from urine and faeces, respectively, during 216 hours after the first dose. Means of 24.2 and 1.2% dose were recovered from skin and dressing, with a total recovery of 68.4% dose, compared to 44.4% after a single dose, meaning that a high proportion was lost due to evaporation. The repeated exposure partially prevented the evaporation issue because the skin was covered for longer time. Radioactivity compound concentrations were detected in the bladder, the eye-related Harderian gland and at the application site. Lower levels were found in the small intestine content, kidney, caecal contents, liver, blood, lung, uterus and nasal mucosa, intestine contents, liver, blood, lung, jawbone, ovary, uterus, eye and fur and mammary tissue. Radioactivity was detected in the foetuses of pregnant rats sacrificed at peak plasma concentration after ten doses at both dose levels. The major metabolite corresponded to 2-phenaceturic acid and accounted for about 80% of the urinary radioactivity. Some urinary radioactivity corresponded to hippuric acid and 2-phenylacetic acid; very little corresponded to 2-phenethyl alcohol.

Lastly the toxicokinetics of 2-phenethyl alcohol was examined in rabbits. 2-Phenethyl alcohol was rapidly and extensively absorbed, biotransformated and eliminated. Half the dose was recovered at both dose levels, mainly from the urine. The remaining dose was lost probably due to evaporation. The initial stage in the biotransformation of 2-phenethyl alcohol in rabbits was similar to that in humans and rats in which the alcohol was oxidised to 2-phenylacetic acid. The biotransformation of 2-phenylacetic acid in the rabbit followed the same pathway as that in the rat.

The absorption and disposition of 2-phenethyl alcohol was studied in female New Zealand White rabbits after topical application of the 14C-compound at dose level of 0.14 and 0.7 ml/kg. Excretion data, plasma concentration data and metabolic data indicated that phenethyl alcohol is rapidly and extensively absorbed, biotransformated and eliminated. Approximately half dose was recovered at both dose levels, mainly from the urine. Most of the remaining dose was lost probably due to evaporation. The initial stage in the biotransformation of 2-phenethyl alcohol in rabbits was similar to that in humans and rats in which the alcohol was oxidised to phenylacetic acid. The biotransformation of 2-phenylacetic acid in the rabbit followed the same pathway as that in the rat where the acid was conjugated with glycine forming 2-phenaceturic acid, rather than that in humans where the acid was conjugated with L-glutamine. In rabbits, as in rats, traces of hippuric acid and conjugated 2-phenethyl alcohol were detected.

Politano et al. (2013) compared the dermal absorption, plasma pharmacokinetics, and excretion of 2-phenethyl alcohol by pregnant and nonpregnant rats, rabbits, and humans. Following dermal (430, 700, or 1,400 mg/kg bw), gavage (430 mg/kg bw), or dietary (430 mg/kg bw) administration of 2-phenethyl alcohol to rats, plasma concentrations of the alcohol were found to be low regardless of the route of administration. The plasma concentrations of the major metabolite, phenylacetic acid, exceeded the concentrations of the parent alcohol and were highest after gavage, followed by dermal then dietary administration.

Absorption, distribution, metabolism, and excretion were compared following topical application of ^{14}C-labeled 2-phenethyl alcohol to rats, rabbits, and humans (specific activities of dosing solutions: 58-580, 164, and 50 µCi/ml, respectively). In rabbits, the plasma concentration–time profile for phenylacetic acid was markedly prolonged compared to rats or humans. In humans, only 7.6% of the applied dose of 2-phenethyl alcohol was absorbed, versus 77% in rats and 50% in rabbits.

2-Phenethyl alcohol and its metabolite 2-phenylacetic acid were rapidly absorbed and excreted. Following oral and dermal administration, approximately 70% and 27%, of the administrated doses was eliminated in urine as 2-phenylacetic acid and its conjugates, respectively. The absorption rate by dermal administration was much lower due to probably to evaporation and loss in dressing. The metabolic clearance is seemed to be reduced at higher plasma concentration due to capacity-limited elimination processes.

Regardless of route of exposure, the metabolism of 2-phenethyl alcohol is oxidised to 2-phenethyl acid before undergoing Phase 2 conjugation to yield hippuric acid or glucuronide derivatives. This metabolic pathway appears to be solely involved in the detoxification of 2-phenethyl alcohol.

In final summary, ester hydrolysis is a universal metabolic step. While the experimental hydrolysis data reveals variations in half-life and % hydrolysed after 2 hours, these differences and not considers to be critical to repeated-dose toxicity. The rate of hydrolysis of straight-chain esters is approximately 100 times greater than that of branched-chain esters and enzyme binding (lower K_m) and maximum velocity (V_{max}) increases with C-chain length of either the alcohol or carboxylic acid component. The ester hydrolysis of acetate derivative may be taken as a worse possible scenario for the three series of esters examined here.

4.3.2.5. Carboxylic acids

Carboxylic or fatty acids are typically unbranched, organic acids with different chain length. The absorption, distribution, metabolism and elimination of these acids have been well-studied (Dawson et al., 1964; Stokke, 1969; Nelson and Cox, 2008). Due to the role as nutritional energy source, carboxylic acids are absorbed from the lumen of the intestine by different uptake mechanisms depending on the chain length. Short- and medium-chain fatty acids (C1 to C12) are rapidly absorbed via intestine capillaries into the blood stream. In contrast, due to physico-chemical properties such as melting temperature, solubility and polarity, fatty acids are in general poorly absorbed through skin.

Carboxylic acids are absorbed through the intestine and transported throughout the body. Short-chain acids are taken up and transported complexed to albumin via the portal vein into the blood vessels supplying the liver. Medium-chain acids are esterified

with glycerol to triacylglycerides and packaged in chylomicrons. The most significant oxidation pathway of carboxylic acids is the β-oxidation pathway. Briefly, the acids are converted to acyl-CoA derivatives (aliphaticacyl-CoA) and transported into cells and mitochondria by specific transport systems. Then, the acyl-CoA derivatives are completely metabolised to acetyl-CoA or other key metabolites by the efficient enzymatic removal of the 2-carbon units from the aliphatic acyl-CoA molecule. The complete oxidation of carboxylic acids via the citric acid cycle leads to H_2O and CO_2. Other pathways for acid catabolism include α- and ω-oxidation (Wanders et al., 2010). Carboxylic acids are not expected to be excreted to any significant amount in the urine or faeces under normal physiological conditions.

Saturated carboxylic acids are rapidly metabolised by endogenous pathways to acetic acid or ketone bodies. Subsequently, metabolites are introduced into other endogenous metabolic cycles (e.g., tricarboxylic acid cycle) or excreted. For example, Medes et al. (1945) report results from *in vitro* experiments where [1^{13}C]butyric acid was incubated with slices of rat liver from fasted rats for two hours at pH 7.3 and 37.5°C. Evaluating the relative amount and distribution of label, two mechanisms for the formation of ketone bodies from butyric acid could be established (cleavage by ß-oxidation and recombination to ketone body or direct ß-oxidation and formation of ketone body with conservation of the C4 structure). In two experiments, butyric acid was utilised to 51 and 32%, respectively. Respiratory CO_2 amounted to 13.5 and 12.3%. Acetoacetic acid (35.7 and 45.8%) and hydroxybutyric acid (11.7 and 9.3%) were demonstrated to be the major metabolites of butyric acid.

The saturated branched-chain aliphatic acids formed via ester hydrolysis are endogenous in humans as intermediary products in the metabolism of amino acids (Voet and Voet, 1990; JCFA, 1998). Short ($\leq C_6$) branched-chain acids undergo ß-oxidation. ß-Cleavage of the resulting acid yields linear acid fragments which are sources of carbon in the fatty acid pathway or tricarboxylic acid cycle (Voet and Voet, 1990). The principal metabolic pathways utilised by branched-chain acids are determined primarily by the position of the methyl substituent. Acids with a methyl substituent located at an even-numbered carbon are extensively metabolised to CO_2 via ß-oxidation and cleavage in the fatty acid pathway and (Michal, 1999). If the methyl group is located at the 3-position, ß-oxidation is inhibited and alpha-oxidation predominates, primarily leading to short-chain acid fragments capable of being completely metabolised (Williams, 1959)

For example, DiVicenzo and Hamilton (1979) investigated the metabolic fate of isobutyric acid following a single oral (gavage) administration. Briefly, groups of four male Charles River CD rats at doses of 4, 40, and 400 mg/kg bw and to four female CD rats at 400 mg/kg bw were administered radiolabelled [1-^{14}C]isobutyric acid. Isobutyric acid was readily absorbed after oral application as demonstrated by the fast excretion in expired air and peak plasma levels after 0.5 to 1 hour. Peak concentrations of isobutyric acid in plasma were 11.4 ± 2.4 µg/ml. Plasma levels decrease to 3.3 µg/ml at 2 hours. By four hours, plasma isobutyric acid was below the limit of detection. In the first four hours after dosing, 67 to 83% of the administered dose was excreted as CO_2 in expired air. Unchanged isobutyric acid was less than 0.1%. The recovery of radioactivity in the breath at 48 hours was 90.1, 96.7, and 90.8% for male rats dosed with 4, 40, and 400 mg/kg, respectively and 86.2% for female rats dosed with 400 mg/kg. Radioactivity excreted in urine over the first hours ranged from 3.21 to 4.61%, while faecal radioactivity was less than 1.0% of the dose. The excretion by female rats was similar to that of male rats. These studies show that isobutyric acid is rapidly metabolised to CO_2.

In Summary, carboxylic acids are almost completely absorbed after oral intake, whereas only limited dermal uptake has to be expected. The major metabolic pathway for linear fatty acids is the β-oxidation pathway for energy generation, while alternatives are the α- and ω-oxidation.

4.3.3. In silico toxicokinetic similarity

All the esters and alcohols include in this study are highly similar in regards to *in silico* toxicokinetics. Based on metabolism simulators in the OECD Toolbox v3.4, rat liver S9 and skin metabolism, all the derivatives are considered to be readily metabolised with no reactive intermediates. Similar predictions were observed with TIME SS, MetaPrint2D-React, SMARTCyp version 2.4.2, and Meteor softwares.

4.3.4. In vivo toxicodynamic similarity

Available experimental toxicodynamic data is summarised in Table 5 of Annex 1. Briefly, based on the results from the sub-chronic and chronic gavage, diet administered or dermal studies, the toxicodynamics of aryl alkyl carboxylic acid esters and their corresponding aryl alcohol are highly similar. LOAEL effects including final mean body weight and clinical signs differences at higher dose/concentrations. Depending on species, sex, exposure scheme and derivative the reported NOAEL values vary from 500 to 100 mg/kg bw/d.

4.3.4.1. Benzyl acetate

A National Toxicology Program study (NTP, 1986), reported the results of 13-week gavage repeated-dose toxicity study of benzyl acetate in both rats and mice. Briefly, F344 rats (10/sex/dose) were administered 0, 62.5, 125, 250, 500, or 1,000 mg/kg bw/d benzyl acetate in corn oil for 5 days a week. The dose range was selected from a prior 14-day gavage study at doses of 0, 250, 500, 1,000, 2,000, 4,000 mg/kg bw/d where mortality was observed at the two highest doses. Briefly, rats were routine observed for mortality, body weight, clinical signs, necropsy and histopathology was conducted on controls, the highest dose group animals and all animals that died before scheduled termination.

In the subchronic study, two male and one female in the highest dosed group died before the end of the study. The final mean body weight in male rats receiving 1,000 mg/kg was about 12% lower than the control group. The only clinical signs attributed to compound administration were observed in male and female rats receiving 1,000 mg/kg and in females receiving 500 mg/kg. These signs included trembling, ataxia and sluggishness. Thickened stomach walls were also observed in 2/9 males and 4/10 females in the highest dosed group. No compound-related histopathologic effects were observed. The NOAEL for subchronic toxicity of benzyl acetate in rats was determined to be 500 mg/kg bw/d and 250 mg/kg bw/d for male and female respectively, based on observed clinical signs of tremor and ataxia among rats treated with benzyl acetate over a period of 13 weeks (NTP, 1986).

In a 13-week gavage GLP study, 10 B6C3F1 mice/sex/dose were administered test material, benzyl acetate 5 days a week in doses of 0, 62.5, 125, 250, 500, or 1,000 mg/kg bw/d for male mice and 0, 125, 250, 500, 1,000, and 2,000 mg/kg bw/d for female mice using corn oil as the vehicle (NTP, 1986). Routine observations (twice daily) for mortality, body weight, clinical signs and necropsy were conducted on controls, the highest dose group animals and all animals dying before termination. The dose range

was selected from a previous 14-day gavage study (0, 125, 250, 500, 1,000 or 2,000 mg/kg bw/d), based on the observed mortality, clinical observations (ataxia, laboured breathing and hyperactivity) and altered stomach morphology (roughened stomach) at the highest dose levels (2,000 mg/kg).

During the 13-week period, mortality (8/10) was reported among the female animals of the highest dose group. Compound-related clinical signs observed in high-dose mice included trembling, inactivity, laboured breathing and lower body temperature among high dose group animals. No compound-related gross or microscopic pathologic effects were observed among the treated groups. The oral gavage NOAEL for mouse subchronic repeated dose toxicity of benzyl acetate was determined to be 500 mg/kg bw/d based on mortality and clinical signs of tremor and inactivity of the 13-week study (NTP, 1986).

Because the NTP was sceptical on the pancreatic tumours observed in the gavage studies, possibly related to administration in corn oil vehicle, the NTP conducted two 2-year carcinogenicity studies on benzyl acetate. One via gavage and one via diet (NTP, 1986, 1993, respectively).

During the gavage 2-year chronic study, 50 F344 rats/sex/dose were administered, benzyl acetate in corn oil, at doses of 0, 250 or 500 mg/kg bw/d (NTP, 1986). There was no significant difference in survival among treated rats. Acinar-cell hyperplasia of the pancreas was observed in all groups of male rats (control: 37/50; low-dose 34/50; high-dose: 36/49), while acinar-cell adenomas occurred with a positive trend with a significant increase of incidence (control: 22/50; low-dose: 27/50; high-dose group 37/49). No acinar-cell hyperplasia or adenoma of the pancreas was observed in female rats.

There was an increase in the incidence of preputial gland tumours in males and the incidence of subcutaneous fibromas was reported among low dose males. However, these incidences were not considered due to test material administration since the combined incidence of adenomas, adenocarcinomas or carcinomas were not increased.

Overall, the exposure to benzyl acetate showed an increased incidence of acinar-cell adenomas of the exocrine pancreas in male rats only, due to the administration of high levels of fat (corn oil vehicle) to experimental animals which have been shown to enhance the development of spontaneous and chemical-induced neoplasms.

Based on the results obtained from the 13-week study, doses of 0, 500 and 1000 mg/kg/day in corn oil were selected for the 2-year chronic study, where groups of 50 B6C3F1 mice/sex/dose were administered benzyl acetate via gavage (NTP, 1986). Findings included the following. There was an increase in survival among the high dose female mice (30/50) as compared to controls (15/50). There were increased incidences of hepatocellular adenomas (males: 0/50, 5/49, 13/50; females: 0/50, 0/50, 6/50) which only reached statistical significance at the highest dose. There was no increase in the incidence of carcinomas among treated mice as compared to controls. Squamous cell neoplasms of the forestomach (male: 4/49, 4/48, 11/49; female: 0/50, 0/50, 4/48) were also reported. Forestomach hyperplasia occurred at increased incidences in dosed mice of either sex (males: 1/49, 7/48, 22/49; females: 1/50, 6/50, 17/48). These neoplasms and hyperplasia of the forestomach were possibly related to the administration of benzyl acetate.

McGinty et al. (2012) reported a toxicologic and dermatologic review of benzyl acetate when used as a fragrance ingredient which agrees with the above.

In summary, the 90-day repeated-dose toxicity of benzyl acetate in rodents is in the 250 - 500 mg/kg bw/d based on clinical signs of tremor and inactivity.

4.3.4.2. Benzyl alcohol

OECD concluded: "Benzyl alcohol, benzoic acid and its sodium and potassium salt can be considered as a single category regarding human health, as they are all rapidly metabolised and excreted via a common pathway within 24 hours (OECD, 2001). In a Cosmetic Ingredient Review (CRI, 2001), it was reported that: 1) the acceptable daily intake for benzyl alcohol established by the World Health Organization is 5 mg/kg bw/d, 2) benzyl alcohol is generally recognised as safe in foods according to the U.S. Food and Drug Administration, and, 3) no adverse effects of benzyl alcohol were seen in chronic exposure animal studies using rats and mice. A toxicologic and dermatologic review of benzyl alcohol when used as a fragrance ingredient is presented by Scognamiglio and co-workers (2012a).

In a 13-week subchronic repeated-dose test F344/N rats (10/sex/dose) received once daily on 5 days/week 0, 50, 100, 200, 400, 800 mg/kg bw/d benzyl alcohol via oral gavage (NTP, 1989). Observations included mortality, body weight, clinical signs, necropsy and selected histopathology. Eight of 10 male rats dosed at 800 mg/kg/d died during weeks 7 and 8; four of these deaths were described as gavage related. Rats dosed with 800 mg/kg/d exhibited clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Haemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. No notable changes in body weight gain or benzyl alcohol-related histopathological lesions were observed in rats for the lower dose groups. Based on clinical signs and reduced body weight development in males and females and histopathological changes in the brain at 800 mg/kg bw/d the NOAEL value of benzyl alcohol was considered to be 400 mg/kg bw/d (NTP, 1989).

In a study equivalent to OECD TG 451 (supervised by NTP) 50 male and female F344/N rats/dose group received daily by gavage 0, 200 and 400 mg/kg bw/d benzyl alcohol diluted in corn oil for 104 weeks (5 days/week) (NTP, 1989). No effect on body weight gain and no compound-related clinical signs were observed throughout the study. Survival was reduced only for female rats, but in many cases deaths were attributed the gavage procedure. Gross necropsy and histopathology revealed no apparent compound-related non-neoplastic responses. Thus, the NOAEL can be considered to be 400 mg/kg bw/d (NTP, 1989).

In a 13-week dose-finding study male and female B6C3F1 mice received benzyl alcohol once daily for 5 days/week at doses of 0, 50, 100, 200, 400, 800 mg/kg bw/d via gavage (NTP, 1989). Observations included mortality, body weight, clinical signs, etc. In mice, deaths were scattered among all dose groups, but none occurred in vehicle controls. Four male and six female mice died after being dosed; all deaths, but one, were described as gavage related. Staggering after dosing also occurred the first 2 weeks of the studies in mice dosed at 800 mg/kg bw/d. Based on clinical signs and reduced body weight development in both sexes the NOAEL value of benzyl alcohol in mice was reported to be 200 mg/kg bw/d (NTP, 1989).

In a study equivalent to OECD TG 451 (supervised by NTP) male and female B6C3F1 mice received daily by gavage 0, 100 and 200 mg/kg bw/d benzyl alcohol diluted in corn oil for 104 weeks (5 days/week) (NTP, 1989). No effect on body weight gain and no compound-related clinical signs were observed throughout the study. Survival was

not affects by benzyl alcohol administration. Gross necropsy and histopathology revealed no apparent compound-related non-neoplastic responses. Thus, 200 mg/kg bw/d was considered the NOAEL value for benzyl alcohol (NTP, 1989).

In summary, clinical signs indicative of neurotoxicity along with reductions in relative weight gain were observed at 800 mg/kg bw/d in both sexes of both species. Moreover, there were reductions in relative weight gain in female rats dosed with 200, and 400, mg/kg bw/d, in male mice dosed with 400 mg/kg bw/d, and in female mice dosed with 200 and 400 mg/kg bw/d. No notable changes in body weight gain nor compound related histopathologic lesions were observed in rats or mice from the lower dose groups. While In three of the repeated dose toxicity studies described above NOAELs of 400, 200 and 200 mg/kg bw/d were reported, to be consistent with the worst possible scenario, the oral gavage NOAEL of benzyl alcohol for both rats and mice was determined to be 200 mg/kg bw/d in males and 100 mg/kg bw/d in females.

4.3.4.3. 2-Phenethyl acetate

No sub-chronic repeated dose toxicity studies of 2-phenethyl acetate were found. Rather in the ECHA registration dossier (https://echa.europa.eu/registration-dossier/-/registered-dossier/5534) the data gap is filled by read-across from benzyl acetate.

4.3.4.4. 2-Phenethyl alcohol

The repeated-dose toxicity findings of 2-phenethyl alcohol have been reported by Owston et al. (1981), CIR (1990) and Scognamiglio et al. (2012b). Owston et al. (1981) conducted a study following a protocol similar to the OECD TG 411. Briefly, 2-phenethyl alcohol was administered at 0, 0.25, 0.5, 1.0 and 2.0 ml/kg bw/d (≈ 250, 500, 1,000, and 2,000 mg/kg bw/d) for 90 days in open application to shaved dorsa of Sprague Dawley rats (15/sex/dose). No attempt was made to prevent inadvertent ingestion by grooming. The animals were observed for daily changes in appearance and behaviour. Body weight and food consumption parameters were measured weekly. Ophthalmic examinations (funduscopic and bio-microscopic) were performed on the eyes of all animals before and after treatment at week 13. Haematology (week 6 and 13) and biochemical analysis (week 13) was conducted on blood samples. Urinalysis was performed on week 6 and 13. The animals were then euthanised on week 13 and subjected to gross necropsy analysis. Histopathological examinations were performed on adrenals, brain, heart, kidney, liver, lung and bronchi, mesenteric lymph nodes, pituitary, sternum, spinal cord, testes with epididymis, ovaries, spleen, urinary bladder and nerve with muscles from all control and high dose group animals.

Significant decreases in body weight gain and body weights were reported for both sexes at the two highest dose levels, although there was no effect on food consumption. Survival rate was unaffected, and ophthalmological examinations were unremarkable. Males dosed at 2.0 ml/kg bw/d had decreased haemoglobin and white blood cell counts at 6 and 13 weeks. Relative liver weights were increased in females at all doses, but there were no differences in absolute values, so the increase in relative liver weight was attributed to the lower body weights. Both absolute and relative liver weights were reduced in the 1.00 ml/kg bw/d males but not in the 2.0 ml/kg bw/d males, and thus, were not considered toxicologically significant. Significant increases ($P<0.05$) in relative brain, kidneys, and gonads (males) weights were reported for both sexes of rats at the 2.0 ml/kg dose level. Histopathological examination of tissues taken from high-

dose and control animals did not show any treatment-related differences between the two groups.

In summary, based on reduction in body weight and body weight gains in the two highest dose groups, the dermal 90-day repeated-dose toxicity NOAEL of 2-phenethyl alcohol was considered to be 0.50 ml/kg bw/d (i.e., 500 mg/kg bw/d). It is worth noting that the NOAEL for 2-phenethyl alcohol was modified to reflect systemic dose using dermal absorption factor derived from an in vivo rat study.

4.3.4.5. Carboxylic acids

In an ECHA registration dossier (https://echa.europa.eu/registration-dossier/-/registered-dossier/14128/7/6/1), reliable repeat dose toxicity studies in rat are reported. Propionic acid was administered via the diet to groups of 20 male and 20 female Sprague Dawley rats at doses of (0, 6,200, 12,500, 25,000 and 50,000 ppm) on daily basis for a period of 90 days. After the administration interval, 10 animals of each sex from the 0, 6,200 and 50,000 ppm dose groups were maintained for an additional 42 days recovery interval to determine the reversibility of potential effects. During the administration interval, no substance related clinical signs of toxicity occurred. No substance related systemic toxicity was exhibited by the test animals. Haematological and clinical chemistry parameters of the treated animals were within physiological limits and comparable to that of the control animals. Gross pathology revealed no adverse effects. In the forestomach of the rats, histopathology revealed a dose dependent increase in the incidence and severity of proliferation-acanthosis and retention-hyperkeratosis of the forestomach mucosa was seen from the 12,500 ppm dose group and above. These effects were more distinctive in females than in males. Reversibility of these effects was noticed after the 42 day post-exposure-observation-period. Based on these results, the systemic toxicity NOAEL is >25,000 ppm (≈2000 mg/kg bw/d) propionic acid in the diet.

Amoore et al. (1978) examined sub-chronic repeated-dose toxicity of isovaleric acid. In a rat feeding study, neutralised isovaleric acid was fed to a group of five to six male SD rats for 90 days at 5% (50,000 ppm) in the diet. In a pilot study with 10%, the food intake and body weight gain were significantly reduced, as compared to control. No effects were seen in the main study for the parameters examined (i.e., food consumption, bodyweight development, organ weights, haematology, blood chemistry, urinalysis, and histopathology of 35 organs). The exception was a more basic urine and decrease in haemoglobin levels in treated rats compared to controls (pH 8.4 versus 7.2). Based on these finding, the NOAEL value for neutralised isovaleric acid is 5% in diet or 5,000 mg/kg bw/d.

Fitzhugh et al. (1960) conducted a feeding study, where dodecanoic acid was given at a concentration of 10% in the diet to 5 male Osborne-Mendel rats for 18 weeks. No clinical signs, no alteration in body weights and no mortality were noted. Moreover, no significant differences between the controls and test animals were noted in either organ weight parameters or histopathology. Based on these finding the NOAEL for dodecanoic acid is ≈10,000 mg/kg bw/d.

In summary, the no adverse effect levels for carboxylic acids following short term toxicity studies via oral route are observed to be > 2,000 mg/kg bw/d. Based on these findings no further analyses of carboxylic acids were undertaken.

4.3.5. High throughput toxicodynamic similarity

Using the USEPA Chemistry Dashboard program https://comptox.epa.gov/dashboard/, available ToxCast data was searched. ToxCast test results were found for five benzyl esters and benzyl alcohol, five 2-phenethyl esters and 2-phenethyl alcohol, as well as one 3-phenpropyl ester and 3-phenpropyl alcohol.

Of the 298 ToxCast molecular screening assays used to screen benzyl acetate an 'active hit call' was only reported for the ACEA_T47D_80hr_Positive assay of the nuclear receptor family. Of the 276 ToxCast screening assays used to screen benzyl propanoate an active hit call was only reported for the ATG_DR5_CIS_dn assay in the nuclear receptor family. Of the 276 ToxCast assays used to screen benzyl butyrate and the 163 ToxCast assays used to screen benzyl isobutyrate no active hit calls were reported. Of the 276 ToxCast molecular screening assays used to screen benzyl isovalerate active hit calls were reported for TOX21_NFkB_BLA_agonist_viability of the cell cycle family, ATG_Ahr_CIS_dn of the DNA binding family, and ATG_PXRE_CIS_up of the nuclear receptor family.

Of the 541 ToxCast screening assays used to screen benzyl alcohol an active hit Calls were reported for NVS_ENZ_oCOX2 of the oxidoreductase family, as well as ATG_PPRE_CIS_up, ATG_RXRa_TRANS_up, ATG_RXRb_TRANS_up and ATG_NURR1_TRANS_up, all of the nuclear receptor family.

Of the276 ToxCast molecular screening assays used to screen 2-phenethyl acetate and 2-phenethyl isobutyrate no active hit calls were reported. Of the 113 ToxCast screening assays used to screen 2-phenethyl propionate three active hit calls were reported-TOX21_Aromatase_Inhibition of the cyp family, TOX21_ARE_BLA_agonist_ratio of the DNA binding family and TOX21_PPARd_BLA_antagonist_ratio of the nuclear receptor family. Of the 276 ToxCast screening assays used to assess 2-phenethyl butyrate, the only active hit call was reported for TOX21_NFkB_BLA_agonist_viability of the cell cycle family. Of the 276 ToxCast assays used to screen 2-phenethyl hexanoate, active hit calls were reported for TOX21_p53_BLA_p3_ch2 (a background measurement) and ATG_Ahr_CIS_dn of the DNA binding family. Of the 296 ToxCast molecular screening assays used to assess 2-phenethyl alcohol an active hit call was reported for OT_ER_ERbERb_1440 and ATG_PPARg_TRANS_dn, both of the nuclear receptor family.

Of the 276 ToxCast molecular screening assays used to evaluate 3-phenpropyl acetate, an active hit call was reported for ATG_PXRE_CIS_up of the nuclear receptor family. Of 276 ToxCast assays used to screen 3-phenpropyl alcohol no active hit calls were reported.

In summary, based on ToxCast results the aryl alkanoates, as well as their corresponding alcohols, are an unhazardous category of chemicals. For the eleven esters and more that 3,600 results in ToxCast, an active hit is reported for only 12 assays, a rate of 0.33%. Moreover, there is no pattern in these positive responses. For the three alcohol metabolites, similar results are observed. Specifically, of the over 1,000 results in ToxCast only seven active hits are reported, a rate of 0.63%.

4.3.6. In silico toxicodynamic similarity

All the aryl alkanoates and alcohols included in this study are highly similar in regards to *in silico* toxicodynamics. Category and subcategory consistence was established using the OECD QSAR Toolbox Version 3.4. Specifically, results from the mechanistic

and endpoint profilers (data not shown) revealed none of the substances considered on this case study had alerts for any activity. Moreover, all analogues are considered to be Cramer Class I chemicals. In addition, none are classified as potential receptor binders by COSMOS (COSMOS profiler available at: http://knimewebportal.cosmostox.eu/webportal). DEREK Nexus predictions for all the category members are no specific organ toxicity (except for "irritation of gastrointestinal track" for two members).

5. STRATEGY FOR AND INTEGRATED CONCLUSION OF DATA GAP FILLING BY READ-ACROSS

5.1. Uncertainty

While earlier IATA meetings have identified the need for developing guidance on addressing uncertainties in category assessments, including read-across, no OECD guidance was found at this time. In an effort to address this deficiency, in this case study, a qualitative assessment (i.e., low medium and high) was assigned to each of several factors (see Table 2). Subsequently an overall uncertainty of the read-across prediction(s), again low medium and high, was derived. An overall uncertainty of "low" is equivalent to doing a standardised *in vivo* test (e.g., OECD TG 408 (Subchronic Oral Toxicity: 90-Day Study)). An overall uncertainty of "medium" means it is likely that the uncertainty will be similar to as doing a standardised *in vivo* test. An overall uncertainty of "high" means it is not possible to assess the uncertainty in relation to as doing a standardised *in vivo* test.

In this case study, a quantitative assessment of overall uncertainty of the read-across prediction(s) was also derived. For "low" overall uncertainty, the *in vivo* experimental NOAEL value, based on a particular LOEAL effect, was read-across as concluded. For "medium" overall uncertainty, the *in vivo* experimental NOAEL value, based on a particular LOEAL effect, was divided by two and multiplied by two to give a range of values. While it is likely that assessors will be tempted to default to the lowest values in the range, it is anticipated that as more read-across categories are developed they will provide assistance and supporting information for using other values within the range. Lastly, for "high" overall uncertainty, no quantification is to be undertaken.

As noted in Table 2, there is low uncertainty associated with each factor for the read-across. An exception may be the number of analogues used for the read-across, especially within each subcategory. However, if ester hydrolysis and alcohol toxicity is accepted as the seminal factors in this case study, then the read-across is dramatically simplified– there are only three possibilities.

Table 2. Summary of Uncertainties.

Factor	Uncertainty (low, medium, high)	Comment
Hypothesis used for the read-across	Low	Strong experimental evidence for hypothesised toxicokinetics and toxicodynamics. There is universal regulatory acceptance for clustering relevant esters, alcohols and acids together.
Structural similarity	Low	Limiting membership to simple aryl alcohol alkyl carboxylic esters limits the structural variability of the category. The structural variability (hydrocarbon chain length) is not toxicologically relevant as the definitive toxicants are either benzyl-, 2-phenethyl- or 3-phenpropyl alcohol.
Similarity of physico-chemical properties	Low	Limiting membership to simple aryl alcohol alkyl carboxylic esters mean variabilities in physico-chemical properties of the category are basically a reflection of hydrocarbon moieties. These variabilities in physico-chemical properties are not toxicologically relevant as definitive toxicants are either benzyl-, 2-phenethyl- or 3-phenpropyl alcohol.
Similarity of toxicokinetics data	Low	Universally accepted ADME processes.
Similarity of other supportive data (e.g. data related to key event)	Not applicable	
Number of analogues used for the read across	Medium	Few but adequate number of derivatives with experimental data.
Quality of the target endpoint data used for the read across	Low	High quality toxicokinetic and toxicodynamic test data.
Similarity of the target endpoint data (among source chemicals)	Low	Qualitative and quantitative agreement among the tested derivatives.
Concordance and weight of evidence of all data used for justifying the hypothesis	Low	Good agreement between and/or among experimental data for esters, alcohols and acids.
Overall uncertainty of the read-across predictions	Low for benzyl esters; Low to moderate for 2-phenethyl esters; Moderate for 3-phenpropyl esters	Key factors in assessing overall uncertainty are: 1) bio-hydrolysis to one of only three corresponding alcohol, 2) experimental ADME and *in vivo* toxicity data for benzyl acetate, benzyl alcohol, 2-phenetyl acetate and/or 2-phenethyl alcohol, 3) very low *in vivo* toxicity of n-carboxylic acids and small branched carboxylic acids, and 4) supporting *in silico* and ToxCast data.

5.2. Integrated conclusion

This study illustrates specific considerations in read-across predictions where *in silico*, *in vitro* and *in vivo* metabolism of all the analogues in the chemical category is extremely similar and plays a key role in toxicity (see italicised paragraphs in Section 4.2). The investigation also illustrates how *in vivo* data, in the form of repeated-dose toxicity derived from various protocols (i.e., routes of exposure/duration of exposure/tested species), as well as for key metabolites, may be used to reduce uncertainties (see italicised paragraphs in Section 4.3). These data also add to mechanistic plausibility and increase the WoE of the read-across arguments. *In silico* and ToxCast results the aryl alkanoates and corresponding alcohols, demonstrate these esters/alcohols are a very safe group of chemicals. Furthermore, these data enhance the mechanistic plausibility and increases the WoE to the read-across arguments.

While the single dermal repeated-dose test reported a NOAEL of 500 mg/kg bw, based on toxicokinetic and other toxicodynamic data for benzyl acetate and benzyl alcohol, a NOAEL of 200 mg/kg bw/d in males and 100 mg/kg bw/d in females, based on reduced body weight gain, can be read across to the other benzyl alkanoates within the subcategory. The overall uncertainty is equivalent to doing a standardised *in vivo* test on the other benzyl alkanoates in the subcategory. These predictions are supported by the concordance with *in vivo* data for 2-phenethyl acetate and 2-phenethyl alcohol.

Based on toxicokinetic of 2-phenethyl acetate and toxicodynamic data for 2-phenethyl alcohol, a NOAEL of 200 mg/kg bw/d in males and 100 mg/kg bw/d in females, based on reduced body weight gain, can be read across to the other 2-phenethyl alkanoates within the subcategory. The overall uncertainty is equivalent to doing a standardised *in vivo* test on the other 2-phenethyl alkanoates within the subcategory. These predictions are supported by the concordance with data for benzyl acetate and benzyl alcohol.

Based on toxicokinetic and/or toxicodynamic considerations for benzyl acetate, benzyl alcohol, 2-phenethyl acetate and 2-phenethyl alcohol, it is likely that the overall uncertainty for the 3-phenpropyl alkanoates will be similar to doing a standardised *in vivo* test. The estimated NOEAL is within 100 to 400 mg/kg bw/d in males and within 50 to 200 mg/kg bw/d in females. Taking into consideration the reasoning and data supporting the read-across predictions of 2-alkyl-1-alkanols (Schultz et al., 2017b), a NOAEL of 200 mg/kg bw/d in males and 100 mg/kg bw/d in females, based on reduced body weight gain, may be read across to the 3-phenpropyl alkanoates within the subcategory. However, the lack of *in vivo* data for any 3-phenpropyl alkanoates or the common metabolite 3-phenpropyl alcohol means these read-across predictions are not likely to be sufficient for risk assessment.

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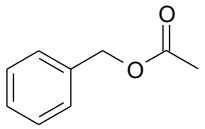
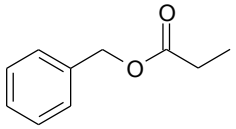
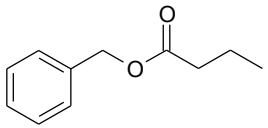
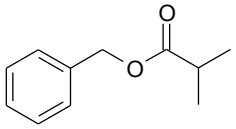
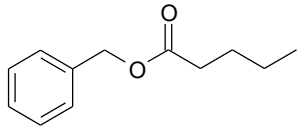
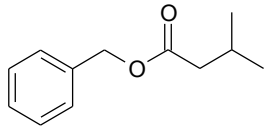
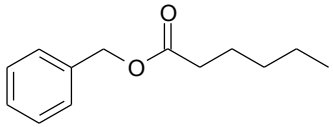
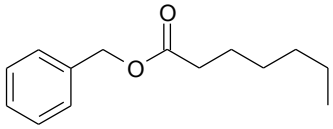
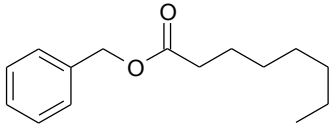
ANNEX TABLES FOR ASSESSING SIMILARITY OF ANALOGUES AND CATEGORY MEMBERS FOR READ-ACROSS

Table 1a: Comparison of Substance Identification, Structure and Chemical Classifications of Benzyl-Derivatives

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
1	Benzyl acetate	140-11-4	c1ccccc1COC(=O)C		C9H10O2
2	Benzyl propionate	122-63-4	c1ccccc1COC(=O)CC		C10H12O2
3	Benzyl butyrate	103-37-7	c1ccccc1COC(=O)CCC		C11H14O2
4	Benzyl isobutyrate	103-28-6	c1ccccc1COC(=O)C(C)C		C11H14O2

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
5	Benzyl valerate	10361-39-4	c1ccccc1COC(=O)CCCC		C12H16O2
6	Benzyl isovalerate	103-38-8	c1ccccc1COC(=O)CC(C)C		C12H16O2
7	Benzyl hexanoate	6938-45-0	c1ccccc1COC(=O)CCCCC		C13H18O2
8	Benzyl heptanoate	5454-21-7	c1ccccc1COC(=O)CCCCCC		C14H20O2
9	Benzyl octanoate	10276-85-4	c1ccccc1COC(=O)CCCCCCC		C15H22O2

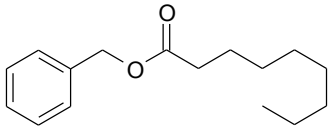
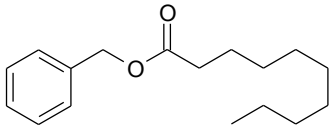
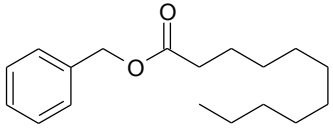
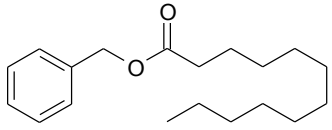
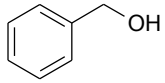
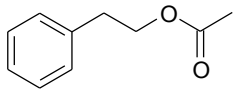
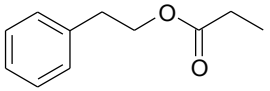
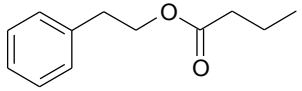
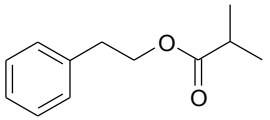
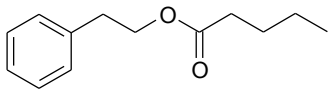
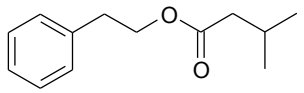
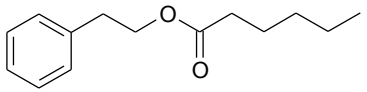
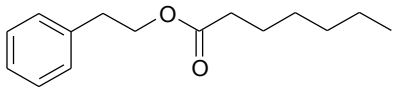
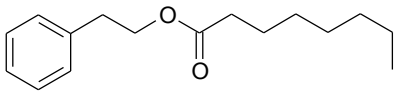
ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
10	Benzyl nonanoate	6471-66-5	c1ccccc1COC(=O)CCCCCCCC		C16H24O2
11	Benzyl decanoate	42175-41-7	c1ccccc1COC(=O)CCCCCCCCC		C17H26O2
12	Benzyl undecanoate	64273-11-6	c1ccccc1COC(=O)CCCCCCCCCC		C18H28O2
13	Benzyl dodecanoate	140-25-0	c1ccccc1COC(=O)CCCCCCCCCCC		C19H30O2
14	Benzyl alcohol	100-51-6	c1ccccc1CO		C7H8O

Table 1b: Comparison of Substance Identification, Structure and Chemical Classifications of 2-Phenethyl-Derivatives

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
1	2-Phenethyl acetate	103-45-7	c1ccccc1CCOC(=O)C		C10H12O2
2	2-Phenethyl propionate	122-70-3	c1ccccc1CCOC(=O)CC		C11H14O2
3	2-Phenethyl butyrate	103-52-6	c1ccccc1CCOC(=O)CCC		C12H16O2
4	2-Phenethyl isobutyrate	103-48-0	c1ccccc1CCOC(=O)C(C)C		C12H16O2

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
5	2-Phenethyl valerate	7460-74-4	c1ccccc1CCOC(=O)CCCC		C13H18O2
6	2-Phenethyl isovalerate	140-26-1	c1ccccc1CCOC(=O)CC(C)C		C13H18O2
7	2-Phenethyl hexanoate	6290-37-5	c1ccccc1CCOC(=O)CCCCC		C14H20O2
8	2-Phenethyl heptanoate	5454-11-5	c1ccccc1CCOC(=O)CCCCCC		C15H22O2
9	2-Phenethyl octanoate	5457-70-5	c1ccccc1CCOC(=O)CCCCCCC		C16H24O2

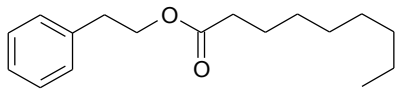
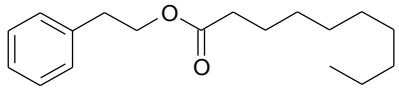
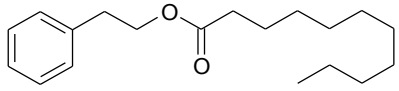
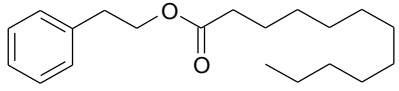
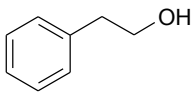
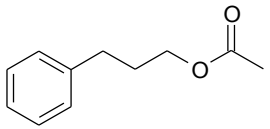
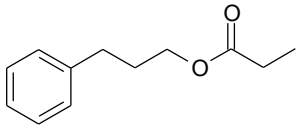
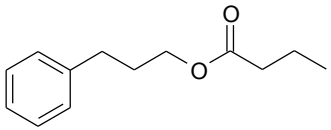
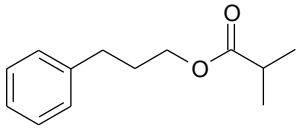
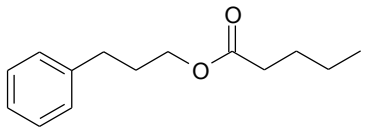
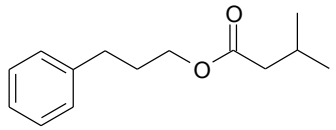
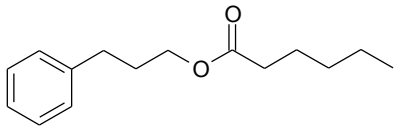
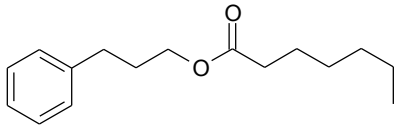
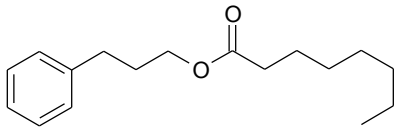
ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
10	2-Phenethyl nonanoate	57943-67-6	c1ccccc1CCOC(=O)CCCCCCCC		C17H26O2
11	2-Phenethyl decanoate	61810-55-7	c1ccccc1CCOC(=O)CCCCCCCCC		C18H28O2
12	2-Phenethyl undecanoate	112690-45-6	c1ccccc1CCOC(=O)CCCCCCCCCC		C19H30O2
13	2-Phenethyl dodecanoate	6309-54-2	c1ccccc1CCOC(=O)CCCCCCCCCCC		C20H32O2
14	2-Phenethyl alcohol	60-12-8	c1ccccc1CCO		C8H10O

Table 1c: Comparison of Substance Identification, Structure and Chemical Classifications of 3-Phenpropyl-Derivatives

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
1	3-Phenpropyl acetate	122-72-5	c1ccccc1CCCOC(=O)C		C11H14O2
2	3-Phenpropyl propionate	122-74-7	c1ccccc1CCCOC(=O)CC		C12H16O2
3	3-Phenpropyl butyrate	7402-29-1	c1ccccc1CCCOC(=O)CCC		C13H18O2
4	3-Phenpropyl isobutyrate	103-58-2	c1ccccc1CCCOC(=O)CC(C)C		C13H18O2

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
5	3-Phenpropyl valerate	5451-88-7	c1ccccc1CCCOC(=O)CCCC		C14H20O2
6	3-Phenpropyl isovalerate	5452-07-3	c1ccccc1CCCOC(=O)CC(C)C		C14H20O2
7	3-Phenpropyl hexanoate	6281-40-9	c1ccccc1CCCOC(=O)CCCCC		C15H22O2
8	3-Phenpropyl heptanoate	856084-16-7	c1ccccc1CCCOC(=O)CCCCCC		C16H24O2
9	3-Phenpropyl octanoate	68141-25-3	c1ccccc1CCCOC(=O)CCCCCCC		C17H26O2

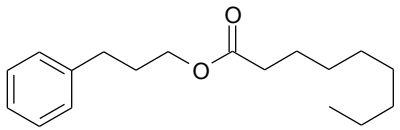
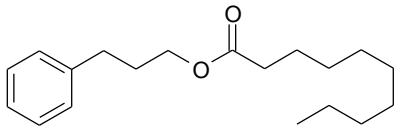
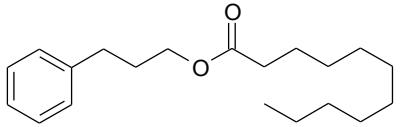
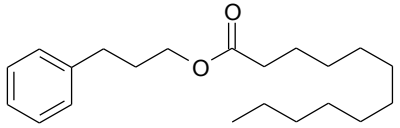
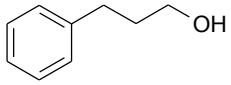
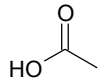
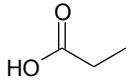
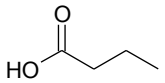
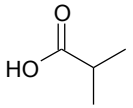
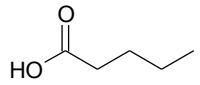
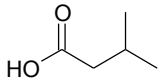
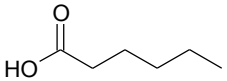
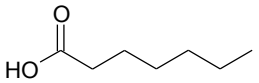
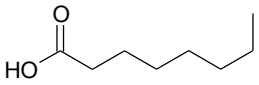
ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
10	3-Phenpropyl nonanoate	1149377-63-8	c1ccccc1CCCOC(=O)CCCCCCCC		C18H28O2
11	3-Phenpropyl decanoate	475385-55-8	c1ccccc1CCCOC(=O)CCCCCCCCC		C19H30O2
12	3-Phenpropyl undecanoate	1149377-65-0	c1ccccc1CCCOC(=O)CCCCCCCCCC		C20H32O2
13	3-Phenpropyl dodecanoate	85377-01-3	c1ccccc1CCCOC(=O)CCCCCCCCCCC		C21H34O2
14	3-Phenpropyl alcohol	122-97-4	c1ccccc1CCCO		C9H12O

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

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
1	Acetic acid	64-19-7	OC(=O)C		C2H4O2
2	Propionic acid	79-09-4	OC(=O)CC		C3H6O2
3	Butyric acid	107-92-6	OC(=O)CCC		C4H8O2
4	Isobutyric acid	79-31-2	OC(=O)C(C)C		C4H8O2

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
5	Valeric acid	109-52-4	OC(=O)CCCC		C5H10O2
6	Isovaleric acid	503-74-2	OC(=O)CC(C)C		C5H10O2
7	Hexanoic acid	142-62-1	OC(=O)CCCCC		C6H12O2
8	Heptanoic acid	111-14-8	OC(=O)CCCCCC		C7H14O2
9	Octanoic acid	124-07-2	OC(=O)CCCCCCC		C8H16O2

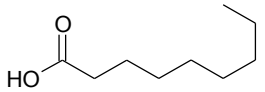
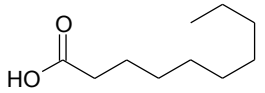
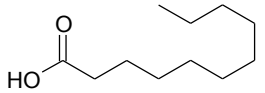
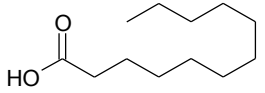
ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
10	Nonanoic acid	112-05-0	OC(=O)CCCCCCCCC		C9H18O2
11	Decanoic acid	334-48-5	OC(=O)CCCCCCCCCC		C10H20O2
12	Undecanoic acid	112-37-8	OC(=O)CCCCCCCCCCC		C11H22O2
13	Dodecanoic acid	143-07-7	OC(=O)CCCCCCCCCCCC		C12H24O2

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

ID	Name	Molecular Weight [g/mol]	Log K_{ow}	Vapor Pressure [Pa at 25°C]	Density[2] [g/cm^3 at 25°C]	Melting Point [°C]	Water Solubility [mg/L]	Boiling Point [°C]
1	Benzyl acetate	150.18	1.96	23.6	1.1±0.1	-51.2	3.1 x 10^4	213
2	Benzyl propionate	164.21	2.57	17.5	1.0±0.1	10.6	416.4	220
3	Benzyl butyrate	178.23	3.06	6.51	1.0±0.1	21.44	136	239
4	Benzyl isobutyrate	178.23	2.99	5.70	1.0±0.1	10.84	157.2	241.5
5	Benzyl valerate	192.26	3.55	1.14	1.0±0.1	32.02	44.15	269.08
6	Benzyl isovalerate	192.26	3.26	4.74	1.0±0.1	21.57	78.74	245
7	Benzyl hexanoate	206.29	4.05	0.398	1.0±0.1	42.34	14.26	285.11
8	Benzyl heptanoate	220.31	4.54	0.144	1.0±0.1	52.39	4.582	300.23
9	Benzyl octanoate	234.34	5.03	0.0542	1.0±0.1	62.17	1.467	314.45
10	Benzyl nonanoate	248.37	5.52	0.0213	1.0±0.1	71.69	0.4678	327.76
11	Benzyl decanoate	262.4	6.01	8.79 x 10^{-3}	1.0±0.1	80.95	0.1487	340.18
12	Benzyl undecanoate	276.24	6.50	3.75 x 10^{-3}	1.0±0.1	90.02	0.04716	351.91
13	Benzyl dodecanoate	290.45	6.99	8.39 x 10^{-3}	0.9±0.1	99.04	0.01491	363.52
14	Benzyl alcohol	108.14	1.10	12.5	1.0±0.1	-15.2	4.29 x 10^4	205

[1]Values typically from https://pubchem.ncbi.nlm.nih.gov or derived from EPISuite v4.1 experimental values (noted in black) where taken over predicted values (noted in purple); [c2]ACD/Lab Percepta Platform - PhysChem Module (from ChemSpider).

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

ID	Name	Molecular Weight [g/mol]	Log K_{ow}	Vapor Pressure [Pa at 25°C]	Density[2] [g/cm^3 at 25°C]	Melting Point [°C]	Water Solubility [mg/L]	Boiling Point [°C]
1	2-Phenethyl acetate	164.20	2.30	4.19	1.0±0.1	-31.1	0.711	232.6
2	2-Phenethyl propionate	178.23	3.06	6.86	1.0±0.1	21.44	0.278	238
3	2-Phenethyl butyrate	192.26	3.55	1.14	1.0±0.1	32.02	44.15	269.08
4	2-Phenethyl isobutyrate	192.26	3.48	3.63	1.0±0.1	21.57	51.02	250
5	2-Phenethyl valerate	206.29	4.05	0.398	1.0±0.1	42.34	14.26	285.11
6	2-Phenethyl isovalerate	206.29	3.97	0.907	1.0±0.1	24.45	16.47	275.55
7	2-Phenethyl hexanoate	220.31	4.54	0.144	1.0±0.1	52.39	4.582	300.23
8	2-Phenethyl heptanoate	234.34	5.03	0.0542	1.0±0.1	62.17	1.467	314.45
9	2-Phenethyl octanoate	248.37	5.52	0.0213	1.0±0.1	71.69	0.4678	327.16
10	2-Phenethyl nonanoate	262.4	6.01	8.79×10^{-3}	1.0±0.1	80.95	0.1487	340.18
11	2-Phenethyl decanoate	276.42	6.50	3.75×10^{-3}	1.0±0.1	90.02	0.04716	351.91
12	2-Phenethyl undecanoate	290.45	6.99	1.60×10^{-3}	1.0±0.1	99.04	0.01491	363.52
13	2-Phenethyl dodecanoate	304.48	7.48	6.78×10^{-4}	0.9±0.1	108.06	4.70×10^{-3}	375.12
14	2-Phenethyl alcohol	122.16	1.36	11.6	1.0±0.1	-27.1	2.22×10^{4}	218.2

[1]Values typically from https://pubchem.ncbi.nlm.nih.gov or derived from EPISuite v4.1 experimental values (noted in black) where taken over predicted values (noted in purple); [2]ACD/Lab Percepta Platform - PhysChem Module (from ChemSpider).

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

ID	Name	Molecular Weight [g/mol]	Log K_{ow}	Vapor Pressure [Pa at 25°C]	Density[2] [g/cm^3 at 25°C]	Melting Point [°C]	Water Solubility [mg/L]	Boiling Point [°C]
1	3-Phenpropyl acetate	178.23	2.85	3.24	1.0±0.1	21.44	136	252
2	3-Phenpropyl propionate	192.26	3.55	1.14	1.0±0.1	32.02	44.15	269.08
3	3-Phenpropyl butyrate	206.29	4.05	0.398	1.0±0.1	42.34	14.26	285.11
4	3-Phenpropyl isobutyrate	206.29	3.97	0.907	1.0±0.1	24.45	16.47	275.55
5	3-Phenpropyl valerate	220.31	4.54	0.144	1.0±0.1	52.39	4.582	300.23
6	3-Phenpropyl isovalerate	220.31	4.46	0.289	0.978±0.06 g/cm3 at Temp. 20 °C Press. 760 Torr	42.26	5.295	291.22
7	3-Phenpropyl hexanoate	234.34	5.03	0.0542	1.0±0.1	62.17	1.467	314.45
8	3-Phenpropyl heptanoate	248.37	5.52	0.0213	0.963±0.06 g/cm3 at Temp. 20 °C Press. 760 Torr	71.69	0.4678	327.76
9	3-Phenpropyl octanoate	262.4	6.01	8.79×10^{-3}	1.0±0.1	80.95	0.1487	340.18
10	3-Phenpropyl nonanoate	276.42	6.5	3.75×10^{-3}	0.950±0.06 g/cm3 at Temp. 20 °C Press. 760 Torr	90.02	0.04716	351.91
11	3-Phenpropyl decanoate	290.45	6.99	1.60×10^{-3}	0.945±0.06 g/cm3 at Temp. 20 °C Press. 760 Torr	99.04	0.01491	363.52
12	3-Phenpropyl undecanoate	304.48	7.48	6.78×10^{-4}	0.940±0.06 g/cm3 at Temp. 20 °C Press. 760 Torr	108.06	4.70×10^{-3}	375.12
13	3-Phenpropyl dodecanoate	318.5	7.99	2.85×10^{-4}	0.9±0.1	117.08	1.48×10^{-3}	386.73
14	3-Phenpropyl alcohol	136.19	1.88	2.65	1.0±0.1	-18.0	5.68×10^{3}	235

[1]Values typically from https://pubchem.ncbi.nlm.nih.gov or derived from EPISuite v4.1 experimental values (noted in black) where taken over predicted values (noted in purple); [2]ACD/Lab Percepta Platform - PhysChem Module (from ChemSpider).

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

ID	Name	Molecular Weight [g/mol]	Log K_{ow}	Vapor Pressure [Pa at 25°C]	Density[2] [g/cm^3 at 25°C]	Melting Point [°C]	Water Solubility [mg/L]	Boiling Point [°C]
1	Acetic acid	60.05	-0.11	2.09×10^3	1.1±0.1	16.6	1.00×10^6	117.9
2	Propionic acid	74.08	0.33	471	1.0±0.1	-20.07	1.57×10^5	141.1
3	Butyric acid	88.11	0.79	220	1.0±0.1	-5.70	6.00×10^4	163.7
4	Isobutyric acid	88.11	0.94	241	1.0±0.1	-46.0	1.67×10^5	154.4
5	Valeric acid	102.13	1.39	26.1	1.0±0.1	-34.0	2.40×10^4	186.1
6	Isovaleric acid	102.13	1.16	58.7	1.0±0.1	-29.3	4.70×10^4	176.5
7	Hexanoic acid	116.16	1.92	5.8	1.0±0.1	-3.00	1.03×10^4	205.2
8	Heptanoic acid	130.19	2.42	1.43	0.9±0.1	-7.30	2.82×10^3	222.2
9	Octanoic acid	144.22	3.05	0.495	0.9±0.1	16.3	789	239
10	Nonanoic acid	158.24	3.42	0.22	0.9±0.1	12.3	284	254.5
11	Decanoic acid	172.27	4.02	4.88×10^{-3}	0.9±0.1	31.9	61.8	268.7
12	Undecanoic acid	186.3	4.42	0.508	0.9±0.1	28.6	52.2	280
13	Dodecanoic acid	200.32	4.6	2.13×10^{-3}	0.9±0.1	43.2	4.81	298.9

[1]Values typically from https://pubchem.ncbi.nlm.nih.gov or derived from EPISuite v4.1 experimental values (noted in black) where taken over predicted values (noted in purple); [2] CD/Lab Percepta Platform - PhysChem Module (from ChemSpider).

Table 3a: Comparison of Key Structural Fragment, Functional Groups and Chemical Classes for Benzyl Derivatives

ID	Name	Key Fragment	Functional Group(s)			Chemical Classes
1	Benzyl acetate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
2	Benzyl propionate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
4	Benzyl isobutyrate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH} [CH2]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
5	Benzyl valerate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
6	Benzyl isovalerate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH] [CH2]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
7	Benzyl hexanoate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
8	Benzyl heptanoate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
9	Benzyl octanoate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
10	Benzyl nonanoate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
11	Benzyl decanoate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
12	Benzyl undecanoate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
13	Benzyl dodecanoate	($C_6H_5COC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Benzyl esters
14	Benzyl alcohol	(C_6H_5CO)	Alcohol [-OH]	Aliphatic Carbon [CH2]	Aromatic Carbon [C]	Aryl alcohols; Benzyl alcohols

Table 3b: Comparison of Key Structural Fragment, Functional Groups, and Chemical Classes for 2-Phenethyl Derivatives

ID	Name	Key Fragment	Functional Group(s)			Chemical Classes
1	2-Phenethyl acetate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
2	2-Phenethyl l propionate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
4	2-Phenethyl isobutyrate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH} [CH2]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
5	2-Phenethyl valerate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
6	2-Phenethyl isovalerate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH] [CH2]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
7	2-Phenethyl hexanoate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
8	2-Phenethyl heptanoate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
9	2-Phenethyl octanoate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
10	2-Phenethyl nonanoate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
11	2-Phenethyl decanoate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
12	2-Phenethyl undecanoate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
13	2-Phenethyl dodecanoate	($C_6H_5CCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenethyl esters
14	2-Phenethyl alcohol	(C_6H_5CCO)	Alcohol [-OH]	Aliphatic Carbon [CH2]	Aromatic Carbon [C]	Aryl alcohols; Phenethyl alcohols

Table 3c: Comparison of Key Structural Fragment, Functional Groups, and Chemical Classes for 3-Phenpropyl Derivatives

ID	Name	Key Fragment	Functional Group(s)			Chemical Classes
1	3-Phenpropyl acetate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
2	3-Phenpropyl propionate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
4	3-Phenpropyl isobutyrate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH} [CH2]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
5	3-Phenpropyl valerate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
6	3-Phenpropyl isovalerate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH] [CH2]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
7	3-Phenpropyl hexanoate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
8	3-Phenpropyl heptanoate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
9	3-Phenpropyl octanoate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
10	3-Phenpropyl nonanoate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
11	3-Phenpropyl decanoate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
12	3-Phenpropyl undecanoate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
13	3-Phenpropyl dodecanoate	($C_6H_5CCCOC(=O)$)	Aliphatic Ester [COC(=O)]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]	Aryl alkyl esters; Phenpropyl esters
14	3-Phenpropyl alcohol	(C_6H_5CCCO)	Alcohol [-OH]	Aliphatic Carbon [CH2]	Aromatic Carbon [C]	Aryl alcohols; Phenpropyl alcohols

Table 3d: Comparison of Key Structural Fragment, Functional Groups and Chemical Classes for Carboxylic Acids

ID	Name	Key Fragment	Functional Group(s)		Chemical Classes
1	Acetic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH3]	Alkyl carboxylic acids
2	Propionic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
4	Butyric acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
5	Isobutyric acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH] [CH2]	Alkyl carboxylic acids
6	Valeric acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
7	Isovaleric acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH] [CH2]	Alkyl carboxylic acids
8	Hexanoic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
9	Heptanoic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
10	Octanoic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
11	Nonanoic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
12	Decanoic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
13	Undecanoic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids
14	Dodecanoic acid	(OC(=O)C)	Aliphatic Acid [OC(=O)]	Aliphatic Carbon [CH2] [CH3]	Alkyl carboxylic acids

ANNEX TABLES FOR ASSESSING TOXICOKINETIC AND TOXICODYNAMIC SIMILARITIES

Table 4: Comparison of Experimental Toxicokinetic Information

Name	*In Vivo* and *In Vitro* Toxicokinetics
Benzyl acetate	Regardless of route of exposure, the metabolism of benzyl acetate proceeds by hydrolysis to benzyl alcohol, the bulk of which is oxidised to benzoic acid before undergoing conjugation to yield hippuric acid or glucuronide[1-5]. Benzyl acetate administrated to rats and mice dosed via gavage or feed is rapidly hydrolysed to benzyl alcohol and then oxidised to benzoic acid and mainly excreted in the urine as hippuric acid [1]. After gavage administration of benzyl acetate in corn oil at 500 mg/kg bw (rats) and 1000 mg/kg bw (mice), high benzoic acid plasma concentrations were observed. In contrast, much lower benzoic acid plasma concentrations were found after feed administration at (10,800 ppm for rats and 2,700 ppm for mice (≈ 615 mg/kg bw/d for rats and ≈ 850 mg/kg bw/d for mice). Although the daily doses of benzyl acetate are comparable bolus gavage administration effectively saturated the benzoic acid elimination pathway whereas dosed feed administration did not. In contrast, hippuric acid plasma concentrations were similar after both gavage and dosed feed administration due to the depletion of the glycine supply pool[2]. These differences may be related to the concentration of intermediate (benzaldehyde) generated, which is postulated to be higher in the gavage study owing to the higher input of benzyl acetate[2]. Other results indicate changes in minor routes of metabolism and excretion of benzyl acetate occur with age, but formation of hippuric acid from benzyl acetate is not affected by aging[3]. Following dermal administration of neat methylene-^{14}C-benzyl acetate compound to rats, 28-48% of the dose was recovered from the application site[4]. Similarly, 28-46% was absorbed and excreted in the 0-24-hr urine. Excretion of ^{14}C in the urine over 0-24 hours accounted for ≈ 95% of absorbed ^{14}C with <4% of the dose present in the carcass at the end of the experiments. The total recovery of radioactivity was 79-84%. The major urinary metabolite (≈95%) was hippuric acid; other metabolites included much smaller amounts of benzoyl glucuronide, benzoic acid and benzylmercapturic acid[4]. While dermal absorption of benzyl acetate is incomplete, there is significant penetration through the skin, which is related to the concentration applied. The failure to obtain a complete recovery of dermally applied ^{14}C is most likely due to loss by evaporation[5]. The various metabolic pathways appear to be solely involved in the detoxication of benzyl acetate[1-5].
Benzyl alcohol	Humans, rabbits and rats readily oxidise benzyl alcohol to benzoic acid, which is subsequently conjugated with glycine prior to being rapidly eliminated as hippuric acid in the urine[6]. Within six hours after the oral administration of 0.40 g benzyl alcohol/kg of body weight, rabbits eliminated 65.7% of the dose as hippuric acid in the urine[7]. Metabolites identified in the urine of rabbits given an oral dose of 0.25 g/kg benzyl alcohol are chiefly glycine conjugate (74%) and glucosiduronic acid (14%)[8].

2-Phenethyl acetate	The *in vitro* potential for hydrolysis of 16 esters including 2-phenethyl acetate was examined[9]. Hydrolysis in rat liver and small intestinal tissue preparations, as well as artificial pancreatic juice and artificial gastro-intestinal juices followed first order rate kinetics. The tissue rates (liver tissue > intestinal mucosal tissue) showed that esters hydrolyse much more readily in tissues than with artificial juices[9].
2-Phenethyl alcohol	The toxicokinetics of 2-phenylethyl alcohol has been extensively studied[10]. In rats and rabbits 2-phenethyl alcohol is rapidly and extensively absorbed, oxidise to 2-phenylacetic acid and excreted[11]. In rats, following oral and dermal administration, approximately 70% and 27%, of the administrated doses was eliminated in urine as 2-phenylacetic acid and its conjugates, respectively. The absorption rate by dermal administration is lower due to probably to evaporation and loss in dressing. The metabolic clearance is seemed to be reduced at higher plasma concentration due to capacity-limited elimination processes. The absorption and disposition of ^{14}C-2-phenethyl alcohol was examined in rats after single and repeated dermal application. After single dermal doses at 0.14 ml/kg means of 80.7 and 1.3% dose were excreted in urine and faeces, respectively. After 5 repeated dermal doses at 0.14 ml/kg, means of 68.6 and 1.1% of the cumulative dose were recovered from urine and faeces, respectively, during 216 hrs after the first dose. After repeated doses, means of 24.2 and 1.2% dose were recovered from skin and dressing; After repeated doses, total recovery was 68.4% dose as compared to 44.4% after a single dose. This difference was contributed to differences due to evaporation. Radioactivity was detected in a variety of organs/tissues. The major metabolite corresponded to 2-phenaceturic acid and accounted for about 80 of the urinary radioactivity[11]. While some urinary radioactivity corresponded to hippuric acid and 2-phenylacetic acid, very little corresponded to 2-phenethyl alcohol[11]. In rabbits, half the dose was recovered, mainly from the urine. The remaining dose was lost probably due to evaporation. In rabbits, the initial stage in the biotransformation of 2-phenethyl alcohol (oxidation to 2-phenylacetic acid) was similar to that in rats and humans.

Short- and medium- chain carboxylic acids	The absorption, distribution, metabolism and elimination of short- and intermediate- chain carboxylic acids are well-studied[12]. Carboxylic acids are absorbed from the GI tract by different uptake mechanisms depending on the chain length. Short- and medium-chain fatty acids (C1 - C12) are rapidly absorbed *via* intestine capillaries into the blood stream. Due to physico-chemical properties such as melting temperature, solubility and polarity, fatty acids are in general poorly absorbed through skin. Short-chain acids are taken up and transported complexed to albumin; medium-chain acids are esterified with glycerol to triacylglycerides and packaged in chylomicrons. The most significant oxidation pathway of carboxylic acids is the β-oxidation pathway leading to H_2O and CO_2, but other pathways for acid catabolism include α- and ω-oxidation[13]. Under normal physiological conditions, carboxylic acids are not expected to be excreted to any significant amount in the urine or faeces[13]. Results from *in vitro* experiments where [1-^{13}C]butyric acid was incubated with slices of rat liver from fasted rats for two hours at pH 7.3 and 37.5°C have been reported[14]. In two experiments, butyric acid was utilised to 51 and 32% respectively. the major *in vitr*o metabolites of butyric acid included respiratory CO_2 (13.5 and 12.3%), acetoacetic acid (35.7 and 45.8%) and hydroxybutyric acid (11.7 and 9.3%)[14]. Investigation of the metabolic fate of isobutyric acid in rats following a single gavage administration of radiolabelled [1-^{14}C]isobutyric acid were reported[15]. Isobutyric acid was readily absorbed after oral application and rapidly excreted in expired air (i.e., peak plasma levels after 0.5 to 1 hr). In the first 4 hours after dosing, 67 to 83% of the administered dose was excreted as CO_2 in expired air. Unchanged isobutyric acid was less than 0.1%. The recovery of radioactivity in the breath at 48 hr was 90.1 to 96.7%. Radioactivity excreted in urine (48hr) ranged from 3.21 to 4.61%, while faecal radioactivity was less than 1.0% of the dose[15].

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Table 5: Comparison of Repeated-Dose *In Vivo* Data

Name	Sub-Chronic and Chronic Repeated Dose Toxicity
Benzyl acetate	Benzyl acetate has a 13-week gavage GLP study for subchronic repeated-dose toxicity conducted on 10 F344 rats/sex/dose administered 0, 62.5, 125, 250, 500, or 1,000 mg/kg test material, benzyl acetate in corn oil for 5 days a week[1]. Tremor, ataxia and sluggishness were reported among the 500 mg/kg/day females and animals of either sex at 1000 mg/kg/day. The body weights among the high dose group animals were significantly lower than the control group animals. The NOAEL was determined to be 500 mg/kg/day for males and 250 mg/kg/day for females based on observed clinical signs of tremor and ataxia among higher dose group animals[1]. In another GLP study, benzyl acetate was administered via gavage to 10 B6C3F1 mice/sex/dose for 13-weeks. The animals were administered benzyl acetate at doses of 0, 62.5, 125, 250, 500, or 1,000 mg/kg for male mice and 0, 125, 250, 500, 1,000, and 2,000 mg/kg for female mice using corn oil as the vehicle. Mortality among the high dose females was reported. Compound-related clinical signs observed in high-dose mice included trembling, inactivity, laboured breathing and lower body temperature among high dose group animals. The NOAEL for repeated dose toxicity was determined to be 500 mg/kg bw/day based on mortality and clinical signs of tremor and inactivity[1]. Also during a 2-year carcinogenicity study conducted on rats and mice, there was no evidence of carcinogenicity among animals treated with benzyl acetate up to the highest dose tested[1]. The most conservative NOAEL of 250 mg/kg/day was selected for the repeated dose toxicity endpoint.
2-Phenethyl acetate	No experimental data

Benzyl alcohol	Benzyl alcohol has a 13-week gavage GLP study for subchronic repeated-dose toxicity conducted in F344/N rats and B6C3F1 mice[2]. Groups of 10 animals/sex/species/dose were gavaged with 0, 50, 100, 200, 400, or 800 mg/kg bw/d benzyl alcohol in a corn oil vehicle 5 days/week for 13 consecutive weeks. Observations included mortality, body weight, clinical signs, necropsy and selected histopathology. Eight of 10 male rats dosed at 800 mg/kg bw/d died during weeks 7 and 8; four of these deaths were described as gavage related. Rats dosed with 800 mg/kg bw/d exhibited clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Haemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. In mice, deaths were scattered among all dose groups, but none occurred in vehicle controls. Four male and six female mice died after being dosed; all deaths but one were described as gavage related. Staggering after dosing also occurred the first 2 weeks of the studies in mice dosed at 800 mg/kg bw/d. Some of the deaths in rats and mice may have been caused by a combination of the gavage procedure and chemical toxicity, since there was evidence that benzyl alcohol induced neurotoxic effects. There were reductions in relative weight gain in male rats dosed at 800 mg/kg bw/d, in female rats dosed with 200, 400, and 800 mg/kg bw/d, in male mice dosed with 400 and 800 mg/kg bw/d, and in female mice dosed with 200, 400, and 800 mg/kg bw/d[2]. No notable changes in body weight gain or compound related histopathologic lesions were observed in rats or mice from the lower dose groups. The NOAEL for both rats and mice was determined to be 100 mg/kg bw/d[2].
2-Phenethyl alcohol	Findings from 13-week dermal repeated-dose studies in rats at at 0, 0.25, 0.5, 1.0 and 2.0 ml/kg bw/d (≈ 250, 500, 1000, and 2000 mg/kg bw/d) 2-phenethyl alcohol[3]. Based on reduction in body weight and body weight gains in the two highest dose groups, the dermal 90-day repeated-dose NOAEL of 2-phenethyl alcohol was 0.50 ml/kg bw/d (i.e., 500 mg/kg bw/d)[3].

Short- and medium- chain carboxylic acids	In a 90-day diet study, rats were dosed with 0 or 0.62%, 1.25%, 2.5%, or 5% (≈312, 625, 1,250 or 2,500 mg/kg bw/d) propionic acid[5]. During the administration interval, there was no mortality and no clinical signs of toxicity. Food consumption was slightly reduced in males in the high dose group and by the end of the study, mean body weights in this group were reduced by 6% relative to controls. There were no significant changes in haematology or clinical chemistry parameters that could be attributed to the test material. There were no differences in absolute organ weights in treated groups relative to controls. Relative kidney weights were decreased in high dose males (12%). In high dose females, there was an increase in the relative weights of the heart (5%) and liver (9%). Examination of tissues revealed no lesions except point-of-contact (mucosa of the forestomach) changes included acanthosis, hyperkeratosis, and proliferation of the epithelium in the high treatment group. These changes were not observed in the post-exposure recovery group, and there were no differences in relative or absolute organ weights. The NOAEL value for local and systemic effects of propionic acid was determined to be ≈1,250 and ≈2,500 mg/kg bw/d for male and female rats, respectively[5]. Amoore et al. (1978) examined 90-day repeated-dose toxicity of isovaleric acid in a rat feeding study with 0, 5% and 10% (0, ≈5,000 and ≈10,000 mg/kg bw/d) neutralised isovaleric acid in the diet[6]. In a pilot study with 10%, the food intake was significantly reduced, rats lost significantly weight, and one rat dies. No effects were seen in the main study, 5% for the parameters examined (i.e., food consumption, bodyweight development, organ weights, haematology, blood chemistry, urinalysis, and histopathology of 35 organs). The exception was a more basic urine in treated rats compared to controls (pH 8.4 versus 7.2). Based on these finding, the NOAEL value for neutralised isovaleric acid is 5% in diet or ≈5000 mg/kg bw/d[6]. Fitzhugh et al. (1960) examined the 18-week repeated-dose toxicity of dodecanoic acid in a feeding study with 10% (≈10,000 mg/kg bw/d) in the diet[7]. No clinical signs and no mortality were noted. No adverse effects on weight gain. Moreover, no significant differences between the controls and test animals were noted in either organ weight parameters or histopathology. Based on these finding the NOAEL for dodecanoic acid is ≈10,000 mg/kg bw/d[7].

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