Firman, J, Patel, A, Date, M, Cronin, MTD and Schultz, TW

Read-across of 90-day rodent repeated-dose toxicity: A case study for selected simple aryl alcohol alkyl carboxylic acid esters

Read-across of 90-day rodent repeated-dose toxicity: A case study for selected simple aryl alcohol alkyl carboxylic acid esters

James W. Firman[a,*], Atish Patel[b], Mihir Date[b], Mark T.D. Cronin[a], Terry W. Schultz[c]

[a] Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, United Kingdom
[b] Research Institute of Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA
[c] The University of Tennessee, College of Veterinary Medicine, 2407 River Drive, Knoxville, TN 378996-4543, USA

* Corresponding author.

E-mail address: j.w.firman@ljmu.ac.uk (James W. Firman).

Postal address:

James Firman
School of Pharmacy and Biomolecular Sciences
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF
United Kingdom

Disclaimer: The views expressed in this document are those of the authors and do not necessarily represent the official views of the Research Institute of Fragrance Materials or its members.

Word Count for Abstract: 167 words

Word Count for Text: 6195 words

Word Count for References: 1344 words

Abstract

Aryl alcohol alkyl carboxylic acid esters constitute a wide-employed class of fragrance materials. Outcomes presented within this study illustrate formulation of a read-across protocol for 90-day, repeat-dose toxicity within a series of these compounds, extending in scope to the dodecanoic acid esters of both benzyl and 2-phenylethyl alcohol. Central to the filling of data gaps is the hypothesis that adverse impact – mediated through mechanisms analogous to narcosis-associated non-specific basal cytotoxicity – is as a consequence of the hydrolysis of parent compounds to their corresponding alcohol. High-quality *in vivo* data (equivalent to either OECD Test Guideline 408 or 411) relating the toxicodynamic and toxicokinetic profiles of benzyl alcohol, 2-phenylethyl alcohol and benzyl acetate within rodents were retrieved, permitting extrapolation to each ester derivative with great confidence. NOAEL values of 250-500 mg/kg/day were assigned to esters, with a greater toxicity present within females. In order to greater enhance reliability, further theoretical support for the read-across prediction is provided through the integration, where appropriate, of *in vitro* and *in silico* data.

Keywords: aryl alcohol alkyl carboxylic acid esters, read-across, similarity, uncertainty, repeated-dose toxicity

Highlights:

- A read-across category including C2 to C12 benzyl and 2-phenylethyl alcohol alkyl carboxylic acid esters.
- Hydrolysis forms toxicants, benzyl or 2-phenylethyl alcohol, which possess non-specific narcosis-associated mode of action.
- NOAEL values of 250-500 mg/kg/day can be read across with confidence as a worst-possible scenario to untested compounds.
- Uncertainty is reduced by the consistency within the category of *in vivo*, *in vitro* and *in silico* data.

1. Introduction

1.1. Read-across

Underpinning the concept of toxicological read-across is the principle that chemical compounds which have similar molecular structures will have a similar toxicodynamic and toxicokinetic profile.[1] Accordingly, given the availability of experimentally-derived toxicological data for one or more source compounds, the corresponding activity of untested, structurally-related target compounds might be rationally inferred and predicted.[2] As research in toxicology continues to trend away from the methods employing widespread use of animals – notably, but not entirely, within the field of cosmetic ingredient testing – the requirement for robust, alternative means to fill gaps in safety data grows more acute.

Read-across methodology has for decades been employed across industry, academia and within regulatory settings.[3] The anticipated increased future reliance upon it as a predictive tool has ensured that more thorough, systematic approaches to chemical category formation, analogue identification and data interpretation need be devised and evaluated.[4] In order to formulate a robust, comprehensive read-across protocol, a series of case studies were undertaken by the authors, covering varying scenarios with regard to the relevance of metabolism to toxicity.[5, 6] These have provided suitable illustrations on how strong, sound groupings can be built considering the structural, physicochemical, toxicodynamic and toxicokinetic properties of compounds and address, in part at least, some of the concerns raised by historical studies.[7]

The aim of this study was to further extend the applicability of the principles established in the previous read-across studies to permit read-across assessment of sub-chronic, repeat-dose toxicity within a series of aryl alcohol alkyl carboxylic acid esters.[5-7] It relates to two separate, but structurally-related, categories: the alkanoates of benzyl alcohol and 2-phenylethyl alcohol. Through consideration of shared mechanistic features between compounds – most notably the metabolic

biotransformation to shared toxicophoric species – a robust case for read-across was presented. Relevant toxicokinetic and toxicodynamic data were searched for, with a focus on no-observed-adverse-effect level (NOAEL) values derived from 90-day rat and mouse studies supported by *in vivo* and *in vitro* metabolic assessment. The read-across arguments were assessed and evaluated by a discussion of appropriate concerns associated with uncertainty and the weight of evidence supporting the claims, in accordance with Organisation for Economic Co-operation and Development (OECD) Integrated Approaches for Testing (IATA) guidelines.[8]

1.2. Aryl alcohol alkyl carboxylic acid esters: overview of existing knowledge

Owing to their utility as fragrance materials, alkyl carboxylic esters of aryl alcohols are used widely within cosmetic preparations.[9] Whilst the acute toxicity of selected benzyl and 2-phenylethyl alkanoates is well characterised, corresponding assessment into the effects of repeat-dose, sub-chronic exposure has been limited.[10, 11] It has been established, however, that such compounds, in common with many related forms of carboxylic acid ester, undergo rapid and near-complete hydrolysis. This metabolism occurs through the action of broad-specification hydrolase enzymes and yields their alcohol and acid substituents (illustrated with reference to formation of benzyl alcohol and acetic acid from benzyl acetate, Figure 1.).[12, 13] Accordingly, it can be postulated that their toxic effects derive solely as a product of their metabolites, and that, given knowledge of the properties of such compounds, the toxicological profiles of untested esters across a category might be inferred.

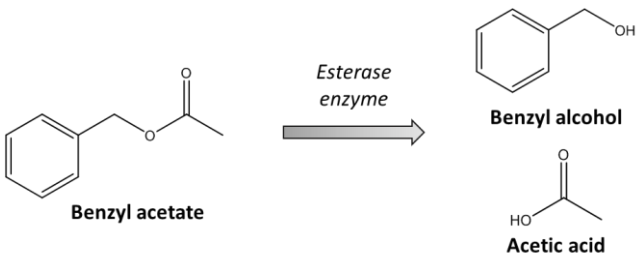


Figure 1. Simplified scheme outlining enzyme-mediated hydrolysis of benzyl acetate to yield aryl alcohol and alkyl carboxylic acid constituents.

The aryl alcohols resulting from metabolism from their alkyl carboxylic esters are associated with toxicity.[6] Mechanisms underpinning acute toxicity are believed to be analogous with the widely accepted concept of non-polar narcosis in environmental species. Broadly speaking, this mechanism centres upon non-specific disruption of cellular membranes, in turn affecting their integrity.[14, 15] In the same manner, appreciation of sub-chronic effects may be achieved by their consideration in the context of a framework of repeat-dose "basal cytotoxicity".[16] The carboxylic acid moiety, on account of ready catabolism and integration into physiological pathways, is conversely deemed to not to contribute towards toxicity. Given the immediacy of hydrolysis, the physicochemical attributes of the esters, which vary reliably and predictably with alkyl chain length from C2 to C12, are thought to be of only minor relevance with regards to their toxicokinetic profiles. It can, nevertheless, be considered reasonable to assume that absorption characteristics, particularly with respect to dermal administration, will display some degree of variance attributable to the heightened hydrophobicity of the compounds with longer alkyl chain lengths.

It therefore follows that although suitable *in vivo* sub-chronic, repeat-dose toxicological data may exist only for a single aryl alcohol alkyl ester within the category, the properties of analogues can be predicted reliably based upon extrapolation of corresponding results both from the source compound and also from the shared aryl alcohol constituent. NOAEL values are available from repeat-dose 90-day rodent studies both for benzyl alcohol and benzyl acetate, and they are thus proposed for use in inferring the corresponding quantities in a wider set of benzyl alcohol alkyl esters extending, for the purposes of this assessment, to benzyl dodecanoate.[17, 18] Similarly, an experimental sub-chronic NOAEL for 2-phenylethyl alcohol exists within the literature, and this might be employed for the same purpose within a category of 2-phenylethyl alkanoates, even in the absence of equivalent data for any such individual compound.[19] As implied above, it is anticipated that owing to shared structural features between categories, their metabolic profile and toxicodynamic impact will show great concordance. The lack of experimental, repeat-dose data for many compounds under consideration nevertheless ensures that is prudent, taking into account the

shared mechanism of metabolism to the alcohol toxicophore, to adopt a "worst-case" approach in assigning toxicological properties to targets.

ID	Name	CAS No.	SMILES
Benzyl alkanoate			
1	Benzyl acetate	140-11-4	c1ccccc1COC(=O)C
2	Benzyl propionate	122-63-4	c1ccccc1COC(=O)CC
3	Benzyl butyrate	103-37-7	c1ccccc1COC(=O)CCC
4	Benzyl isobutyrate	103-28-6	c1ccccc1COC(=O)C(C)C
5	Benzyl valerate	10361-39-4	c1ccccc1COC(=O)CCCC
6	Benzyl isovalerate	103-38-8	c1ccccc1COC(=O)CC(C)C
7	Benzyl hexanoate	6938-45-0	c1ccccc1COC(=O)CCCCC
8	Benzyl heptanoate	5454-21-7	c1ccccc1COC(=O)CCCCCC
9	Benzyl octanoate	10276-85-4	c1ccccc1COC(=O)CCCCCCC
10	Benzyl nonanoate	6471-66-5	c1ccccc1COC(=O)CCCCCCCC
11	Benzyl decanoate	42175-41-7	c1ccccc1COC(=O)CCCCCCCCC
12	Benzyl undecanoate	Not found	c1ccccc1COC(=O)CCCCCCCCCC
13	Benzyl dodecanoate	140-25-0	c1ccccc1COC(=O)CCCCCCCCCCC
14	Benzyl alcohol	100-51-6	c1ccccc1CO
2-Phenylethyl alkanoate			
15	2-Phenylethyl acetate	103-45-7	c1ccccc1CCOC(=O)C
16	2-Phenylethyl propionate	122-70-3	c1ccccc1CCOC(=O)CC
17	2-Phenylethyl butyrate	103-52-6	c1ccccc1CCOC(=O)CCC
18	2-Phenylethyl isobutyrate	103-48-0	c1ccccc1CCOC(=O)C(C)C
19	2-Phenylethyl valerate	7460-74-4	c1ccccc1CCOC(=O)CCCC
20	2-Phenylethyl isovalerate	140-26-1	c1ccccc1CCOC(=O)CC(C)C
21	2-Phenylethyl hexanoate	6290-37-5	c1ccccc1CCOC(=O)CCCCC
22	2-Phenylethyl heptanoate	5454-11-5	c1ccccc1CCOC(=O)CCCCCC
23	2-Phenylethyl octanoate	5457-70-5	c1ccccc1CCOC(=O)CCCCCCC
24	2-Phenylethyl nonanoate	57943-67-6	c1ccccc1CCOC(=O)CCCCCCCC
25	2-Phenylethyl decanoate	61810-55-7	c1ccccc1CCOC(=O)CCCCCCCCC
26	2-Phenylethyl undecanoate	Not found	c1ccccc1CCOC(=O)CCCCCCCCCC
27	2-Phenylethyl dodecanoate	Not found	c1ccccc1CCOC(=O)CCCCCCCCCCC
28	2-Phenylethyl alcohol	60-12-8	c1ccccc1CCO

Table 1. Aryl alcohol alkyl carboxylic acid esters considered in this study, grouped within read-across categories alongside their shared aryl alcohol substituent. Further description of these compounds, including their chemical structures and molecular formulae, are listed in Supplementary Table 1.

2. Method and materials

Compounds were evaluated through a read-across protocol drawing extensively on the workflows proposed by Schultz *et al.* and Przybylak *et al.*[5, 6, 20] Analysis was further expanded to include consideration of the categories under existing OECD IATA guidelines.[8] Relevant *in vivo* data were accrued from literature sources, including the ECHA REACH Registered Substances database.[21] Mechanistic relevance, alongside toxicokinetic and toxicodynamic similarity of the category analogues, was established using appropriate non-animal data.

2.1. Source compounds and category members

Two structurally related categories were considered within this study, each consisting of 13 aryl alcohol alkyl carboxylic acid esters and a single parent aryl alcohol. The members of each category are detailed in Table 1. Within the benzyl alcohol ester category, two source compounds – benzyl alcohol and benzyl acetate – were identified based upon the availability of repeat-dose *in vivo* data. The 2-phenylethyl ester category similarly comprised two source compounds: 2-phenylethyl alcohol and 2-phenylethyl acetate. Whilst *in vitro* toxicokinetic data were present in this instance for the ester, toxicodynamic outcomes were not.

2.2. Endpoint

The endpoint for this read-across was repeat-dose, sub-chronic toxicity. This was assessed using data from protocols equivalent either to OECD Test Guideline 408 (Repeated Dose 90-Day Oral Toxicity Study, Rodents) or to OECD Test Guideline 411 (Sub-chronic Dermal Toxicity: 90-day Study).[22, 23] NOAEL values assigned from these reports provided the quantitative expression of toxic potency. In the instance of a single source compound (benzyl acetate), further supporting evidence was

provided through a procedure performed in line with the United States' National Toxicological Program (NTP) carcinogenicity 2-year study guidelines.[18]

2.3. Similarity hypothesis for category formation

- Aryl alcohol unsaturated alkyl esters constitute a class of indirect toxicants, the activity of which is dependent exclusively upon narcosis-related unspecific basal toxicity as induced through their aryl alcohol hydrolysis products.
- Owing to the rapid nature of hydrolysis, the varying intra-category physicochemical properties of the compounds, dictated solely by the length of the alkyl chain (from C2 to C12), hold minor, albeit not negligible with respect to absorption, toxicological relevance.
- Nevertheless, an absence of data for many esters ensures that uncertainty exists regarding the precise impact of the incremental variation in molecular structure upon quantitative toxicological outcome – as such, a "worst-case" approach may be adopted to infer properties from the source esters and, where necessary, alcohols.
- The toxic profiles of the each of the benzyl alcohol esters can be inferred from those of benzyl alcohol, and the 2-phenylethyl alcohol esters from 2-phenylethyl alcohol, and, where appropriate, also benzyl acetate.
- Similarity in physicochemical and toxicological properties between alcohols permits cross-category inference of effects in the absence of relevant data.
- Carboxylic acid metabolites exert no contribution towards toxicity, being primarily catabolised to substrates which participate with various physiological energy-producing pathways.

3. Results and discussion*3.1. Compilation of data*

Data were accrued from relevant sources in accordance with the methodology outlined in Section 2. Tables detailing in full the structure and identity of the category members, alongside their experimentally-derived physicochemical (Supplementary Table 2), toxicodynamic (Table 2 and Supplementary Table 3) and toxicokinetic properties (Supplementary Table 4), are provided. A discussion of the recovered toxicodynamic and toxicokinetic outcomes is presented below:

3.1.1. Benzyl acetate

Several assessments have been conducted examining the repeated-dose toxicological profile of benzyl acetate *in vivo*. Male and female F344 rats, dosed for a period of 13 weeks *via* gavage with 0, 62.5, 125, 250, 500 and 1000 mg/kg/day test compound, were observed for clinically relevant signs.[18] Combined with reduced body weight, evidence of ataxia in tremor in higher-dose groups prompted the assignment of values for NOAEL of 500 mg/kg/day (males) and 250 mg/kg/day (females).

A similar study was performed in $B6C3F_1$ mice. Gavage administration of 0, 62.5, 125, 250, 500 and 1000 mg/kg/day benzyl acetate (males) and 0, 125, 250, 500, 1000, 2000 mg/kg/day benzyl acetate (females) proceeded for 13 weeks.[18] Tremor, inactivity and lowered body temperature were evident at higher doses in both sexes, with mortality in females being notably elevated. NOAEL was universally determined as 500 mg/kg/day.

A concurrent 2-year carcinogenicity analysis utilising identical species and strains yielded negative results at all doses examined (up to 250 mg/kg/day in rats, 500 mg/kg/day in mice).[18]

3.1.2. Benzyl alcohol

In a 13-week study, male and female F344/N rats were administered benzyl alcohol by gavage at concentrations of 0, 50, 100, 200, 400 or 800 mg/kg/day.[17] Mortality was evident within the 800

mg/kg/day cohort, with surviving animals exhibiting a variety of clinical signs including staggering and respiratory dysfunction (indicative of neurotoxicity), localised haemorrhaging and histological abnormality. Decreased body mass was further apparent in many of the dosed groups. B6C3F_1 mice treated under identical experimental conditions were subject to a similar pattern of effects, albeit with mortality evident across a wider dose range. Each species was assigned a NOAEL of 100 mg/kg/day.

3.1.3. 2-Phenylethyl alcohol

A 13-week study assessed the impact of dermally-administered 2-phenylethyl alcohol upon male and female Sprague-Dawley rats.[19] Doses of 0, 0.25, 0.5, 1.0 and 2.0 ml/kg/day (corresponding approximately to 250, 500, 1000 and 2000 mg/kg/day) were provided. On account of body weight abnormalities observed at the higher concentrations, a NOAEL of 0.50 ml/kg/day (500 mg/kg/day) was assigned.

3.1.4. Saturated short- to medium-chain carboxylic acids

The repeat-dosed toxicity of propionic acid has been examined in male and female Sprague-Dawley rats.[24] Animals were exposed to the compound through their diet for a period of 13 weeks, at concentrations of 0, 0.62%, 1.25%, 2.5% or 5.0% (approximating to 312, 625, 1250 or 2500 mg/kg/day). A minor decrease in body weight was apparent within the higher-dose male group, with a 12% reduction in kidney size being the most notable single-organ alteration. High-dose females displayed increased mass in heart and liver. Neither sex exhibited histological abnormalities, and lesions were observed only within forestomach mucosa. NOAEL values were assigned as 1250 mg/kg/day and 2500 mg/kg/day for males and females respectively.

A 13-week assessment of isovaleric acid toxicity was performed in male Sprague-Dawley rats.[25] Neutralised isovaleric acid was incorporated into the diet at concentrations of 0, 5% and 10% (approximately corresponding to 0, 5000 and 10000 mg/kg/day). Whilst a single instance of

mortality was observed at the highest dose, no instances of unusual histopathological or gross effect, aside from alkalisation of urine, were noted at 5%. The NOAEL can thus be considered to be 5000 mg/kg/day.

Dodecanoic acid was analysed for toxic effects in male and female Osborne-Mendel rats.[26] Test animals were administered the carboxylic acid in their diet over a period of 18 weeks, at concentrations 0 and 10% (approximating to 10000 mg/kg/day). No clinical effects were observed across all parameters examined, ensuring that a NOAEL of 10000 mg/kg/day could be attributed.

Compound	NOAEL (mg/kg/day)			
	Rat		Mouse	
	Male	Female	Male	Female
Benzyl acetate	**500**	**250**	**500**	**500**
Benzyl propionate	*500*	*250*	*500*	*500*
Benzyl butyrate	*500*	*250*	*500*	*500*
Benzyl isobutyrate	*500*	*250*	*500*	*500*
Benzyl valerate	*500*	*250*	*500*	*500*
Benzyl isovalerate	*500*	*250*	*500*	*500*
Benzyl hexanoate	*500*	*250*	*500*	*500*
Benzyl heptanoate	*500*	*250*	*500*	*500*
Benzyl octanoate	*500*	*250*	*500*	*500*
Benzyl nonanoate	*500*	*250*	*500*	*500*
Benzyl decanoate	*500*	*250*	*500*	*500*
Benzyl undecanoate	*500*	*250*	*500*	*500*
Benzyl dodecanoate	*500*	*250*	*500*	*500*
Benzyl alcohol	**100**	**100**	**100**	**100**
2-Phenylethyl acetate	*500*	*500*		
2-Phenylethyl propionate	*500*	*500*		
2-Phenylethyl butyrate	*500*	*500*		
2-Phenylethyl isobutyrate	*500*	*500*		
2-Phenylethyl valerate	*500*	*500*		
2-Phenylethyl isovalerate	*500*	*500*		
2-Phenylethyl hexanoate	*500*	*500*		
2-Phenylethyl heptanoate	*500*	*500*		
2-Phenylethyl octanoate	*500*	*500*		
2-Phenylethyl nonanoate	*500*	*500*		
2-Phenylethyl decanoate	*500*	*500*		
2-Phenylethyl undecanoate	*500*	*500*		
2-Phenylethyl dodecanoate	*500*	*500*		
2-Phenylethyl alcohol	**500**	**500**		

Table 2. Summary of NOAEL values, both experimental and inferred. Quantities in bold print correspond to experimentally-determined 90-day NOAEL. Those in italics represent values read across in instances of data absence.

3.2. Justification of read-across

The acceptance of the validity of a read-across hypothesis is dependent not solely upon identification of shared structural features between source and target compound, but also on establishing their similarity in physicochemical, metabolic, toxicodynamic and toxicokinetic properties.[20] It is furthermore a requirement that a firm rationale for mechanistic plausibility be developed and supported. Accordingly, relevant concordance and variation within such parameters across the ester categories are outlined and considered:

3.2.1. Applicability domain

The applicability domain of the study incorporates the alkyl esters of benzyl and 2-phenylethyl alcohol, possessing chain lengths on the carboxylic acid moiety of C2 to C12.

3.2.2. Structural Similarity

All aryl alcohol esters considered display substantial structural similarity. Notably, they belong: (1) to a common chemical class in the form of carboxylate esters and (2) the subclass of primary aryl alcohol alkyl esters. Variability in structure within category is limited to the composition of alkyl chain, which ranges in length from 2 to 12 carbon atoms. Between categories, the length of carbon linker adjoining the aromatic unit varies from 1 to 2 carbon atoms. Accordingly, the composition of the toxic aryl alcohol metabolite exhibits only very minor inter-category variation.

3.2.3. Chemical property similarity

The physicochemical properties for each respective category member are reported in Supplementary Table 2. Experimentally-derived quantities are used as available, although for the great majority of the aryl alcohol esters such parameters were not available. Accordingly, predicted values for physicochemical properties were employed where appropriate. Data pertaining the relevant carboxylic acids, predominantly experimental in source, are included for completeness. It can be observed that there is, across all properties with the sole exception of density, an intra-

category variance correlated strongly with alkyl chain length and molecular size. The molecular weights of the esters range from 150.2 to 304.5. With increasing molecular mass comes increase in the logarithm of the octanol-water partition coefficient (log K_{OW}), melting point and boiling point, and a concurrent decrease in water solubility and vapour pressure.

3.2.4. Chemical constituent similarity

All aryl alcohol alkyl esters included within this analysis contain common constituents limited to: (1) a polar aliphatic ester functional unit, -[COC(=O)]-, (2) aliphatic secondary and primary carbon -[CH2]- and -[CH3] structural fragments, and (3) aromatic carbon -[CH]-. Also present on branched substituents is the tertiary aliphatic carbon -[CH]-. Primary aryl alcohols are each composed of: (1) a polar functional substituent -[OH], (2) the aliphatic secondary carbon fragment -[CH2]- and (3) the aromatic carbon unit -[CH]-. Alkyl carboxylic acids are constituted from (1) the polar aliphatic carboxylic acid functional moiety [OC(=O)]- and (2) the structural fragments aliphatic secondary and primary carbon -[CH2]- and -[CH3]. Also present on branched substituents is the tertiary aliphatic carbon -[CH]-.

3.2.5. Toxicokinetic similarity

Whilst experimental toxicokinetic data are not available for all the esters considered within this study, the premise upon which their activity is believed to rest – namely, metabolism to toxicophoric aryl alcohol and non-toxic alkyl carboxylic acid species – can be considered conserved across all category members. Generally, such compounds are rapidly absorbed, distributed via the blood, metabolised and excreted. As compared with dermal exposure, there is evidence of more extensive absorption from the gut. In the latter case, toxicokinetic potency is greater *via* gavage than through dosed feed administration. The results of studies of ester hydrolysis *in vitro* indicate it to be a universal metabolic step.[27, 28] The hydrolysis rate of straight-chain esters is approximately 100 times greater than that of their branched-chain counterparts.[29] 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.[12]

Evidence pertaining to the *in vivo* metabolic profile of benzyl acetate suggests that hydrolysis of the parent compound proceeds rapidly, yielding substrates which undergo ready conjugation and elimination. The outcomes of oral administration studies in both rats and mice indicate that formation of benzyl alcohol occurs as an initial step.[30] Oxidation of this compound, through a benzaldehyde intermediate, to benzoic acid is followed by conjugation with glycine, creating hippuric acid. It is this which ultimately constitutes the dominant metabolite present within urine.[31] The toxicokinetics of ^{14}C-radiolabelled benzyl acetate following dermal application have been examined further in rats. 28-46% of the administered dose was absorbed into the circulation, of which 95% was excreted in urine within 24 hours of dosing. Hippuric acid was once again found to be the principal species expelled, accounting for 95% of metabolites detected.[32] Dedicated examinations of the fate of benzyl alcohol have been performed in several species, with consistency in disposition displayed in each.[33] Within rabbits, 65.7% of an oral dose was found eliminated in urine as hippuric acid within 6 hours of treatment.[34]

Whilst *in vivo* assessment of 2-phenylethyl acetate toxicokinetics have not been reported, evidence derived from *in vitro* studies, utilising both rat liver and intestine tissue samples and artificial gastric juices, strongly indicates that the ester readily undergoes hydrolysis to its constituent alcohol and acid.[27] The properties of 2-phenylethyl alcohol have been subject to extensive *in vivo* examination, with the compound displaying rapid absorption within both rabbits and rats.[35] Rat studies demonstrate that 70% of the dose may be recovered following oral administration – predominantly in the form of 2-phenylethylacetic acid and its conjugates.[36] Recovery following topical application was considerably lower, at 27%. Further dermal investigations using ^{14}C-radiolabelled 2-phenylethyl alcohol revealed that proportion of total substrate recovery was greater following repeat-dosing,

reaching 68.4%, primarily as the metabolite 2-phenylethylacetic acid, at the conclusion of a 9-day assessment.

Short-to-medium chain carboxylic acids are well-absorbed orally, although owing to unfavourable physicochemical properties, they generally do not permeate the skin.[37] Depending upon chain length, they may be bound to albumin within the bloodstream, or incorporated into triglyceride structures. Participation in catabolic pathways is a common fate.[38] *In vitro* examination into the metabolism of specific, radio-labelled variants revealed that the predominant pathways were the oxidative breakdown into both shorter-chained derivatives and CO_2, whilst overall urinary excretion was low.[39, 40]

3.2.6. Metabolic similarity

It can be assumed with high confidence that, due to the structural similarity, all esters analysed within this study will be subject to carboxylesterase enzyme-mediated initial hydrolysis which in turn yields both the aryl alcohol and alkyl carboxylic acid metabolites. Evidence suggests that such alcohols undergo a two-stage process of oxidation – first to their equivalent aldehyde and then further to the carboxylic acid – through transformations conducted respectively by alcohol and aldehyde dehydrogenase enzymes.[41] Conjugation of the resultant carboxylic acid to either glycine, glucuronic acid derivatives or L-glutamine produces metabolites excreted primarily in urine (refer to Supplementary Figure 1 for schematic representation). The alkyl carboxylic acids produced through hydrolysis are integrated as substrates within physiological processes. Following ligation to coenzyme A, translocation to the mitochondrion precedes catabolism through β-, α- or ω-oxidation pathways. Resulting acetyl-CoA units are incorporated into the citric acid cycle, from which water and CO_2 are by-products.

All esters and alcohols included show great similarity with regards to *in silico* toxicokinetics. Based upon simulators present within the OECD Toolbox v3.4, (rat liver S9 and skin metabolism), all derivatives are considered to be readily metabolised through safe pathways. Similar predictions

were observed with TIMES-SS (v. 2.28.), MetaPrint2D-React, SMARTCyp (v. 2.4.2.), and Meteor Nexus (v. 3.0.) (Lhasa Limited, UK) softwares – each of which may be described broadly as an "expert system", drawing upon existing structure-activity knowledge in order to predict biotransformative fate (refer to Supplementary Table 6 for summary of outcomes, as available).

3.2.7. In silico toxicodynamic similarity

All aryl alkanoates and alcohols considered are highly similar with regards to *in silico* toxicodynamics. Category and subcategory consistency was established using the OECD QSAR Toolbox (v. 3.4.). Specifically, results from the mechanistic and endpoint profilers (data not shown) revealed none of the compounds possessed alerts indicative of potential toxicity. Moreover, all compounds are deemed Cramer Class I chemicals. In addition, none are classified as potential receptor binders by COSMOS (COSMOS profiler available at: http://knimewebportal.cosmostox.eu/webportal). No alerts were fired in Derek Nexus (v. 2.1.) (Lhasa Limited, UK), with the exception in two compounds associated with "irritation of gastrointestinal tract" (refer to Supplementary Table 6 for summary of outcomes, as available).

3.2.8. High throughput toxicodynamic similarity

Available ToxCast data were searched through the US EPA Chemistry Dashboard https://comptox.epa.gov/dashboard/ [accessed 10/10/2017]. Test results were recovered for five benzyl esters and benzyl alcohol, alongside five 2-phenylethyl esters and 2-phenylethyl alcohol (refer to Table 3 for additional detail).

Compound	Assays run	Active hits	Assay ID	Target family
Benzyl alkanoate				
Benzyl acetate	298	1	ACEA_T47D_80hr_Positive	Nuclear receptor
Benzyl propionate	276	1	ATG_DR5_CIS_dn	Nuclear receptor
Benzyl butyrate	276	0		
Benzyl isobutyrate	163	0		
Benzyl isovalerate	276	3	TOX21_NFkB_BLA_agonist_viability	Cell cycle
			ATG_Ahr_CIS_dn	DNA binding
			ATG_PXRE_CIS_up	Nuclear receptor
Benzyl alcohol	541	5	NVS_ENZ_oCOX2	Oxidoreductase
			ATG_PPRE_CIS_up	Nuclear receptor
			ATG_RXRa_TRANS_up	Nuclear receptor
			ATG_RXRb_TRANS_up	Nuclear receptor
			ATG_NURR1_TRANS_up	Nuclear receptor
Phenylethyl alkanoate				
2-Phenylethyl acetate	276	0		
2-Phenylethyl isobutyrate	276	0		
2-Phenylethyl propionate	113	3	TOX21_Aromatase_Inhibition	Cytochrome
			TOX21_ARE_BLA_agonist_ratio	DNA binding
			TOX21_PPARd_BLA_antagonist_ratio	Nuclear receptor
2-Phenylethyl butyrate	276	1	TOX21_NFkB_BLA_agonist_viability	Cell cycle
2-Phenylethyl hexanoate	276	2	TOX21_p53_BLA_p3_ch2	Background
			ATG_Ahr_CIS_dn	DNA binding
2-Phenylethyl alcohol	296	2	OT_ER_ERbERb_1440	Nuclear receptor
			ATG_PPARg_TRANS_dn	Nuclear receptor

Table 3. Summary of output related to ToxCast screening of aryl alkyl alkanoates.

Based on ToxCast results the benzyl, 2-phenylethyl and 3-phenpropyl alkanoates, as well as their corresponding alcohols, do not appear to be associated with specific toxicities. For the ten esters and more that 3,600 results in ToxCast, an active hit was reported for only eleven assays, a rate of 0.33%. Moreover, there is no pattern in these positive responses. Similar results were observed for the two alcohol metabolites; specifically, of the over 1,000 results in ToxCast only seven active hits were reported, a rate of 0.63%.*3.2.9. Plausibility of mechanistic similarity*

On account of the high levels of structural conformity evident for all category members, a shared general mode of action may be assumed. It is postulated that this is centred upon the metabolism of parent compounds to their shared primary aryl alcohol substituents, which themselves proceed to impart toxicity through a mechanism of unspecific basal cytotoxicity. Extrapolation of the concept of narcosis – a phenomenon held to arise as a consequence of reversible non-covalent interference with the structure and function of the phospholipid bilayers constituting cell membranes, and

391 further closely associated with the acute toxicity of such compounds – may be considered in order to
392 rationalise the sub-chronic effects.[42, 43]

393 There are several lines of evidence that support the hypothesis that all the analogues considered act
394 in a similar manner, that is, through simple anaesthesia or basal cytotoxicity. Specifically, while there
395 is no mammalian adverse outcome pathway for the hypothesised mode of action, it is generally
396 accepted that the toxicity of saturated esters is based on their alcohol metabolite and thus is due to
397 a non-specific narcotic effect.[6] There is both theoretical and biochemical evidence for the cell
398 membrane functioning as the site of action for anaesthetic-like compounds such as saturated
399 alcohols.[14, 15, 44] These findings are supported by animal data.[45-48] Narcosis, in the broadest sense, is
400 the reversible, non-covalent disruption of hydrophobic interactions within membranes with a
401 particular volume fraction, rather than molar fraction.[49] While the precise mechanism has yet to be
402 elucidated, it is the accumulation of alcohols in cell membranes which leads to disturbance in
403 cellular function. With repeated exposure, likelihood of cumulative damage to tissue within
404 susceptible organ systems grows to constitute credible concern.

405 McCloskey *et al.* reported an attempt to alter benzyl alcohols toxicity by using pyrazole and
406 disulfiram to inhibit the activities of alcohol dehydrogenase and aldehyde dehydrogenase,
407 respectively.[41] Treatment with pyrazole, before benzyl alcohol exposure, resulted in an increase in
408 benzyl alcohol levels to 203% of controls concomitant with a marked increase in toxicity. Although
409 pre-treatment with disulfiram led to benzaldehyde levels which were 368% of controls, toxicity was
410 unchanged. These data imply that the acute toxicity of benzyl alcohol, which includes sedation,
411 dyspnoea and loss of motor function is due to the alcohol itself and not to its metabolite,
412 benzaldehyde.

413 While an AOP for narcosis is under development
414 (http://www.oecd.org/chemicalsafety/testing/projects-adverse-outcome-pathways.htm) no
415 pathway has been evaluated. However, the basic premise of such a sequence (i.e., partitioning of

416 unreactive compounds into cellular membranes leading to decreased physiological performance) is
417 consistent with the mode of action proposed in a previous read-across for saturated alcohols.[6] As
418 previously noted, the ToxCast data show the aryl alkanoates and corresponding alcohols to be a
419 seemingly innocuous group of compounds not consistently associated with receptor binding.

420 The applicability of the mechanism to the primary aryl alcohols in this analysis can be inferred from
421 the properties of related chemical species, including n-alkanols, supported by mammalian *in vivo*
422 analysis.[6, 46] One such study indicated that administration of analogous compounds induces
423 depressant effects within rodents, generally additive to those observed following co-treatment with
424 the structurally-unrelated volatile anaesthetic desflurane.[14] Although evidently non-mammalian,
425 further support for such hypotheses can be derived through consideration of the mechanism
426 proposed to underpin alcohol-induced non-specific toxicity within aquatic species.[45] Conversely, the
427 alkyl carboxylic acids liberated through hydrolysis are, irrespective of chain length, proposed to play
428 no contributory role towards toxic effects. Their participation in catabolic pathways ensures their
429 ready integration into physiological processes, whilst the major breakdown product, CO_2, is excreted
430 harmlessly.

Assessment Factor	Uncertainty	Comment
Hypothesis used for read-across	**Low**	The hypothesis underpinning the read-across – namely that toxicity in aryl alkanoate esters emerges as a consequence of their rapid hydrolysis into aryl alcohol and carboxylic acid substituents, the former of which proceeds to stimulate adverse effects through a mechanism akin to basal cytotoxicity or non-polar narcosis – has convincing theoretical and experimental support from *in vivo, in vitro* and *in silico* toxicodynamic and toxicokinetic evidence.
Structural similarity	**Low**	All compounds included within this study belong to the class of aryl alcohol unsaturated carboxylic acid esters. Compounds are subdivided into two categories, each composed of 13 esters alongside their common primary aryl alcohol constituent. Functional groups are consistent across all entries, with liability towards metabolism to toxicologically-relevant alcohol moiety universally conserved. A category is thus defined by structure of shared alcohol – either benzyl or 2-phenylethyl. Intra-category variation in molecular structure is dependent solely upon the length of carboxylic acid alkyl chain, which increases from C2 to C12.
Similarity of physicochemical properties	**Low**	Structural variation within each respective category is defined solely by length of alkyl chain present upon the carboxylic acid functionality. As such, whilst experimentally-derived physicochemical properties are reported for only a comparatively small number of target compounds, variation in parameters such as melting point, boiling point, vapour pressure and solubility may be predicted reliably using existing *in silico* models.

		Conservation of relevant functional groups across members further enables is also consistent with the variation in physicochemical properties.
Similarity of toxicokinetic data	**Low to medium**	Despite paucity of experimental data, there is concordance in the ADME profiles of the esters and alcohols examined. This is evidence from *in vivo* and *in vitro* outcomes, which indicate the commonality of the key hydrolysis step, followed by both alcohol conjugation and excretion and carboxylic acid integration into catabolic pathways.
Similarity of other supportive data	**Low**	Extensive *in silico* evidence, derived from a variety of established computational approaches, reveals commonality in toxicodynamic and toxicokinetic effects for all compounds.
Number of analogues used for read-across	**Low to medium**	Experimental toxicodynamic data were obtained for only one of the thirteen benzyl esters. Within the 2-phenylethyl category, this number falls to zero. Proposed common alcohol metabolites, from which relevant toxicological effects originate are, however, well characterised – in turn serving to mitigate the low number of toxicity data for source compounds. As a result, greater uncertainly (medium) is associated with the 2-phenylethyl constituents as compared to their benzyl derivatives (low).
Quality of target endpoint data used for read-across	**Low**	Where present, *in vivo* characterisation of toxicodynamic effects was performed using consistent methodology, in line with recognised and accepted protocol. Namely, this consisted of 90-day repeat-dose administration in rodents, either through oral or dermal route, in accordance with procedures corresponding to OECD Test Guidelines 408 or 411.
Similarity of target endpoint data	**Low**	Within the single ester, benzyl acetate, for which experimental data for repeat-dose *in vivo* toxicity were available, general concordance was evident with respect to effect across species and sex. NOAELs of 250-500 mg/kg/day were assigned, with higher susceptibility present within females. Benzyl and 2-phenylethyl alcohols similarly each induce toxicity within rodent species at moderately elevated dose. Neither short nor medium-chained carboxylic acids exhibited signs of stimulating sub-chronic toxicity at doses below 1000 mg/kg/day.
Concordance and weight-of-evidence of all data used in justifying hypothesis	**Low**	Consistency is present with respect to both *in vivo* and *in vitro* toxicokinetic and toxicodynamic data. Supported by predictions performed *in silico*, they each serve to provide weight to the hypothesis that the aryl alkanoate compounds investigated undergo swift metabolism to their toxicophoric alcohol and essential non-toxic carboxylic acid constituents, each of which proceed to undergo metabolism and excretion through shared pathways. As such, mitigation for the comparatively small number of toxicity data available for the source material may be assumed with confidence.
Overall uncertainty of read-across	**Low**	Despite the necessity in extrapolating outcomes from a comparatively small pool of source data, robust theoretical backing – assisted in the form *in silico* screening – can be provided for the hypothesis that such toxic effects emerge through single shared common products of metabolism (aryl alcohols), themselves characterised through high quality and consistent experimental study. As such, it may be inferred with confidence that cross-category consistency is present with respect to toxicological profile, and that minor physicochemical variance amongst members has minimal impact on toxicity. It should be noted that the wider data availability apparent across the benzyl ester category lends reduced uncertainty to predictions performed across it, relative to those within the 2-phenyethyl family.

Table 4. Assessment of the read-across in terms of uncertainty, characterised in accordance with OECD IATA guidelines.

4. Statement of uncertainty

Levels of confidence attributable to several aspects key to establishing the validity of the read-across are expressed in terms of OECD IATA "Uncertainty" rating, as detailed within Table 4.[8] Within each set considered, experimental toxicodynamic data may be found for only a comparatively small proportion of individual compounds (one benzylic alcohol ester and zero 2-phenylethyl alcohol esters each from thirteen, also shared alcohol constituents). Whilst this might appear insufficient for reliable prediction across such a large number of potential targets, the great similarity in structure between compounds ensures that an identical mechanism of action – one dependent upon swift enzyme-mediated hydrolysis to the toxicophoric aryl alcohol unit – may be assumed to be applicable throughout. Considering this mechanism further, it is necessary to discuss the potential influence which structural variations across the parent compounds might have with respect to their absorption and distribution. Although there are few experimental data, physicochemical properties, which vary with alkyl chain length, may be reliably predicted. Whilst it is feasible to presume that such subtle alterations in lipophilicity and volatility across the category are inconsequential due to the rapid activation towards the alcohol toxicophore, a level of uncertainty remains on account of the lack of experimental evidence directly confirming the properties of the longer chain analogues. Through adoption of a "worst case" approach, reading across directly from the aryl alcohol, such concerns may be allayed. Naturally, a heightened degree of doubt will continue to exist within the 2-phenylethyl category on account of the comparative lack of direct evidence confirming, or otherwise, the behaviour of its members.

Where present, the experimental assessments of toxicokinetic and toxicodynamic properties are themselves of high quality, lending credibility to the values and effects extrapolated across. The shared alcohols within the benzyl and 2-phenylethyl categories are notably well characterised, standing to provide dependable prediction of toxicokinetics and toxicodynamics across their respective esters with adoption of a "worst-case" reading. With respect to carboxylic acids, data are

459 recovered across a range of chain compositions extending to C12. As such, interpolation towards
460 properties within intermediate-length analogues may be performed with confidence.

461

462 **5. Conclusion**

463 The outcomes presented illustrate the formulation of a read-across protocol for 90-day, repeat-dose
464 toxicity within a series of aryl alcohol alkyl carboxylic acid esters, framed to comply with the
465 guidelines within the OECD IATA scheme.[7] The nature of the principles underpinning the read-
466 across – namely that a category of similar compounds, possessing a minor structural variation
467 accompanied by predictable alteration in physicochemical properties, produce toxicity upon
468 activation to a shared toxicophoric species – is in accordance with Scenario 3 of the European
469 Chemical Agency's Read-Across Assessment Framework.[50]

470 Two distinct sub-categories of esters were formed, unified by a shared aryl alcohol substituent and
471 with variance within confined solely to the length of carboxylic acid alkyl chain. Levels of read-across
472 certainty, and therefore OECD IATA "Uncertainty" ranking, differ only slightly between both,
473 reducing with the availability of suitable experimental data attesting the properties of source
474 compounds. High-quality *in vivo* assessments for both the toxicodynamic and toxicokinetic
475 properties of benzyl alcohol and acetate were available from the literature. Conversely, whilst 2-
476 phenylethyl alcohol has been subject to wide examination, data relating its acetate are less robust.

477 In order to provide necessary theoretical support, a mechanistic hypothesis was proposed – namely
478 that the parent esters undergo rapid hydrolysis to their aryl alcohol and alkyl carboxylic acid units:
479 the former inducing toxicity through a basal cytotoxicity, or non-polar narcosis, mechanism prior to
480 metabolism and excretion, the latter being causative of, and contributory to, no adverse effect. On
481 account of these shared characteristics, NOAEL values and specific effects noted for the alcohol can
482 hence be expanded across its alkanoate category with confidence through adoption of a "worst-case"

approach. Such a methodology can be further extended between categories, with the great structural similarities in toxicophoric units permitting the inference of properties across benzyl and 2-phenylethyl counterparts. Whilst some degree of uncertainty exists concerning the relatively small number of compounds for which experimental data may be recovered, and for the impact which the greater lipophilicity of category members with longer chain lengths might have upon their absorption and distribution, the quality of the data available, the similarity in chemical structure and the theoretical basis for the proposed shared mechanism are sufficient as they stand to support the plausibility and applicability of the approach adopted.

Expansion of experimental evidence would have immediately obvious potential in enhancing the confidence in the read-across validity. Within the scope of this study, it would appear feasible to develop greater certainty in the existence of shared toxicokinetic profiles through extension *in vitro* assessment of ester hydrolysis susceptibility, as recorded in 2-phenylethyl acetate, so that it may encompass a wider range of category members.[27] More challenging, but at the same time of greater utility, would be performance of further repeat-dose *in vivo* toxicity analysis, perhaps over a shortened time-scale of 28 days, upon selected compounds.

6. Acknowledgements

TWS gratefully acknowledges funding from The International Council for Animal Protection in OECD Programmes (https://www.icapo.org) in partial support of this study.

7. References

1. Dimitrov, S., Mekenyan, O., *An Introduction to Read-Across for the Prediction of the Effects of Chemicals*, in *In Silico Toxicology : Principles and Applications*, M.T.D. Cronin, Madden, J.C., Editor. 2010, p. 372-384. The Royal Society of Chemistry, Cambridge, UK
2. van Leeuwen, K., et al., *Using chemical categories to fill data gaps in hazard assessment.* SAR and QSAR in Environmental Research, 2009. **20**(3-4): p. 207-220.

3. Cronin, M.T.D., Madden, J.C., Enoch, S.J., Roberts, D.W., *Chemical Toxicity Prediction: Category Formation and Read-Across Applications*. 2013, The Royal Society of Chemistry, Cambridge, UK
4. Teubner, W., Landsiedel, R., *Read-across for hazard assessment: The ugly duckling is growing up.* Altern. Lab. Anim. , 2015. **43**: p. 67-71.
5. Przybylak, K.R., et al., *Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected β-olefinic alcohols.* Computational Toxicology, 2017. **1**: p. 22-32.
6. Schultz, T.W., et al., *Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected n-alkanols.* Computational Toxicology, 2017. **2**: p. 12-19.
7. Ball, N., et al., *The challenge of using read-across within the EU REACH regulatory framework; how much uncertainty is too much? Dipropylene glycol methyl ether acetate, an exemplary case study.* Regulatory Toxicology and Pharmacology, 2014. **68**(2): p. 212-221.
8. Organization for Economic Co-Operation and Development (OECD). *Report on considerations from case studies on integrated approaches for testing and assessment (IATA)*, E. Directorate, Editor. 2016.
9. Belsito, D., et al., *A toxicological and dermatological assessment of aryl alkyl alcohol simple acid ester derivatives when used as fragrance ingredients.* Food and Chemical Toxicology, 2012. **50**: p. S269-S313.
10. Api, A.M., et al., *RIFM fragrance ingredient safety assessment, Benzyl acetate, CAS Registry Number 140-11-4.* Food and Chemical Toxicology, 2015. **84**: p. S15-S24.
11. McGinty, D., et al., *Fragrance material review on phenethyl acetate.* Food and Chemical Toxicology, 2012. **50**: p. S491-S497.
12. Heymann, E., *Carboxylesterases and amidases*, in *Enzymatic Basis of Detoxication*, W.B. Jakoby, Editor. 1980, Academic Press: New York. p. 291-323.
13. Heymann, E., *Hydrolysis of carboxylic esters and amides*, in *Metabolic Basis of Detoxication*, W.B. Jakoby, Bend, J.R., Caldwell, J., Editor. 1982, Academic Press: New York. p. 229-245.
14. Fang, Z., et al., *Anesthetic potencies of n-alkanols: Results of additivity and solubility studies suggest a mechanism of action similar to that for conventional inhaled anesthetics.* Anesthesia and Analgesia, 1997. **84**(5): p. 1042-1048.
15. McKarns, S.C., et al., *Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity.* Fundamental and Applied Toxicology, 1997. **36**(1): p. 62-70.
16. Vinken, M. and B.J. Blaauboer, *In vitro testing of basal cytotoxicity: Establishment of an adverse outcome pathway from chemical insult to cell death.* Toxicology in Vitro, 2017. **39**: p. 104-110.
17. National Toxicology Program (NTP). *Toxicology and carcinogenesis studies of benzyl alcohol in F344/N rats and B6C3F$_1$ mice. NTP TR-343*. 1989, NIH Publication No. 89-2599: USA.
18. National Toxicology Program (NTP). *Toxicology and carcinogenesis of benzyl acetate (CAS NO. 140-11-4) in F344/N rats and B6C3F$_1$ mice (gavage studies). NTP TR-250*. 1986, NIH Publication No. 86-2506: USA.
19. Owston, E., R. Lough, and D.L. Opdyke, *A 90-day toxicity study of phenylethyl alcohol in the rat.* Food and Cosmetics Toxicology, 1981. **19**(6): p. 713-715.
20. Schultz, T.W., et al., *A strategy for structuring and reporting a read-across prediction of toxicity.* Regulatory Toxicology and Pharmacology, 2015. **72**(3): p. 586-601.
21. Gauhar, Z., et al., *Genomic mapping of binding regions for the Ecdysone receptor protein complex.* Genome Research, 2009. **19**(6): p. 1006-1013.
22. Organization for Economic Co-Operation and Development (OECD). *OECD Guideline for the Testing of Chemicals: Repeated Dose 90-day Oral Toxicity Study in Rodents (408)*. 1998.
23. Organization for Economic Co-Operation and Development (OECD). *OECD Guideline for Testing of Chemicals: Subchronic Dermal Toxicity: 90-day Study (411).* 1981.

24. Organization for Economic Co-Operation and Development (OECD). *SIDS Initial Assessment Profile: Propionic Acid*. 2007, United Nations Environmental Program (UNEP).
25. Amoore, J.E., et al., *Synthetic flavors - efficiency and safety factors for sweaty and fishy odorants.* Chemical Senses & Flavour, 1978. **3**(3): p. 307-317.
26. Fitzhugh, O.G., Schouboe, P.J., Nelson, A.A., *Oral toxicities of lauric acid and certain lauric acid derivatives.* Toxicol. Appl. Pharmacol., 1960. **2**: p. 59-67.
27. Longland, R.C., W.H. Shilling, and S.D. Gangolli, *Hydrolysis of flavoring esters by artificial gastrointestinal juices and rat tissue preparations.* Toxicology, 1977. **8**(2): p. 197-204.
28. Leegwater, D.C., van Straten, S., *In vitro study on the hydrolysis of twenty-six organic esters by pancreatin.* TNO Central Institute for Nutrition and Food Research, 1974. **Report 4319**.
29. Drake, J.J.P., et al., *Short-term toxicity of isoamyl salicylate in rats.* Food and Cosmetics Toxicology, 1975. **13**(2): p. 185-193.
30. Yuan, J.H., et al., *Effects of gavage versus dosed feed administration on the toxicokinetics of benzyl acetate in rats and mice.* Food and Chemical Toxicology, 1995. **33**(2): p. 151-158.
31. Bray, H.G., Thorpe, W.V., White, K., *Kinetic studies of the metabolism of foreign organic compounds. 1. The formation of benzoic acid from benzamide, toluene, benzyl alcohol and benzaldehyde and its conjugation with glycine and glucuronic acid in the rabbit.* Biochem J., 1951. **48**(1): p. 88-96.
32. Caldwell, J., Kennedy, J.F., Chidgey, M.A.J., *Absorption and disposition of topically-applied Methylene-carbon-14 benzyl acetate in the rat.* Pharmacology and the Skin: Skin Pharmacokinetics, 1987. **1**: p. 209-213.
33. Williams, R.T., *Detoxification Mechanisms: The Metabolism and Detoxification of Drugs, Toxic Substances and Other Organic Compounds*. 1959, New York: John Wiley and Sons, Inc.
34. Diack, L.D., Lewis, H.B., *Studies in the synthesis of hippuric acid in the animal organism. VII. A comparison of the rate of elimination of hippuric acid after the ingestion of sodium benzoate, benzyl alcohol, and benzyl esters of succinic acid.* J. Biol. Chem., 1928. **77**: p. 89-95.
35. Gunamalai, V., R. Kirubagaran, and T. Subramoniam, *Hormonal coordination of molting and female reproduction by ecdysteroids in the mole crab Emerita asiatica (Milne Edwards).* General and Comparative Endocrinology, 2004. **138**(2): p. 128-138.
36. Politano, V.T., et al., *The Pharmacokinetics of Phenylethyl Alcohol (PEA): Safety Evaluation Comparisons in Rats, Rabbits, and Humans.* International Journal of Toxicology, 2013. **32**(1): p. 39-47.
37. Nelson, D.L., Cox, M.M., *Principles of Biochemistry*. Fifth ed. 2008: W.H. Freeman.
38. Wanders, R.J.A., et al., *Peroxisomes, lipid metabolism and lipotoxicity.* Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids, 2010. **1801**(3): p. 272-280.
39. Medes, G., S. Weinhouse, and N.F. Floyd, *Fatty acid metabolism: II. The breakdown of carboxyl-labeled butyric acid by liver tissue.* Journal of Biological Chemistry, 1945. **157**: p. 35-42.
40. Divincenzo, G.D. and M.L. Hamilton, *Metabolic-fate of isobutyric-C-14 acid in the rat.* Toxicology and Applied Pharmacology, 1979. **47**(3): p. 609-612.
41. McCloskey, S.E., et al., *Toxicity of Benzyl Alcohol in Adult and Neonatal Mice.* Journal of Pharmaceutical Sciences, 1986. **75**(7): p. 702-705.
42. He, J., et al., *Investigation on baseline toxicity to rats based on aliphatic compounds and comparison with toxicity to fish: Effect of exposure routes on toxicity.* Regulatory Toxicology and Pharmacology, 2014. **70**(1): p. 98-106.
43. Edelfors, S. and A. Ravn-Jonsen, *The Effects of Alcohols in Vitro on the Nervous Cell Membrane Measured by Changes in the (Ca2+/Mg2+) ATPase Activity and Fluidity of the Synaptosomal Membrane.* Pharmacology & Toxicology, 1990. **67**(1): p. 56-60.
44. McCreery, M.J. and W.A. Hunt, *Physicochemical correlates of alcohol intoxication.* Neuropharmacology, 1978. **17**(7): p. 451-461.

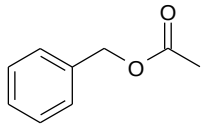
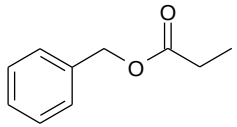
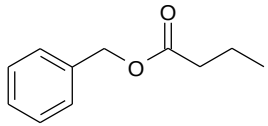
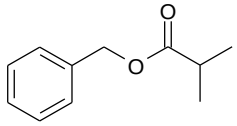
45. McKim, J.M., S.P. Bradbury, and G.J. Niemi, *Fish acute toxicity syndromes and their use in the QSAR approach to hazard assessment.* Environmental Health Perspectives, 1987. **71**: p. 171-186.
46. Koleva, Y.K., et al., *Modelling acute oral mammalian toxicity. 1. Definition of a quantifiable baseline effect.* Toxicology in Vitro, 2011. **25**(7): p. 1281-1293.
47. Munch, J.C., *Aliphatic alcohols and alkyl esters - narcotic and lethal potencies to tadpoles and to rabbits.* Industrial Medicine and Surgery, 1972. **41**(4): p. 31-&.
48. Veith, G.D., E.P. Petkova, and K.B. Wallace, *A baseline inhalation toxicity model for narcosis in mammals.* Sar and Qsar in Environmental Research, 2009. **20**(5-6): p. 567-578.
49. Alifimoff, J.K., L.L. Firestone, and K.W. Miller, *Anesthetic potencies of primary alkanols - implications for the molecular dimensions of the anesthetic site.* British Journal of Pharmacology, 1989. **96**(1): p. 9-16.
50. European Chemicals Agency (ECHA). *Read-Across Assessment Framework*. 2017, European Chemicals Agency.

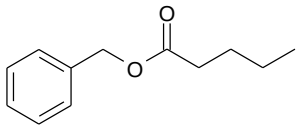
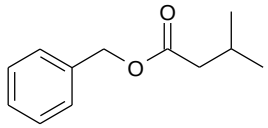
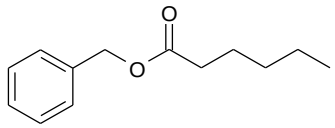
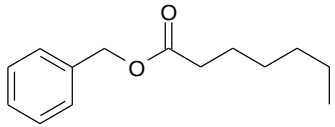
Supplementary Material

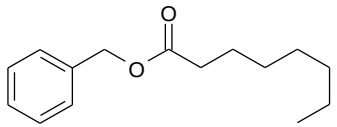
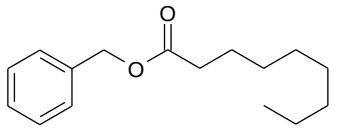
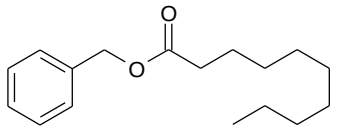
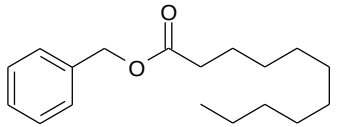
Read-across of 90-day rodent repeated-dose toxicity:

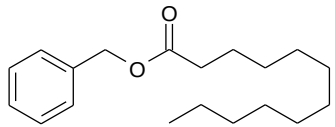
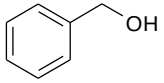
A case study for selected simple aryl alcohol alkyl carboxylic acid esters

643 **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

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
9	Benzyl octanoate	10276-85-4	c1ccccc1COC(=O)CCCCCCC		C15H22O2
10	Benzyl nonanoate	6471-66-5	c1ccccc1COC(=O)CCCCCCCC		C16H24O2
11	Benzyl decanoate	42175-41-7	c1ccccc1COC(=O)CCCCCCCCC		C17H26O2
12	Benzyl undecanoate	Not found	c1ccccc1COC(=O)CCCCCCCCCC		C18H28O2

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
13	Benzyl dodecanoate	140-25-0	c1ccccc1COC(=O)CCCCCCCCCCC		C19H30O2
14	Benzyl alcohol	100-51-6	c1ccccc1CO		C7H8O

644

645

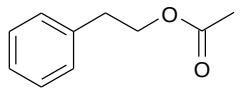
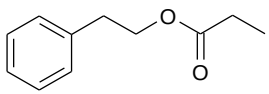
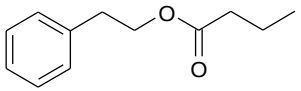
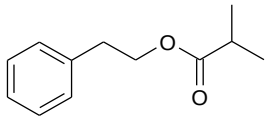
646

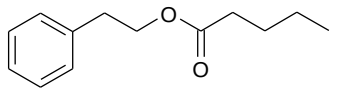
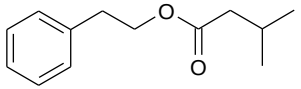
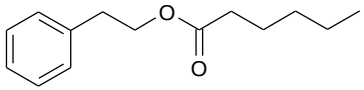
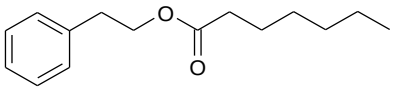
647

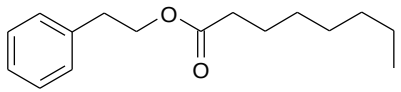
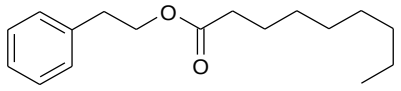
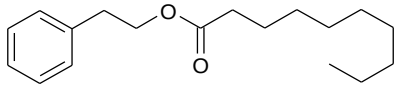
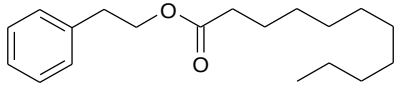
648

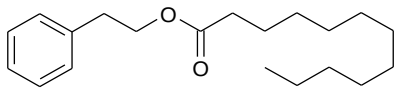
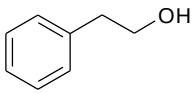
649 **Table 1b: Comparison of Substance Identification, Structure and Chemical Classifications of 2-Phenylethyl Derivatives**

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula

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

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

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
9	2-Phenylethyl octanoate	5457-70-5	c1ccccc1CCOC(=O)CCCCCCC		C16H24O2
10	2-Phenylethyl nonanoate	57943-67-6	c1ccccc1CCOC(=O)CCCCCCCC		C17H26O2
11	2-Phenylethyl decanoate	61810-55-7	c1ccccc1CCOC(=O)CCCCCCCCC		C18H28O2
12	2-Phenylethyl undecanoate	Not found	c1ccccc1CCOC(=O)CCCCCCCCCC		C19H30O2

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
13	2-Phenylethyl dodecanoate	Not found	c1ccccc1CCOC(=O)CCCCCCCCCCC		C20H32O2
14	2-Phenylethyl alcohol	60-12-8	c1ccccc1CCO		C8H10O

650

651

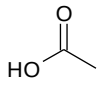
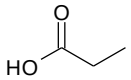
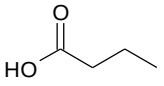
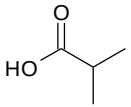
652

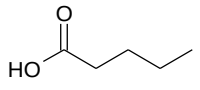
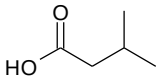
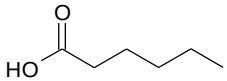
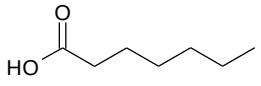
653

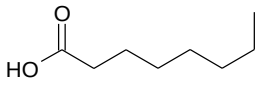
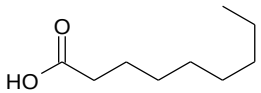
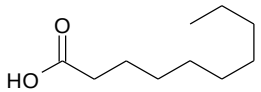
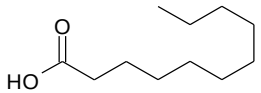
654

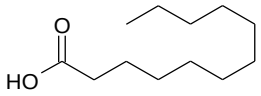
655 **Table 1c: Comparison of Substance Identification, Structure and Chemical Classifications of Carboxylic Acids**

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula

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

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
9	Octanoic acid	124-07-2	OC(=O)CCCCCCC		C8H16O2
10	Nonanoic acid	112-05-0	OC(=O)CCCCCCCC		C9H18O2
11	Decanoic acid	334-48-5	OC(=O)CCCCCCCCC		C10H20O2
12	Undecanoic acid	112-37-8	OC(=O)CCCCCCCCCC		C11H22O2

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula
13	Dodecanoic acid	143-07-7	OC(=O)CCCCCCCCCCC		C12H24O2

656

657

Table 2a: Comparison of Physicochemical and Molecular Properties of Benzyl Derivatives[1]

ID	Name	Molecular Weight [g/mol]	Log K_{ow}	Vapor Pressure [Pa at 25°C]	Density[c] [g/cm³ 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 derived from https://pubchem.ncbi.nlm.nih.gov or EPISuite v4.1.. Experimental outcomes (denoted *) displayed in preference to predicted (denoted †) as available.

661 **Table 2b: Comparison of Physicochemical and Molecular Properties of 2-Phenylethyl Derivatives[1]**

ID	Name	Molecular Weight [g/mol]	Log K_{ow}	Vapor Pressure [Pa at 25°C]	Density[c] [g/cm³ at 25°C]	Melting Point [°C]	Water Solubility [mg/L]	Boiling Point [°C]
1	2-Phenylethyl acetate	164.20*	2.30*	4.19*	1.0±0.1†	-31.1*	0.711†	232.6*
2	2-Phenylethyl propionate	178.23*	3.06†	6.86†	1.0±0.1†	21.44†	0.278†	238*
3	2-Phenylethyl butyrate	192.26*	3.55†	1.14†	1.0±0.1†	32.02†	44.15†	269.08†
4	2-Phenylethyl isobutyrate	192.26*	3.48†	3.63†	1.0±0.1†	21.57†	51.02†	250*
5	2-Phenylethyl valerate	206.29*	4.05†	0.398†	1.0±0.1†	42.34†	14.26†	285.11†
6	2-Phenylethyl isovalerate	206.29*	3.97†	0.907†	1.0±0.1†	24.45†	16.47†	275.55†
7	2-Phenylethyl hexanoate	220.31*	4.54†	0.144†	1.0±0.1†	52.39†	4.582†	300.23†
8	2-Phenylethyl heptanoate	234.34*	5.03†	0.0542†	1.0±0.1†	62.17†	1.467†	314.45†
9	2-Phenylethyl octanoate	248.37*	5.52†	0.0213†	1.0±0.1†	71.69†	0.4678†	327.16†
10	2-Phenylethyl nonanoate	262.4*	6.01†	8.79×10^{-3}†	1.0±0.1†	80.95†	0.1487†	340.18†
11	2-Phenylethyl decanoate	276.42*	6.50†	3.75×10^{-3}†	1.0±0.1†	90.02†	0.04716†	351.91†
12	2-Phenylethyl undecanoate	290.45*	6.99†	1.60×10^{-3}†	1.0±0.1†	99.04†	0.01491†	363.52†
13	2-Phenylethyl dodecanoate	304.48*	7.48†	6.78×10^{-4}†	0.9±0.1†	108.06†	4.70×10^{-3}†	375.12†
14	2-Phenylethyl alcohol	122.16*	1.36*	11.6*	1.0±0.1†	-27.1*	2.22×10^{4}*	218.2*

662 [1]Values typically derived from https://pubchem.ncbi.nlm.nih.gov or EPISuite v4.1.. Experimental outcomes (denoted *) displayed in preference to predicted (denoted †) as available.

663 **Table 2c: Comparison of Physicochemical and Molecular Properties of Carboxylic Acids[1]**

ID	Name	Molecular Weight [g/mol]	Log K_{ow}	Vapor Pressure [Pa at 25°C]	Density[c] [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 x 10^{3}*	1.1±0.1†	16.6*	1.00 x 10^{6}*	117.9*
2	Propionic acid	74.08*	0.33*	471*	1.0±0.1†	-20.07*	1.57 x 10^{5}*	141.1*
3	Butyric acid	88.11*	0.79*	220*	1.0±0.1†	-5.70*	6.00 x 10^{4}*	163.7*
4	Isobutyric acid	88.11*	0.94*	241*	1.0±0.1†	-46.0*	1.67 x 10^{5}*	154.4*
5	Valeric acid	102.13*	1.39*	26.1*	1.0±0.1†	-34.0*	2.40 x 10^{4}*	186.1*
6	Isovaleric acid	102.13*	1.16*	58.7*	1.0±0.1†	-29.3*	4.70 x 10^{4}*	176.5*
7	Hexanoic acid	116.16*	1.92*	5.8*	1.0±0.1†	-3.00*	1.03 x 10^{4}*	205.2*
8	Heptanoic acid	130.19*	2.42*	1.43*	0.9±0.1†	-7.30*	2.82 x 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 x 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 x 10^{-3}*	0.9±0.1†	43.2*	4.81*	298.9*

664 [1]Values typically derived from https://pubchem.ncbi.nlm.nih.gov or EPISuite v4.1.. Experimental outcomes (denoted *) displayed in preference to predicted (denoted †) as available.

665 **Table 3: Comparison of Experimental Toxicodynamic Information**

Name	*In Vivo* and *In Vitro* Toxicodynamic Data
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, labored 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-Phenylethyl 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. Hemorrhages 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-Phenylethyl 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-phenylethyl 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-phenylethyl 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.[4] 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 hematology 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.[4] 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.[5] 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.[5] 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.[6] 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.[6]

References

[1] National Toxicology Program (NTP) 1986. Toxicology and carcinogenesis of benzyl acetate (CAS NO. 140-11-4) in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program TR250, NIH Publication No. 86-2506, USA.

[2] National Toxicology Program 1989. Toxicology and carcinogenesis studies of benzyl alcohol in F344/N rats and B6C3F1 mice. NTP-TR-343; PB-89-2599.

[3] Owston, E., Lough, R. and Opdyke, D.L. 1981. A 90-day study of phenylethyl alcohol in the rat. Food and Cosmetics Toxicology; 19: 713-715.

[4] ECHA registration dossier (https://echa.europa.eu/registration-dossier/-/registered-dossier/14128/7/6/1).

[5] Amoore, J.E., Gumbmann, M.R., Booth, A.N. and Gould, D.H. 1978. Synthetic flavors: Efficiency and safety factors for sweaty and fishy odorants. *Chem. Senses Flavour* 3: 307-317.

[6] Fitzhugh, O.G., Schouboe, P.J. and Nelson, A.A. 1960. Oral toxicities of lauric acid and lauric acid derivatives. Toxicol. Appl. Pharmacol. 2: 59-67.

Table 4: Comparison of Experimental Toxicokinetic Information

Name	*In Vivo* and *In Vitro* Toxicokinetic Data
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 detoxification 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-Phenylethyl acetate	The *in vitro* potential for hydrolysis of 16 esters including 2-phenylethyl 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-Phenylethyl alcohol	The toxicokinetics of 2-phenylethyl alcohol has been extensively studied.[10] In rats and rabbits 2-phenylethyl 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-phenylethyl 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-phenylethyl 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-phenylethyl 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 physicochemical 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 vitro* 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]

713

References

[1] Chidgey, M.A.J. and Caldwell, J. 1986. Studies on benzyl acetate I. Effect of dose size and vehicle on the plasma pharmacokinetics and metabolism of [methylene-(14)C]benzyl acetate in the rat. Fd. Chem. Toxicol. 24: 1257-1265.

[2] Yuan, J.H., Goehl, T.J., Abdo, K., Clark, J., Espinosa, O., Bugge, C. and Garcia, D. 1995. Effects of gavage versus dosed feed administration on the toxicokinetics of benzyl acetate in rats and mice. Fd. Chem. Toxic. 33: 151-158.

[3] McMahon, T.F., Diliberto, J.J. and Birnbaum, L.S. 1989. Age-related changes in the disposition of benzyl acetate: A model compound for glycine conjugation. Drug Metab. Dispos. 17: 506-512.

[4] Caldwell, J., Kennedy, J.F. and Chidgey, M.A.J. 1987. Absorption and Disposition of Topically-applied Methylene-carbon-14 Benzyl Acetate in the Rat. In Pharmacology and the Skin; Vol. 1. Skin Pharmacokinetics, 209-213.

[5] Chidgey, M.A.J., Kennedy, J.F. and Caldwell, J. 1987. Studies on benzyl acetate. III. The percutaneous absorption and disposition of [Methylene{14}C]benzyl acetate in the rat. Fd. Chem. Toxicol.25: 521-525.

[6] Williams, R.T. 1959. Detoxification Mechanisms. Chapter 10, pp.318-347. John Wiley and Sons Inc. New York.

[7] Diack, S.L. and Lewis, H.B. 1928. Studies in the synthesis of hippuric acid in the animal organism: A comparison of the rate of elimination of hippuric acid after the ingestion of sodium benzoate, benzyl alcohol and benzyl esters of succinic acid. J. Biol. Chem. 77: 89-95.

[8] Bray, H.G., Thorpe, W.V. and White, K. 1951. Kinetic studies of the metabolism of foreign organic compounds. Biochem. J. 48: 88-96.

[9] Longland, R.C., Shilling, W.H. and Gangolli, S.D. 1977. The hydrolysis of flavouring esters by artificial gastrointestinal juices and rat tissue preparations. Toxicology 8: 197-204.

[10] ECHA phenylethyl alcohol (https://echa.europa.eu/registration-dossier/-/registered-dossier/13615/7/2/2).

[11] Politano, V.T., Diener, R.M., Christian, M.S., Hawkins, D.R., Ritacco, G. and Api A.-M. 2013. The pharmacokinetics of phenylethyl alcohol (PEA): Safety evaluation comparisons in rats, rabbits, and humans. International Journal of Toxicology; (formerly Journal of the American College of Toxicology), 32(1), 39-47.

[12] Nelson, D.L. and Cox, M.M. 2008. Lehninger Principles of Biochemistry, Fifth Edition. W. H. Freeman.

[13] Wanders, R.J.A., Ferdinandusse, S., Brites, P. and Kemp, S. 2010. Peroxisomes, lipid metabolism and lipotoxicity. Biochim Biophys Acta 1801: 272-280.

[14] Medes, G., Weinhouse, S., and Floyd, N.F. 1945. Acid metabolism II. The breakdown of carboxyl-labeled butyric acid by liver tissue. J. Biol. Chem. 157: 35-41.

[15] DiVicenzo, G.D. and Hamilton, M.L. 1979. Metabolic fate of [1-^{14}C]isobutyric acid in the rat. Toxicology and Applied Pharmacology 47: 609-612.

Table 5: Summary of *in silico* Toxicodynamic Screening Outcomes

Name	Cramer classification	OECD QSAR Toolbox	COSMOS profiling	Derek Nexus
Benzyl alkanoate esters*	Class I	No alert	No alert	No alert
Benzyl alcohol	Class I	No alert	No alert	Irritation GI tract
2-Phenylethyl alkanoate esters*	Class I	No alert	No alert	No alert
2-Phenylethyl alcohol	Class I	No alert	No alert	Irritation GI tract

* Denotes all considered aryl alcohol esters within category, from acetate to dodecanoate.

761 **Table 6: Summary of *in silico* Toxicokinetic and Metabolic Screening Outcomes**

762

Name	OECD QSAR Toolbox		MetaPrint 2D-React	TIMES-SS	Meteor Nexus
	Rat liver S9	Skin metabolism			
Benzyl alkanoate esters*	Benzyl alcohol Benzoic acid Benzaldehyde *Carboxylic acid*	Benzyl alcohol Carboxylic acid	Not available†	Not available†	Not available†
Benzyl alcohol	Benzoic acid Benzaldehyde	Benzoic acid Benzaldehyde	Not available†	Not available†	Not available†
2-Phenylethyl alkanoate esters*	2-Phenylethyl alcohol 2-Phenylacetic acid 2-Phenylacetaldehyde 2-Keto-2-phenylethyl alcohol *2-Hydroxy-2-phenylethyl alkanoate* *2-Keto-2-phenylethyl alkanoate* *Carboxylic acid*	2-Phenylethyl alcohol *Carboxylic acid*	Not available†	Not available†	Not available†
2-Phenylethyl alcohol	2-Phenylacetic acid 2-Phenylacetaldehyde 4-Hydroxyphenylethanol 3,4-Dihydroxyphenylethanol	2-Phenylacetic acid 2-Phenylacetaldehyde	Not available†	Not available†	Not available†

763 * Denotes all considered aryl alcohol esters within category, from acetate to dodecanoate.

764 † Data not presented owing to proprietary nature of software.

765

766

Figure 1. Schematic representation of metabolic pathways concerning benzyl alcohol esters