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

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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.

Introduction

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)

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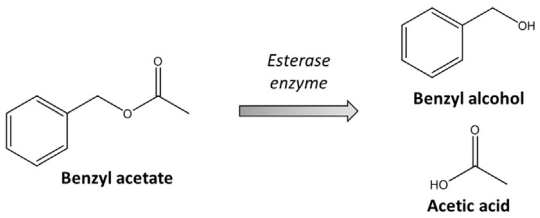

Fig. 1. Simplified scheme outlining enzyme-mediated hydrolysis of benzyl acetate to yield aryl alcohol and alkyl carboxylic acid constituents.

guidelines [8].

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, Fig. 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.

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.

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.

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.

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].

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,