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Read-across for rat oral gavage repeated-dose toxicity for short-chain mono-alkylphenols: A case study

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ABSTRACT

Short-chain mono-alkylphenols provide an example of where a category-approach to read-across may be used to estimate the repeated-dose endpoint for a number of derivatives. Specifically, the NOAELs of 50 mg/kg bw/d for mono-methylphenols based on a LOAEL of very low systemic toxicity can be read across with confidence to untested mono-alkylphenols in the category. These simple alkylphenols are non-reactive and exhibit an unspecific, reversible polar narcosis mode of toxic action. Briefly, polar narcotics act via unspecific, reversible interactions with biological membranes in a manner similar to cataleptic anaesthetics. The read-across premise includes rapid and complete absorption via the gastrointestinal tract, distribution in the circulatory system, first-pass phase II metabolism in the liver and elimination of sulphates and glucuronides in the urine. Thus, toxicokinetic parameters are considered to be similar and have the same toxicological significance. Six analogues have high quality experimental oral repeated-dose toxicity data (i.e., OECD TG 408 or OECD TG 422). These repeated-dose toxicity test results exhibit qualitative consistency in symptoms. Typical findings include decreased body weight and slightly increased liver and kidney weights which are generally without concurrent histopathological effects. The sub-chronic findings are quantitatively consistent with the No Observed Adverse Effect Level (NOAEL) of $\geq$50 mg/kg bw/d.

Chemical similarity between the analogues is readily defined and data uncertainty associated with the similarities in toxicokinetic properties, as well as toxicodynamic properties, are low. Uncertainty associated with mechanistic relevance and completeness of the read-across is low-to-moderate, largely because there is no adverse outcome pathway or intermediate event data. Uncertainty associated with mechanistic relevance and completeness of the read-across is reduced by the concordance of *in vivo*, *in vitro*, US EPA toxicity forecaster (ToxCast) results, as well as *in silico* data. The rat oral repeated-dose NOAEL values for the source substances can be read across to fill the data gaps of the untested analogues in this category with uncertainty deemed equivalent to results from a TG 408 assessment.

Introduction

Read-across

Grouping of organic chemicals with the intention of conducting read-across is a method that has application in regulatory toxicology. The principle philosophy of a toxicological read-across is that chemicals that are similar in molecular structure exhibit similar chemical properties and, in-so-doing, demonstrate similar toxicokinetic and toxicodynamic properties. As a consequence, experimentally-derived toxicokinetic and toxicodynamic properties from one or several substance(s), the source chemical(s), can be read across to fill the data gap for other substances, the target chemicals. This type of data gap filling is particularly useful for cosmetic ingredients, where *in vivo* testing in Europe is legislatively prohibited [1].

Read-across arguments can be used for different purposes. The style of the read-across often differs with purpose. A wide-domain style is typically associated with screening and priority setting. Wide-domain applications have multiple target chemicals, often one, but generally three or fewer source substances. In contrast, narrow-domain read-across exercises include those associated with the development of a substance-specific assessment, such as with a REACH dossier. In this case study, a wide-domain approach is used.

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Short-chain alkylphenols: an overview of existing knowledge

Alkyl-substituted phenols are a structurally complex group of compounds, which differ in both the size and shape of their substituents and their position(s) on the phenolic ring. They are hypothesised to act as polar narcotics by way of unspecific, reversible interactions with biological membranes in a manner similar to cataleptic anaesthetics. There are sufficient *in vivo* data available and there are also *in vitro* data from ToxCast for several of the chemicals in this class [2]. In a preliminary investigation of alkylphenols, it was revealed that *in vivo* oral repeated-dose exposure to alkyl-substituted phenols gives rise to a variety of toxicity symptoms including toxicities involving the liver, kidney, blood and whole body effects with No Observed Adverse Effect Level (NOAEL) values ranging from >100 to <10 mg/kg bw/d [3]. Moreover, experimental results of toxicokinetics parameters are inconsistent. These toxicokinetic and toxicodynamic differences increase uncertainty associated with read-across [4]. Endpoint specific factors affecting prediction uncertainties include how molecular structure impacts metabolism and clearance, as well as repeated-dose potency.

Goal and aims

From our preliminary investigation, we conclude that alkylphenols are not likely to form a single category for repeated-dose toxicity read-across. Further, we hypothesised based on bioavailability, and distribution, and mechanistic considerations, it was highly likely that a single category could be formed for mono-alkylphenols, especially short-chain (i.e., C4 or less) derivatives. It is the intent of this case study to demonstrate that short-chain, mono-alkylphenols provide a high-quality example whereby the category approach to read-across may provide predictions for data gap filling for the oral gavage sub-chronic repeated-dose endpoint. In this scenario, the chemical category represents analogues which are non-reactive and exhibit no specific mode of toxic action and metabolism being consistent across the domain has minimal toxicological relevance.

The particular aims in this read-across case study were: 1) the use of online ECHA registrations information as a primary guide to, and source of, toxicokinetic and toxicodynamic data, 2) the incorporation of sub-chronic repeated dose toxicity data and data for alkylphenols residing outside the applicability domain of the case study, and 3) the incorporation of high-through-put screening (HTS) data in the form of ToxCast data [5,6] and of *in silico* nuclear receptor binding predictions [7]. The specific aim of using all sub-chronic repeated-dose toxicity data (e.g., data from Organization for Economic Co-Operation and Development (OECD) test guidelines (TG) 408, TG 422 and TG 407 studies) was to increase the *in vivo* weight-of-evidence (WoE) and thereby reduce toxicokinetic and toxicodynamic uncertainties. The specific aim of the HTS data and *in silico* predictions was to reduce uncertainty associated with mechanism plausibility.

As a case study, this category assessment is designed to illustrate specific issues associated with predicting sub-chronic health effects [8]. It is not intended to be related to any regulatory discussions on this chemical group.

Preliminary investigations

Toxicokinetic differences

A preliminary examination of data revealed that the alkyl substitution pattern of phenol impacts toxicokinetics. In particular, the size and number of the ortho-substitution impact metabolism, as well as substitution in the para position. While species differences in metabolism of phenol have been shown, humans and rats showed similar metabolic pathways and quantities of metabolites in urine [9]. It was concluded that the rat is likely to be a good surrogate for human metabolism of phenol.

Hughes and Hall investigated the metabolism and clearance of phenol in rats [10]. The study was comparable to OECD TG 417 with acceptable restrictions. Briefly, female F344 rats (3–4/group) received 0.03 mg/kg bw ^{14}C-labelled phenol via oral administration. Radioactivity in urine and faeces was analysed after sampling in metabolism cages; the animals were sacrificed 72 h after application and radioactivity in organs, carcass and washings determined.

Phenol showed rapid and complete absorption and was distributed throughout the body after oral exposure. Of the administered radioactivity, 70–85% of the recovered dose was excreted in urine 4 h after administration and urinary elimination was essentially complete by 12 h. After 72 h, 95% of the applied dose was excreted via urine and only 1–3% was excreted via faeces. Specifically, after oral dosing 63.4 ± 2.3% was excreted as phenyl sulphate and 26.8 ± 2.7% was excreted as phenyl glucuronides. Similar findings are reported for methylphenols [11].

Takahashi and Hiraga conducted an investigation of the metabolism and clearance of 2,4,6-tri-*tert*-butylphenol in rats [12]. Clearance studies (dosed by oral gavage and in the diet) and the analysis of urinary and faecal metabolites (dosed via the diet) took place. For clearance studies, male Sprague-Dawley rats received oral doses (260 mg/kg) of 2,4,6-tri-*tert*-butylphenol by gavage in soy bean oil following overnight starvation; rats given 2,4,6-tri-*tert*-butylphenol via the diet *ad libitum* were also used for clearance studies. At various times, rats were killed and blood, liver, spleen, kidneys, testes and samples of epididymal adipose tissue were collected for analysis. For the analysis of biliary excreted metabolites, the bile duct was cannulated with polyethylene tubing for the collection of bile. For the analysis of urinary and faecal metabolites, rats were fed a diet containing 0.2% test material for two days, and urine and faeces were collected. Single oral doses were well-absorbed in the rat. Peak blood levels of the test material were reached in 15–60 min. The blood elimination half-lives were 18.2 min for the α-phase and 11.8 h for the slower β-phase. Maximum tissue concentrations were reached after 2–3 h in the liver, 2–6 h in the kidneys, 1.5–2.5 h in the spleen and >24 h in epididymal adipose tissues. 2,4,6-Tri-*tert*-butylphenol and its metabolites were not excreted in the urine; a metabolite, but not the parent compound, was detected in the faeces. The faecal metabolite had a molecular weight of 261 g/mol and was considered to be a 2,4,6-tri-*tert*-butylphenoxy radical. The phenoxy radical was also detected in the bile of rats.

Several metabolic pathways and numerous metabolites of 2,6-di-*tert*-butyl-4-methylphenol are known. The main metabolic pathway leads to the alcohol, aldehyde and acid derivatives by stepwise oxidation of the 4-methyl group [13]. However, a cyclic metabolic pathway via quinoid metabolites (i.e., 2,6-di-*tert*-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadienone and 2,6-di-*tert*-buty-4-hydroxy-4-methyl-2,5-cyclohexadienone) has been described in rat liver microsomes [14]. Yamamoto et al. detected reactive metabolites (i.e., 2,6-di-*tert*-butyl-p-benzoquinone and 2,6-di-*tert*-butylhydroquinone) possibly also as a result of this pathway [15]. A further quinoid metabolite, 2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone is considered to be a possible reactive metabolite [16].

Conning and Phillips studied the toxicokinetics of 2,6-di-*tert*-butyl-4-methylphenol following oral administration [17]. For most species, hindered phenols (*ortho*-substituted) are cleared more slowly than unhindered phenols, due to increased enterohepatic circulation. Oxidative metabolism (i.e., phase 1 reactions) is mediated by the microsomal monooxygenase system; oxidation of the