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Metabolomics as read-across tool: A case study with phenoxy herbicides

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ABSTRACT

New technologies, such as metabolomics, can address chemical grouping and read across from a biological perspective. In a virtual case study, we selected MCPP as target substance and MCPA and 2,4-DP as source substances with the goal to waive a 90-day study with MCPP. In order to develop a convincing case to show how biological data can substantiate read across, we used metabolomics on blood samples from the 28-day studies to show the qualitative and quantitative similarity of the substances. The 28-day metabolome evaluation of source substances and the target substance indicate liver and kidneys as target organs. 2,4-DP was identified as the best source substance. Using the information of the 90-day 2,4-DP study, we predicted MCPP's toxicity profile at 2500 ppm: reduced food consumption and body weight gain, liver and kidney weight increases with clinical-pathology changes and a moderate red blood cell parameter reduction. NOEL prediction for MCPP was below that of 2,4-DP (<500 ppm), and similar to that of MCPA (≥150 ppm). Qualitatively, these predictions are comparable to the results of the real MCPP 90-day study in rats (reduced food consumption and body weight gain, weight increases and clinical-pathology changes in liver and kidneys, reduced red blood cells values). Quantitatively, the predicted NOAEL (150 ppm) is similar to the actual study (NOEL = 75 ppm, NOAEL ≤ 500 ppm). Thus, the 90-day rat toxicity study of MCPP could have been waived and substituted by the 90-day results of 2,4-DP by using metabolome data of 28 day studies.

1. Introduction

The information requirements according to the REACH legislation, and the number of chemicals involved, lead to a very significant increase of animal testing. The REACH legislation, in principle notes that animal testing should be the last resort and promotes the development and use of alternative methods. However, with the exception of some less complex studies (e.g. skin and eye irritation), very little progress has been made to have validated and regulatory acceptable alternative methods in place for REACH testing (Hoefer et al., 2004; Hartung and Leist, 2008). Grouping of chemicals and subsequent read across from data rich chemicals belonging to the group is probably the most efficient way to provide the required safety information, while keeping the amount of animal testing to an absolute minimum. The big question here is the quality of the grouping process. Read-across entails the use of relevant information from analogous substances (the 'source' information) to predict properties for the 'target' substance(s) under consideration. Grouping and read-across may be based purely on structural similarity, however, with some risk of error. It would therefore seem prudent to include and take into account some biological data in the grouping process, whenever possible (van Ravenzwaay et al., 2012). These may come from in vitro studies, or could be derived from a limited number of base set animal studies. Omics technologies could serve as an important tool to enhance the quality and quantity of data obtained during regulatory toxicity testing (ECETOC, 2008; ECETOC, 2010).

ECHA has the obligation to evaluate if submitted read-across cases are sufficiently convincing to substitute these for standard tests. In response to this challenge ECHA developed and published the Read-Across Assessment Framework (RAAF) http://echa.europa.eu/documents/10162/13628/raaf_en.pdf. In this framework read-across approaches are assessed through the use of different scenarios and the quality of the case is consistently evaluated based on a number of predefined criteria. If supporting evidence is provided for a read-across case then this may be taken into account

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when conducting an assessment according to the RAAF. Many new approaches and methodologies for investigating properties of chemicals have been developed over the past years. To assess the value of these new approach methodologies (NAMs), ECHA organized a workshop in Helsinki in April 2016 called "Topical Scientific Workshop on New Approach Methodologies in Regulatory Science". The present paper was prepared as a case study for this workshop using metabolomics as a NAM to support read across.

In this paper we describe how metabolomics, can be used to address chemical grouping and read across from a biological perspective. The goal was to provide a convincing case to waive a 90-day rat study for the target substance MCPP (also referred to as Mecoprop or Mecoprop-p). Two other phenoxy-herbicides, MCPA and 2,4-DP (also referred to as Dichlorprop or Dichlorprop-P) were selected as source substances. It should be noted here that one of the requirements to serve as a case study was a substantial chemical similarity as specified in the RAAF. MCPP and 2,4-DP are phenoxypropionic acids and have a chiral centrum. In the past, these compounds were produced as racemic (50:50) mixtures of the two enantiomers. Since the 1990's the production has been modified to only produce one enantiomer (in documents generally specified by the addition of -p to the name of the compounds, e.g. mecoprop-p) which has the highest herbicidal activity. As the herbicidal activity is related to a plant specific receptor, not present in animals, the toxicity of racemic mixture and single enantiomer was shown to be identical. The modern toxicological package for both compounds has been generated in the 1990's and 2000's with the single enantiomer. The metabolome studies presented here were also performed with the single "−p" isomer. It should also be noted, that there are more phenoxy herbicides than the ones used for this case study, in particular 2,4-D, these, however were not included here, because of a lack of appropriate metabolomics data.

Within the context of ECHA's RAAF we work with the category approach, scenario 4 or 6. This scenario covers the category approach for which the hypothesis is based on different compounds, which have the same type of effect(s). For the REACH information requirement under consideration, the effects obtained in studies conducted with different source substances are used to predict the effects that would be observed in a study with the target substance if it were to be conducted. Concerning the strength of the effect (i.e. the major differences between cat 4 and 6) we would like the data to speak for itself and make a reasonable conclusion when all data are considered together. The overall purpose of this paper is to demonstrate the possibilities to assess toxicity by means of multi-parameter 'omics sciences', in this case particularly metabolomics.

For the read across case, the situation is as follows: there is an adequate 28-day rat study with MCPA, but only limited 28-day information for 2,4-DP. For all three substances, metabolome data from 28-day studies are available. The metabolome information is used for two purposes: 1) to predict the toxicological profile of each of the compounds, and 2) to compare the similarity of the metabolome of the source substances with the target substance and select the most appropriate one, to make a prediction of the 90-day toxicity in rats of the target compound. For both source substances 90-day studies are available. Finally, we compare the predicted outcome for the target substance with the real outcome.

1.1. Identity of the target substance

Structural information as well as phys-chem data on the target substance MCPP as well as on the source substances MCPA and 2,4-DP are depicted in Fig. 1.

The target substance and source substances are structurally similar. The target substance MCPP is a phenoxypropionic acid, and as such comparable with phenoxypropionic acid 2,4-DP. The target substance has a methyl and chlorine substituent in the 2,4-position, and this part of the molecule is thus most similar with MCPA. The structural similarities of the compounds can be quantified by Tanimoto Scores (Fig. 2).

The different parameters of acute toxicity for the target substance and the source substances are listed in Table 1.

1.2. Conclusion

Acute Toxicity: the acute toxicity of the target substance and the source substances are comparable.

Mutagenicity: overall there are no concerns about the genotoxicity of the target and source compounds.

2. Absorption, distribution, metabolism & excretion

Absorption, distribution, metabolism and excretion, in short ADME, parameters are available for all three substances (MCPP was reviewed by California Environmental Protection Agency, 1999; MCPA was reviewed by JMPR, 2012; 2,4-DP was reviewed by California Environmental Protection Agency, 2002). The ADME results are summarized in Table 2. For all substances ^{14}C − phenyl labelled test substance was administered once by gavage as an aqueous CMC suspension to rats. Animals were maintained in metabolism cages. Studies were basically performed according to the respective OECD and US_EPA test guidelines for kinetics and metabolism.

Overall, bioavailability for target and source substances is high (>90% at low dose levels), to a somewhat lesser extent at higher dose levels. For all substances there is rapid elimination predominantly through the urine (low dose levels 80–90%) at high dose levels to a slightly lesser extent. Fecal elimination accounts for ca. 10% or less at low dose levels, and increases up to ca. 20% at high dose levels. Reduced urinary excretion and increased fecal excretion at high dose levels indicate a slightly reduced bioavailability at high dose levels. There is no elimination through the expired air. Fast elimination is reflected in relatively short, less than 8 h, and comparable half-lives. The unchanged parent compound is for all three substances by far the major component in the blood. Metabolism is limited to the production of one or a few minor metabolites (e.g. for MCPA: hydroxylation of the methyl group of the alcohol (HMCPA), followed by a second hydroxylation to form the acid (CCPA)), some of which have been tested for systemic toxicity and shown to be less toxic (van Ravenzwaay et al., 2005). There were no major differences between male and female animals. In conclusion, the ADME properties of the target and sources substances are substantially similar.

3. Twenty-eight-Day toxicity studies

There are only few 28-day toxicity studies available in the public literature and most of these studies have been performed at relatively low dose levels. Hence, the toxicological profiles following 28 days of compound administration are not very well defined (with the exception of MCPA). The findings of these studies have been summarized in Table 3.

3.1. MCPA (van Ravenzwaay et al., 2005)

Five male and five female Wistar rats received MCPA at a dietary concentration of 2000 ppm for four weeks, with examinations according to OECD guideline 407. Test substance intake was 166 and 172 mg/kg body weight/day for males and females respectively.

MCPA caused no clinical signs either during the study or in the