PHENOXY HERBICIDES CASE STUDY CONSIDERED IN CONTEXT OF ECHA RAAF

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Disclaimer:
The assessment document has been prepared to facilitate the discussion at the Topical Scientific Workshop and does not represent ECHA's position. The assessors conducted the assessment of the scientific case study also based on data/information available for the respective phenoxy herbicides which could not be shown/included in the case study for various reasons.

Case Study:
Metabolomics as read-across tool: a case study with phenoxy herbicides. The purpose of this case study is to demonstrate the usefulness of a biology based tool to provide qualitative and quantitative information to improve chemical grouping for read-across purposes. The case study is not made to substitute any data, because the compounds used in this case have a full, agrochemical, toxicological data base.

Phenoxy herbicides Case Study Read-Across Hypothesis Summary:
Read-across is proposed to fill a data gap for 90-day oral repeated dose toxicity of the phenoxy herbicide MCPP. The read-across hypothesis is that as a result of their chemical structural similarity and a similar mode of action (based on available metabolomics data for the three substances and [partially limited] repeated dose studies), the toxicological properties of the category members are likely to be similar.

Read Across scenario according to ECHA RAAF:
This read-across is consistent with RAAF Scenarios # 4 – i.e. category approach with a read-across hypothesis based on different compounds which have the same type of effect(s).

Assessment of Read Across according to ECHA RAAF:
1. Evaluate the read-across hypothesis.
2. Identify the appropriate RAAF read-across 'Scenario' based on the hypothesis.
3. Evaluate all relevant read-across 'Assessment Elements' for that Scenario as detailed in RAAF. (Assessment elements are crucial scientific aspects to judge validity and reliability of the read-across.)
4. Assign a specific 'Assessment Option' for each assessment element. (Assessment options are pre-defined response/scores assigned based on the strength of information/evidence provided in the read-across to address the given assessment element.)
5. Determine outcome of the read-across assessment based on totality of 'Assessment Option' responses to all elements.

Phenoxy herbicide Case Study Considered in Context of the ECHA RAAF:

The table represents consideration of the phenoxy herbicide case study in the context of the ECHA RAAF. The table is structured as follows:
In first columns: the RAAF Assessment Elements (AE) that apply to RAAF Scenario #4.
In middle columns: the location (by page) and text from the phenoxy herbicide case study that addresses the AE features (described in the RAAF).
In last columns: an assessment option (AO) score for each AE based on consideration of the information provided in the case study. For AOs < 5, the rationale for the AO selection is provided along with suggested additions to the case study that would result in an improved AO score.

TABLE

RAAF SCENARIO #4		PHENOXY HERBICIDES Case Study		RAAF Assessment Option	
AE#	**Assessment Element/** Details	**Page#**	**Relevant Text and Tables in Case Study Report**	**AO#**	**Rationale**
C.1	**Identify/characterize substances which are members of the category, including impurity profile**		All data available, high purity, impurities known, no reason for concern.	5	
C.2	**Describe the structural similarity and allowable differences for category**	p 3 and 4	The target substance and source substances are structurally similar. The target substance MCPP is a phenoxy-propionic acid, and as such comparable with phenoxy propionic 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 can be quantified by Tanimoto Scores (Figure 1). A value of 0.6 indicates that two structures are similar; structures with a score > 0.6 are substantially similar.	5	
C.3	**Explain the link between the structural similarities/differences and the proposed prediction of property**		The target substance as well as the source substances consist of a phenoxy core structure with is methyl and chlorine substituted in the 2,4-position. Metabolism and toxicological properties of these compounds are expected to be comparable due to the high structural similarity (Tanimoto Score ≥ 0.75).	4	
C.4	**Demonstrate/discuss consistency of effects in data matrix**			5	
	Documentation includes discussion of consistency of data for predicted property, and any inconsistencies are explained		Is given for acute and local toxicity as well as genotoxicity. Repeated dose toxicity data is given as narrative.		
	Documentation includes discussion of occurrence of any other relevant effects (than predicted property)		There are no other relevant effects.		

	Consistency between predicted and related properties is demonstrated		ADME for the three substances is similar. Effects in the repeated dose study are comparable. Metabolome data shows that the three substances have the same target organs (liver, kidney).		
	Any clustering of effects w/ across structural features of category (or subcategories) is characterized		Structures and effects cluster.		
C.5	**Demonstrate adequacy of the source data to meet the info requirements**			5	
	Read-across source study is of adequate design		Source studies are regulatory toxicity studies for agro chemicals according to OECD guideline and GLP.		
	Test material used represents source		Test material represents with impurity spectrum the commercial and registered material.		
	Results of read-across source study are sufficient for C&L purposes		Dose levels and target organs are comparable and thus sufficient for classification and labelling purposes.		
4.1	**Identify all compounds to which the test organism is exposed**			5	Based on the toxicokinetics data provided in the case study it safe to assume that the unchanged parent compound is responsible for the toxicity observed
	Substances to which organism is exposed (for target and all sources) are identified, as well as how they are formed	p 5-8	Bioavailability for target and source substances is high (> 90% at low dose levels), to a somewhat lesser extent at higher dose levels. For all three 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. There is no elimination through the expired air. Fast elimination is reflected in relatively short 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. Overall, the ADME properties of the target and sources substances are substantially similar.		
	Supporting evidence of qualitative or quantitative kinetics is provided	p 5-8	See above		
4.2	**Identify the common underlying mechanism, qualitative aspects**			5	The metabolomics data together with the repeated dose studies provided establish a clear
	The mechanism that links the structures of the category members with the predicted property/effect is explained	p 15-16	All three compounds showed a clear effect on the liver, matching patterns for liver peroxisome proliferation and fibrate as well as phthalate induced liver toxicity, the MoA of these compounds being related to a lipid reducing effect, based on PPAR-alpha induction and subsequent peroxisome proliferation (table 5). Moreover, compounds		

			which matched well with the overall prolife of MCPP belonged to the group of fibrates and phthalates, both known to be PPAR-alpha agonists. In addition to the liver also the kidney was identified as a target organ. All treatments generated at least a weak match with the pattern for the inhibition of the transport of weak organic acids in the kidneys. Mechanistic studies with MCPA and related compounds have shown that this group inhibits the weak organic acid transporter in the kidney.		common underlying mechanism for liver and kidney toxicity.
	Supporting qualitative evidence from in vivo or in vitro studies or for uptake/kinetics (for negative read-across) is provided		ADME studies are OECD guideline, GLP studies performed for registration of agrochemicals. No further supporting evidence necessary.		
4.3	**Describe the quantitative aspects of the common underlying mechanism**			5	
	Prediction model, which defines the independent variable (structural feature or physchem property), is clearly documented. Prediction model is based on either a regular pattern or worst case.		Prediction is based on structural similarity of the compounds and of effects. Prediction is enhanced by full metabolome analysis of the three compounds and a qualitative and quantitative comparison thereof.		
	Explanation provided for how chemical structures influence kinetics and/or potency to determine the differences in strength of effects across category		The metabolome analysis provides quantitative information concerning potency differences in strength of effects across category, based on rounded down average of absolute medians of t-values of all metabolite changes as well as number of statistically significantly regulated metabolites.		
	Supporting evidence for the explanation and prediction model is provided		The additional prediction model based on metabolomics is comprehensibly described in the paper provided in the annex.		
	Any uncertainty for targets at the boundary of the category is addressed and/or worst-case approach is justified		This is a case with only three structurally similar compounds.		
4.4	**For any compounds not linked to the prediction (i.e. non-common compounds such as intermediates, metabolites, impurities of category members), characterize (or demonstrate no) influence on the prediction**			5	The toxicokinetic data provided in the case study suggest with high
	Documentation indicates whether other compounds	p 5-8	Bioavailability for target and source substances is high (> 90% at low dose levels), to a somewhat lesser extent at higher dose levels. For all three substances there is rapid		

	not linked to the prediction are present		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. There is no elimination through the expired air. Fast elimination is reflected in relatively short 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. The metabolism, mainly oxidation, results in an increased water solubility and a better renal elimination. The toxicity of one of such metabolites of MCPA, called CCPA, was studied. The results indicate that the metabolite was less toxic than the parent compound MCPA. Overall the ADME properties of the target and sources substances are substantially similar. There is no evidence, or reasons for concern that the metabolites formed would have a higher toxicity potential compared to the unchanged parent compounds.		certainty that the parent substances are responsible for the effects. There is no reason to suspect that one of few of the minor metabolites would have a higher toxicity potential than the parent
	Explanation is provided for how/why any other compounds formed lack influence on the predicted property		See above		
	Supporting evidence based on kinetics or lack of other effects in data matrix is provided (at minimum for target and RA source)		Effects and ADME for all three substances are substantially similar.		
4.5	**Characterize (or demonstrate no) occurrence of other effects than those covered by the read-across hypothesis and justification**			5	
	Documentation indicates whether other effects not linked to the prediction are present		The data provided establish a clear common underlying mechanism for liver and kidney toxicity together with effects on food consumption, body weight gain and blood parameters. There are no other effects observed other than those predicted.		
	Any other effects are evaluated on a case-by-case basis and it is explained why they are either irrelevant OR possibly indicative of additional mechanisms not identified in the hypothesis	Not relevant for this case study	Not relevant for this case study		
	Any uncertainty arising from possibility of additional	Not releva	Not relevant for this case study		

	mechanisms is addressed	nt for this case study			
4.6	**Demonstrate there is no bias influencing the prediction**			5	
	Criteria used in selection of sources is described and no otherwise suitable members have been excluded (or if so a justification is provided)		The three category members were chosen based on their structural similarity, and availability of toxicity and metabolomics data. One compound, which normally would have been included is 2,4-D. This compound is structurally similar to the other three compounds. It was not selected because of the unavailability of appropriate metabolomics data. For this particular case, the demonstration of the usefulness of a new technology to improve chemical grouping and read across, it was therefore not considered useful. The toxicity profile of 2,4-D however is substantially similar to the one of the compounds used for this case-study; i.e. liver and kidney as primary target organs. As such the omission of 2,4-D should not affect the validity of the present case study.		
	Conservative 'worst case' (highest concern) studies available on source(s) are used in RA or if not a justification is provided		Not relevant		

B. van Ravenzwaay, W. Mellert, K. Deckardt, K. Küttler: "The comperative toxicology of 4-chloro-2-methylphenoxyacetic acid and its plant metabolite 4-chloro-2-carboxyphenoxyacetic acid in rats", Regulatory Toxicology and Pharmacology 42 (2005) 47-54

ANNEX

Other relevant information from RAAF for this exercise:

Definitions

Property under consideration = outcome of a relevant study used to fulfil a REACH info requirement for the endpoint being read across.

Analogue approach = read-across is employed between a small number of structurally-similar substances; there is no trend in the property. If analogue approach uses more than one source or target substance, the assessment of the read-across approach has to be repeated for each source and/or target substance. (RAAF p6)

Category approach = read-across is employed between several substances that have structural similarity. The toxicological properties will either all be similar or follow a regular pattern. It may be possible to make predictions within the group for the target substance(s) on the basis of a demonstrable regular pattern or alternatively when there is more than one source substance in the category and no regular pattern is demonstrated for the property the prediction may be based on a read-across from a category member with relevant information in a conservative manner (worst case). (RAAF p6)

Trend = for category approach, there is a need to further take account of whether or not quantitative variations in the effects are observed among the category members. (RAAF p10)

Worst-case approach = the strength of effects in the target substance is actually expected to be lower than the strength of effects observed for the source substance. Scientific explanations for such situations may be based on kinetics (e.g. evidence for differences in bioavailability) or potency (e.g. evidence that structural features lead to higher potency for the source substance). (RAAF p6)

Biotransformation to common compounds = different substances (i.e. source and target chemicals) give rise to (the same) common compounds to which the organism is exposed. The common compound may be the unchanged form of one of the parent substances and the biotransformation product of the other substance OR a biotransformation product formed from both substances. (RAAF p9)

Different compounds have the same type of effect = as a result of structural similarity, different compounds cause the same type of effects. The different compounds may be the source and target substances themselves OR one or more of their biotransformation products. (RAAF p10)