Topical Scientific Workshop on
New Approach Methodologies in Regulatory Science
19 – 20 April, 2016

Break-out session 3

Case study from BASF

Read-across with metabolomics for phenoxy herbicides

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Question block 1: Case description

1. Are the chemical structures from the source substances and target substances sufficiently described to determine the structural (dis)similarities?

No real problems. It is clear that the substances are not the racemic mixtures of the past. However, it is not clear from the text which optical isomers are the subject of the read-across. This can easily be addressed. One participant desired a better underpinning of the choice of the substances based on chemometrics.

2. What is the regulatory purpose for which the case is prepared?

It is not clear from the document what the regulatory purpose is. The substances are herbicides with a lot of data. They have just been chosen to demonstrate the possibilities of the NAM. So in itself the case does not serve a regulatory purpose. However, the case was discussed as if it was aimed at meeting a regulatory data requirement, and not at screening or prioritization.

3. What is the property for which a prediction is attempted?

Ninety day repeated-dose oral toxicity with the rat. OECD 408

Question block 1: Case description

4. What is the hypothesis under which the prediction is attempted?

The scenario used for the RAAF assessment was either 2 for analogue approach or scenario 4 for category approach.

The actual mechanisms by which the three substances are expected to cause the same effects ***are established by metabolome pattern ranking****. This pointed clearly to liver toxicity (PPAR alpha) and kidney toxicity. This toxicity was observed for both sources. About the same metabolome changes were found with the target substance. It was therefore concluded that this substance would cause the same effects via the same mechanisms.*

The metabolome analysis combines the formulation of the hypothesis, its confirmation to an acceptable level of confidence and, in this case, a quantitative prediction.

Question block 1: Case description

5. What information obtained in animal studies is provided to support the prediction?

28-day studies with the two sources and the target. However, the doses of two of them (one source and the target) were too low. ADME studies.

6. What are the weak points of the justification, which are identified by the assessment according to the RAAF?

*Uncertainty about the occurrence of other effects of the target that are not reflected in its metabolome profile**. The presenter emphasized during the BOS that most (nearly all?) of the effects observed in 90-day RDT studies give rise to metabolome changes and that these changes are more sensitive than the effects.** However, this is not really elaborated upon in the case-study report.*

Question block 1: Case description

*Metabolome analysis was based on 28-day studies and used for RA between 90-day studies**. According to the presenter, all effects that can be seen in a 90–day study give already metabolome changes within about 14 days.***

An other uncertainty was caused by the low dose-levels applied in two crucial underpinning 28-day studies.

Moreover, there was some discussion about the of effects observed. In particular effects on the testes and the adrenals, which did not fit the mechanistic explanation. However, the presenter attributed the former to reduced food intake and did not regard the latter (discoloration) as relevant/adverse.

Question block 1: Case description

7. What supporting evidence is still missing or could be added to increase the confidence in the prediction?

Information on the general qualitative and quantitative sensitivity of the metabolome analysis to reflect the effects that can be observed in an oral 90-day RDT with rats.

This to build more confidence that the target will not show 'other'effects that are not reflected in the metabolome. A validation point.

Detailed insight in the results of the/a 90-day study with 2,4 D. The absence of 'other'effects for this substance would make the case stronger.

Combination with other NAM (bottom-up/top-down).

Etc.

Question block 2: Contribution of NAM information

8. What types of NAM information have been used in the case to increase the confidence in the prediction?

Metabolome profiling formed the core of the case. It was not an extra. Without the NAM there would not have been a viable hypothesis/mechanistic explanation.

9. Were the weak points in the prediction addressed?

Without the NAM, the case is 'empty'. It completely depends on the NAM.

Question block 3:
Detailed characteristics of the NAM information provided

10. Are the NAM methods used in the case study generally available?

The NAM depends on advanced AC analysis, statistical 'processing' and the availability of a database. The first two can be implemented by a well-equipped CRL. ***The database is not available.***

More transparency is necessary to get the NAM broadly accepted for read-across purposes. The assessing scientists from regulatory agencies find it important that they can judge the validity of the methods in (much) more detail.

The current document does not provide this level of detail. This holds in particular for the database on which the NAM is used.

In this respect it is also important to mention the need of standardization.

Question block 3:
Detailed characteristics of the NAM information provided

11. What scientific limitations are evident for the NAM information in the case study?

*Assuming that a detailed assessment of the underlying information would not give rise to problems (see Question 10), the scientific limitations of the NAM are in **THIS PARTICULAR CASE OF POSITIVE READ-ACROSS** limited. The outspoken character of the results gives confidence.*

Only the uncertainty of the occurrence of 'other' effects remains. Based on the statements of the presenter it seems that this uncertainty is not great.

Question block 3:
Detailed characteristics of the NAM information provided

12. What could be the barriers in using or generating the necessary NAM data?

Lack of transparency and detailed info;

Dependence of profile changes on experimental conditions and strain (see below);

The toxicological scope;

Need for standardization;

Need for validation;

Costs?????

Required technical facilities?

Required expertise (also for assessment)?

Additional discussion points

The relevance of high Tanimoto scores;

Chemical read-across versus biological read-across;

Dependence of metabolome profiles on experimental conditions, in particular animal strains,

Targeted and untargeted (fingerprinting) metabolome analysis; this case is a mixture;

Acceptance of the case and scoring; large majority in favour of acceptance.

Conclusions from break-out session 3

- The case convincingly demonstrates the great potential value of metabolome analyses to support read-across;
- The analysis shows that it is highly likely that the target will cause the same effects as were observed for the sources at about the same dose level;
- There is uncertainty about possible other effects of the target that are not reflected in the metabolome;
- Implementation requires more transparency on the methods and database, additional validation studies and standardization.