LOA Webinar

Utility of Metabolomics to Support Read-Across for UVCB substances under REACH

Questions and Answers

When do you take blood samples in the different studies? At the end? Is the time of sample collection critical?

For building up the MetaMap®Tox database, blood samples were taken on Days 7, 14 and 28, for the reference compounds tested. Overall, the observed metabolome changes were quite similar on Days 7, 14 and 28. However, it has to be taken into account that some effects need some time to manifest and therefore are detectable only after longer treatment periods (i.e., 14 or 28 days).

In standard study designs, samples would be collected on Days 7 or 14 (Dose Range-Finding studies) and Day 28 for regulatory (GLP) studies. For 90-day studies, intermediate samples could also be collected on Day 28, however, we have limited experience with this setup and one should consider that the overnight fasting will slightly impact the body weight data.

For OECD 421 or 422 studies, blood samples should be taken at the end of the treatment period. Note that only the males can be fully compared to the BASF data base. Females (due to pregnancy/ delivery) can still be used for comparison within a group of compounds.

How long were the rats exposed to the test substance? Why did you choose this duration?

For the reference compounds in the MetaMap-Tox® database, the rats were treated for a total of 28 days to comply with the OECD 407 Repeated Dose 28-day Oral Toxicity Study in Rodents test guideline. The LOA substances were tested in a 14-day dose range-finding study to provide dual purpose (dose selection and metabolomics) with blood sampling on Day 14, as the metabolome is sufficiently predictive after 14 days of exposure[1,2]. Based on statistical analysis performed in another rodent study, blood sampling on Day 14 produced more statistically significantly changed metabolites than other timepoints (Day 7 and Day 28) and could therefore be a more sensitive timepoint for assessing patterns of metabolome change associated with organ toxicity or a particular mode of action in rats. This may be related to the fact that 14 days is a sufficient time-period in which organ toxicity could occur (compared to a 7-day treatment), yet it is short enough to represent the organism in a non-homeostatic state. Limited experience with 90-day metabolome studies shows an overall reduced metabolome response towards the end of these studies. The beginning of such a reduction in response may be the reason for a less pronounced metabolome response at Day 28, although the effects observed in 28-day metabolome studies are usually the same as those seen in 14-day dose range-finding studies. Therefore,

[1] B. van Ravenzwaay, G.A. Montoya, E. Fabian, M. Herold, G. Krennrich, R. Looser, W. Mellert, E. Peter, V. Strauss, T. Walk, H. Kamp (2014) The sensitivity of metabolomics versus classical regulatory toxicology from a NOAEL perspective. *Toxicology Letters*, 227: 20–28

[2] B. van Ravenzwaay, H. Kamp, G.A. Montoya, V. Strauss, E. Fabian, W. Mellert, G. Krennrich, T. Walk, E. Peter, R. Looser, M. Herold (2015) The development of a database for metabolomics – looking back on ten years of experience. *Int. J. Biotechnology*, Vol. 14, No. 1

the metabolome pattern after 14 days provides a better dataset and, importantly, it is also preferred from an animal welfare perspective due to a shorter in-life phase. However, it must be noted that there are forms of toxicity (e.g., mild forms of reduced bone marrow activity and some forms of renal toxicity, such as inhibition of the antidiuretic hormone or tubular necrosis), which require more than 14 days to manifest in the animals and should therefore be considered.

When would you blood sample in an OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test?

For OECD 421 and 422 studies, blood samples should be taken at the end of the treatment period. Note that only the males can be fully compared to the BASF data base. Females (due to pregnancy/delivery) can still be used for comparison within a group of compounds. Metabolomic analysis was not conducted in the OECD 422 studies performed with the LOA substances since the database was built and annotated to address in particular the repeat-dose toxicity endpoints (including those on the endocrine system), not the endpoints specific to reproductive toxicity. We are currently conducting OECD 422 studies for LOA Category L (Resin Oils and Cyclic Dienes) to assist in the use of the read-across approach for the category. The results from these studies will help us determine which actions to make in the future, such as annotation of the database for reproductive toxicity.

Is MetaMap-Tox® accessible to external users and, if so, can it be used to compare their data with the MetaMap-Tox® database?

The BASF MetaMap-Tox® database has been developed as an internal tool and is being used by BASF for compound development and regulatory purposes. Therefore, MetaMap-Tox® is currently unavailable to external users. BASF can serve as a contract research organisation evaluating the metabolome from third parties, using MetaMap-Tox®. In such a case BASF will grant regulatory agencies access to MetaMap-Tox® to be able to comprehend how conclusions were derived. Academic questions could be specifically addressed if there is a mutual interest.

One test animal gives a series of data points in isolation. Are tests replicated in order to validate the data?

Exact repetitions were performed (i.e. same compounds, same dose levels) which showed that treatments were highly reproducible, despite slight biological and technical variability between each study. Reproducibility decreases if you have 'no' or 'very slight' changes induced on the metabolome. For reference, see Kamp et al., 2012: doi: 10.1016/j.toxlet.2012.09.015.

Metabolomics looks at one substance and its effects on the metabolome. How can this be applied to UVCBs?

Metabolomics can be used to investigate the effects of a substance on the metabolome, regardless of the substance composition (i.e. monomer, polymer and UVCB). As long as the UVCB substance is absorbed, is (bio)available to the test system (e.g rat) and is well characterised, then it is likely that any effect seen on the metabolome is attributed to the substance. The use of concurrent controls and at least two dose levels of test substances were used to clearly show that the test substance affected the metabolome signature.

How do you deal with the variability in the composition of UVCBs when using metabolomics? How variable are LOA substances?

Boundary conditions are in place, based on analytical data, for each category of LOA substances. Although there may be some variability between the composition of UVCBs in a specific LOA category, their activity in biological systems and effects on the metabolome can be quite similar, as Prof. Dr. Hennicke Kamp demonstrated in his presentation.

Although, as explained, metabolomics looks at comparing/predicting phenotypic changes of chemicals, can it also be used as a surrogate for exposure data for UVCBs (e.g. looking at signal strength changes with dose, changes over time etc)?

Theoretically, yes; if a compound (e.g. UVCB) can produce metabolites unique to that compound, then the quantitative changes seen correlate to exposure. If the composition of the UVCB is defined, one or more well-described constituents of the UVCB in plasma could also be measured. However, in practice, such a pure metabolic signature so uniquely tied to a UVCB is less likely to be found.

Was blood serum level of substances recorded? If so, did this demonstrate that substance uptake was the same between animal sexes?

The constituents in the plasma were not measured in this study. Therefore, substance uptake between males and femles was not assessed. To analyse a plasma sample for metabolites of a UVCB would be challenging due to the highly complex composition of UVCBs.

From the Bootstrapping slides (male animals) the BASF LOA stream containing naphthalene / indene do overlap with the pure substance streams. What, if any, conclusions have you drawn from this?

These data show that the effects induced by the marker compounds are relevant to the streams, especially those streams which contain higher parts of the marker compounds. However, the clustering in the PCA also shows that the effects of the LOA streams cannot be completely explained by the effects of the marker compounds. Consequently, also the other components of the UVCBs have an impact. It has to be noted though that, in the case of the LOA study, all effects observed for the streams have also been observed for the marker compounds.

Do you expect the same PCA metabolome similarities / clusters in 90-day studies, relative to a 14- or 28-day study?

Yes, since the findings were considered to be a surrogate of systemic toxicity caused by a particular substance. However, when focusing on specific modes of action, such as liver enzyme induction, we know induction is higher over a shorter exposure period. Therefore, some differences such as this may be seen between a 14-day and 90-day study.

How do these results on LOA substances relate to other types of chemicals tested using this methodology?

When we compare the LOA streams with the MetaMap-Tox® database, reference compounds which induce similar metabolome changes and toxic effects are shown. The UVCB substances and the marker chemical substances show mainly effects on the liver, thyroid, red blood cells and kidney (males). Chemicals in the database cover a very broad range of target organ toxicity and mode of action (MoA) patterns. As mentioned during the webinar, hundreds of pathways have been covered, of which the LOA substances and the markers hit approximately 10 %.

Do you consider that the scope of metabolomics parameters 'covers' equally well all potential organ and systems toxicity?

The BASF covers a wide range of different target organ toxicities. However, some target organ effects such as cardiovascular changes and types of neurotoxicity have not yet been fully identified. Generally, although it is assumed that a mode of action (MoA) that changes the biochemistry of an organ can be observed in the metabolome, when plasma is used (as is the case at BASF), effects might overlap and mask each other in this 'surrogate matrix'.

Is it too early to provide estimates on how many animals can be potentially saved from higher tier testing as a result of the clustering and improved biological basis for read-across from the metabolomics analysis on the LOA substances?

Since this LOA Category L has been selected as a pilot for this technology, it is indeed too early to be precise here, but when data from the OECD 422 studies become available, we should be able to assess the category justification again. Within this category, we are currently targeting approximately 50% of the substances for higher-tier testing. In our view, the power of this approach allows for well-informed decisions to be made on which substances to select for higher-tier testing, such as those in the presented clusters (e.g. naphthalene-/Indene-rich, DCPD-rich etc) and the dose levels to be applied.

Was metabolome analysis also performed on rabbit blood samples or are there any plans for these? If yes, could the metabolome analysis also be used for species comparisons to identify the presence or absence of species differences?

Some OECD 414 Prenatal Developmental Toxicity studies in the rabbit have been performed at BASF and the data from these studies are currently being analysed. However, metabolome analysis on rabbit blood samples was out of scope of the work conducted by the LOA Consortium and the LOA Consortium does not intend to perform such an analysis.