The use of toxicokinetic data for assessing bioaccumulation

21 February 2018 (09:00 – 15:00 CET)

Helmholtz Centre for Environmental Research GmbH - UFZ, Leipzig, Germany

Setting the scene

Welcome and objectives of the workshop [Christian Schlechtriem, Chair of the day]

Participants in the room and on the webinar were welcomed to the Workshop.

The Workshop originates from Cefic LRI project ECO 40 'Investigations on the bioconcentration of xenobiotics in the freshwater amphipod *Hyalella azteca* and inter-laboratory comparison of a new BCF test protocol'. Phase 1 of this project is complete, and the second phase (ring test for *H. azteca* BCF study) is currently under consideration. Phase 1 of the project also included investigations into use of *in vitro* data for assessing bioaccumulation of aquatic and terrestrial organisms.

The objectives of this Workshop are to share recent developments in relation to the use of invertebrate species, TK data and *in vitro* data in bioaccumulation assessment under regulatory regimes, and to make recommendations for next steps in this area of science.

Presentation: Challenges and needs in bioaccumulation assessment from a regulator's perspective [Caren Rauert, German Environment Agency, UBA, D]

The current requirements for bioaccumulation assessment under regulatory regimes was set out, along with the specification from a historical perspective as to the origins of the cut-off metrics presently applied.

Gaps in the knowledge were highlighted. E.g. mainly aquatic data is available at present, whilst data/guidelines/metrics for assessing bioaccumulation potential in terrestrial organisms and for ionic chemicals is lacking. The application of in vivo vertebrate tests for assessing terrestrial bioaccumulation is unlikely to be considered an appropriate option.

Some ways forward were proposed:

- Move beyond the physicochemical screening data by the application of *in vitro* metabolism and PBTK data. Such data could include from k_2/ADME data from existing mammalian toxicology studies.
- Further development is needed with regard to fish *in vitro* methods, IVIVE and mass-balance/PBTK models.
- The *in vitro* methods and IVIVE/PBTK could be extended to terrestrial organisms.
- *H. azteca* BCF testing could be a useful way forward in terms of screening, weight of evidence or an alternative to *in vivo* fish studies.
- Further guidance for weight of evidence approach required (e.g. data quality).

Invertebrates

Presentation: Acquisition of toxicokinetic information in aquatic and terrestrial bioaccumulation studies [Christian Schlechtriem, Fraunhofer IME, Schmallenberg, D]

Invertebrate bioaccumulation testing according to OECD 315 'Bioaccumulation in Sediment-dwelling Benthic Oligochaetes' is difficult to apply in a regulatory context due to lack of relevant criteria. In addition, such tests can include exposure via diet and soil pore water.

H. azteca BCF testing is being investigated as an alternative to vertebrate (fish) BCF testing.

A comparison of available measured *H. azteca* BCF values versus measured fish BCF values (from a range of species) indicates reasonably good correlation, and the potential for false negatives (i.e. *H. azteca* BCF value indicates not B and fish BCF value indicates B for same test substance) is absent for the dataset.

Regarding terrestrial bioaccumulation assessment, the isopod *Porcellio scaber* is being investigated as an alternative to OECD 317 'Bioaccumulation in Terrestrial Oligochaetes'. OECD 317, as for OECD 315, likely represents a combination of dietary and water exposure. Initial work on *Porcellio scaber* indicates that the uptake/depuration kinetics are very different to oligochaetes.

Discussion: Are invertebrate methods suitable to provide reliable information on bioaccumulation and how to use the information in a regulatory context [Moderator: Heike Laue]

- It was noted that trigger (or cut-off) values in a regulatory context will take some time to develop for tests with new species
- Species differences need consideration – for aquatic exposure, fish is assumed to represent a reasonable worst case (high performance gill-breather)
- Agreed that BCF and BMF represent two different assessment processes, and should be considered separately (even though cross-calculations of the two values can be made with various assumptions)
- More data is needed, across a range of test substances, to assess whether *H. azteca* BCF are more conservative than fish BCF.

Alternative approach

Presentation: The use of toxicokinetic information/k2 in the assessment of aquatic and terrestrial bioaccumulation [Kai-Uwe Goss, UFZ, Leipzig, D]

A regulatory approach for a consistent bioaccumulation assessment for aquatic **and** terrestrial was proposed:

1. Expose fish via food and water
2. Expose terrestrial organism via food

If fugacity of test substance in organism < fugacity of test substance in exposure media, then the conclusion is 'not B'.

An interim approach should consist of fish BCF > 2000; terrestrial BMF >1 indicates 'B'.

For the aquatic compartment, BCF can be calculated from measured k_2 data (k_1 can be calculated based on fish size and assumed uptake efficiency). A previous plot of fish log BCF vs log k_2 values determined in the same experiment (Brooke and Crookes, 2012[1]) indicated some correlation over a wide range of values, but mainly scatter.

[1] Brooke D, Crookes M (2012) Depuration rate constant: growth correction and use as an indicator of bioaccumulation potential. Environment Agency, UK, Bristol, UK

A plot of measured log BCF versus calculated log BCF (from measured k_2) gives a better correlation. However, the size-dependence (ventilation rate is determining factor) of k_1 should be considered if calculated k_1 are combined with measured k_2 estimates. The remaining scatter in the plot is likely associated with both the uncertainty inherent in the measured BCF data and the k_2 approach and should not be interpreted as uncertainty of the k_2 approach alone. Comparison of k_2 from BCF experiments (i.e. aqueous exposure) and k_2 from a feeding study show very good agreement, which lends further support to the usefulness of k_2 data.

For the terrestrial compartment, BMF data could potentially be obtained via an extension to the rat repeat dose oral toxicity 28-d/90-d studies. Alternatively, in a first step, biotransformation rate from hepatocyte data could be extrapolated to the whole organism and combined with calculated k_1 (assuming uptake efficiency of 100% as a first worst-case assumption unless respective data for the uptake efficiency are available).

Discussion: Current status of the use of toxicokinetic information in bioaccumulation assessment from a scientific, practical and regulatory view [Moderator: Caren Rauert]

- Agreed that applicability domain of fish k_1 calculation needs to be defined (e.g. max molecular size and K_{ow})
- For terrestrial organisms, a more complex model than 1-box is likely required
- Uptake phase in repeat dose oral toxicity 28-d/90-d studies may be too short to obtain environmentally-realistic BMF data
- Rat may not represent a worst-case organism for terrestrial bioaccumulation testing. Other terrestrial organisms may exhibit slower metabolic rates.
- There is an ongoing JRC project being conducted by Dr Jon Arnot (ARC, CA) to collate available *in vitro* and *in vivo* aquatic and terrestrial biotransformation/TK data.

In vitro methods

Presentation: Practical aspects of in vitro assays using liver S9 fractions and hepatocytes to determine in vitro intrinsic clearance [Heike Laue, Givaudan, CH]

A multi-laboratory ring-trial has been conducted to inform the development of two OECD Test Guidelines (TGs) for determination of *in vitro* intrinsic clearance rates in rainbow trout hepatocytes (RT-HEP) and rainbow trout liver S9 fractions (RTS9). The results from the substrate depletion assays demonstrate that both methods are highly reliable.

$CL_{IN\ VITRO,\ INT}$ values can be extrapolated to whole body k_m (via IVIVE) which can then be input into mass-balance models to calculate BCF values. The Test Guidelines only describe determination of $CL_{IN\ VITRO,\ INT}$ values and do not discuss IVIVE processes. The associated proposed OECD Guidance Document to the TGs provides guidance on how to best perform these methods and information on how $CL_{IN\ VITRO,\ INT}$ can be used to inform IVIVE models to predict bioaccumulation in fish. Incorporation of *in vitro* biotransformation rates enhances the reliability of *in silico* models for BCF predictions. Possible applications of BCF predictions based on measured $CL_{IN\ VITRO,\ INT}$ were proposed:

- As screening tool to decide whether a chemical is B or not B according to the regulatory framework
- To access the bioaccumulation potential as a part of a weight of evidence approach for read across
- As a screening tool to decide whether a full OECD TG 305 study is warranted to mitigate unnecessary testing requirements based solely on K_{ow}
- May be used for cases where BCF testing is not technically feasible or prohibited by a regulatory framework.

Limitations and uncertainties of the *in vitro* test systems are the use of liver systems, which do not take into account potential extrahepatic metabolism, and the limited working lifetime of the test systems. More data are needed to establish acceptable ranges of activities of the biological materials. Furthermore, supporting work is needed for the regulatory acceptance of the *in vitro* methods, such as improvement of IVIVE models, especially protein binding, and generation of more *in vivo* BCF data (OECD 305) for comparison.

***Presentation*: In vitro-in vivo extrapolation of hepatic metabolism – conceptual and theoretical issues raised by the recent OECD ring study [Sophia Krause, UFZ, Leipzig, D]**

An alternative IVIVE calculation, to those cited in the proposed OECD Guidance Document associated with the OECD Test Guidelines RT-HEP and RTS9, was presented.

The sorption correction is considered a key part of the IVIVE. Using $f_{u,\ plasma}/f_{u,\ assay} = 1$ is not a worst-case assumption, as $f_{u,\ plasma}/f_{u,\ assay}$ is always <<1. Using $f_{u,\ plasma}/f_{u,\ assay}$ appears to be worst-case, but the mathematics behind this approach appear questionable. An alternative mathematical approach for the extrapolation procedure was presented.

It was noted that even with this alternative IVIVE method, there are still uncertainties in the IVIVE due to non-consideration of extra-hepatic metabolism.

***Discussion*: Are in vitro methods suitable to provide reliable information on bioaccumulation how to use the information in a regulatory context [Moderator: Kai-Uwe Goss]**

- Recommended that UFZ reach out to be involved in discussions at OECD level regarding IVIVE approaches
- Would be useful to compare *in vivo* elimination rates with k_2 values calculated from *in vitro* data. Prof Frank Gobas (SFU, CA) is already involved in such a project.
- Mass balance models used to calculate BCF from whole body k_m (from $CL_{IN\ VITRO,\ INT}$ values) are still based on K_{ow}, and adsorption to other biological matter (e.g. proteins) also needs consideration
- *In vitro* data from mammalian cell lines should be investigated. Cefic LRI project ECO 41 (due to start this year) includes development and testing of an *in vitro* screening approach for assessing bioaccumulation for air-breathing organisms
- The costs, complexity and accessibility of *in vitro* testing is a foreseen challenge. More *in vitro* data is needed for regulatory acceptability, leading to a Catch 22 situation.
- *In vitro* methods could aid in metabolite evaluation, where applicable.

General discussion and workshop recommendations

Sum up of previous discussions, general discussion, workshop recommendations, next steps [Moderator: Peter Dohmen, with notes by Caren Rauert]

Discussion points from each of the three morning sessions (1. Invertebrate methods, 2. Use of TK/k_2 data, 3. *In vitro* assays) were revisited, and the following recommendations for ways forward were agreed and documented:

1st recommendation: Use established trigger values, but consider all available information (in a weight of evidence approach)

It is agreed that the BCF is not sufficient for bioaccumulation assessment and that terrestrial assessment needs to be added. However, the development of new/additional trigger values will take time and a discussion process needs to be initiated to reach agreement. The use of new trigger values in a weight of evidence approach needs guidance to reach general acceptance. The applicability domain of k_1 estimation for fish BCF needs to be defined in combination with experimental k_2.

2nd recommendation: Terrestrial bioaccumulation is to be considered separately from aquatic bioaccumulation

Investigations on bioaccumulation in terrestrial organisms should be based on existing vertebrate (toxicokinetic) data. A Cefic LRI project (ECO 41), which is due to start in 2018, includes development and testing of an *in vitro* screening approach for assessing bioaccumulation in air-breathing organisms. It was noted that the uptake efficiency in terrestrial organisms could be significantly less than one (the current worst-case assumption), as data from studies on pesticides show.

3rd recommendation: Provided that an accepted test guideline is available, the *H. azteca* BCF test can help to avoid fish studies in bioaccumulation assessment.

The limited amount of substances tested so far show more conservative BCF values for *H. azteca* than fish. Therefore, the *H. azteca* BCF test could be used in a tiered approach to assess the need for further fish *in vivo* testing. However, a definition of the applicability domain is required. The metabolism of *H. azteca* has been investigated in Cefic LRI project ECO 40, but further investigation on species differences (fish vs. *H. azteca*) is needed.

4th recommendation: Quality data for *in vitro* tests to be developed

Reliable *in vitro* biotransformation rates can be determined for fish. However, the cost of *in vitro* tests, as well as their acceptability and complexity, need to be considered. Benchmarking should be included to provide mechanistic insight and to reduce variance and uncertainty. The mathematical extrapolation procedure needs to be improved. Investigations on metabolism as part of *in vitro* studies are suggested.

5th recommendation: Disseminate the recommendations from this workshop, and endeavour to gain acceptance for alternative approaches to vertebrate testing for bioaccumulation assessment

Close of workshop

The workshop was closed at 15:00 with thanks to the participants and special thanks to the presenters and moderators. Cefic LRI was thanked for funding the ECO 40 project.

Abbreviations

ADME	Adsorption, distribution, metabolism and excretion
BCF	Bioconcentration factor
BMF	Biomagnification factor
$CL_{IN\ VITRO,\ INT}$	*In vitro* intrinsic clearance
f_u	Fraction unbound
IVIVE	*In vitro* to *in vivo* extrapolation
k_2	Depuration rate
k_1	Uptake rate
km	Metabolism rate
PBTK	Physiologically based toxicokinetic
TG	Test Guideline

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