Concawe Online Workshop on testing and assessment of petroleum UVCB substances

21st June 2021

Summary of Discussions

Concawe organized on 21st June 2021 a workshop to which representatives of Member States, ECHA, European Commission, Academia, Consultants and Industry participated. After a general introduction by Concawe and ECHA, the workshop focused on two main challenges: that of developing an adequate testing program for Human Health and petroleum UVCB substance PBT assessment. Each challenge was introduced by presentations from Concawe and ECHA. All slides and video-recorded presentations are available for download from the Concawe website. The discussions between participants that followed are summarised below.

1. **Human Health Challenge:**

How to overcome the practical challenges for petroleum UVCB substances in delivering a testing program, which takes into account animal welfare considerations as well as an acceptable time to complete evaluation while avoiding underestimation of human health hazards?

Substance compositional data for read across

There are changes to the REACH legal text, notably for read-across, which must be taken into account when developing a testing program for UVCB substances. The European Commission has adopted changes to Annexes VI-XI of REACH as published in the EU Official Journal on 18 June 2021. The measures will enter into force on 8 July 2021 and will start to apply as of 8 January 2022. The structural similarity of UVCBs shall be based on similarities in the structures of the constituents and their concentration. Specifically:

"*Structural similarity for UVCB substances shall be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents. If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the structural similarity may be demonstrated by other means, to enable a quantitative and qualitative comparison of the actual composition between substances*."

ECHA will issue guidance on these changes. Registrants need to demonstrate an understanding of the identity, concentration and variability of substance constituents and justify the data provided to enable a quantitative and qualitative approach to read-across. As it may not be technically possible to characterise each constituent, justification is also required when identification/measurement is not feasible. Information should also be provided

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on constituents that have been analysed and found not to be present. More compositional data will be needed for those constituents that drive hazard properties, as compared with constituents that are known to be non-hazardous, since the aim is to support read across. Likewise, the characterisation of variability may require more compositional data for those constituents that drive the hazard profile, as compared with those that do not. The agreed variation within a joint submission is described in a Substance Identity Profile (SIP). Note that the aims of Substance Identification and Read-across are distinct, with separate legal constraints. Consequently, the compositional information that is necessary to support read across in hazard assessment may well be broader and more granular than the information required for Substance Identification. Justification is also required as to why the test material constituent profile is representative of the substance on the EU market.

Hazard assessment testing

Given the low solubility of petroleum UVCBs, further work is needed to define options for delivering test substances into test models and animals.

ECHA confirmed that standard information requirements may be fulfilled with alternative (e.g. read-across adaptation) and/ or in vitro approaches, as described in Annex XI. Mechanistic information, including in vitro and in silico methods, can be used to support alternative test approaches within demonstrated fit for purpose integrated testing strategies. If grouping and read-across is correct and scientifically valid, there is a straightforward legal basis to accept the read-across. ECHA are already working towards the use of alternative methods, demonstrated by the recent integrated testing strategy for skin sensitization. Realistically it has to be acknowledged that in vitro approaches do not appear to be accepted as sufficiently mature to replace in vivo testing for higher tier endpoints.

Concawe is currently developing a holistic approach addressing the full hydrocarbon space to overcome the practical challenges of a substance-by-substance approach for UVCBs. Improvements should be explored that could increase the confidence (and decrease the remaining uncertainties) in the chemical-biological similarity assessment under study. To this end, the need for collaboration is recognised, aiming at building a constructive approach to address the challenging regulatory assessment of petroleum UVCB substances under REACH.

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2. **Environmental Challenge:**

How to progress PBT assessment of petroleum UVCB substances in a scientifically sound, regulatory-acceptable and timely manner, with a testing program taking into account that state-of-the-art analytical techniques cannot identify all constituents?

What metrics can be used to define similarity of constituents within a hydrocarbon block, in the context of PBT assessment?

The response from the authorities emphasized the need for consistent PBT properties of the constituents within a block. While regulation calls for structural similarity, there are no specific metrics; it is up to the registrant to provide a clear justification for the structural similarity of the constituents within the block. However, if you have clear differences in structures of constituents, then it is difficult to claim that there are similar PBT properties for the whole block, in particular to conclude that the whole block is not PBT.

What are the issues encountered when trying to dose UVCB substances into biodegradation test systems?

Issues with dosing UVCBs or their constituents were raised by industry, consultants, and academia. It was noted that constituents which are separated from the whole substance behave differently than when they are in their original matrix. Determining the in vitro bioavailable concentration is a critical, yet unmet need to refine in vitro-to-in vivo extrapolation for UVCBs. For example, what is in DMSO extract versus whole substance (https://pubmed.ncbi.nlm.nih.gov/32040194/), which was an issue during the CatApp work. The question is how to introduce a mixture into a test system: spiking, WAF (water-accommodated fraction) or passive dosing. Passive dosing can be used to keep constant concentrations in the test but different matrices can be used with different capabilities of liberating chemicals. There are recent publications on the dosing principle available (DOI: 10.1021/acs.est.9b06062, DOI: 10.1021/acs.est.1c00343). Analytical information should be provided to assess the bioavailability of the substance or constituents of interest.

How can UVCBs be tracked in testing systems?

Being able to track what is in the test system is a recognized need. There is a Peter Fisk Associates report for ECHA on the feasibility of analytical methods for environmental fate studies (e.g. OECD Test Guidelines 305, 307, 308 and 309) summarizing the recent scientific developments, pros and cons of available analytical tools and guidance on how to improve the quality of the data obtained from environmental fate studies (https://echa.europa.eu/-/echa-weekly-10-march-2021). The report highlights the difficulties of analytical methods for environmental testing.

Bioavailability controls how chemicals degrade in the environment, and the composition of a mixture composition affects the bioavailability. There should be an alternative to radiolabelling to track constituents in UVCBs during biodegradation testing, as further data is needed on these individual constituents. Additionally, radiolabelling generates side products, that would be indistinguishable from degradation products. One suggested alternative to

radiolabelling was the use of stable isotope analysis, based on the idea that the 12C/13C ratios in petroleum substances differ from different sources and this ratio could be used to track substance biodegradation. However, there were concerns raised as to the sensitivity and feasibility of the method, due to biological preference for certain isotopes.

What criteria should be used to choose representative constituents of a block?

Blocks are a pragmatic solution for a complex problem. One could in practice even have different block definitions depending on different properties for the same substance. The authorities reiterated that consistent PBT properties and a strong read-across justification is needed for a block. It is also possible to demonstrate a trend within the block and read-across from the worst-case scenario, which may differ based on the particular environmental endpoint in question. The level of alkylation or branching may be an important structural feature. Representative constituents for a block should bookend the properties of the block. It will be difficult to demonstrate that a full block is not PBT; however, it is easier to accept that everything in a block is PBT. If all individual members of block are not known, it hampers choosing worst case. Concawe mentioned that it is impossible to know all constituents for every block. For practical reasons it seems necessary to acknowledge that the granularity of blocks will be limited by our ability to quantify them using currently available analytical methods.

What are some options for analysis of petroleum substances to identify constituents, particularly during testing?

It was stated that GCxGC FID is very cost-effective to quantify blocks, although there was some question as to the maturity of the method. It does not give complete analyte separation and the elution is upset by the presence of heteroatoms, but it is overall a very good technique to quantify and provide tracked peaks. One could use GCxGC FID together with analyte-tracking (GCxGC coupled to HR TOFMS) to identify particular peaks (as well as block concentration). This approach could be used to screen the P and B properties of constituent structures that can then be analysed for similarity; this allows organization of the constituents into appropriate blocks based on their behaviours and to verify that the available structure libraries are representative of the products.

Identifying actual PS constituents is important, because we want to avoid spending time assessing structures that do not appear in PS, and we also want to avoid overlooking relevant analyte structures. For further structural elucidation, there are many papers on petroleum MS analysis – direct infusion, single ion monitoring. The problem with biodegradation testing with HR TOF MS is that the metabolites have a very different polarity.

Depending on the analytical technique, it can take significant time for analysis. Not every High-resolution MS is rapid to perform and process the data. One could also use statistical approaches to describe a block, such as theoretical assessments generating all possible structures within a block, as it is otherwise impossible to know all constituents. With the advancement of analytical chemistry, we observe higher complexity of the petroleum

samples. Here is an interesting example of the exotic structures in petroleum: https://onlinelibrary.wiley.com/doi/full/10.1002/anie.202005449

Issues with Log Kow assessment of PS

Log Kow is a ratio of substance concentration in water vs octanol. N-octanol may not be the perfect lipid surrogate. It does not work well for all PS constituents, as there are substances insoluble in water and octanol, so it would not be a valid descriptor. More research is needed to identify a good way to measure partition coefficient. However, the authorities stated that log Kow is the metric in the guidance R.11, although they understand that there are limitations and does not work for all substances. If you believe it is not applicable to your substance or part of substance (explain mechanistically), ECHA will look at it.

Acronyms:

B: Bioaccumulative

DMSO: dimethyl sulfoxide

DOI: Digital Object Identifier

ECHA: European Chemicals Agency

EU: European Union

FID: Flame Ionisation Detection

GC x GC: 2 dimensional Gas Chromatography

HR TOF MS: High Resolution Time of Flight Mass Spectrometry

Kow: n-octanol/water partition coefficient

MS: Mass Spectrometry

OECD: Organisation for Economic Co-operation and Development

P: Persistent

PBT: Persistent, Bioaccumulative and Toxic

PS: Petroleum Substance

REACH: Registration Evaluation Authorisation and Restriction of Chemicals

SIP: Substance Identity Profile

UVCB: Unknown, Variable, Complex and Biological

WAF: Water Accommodated Fraction

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