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WE ARE THE CENTRE FOR CHEMICAL SAFETY ASSESSMENT

Workshop background and objectives:

In June 2017 ECHA revised its Chapter R11: PBT / vPvB Assessment guidance document including new screening threshold criteria for bioaccumulation in air-breathing organisms linked to K_{OW} and K_{OA} (log K_{OW} >2 in conjunction with log K_{OA} >5). From initial evaluation, a significant percentage of REACH registered substances would fall within the threshold parameters. However, no technical guidance accompanied the updated criteria to outline methodologies and approaches to assess the newly included bioaccumulation (B) criteria, although complementary information on the criterion is already being requested from Registrants.

Several multi-stakeholder research initiatives have been initiated since the 2017 publication of the R11 document. ECHA established a 'Toxicokinetics for B assessment' Working Group to discuss the use of toxicokinetic data for the prioritisation of substances which may bioaccumulate in terrestrial organisms. Cefic LRI has also initiated projects aiming to develop approaches and tools to support assessment of bioaccumulation in air-breathing organisms, including Cefic LRI ECO41 'Enhanced Screening Methods to Determine Bioaccumulation Potential of Chemicals in Air-Breathing Species' and Cefic LRI ECO 44 'A toxicokinetic mammalian modelling framework for bioaccumulation assessment'

This workshop will build upon the recent effort to advance this topic and aims to:

- Address key open questions
- Identify data gaps/research needs

Workshop format – Following a brief introduction and two presentations providing an overview of the regulatory landscape and state of the science related to bioaccumulation in air breathing organisms, the principal activity of the workshop will comprise two focused breakout group sessions to cover the theoretical aspects (morning) and practical aspects (afternoon) of key

This workshop is a satellite event of the 2022 SETAC Europe Annual Meeting. For more information on the SETAC Conference, please see below:

questions related to developing a regulatory evaluation approach. The workshop has an overall objective to develop a blueprint for a practical tiered testing and assessment approach for bioaccumulation in air-breathing species.

At least one week prior to the date of the workshop, participants will receive the following:

- A list of open questions/topics per breakout group, intended as a thought-starter;
- Where applicable, additional information and a pre-read list.

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Workshop programme

Sunday 15 May 2022 – Crowne Plaza Copenhagen Towers, Copenhagen			
08.00 – 08.30	Arrival and registration; Coffee	Everest Foyer	
08.30 – 08.40	Welcome, introduction and workshop objectives	Room Lake Geneva	**Gordon Sanders** (Givaudan, CH; ECETOC Scientific Committee member)
08.40 – 09.05	Regulatory context and background to/status of Toxicokinetics for B assessment Working Group	Room Lake Geneva	**Caren Rauert** (UBA, DE)
09.05 – 09.30	Enhanced screening methods to determine the bioaccumulation potential of chemicals in air-breathing species (Cefic LRI ECO 41)	Room Lake Geneva	**Frank Wania** (University of Toronto, CA)
09.30 – 09.45	*Coffee - Everest Foyer*		
09.45 – 11.15	**Morning breakout session (theoretical aspects)**		
	Breakout group 1: Tier 1 screening thresholds for B in air breathing organisms	Room Lake Geneva	Moderator: **Frank Wania** (University of Toronto, CA); Rapporteur: **Caren Rauert** (UBA, DE)
	Breakout group 2: Tier 2 *In silico* approaches and incorporating biotransformation information in the B assessment	Room Loch Ness 1	Moderator: **Ester Papa** (University of Insubria, IT)**;** Rapporteur: **Miriam Leon Paumen** (ExxonMobil, BE)
	Breakout group 3: Tier 3 *In vitro* assays	Room Loch Ness 2	Moderator: **Heike Laue** (Givaudan, CH); Rapporteur: **Gordon Sanders** (Givaudan, CH)
11.15 – 12.00	Plenary: Rapporteurs feedback on breakout group discussions and Q&A	Room Lake Geneva	**Caren Rauert, Miriam Leon Paumen, Gordon Sanders**
12.00 – 12.45	*Lunch - Restaurant*		
12.45 – 14.15	**Afternoon breakout session (practical aspects)**		
	Breakout group 4: How to include IVIVE modelling in the assessment?	Room Lake Geneva	Moderator: **Kai-Uwe Goss** (UFZ, DE); Rapporteur: **Frank Gobas** (Simon Fraser University, CA)
	Breakout group 5: Which data from other areas (e.g. Toxicology data, modelling etc.) will be helpful for the WoE?	Room Loch Ness 1	Moderator: **Maike Habekost** (BASF, DE); Rapporteur: **Gordon Sanders** (Givaudan, CH)
	Breakout group 6: Animal Welfare and *In vivo* testing - how to get the most out of any new testing? (if higher tier studies required)	Room Loch Ness 2	Moderator: **Fiona Sewell** (NC3Rs, UK); Rapporteur: **Heike Laue** (Givaudan, CH)
14.15 – 14.30	*Coffee - Everest Foyer*		
14.30 – 15.15	Plenary: Rapporteurs feedback on breakout group discussions and Q&A	Room Lake Geneva	**Frank Gobas, Gordon Sanders, Heike Laue**
15.15 – 15.30	Closing remarks/Next steps	Room Lake Geneva	**Gordon Sanders** (Givaudan, CH)

Organising Committee

Kai-Uwe Goss (UFZ, DE)
Maike Habekost (BASF, DE)
Sylvia Jacobi (Albemarle, BE)
Heike Laue (Givaudan, CH)
Miriam Leon Paumen (ExxonMobil, BE)
Gordon Sanders (Givaudan, CH)
Frank Wania (University of Toronto, CA)

Lucy Wilmot (ECETOC, BE)
Francesca Uguccioni (ECETOC, BE)
Blanca Serrano Ramon (ECETOC, BE)

Venue

Crowne Plaza Copenhagen Towers (see here for more info)
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Attendee List

Sivani Baskaran, University of Toronto, CA
Natalie Burden, NC3Rs, UK
Sandrine Deglin, HESI, US
Steven Droge, Wageningen Environmental Research, NL
Frank Gobas, Simon Fraser University, CA
Kai-Uwe Goss, Helmholtz Centre for Environmental Research (UFZ), DE
Maike Habekost, BASF SE, DE
Doris Hirmann, ECHA, FI
Tim Hofer, Norwegian Institute of Public Health (NIPH), NO
Anu Kapanen, ECHA, FI
Jaeshin Kim, Dow Chemical, US
Heike Laue, Givaudan, CH
Miriam Leon Paumen, ExxonMobil, BE
Delina Lyon, Concawe, BE
Michael McLachlan, Stockholm University, SE
Ester Papa, University of Insubria, IT
Julia Pletz, Exponent International, CH
Kathy Plotzke, Dow, US
Caren Rauert, German Environment Agency (UBA), DE
Gordon Sanders, Givaudan, CH
Alessandro Sangion, University of Toronto, CA
Leslie Saunders, University of Toronto, CA
Christian Schlechtriem, Fraunhofer, DE
Blanca Serrano Ramon, ECETOC, BE
Fiona Sewell, NC3Rs, UK
Gabriele Treu, UBA, DE
Nathalie Vallotton, Dow Europe, CH
Frank Wania, University of Toronto, CA

Invited speakers: bios and abstracts

Key note speaker - Caren Rauert, UBA (Umweltbundesamt; German Environment Agency), Germany

Caren received a Diplom-Ingenieur in environmental engineering from the Universität-Gesamthochschule Paderborn (Germany) and has since worked at the German Environment Agency (Umweltbundesamt; UBA) in various units. Caren currently works in the unit 'International Chemicals Management' mainly on issues relating to the Stockholm Convention and on bioaccumulation assessment. She is also a member of the POP Review Committee under the Stockholm Convention.

Abstract: ***'Regulatory context and background to/status of Toxicokinetics for B assessment Working Group'***

For most substances, a bioaccumulation assessment that focuses on aquatic species is adequate and sufficient. The bioconcentration factor is used as criterion in PBT and POP assessment. Only for some substances, this is not the case. Among these are endosulfan, many perflourinated alkyl substances or highly lipophilic compounds, which accumulate substantially in air-breathing organisms and may not be recognised as bioaccumulative if the assessment is based on aquatic testing only.
An ECHA working group is developing an approach to use toxicokinetic data for the prioritisation and assessment of substances potentially bioaccumulating in air-breathing organisms. The aim is to integrate the developed tiered assessment approach into the weight-of-evidence assessment of bioaccumulation potential of air-breathing organisms and provide further guidance on its implementation in regulatory context.
This presentation will give an update on the current state of the work.

Key note speaker - Prof. Frank Wania, Department of Physical and Environmental Sciences, University of Toronto, Canada

Prof Frank Wania studied Environmental Science at the University of Bayreuth in Germany and received his Doctorate in Chemical Engineering and Applied Chemistry from the University of Toronto in 1995. After two years as a scientist at the Norwegian Institute for Air Research, and three years as an independent researcher, he joined the University of Toronto Scarborough in 1999, where he is currently a professor of environmental chemistry. He has wide-ranging research interests related to environmental contaminant fate, with a focus on gaining a mechanistic understanding of contaminant enrichment processes through a combination of fieldwork, laboratory experimentation and model simulations.

Abstract: ***'Enhanced screening methods to determine the bioaccumulation potential of chemicals in air-breathing species (CEFIC LRI ECO41)'***

It is known for two decades that bioaccumulation (B) assessment of organic chemicals that relies solely on aquatic biota is insufficient, because some moderately hydrophobic chemicals may bioaccumulate in air-breathing organisms while being readily eliminated from organisms respiring water. Little regulatory guidance on B assessment in air-breathing organisms exists, beyond the K_{OA} and K_{OW} thresholds for bioaccumulation that were derived from model calculations of persistent chemicals in food chains comprising air-breathing organisms. These thresholds are largely

ineffective for screening large numbers of commercial organic chemicals for B, because the fraction of chemicals that are sufficiently volatile and/or water soluble to fall below these thresholds is quite small. CEFIC LRI project ECO41 sought to advance B assessment in air-breathing organisms by exploring how: (a) K_{OA} and K_{OW} values can be reliably predicted (or measured) and used in an initial, low-effort screening that accounts for the uncertainty of the predictions (or measurements), (b) information on biotransformation rates in air-breathing organisms, generated by a variety of *in silico* methods, can enhance low tier B assessments, and (c) biotransformation data for an air-breathing organism can be reproducibly generated *in vitro* and then combined with IVIVE methods to constitute a second tier of B assessment. The presentation will summarize the findings of the project and identify unresolved issues surrounding a tiered B assessment in air-breathing organisms.

Breakout groups – morning session (theoretical aspects)

Breakout group 1: Tier 1 screening thresholds for B in air breathing organisms

Location: Room Lake Geneva

Participants:

First name	Surname	Affiliation
Frank	**Wania (Moderator)**	University of Toronto
Caren	**Rauert (Rapporteur)**	UBA
Sivani	Baskaran	University of Toronto
Sandrine	Deglin	HESI
Maike	Habekost	BASF
Tim	Hofer	NIPH
Anu	Kapanen	ECHA
Michael	McLachlan	University of Stockholm
Kathy	Plotzke	Dow

Open questions/topics for discussion:

1. Is there something like a consensus on the use of a steady state biomagnification factor (BMF, defined as the ratio of fugacities in organism and diet, approximated for non-polar substances as the ratio of lipid-normalised concentrations) of 1 as a suitable threshold for bioaccumulation in air-breathing organisms? If not, why and what would be a better alternative?

2. In some regulatory contexts a categorization of chemicals into not B, B or vB is required. Is it possible to use the metric of the BMF in an air-breathing organism for such a categorization? What would this look like?

3. The currently recommended K_{OA} and K_{OW} thresholds for bioaccumulation in air-breathing organisms are based on early food chain modelling results by Kelly et al. (2007). Are these thresholds still appropriate or should they be revisited? If yes, what would be a defendable procedure for deriving those thresholds?

4. How to account for the fact that BMF and the partitioning ratio thresholds vary with an organism's physiological characteristics (breathing rate, urination rate, dietary intake and composition, digestion efficiency, assimilation kinetics)? Is it acceptable to use the laboratory rat as the reference organism?

Breakout group 2: Tier 2 *In silico* approaches and incorporating biotransformation information in the B assessment

Location: Room Loch Ness 1

Participants:

First name	Surname	Affiliation
Ester	**Papa (Moderator)**	University of Insubria
Miriam	**Leon Paumen (Rapporteur)**	ExxonMobil
Steven	Droge	University of Wageningen
Kai-Uwe	Goss	UFZ
Doris	Hirmann	ECHA
Jaeshin	Kim	Dow
Julia	Pletz	Exponent
Alessandro	Sangion	University of Toronto
Gabriele	Treu	UBA

Open questions/topics for discussion:

1. Are appropriate tools available for biotransformation estimation (*in vivo/in vitro*) to incorporate them in the B assessment or is this premature?
2. Have people used available tools? What was their experience, could they be improved?
3. How should *in vitro* information be incorporated into a definitive B assessment (which is *in vivo*)?
4. How trustworthy is the *in silico* estimation of biotransformation rate constants? Is data quality/uncertainty the most important factor?
5. How is the *in silico* biotransformation information going to be used – screening or assessment, or as part of a WoE? If the data is going to be used in higher tiers, the quality will potentially have to be higher.

Breakout group 3: Tier 3 *In vitro* assays

Location: Room Loch Ness 2

Participants:

First name	Surname	Affiliation
Heike	**Laue (Moderator)**	Givaudan
Gordon	**Sanders (Rapporteur)**	Givaudan
Natalie	Burden	NC3Rs
Frank	Gobas	Simon Fraser University
Delina	Lyon	Concawe
Leslie	Saunders	University of Toronto
Christian	Schlechtriem	Fraunhofer
Blanca	Serrano Ramon	ECETOC
Fiona	Sewell	NC3Rs
Nathalie	Vallotton	Dow

Open questions/topics for discussion:

1. Current status of *in vitro* assays: What exists, comparing *In vitro* results to *In vivo* data, applicability within a regulatory context
2. What is needed regarding *in vitro* assays to be applicable to assess bioaccumulation in air-breathing organisms?
3. Key points for regulatory application of the *in vitro* method in future

Breakout groups – afternoon session (practical aspects)

Breakout group 4: How to include IVIVE modelling in the assessment?

Location: Room Lake Geneva

Participants:

First name	Surname	Affiliation
Kai-Uwe	**Goss (Moderator)**	UFZ
Frank	**Gobas (Rapporteur)**	Simon Fraser University
Steven	Droge	University of Wageningen
Doris	Hirmann	ECHA
Jaeshin	Kim	Dow
Miriam	Leon Paumen	ExxonMobil
Michael	McLachlan	University of Stockholm
Ester	Papa	University of Insubria
Kathy	Plotzke	Dow
Alessandro	Sangion	University of Toronto
Leslie	Saunders	University of Toronto

Open questions/topics for discussion:

For regulators: How often do you find in vitro evidence as part of a dossier (presumably only for fish)? How do you assess this? Do you feel confident to evaluate the quality of such data? Do you check the provided IVIVE calculations? Do you generally believe in the idea of obtaining missing quantitative information on biotransformation from in vitro experiments? Do you see this as a useful approach in the assessment also for terrestrial B?

For registrants: How useful is the IVIVE approach for registration? What are the pros and cons from your point of view? Is the experimental work done in-house? What tool do you use for the extrapolation calculations? Do you use IVIVE evidence as a complete replacement of OECD 305 or only as additional information in a WoE in the B-assessment of fish? Do you have any experience with IVIVE in the assessment of terrestrial B? What do you expect for the future?

General:

1. Would you expect the IVIVE approach to give a systematic over- or underestimation of the *in vivo* biotransformation kinetics? If so, why?
2. Do you feel that further guidance in the IVIVE extrapolation procedure is needed?
3. Do you have suggestions for an improvement of the IVIVE procedure?
4. Are there specific cases where you would expect IVIVE not to be applicable?
5. Should IVIVE become more important for terrestrial B-assessment than for fish or less important? What other source of experimental information on biotransformation kinetics in terrestrial animals is conceivable?

Breakout group 5: Which data from other areas (e.g. Toxicology data, modelling etc.) will be helpful for the WoE?

Location: Room Loch Ness 1

Participants:

First name	Surname	Affiliation
Maike	**Habekost (Moderator)**	BASF
Gordon	**Sanders (Rapporteur)**	Givaudan
Sivani	Baskaran	University of Toronto
Sandrine	Deglin	HESI
Delina	Lyon	Concawe
Christian	Schlechtriem	Fraunhofer
Gabriele	Treu	UBA
Nathalie	Vallotton	Dow
Frank	Wania	University of Toronto

Open questions/topics for discussion:

1. Can already existing data on bioconcentration and metabolization (fish BCF from an OECD 305 study, ADME data) be applied for terrestrial bioaccumulation assessment? If yes, how?
2. Which role can food chain modelling play in a WoE?
3. What else is needed for a WoE approach?

Breakout group 6: Animal Welfare and *In vivo* testing - how to get the most out of any new testing? (if higher tier studies required)

Location: Room Loch Ness 2

Participants:

First name	Surname	Affiliation
Fiona	**Sewell (Moderator)**	NC3Rs
Heike	**Laue (Rapporteur)**	Givaudan
Natalie	Burden	NC3Rs
Tim	Hofer	NIPH
Anu	Kapanen	ECHA
Julia	Pletz	Exponent
Caren	Rauert	UBA
Blanca	Serrano Ramon	ECETOC

Open questions/topics for discussion:

1. What are the main challenges for conducting *in vivo* studies to gain information on Air-Breather B properties in terms of animal use and animal welfare?
2. Which typical *in vivo* study requirements could be adapted to gain sufficient information on Air-Breather B properties in order to avoid use of additional vertebrate test animals?
3. If it is possible to integrate B measurements into existing *in vivo* studies, what TG revisions and/or guidance documents would be needed, and are there any implications for GLP?

Literature

Arnot JA, Toose L, Armitage JM, Embry M, Sangion A, Hughes L (2022) A weight of evidence approach for bioaccumulation assessment. Integr Environ Assess Manag doi:10.1002/ieam.4583
BAT tool

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Comparison of in vitro biotransformation rates in hepatocytes from different species (humans, rat and trout)

Burden N et al. (2017) Reducing the number of fish in regulatory bioconcentration testing: Identifying and overcoming the barriers to using the 1-concentration approach. Integrated Environmental Assessment and Management, 13(1):212-214. doi:10.1002/ieam.1851
Example of a data-driven project to support the 3Rs in fish bioaccumulation testing, which led to evidence-based policy change.

Goss KU, Linden L, Ulrich N, Schlechtriem C (2018) Revisiting elimination half live as an indicator for bioaccumulation in fish and terrestrial mammals. Chemosphere 210:341-346 doi:10.1016/j.chemosphere.2018.07.017
K2 as metric for B assessment

Hofer T, Myhre O, Peltola-Thies J, Hirmann D (2021) Analysis of elimination half-lives in MamTKDB 1.0 related to bioaccumulation: Requirement of repeated administration and blood plasma values underrepresent tissues. Environ Int 155:106592 doi:10.1016/j.envint.2021.106592
Data analysis of elimination half-lives in mammals from single and repeated dose studies

Hoke R, Huggett D, Brasfield S, et al. (2016) Review of laboratory-based terrestrial bioaccumulation assessment approaches for organic chemicals: Current status and future possibilities. Integr Environ Assess Manag 12(1):109-22 doi:10.1002/ieam.1692
Review from the HESI sponsored Workshop on environmental assessment of organic chemicals bioaccumulation in terrestrial ecosystems (Jan 2013)

Knudsen GA, Sanders JM, Birnbaum LS (2016) Disposition of the Emerging Brominated Flame Retardant, 2-Ethylhexyl 2,3,4,5-Tetrabromobenzoate, in Female SD Rats and Male B6C3F1 Mice: Effects of Dose, Route, and Repeated Administration. Toxicol Sci 154(2):392-402 doi:10.1093/toxsci/kfw176
In vivo study with a flame retardant in rats and mice comparing single vs. multiple administration

Lee Y-S, Otton SV, Campbell DA, Moore MM, Kennedy CJ, Gobas FAPC (2011) Measuring in vitro biotransformation rates of super hydrophobic chemicals in rat liver S9 fractions using thin-film sorbent-phase dosing. Environ Sci Technol 46(1):410-418 doi:10.1021/es203338h
In vitro biotransformation of PAHs in rat liver S9 fractions using passive dosing

Richardson SJ, Bai A, Kulkarni AA, Moghaddam MF (2016) Efficiency in Drug Discovery: Liver S9 Fraction Assay As a Screen for Metabolic Stability. Drug Metab Lett 10(2):83-90
Rat liver S9 assay used as screening assay in drug discovery