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PBT assessment of substances – Proposal of a trigger value for bioaccumulation in terrestrial oligochaetes

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1. Introduction

The identification and regulation of persistent (P), bioaccumulative (B) and toxic (T) or very persistent (vP) and very bioaccumulative (vB) substances (PBT/vPvB substances) is necessary to ensure a high level of protection of human and animal health, and of the environment. The biocentration factor (BCF) in aquatic organisms estimated in flow-through fish tests according to OECD 305 is the key factor for the assessment of the bioaccumulation behaviour of chemicals. Until now, there are no appropriate criteria for the assessment of bioaccumulation in terrestrial organisms.

The OECD guideline 317 (2010) for testing of chemicals relating to bioaccumulation in terrestrial oli-gochaetes is an internationally accepted, standardized test guideline for the determination of bioaccu-mulation of soil bound substances in terrestrial soil organisms (Lumbricid Earthworm, Enchytraeid Potworm). However, the criteria indicated for identification of PBT and vPvB, respectively, in Annex XIII of the REACH regulation do not define trigger values regarding accumulation in terrestrial organisms. The existing BCF trigger values of 2000 and 5000 for aquatic organism cannot be converted to BAF or BSAF values due to lack of suitable data and different processes of uptake and depuration in terrestrial invertebrates. The objective of this project is to provide adequate data which will help to clarify how to address terrestrial bioaccumulation in the B assessment and to define trigger values for BAF data obtained from OECD 317 tests that are comparable to the B/vB criteria in the scope of the PBT assessment. The results might be used for future guideline revision (e.g. ECHA R.11 PBT/vPvB assessment [1]).

2. Materials and methods

The project is divided in three parts. The first part comprises a literature search regarding terrestrial bioaccumulation, fish bioconcentration and fish magnification studies. ICS (Information system Chemical Safety) data sets for 21 plant protection substances containing the complete list of endpoints and reports from bioaccumulation studies with oligochaetes were provided by the German UBA for evaluation. Based on the results obtained, possible correlations of bioaccumulation factors with physical-chemical properties of the contaminants (K_{OW}, K_{OC}) and between BCF/BMF in fish and BAF/BSAF in worms were investigated. Subsequently, an experimental part followed: bioaccumulation studies with the terrestrial oligochaete *Eisenia andrei* were conducted according to the OECD guideline 317 applying four representative substances covering log K_{OW} values of 3.5 – 8.4. Finally, all available data and information were evaluated and a proposal for a suitable trigger value for assessing the bioaccumulation of terrestrial organism in the framework of a PBT assessment was developped.

3. Results and discussion

3.1. Results

In total, 85 publications on lumbricidae presenting 92 BAF for 123 individual substances were identified as relevant. However, only 41 lumbricid BAF for seven substances were derived from only four guideline-compliant or guideline-based studies. The ICS data sets provided data for 9 substances. Regarding fish BCF and BMF, in total 50 publications were identified as relevant. Only 20% followed the OECD 305 test guideline as noted by the author, the others did not refer to any guideline. The ICS data sets provided fish BCF values for all substances.

The terrestrial BAF values for lumbricids from the guideline-compliant or guideline-based studies range from 0 to 22. BSAF values range from 0.25 to 14.8. The theoretical values calculated according to the "Simple worst case assessment" are within the range of 0.01 and 429. The theoretical values calculated according to the "pore water aproach" are within the range of 0.875 and 0.937. No correlation between experimental BAF/BSAF

values and theoretical BAF/BSAF values were obtained. The fish BCF values for the ICS substances are within a range of 3.2 und 8200. No correlation between experimental BAF/BSAF values for earthworms and fish BCF values was obtained. Consequently, the equation is not suitable to estimate the BSAF based on a BCF value. With kinetic and steady state BAF and BSAF values of 0.6 – 3.0 derived for the four substances, the results of the studies conducted in the experimental part of this project confirm the general impression of numerically low values in earthworms, compared with the BCF values in fish.

3.2. Discussion

In the guidance document R.11 on PBT/vPvB assessment of the European Chemicals Agency (ECHA, 2014) it is concluded that lipid- and organic carbon-normalised BSAF values of 0.5 and higher are indicating high bioaccumulation. A $BSAF_k$ of 1.0 is in agreement with the concept of bioaccumulation, where bioaccumulation is acknowledged if the ratio of C(organism)/C(medium) is above 1.0. This rationale is also outlined in Weisbrod et al. (2009 [2]): These authors suggested a lipid/carbon-normalised BSAF of 1.0 as a trigger value for identification of potentially bioaccumulating chemicals. Also, Gottardo et al. (2014 [3]) mention the factor of >1.0 as an indicator of bioaccumulation and biomagnification.

Eight of the evaluated 19 BSAF are above a value of 1.0, and the respective dish BCF exceed the ECHA trigger-BCF of 2000 or 5000 for bioaccumulative substances. For the substances showing earthworm BSAF below or around 1.0, the fish BCF values show an ambiguous picture: While one is below the ECHA trigger of 2000, two of these three substances show fish BCF above the ECHA trigger of 2000, or even above 5000.

Applying the proposed trigger value on the experimentally determined BSAF values of this project, and on 11 potentially bioaccumulating chemicals as based on phys.-chem. properties (ICS data provided by UBA), one chemical classified as non-accumulating in fish would be assessed as bioaccumulative in terrestrial organisms (>1.0). Six compounds would not be assumed to accumulate in fish or in earth-worms; four substances would be considered bioaccumulative in both environments, while four chemicals would be seen as bioaccumulating in fish only, but not in earthworms (Figure 1).

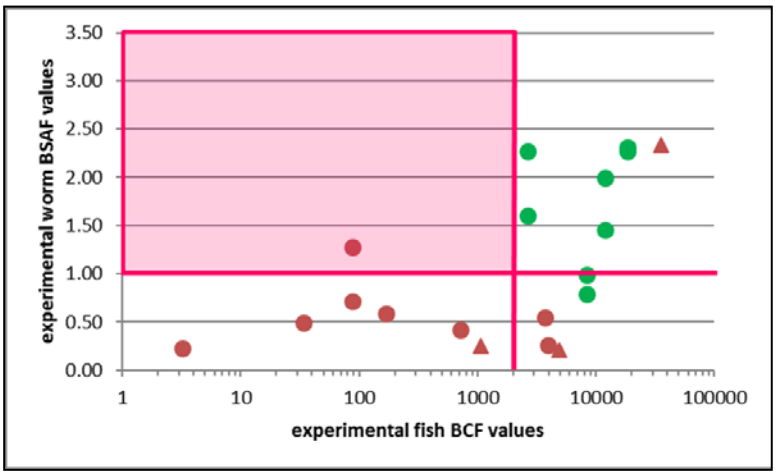

Figure 1: Experimentally-derived terrestrial BSAF vs. BCF (n = 19). Green circles: this study (four substances with two values each); brown circles: ICS data set; brown triangles: literature data.

4. Conclusions

Based on the experimental data of this research project, literature originated data and the considerations above, a BSAF of 1.00 is therefore proposed as a general trigger to classify a substance as potentially bioaccumulative in terrestrial oligochaetes in the scope of PBT assessment.

5. References

[1] European Chemicals Agency (2014): Guidance on information requirements and chemical safety assessment, chapter R.11: PBT/vPvB assessment. ECHA-14-G-07-EN; ISBN: 978-92-9244-760-1. November 2014.

[2] Weisbrod A.V., Woodburn K.B., Koelmans A.A., Parkerton T.F. McElroy A.E. and Borga K. (2009): Evaluation of Bioaccumulation Using In Vivo Laboratory and Field Studies. Integrated Environmental Assessment and Management, Vol. 5, No. 4, pp. 598 – 623.

[3] Gottardo S., Hartmann N.B. and Sokull-Klüttgen B. 2014. Review on available criteria for non-aquatic organisms within PBT/vPvB frameworks. Part I: Bioaccumulation assessment. JRC Science and Policy Report. EUR 26802 EN, ISBN 978-92-79-39659-5

PBT assessment of substances

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Proposal of a trigger value for bioaccumulation in terrestrial oligochaetes

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Background

- Identification and regulation of bioaccumulative (B) / very bioaccumulative (vB) substances (PBT/vPvB substances) is necessary to ensure protection of human and animal health, and of the environment
- The biocentration factor (BCF) in aquatic organisms estimated in fish tests according to OECD 305 is the key factor for the assessment of the bioaccumulation behaviour of chemicals (>2000 B and >5000 vB)
- Until now, there are no appropriate criteria for the assessment of bioaccumulation in terrestrial organisms (Annex XIII, REACH regulation)
- There is an adopted OECD guideline for the determination of bioaccumulation of soil bound substances in terrestrial soil organisms (OECD guideline 317 (2010))
- Existing BCF trigger values for aquatic organism cannot be converted to BAF or BSAF values for terrestrial organisms due to lack of suitable data and different processes of uptake and depuration

UBA-Research project

Bioaccumulation of contaminants in soil organisms - derivation and verification of suitable criteria for assessment of terrestrial bioaccumulation (FKZ 3713 64 419)

- UBA-Research project „Bioaccumulation of contaminants in soil organisms - derivation and verification of suitable criteria for assessment of terrestrial bioaccumulation“ (FKZ 3713 64 419)

  funded to provide adequate data which will help to

  - clarify how to address terrestrial bioaccumulation in the B assessment
  - define trigger values for BAF data obtained from OECD 317 tests that are comparable to the B/vB criteria in the scope of the PBT assessment

UBA-Research project

Bioaccumulation of contaminants in soil organisms - derivation and verification of suitable criteria for assessment of terrestrial bioaccumulation (FKZ 3713 64 419)

- Literature search and assessment of the data derived
  - Terrestrial bioaccumulation (BAF), OECD 317 or ASTM 1998,
  - Bioconcentration (BCF) and biomagnification (BMF) in fish, OECD 305
    Publications, ICS (Information system Chemical Safety)

- Experimental part (Earthworm bioaccumulation studies (OECD 317))
  - *Eisenia andrei* (Lumbricidae, Oligochaeta)
  - 4 substances (Endosulfan, Methoxychlor, o-Terphenyl, PCB153; log K_{OW} 4.5 – 7.8)
  - 2 artificial soils (5% and 10% peat corresponding with 2.0% and 4.0% C_{org})

- Proposal for a trigger value for assessing the bioaccumulation of terrestrial organism in the framework of a PBT assessment

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Literature search “Terrestrial bioaccumulation”

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)

- ICS data: reports for 22 substances (confidential, provided by UBA).
  - For 10 substances, study design was according to or following OECD 317
  - For 9 substances data for kinetic uptake and elimination rate constants *recommended by the guideline*
  - For 8 substances lipid/C_{org} standardized BSAF values *recommended by the guideline*

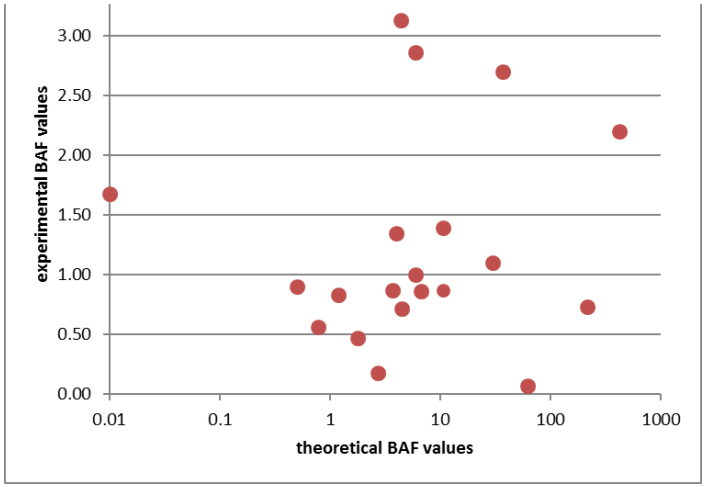
- Free literature: 85 publications regarding accumulation in lumbricids.
  - For 7 substances, study design was according to or following OECD 317
  - For all 7 substances data for kinetic uptake and elimination rate constants *recommended by the guideline*
  - No lipid/C_{org} standardized BSAF values in guideline conform studies *recommended by the guideline*

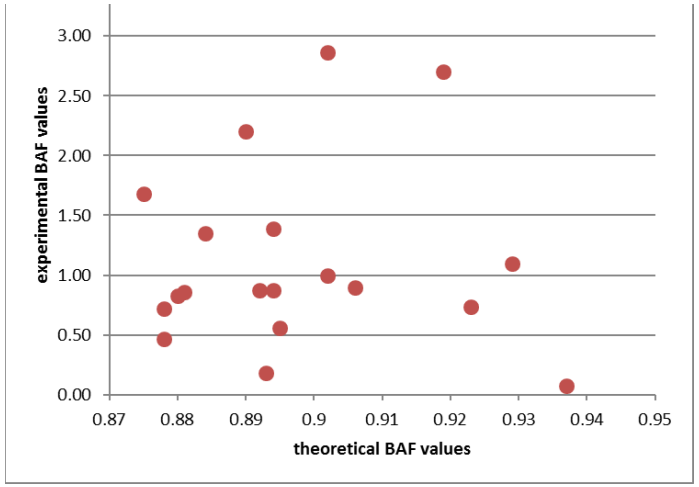
Literature search “Terrestrial bioaccumulation”

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)

- Range of lumbricid BAF in guideline conform studies
  - Experimental values (publications/reports): 0 - 22
  - Theoretical values “dry soil approach“: 0.01 - 429
  - Theoretical values "pore water aproach“: 0.88 - 0.94

No correlation between experimental and theoretical BAF values occurs





European Food Safety Authority 2009: Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA
Dry soil approach (BAF = (0.84+0.012 x K_{OW})/f_{OC} x K_{OC}; using f_{OC} = 0.02 as default value)
Pore water approach for soft bodied soil organisms (BAF = (0.84+0.012 x K_{OW})/ RHOearthworm; using RHO= 1 [$kg_{wwt} \times L^{-1}$] as default))

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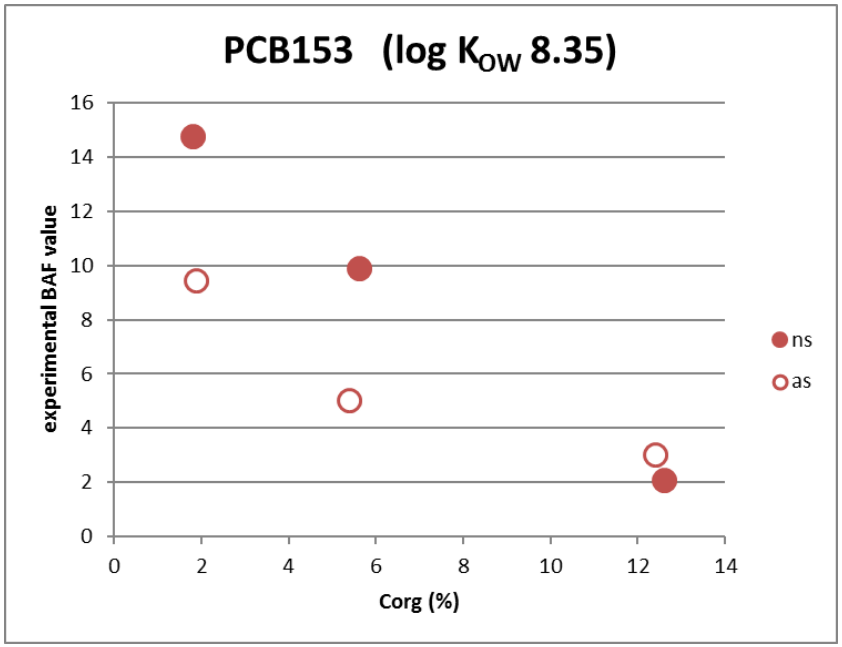
Literature search “Terrestrial bioaccumulation”

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)

- Vlčková and Hofmann (2012)

  Experimental values for five substances in natural and artificial soils at 3 C_{org} levels

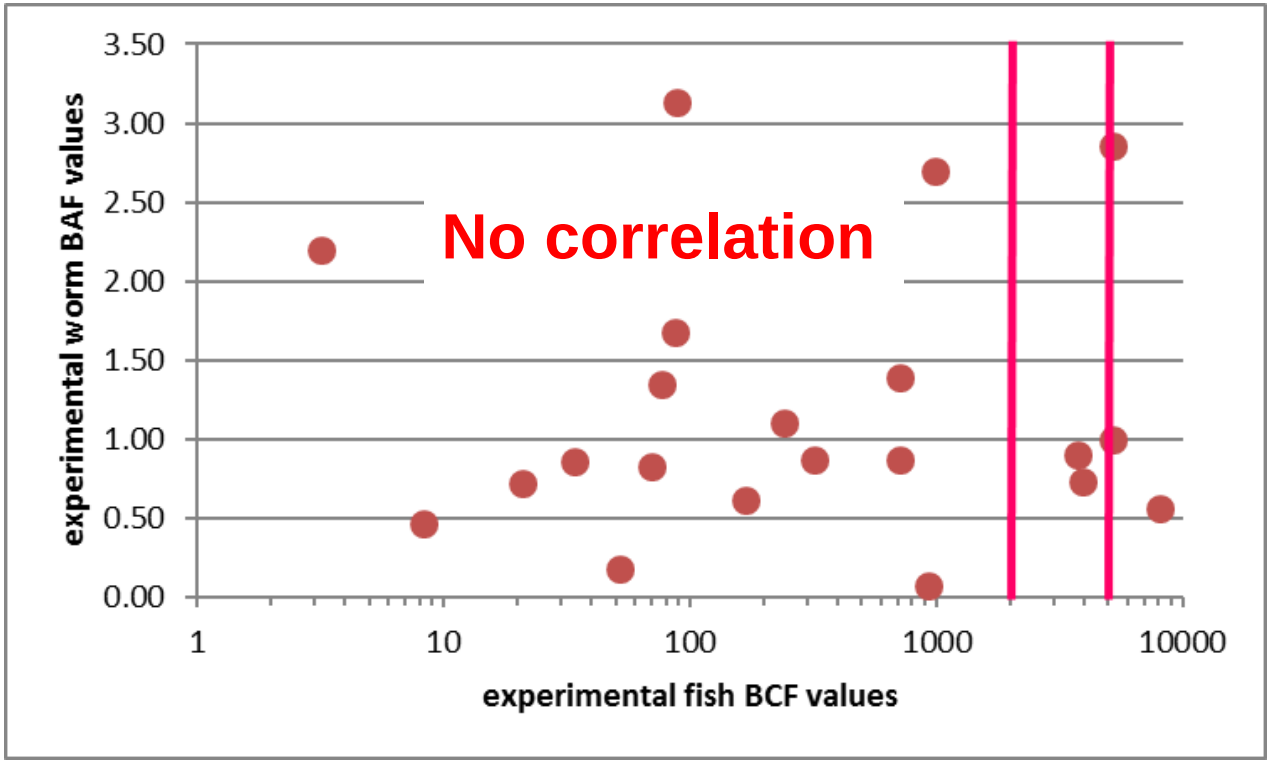


BAF depends on C_{org} - but also on other matrix properties

Literature search “Terrestrial bioaccumulation”

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)

- Dependency between experimental BAF and fish BCF values (ICS data)



The vertical lines at BCF 2000 and 5000 indicate the trigger values for “B” and “vB” for fish.

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Literature search “Terrestrial bioaccumulation”

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)

- Soil composition affects BAF values
  - Different soils with comparable C_{org} result in different BAF values
  - Natural soils result in higher BAF values
  - Log K_{OW} and/or K_{OC} affect the BAF, but they are not the only parameters

- A prediction on the basis of Log K_{OW} and/or K_{OC} is not possible

- A prediction on the basis of fish BCF is not possible

What is the conclusion?

Actually, there is no replacement for real bioaccumulation studies!

However, it is strictly recommended to follow the OECD 317

- OECD artificial soil with know C_{org} content
- Standardisation of BAF to BSAF (lipid content worms and C_{org} content soil)

to get representative results under standardized and reproducible conditions

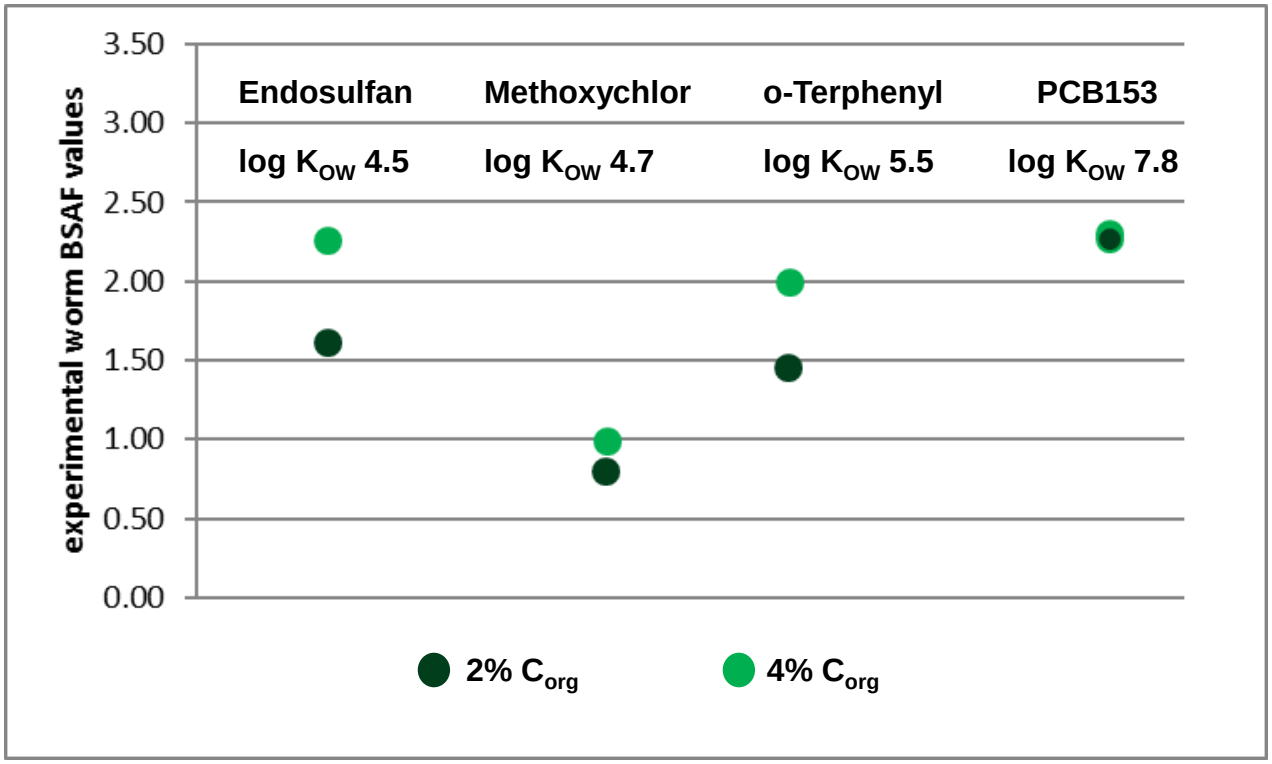
Experimental part “Earthworm bioaccumulation studies (OECD 317)”

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)

- Experimental part (Earthworm bioaccumulation studies (OECD 317))
  - *Eisenia andrei* (Lumbricidae, Oligochaeta)
  - 4 substances
    Endosulfan, Methoxychlor, o-Terphenyl, PCB153; log K_{OW} 4.5 – 7.8
    For all substances experimental fish BCF and BMF values are available
  - 2 artificial soils (5% and 10% peat corresponding with 2.0% and 4.0% C_{org})
  - Evaluation as kinetic BSAF

Experimental part “Earthworm bioaccumulation studies (OECD 317)”

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)



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Experimental part “Earthworm bioaccumulation studies (OECD 317)”

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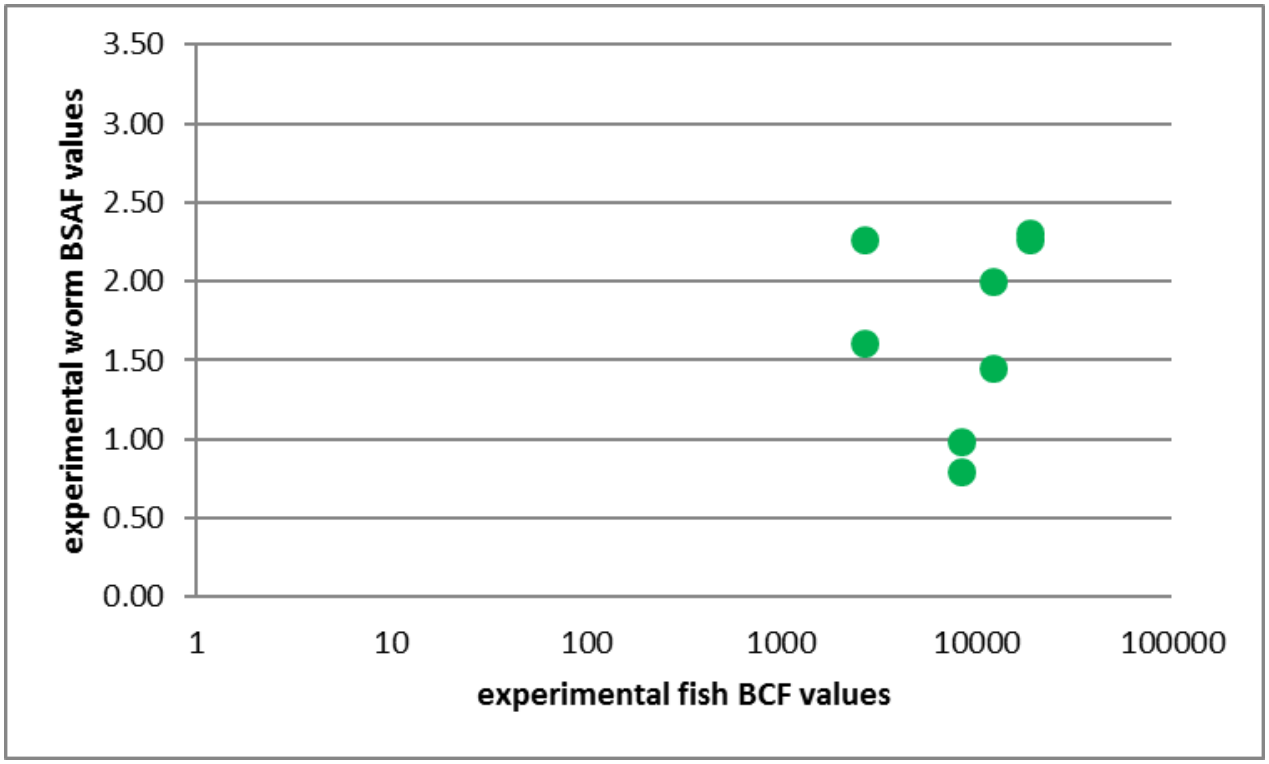

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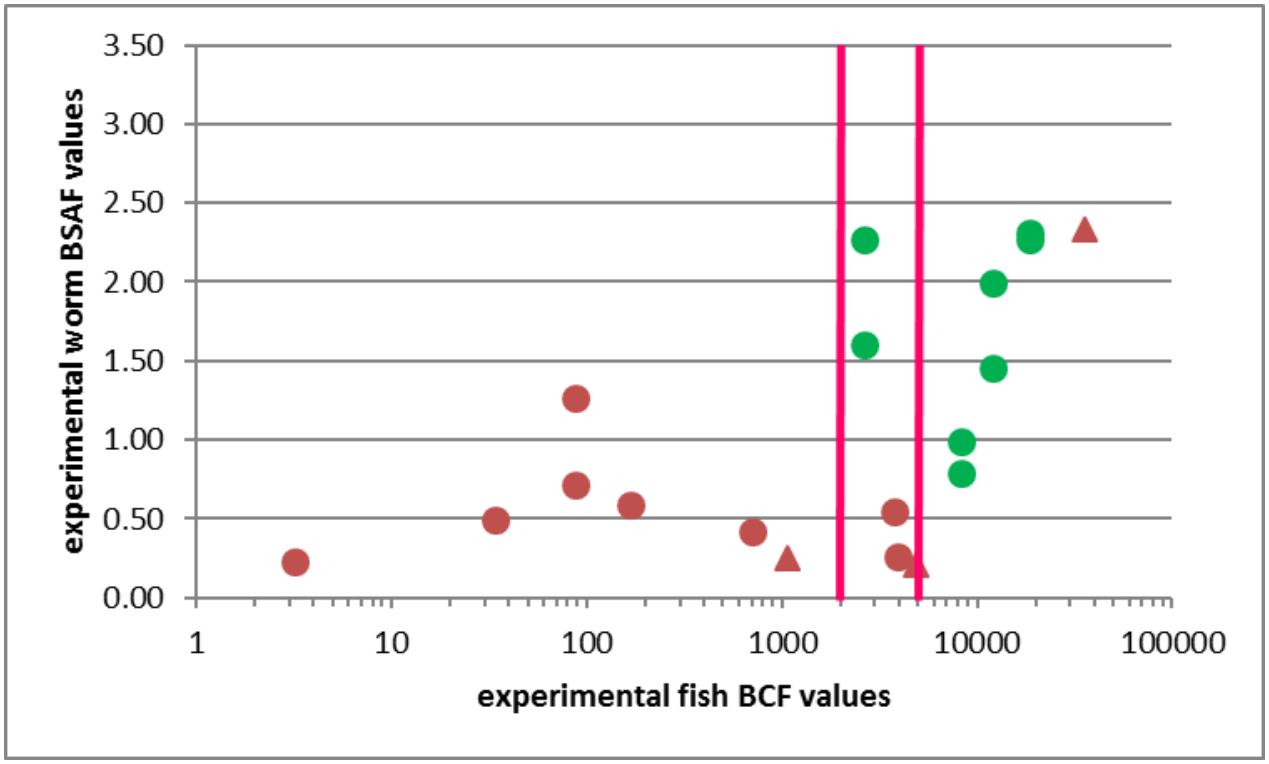
Proposal for a trigger value for terrestrial organism for PBT assessment

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)



The vertical lines at BCF 2000 and 5000 indicate the trigger values for “B” and “vB” for fish.

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Proposal for a trigger value for terrestrial organism for PBT assessment

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)

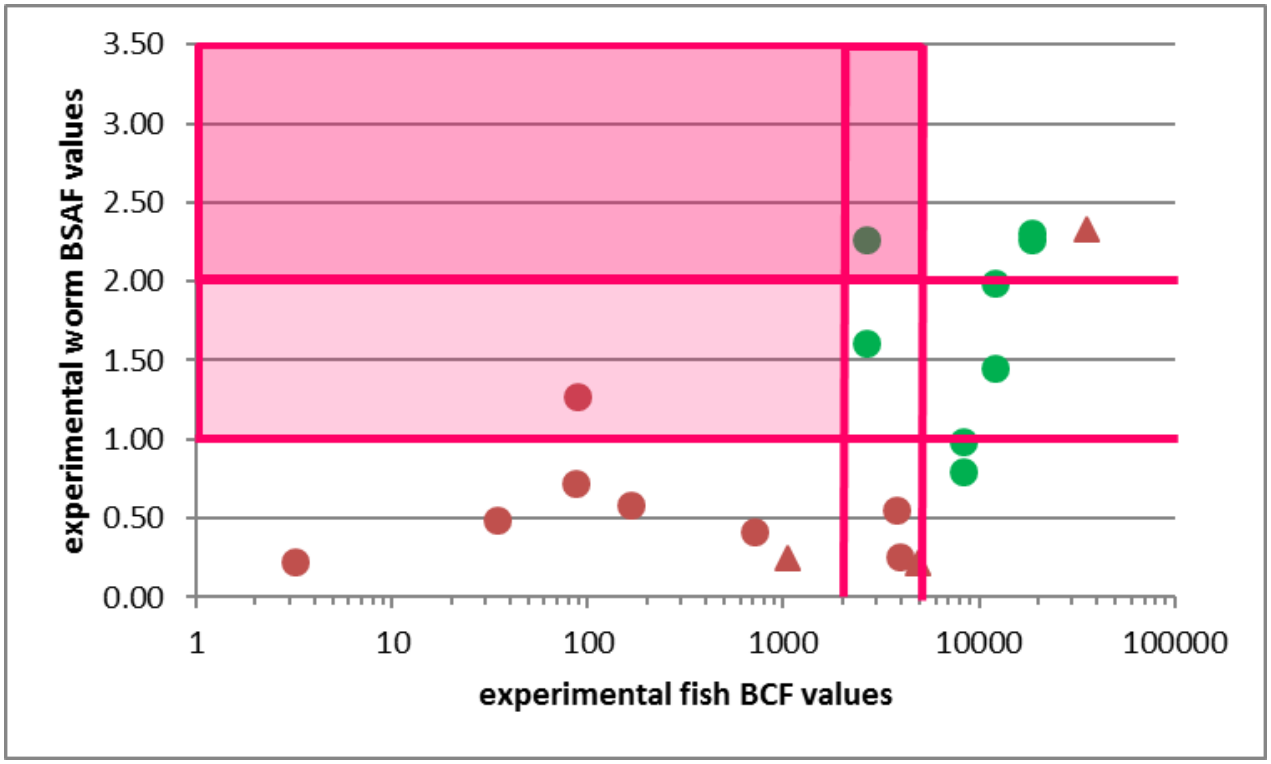
- ECHA guidance R.11 for the assessment of PBT/vPvB (2014)
  BSAF values **≥ 0.5** are indicating **high** bioaccumulation.

Basic assumption for bioaccumulation

Bioaccumulation is given in cases, where a chemical's concentration in the organism is **higher** than the concentration in the respective medium or the organism's feed, i.e., the ratio of C(organism)/C(medium) is **> 1.0**.

Proposal for a trigger value for terrestrial organism for PBT assessment

UBA-Research project „Bioaccumulation of contaminants in soil organisms …“ (FKZ 3713 64 419)



- Considering this basic assumption and the data of this research project, we propose a BSAF of 1.00 as a general trigger for bioaccumulation (B) potential in terrestrial oligochaetes (potentially 2.00 for vB).

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Take home message

- No realistic estimation of an earthworm BAF/BSAF is possible on the basis of Log K_{OW}, K_{OC} or fish BCF
- Lipid- and C_{org}-normalized BSAF should be used for the assessment of terrestrial bioaccumulation
- ECHA guidance concluded that BSAF values of ≥ 0.5 indicate high bioaccumulation
- Based on the data obtained and evaluated in the present work, a ***BSAF trigger value of 1.00*** is proposed as a general trigger to indicate bioaccumulation in terrestrial organisms in the PBT assessment framework

These conclusions are the result of a national workshop (Dessau, Germany) hosted by the German Federal Environment Agency

Slide 1

PBT assessment of substances –
Proposal of a trigger value for bioaccumulation in terrestrial oligochaetes

Slide 2

Assessment and regulation of bioaccumulative (“B”) and very bioaccumulative (“vB”) substances are necessary to ensure a high level of protection of human and animal health, and of the environment.

In aquatic organisms, trigger values for the identification of bioaccumulative (“B”) and very bioaccumulative (“vB”) substances are bioconcentration factors (BCF) of >2000 and >5000, respectively, obtained from fish flow-through studies according to OECD 305.

However, exposure of terrestrial organisms e.g. via spreading of manure or application of plant protection products is also a realistic pathway.
The criteria indicated for identification of B/vB substances in Annex XIII of the REACH regulation do not define trigger values regarding bioaccumulation in terrestrial organisms despite of an adopted internationally accepted, standardized OECD guideline regarding bioaccumulation in terrestrial oligochaetes

Existing BCF trigger values for aquatic organism cannot be converted to BAF or BSAF values for terrestrial organisms due to lack of suitable data and different processes of uptake and depuration.

Slide 3

The objective of this project is to provide a suitable data base which will help to clarify how to address terrestrial bioaccumulation in the B assessment and to define trigger values for the bioaccumulation factor (BAF) obtained from bioaccumulation studies with terrestrial oligochaetes according to OECD 317 that are comparable to the B/vB criteria in the scope of the PBT guidance revision.

Slide 4

For this aim, the project comprised the following three steps:

1) Literature research on available bioaccumulation factors (BAFs) both in open scientific literature and in regulatory data from several OECD 317 studies and performance of correlation analysis between soil-/substance-properties, BCF and BAF values.

2) Performance of bioaccumulation studies according to OECD 317 with the earthworm *Eisenia andrei* using the four model substances endosulfan, methoxychlor, o-terphenyl and PCB153 in artificial soil with two different Corg contents.

3) Proposal of a trigger value for identification of bioaccumulative substances in terrestrial organisms based on the literature research and the performed experimental results.

Slide 5

In the scope of administrative enforcement, data sets from the Information system Chemical Safety (ICS) for 21 plant protection substances containing the complete list of endpoints and reports from bioaccumulation studies with oligochaetes were provided by the UBA for evaluation. For 10 substances, study design was according to or at least following the OECD 317 guideline. For 9 substances data for kinetic uptake and elimination rate constants for terrestrial oligocheates are available and for 8 substances BSAF values for terrestrial oligochaetes are available – which is recommended by the guideline. All studies were performed using lumbricid species.

In a literature search regarding terrestrial bioaccumulation, in total, 85 publications with studies on lumbricidae were identified. For 7 substances, study design was according to or at least following the OECD 317 guideline. For all 7 substances data for kinetic uptake and elimination rate constants are available. However, for none of them BSAF values are available – due to the lack of lipid measurements in worms or Corg measurements in the soil.

Slide 6

The terrestrial BAF values from the guideline-compliant or guideline-based studies range from 0 to 22.

The European Food Safety Authority (EFSA) provides two calculation models based on Kow, Koc and Corg values. The first is a general model based on substance concentration in dry soil. The second is recommended for soft bodied soil organisms like earthworms and is based on the pore water concentration.

Figure 1: The theoretical BAF values calculated according to the "dry soil approach" are within the range of 0.01 and 429 which overestimate the experimental data.
Figure 2: The theoretical BAF values calculated according to the "pore water aproach" are within the range of 0.875 and 0.937 and underestimate the experimental data.
No correlation between experimental BAF values and theoretical BAF values were obtained.

Slide 7

How can it be that no correlation occurs even when it is known that Kow and Koc of a substance and the Corg content of the matrix affect the bioaccumulation of – at least lipophilic – substances?

Vlčková and Hofmann demonstraded in a well documented publication the influence of soil Corg content on the bioaccumulation factor in earthworms five substances with a range of Kow.
Figure 1: As an example the results for PCB153 are shown. There is a clear correlation between the BAF value and the soil Corg content. However, comparing the results for artificial soil and natural soil, it can be seen that other matrix properties affect the BAF.

The BAF depends on Corg - but also on other matrix properties.

Slide 8

The European Food Safety Authority (EFSA) suggest to equate lipid/Corg standardized BAF values to a lipid-normalised BCF. Consequently, a correlation should exist.

However no correlation can be seen for the data found.

Slide 9

To sum up, we can say that

Soil composition affects BAF values
but it is not possible to predict the BAF on the basis of Log Kow and/or Koc of a substance
and it is also not possible to predict the BAF on the basis of the fish BCF.

Slide 10

What is the conclusion?

Actually, there is no replacement for real bioaccumulation studies!

However, it is strictly recommended to follow the OECD 317 guideline regarding the use of OECD artificial soil with documented Corg content and the calculation of lipid/Corg standardized Biota-Soil-Accumulation Factors (BSAF) – which requires also lipid measurements of the worms. This is absolutely essential to get results under standardized and reproducible conditions.

Slide 11

To increase the number of guideline compliant data, bioaccumulation studies were conducted with the earthworm Eisenia andrei for 4 representative substances covering a log Kow range of 4.5 – 7.8. To verify the influence of soil Corg on the BAF and to check if lipid/Corg standardization balance the possible effect, the studies were conducted with artificial soil at two Corg contents. To get kinetic BSAF values, data from the uptake and the depuration phase were applied.

Slide 12

The results show that – based on the data for these four substances - there is no clear correlation between log Kow and the BSAF values.
The results confirmed the effect of the soil Corg content on the accumulation factor. However, lipid/Corg standardization balanced this effect or even turned it around.

Let’s have a look on the results in the context of the literature data.

Slide 13

These are the experimental BSAF values compared with the respective fish BCF values for the substances.

Slide 14

Here, BSAF/BCF data pairs from literature for 11 other substances are included. Circles are ICS data and triangles are from open literature. The vertical lines at BCF 2000 and 5000 indicate the trigger values for “B” and “vB” for fish.

Slide 15

Even when the REACH regulation do not define trigger values regarding bioaccumulation in terrestrial organisms, ECHA guidance for the assessment of PBT/vPvB (2014) suggest that BSAF values equal or higher 0.5 are indicating high bioaccumulation.

But the basic assumption for bioaccumulation ist hat

Bioaccumulation is given in cases, where a chemical's concentration in the organism is higher than the concentration in the respective medium or the organism's feed, i.e., the ratio of C(organism)/C(medium) is higher than 1.0.

Slide 16

Considering this basic assumption and the data obtained in this research project, we propose a BSAF of 1.00 as a general trigger for bioaccumulation potential in terrestrial oligochaetes and potentially a trigger of 2.00 for very bioaccumulating.

Slide 17

To sum up, the main statements of this project are

No realistic estimation of an earthworm BAF/BSAF is possible on the basis of Log Kow, Koc or fish BCF

Lipid- and Corg-normalized BSAF values should be used for the assessment of terrestrial bioaccumulation

ECHA guidance concluded that BSAF values equal or higher than 0.5 indicate high bioaccumulation.

However, based on the data obtained and evaluated in the present work, a
BSAF trigger value of 1.00 is proposed as a general trigger to indicate bioaccumulation in terrestrial organisms in the PBT assessment framework.