

'B-compass fish' calculation tool for calculation of bioconcentration factors

MANUAL

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For further information, we refer to the paper associated with B-compass fish:

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Check www.ufz.de/b-compass-fish for updates of B-compass fish.

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1. BACKGROUND

The assessment of the bioaccumulation potential of chemicals is an important aspect in the identification of substances of potential concern for humans and the environment. Typically, the bioaccumulation potential of a chemical for aquatic organisms is quantified by the fish bioconcentration factor (BCF). The BCF is the steady state concentration of the chemical in the fish divided by the concentration of the chemical in the surrounding water phase. According to the European chemicals regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), a chemical with a BCF > 2000 L/kg is considered bioaccumulative, a chemical with a BCF > 5000 L/kg is considered very bioaccumulative. In most cases, BCFs are determined using *in vivo* fish tests (OECD Test No. 205 [1]).

In accordance with the 3Rs principle (replacement, reduction, refinement), alternatives to animal testing are also required for the determination of BCFs. The prediction of BCFs by suitable models is considered a promising approach. Very simple prediction models calculate the BCF of a chemical solely on the basis of its hydrophobicity (expressed by the octanol-water partition coefficient KOW), but these models fail when the chemical is actively eliminated in fish. More advanced predictive models therefore calculate the BCF considering elimination or biotransformation of the chemical in the organism. To consider biotransformation in models, knowledge of the corresponding biotransformation rate constant is required. In contrast to other elimination processes, however, the rate of biotransformation cannot be easily estimated from the physicochemical properties of the chemical. Instead, the current method of choice is to perform *in vitro* biotransformation studies with hepatocytes or S9 fractions and then predict the corresponding *in vivo* kinetics from the collected data (*in vitro-in vivo*-extrapolation). Standardized OECD test guidelines (OECD Test No. 319A and 319B [2, 3]) have already been developed for the performance of *in vitro* biotransformation studies.

2. GENERAL INFORMATION ON „B-COMPASS FISH“

The B-compass fish tool presented here provides models for the calculation of BCFs using *in vitro* biotransformation data. The implemented models are further developments of already existing models [4, 5], because extrahepatic biotransformation (e.g. in the gills or gastrointestinal tract) can also be taken into account. To calculate the BCF, B-compass fish uses two models simultaneously: a simple one-compartment fish model and a more complex multi-compartment fish model. The purpose of using two models simultaneously is to ensure a worst case assessment of the bioaccumulation potential of a chemical. The multi-compartment model differs from the single-compartment model in that blood flow limitation as well as the potential first pass effect in the gills and direct GIT-to-liver blood flow are represented. The one-compartment model, in contrast, neglects these processes. For a detailed mathematical description of the two models we refer to the corresponding paper [6]. *In vitro-in vivo*-extrapolation is performed in B-compass fish according to the recently published revised extrapolation formalism [7].

The results of both models are output simultaneously in B-compass fish. Both model calculations are based on the same minimum data input. The additional physiological information required by the multi-compartment model is already provided in the calculation tool as default values. By comparing the results of the two models, the user can evaluate if blood flow limitation, first-pass effect and direct GIT-to-liver blood flow are relevant for calculating the BCF of the chemical of interest (i.e. if there are differences between the results of both models).

In addition to the calculation of BCFs, B-compass fish also allows the calculation of bioaccumulation factors (BAFs) that differ with respect to the exposure scenario of BCFs. In the BCF scenario the contamination occurs exclusively via ventilation, while in the BAF scenario the contamination occurs via ventilation and food. Thus, the BAF represents a more realistic exposure scenario due to the

simultaneous contamination via food and ventilation, so that it might become interesting as an assessment criterion in the future.

3. APPLICATION OF B-COMPASS FISH

For application of B-compass fish it is essential to activate all macros in the macro settings of Microsoft Excel (for details see [HOW TO ACTIVATE MACROS?](#)).

As described above, B-compass fish contains both a one-compartment model and a multi-compartment model for BCF calculation. The user does not have to decide which of the two models is to be used, but the results of both models are provided simultaneously without any additional effort for the user. All required rate constants, in case of the one-compartment model these are whole body rate constants, in case of the multi-compartment model organ rate constants, are calculated automatically from the input data.

To ensure clarity, B-compass fish is presented in the form of several spreadsheets. For the standard application of B-compass fish, only the "Compound input data" and "Organism input data" sheets require input from the user. The calculated BCF values can then be accessed by the user in the next sheet called "BCF".

The other spreadsheets show details of the calculation of the individual uptake and elimination processes. Here, changes by the user are only necessary if desired.

In the following, the individual spreadsheets are briefly explained:

INTRODUCTION

This spreadsheet contains a brief overview of the model structures and application of B-compass fish.

COMPOUND INPUT DATA

This spreadsheet contains a list of all substance-specific input parameters. To be entered by the user:

- Partition information

The partition information is calculated either via correlations with the octanol-water partition coefficient ($\log K_{ow}$) of the chemical or via poly-parameter linear free energy relationships (pp-LFERs). Accordingly, there are two variants of the B-compass fish tool, which differ only in the required partition input parameters and require either the $\log K_{ow}$ of the chemical or pp-LFER descriptors (from the UFZ-LSER database www.ufz.de/lserd) of the chemical as input.

- *in vitro*-assay information

To enter the *in vitro* information, you must first select the appropriate option from the available option fields:

- > „S9-assay“, if *in vitro* data from S9 assays should be used
- > „cell assay“, if *in vitro* data from cell assays should be used
- > „N/A“, if no *in vitro* data are available

Depending on the selected option field, input fields for assay rate constant, S9 or cell concentration, total assay volume and, in the case of cell assays, also cell viability in the assay will then be visible. From the entered data unbound fractions *in vitro* and *in vivo* as well as partition coefficients are calculated automatically.

In addition, default values for the content of dissolved organic carbon (DOC) and particulate organic carbon (POC) in surface water are provided in this spreadsheet to allow the sorption of the chemical to DOC/POC to be considered in the BCF calculation.

ORGANISM INPUT DATA

This spreadsheet contains a list of all required fish physiological input parameters. To be entered by the user:

- bodyweight
- lipid content
- and surrounding temperature of the modeled fish

For numerous other physiological information (blood flow rates, compositions, scaling factors) typical standard values for rainbow trout are already stored, which do not need to be changed by the user (unless another fish species is to be calculated).

BCF

This spreadsheet presents the results of the BCF calculation:

- 1) partition based BCF
i.e. the BCF resulting from the partition properties of the chemical without considering biotransformation
- 2) „kinetic“ BCF based on total chemical concentration in the surrounding water
i.e. calculated on the basis of uptake and elimination kinetics
(this BCF is consistent with experimental BCFs determined according to the OECD 305 Guideline)
 - a) calculated with the one-compartment model
 - b) calculated with the multi-compartment model
- 3) „kinetic“ BCF based on freely dissolved chemical concentration in the surrounding water
 - a) calculated with the one-compartment model
 - b) calculated with the multi-compartment model
- 4) Whole-body elimination rate constant
Sum of the whole body rate constants of all elimination pathways, i.e. ventilation, biotransformation in liver, GIT and gills and excretion via feces
- 5) in addition, this sheet contains two graphs illustrating the steady-state mass fluxes of the individual elimination paths relative to the mass taken up

k_1

This spreadsheet contains two allometric equations for calculating the uptake rate constant k_1 via ventilation in the gills. The Sijm-method [8] is preselected as the standard equation because this equation is also referred to in the OECD guideline. However, the user can change this selection if necessary.

k_2 – GILLS

This spreadsheet calculates the whole body rate constant k_v for elimination via ventilation (branchial elimination) using the organism water partition coefficient and k_1 . In addition, the whole-body rate constant k_v is converted to a corresponding gill elimination rate constant $k_{\text{gills,ventilation}}$, which is required in the multi-compartment model.

k_2 – HEPATIC BIOTRANSFORMATION

If the user has entered S9 or cell assay *in vitro* data in the "compound input data" sheet, the hepatic *in vivo* biotransformation rate constant is calculated in the "k2 - hepatic biotransformation" sheet by *in vitro-in vivo*-extrapolation from the entered *in vitro* rate constant. Thereby, both an *in vivo* whole-body biotransformation rate $k_{\text{B,LIVER}}$ for the one-compartment model and an *in vivo* liver biotransformation rate constant $k_{\text{liver,biotransf}}$ for the multi-compartment model are calculated. In addition, this spreadsheet converts the liver blood flow rate entered in the "organism input data" sheet into rate constants that describe the transport of the chemical into and out of the liver with the blood flow.

k₂ – GILL BIOTRANSFORMATION

This spreadsheet is analogous to the "k₂ - hepatic biotransformation" sheet, except that here *in vitro* data for gill biotransformation are extrapolated.

k₂ – GIT BIOTRANSFORMATION

This spreadsheet is analogous to the "k₂ - hepatic biotransformation" sheet, except that here *in vitro* data for GIT biotransformation are extrapolated.

k₂ – FECES

This spreadsheet calculates the whole body rate constant k_E for the excretion of the chemical with the feces using an equation of Arnot and Gobas [9]. In addition, the whole-body rate constant k_E is converted to a corresponding GIT elimination rate constant $k_{GIT,feces}$, which is required in the multi-compartment model.

REFERENCES

This sheet contains references for the equations and physiological parameters used.

GLOSSARY

This sheet contains explanations of all variables and color codes used.

BAF

This spreadsheet contains additional input data, which are necessary for the BAF calculation, and presents the results of the BAF calculation.

For the calculation of the BAF as additional input data the concentration of the chemical in the food as well as the feeding rate is necessary. For the feeding rate a default value is stored in the "BAF" sheet and the concentration of the chemical is calculated in the "BAF" sheet based on the partition properties of the chemical (i.e. equilibrium between food and the surrounding water is assumed).

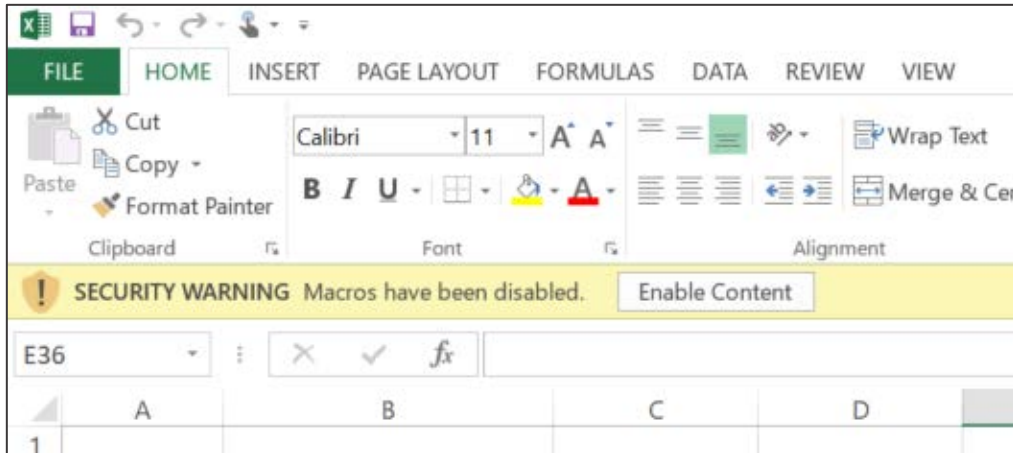
The following values are given as results:

- 1) "Kinetic" BAF based on the total concentration of the chemical in the surrounding water
 - a) calculated with the one-compartment model
 - b) calculated with the multi-compartment model
- 2) "Kinetic" BAF based on the free dissolved concentration of the chemical in the surrounding water
 - a) calculated with the one-compartment model
 - b) calculated with the multi-compartment model
- 3) in addition, this spreadsheet contains two graphs illustrating the steady-state mass fluxes of the individual elimination paths relative to the mass taken up

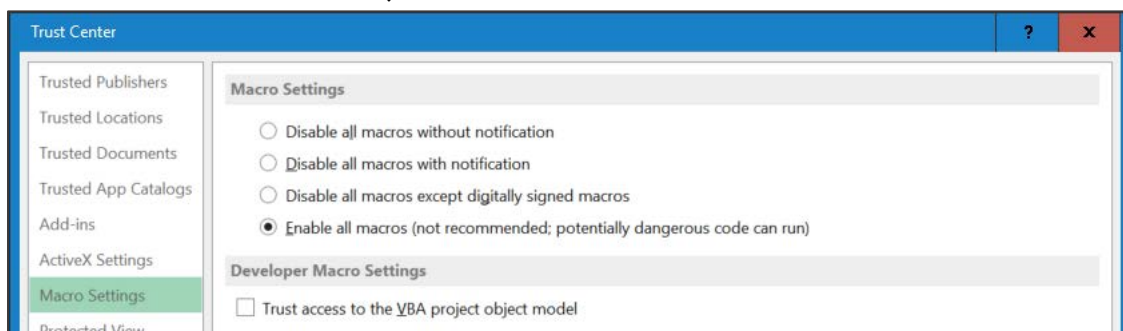
HOW TO ACTIVATE MACROS?

You can activate the macros after opening B-Compass Fish

- a) by either confirming macro activation in the security warning that appears automatically by clicking on "Enable content"



- b) or by opening the „Macro Security“ dialog box via the “Developer“ tab and setting the macro settings there to "Enable all macros".



VERSIONS OF B-COMPASS FISH

The B-compass fish tool was first published in May 2020. Later on, the following updates were released:

- Version 1.1 (March 2021)

The default value for the scaling factor for intestinal S9 was updated as new data became available. Furthermore, there were minor changes in design (colors, fonts).

- Version 1.2 (April 2021)

After the workshop “Advancing Bioaccumulation Assessment with IVIVE”, new default values for the concentrations of dissolved and particular organic carbon present in water during OECD 305 BCF fish tests were implemented. The new defaults now correspond to the upper acceptable limits indicated in the OECD 305 test guideline [1]. The values used previously were standard values for surface waters, which are also used in the model by Nichols et al. 2013 [4]. These values were higher than the values now implemented. These differences affect the BCF prediction particularly for highly hydrophobic chemicals.

ABBREVEATIONS AND VARIABLE NAMES

k_1	gill uptake rate constant [L/kg/d]
k_{assay}	first-order biotransformation rate constant determined in assay [1/h]
BAF	bioconcentration factor [$L_{\text{water}}/kg_{\text{fish}}$]
BCF	bioaccumulation factor [$L_{\text{water}}/kg_{\text{fish}}$]
DOC	dissolved organic carbon concentration [kg/L]
GIT	gastrointestinal tract
k_2	elimination rate constant [L/d]
$k_{\text{B,LIVER}}$	whole-body biotransformation rate constant [1/d]
k_{E}	fecal egestion rate constant [1/d]
$k_{\text{gills,ventilation}}$	"gill-based" ventilation rate constant [1/d]
$k_{\text{GIT,feces}}$	GIT fecal egestion rate constant [1/d]
$k_{\text{liver,biotransf}}$	liver biotransformation rate constant referring to liver concentration [1/d]
K_{OW}	octanol-water partition coefficient [$L_{\text{H}_2\text{O}}/L_{\text{octanol}}$]
k_{V}	whole-body ventilation elimination rate constant [1/d] (also called branchial elimination rate constant)
POC	particulate organic carbon concentration [kg/L]
pp-LFER	polyparameter linear free energy relationship

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