A new data set for PBTK modelling to improve the in-silico assessment of the bioaccumulation and toxicity potential of chemicals

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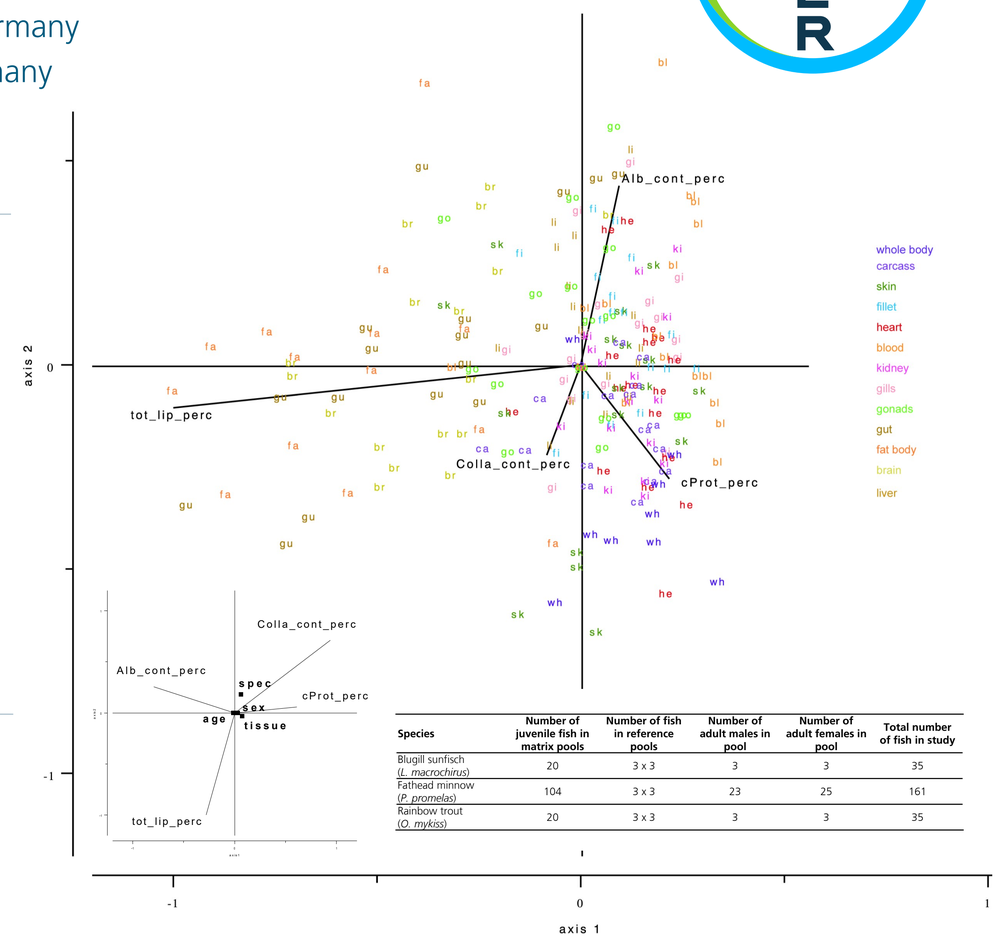
Introduction

PBTK (physiologically based toxicokinetic) modelling is an important tool with increasing relevance for the assessment of the bioaccumulation and toxicity potential of chemicals in the aquatic environment. For the refinement of PBTK models, we provide a holistic data set for the warm-water species *Danio rerio* and *Pimephales promelas* (both *Cyprinidae*), *Oryzias latipes* (*Adrianichthyidae*) and *Lepomis macrochirus* (*Centrarchidae*), as well as for *Oncorhynchus mykiss* (*Salmonidae*) as a representative of cold-water fish species. The dataset comprises information on allometry (age, size, sex), physiological measurements (respiratory rates), as well as composition of the different organ matrices with respect to lipid and protein content.

Model concept and improvement with measured data

To improve modelling of critical assessment parameters, a deep parameterisation of physiological processes and compositional aspects of organs is inevitable. The basic model from Nichols [1] was based on lumped parameters. The model was further extended to a fully mechanistical multicompartment model by Larisch at al. [2] and provides a detailed description of physiological processes. Being extendable to different organisms, as shown for a less complex model by Brinkmann et al. [3], made this model the model of choice. However, data of organ composition with regard to protein and lipid contents were rarely available. With our screening, we generated a broad dataset to improve the data basis on a molecular level (Figure 1) for compartments considered in the model (Figure 2) after Larisch at al. [2]. At physiological level, previous studies have shown that the ventilation rate and the resulting respiration rate are crucial for the uptake and elimination kinetic of compounds via gills. Recent attempts to consider the ventilation rate of fish are based on allometric scaling, yet this scaling factor for fish is very uncertain [2] and results in different kinetics and, finally, modelling results (Figure 3). With a broad respiration study, we provide weight-normalized respiration data from species typically used in in–vivo tests to improve the data basis at physiological level (Figure 4).



Species	Number of juvenile fish in matrix pools	Number of fish in reference pools	Number of adult males in pool	Number of adult females in pool	Total number of fish in study
Blugill sunfisch (*L. macrochirus*)	20	3 x 3	3	3	35
Fathead minnow (*P. promelas*)	104	3 x 3	23	25	161
Rainbow trout (*O. mykiss*)	20	3 x 3	3	3	35

Figure 1: Correspondence analysis (CA) *allows a broad overview on measured data of protein parameters and whole lipid content across tissues. Total lipids were determined gravimetrically from tissue samples after solvent extraction according to a method after Smedes, 1999 [5]. For total protein isolation T-PER™ reagent was used before determination of the total protein content using a Pierce BCA assay kit. Collagen content was determined from protein extracts with the EnzyFlou™ assay kit, the albumin content was determined using a bromocresol purple (BCP) assay kit. The holistic analysis of all measured protein and lipid data via CA reveal the both axis 1 and 2 representing the eigenvalues defined by the assessed parameters. The four vectors show the effect size (by length of the vector) and if parameters are correlated (by angle between vectors) with each other. Positions of the tissue shortcuts represent single samples with the joint information from all measured data. In addition, the canonical CA (small plot) displays the effect size of species, tissue age and sex. Results from single statistical testing are summarized below.*

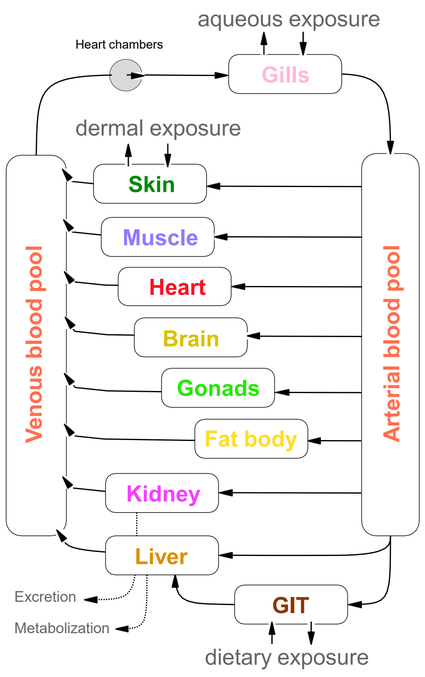


Figure 2: Flowchart of the toxico-kinetic fish model, adapted from [2].

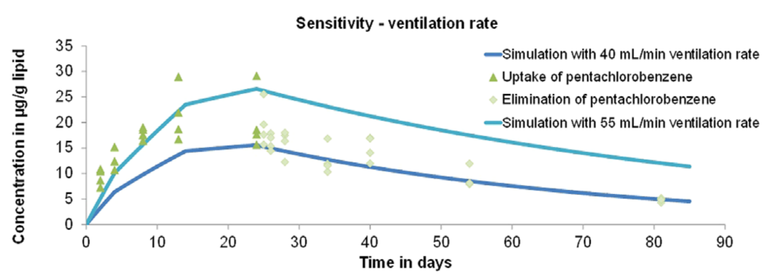
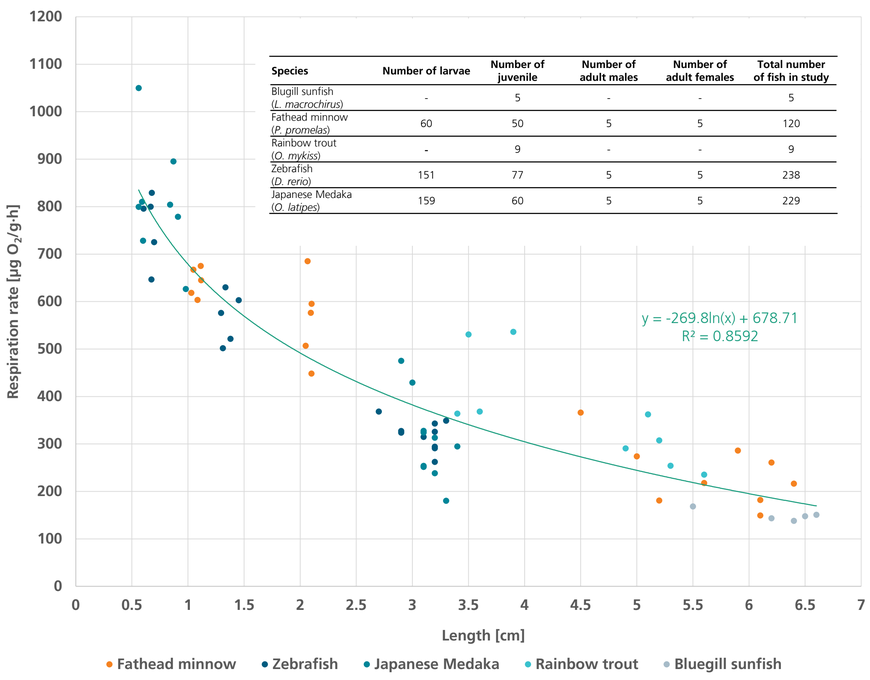


Figure 3: Influence of ventilation rate on modelling of the uptake of pentachlorobenzene in rainbow trout. Figure from [2], data from [4].



Species	Number of larvae	Number of juvenile	Number of adult males	Number of adult females	Total number of fish in study
Blugill sunfish (*L. macrochirus*)	-	5	-	-	5
Fathead minnow (*P. promelas*)	60	50	5	5	120
Rainbow trout (*O. mykiss*)	-	9	-	-	9
Zebrafish (*D. rerio*)	151	77	5	5	238
Japanese Medaka (*O. latipes*)	159	60	5	5	229

Figure 4: Respiration rates of five different fish species *at different ages in relation to their body length. Method: Different life stages from five species were assessed in respiration experiments. Therefore fish were introduced into respiration chambers to record the oxygen consumption. One day before test start, fish were transferred to the test chambers for acclimation. The oxygen consumption was measured then continuously for 24 h. Dots represent the mean of the measurement of single or pools of fish depending on the fish size. The data represent a cross-species dataset for implementation and improvement of physiological data for model enrichment.*

Statistical analysis of organ composition - multivariate linear regression and Tukey Post-hoc test

Lipid content

- Fat body > all other tissues
- Gut > liver, heart, blood, gills, gonads (borderline case), fillet, skin
- Brain > blood
- Bluegill sunfish > fathead minnow and rainbow trout
- No significant differences between sex or age detected

Protein content

- Fat body < all other tissues
- Liver > all other tissues except gonads and kidney
- Kidney > heart, brain, gills, fillet, skin, fat and carcass
- Gonads > brain, skin, fat and carcass
- Blood > skin and fat
- No significant differences found between species, sex or age

Albumin and collagen

- Fat body < kidney, blood, gonads, carcass
- Kidney > heart, brain, gills, gut, skin and fat body
- Fathead minnow significantly higher overall levels than rainbow trout and bluegill sunfish

Outlook

The obtained data will be implemented into the model by Larisch et al. [2] and further validated for different fish species by comparing model predictions with experimental data.

Use of the model

- Visualization of kinetic processes and distribution of compounds between different tissues
- Species extrapolation
- Application in risk assessment for a better mechanistic understanding regarding kinetic processes and observed toxicity
- By application of the model, animal experiments could be reduced in future following the 3R principles

1. Nichols, J. W., et al. (1990). "A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish." Toxicology and Applied Pharmacology 106(3): 433-447.
2. Larisch, W., et al. (2017). "A toxicokinetic model for fish including multiphase sorption features." Environmental Toxicology and Chemistry 36(6): 1538-1546.
3. Brinkmann, M., et al. (2016). "Cross-Species Extrapolation of Uptake and Disposition of Neutral Organic Chemicals in Fish Using a Multispecies Physiologically-Based Toxicokinetic Model Framework." Environmental Science & Technology 50(4): 1914-1923.
4. Adolfsson-Erici, M., et al. (2012). "Measuring bioconcentration factors in fish using exposure to multiple chemicals and internal benchmarking to correct for growth dilution." Environmental Toxicology and Chemistry 31(8): 1853-1860.
5. Smedes, F. (1999). "Determination of the total lipid using non-chlorinated solvents." Analyst, 124, 1711-1718.