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Generic physiologically-based toxicokinetic modelling for fish: Integration of environmental factors and species variability

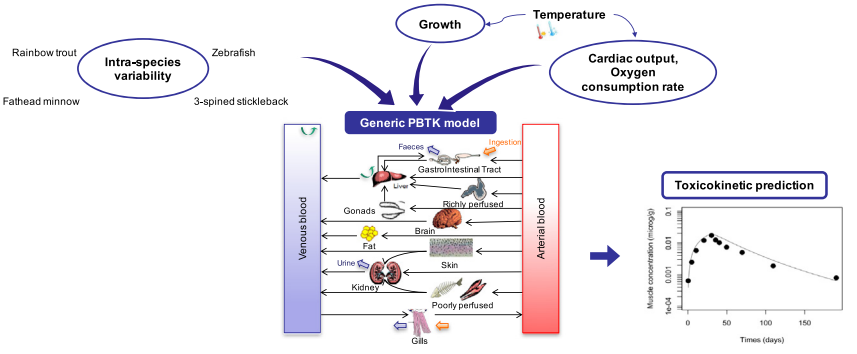
Audrey Grech [a,b,c], Cleo Tebby [a], Céline Brochot [a], Frédéric Y. Bois [a], Anne Bado-Nilles [c], Jean-Lou Dorne [d], Nadia Quignot [b], Rémy Beaudouin [a,c,*]

[a] Institut National de l'Environnement Industriel et des Risques (INERIS), Models for Ecotoxicology and Toxicology Unit, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France
[b] Analytica Laser, 3 rue de l'arrivée, 75015 Paris, France
[c] Institut National de l'Environnement Industriel et des Risques (INERIS), UMR-I 02 SEBIO, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France
[d] European Food Safety Authority (EFSA), Scientific Committee and Emerging Risks Unit, Via Carlo Magno 1A, 43126 Parma, Italy

HIGHLIGHTS

- Development of a PBTK model for rainbow trout, zebrafish, fathead minnow, stickleback.
- New mechanistic sub-models for parameter dependence on temperature and life cycle.
- Intra-species variability was assessed using extensive literature searches.
- Application of PBTK to case studies including different organic substances.
- Adequate PBTK model predictions despite large uncertainties on some parameters

GRAPHICAL ABSTRACT



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ABSTRACT

One of the goals of environmental risk assessment is to protect the whole ecosystem from adverse effects resulting from exposure to chemicals. Many research efforts have aimed to improve the quantification of dose-response relationships through the integration of toxicokinetics. For this purpose, physiologically-based toxicokinetic (PBTK) models have been developed to estimate internal doses from external doses in a time-dependent manner. In this study, a generic PBTK model was developed and adapted for rainbow trout (*Onchorhynchus mykiss*), zebrafish (*Danio rerio*), fathead minnow (*Pimephales promelas*), and three-spined stickleback (*Gasterosteus aculeatus*). New mechanistic approaches were proposed for including the effects of growth and temperature in the model. Physiological parameters and their inter-individual variability were estimated based on the results of extensive literature searches or specific experimental data. The PBTK model was implemented for nine environmental contaminants (with log k_{ow} from −0.9 to 6.8) to predict whole-body concentrations and concentrations in various fish's organs. Sensitivity analyses were performed for a lipophilic and a hydrophilic compound to identify which parameters have most impact on the model's outputs. Model predictions were compared with experimental data according to dataset-specific exposure scenarios and were accurate: 50% of predictions were within a 3-fold factor for six out of nine chemicals and 75% of predictions were within a 3-fold factor for three of the most lipophilic compounds studied. Our model can be used to assess the influence of physiological and environmental factors on the toxicokinetics of chemicals and provide guidance for assessing the effect of those critical factors in environmental risk assessment.

* Corresponding author.
E-mail address: remy.beaudouin@ineris.fr (R. Beaudouin).

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1. Introduction

Environmental risk assessment (ERA) of chemicals is a well-established regulatory and scientific research field which is undergoing constant development (EFSA, 2015). Classically, ERA for a chemical is performed by comparing an exposure metric to a hazard metric for risk characterisation. Most often, the "external dose" (*i.e.* the amount of chemical that reaches an organism) is used to quantify exposure. Over the last two decades, many research efforts have aimed at improving the quantification of the dose-response relationship for the chemical in a given species through the use of internal dose metrics taking into account the toxicokinetics (TK) (EFSA, 2015). TK characterise the time-course of chemical concentrations in the organism based on a description of absorption, distribution, metabolism, and excretion (ADME) processes (Ashauer and Escher, 2010). Ideally, such a description of ADME processes should be quantitative to provide a sound basis for the analysis of an internal dose-response relationship. Internal dose depends partly on the uptake efficiency and bioconcentration potential of the chemical in the organism (Escher and Hermens, 2004). The bioconcentration factor (BCF) is one of the most used and simplest approach to account for chemical bioconcentration quantitatively at steady-state. The BCF is defined as the ratio of the chemical concentration in the organism over the chemical concentration in water or any other biological media at steady-state (Landrum et al., 1992). Under environmental conditions, chemical occurrence and exposure may vary considerably over time and a steady-state assumption may not be relevant or appropriate (Ashauer et al., 2006) since various abiotic and biotic factors can drastically change the TK (*e.g.* water temperature or dilution by growth Stow and Carpenter (1994)). In ERA, TK models have been developed to estimate internal doses based on external doses in a time-dependent manner, and to extrapolate between laboratory and environmental conditions or between species, taking into account abiotic and biotic variation factors (IPCS, 2010; U.S. EPA, 2006).

Physiologically-based toxicokinetic (PBTK) models provide a quantitative mechanistic framework to predict the time-course of the concentration of chemicals in various organs and body fluids. PBTK models are based on the description of the ADME processes, the physiology and anatomy of the organism (Bois and Brochot, 2016). The first fish PBTK model was developed by Nichols et al. (1990) for rainbow trout and was subsequently improved (Nichols et al., 2004; Nichols et al., 1996) and adapted to other species such as channel catfish (*Ictalurus punctatus*) (Nichols et al., 1993), lake trout (*Salvelinus namaycush*) (Lien et al., 2001), fathead minnow (Stadnicka et al., 2012), roach (*Rutilus rutilus*) (Brinkmann et al., 2016) and zebrafish (Brinkmann et al., 2016; Pery et al., 2014). In fish, PBTK models have been developed mostly for adults and have yet rarely considered the impact of physiological processes, such as growth or reproduction, or of environmental conditions on TK (Grech et al., 2017). PBTK models enable dose to dose as well as cross-species extrapolation when species-specific parameters are available, thus bridging gaps on whole-body and tissue bioconcentration, while limiting the need for additional animal experiments. Tissue concentrations in target organs provide better understanding of toxicity, and concentrations at biotransformation sites allow the integration of *in vitro* data (Bois and Brochot, 2016; Kleinow et al., 2008). On the downside, PBTK models require a large amount of information for their parameterisation including physiological data on anatomical, biological, and biochemical entities (*e.g.*, organ volume, cardiac output, and enzyme concentrations) and such information is most often sparse for fish (Grech et al., 2017).

The aim of this study is to improve fish PBTK models through the provision of a more precise description of fish physiology, integrating in particular the effects of growth and temperature changes, for more realistic ERAs. We focus on four teleost freshwater fish species commonly used in ecotoxicology (Yancheva et al., 2015), originating from different ecosystems, with different physiological characteristics. Rainbow trout (*Oncorhynchus mykiss*) and three smaller fish species, zebrafish (*Danio rerio*), fathead minnow (*Pimephales promelas*), and three-spined stickleback (*Gasterosteus aculeatus*) are used worldwide in a variety of biological disciplines including ecotoxicology (Harmon et al., 2009; Lien et al., 1994; Scholz, 2012). First, a meta-analysis of physiological data was performed to measure the uncertainty affecting physiological parameters values and to characterise their inter-individual variability. Second, a sub-model for fish growth was implemented, using equations from the Dynamic Energy Budget (DEB) theory (Kooijman, 2010). Mechanistic equations for the impact of temperature and mass on physiological process were also defined. The influence of temperature, growth, and physiological parameters on TK was investigated by sensitivity analyses using both a lipophilic and a hydrophilic compound. Finally, the model predictions were critically examined in the context of nine case studies.

2. Materials and methods

2.1. PBTK model description

2.1.1. Structure

The general structure of the PBTK model is similar for all four fish species and is presented in Fig. 1. The model comprises twelve compartments: arterial and venous blood, gills, gastrointestinal tract (GIT), skin, kidney, fat, liver, gonads, brain, poorly perfused tissues (PPT), and richly perfused tissues (RPT). All the organs/tissues are modelled as well-mixed compartments with a blood flow-limited distribution. The venous blood that flows out of the RPT, GIT, and gonads collects in the portal vein and enters the liver. A fraction of the venous blood draining the skin and PPT was assumed to flow directly to the kidney, and the remaining blood flow returns to the mixed venous circulation (Nichols et al., 1996; Satchell, 1991; Satchell, 1992; Steffensen and Lomholt, 1992).

Both gastro-intestinal (in case of food ingestion) and branchial absorption are modelled. Exchanges with water occur in the gills and are modelled using an exchange coefficient as suggested by Nichols et al. (1990) and Erickson and McKim (1990b). Chemical exchanges are limited either by the effective respiratory volume or by cardiac output (detailed in Supporting Information, SI, part 1). Absorption from contaminated food is modelled in the GIT as a first order process. Chemical binding to plasma proteins is considered by introducing an unbound fraction of the chemical in plasma. Metabolism is modelled in the liver using either a first order equation, or the Michaelis-Menten equation in case the literature indicated saturable metabolism. Excretion can occur *via* urine, expired water, and faeces (as unabsorbed fraction or by biliary excretion). Compounds excreted by the gills and urine are released in the water and can be reabsorbed in static water conditions. The model equations and parameter abbreviations are provided in the supporting information (SI, part 1).

2.1.2. Dynamic sub-models for PBTK parameters

Water temperature is assumed to affect fish growth, cardiac output, and oxygen consumption rate according to Arrhenius' equation. This equation is commonly used to predict the increase in rate of a physiological process due to increasing temperatures in the vicinity of the optimum temperature for each species (Kooijman, 2010):

$$\dot{k}_T = \dot{k}_r \times \dot{A}_T \text{ with } \dot{A}_T = exp\left(\frac{T_A}{T_r} - \frac{T_A}{T}\right) \quad (1)$$

where T is the water absolute temperature (Kelvin), T_r the reference temperature (in Kelvin) at which the reference value of the process was recorded, T_A the Arrhenius temperature (in Kelvin), $\dot{k}_T$ a reaction rate and $\dot{k}_r$ its value at temperature T_r (the dot indicates a rate, which merely indicates the dimension "per time").