Adv Biochem Eng Biotechnol
DOI: 10.1007/10_2015_5015

Advancing In Vitro–In Vivo Extrapolations of Mechanism-Specific Toxicity Data Through Toxicokinetic Modeling

Markus Brinkmann, Thomas G. Preuss, and Henner Hollert

Abstract International legislation, such as the European REACH regulation (registration, evaluation, authorization, and restriction of chemicals), mandates the assessment of potential risks of an ever-growing number of chemicals to the environment and human health. Although this legislation is considered one of the most important investments in consumer safety ever, the downside is that the current testing strategies within REACH rely on extensive animal testing. To address the ethical conflicts arising from these increased testing requirements,

M. Brinkmann (✉)
Department of Ecosystem Analysis, Institute for Environmental Research, Aachen Biology and Biotechnology – ABBt, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany
e-mail: markus.brinkmann@bio5.rwth-aachen.de

T.G. Preuss
Chair of Environmental Biology and Chemodynamics, Institute for Environmental Research, Aachen Biology and Biotechnology – ABBt, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

Bayer CropScience AG, Monheim am Rhein, Germany

H. Hollert (✉)
Department of Ecosystem Analysis, Institute for Environmental Research, Aachen Biology and Biotechnology – ABBt, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

College of Resources and Environmental Science, Chongqing University, 1 Tiansheng Road Beibei, Chongqing 400715, China

College of Environmental Science and Engineering and State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, 1239 Siping Road, Shanghai, China

State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, China
e-mail: henner.hollert@bio5.rwth-aachen.de

decision-makers, such as the European Chemicals Agency (ECHA), are committed to Russel and Burch's 3R principle (i.e., reduction, replacement, refinement) by demanding that animal experiments should be substituted with appropriate alternatives whenever possible. A potential solution of this dilemma might be the application of in vitro bioassays to estimate toxic effects using cells or cellular components instead of whole organisms. Although such assays are particularly useful to assess potential mechanisms of toxic action, scientists require appropriate methods to extrapolate results from the in vitro level to the situation in vivo. Toxicokinetic models are a straightforward means of bridging this gap. The present chapter describes different available options for in vitro-in vivo extrapolation (IVIVE) of mechanism-specific effects focused on fish species and also reviews the implications of confounding factors during the conduction of in vitro bioassays and their influence on the optimal choice of different dose metrics.

Keywords Bioassay, IVIVE, PBPK, PBTK, Predictive toxicology, Toxicokinetics

Contents

1 Introduction

The emission of anthropogenic chemicals into the environment is a key determinant for water quality and an issue of increasing public and scientific interest. To protect prospectively the environment and ultimately also us humans from the negative consequences of exposure to environmental chemicals, legislation of varying rigor such as the European REACH regulation (which concerns the registration, evaluation, authorisation, and restriction of chemicals) or the United States Toxic Substances Control Act (TSCA) has been established around the world [1–3].

Unlike in previous national legislation, the responsibility to guarantee that chemicals produced in or imported to the European Union are safe in use is assigned solely to industry under REACH following the guiding principle "No data, no market" [2]. To meet this mandate, producers and importers are obliged to register

chemicals in a central database of the European Chemicals Agency (ECHA), along with information on their physicochemical properties and the risk of possible human or environmental health effects. Apart from information on the potential exposure to a chemical, estimating such risks requires information on its toxicity. In the aquatic risk assessment process, toxicity data for representative species of all trophic levels, i.e., destruents (bacteria), producers (algae), and invertebrate and vertebrate consumers (daphnids and fish, respectively), must be provided. With increasing production volumes, these trophic levels need to be covered with different testing requirements [4, 5]. Regardless of the production volume, carcinogenic, mutagenic, and reprotoxic (CMR) substances, persistent, bioaccumulative, and toxic (PBT), and very persistent and very bioaccumulative (vPvB) substances need to be identified and authorized by ECHA [6, 7].

REACH has been estimated to concern approximately 30,000 compounds out of 100,000 chemicals already in use in Europe [8]. Up to May 2015, 13,149 unique substances have been registered [9]. The downside of REACH is that it potentially requires an enormous number of animal experiments [10]. To address this ethical conflict of interest, ECHA is committed to Russell and Burch's 3R principle (i.e., reduction, replacement, refinement) by requiring animal experiments to be substituted with appropriate alternatives whenever possible [11–14]. Non-experimental methods, such as quantitative structure-activity relationships (QSARs), read-across, grouping, or weight-of-evidence approaches are mostly based on previous knowledge about a chemical, and attempt to predict its toxicological effects based on physicochemical characteristics or by assuming that similar chemical structures result in similar effects [12–14]. Experimental animal alternatives, mostly in vitro bioassays, use cells or preparations of biological materials outside their biological context to study the effects of chemicals on biological processes without performing experiments on live animals [15]. The results generated using in vitro bioassays generally cannot easily be transposed to the reaction of whole organisms in vivo [16], which is one reason why they are currently not as widely accepted in regulatory ecotoxicology as would be desirable from an ethical perspective.

To overcome these current limitations, reliable and robust methods for quantitative in vitro-in vivo extrapolation (IVIVE) are urgently needed to face the challenge of increased testing requirements. IVIVE can be roughly subdivided into two distinct areas: (1) IVIVE of pharmaco-/toxicokinetics (PK/TK), i.e., the fate of a chemical within an animal's body and (2) IVIVE of pharmaco-/toxicodynamics (PD/TD), i.e., the effects of a chemical at the site of action [16].

Extrapolations of PK/TK processes generally utilize in vitro bioassays to generate experimental data on individual aspects regarding the processes of absorption, distribution, metabolism, and excretion (ADME). On the one hand these might include the study of active transport phenomena, e.g., at intestinal epithelia using the heterogeneous human epithelial colorectal adenocarcinoma cell line Caco-2 [17] or at the hepatobiliary interface using sandwich-cultured hepatocytes (SCH) assays [18, 19]. On the other hand, they might comprise in vitro assays with hepatocytes or liver subcellular fractions (microsomes or S9 fractions) to study

the metabolic clearance of a chemical from the system through biotransformation enzymes.

By definition, IVIVE of PK/TK requires the application of quantitative PK/TK models that describe the "*baseline disposition*" of a chemical, i.e., its disposition under conditions not affected by active transport or biotransformation phenomena, to be able subsequently to extrapolate the relevance of the process studied in vitro to the in vivo level [20]. The IVIVE of toxicokinetics is currently a very active field of dedicated scientific research, particularly for the assessment of a chemical's bioaccumulation potential [21, 22]. Currently, laborious and expensive exposure studies with fish under flow-through conditions are required to determine the bioconcentration factor (BCF) of a compound as the metric of central regulatory importance with regard to bioaccumulation [23, 24]. Toxicokinetic models for fish typically work sufficiently well for neutral organic substances with low to intermediate *n*-octanol–water partitioning coefficients ($\log K_{ow}$) ranging from 1.5 to 4.5 [25]. If a chemical is readily biotransformed in fish, the actual measured accumulation of that chemical would be lower than predicted by the model. IVIVE of biotransformation can add this extra information to the model and thus has the potential to obviate the need for animal experiments in the context of bioaccumulation assessments. As proof of the importance of such protocols, the Organization for Economic Co-operation and Development (OECD) is currently conducting a project (project 3.13) to establish a new test guideline for in vitro determination of hepatic biotransformation in fish. Nonetheless, this chapter does not go into further detail concerning methods to extrapolate PK/TK processes and parameters from in vitro to in vivo, but capitalizes on methods to extrapolate mechanism-specific effects, i.e., PD/TD processes, from in vitro to in vivo by use of toxicokinetic models.

Following the introduction of the already mentioned Russell and Burch's 3R principle [11], the development of in vitro alternatives to animal experiments has been an active and rapidly progressing field in toxicological research. It is obvious that in vivo outcomes cannot necessarily be directly predicted from effects in vitro. However, the results of in vitro bioassays for mechanism-specific endpoints in particular have often been demonstrated to be highly correlated with the results of in vivo injection studies in rats and mice [26–29]. Unlike in toxicology, chemicals in ecotoxicological research with fish are most often administered through the aqueous phase. Because of differences in physicochemical properties of different chemicals, they can be absorbed at different rates and accumulated to various extents in different tissues and organs [30]. For many studies of this type, no correlation was observed between in vitro and in vivo data [31–33].

It had already been acknowledged in the early 1990s by the critical body residue (CBR) concept that the internal chemical concentration in the organism is a central factor for acute toxicity [34, 35]. Later, this methodology was extended to be able to relate the effects of a chemical to its corresponding concentration in the target tissue; this concept is commonly referred to as the "tissue residue approach for toxicity assessment" (TRA); [36]. Both CBR and TRA are important improvements of our mechanistic understanding of differences in toxicity of chemicals and the

sensitivity of different species. Nonetheless, a major disadvantage is that both concepts are based on either whole-body or tissue-specific BCFs, respectively, which is why they can only be applied under equilibrium conditions [30]. In contrast, toxicokinetic models can be used to predict kinetically the bioconcentration of chemicals and have been demonstrated to be particularly useful as tools for "retrospective" or "reverse" toxicokinetics, i.e., the prediction of toxicokinetics if the analytical information provided with the originally published toxicity data was insufficient [37, 38].

The present chapter presents confounding factors and dose metric considerations which need to be acknowledged when conducting or interpreting in vitro bioassays and IVIVE, summarizes recent approaches to apply toxicokinetic models to problems of IVIVE, and provides examples on how IVIVE can be of practical use in chemical risk assessments of the twenty-first century.

2 Confounding Factors and Dose Metrics Used for In Vitro Testing

When conducting in vitro to in vivo extrapolations, it is evident that not only are the concentrations of chemicals in whole organisms time-variable and variable between different organs and tissues but also the concentration of a chemical test item in in vitro bioassays may, depending on its physicochemical properties, also follow complex temporal variations and differ significantly from the nominal concentration [39, 40]. Theoretically, the most relevant fraction of a chemical for toxicity assessments is the target dose/concentration, often referred to as the biologically effective dose (BED), i.e., the dose or concentration of a chemical reaching the biological site of action [41]. Practically, however, this concentration is difficult to determine experimentally, which is why surrogate dose metrics are used in in vitro research.

Figure 1 illustrates a number of processes which affect the effectively (freely) available chemical concentrations in exposure media, which is acknowledged by the majority of scientists as the only fraction of a chemical readily available for uptake into organisms and cells [40, 43–45], and the freely dissolved internal concentration in cells, which probably shows the greatest correlation with the target dose.

These confounding factors are reflected to a different extent by the most frequently applied dose metrics, i.e., measures of the chemical dose or concentration relative to different reference values, among others the amount of chemical added per volume of exposure medium (nominal concentration), the amount of chemical determined analytically in the exposure medium (total concentration), or the unbound concentration in the medium (freely available concentration). Figure 2 (top) illustrates three different approaches forming the theoretical foundation of the different dose metrics. When nominal concentrations are used as the dose metric,

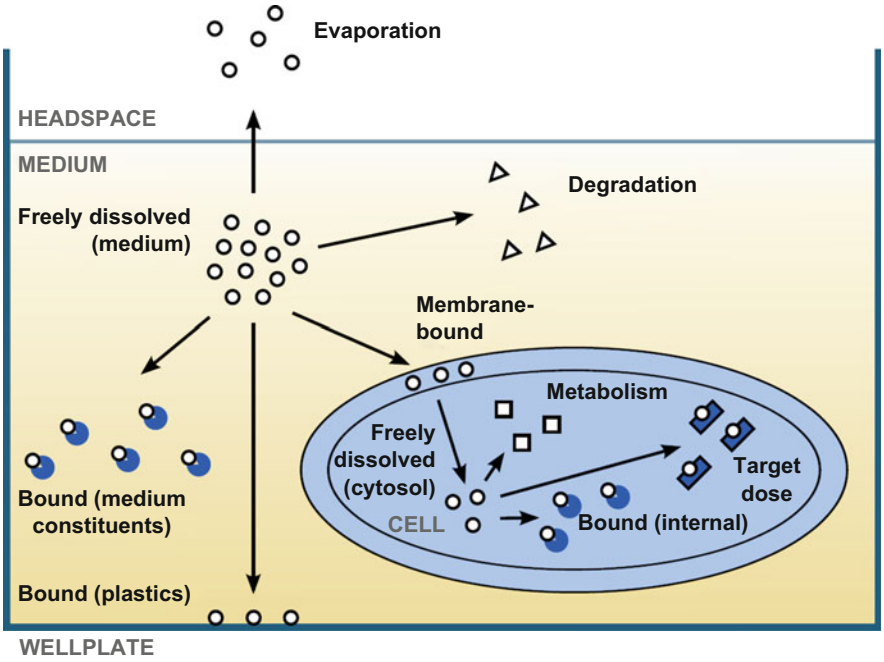

Fig. 1 Illustration of the processes which determine the amount of chemical freely available for uptake into cells and reaching the biological target site, i.e., the target dose. Chemicals within the medium may be subject to evaporation, degradation, as well as binding to plastics or constituents of cell culture media. Within the cell, the chemical might partition into the membrane, be metabolized or bind to cellular constituents. Adapted from Groothuis et al. [39] and Heringa et al. [42]

fractions of the compound that dissipated through binding to plastic materials such as pipette tips and multiwell plates [46–48], evaporation [49–51], degradation, and binding to constituents of the cell culture medium [48, 52] are not accounted for. Choosing total concentration as the dose metric accounts for losses through volatilization, degradation, and binding to plastics, but not for the fraction bound to proteins and other constituents of the exposure media. The latter fraction is only accounted for by the freely available concentration. These discrepancies between nominal, total, and freely available compound concentrations also result in differences of the fraction available for uptake into the cell, and consequently to different measured effect concentrations (Fig. 2, bottom).

To be able to account for these differences appropriately, it appears advisable always to measure or control the freely available concentration of a chemical test item when conducting in vitro bioassays. Analytical methods to measure freely available concentrations comprise equilibrium dialysis, ultrafiltration, centrifugation, and solid-phase micro extraction (SPME) [39, 53, 54]. Furthermore, passive dosing techniques –described in Chap. 5017 in more detail – have been developed to maintain relatively stable free concentrations of the chemicals of interest in

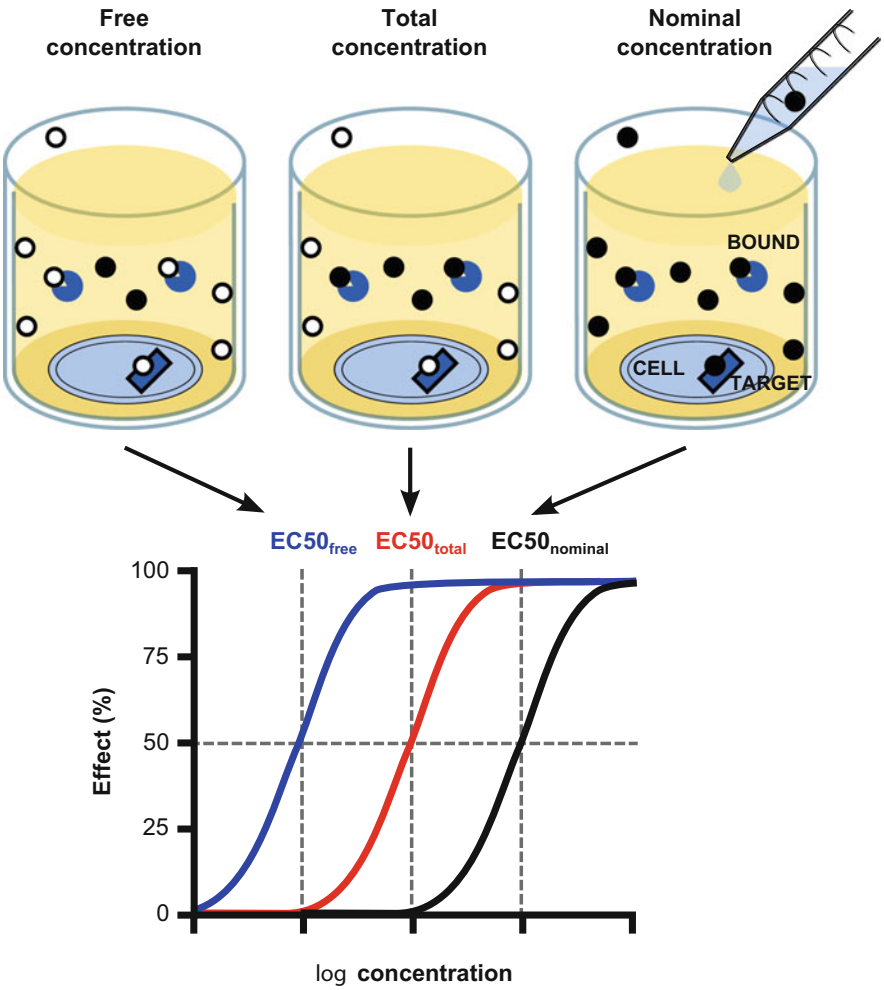

Fig. 2 Illustration of different dose metrics often used in toxicological and ecotoxicological in vitro bioassays, i.e., nominal concentrations, total concentrations, and free concentrations, and the hypothetical influence of applying these different dose metrics on the resulting median effect concentrations (EC_{50}s). *Filled circles*: fraction of molecules included in the dose metric. *Open circles*: fraction of molecules not included in the dose metric. Redrawn from Groothuis et al. [39] and Escher and Hermens [43]

exposure media [55, 56]. Although the analytical determination of the freely available concentration is feasible from an experimental point of view, to maintain the high throughput capability of the different in vitro bioassays it would be desirable to use computational models to predict the free concentration instead if measuring it. Several mathematical approaches for estimating the freely available compound concentration have been proposed, most of which are based on the

partitioning of chemicals to the protein and lipid fraction of exposure media and/or the description of other routes of dissipation [46, 48, 57].

3 Simple IVIVE Methods

In toxicological research on mice and rats, chemicals are often administered through intraperitoneal injection. If the experimental conditions are chosen appropriately (e.g., the exposure time is sufficiently high to reach constant chemical concentrations in the organ of interest) and the variations of physicochemical properties of the investigated compounds are relatively small (e.g., all compounds originate from the same chemical class), in vivo EC_{50}s for mechanism-specific effects may be linearly correlated with in vitro EC_{50}s [26–29]. This assumption was also confirmed to be valid for such effects following intraperitoneal injections in fish by a collection of literature data from our own group (cf. Fig. 6a; [58]).

Castano et al. [59] reviewed cytotoxicity data from fish and mammalian cell lines and found a reasonably good correlation with acute toxicity in fish. They speculate that this good correlation, also between different cell lines, results from the unspecific mode of action responsible for baseline cytotoxicity/narcosis [60, 61]. Following this line of argument, narcotic chemicals cause acute toxicity by unspecifically interfering with biological macromolecules and lipid membranes which are common to all cells and organs. Schirmer et al. [62] thus concluded that cytotoxicity assays with fish cell lines could be a reasonably predictive alternative for the fish acute toxicity test. As detailed in the previous section, Gülden and Seibert [40] found that the predictive power of such correlations is even enhanced when the effective concentrations in cytotoxicity assays are calculated based on freely available chemical concentrations rather than on nominal or total concentrations.

A multi-national research project organized by the Scandinavian Society of Cell Toxicology in the early 1990s under the title “Multicenter Evaluation of In Vitro Cytotoxicity” (MEIC) found a similar correlation between cytotoxicity in mammalian/human cell lines and acutely lethal concentrations in blood [63, 64].

It should be emphasized, however, that such correlative methods are based on a mathematical rather than on a mechanistic foundation, which is why the applicability domain of these methods for IVIVE needs to be evaluated carefully on a substance-by-substance basis. Furthermore, these methods only account for the toxicokinetics in both, cells and animals, to a very limited extent (mainly by choosing specific exposure conditions and durations), which is why they cannot be used for IVIVE of the effects of time-variable exposures or to extrapolate beyond the calibrated range of compounds and/or organisms. In the subsequent sections, we describe how toxicokinetic modeling can be applied to overcome these shortcomings.

4 IVIVE Using Toxicokinetic Modeling

As with the free concentration in cells, one frequently overlooked factor that determines the difference between the reactions of in vitro systems compared to in vivo systems is the by far more complex toxicokinetics in whole organisms, i.e., the processes of absorption, distribution, metabolism, and excretion (ADME). These processes result in complex temporal variations of a compound's concentration at the target site, and in differences of the internal concentrations between the various organs and tissues [43, 65]. When comparing the toxicokinetics of a compound among different species and genera, differences arise from variations in body size, total lipid content, biotransformation capacity, and/or respiratory strategy [66–69]. For example, Nyman et al. [70] experimentally demonstrated the importance of toxicokinetics for interspecies variations in sensitivity of the aquatic invertebrates *Gammarus pulex*, *Gammarus fossarum*, and *Lymnaea stagnalis* exposed to the pesticide diazinon. *L. stagnalis* accumulated a higher whole-body concentration of diazinon than the two gammarids on the basis of whole-body concentrations, but less in target tissues (i.e., the nervous system), thereby explaining the greater tolerance of *L. stagnalis* to diazinon. The same underlying principle has been previously demonstrated by Meador [71], who found that inter-species variation in the acute toxicity of tributyltin to four marine invertebrate and one marine fish species were related to differences in the concentrations in the target organ.

Unlike in the two mentioned examples, it is not always possible to measure the tissue concentrations in organisms, or even directly at the target site [72]. Toxicokinetic models, which are quantitative mathematical descriptions of the ADME processes in biota, are thus increasingly used and valued as powerful tools in ecotoxicology [73, 74].

4.1 Compartmental Toxicokinetic Models

Toxicokinetic models often describe organisms based on one of two strategies: in one-compartment models, the chemical concentration is assumed to be equal throughout the organism, whereas multi-compartment models assume that organisms are composed of different compartments (usually corresponding to organs or tissues) which may differ in their characteristics and the resulting chemical concentrations [75]. Furthermore, they can be differentiated between equilibrium and kinetic models [76], as well as empirical and mechanistic models [30] – all of which have certain advantages and disadvantages. The most widely used toxicokinetic models in aquatic ecotoxicology, probably also because they are recommended by the international guideline OECD 305 [23], are empirical kinetic one-compartment models. Figure 3 depicts a conceptual representation of such a model, which considers the major routes of uptake and elimination. Similar models are frequently

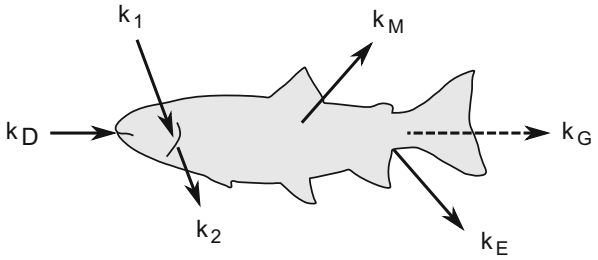

Fig. 3 Conceptual representation of a one-compartment model for fish considering the major routes of chemical uptake and elimination. k_D: dietary uptake rate constant; k_1: gill uptake rate constant; k_2: gill elimination rate constant; k_M: metabolic transformation rate constant; k_E: fecal egestion rate constant; k_G: growth dilution rate constant. Redrawn from Arnot and Gobas [77]

applied in toxicological research on mammals and in pharmacological research on mammals and humans [78], where they have been used with good success for IVIVE [79].

Such models are developed by fitting mathematical equations, e.g., (1), to experimental data of the time-dependence of the chemical concentration in fish exposed to a certain compound [75, 80]. The presented example only takes into consideration uptake and elimination through aqueous routes of exposure, i.e., pure bioconcentration.

$$\frac{d}{dt} C_{\text{int}}(t) = k_1 \cdot C_{\text{w}}(t) - k_2 \cdot C_{\text{int}}(t), \tag{1}$$

where $C_{\text{int}}(t)$ is the internal concentration in the fish per unit body mass, $C_{\text{w}}(t)$ is the chemical concentration in the water per volume, k_1 is the uptake rate constant (volume per unit body mass and time), and k_2 is the elimination rate constant per unit time.

These models can be used with great confidence to interpolate internal chemical concentrations, but they are suitable neither for extrapolation beyond the range of measured values with regard to exposure conditions, species or routes of exposure, nor for predicting a chemical's concentration in specific target organs or tissues [72, 75, 81].

4.2 Physiologically-Based Toxicokinetic Models

Many of the shortcomings of empirical kinetic one-compartment models can be addressed by physiologically-based toxicokinetic (PBTK) models, which are often referred to as physiologically-based pharmacokinetic (PBPK) models in pharmacological research [72]. This model type is based on the physiology of animals or humans rather than on descriptive mathematics, thus providing higher confidence for extrapolations beyond the range of measured concentrations in a toxicokinetic

experiment [75, 82]. Organs and tissues are explicitly represented as individual compartments or as tissue groups within PBTK models, each of which is characterized by its volume (fraction of total body weight), its total lipid and water contents (fraction of tissue wet weight), and the blood flow to the compartment (Fig. 4). Uptake and disposition, i.e., changes of chemical concentrations in each of

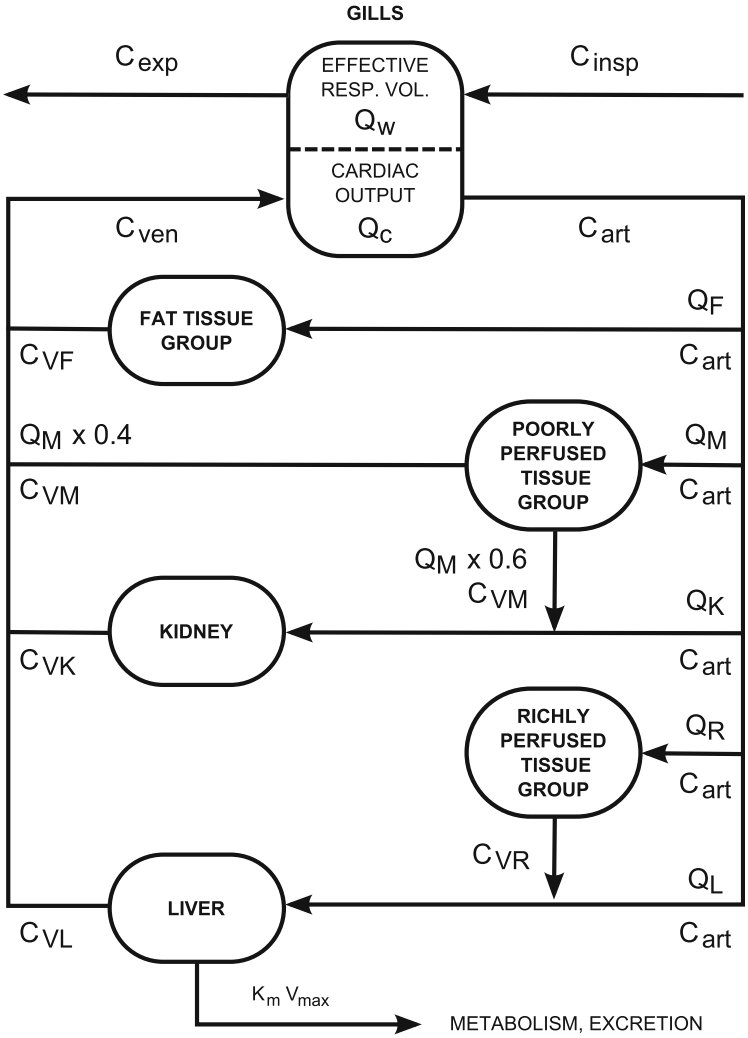

Fig. 4 Conceptional representation of the PBTK model for rainbow trout developed by Nichols et al. [81]. C_{insp}: inspired chemical concentration; C_{exp}: expired chemical concentration; C_{art}: chemical concentration in arterial blood; C_{ven}: chemical concentration in venous blood; Q_F, Q_M, Q_K, Q_R, and Q_L: arterial blood flow to fat tissue group, poorly perfused tissue group, kidney, richly perfused tissue group and liver, respectively (fraction of Q_c); C_{VF}, C_{VM}, C_{VK}, C_{VR}, and C_{VL}: chemical concentration in venous blood leaving fat tissue group, poorly perfused tissue group, kidney, richly perfused tissue group and liver, respectively; K_m: Michaelis–Menten constant of saturable metabolism, V_{max}: maximum velocity of saturable metabolism

Table 1 Compilation of examples of PBPK/TK models for different genera and species

Genus/species	References
Humans	[84–87]
Mammals	
Cattle (*Bos taurus*)	[88]
Sheep (*Ovis aries*)	[89]
Domestic pig (*Sus scrofa domesticus*)	[90]
Rat (*Rattus norvegicus*)	[91]
Mice (*Mus musculus*)	[92]
Syrian hamster (*Mesocricetus auratus*)	[93]
Harbor porpoise (*Phocoena phocoena*)	[94]
Birds	
Chicken/laying hen (*Gallus gallus domesticus*)	[95]
American kestrel (*Falco sparverius*)	[96]
Fish	
Dogfish shark (*Squalus acanthias*)	[97]
Rainbow trout (*Oncorhynchus mykiss*)	[58, 75, 81, 98, 99]
Brook trout (*Salvelinus fontinalis*)	[100]
Lake trout (*Salvelinus namaycush*)	[101]
Atlantic salmon (*Salmo salar*)	[102]
Channel catfish (*Ictalurus punctatus*)	[103]
Fathead minnows (*Pimephales promelas*)	[75]
Tilapia (*Oreochromis mossambicus*)	[104]
Zebrafish (*Danio rerio*)	[105]
Japanese medaka (*Oryzias latipes*)	[106]
Striped mullet (*Mugil cephalus*)	[107]

these compartments, are described by a number of differential equations. Thus, PBTK models are capable of predicting the concentrations of neutral organic pollutants in the whole organism and in different tissues at any time during exposure [16, 83]. Depending on the complexity of the underlying ADME processes and the available experimental data for parameterization and calibration, the level of complexity and sophistication of different PBTK models varies greatly. Although some models are relatively generic in nature and can be applied to a large variety of chemicals, the applicability domain of other models is relatively narrow, e.g., limited to only one specific chemical [72]. The explicit representation of organs and tissues and the high level of mechanistic complexity of PBTK models render them suitable tools for numerous applications in the context of chemical risk assessment and particularly for IVIVE. PBTK models have been developed for a range of different organisms and species, which are exemplarily summarized in Table 1.

Only a limited number of studies have so far used PBTK/PBPK models for IVIVE [16, 108]. De Jongh et al. [109] used in vitro data on biotransformation and tissue-blood partitioning to calibrate a PBTK model for eight neurotoxic compounds (benzene, toluene, lindane, acrylamide, parathion/oxon, caffeine, diazepam, and phenytoin). Subsequently, in vivo neurotoxicity was estimated from in vitro neurotoxicity studies by use of this calibrated model and compared to in vivo data from the literature. This study demonstrated the possibilities and limitations of this approach for the eight reference compounds: although predictions were generally accurate for compounds with low neurotoxicity (approximately twofold deviation from measured values), the accuracy was lower for compounds with higher neurotoxic potency (with deviations up to tenfold). Nonetheless, the study laid the foundation for and defined the direction of research using PBTK models for IVIVE.

Verwei et al. [110] investigated seven compounds with well-described in vivo effects on development. These chemicals were tested in the embryonic stem cell test (EST), which qualitatively classified 5-fluorouracil, methotrexate, retinoic acid, 2-ethoxyacetic acid, and 2-methoxyacetic acid correctly with regard to their in vivo embryotoxic potential. The embryotoxicity of 2-methoxyethanol and 2-ethoxyethanol was underestimated because these compounds require metabolic activation, which is not accounted for in the EST. Next, the authors used a PBTK model to extrapolate the in vitro effect concentrations to the in vivo level. A comparison of the resulting predicted effect values with effect levels measured in rodents resulted in correct predictions for 2-methoxyethanol, 2-ethoxyethanol, methotrexate, and retinoic acid by use of the IVIVE method, although the embryotoxicity of 5-fluorouracil was overestimated. A very similar approach was used by Louisse et al. [111] with good success to predict the developmental toxicity of four different glycol ethers.

The following sections describe how PBTK models for fishes can be used in ecotoxicological research for IVIVE and cross-species extrapolation of bioaccumulation and toxicity, and potentially even in combination with the adverse outcome pathway (AOP) concept.

5 Example: IVIVE of Receptor-Mediated Effects in Rainbow Trout

In a recent study published by our own group, we approached the question of whether the results of in vitro bioassays using primary fish hepatocytes for two receptor-mediated effects can be predictive of effects in rainbow trout in vivo [58]. Endpoints comprised the induction of 7-ethoxyresorufin-*O*-deethylase (EROD) activity which is mediated via the cytosolic aryl hydrocarbon receptor (AhR), and the estrogen receptor (ER)-mediated induction of Vitellogenin (Vtg) expression. EROD activity is a common biomarker of exposure to dioxin-like chemicals (DLCs), whereas Vtg is a biomarker for estrogenic effects in fish,

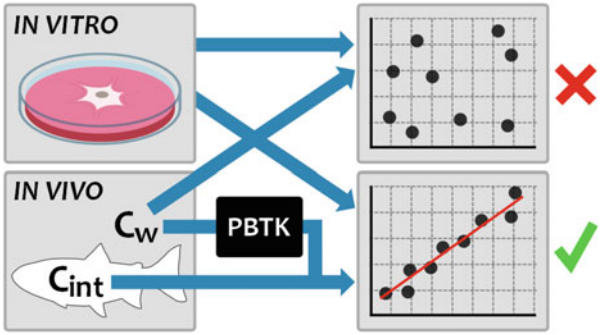

Fig. 5 Outline of the IVIVE study of Brinkmann et al. [58], in which a PBTK model for rainbow trout was used as a tool for reversed toxicokinetics. In vivo EC_{50}s for EROD and Vtg induction based on aqueous concentrations were recalculated to internal concentrations in the liver of exposed fish and then correlated with in vitro data generated by use of fish hepatocytes. Reprinted with permission from Brinkmann et al. [58]. Copyright 2014 American Chemical Society

belonging to the much wider group of endocrine disrupting effects that are a central mode of action (MOA) under REACH [1, 112]. To answer the question raised above, a quantitative framework for IVIVE applying a PBTK model for rainbow trout originally developed by Nichols et al. [82], with modifications by Stadnicka et al. [75], was used (cf. Fig. 4). Five compartments (richly perfused tissues, poorly perfused tissues, liver, kidney, and fat) were explicitly represented in the model. The accuracy of the predictions of the reimplemented model was verified by use of a dataset published by Stadnicka et al. [75].

The original model was extended for the option to simulate injections and an algorithm for saturable metabolism [98]. A comprehensive dataset for the two above-mentioned receptor-mediated MOAs in rainbow trout (EROD and Vtg), was collected, which comprised both in vitro and in vivo data. Using in vivo EC50 values from the literature, the corresponding internal concentrations in the whole body and the liver were calculated using the PBTK model. Both measured and modeled in vivo EC50s were then correlated with the respective in vitro EC50 values (Fig. 5).

Following this approach, it was possible to demonstrate that predicted concentrations of different DLCs in the liver of fish at the corresponding aqueous in vivo EC_{50} showed an excellent correlation with in vitro EC_{50} values. This observation was established on a robust data basis for hepatic activities of EROD (Fig. 6), and confirmed with a smaller and thus weaker dataset for Vtg induction (Fig. 7). Shortly after publication of the research presented in this section, a publication by Stadnicka-Michalak et al. [113] demonstrated that the same methodology was also applicable to predict the acute toxicity of chemicals in fish from cytotoxicity experiments with fish cells with good success.

Together with the results of the present study, this mechanistic link between in vitro alternatives with the corresponding in vivo experiments with fish can be considered an important step towards a broader acceptance of acute and

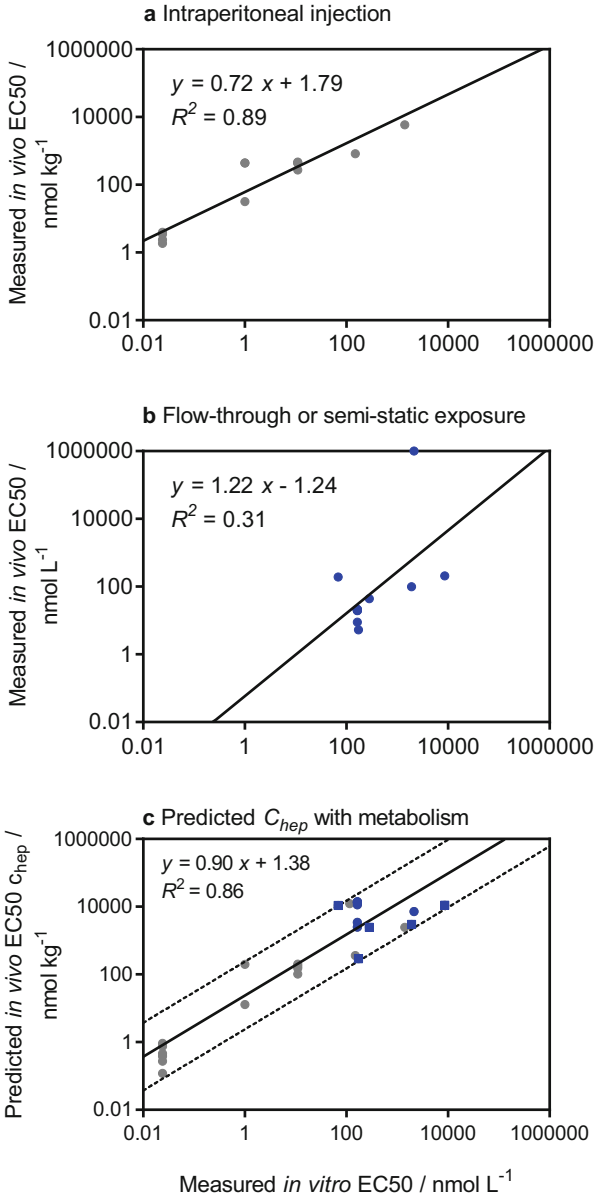

Fig. 6 Correlation between in vitro and in vivo data for EROD induction in rainbow trout on the basis of experimental in vivo EC_{50}s following intraperitoneal injection (**a**) or aqueous exposure (**b**), as well in vivo EC_{50}s from both datasets (**a**, **b**) recalculated to EC_{50}s-based internal hepatic concentrations (IEC_{50}s) by use of the PBTK model (**c**). *Solid lines* represent linear regression line, and *dashed lines* indicate a tenfold difference from the regression line. The coefficient of determination (R^2) and equations for the regression lines using log-transformed data are provided in the graphs. Modified with permission from Brinkmann et al. [58]. Copyright 2014 American Chemical Society

mechanism-specific cell-based bioassays in aquatic risk assessment, and has the potential to result in a major reduction of animals used for toxicity tests with fish.

6 Cross-Species Extrapolation

The next logical step when developing methods for IVIVE is applying the same methods to extrapolate between different organisms, e.g., species of fishes. In the context of most regulatory frameworks, bioaccumulation is considered an inherent substance property that is independent of the actual chemical concentration in the environment [114]. Nonetheless, bioaccumulation in some cases should be viewed with special emphasis on environmental exposure of biota, particularly because bioaccumulation represents the link between the environmental concentration of a chemical and its internal concentration in exposed wildlife [115, 116]. The internal concentration in the target tissue is a key aspect of inter-species differences in sensitivity because it represents the compound fraction which ultimately provokes the biological effects [34, 43].

To be able to account for differences in bioconcentration and toxicokinetics of chemicals between different species of fishes, several approaches have been proposed. Probably the most frequently used method to predict concentrations in biota is the equilibrium partitioning model [117]. In this model it is assumed that the internal concentration of a chemical in an organism depends solely on its concentration in the water phase and the whole-body total lipid content of the organism [118]. There are several factors not taken into account by this simple practitioner's model, including active transport, the influence of the diffusion behavior through cell membranes, different rates of metabolism in various organisms, accumulation behavior of the metabolites, accumulation in specific organs and tissues, special chemical properties such as amphiphilic or ionogenic substances leading to multiple equilibrium processes, uptake and depuration kinetics, and the remaining level of parent compounds or metabolites after depuration [117, 119]. Many different models have been developed to overcome these limitations, including models based on bioenergetics and food web accumulation, and the life-cycle of different organisms [120–123].

The PBTK modeling approaches presented within this chapter attempt to overcome the limitations of the equilibrium partitioning model by specifically considering a number of physiological processes which are the mechanistic foundation for inter-species differences in toxicokinetics. In this way, such models, although based on the partitioning of chemicals into the lipid fraction of an organism, provide fairly

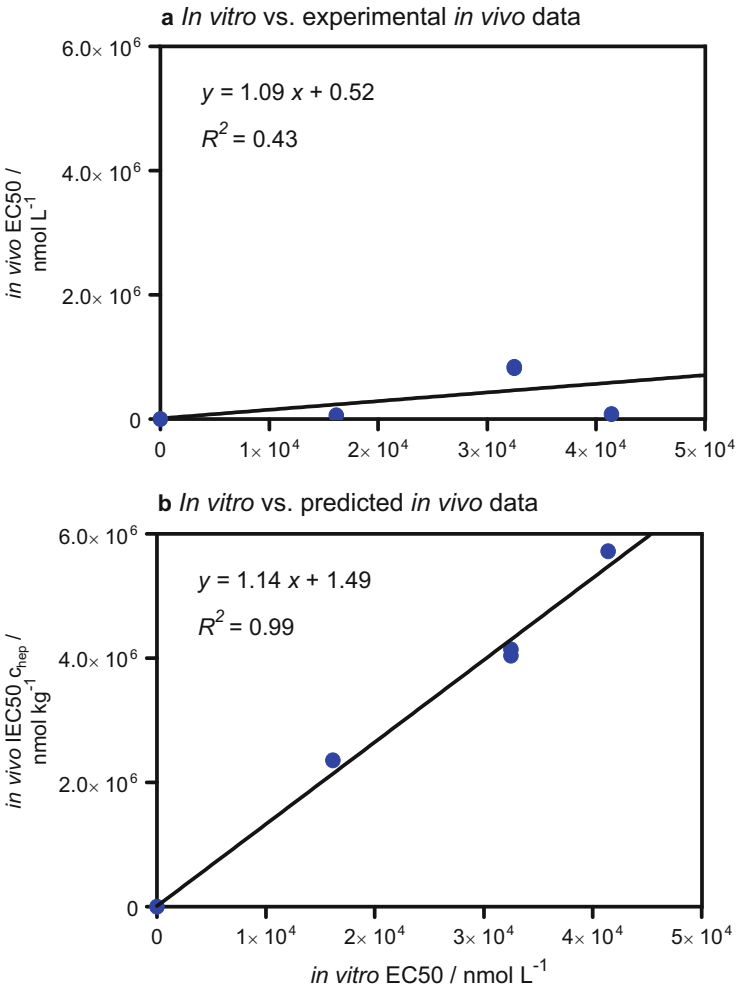

Fig. 7 Correlation between in vitro and in vivo data for Vtg induction in rainbow trout. The correlations were either based on experimental in vivo EC_{50}s (**a**) or derived through EC_{50}s based on modeled internal hepatic in vivo concentrations (IEC_{50}s) (**b**). *Solid line* represents the linear regression line. The coefficient of determination (R^2) and when applicable, the equation for the regression line using log-transformed data are provided in the graphs. Reprinted with permission from Brinkmann et al. [58]. Copyright 2014 American Chemical Society

exact estimates of accumulation and elimination rates, not only bioconcentration factors (BCFs). Furthermore, because organs and tissues are explicitly represented within their structure, PBTK models are powerful tools for predicting a chemical's distribution in exposed organisms. Developing and combining a variety of different PBTK models for different species of fishes would, apart from increased capabilities for IVIVE, also result in powerful options for cross-species extrapolation [124].

7 Example: Integration with the AOP Concept

Both regulators and industry are faced with the challenge to assess the environmental and human health risks associated with an ever-increasing number of chemicals and simultaneously reducing costs, animal use, and time required for chemical testing. To face this challenge, there has been an increasing effort to use mechanistic data (in vivo and in vitro) in support of chemical risk assessments, such as molecular biology methods and omics techniques [125]. This type of data can be generated more rapidly and cost-effectively [126]. One recent approach proposed to integrate such information in the risk assessment process of chemicals is that of the adverse outcome pathway (AOP). AOPs are conceptual frameworks that establish biologically plausible links between molecular-level perturbation of a biological system and an adverse outcome at a level of biological organization of regulatory relevance [125]. AOPs are applicable across species and are not chemical specific, but rather describe the progression from a molecular initiating event (MIE, first interaction of a chemical with a molecular target) that groups of chemicals have in common (e.g., binding to hormone receptors) to an apical outcome (e.g., disruption of reproduction or development). Thus, AOPs allow assessing toxicity across groups of chemicals and species without the need to test each chemical in each species [127]. It has recently been emphasized by Groh et al. [128] that PBTK models are highly useful tools to link toxicokinetic information to the mechanistic knowledge represented by AOPs. Specifically, PBTK models could be used to establish the cause-effect chain between external exposure, internal exposure, and MIEs. This combination surely results in quantitative models for predictive toxicology with a broad applicability domain in chemical risk assessment. It is useful to achieve an overall reduction of animal experiments, at the same time reducing the uncertainties associated with the current risk assessment strategies.

8 Conclusions

We conclude that toxicokinetic models, particularly those based on the physiology of an animal rather than on descriptive mathematics, are one piece of the puzzle which results in the development of scientifically sound integrated testing and risk assessment strategies. Toxicokinetic modeling today already plays an important role as a tool to deepen our understanding of processes that result in differences in uptake and disposition of chemicals in different species, life stages, and under varying environmental conditions. Numerous studies have demonstrated that such models can be conveniently used for extrapolating the results of mechanistic in vitro bioassays to the in vivo level, concerning both effects and biotransformation rates of a chemical. The next logical step is to synergize toxicokinetic models with the enormous amount of toxicological data generated using molecular and omics techniques, and with adverse outcome pathways (AOPs). The resulting

advanced approaches are of enormous value to regulators and industry, and significantly reduce the uncertainties of the risk assessment process, at the same time being more economic and reducing the need for animal testing.

Acknowledgements The authors acknowledge funding through the project "DioRAMA – Assessment of the dioxin-like activity in sediments and fish for sediment evaluation "that was financed by the German Federal Ministry of Transport, Building and Urban Development, as well as a personal scholarship of the German National Academic Foundation ("Studienstiftung des deutschen Volkes") which was granted to the first author.

References

1. Schwarzman MR, Wilson MP (2009) New science for chemicals policy. Science 326:1065–1066
2. EC (2006) Regulation no. 1907/2006 of the European Parliament and of the Council concerning the registration, evaluation, authorization and restriction of chemicals
3. Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, Wehrli B (2006) The challenge of micropollutants in aquatic systems. Science 313:1072–1077
4. ECHA (2008) Guidance on information requirements and chemical safety assessment. European Chemicals Agency, Helsinki, Finland
5. Schulte C, Tietjen L, Bambauer A, Fleischer A (2012) Five years REACH – lessons learned and first experiences. I. an authorities' view. Environ Sci Eur 24:31
6. Hansson SO, Rudén C (2006) Priority setting in the REACH system. Toxicol Sci 90:304–308
7. Zarfl C, Matthies M (2013) PBT borderline chemicals under REACH. Environ Sci Eur 25:11
8. Wolf O, Delgado L (2003) The impact of REACH on innovation in the chemical industry. European Commission, Joint Research Centre, report EUR 20999
9. ECHA (2015) Registered substances. European Chemicals Agency (ECHA); retrieved 28 May 2015, from http://echa.europa.eu/information-on-chemicals/registered-substances; last updated: 24 April 2015, Helsinki
10. Hartung T, Rovida C (2009) Chemical regulators have overreached. Nature 460:1080–1081
11. Russell WMS, Burch RL (1959) The principles of humane experimental technique; special edition, reprinted 1992. UFAW, London
12. ECHA (2011) The use of alternatives to testing on animals for the REACH regulation. European Chemicals Agency, Helsinki, Finland
13. Spielmann H, Sauer UG, Mekenyan O (2011) A critical evaluation of the 2011 ECHA reports on compliance with the REACH and CLP regulations and on the use of alternatives to testing on animals for compliance with the REACH regulation. Altern Lab Anim 39:481–493
14. Gilbert N (2011) Data gaps threaten chemical safety law. Nature 475:150–151
15. Lilienblum W, Dekant W, Foth H, Gebel T, Hengstler JG, Kahl R, Kramer PJ, Schweinfurth H, Wollin KM (2008) Alternative methods to safety studies in experimental animals: role in the risk assessment of chemicals under the new European chemicals legislation (REACH). Arch Toxicol 82:211–236
16. Yoon M, Campbell J, Andersen M, Clewell H (2012) Quantitative in vitro to in vivo extrapolation of cell-based toxicity assay results. Crit Rev Toxicol 42:633–652
17. Pinto M, Robine-Leon S, Appay MD, Kedinger M, Triadou N, Dussaulx E, Lacroix B, Simon-Assmann P, Haffen K, Fogh J, Zweibaum A (1983) Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Biol Cell 47:323–330

18. Liu X, Chism JP, LeCluyse EL, Brouwer KR, Brouwer KL (1999) Correlation of biliary excretion in sandwich-cultured rat hepatocytes and in vivo in rats. Drug Metab Dispos 27:637–644
19. Swift B, Pfeifer ND, Brouwer KL (2010) Sandwich-cultured hepatocytes: an in vitro model to evaluate hepatobiliary transporter-based drug interactions and hepatotoxicity. Drug Metab Rev 42:446–471
20. Balaz S (2009) Modeling kinetics of subcellular disposition of chemicals. Chem Rev 109:1793–1899
21. Nichols JW, Fitzsimmons PN, Burkhard LP (2007) In vitro–in vivo extrapolation of quantitative hepatic biotransformation data for fish. II. Modeled effects on chemical bioaccumulation. Environ Toxicol Chem 26:1304–1319
22. Nichols JW, Schultz IR, Fitzsimmons PN (2006) In vitro–in vivo extrapolation of quantitative hepatic biotransformation data for fish: I. A review of methods, and strategies for incorporating intrinsic clearance estimates into chemical kinetic models. Aquat Toxicol 78:74–90
23. OECD 305 (2012) Test no. 305: bioaccumulation in fish: aqueous and dietary exposure. OECD Publishing, Paris
24. Schlechtriem C, Fliedner A, Schafers C (2012) Determination of lipid content in fish samples from bioaccumulation studies: contributions to the revision of guideline OECD 305. Environ Sci Eur 24:13
25. Nichols J (2002) Modeling the uptake and disposition of hydrophobic organic chemicals in fish using a physiologically based approach. In: Verhaar HM, de Raat WK, Krüse J (eds) The practical applicability of toxicokinetic models in the risk assessment of chemicals. Springer, Netherlands, pp 109–133
26. Mason G, Zacharewski T, Denomme MA, Safe L, Safe S (1987) Polybrominated dibenzo-*p*-dioxins and related compounds: quantitative in vivo and in vitro structure-activity relationships. Toxicology 44:245–255
27. Safe S, Bandiera S, Sawyer T, Zmudzka B, Mason G, Romkes M, Denomme MA, Sparling J, Okey AB, Fujita T (1985) Effects of structure on binding to the 2,3,7,8-TCDD receptor protein and AHH induction - halogenated biphenyls. Environ Health Perspect 61:21–33
28. Fricker G, Drewe J, Huwyler J, Gutmann H, Beglinger C (1996) Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation. Br J Pharmacol 118:1841–1847
29. Dahan A, Hoffman A (2006) Use of a dynamic in vitro lipolysis model to rationalize oral formulation development for poor water soluble drugs: correlation with in vivo data and the relationship to intra-enterocyte processes in rats. Pharm Res 23:2165–2174
30. Mackay D, Fraser A (2000) Bioaccumulation of persistent organic chemicals: mechanisms and models. Environ Pollut 110:375–391
31. Segner H, Navas JM, Schäfers C, Wenzel A (2003) Potencies of estrogenic compounds in in vitro screening assays and in life cycle tests with zebrafish in vivo. Ecotox Environ Saf 54:315–322
32. Van den Belt K, Berckmans P, Vangenechten C, Verheyen R, Witters H (2004) Comparative study on the in vitro/in vivo estrogenic potencies of 17β-estradiol, estrone, 17-α-ethynylestradiol and nonylphenol. Aquat Toxicol 66:183–195
33. Legler J, Zeinstra LM, Schuitemaker F, Lanser PH, Bogerd J, Brouwer A, Vethaak AD, de Voogt P, Murk AJ, van der Burg B (2002) Comparison of in vivo and in vitro reporter gene assays for short-term screening of estrogenic activity. Environ Sci Technol 36:4410–4415
34. McCarty LS, Mackay D (1993) Enhancing ecotoxicological modeling and assessment body residues and modes of toxic action. Environ Sci Technol 27:1718–1728
35. Barron MG, Anderson MJ, Lipton J, Dixon DG (1997) Evaluation of critical body residue QSARS for predicting organic chemical toxicity to aquatic organisms. SAR QSAR Environ Res 6:47–62

36. Meador JP, Adams WJ, Escher BI, McCarty LS, McElroy AE, Sappington KG (2011) The tissue residue approach for toxicity assessment: findings and critical reviews from a society of environmental toxicology and chemistry Pellston workshop. Integr Environ Assess Manage 7:2–6
37. Takacs AR (1995) Ancillary approaches to toxicokinetic evaluations. Toxicol Pathol 23:179–186
38. Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, Freeman K, Clewell HJ, Dix DJ, Andersen ME, Houck KA, Allen B, Judson RS, Singh R, Kavlock RJ, Richard AM, Thomas RS (2012) Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol Sci 125:157–174
39. Groothuis FA, Heringa MB, Nicol B, Hermens JL, Blaauboer BJ, Kramer NI (2013) Dose metric considerations in in vitro assays to improve quantitative in vitro–in vivo dose extrapolations. Toxicology 332:30–40
40. Gülden M, Seibert H (2005) Impact of bioavailability on the correlation between in vitro cytotoxic and in vivo acute fish toxic concentrations of chemicals. Aquat Toxicol 72:327–337
41. Paustenbach DJ (2000) The practice of exposure assessment: a state-of-the-art review. J Toxicol Environ Health B 3:179–291
42. Heringa MB, Schreurs RH, Busser F, Van Der Saag PT, Van Der Burg B, Hermens JL (2004) Toward more useful in vitro toxicity data with measured free concentrations. Environ Sci Technol 38:6263–6270
43. Escher BI, Hermens JLM (2004) Internal exposure: linking bioavailability to effects. Environ Sci Technol 38:455A–462A
44. Hervé F, Urien S, Albengres E, Duché J-C, Tillement J-P (1994) Drug binding in plasma. Clin Pharmacokinet 26:44–58
45. Gülden M, Mörchel S, Seibert H (2001) Factors influencing nominal effective concentrations of chemical compounds in vitro: cell concentration. Toxicol In Vitro 15:233–243
46. Riedl J, Altenburger R (2007) Physicochemical substance properties as indicators for unreliable exposure in microplate-based bioassays. Chemosphere 67:2210–2220
47. Schirmer K, Chan AGJ, Greenberg BM, Dixon DG, Bols NC (1997) Methodology for demonstrating and measuring the photocytotoxicity of fluoranthene to fish cells in culture. Toxicol In Vitro 11:107–119
48. Kramer NI, Krismartina M, Rico-Rico Á, Blaauboer BJ, Hermens JLM (2012) Quantifying processes determining the free concentration of phenanthrene in basal cytotoxicity assays. Chem Res Toxicol 25:436–445
49. Knöbel M, Busser FJM, Rico-Rico Á, Kramer NI, Hermens JLM, Hafner C, Tanneberger K, Schirmer K, Scholz S (2012) Predicting adult fish acute lethality with the zebrafish embryo: relevance of test duration, endpoints, compound properties, and exposure concentration analysis. Environ Sci Technol 46:9690–9700
50. Hinger G, Brinkmann M, Bluhm K, Sagner A, Takner H, Eisenträger A, Braunbeck T, Engwall M, Tiehm A, Hollert H (2011) Some heterocyclic aromatic compounds are Ah receptor agonists in the DR-CALUX assay and the EROD assay with RTL-W1 cells. Environ Sci Pollut Res 18:1297–1304
51. Brinkmann M, Maletz S, Krauss M, Bluhm K, Schiwy S, Kuckelkorn J, Tiehm A, Brack W, Hollert H (2014) Heterocyclic aromatic hydrocarbons show estrogenic activity upon metabolization in a recombinant transactivation assay. Environ Sci Technol 48:5892–5901
52. Kramer NI, van Eijkeren JCH, Hermens JLM (2007) Influence of albumin on sorption kinetics in solid-phase microextraction: consequences for chemical analyses and uptake processes. Anal Chem 79:6941–6948
53. Oravcova´ J, Böhs B, Lindner W (1996) Drug-protein binding studies new trends in analytical and experimental methodology. J Chromatogr B Biomed Sci Appl 677:1–28
54. Heringa MB, Hermens JLM (2003) Measurement of free concentrations using negligible depletion-solid phase microextraction (nd-SPME). Trends Analyt Chem 22:575–587

55. Kramer NI, Busser FJM, Oosterwijk MTT, Schirmer K, Escher BI, Hermens JLM (2010) Development of a partition-controlled dosing system for cell assays. Chem Res Toxicol 23:1806–1814
56. Smith KEC, Oostingh GJ, Mayer P (2010) Passive dosing for producing defined and constant exposure of hydrophobic organic compounds during in vitro toxicity tests. Chem Res Toxicol 23:55–65
57. Gülden M, Seibert H (2003) In vitro–in vivo extrapolation: estimation of human serum concentrations of chemicals equivalent to cytotoxic concentrations in vitro. Toxicology 189:211–222
58. Brinkmann M, Eichbaum K, Buchinger S, Reifferscheid G, Bui T, Schäffer A, Hollert H, Preuss TG (2014) Understanding receptor-mediated effects in rainbow trout: in vitro–in vivo extrapolation using physiologically based toxicokinetic models. Environ Sci Technol. doi:10.1021/es4053208
59. Castaño A, Bols N, Braunbeck T, Dierickx P, Halder M, Isomaa B, Kawahara K, Lee L, Mothersill C, Pärt P (2003) The use of fish cells in ecotoxicology. The report and recommendations of ECVAM workshop 47. Alternatives to laboratory animals: ATLA 31, 317
60. Ekwall B (1980) Preliminary studies on the validity of in vitro measurement of drug toxicity using HeLa cells II. Drug toxicity in the MIT-24 system compared with mouse and human lethal dosage of 52 drugs. Toxicol Lett 5:309–317
61. Schultz TW (1989) Nonpolar narcosis: a review of the mechanism of action for baseline aquatic toxicity. Aquat toxicol hazard assess 12:104–109
62. Schirmer K (2006) Proposal to improve vertebrate cell cultures to establish them as substitutes for the regulatory testing of chemicals and effluents using fish. Toxicology 224:163–183
63. Clemedson C, Ekwall B (1999) Overview of the final MEIC results: I. The in vitro--in vitro evaluation. Toxicol In Vitro 13:657–663
64. Ekwall B (1999) Overview of the final MEIC results: II. The in vitro–in vivo evaluation, including the selection of a practical battery of cell tests for prediction of acute lethal blood concentrations in Humans 1. Toxicol In Vitro 13:665–673
65. Escher BI, Hermens JLM (2002) Modes of action in ecotoxicology: their role in body burdens, species sensitivity, QSARs, and mixture effects. Environ Sci Technol 36:4201–4217
66. Baird DJ, Van den Brink PJ (2007) Using biological traits to predict species sensitivity to toxic substances. Ecotoxicol Environ Saf 67:296–301
67. Rubach MN, Ashauer R, Maund SJ, Baird DJ, Van den Brink PJ (2010) Toxicokinetic variation in 15 freshwater arthropod species exposed to the insecticide chlorpyrifos. Environ Toxicol Chem 29:2225–2234
68. Buchwalter D, Jenkins J, Curtis L (2002) Respiratory strategy is a major determinant of [3H] water and [14C] chlorpyrifos uptake in aquatic insects. Can J Fish Aquat Sci 59:1315–1322
69. Livingstone D (1998) The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. Comp Biochem Physiol A Mol Integr Physiol 120:43–49
70. Nyman A-M, Schirmer K, Ashauer R (2014) Importance of toxicokinetics for interspecies variation in sensitivity to chemicals. Environ Sci Technol 48:5946–5954
71. Meador JP (1997) Comparative toxicokinetics of tributyltin in five marine species and its utility in predicting bioaccumulation and acute toxicity. Aquat Toxicol 37:307–326
72. Krishnan K, Peyret T (2009) Physiologically based toxicokinetic (PBTK) modeling in ecotoxicology. Ecotoxicology modeling. Springer, Newyork, pp 145–175
73. US-EPA (2006) Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (Final Report). US Environmental Protection Agency, Washington

74. Chiu WA, Barton HA, DeWoskin RS, Schlosser P, Thompson CM, Sonawane B, Lipscomb JC, Krishnan K (2007) Evaluation of physiologically based pharmacokinetic models for use in risk assessment. J Appl Toxicol 27:218–237
75. Stadnicka J, Schirmer K, Ashauer R (2012) Predicting concentrations of organic chemicals in fish by using toxicokinetic models. Environ Sci Technol 46:3273–3280
76. Landrum PF, Lydy MJ, Lee H (1992) Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ Toxicol Chem 11:1709–1725
77. Arnot JA, Gobas FAPC (2004) A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environ Toxicol Chem 23:2343–2355
78. Mager DE, Wyska E, Jusko WJ (2003) Diversity of mechanism-based pharmacodynamic models. Drug Metab Dispos 31:510–518
79. Punt A, Brand W, Murk AJ, van Wezel AP, Schriks M, Heringa MB (2013) Effect of combining in vitro estrogenicity data with kinetic characteristics of estrogenic compounds on the in vivo predictive value. Toxicol In Vitro 27:44–51
80. Hendriks AJ, van der Linde A, Cornelissen G, Sijm DT (2001) The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to octanol-water partition ratio and species weight. Environ Toxicol Chem 20:1399–1420
81. Nichols JW, McKim JM, Andersen ME, Gargas ML, Clewell Iii HJ, Erickson RJ (1990) A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish. Toxicol Appl Pharmacol 106:433–447
82. Nichols JW, McKim JM, Lien GJ, Hoffman AD, Bertelsen SL (1991) Physiologically based toxicokinetic modeling of three waterborne chloroethanes in rainbow trout (*Oncorhynchus mykiss*). Toxicol Appl Pharmacol 110:374–389
83. Louisse J, Verwei M, Woutersen RA, Blaauboer BJ, Rietjens IM (2012) Toward in vitro biomarkers for developmental toxicity and their extrapolation to the in vivo situation. Expert Opin Drug Metab Toxicol 8:11–27
84. Timchalk C, Nolan R, Mendrala A, Dittenber D, Brzak K, Mattsson J (2002) A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci 66:34–53
85. Barrett J, Della Casa Alberighi O, Läer S, Meibohm B (2012) Physiologically based pharmacokinetic (PBPK) modeling in children. Clin Pharmacol Ther 92:40–49
86. Bois FY, Jamei M, Clewell HJ (2010) PBPK modelling of inter-individual variability in the pharmacokinetics of environmental chemicals. Toxicology 278:256–267
87. Jongeneelen FJ, Berge WFT (2011) A generic, cross-chemical predictive PBTK model with multiple entry routes running as application in MS excel; design of the model and comparison of predictions with experimental results. Ann Occup Hyg 55:841–864
88. Freijer JI, van Eijkeren JCH, Sips AJAM (1999) Model for estimating initial burden and daily absorption of lipophilic contaminants in cattle. RIVM Report 643810005. Dutch National Institute for Public Health and the Environment (RIVM). pp. 59
89. Craigmill A (2003) A physiologically based pharmacokinetic model for oxytetracycline residues in sheep. J Vet Pharmacol Ther 26:55–63
90. Buur JL, Baynes RE, Craigmill AL, Riviere JE (2005) Development of a physiologic-based pharmacokinetic model for estimating sulfamethazine concentrations in swine and application to prediction of violative residues in edible tissues. Am J Vet Res 66:1686–1693
91. Tardif R, Lapare S, Krishnan K, Brodeur J (1993) Physiologically based modeling of the toxicokinetic interaction between toluene and m-xylene in the rat. Toxicol Appl Pharmacol 120:266–273
92. Garg A, Balthasar JP (2007) Physiologically-based pharmacokinetic (PBPK) model to predict IgG tissue kinetics in wild-type and FcRn-knockout mice. J Pharmacokinet Pharmacodyn 34:687–709
93. Thrall KD, Vucelick ME, Gies RA, Benson J (2000) Comparative metabolism of carbon tetrachloride in rats, mice, and hamsters using gas uptake and PBPK modeling. J Toxicol Environ Health A 60:531–548

94. Weijs L, Yang RS, Das K, Covaci A, Blust R (2013) Application of Bayesian population physiologically based pharmacokinetic (PBPK) modeling and Markov chain Monte Carlo simulations to pesticide kinetics studies in protected marine mammals: DDT, DDE, and DDD in harbor porpoises. Environ Sci Technol 47:4365–4374
95. Van Eijkeren JC, Zeilmaker MJ, Kan C, Traag WA, Hoogenboom L (2006) A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs. Food Addit Contam 23:509–517
96. Nichols JW, Bennett RS, Rossmann R, French JB, Sappington KG (2010) A physiologically based toxicokinetic model for methylmercury in female American kestrels. Environ Toxicol Chem 29:1854–1867
97. Bungay P, Dedrick R, Guarino A (1976) Pharmacokinetic modeling of the dogfish shark (*Squalus acanthias*): distribution and urinary and biliary excretion of phenol red and its glucuronide. J Pharmacokinet Biopharm 4:377–388
98. Law FCP, Abedini S, Kennedy CJ (1991) A biologically based toxicokinetic model for pyrene in rainbow trout. Toxicol Appl Pharmacol 110:390–402
99. Brinkmann M, Eichbaum K, Kammann U, Hudjetz S, Cofalla C, Buchinger S, Reifferscheid G, Schüttrumpf H, Preuss T, Hollert H (2014) Physiologically-based toxicokinetic models help identifying the key factors affecting contaminant uptake during flood events. Aquat Toxicol 152:38–46
100. Nichols JW, Jensen KM, Tietge JE, Johnson RD (1998) Physiologically based toxicokinetic model for maternal transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in brook trout (*Salvelinus fontinalis*). Environ Toxicol Chem 17:2422–2434
101. Lien GJ, McKim JM, Hoffman AD, Jenson CT (2001) A physiologically based toxicokinetic model for Lake trout (*Salvelinus namaycush*). Aquat Toxicol 51:335–350
102. Brocklebank JR, Namdari R, Law F (1997) An oxytetracycline residue depletion study to assess the physiologically based pharmokinetic (PBPK) model in farmed Atlantic salmon. Can Vet J 38:645
103. Nichols JW, McKim JM, Lien GJ, Hoffman AD, Bertelsen SL, Gallinat CA (1993) Physiologically-based toxicokinetic modeling of three waterborne chloroethanes in channel catfish, *Ictalurus punctatus*. Aquat Toxicol 27:83–111
104. Liao C-M, Liang H-M, Chen B-C, Singh S, Tsai J-W, Chou Y-H, Lin W-T (2005) Dynamical coupling of PBPK/PD and AUC-based toxicity models for arsenic in tilapia *Oreochromis mossambicus* from Blackfoot disease area in Taiwan. Environ Pollut 135:221–233
105. Péry ARR, Devillers J, Brochot C, Mombelli E, Palluel O, Piccini B, Brion F, Beaudouin R (2013) A physiologically based toxicokinetic model for the zebrafish Danio rerio. Environ Sci Technol 48:781–790
106. Lien GJ, McKim JM (1993) Predicting branchial and cutaneous uptake of 2,2′,5,5-′-tetrachlorobiphenyl in fathead minnows (*Pimephales promelas*) and Japanese medaka (*Oryzias latipes*): rate limiting factors. Aquat Toxicol 27:15–31
107. Woofter RT, Brendtro K, Ramsdell JS (2005) Uptake and elimination of brevetoxin in blood of striped mullet (Mugil cephalus) after aqueous exposure to Karenia brevis. Environ Health Perspect 113:11–16
108. Caldwell JC, Evans MV, Krishnan K (2012) Cutting edge PBPK models and analyses: providing the basis for future modeling efforts and bridges to emerging toxicology paradigms. J Toxicol 2012
109. DeJongh J, Forsby A, Houston JB, Beckman M, Combes R, Blaauboer BJ (1999) An integrated approach to the prediction of systemic toxicity using computer-based biokinetic models and biological in vitro test methods: overview of a prevalidation study based on the ECITTS1 project. Toxicol In Vitro 13:549–554
110. Verwei M, van Burgsteden JA, Krul CAM, van de Sandt JJM, Freidig AP (2006) Prediction of in vivo embryotoxic effect levels with a combination of in vitro studies and PBPK modelling. Toxicol Lett 165:79–87

111. Louisse J, de Jong E, van de Sandt JJM, Blaauboer BJ, Woutersen RA, Piersma AH, Rietjens IMCM, Verwei M (2010) The use of in vitro toxicity data and physiologically based kinetic modeling to predict dose–response curves for in vivo developmental toxicity of glycol ethers in rat and man. Toxicol Sci 118:470–484
112. Hecker M, Hollert H (2011) Endocrine disruptor screening: regulatory perspectives and needs. Environ Sci Eur 23:15
113. Stadnicka-Michalak J, Tanneberger K, Schirmer K, Ashauer R (2014) Measured and modeled toxicokinetics in cultured fish cells and application to in vitro-in vivo-toxicity extrapolation. PLoS One 9, e92303
114. Gobas FAPC, de Wolf W, Burkhard LP, Verbruggen E, Plotzke K (2009) Revisiting bioaccumulation criteria for POPs and PBT assessments. Integr Environ Assess Manag 5:624–637
115. Swackhamer DL, Needham LL, Powell DE, Muir DCG (2009) Use of measurement data in evaluating exposure of humans and wildlife to POPs/PBTs. Integr Environ Assess Manag 5:638–661
116. Schafer S, Buchmeier G, Claus E, Duester L, Heininger P, Korner A, Mayer P, Paschke A, Rauert C, Reifferscheid G, Rudel H, Schlechtriem C, Schroter-Kermani C, Schudoma D, Smedes F, Steffen D, Vietoris F (2015) Bioaccumulation in aquatic systems: methodological approaches, monitoring and assessment. Environ Sci Eur 27:5
117. Van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149
118. Van der Kooij L, Van De Meent D, Van Leeuwen C, Bruggeman W (1991) Deriving quality criteria for water and sediment from the results of aquatic toxicity tests and product standards: application of the equilibrium partitioning method. Water Res 25:697–705
119. Franke C (1996) How meaningful is the bioconcentration factor for risk assessment? Chemosphere 32:1897–1905
120. Gobas FA (1993) A model for predicting the bioaccumulation of hydrophobic organic chemicals in aquatic food-webs: application to Lake Ontario. Ecol Model 69:1–17
121. Morrison HA, Gobas FA, Lazar R, Whittle DM, Haffner GD (1997) Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in western Lake Erie. Environ Sci Technol 31:3267–3273
122. Sijm DT, Seinen W, Opperhuizen A (1992) Life-cycle biomagnification study in fish. Environ Sci Technol 26:2162–2174
123. Thomann RV, Connolly JP (1984) Model of PCB in the Lake Michigan lake trout food chain. Environ Sci Technol 18:65–71
124. Brinkmann M, Schlechtriem C, Reininghaus M, Eichbaum K, Buchinger S, Reifferscheid G, Hollert H, Preuss TG (2016) Cross-species extrapolation of uptake and disposition of neutral organic chemicals in fish using a multispecies physiologically-based toxicokinetic model framework. Environ Sci Tech 50:1914–1923
125. Ankley G, Bennett R, Erickson R, Hoff D, Hornung M, Johnson R, Mount D, Nichols J, Russom C, Schmieder P, Serrano J, Tietge J, Villeneuve D (2010) Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29:730–741
126. Villeneuve DL, Garcia-Reyero N (2011) Vision & strategy: predictive ecotoxicology in the 21st century. Environ Toxicol Chem 30:1–8
127. Kramer VJ, Etterson MA, Hecker M, Murphy CA, Roesijadi G, Spade DJ, Spromberg JA, Wang M, Ankley GT (2011) Adverse outcome pathways and ecological risk assessment: bridging to population-level effects. Environ Toxicol Chem 30:64–76
128. Groh KJ, Carvalho RN, Chipman JK, Denslow ND, Halder M, Murphy CA, Roelofs D, Rolaki A, Schirmer K, Watanabe KH (2015) Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: I challenges and research needs in ecotoxicology. Chemosphere 120:764–777