CHAPTER X

Integrated Translation Framework for Endocrine Disruptors in the area of computational toxicology

V Kumar,[ab]*, Deepika Deepika[a] and R. P. Sharma[a]

[a] *Environmental Engineering Laboratory, Departament d' Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain*

[b] IISPV, Hospital Universitari Sant Joan de Reus, Universitat Rovira I Virgili, Reus, Spain

*Corresponding contributor. E-mail: vikas.kumar@urv.cat

Abstract

In the recent past, there has been a tremendous increase in the kind of data being generated by high-throughput analysis (OMICS) for endocrine disruptors (EDs). In parallel, several *in silico* tools (PBPK, PD, Systems biology and AOPs) offer an opportunity to understand the biological complexity of EDs and environmental risk assessment. Along with the development of new tools and techniques in toxicological research, it is also necessary to have a continuous re-evaluation of existing data, data integration, and knowledge-based translation that might enable to assess the human health risk of EDs. There is a need for a platform that integrates *in vitro*, *in vivo*, and several *in silico* models into one framework to directly tie the results to a predictive adverse outcomes model. The objective of this chapter is to introduce *in silico* framework that integrates several models at the organ, molecular, cellular and genetic scale, we hereby called it as integrative systems toxicology approaches that could be used in the human health risk assessment for EDs. This integrative systems toxicology will offer a quantitative understanding of the EDs adverse effects on a biological system, through the integration of exposome-internal exposure-molecular/cellular response to the adverse stimulus. Such *in silico* platform will be a dynamic tool to efficiently reduce the risk of EDs for public health.

Abbreviations

AMP	Adenosine Monophosphate	KER	Key event Relationship
AO	Adverse Outcome	LPS	Lipopolysaccharide
AOP	Adverse Outcome Pathways		
BPA	Bisphenol A	MCA	Metabolic Control Analysis
BPAF	Bisphenol AF	MEHP	Monoethylhexyl phthalate
BPF	Bisphenol F	MFA	Metabolic Flux Analysis
BPR	Biocidal Product Regulation	MIE	Molecular initiating events
BPS	Bisphenol S	NCA	Non-compartment Analysis
DDD	Dichlorodiphenyldichloroethane	NRC	National Research Council
DDE	Dichlorodiphenyldichloroethylene	ODE	Ordinary Differential Equation

DDT	Dichlorodiphenyltrichloroethane	ODN	Oligodeoxynucleotides
DEHP	Diethylhexyl phthalate	OECD	Organisation for Economic Co-operation and Development
DNA	Deoxyribonucleic acid	PBK	Physiologically Based Kinetic Model
DR	Dose-response	PBPK	Physiologically Based Pharmacokinetics Model
ECF	Extracellular fluid	PBTK	Physiologically based Toxicokinetic Model
EDs	Endocrine Disruptors	PCBs	Polychlorinated biphenyls
EDSP	Endocrine Disruptor Screening Program	PD	Pharmacodynamics
EEE	Electroencephalogram	PFOA	Perfluorooctanoic acid
EFSA	European Food Safety Authority	PFOS	Perfluorooctane sulfonic acid
EMA	Elementary model analysis	PK	Pharmacokinetics
EU	European Union	P-PBPK	Pregnancy-Physiologically Based Pharmacokinetics Model
FBA	Flux Balance Analysis	PPPR	Plant Protection Products Regulation
FCC	Flux control coefficient	QIVIVE	Quantitative *in vitro*-to-*in vivo* extrapolation
IATA	Integrated assessment and testing approaches	QSPR	Quantitative Structural-Property-Relationship
HCA	Hierarchical Regulation analysis	QSAR	Quantitative Structure -Activity Relationships
IVIVE	*In vitro*-to-*in vivo* extrapolation	QSP	Quantitative Systems Pharmacology Model
KE	Key events	REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROS	Reactive Oxygen Species	TD	Toxicodynamic
SAR	Structural-Activity Relationship	USEPA	United States Environmental Protection Agency
SB	Systems biology	VPH	Virtual Physiological Human
SBW	Systems biology workbench	WHO	World Health Organization
ST	Systems Toxicology		

1. Endocrine disruptors (EDs) and Human Health

Endocrine disruptors (EDs) are natural or anthropogenic substances in the environment, food, or consumer products that can disrupt hormonal balances in humans and wildlife, causing adverse health effects even at low dosage. USEPA defined EDs as exogenous agents that interfere with the synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process [1]. Some of these compounds are stable and difficult to eliminate from the environment. These agents may disturb the hormone signalling networks depending on gender, genome sequence, or lifestyle of each individual.

To date, many test methods have been developed under EU and OECD guidance with the vision that Quantitative Risk Assessments for these chemicals should enable the definition of acceptable

environmental levels and drive policies, which provide that these levels are not exceeded. In the regulation on industrial chemicals (Registration, Evaluation, Authorisation, and restriction of Chemicals, EC 1907/2006, REACH), a chemical identified as an EDs could be subject to authorisation based on risk assessment and socio-economic analysis. The current version of the EDs criteria for both the Biocidal Product Regulation (No 528/2012) (BPR) and the Plant Protection Products Regulation (No 1107/2009) (PPPR) is based on the WHO definition from 2002. The European Commission and its working group have discussed the criteria for EDs since the implementation of the PPPR in 2007.

Quantitative prediction of EDs adverse effect on human health poses a large number of challenges particularly due to involvement of hundreds of chemicals and their metabolites, as well as their associated pattern of exposure, retention time in the body, generation of toxic metabolites and their wide range action via multiple mechanisms [2–4]. *In vitro*, *in vivo* and epidemiological studies have been performed for a better understanding of EDs mechanism, toxicity and health risk (Figure 1). The advancement in current analytical methods of *in vitro*, high throughput screening, genomics, proteomics, and metabolomics have generated a huge amount of data on toxicological profiles. With a lot of information available in public database, *in silico* prediction tools for determining toxicity risk are of great significance and potency.

Nevertheless, many EDs are produced every year and it is not possible to test all of them for risk assessment. Current approaches are time- money- and effort-consuming. There is a need for cost-effective and efficient tools to meet regulatory toxicology needs. One way for this is a hypothesis-driven integrated approach, which includes testing by *in silico* tools and further validation of research based on experimental data. EPA's Toxicity Forecaster (ToxCast) is one of the *in silico* tool for screening thousands of chemicals and today have data for 1800 chemicals from industry to consumer products [5] (https://www.epa.gov/chemical-research/toxicity-forecasting). Now, the Tox 21 focuses on developing methods to efficiently analyse the toxicity of chemicals and integration of *in silico* tools [6]. Integrated assessment and testing approaches (IATAs) focus on an integrated approach for chemical hazard characterization based on existing methods and new information. This integrated approach includes different models such as cheminformatics models, *in silico, in vitro, in vivo* models, etc. AOP is one of the frameworks for developing IATA [7].

Recent, EFSA projects on *in silico* for dermal absorption of pesticides and protein toxicity confirmed that the computational approach has been widely accepted [8]. In parallel to this, the recent development of systems biology and multiscale modelling has increased the understanding of the physiological endogenous pathway and impacts of toxicant on the temporal behaviour of the cell, tissue, and whole organ system.

2. From classical Dose-response to Integrative Translational toxicology

The study of toxicology has been focused on quantifying/predicting chemical-induced adverse effects to the biological system. The major challenges in predicting adverse effects of chemicals on human health are; the inheritance complexity within the biological system, the chemical's complex mechanism(s), and the complex responses of organisms over different life stages or time scales. Translational Toxicology is an area, which integrates classic toxicology (empirical fitting of the dose-response curve) with the quantitative analysis of molecular and functional changes that occurs across multiple levels of biological organization [9]. The fact that adverse effects cannot be predicted individually by animal testing or *in vitro* testing or existing modelling methodologies was the first step on the road of the concept of integrative translational toxicology. An integrative translational approach to predict the adverse effects on human health can be described as "viewing the problem in its entirety as an interconnected system of component operations and functions" and therefore recognizing the full complexity of predicting adverse effects on human health. A Systems Biology is aimed at a mechanistic understanding of chemical interaction with living systems versus conventional empirical endpoints and animal-based testing. Several Systems biology modelling

approaches have been developed to predict the adverse effects of drugs/chemicals on human health [10]. Information regarding the body physiology, pharmacokinetics, pharmacodynamics, chemical exposures, inter-individual variability and covariates relating to toxicity are an integral part of translational toxicology**.**

The classical toxicology involves empirical fitting of external dose (not internal dose) and response (endpoints) as the basis for the dose-response assessment. This approach lacks the mechanistic understanding of the influence of body physiology onto the chemical's fate and the biological changes at the molecular and functional levels due to chemical interaction with a biological target. These processes are described as Pharmacokinetics (PK) i.e. "what body does to the drug/chemical" and Pharmacodynamics (PD) "what drug/chemical does to the body" respectively. Pharmacokinetics encompasses the four elements absorption, distribution,; metabolism and elimination (ADME) that describes the fate of the chemical inside the body. Pharmacodynamics describes the interactions of drugs with biological targets and consequently observed effects.

There have been successive developments of several PK models describing ADMEs of drugs/chemicals. Major types of PK models are Non-compartment Analysis (NCA) and compartment physiological analysis [11]. NCA empirically fits experimental data on the time course of plasma drug concentrations. This allows measuring the elimination and volume of the distribution of chemicals inside the body [12]. Compartment models can be semi-mechanistic adding improved insights into the distribution properties of drugs and physiological properties of organisms. Physiologically based Pharmacokinetics (PBPK) models are systems models where the body is divided into several compartments corresponding to each organ. Organs are connected with each other via the blood circulatory system. The parameters in the model are assigned using physiological measurements (blood flow, organ sizes) and resolved by direct analysis of plasma concentrations and tissue transport, binding, and metabolic properties [11]. The integration of a dynamic change in physiological states related to age, disease, and pregnancy into the PBPK leads to the development of person/population-specific PBPK models.

Pharmacodynamic (PD) models are empirically fitting dose and response relationships at the tissue level. PD models are categorized into five types but mainly they are two types; one is direct effect model that assumes chemical effects are directly proportional to receptor occupancy (i.e. linear transduction), and the other is the indirect effect model in which response is due to chemicals indirect effect to the synthesis or degradation of a response variable [13].

Both PK and PD can be developed individually and linked together which often referred to as PBPK/PD models for chemicals like EDs [14,15]. PBPK describes the internal concentrations rather than external exposure; the key metabolites and their linkage to PD provide a more accurate dose-response relationship. Such integrated PBPK/PD can be able to simultaneously describe chemical ADME at the whole-body level and the resulting EDs effect at the cellular or tissue scale [16]. PBPK/PD has long been used for route-to-route and species-to-species extrapolations and *in vitro*-to-*in vivo* extrapolation (IVIVE) [17]. QIVIVE (quantitative *in vitro in vivo* extrapolation) along with PBPK/PD is used to predict the *in vivo* adverse effects based on *in vitro* dose-response data [18,19]. However, this model has the limitation of not taking into account the process of molecular initiating events (MIEs) to adverse effects and, very often, the endpoints are single biomarkers at cell/tissue level with no clear link to downstream adverse effect(s). To address these challenges the concept of adverse outcomes pathways (AOPs) and systems biology have been developed. Recently, the concept of AOP has been drawn upon a systems biology approach. AOP is defined as "A linear sequence of events commencing with initial interaction(s) of a stressor with a biomolecule within an organism that causes a perturbation in its biology (i.e., molecular initiating event, MIE), which can progress through a dependent series of intermediate key events (KEs) and culminate in an adverse outcome (AO) considered relevant to risk assessment or regulatory decision-making" [20]. AOPs do not, however, address the question of what dose of a chemical will cause sufficient perturbation to drive the pathways to the adverse outcomes [20]. In contrast, Systems biology quantifies the effect of chemical interaction on biological systems across the cellular and multi-tissue level and the observed toxicological effects in relation to the exposure to the chemical under investigation [9]. These

biological model systems could be comprised of linear signalling pathways such as AOPs to detailed complex biological pathways (Systems biology) leading to an integrated approach. Systems biology comprises genomics, metabolomics, and proteomics rationalizing the functional interaction of biological components in a time-dependent fashion [21,22]. Coupling a PBPK/PD model and Systems biology together can form a mechanistic framework that enhances the understanding both of biology and of adverse effects due to chemically induced perturbation to the biological systems (Figure 1) [23,24].

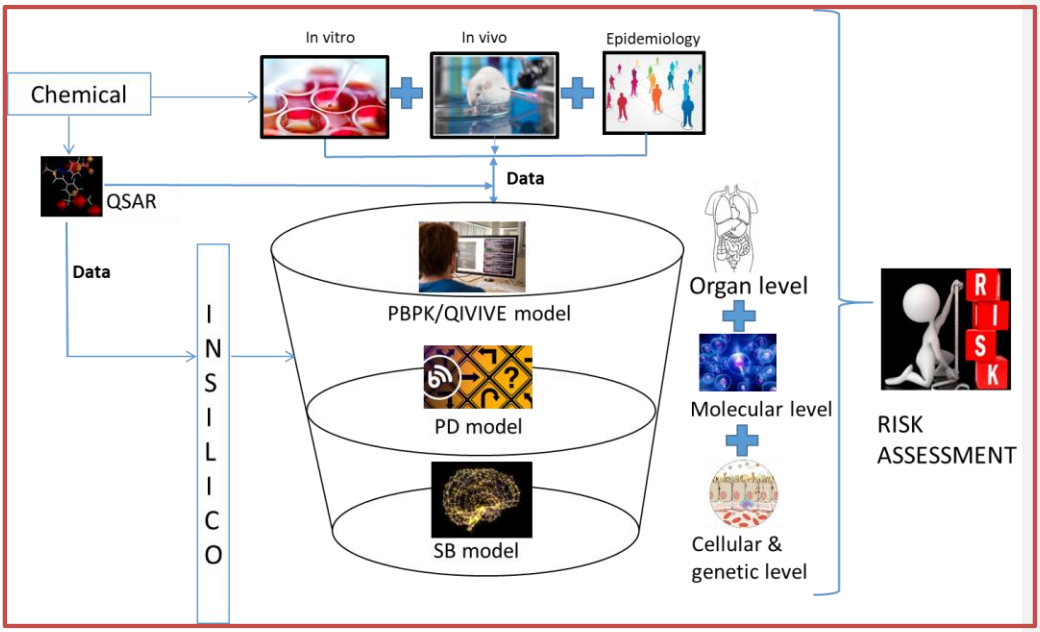


Figure 1: Integrated translational framework for chemical induced toxicity. ~~Modified~~ Adapted from original source~~from original image~~ [25]

3. PBPK (Physiologically Based Pharmacokinetic~~s~~) Model

3.1 Introduction

Physiologically based pharmacokinetic (PBPK) models consist of a series of mathematical representations of biological tissues and physiological processes in the body of target species aimed at describing the absorption, distribution, metabolism, and excretion of chemicals (Figure 2) [26]. When a chemical substance enters the organism, it is usually distributed to various tissues and organs by blood flow [27]. Following its distribution in tissues, the substance can bind to various proteins and receptors, undergo metabolism, or can be eliminated unchanged. The concentration vs. time profiles of the xenobiotic in different tissues, or the number of metabolites, is often used as surrogate markers of its internal dose or biological activity [28]. In a sense, PBPK modelling is an integrated systems approach to both understanding the PK behaviour of compounds and predicting concentration vs time profiles in plasma and tissues [29].
The biological response results from the interaction between the toxicant and the target tissue. For this reason, models that can predict the target tissue concentration of the toxicologically-active

chemical species (parent compound or metabolite) are especially useful and have been applied in the “exposure–dose–response” paradigm. The internal dose metrics (sometimes also referred to as biological effective dose) replaces the external exposure dose in the derivation of the quantitative dose-response relationship, with the intent of reducing the uncertainty inherent in human health risk assessments based on external exposure dose estimation. With the advancements in modelling, now PBPK is extended to species-specific, age-specific, population-specific and organ-specific models. Age kinetics is being included in the PBPK for assessing risk through lifetime exposure. It includes age-dependent changes in absorption, distribution, metabolism, and excretion of different chemicals from the body. For instance, IVIVE along with the PBPK model approach was used to simulate age-related pyrethroid kinetic differences in rats [30]. PBPK models are available for different EDs like Bisphenol A, PFOS, PFOA, and PCBs. Several examples are discussed in the following pages. They include both perfusion-limited and permeability-limited model. Now, QSAR, IVIVE, and other models are being integrated to better predict risks from exposure to EDs.

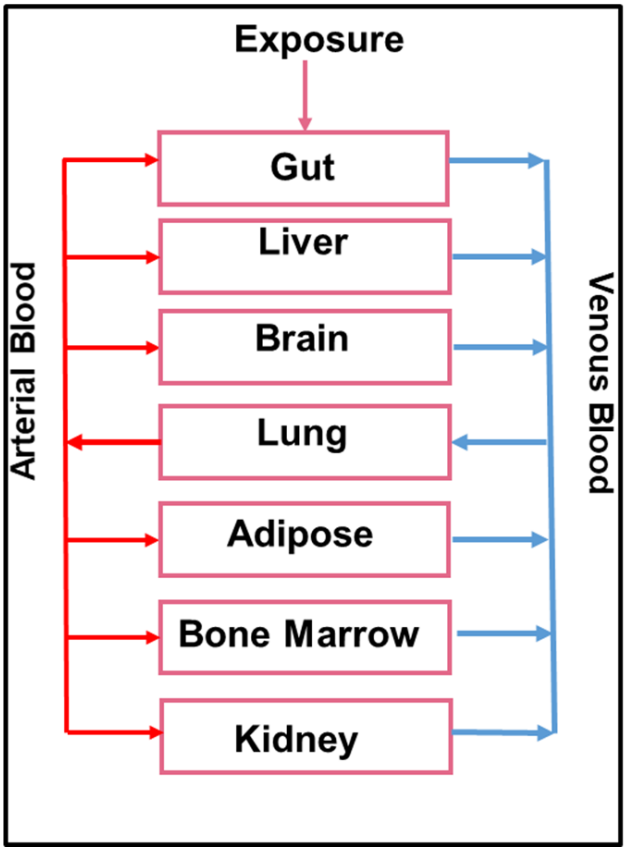


Figure 2: General structure of PBPK model representing different compartments

3.2 Approaches to building PBPK models

Building a PBPK model requires gathering a considerable amount of data which can be categorised in three groups, namely: the model structure, which refers to the arrangement of tissues and organs included in the model; the system's data (physiological, anatomical, biochemical data) and chemical-specific data (physicochemical) (Figure 3). The transport of xenobiotics in several tissues is determined by two different approaches: (i) permeability limited (also called as flow limited), and (ii) perfusion limited (also called as diffusion-limited) [31,32].

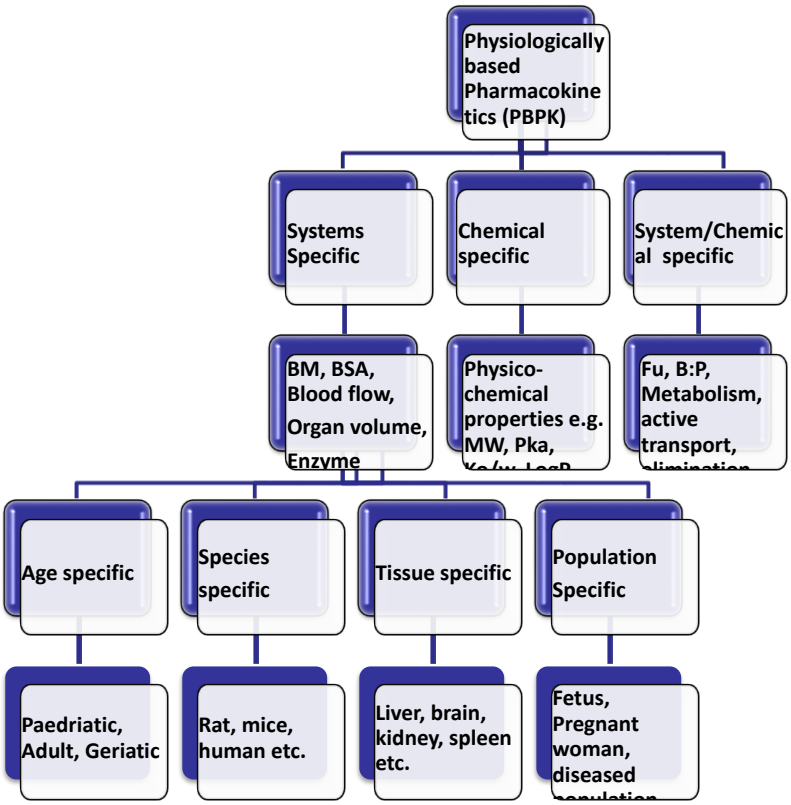


Figure 3: PBPK model structure and approach: representing the model developmental approach and the required parameters input. BM: Body mass; BSA: Body surface area; MW: Molecular weight; $K_{o/w}$: octanol: water partition coefficient; Fu: Fractional unbound concentrations

Permeability-rate –limited Model: This model is also called diffusion-limited and could be applied when the distribution of the substance to tissue is rate-limited by the drug's permeability across the tissue membrane. That condition is more common with polar compounds and large molecular structures. Consequently, the related PBPK models may exhibit different degrees of complexity. Below are some examples of EDs and other chemicals where researchers have used permeability limited PBPK models for quantitative assessment of the time course of chemicals.
A permeability limited PBPK model has been used to predict PK and tissue distribution of PFOA. The model performance has been assessed through seven experimental data sets. Cellular uptake of PFOA was considered to occur both through passive diffusion and active transport by membrane transporters and serum albumin [33]. Lactational transfer of PCB 153 with or without PCB126 in mice was simulated through the PBPK model. For the fat tissue compartment, chemical uptake was defined by the diffusion-limited model. Simulation results were compared and validated with experimental data for PCB. Such a model provides a mechanistic tool for estimating PCB disposition [34]. A PBPK model for the mixture effect of PCB153 and PCB 126 was developed for explaining PK interaction within PCB congeners. The fat compartment was defined as diffusion-limited and added in the PBPK model to describe lactational transfer [35].
The model is applied to many problems, other than EDs. Plasma and pulmonary concentration of seven anti-tuberculosis drugs were simulated by multicompartment permeability limited lung PBPK model. IVIVE approach was used to predict the passive permeability of drugs within the lung. This PBPK IVIVE model predicted the concertation which was in agreement with the experimental clinical data [36].

Perfusion-rate-limited Model: This model is also called flow limited kinetics and could be applied when the tissue membrane presents no barrier to distribution. Here, each tissue is considered a well-stirred compartment in which the substance distribution is simply limited by blood flow. Thus, the chemical will be delivered to the tissue via the blood and is assumed to mix throughout the volume of that compartment immediately and completely and normally partition coefficient is used for the distribution of the chemical. Concentrations in the flow limited compartments generally estimated by applying the following equation:

$$\frac{\text{Dd}C_i}{dt} = \frac{Q_{\text{ti}} * \left(C_a - \frac{C_i}{K_{i:p}}\right)}{Vi\,V_t} \quad (1)$$

Where C_i is the concentration in the tissue i (nM), Q_i is the blood flow in the tissue i (L/h), Ca is the arterial concentration (nM), $K_{i:p}$ is the partition coefficient of tissue i, and V_i is the volume of the tissue i (L).

These are a few examples of perfusion rate-limitedrate limited PBPK model in the case of EDs and other chemicals. A PBPK model for lifetime accumulation of p,p′-DDT, p,p′-DDE, and p,p′-DDD in harbor porpoises was developed to define the kinetics and metabolism of these chemicals. Bayesian approach with Markov chain Monte Carlo simulations was used to take into account uncertainty and estimate species-specific parameters. Such an approach can provide confidence and integrated approach Bayesian population PBPK and Monte Carlo simulation can lead to reliable species-specific modelling [37]. Pharmacokinetic behaviour of different bisphenols (BPA, BPS, BPF and BPAF) was simulated through PBPK in different age groups. It also included oral and dermal exposure along with Monte Carlo uncertainty analysis [38]. DEHP and its metabolite MEHP was estimated in human plasma and urine using chimeric mice and through a simplified PBPK model. Animal biomonitoring equivalents from mice were scaled to human biomonitoring equivalents trough allometric scaling factor and *in vitro* metabolic clearance data. This biomonitoring strategy is also capable of reverse dosimetry [39]. Bisphenol A concentration in the fetus during gestation time was estimated by pregnancy PBPK (P-PBPK) model and the developed model was validated using biomonitoring data from different pregnancy cohort studies. Oral and dermal exposure was included in the model to simulate total internal exposure [40].

3.3 Model Parameterization

There are two approaches of PBPK model building or parameterization: **bottom-up** and **top-down**. In a bottom-up approach, model parameterization is done based on *in silico* prediction or *in vitro* understanding of chemical-related ADME mechanisms. It mainly depends on tools for translation of in-vitro data to in-vivo such as IVIVE (*in vitro- in vivo* extrapolation) and several *in silico* tools such as QSAR, which is in a sense purely predictive model. In contrast, top-down approaches rely on the estimation of model parameters by fitting to the observed experimental data. Model parameterization requires two specific parameters namely; System's and Chemical's specific input parameters.

System-specific parameters: This comprises of both physiological parameters and biochemical parameters.

Physiological parameters: These parameters are species-specific constants. These includes tissues/organs volume (or weight) and tissues blood flow rate which are specific to the species of interest. The parameters are used to develop species-specific PBPK models, the most common being rat, mouse, dog, and human. Physiological parameters for developing such models are routinely available in the literature [41–44].

Biochemical parameters

Biochemical parameters are the hybrid parameters that depend on both chemical and physiology. Among biochemical parameters, chemical metabolism is considered to be a very important parameter, which generally derived from *in vitro* data using IVIVE methodology. The schema of IVIVE has been provided in figure 4.

IVIVE generally involves the scaling of *in vitro* Vmax parameter based on the microsomal protein content per gram of tissue and weight of tissue per kg body weight. Vmax is scaled to *in vivo* per kg BW from *in vitro* cell line studies by using the following equation:

$$Vmax_{invivo} = \frac{Vmax_{invitro} * MPPGT * V_{tissue}}{BW^{.75}} \quad (2)$$

Where, $Vmax$ = Maximum metabolic capacity in per gram of microsomal protein, $MPPGT$ = the microsomal protein per gram of tissue, V_{tissue} = the total tissue weight in gram, and BW =is the whole-body weight in kg.

Chemical-specific parameters

These parameters can be derived by *in vivo* or *in vitro* experiment. However, in certain cases when we lack these data, various *in silico* approaches can be useful. Among physicochemical parameters, the partition coefficient is considered one of the most important parameters. It describes the distribution of the chemical between plasma and different organs. There are various tissue composition based algorithms methods to generate partition coefficient data [45–49].

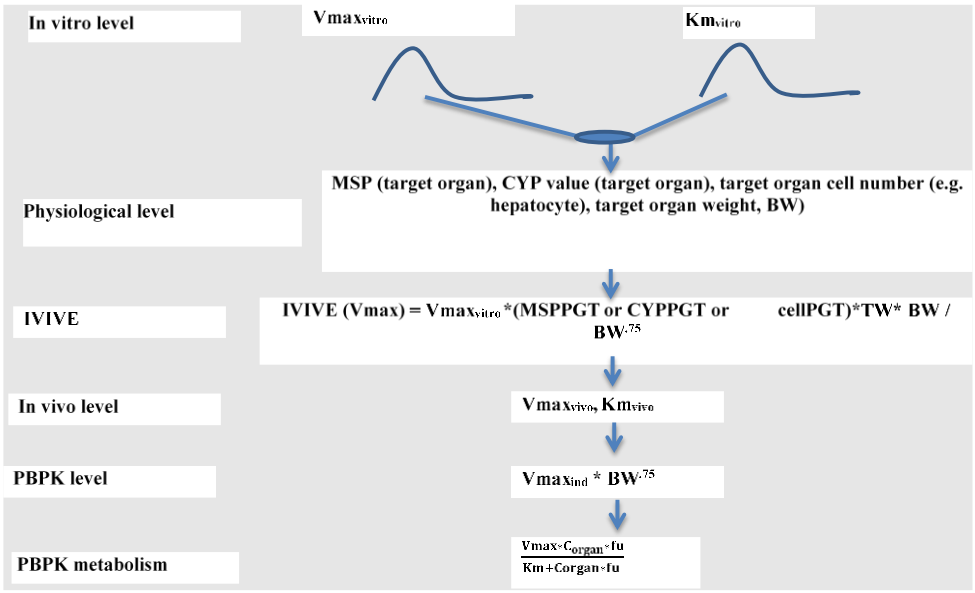


Figure 4: Illustration of Hierarchical structure model approach for metabolic uncertainty and variability in IVIVE scaling.

3.4 Cheminformatics and toxicokinetic models for chemical assessment

PBPK models generally require many inputs like physiological parameters, biochemical and chemical-specific parameters. With many new chemicals arising in the market, it is not feasible to rely only on *in vitro* and in *vivo* data for the PBPK parameters. Value of some parameters is derived through cheminformatics models like SAR, QSAR, and QSPR [50,51]. These models can be used to predict the value of certain chemical parameters like V_{max}, K_m, partition coefficients, etc. based on the molecular structure for data-poor chemicals [52]. Additionally, it is also of great help for predicting the value of some virtual compounds in the early drug discovery phase [53]. However, these predictions may not be accurately correct, but they provide a framework for the classification of chemicals and the results can be further validated by experimental data. QSAR can predict partition coefficient and metabolic constants for chemical and the estimation of pharmacokinetic parameters and internal dose can be done by PBPK modelling. Price et al. conducted a study for predicting the inhalational toxicokinetics of chemicals in a mixture using the QSAR-PBPK model. It involved the determination of partition coefficient, V_{max} and K_m for volatile organic compounds by QSAR. These predictions were used as input for PBPK model to predict pharmacokinetics of chemicals mixture. The study provides an initial evaluation of kinetics of chemicals in mixture of increasing complexity based on structure and toxicity [54]. Karrer et al. readjusted developed PBPK model for one of the endocrine disruptors - bisphenol A analogs (BPS, BPF, BPAF) for peroral and dermal exposure. PBPK model was parametrized by QSAR model for calculating partition coefficient for several BPA analogs [38]. QSAR model was developed for calculating adipose/blood partition coefficient of 67 environmental contaminants including endocrine disruptors and others (alcohols, polybrominated diphenyl ethers, PCBs etc) [55]. These chemical-specific parameters are of immense importance for developing a PBPK model [55]. In another example, the human PBPK model of DEHP with its major metabolites was developed using a bottom up modelling approach to predict the time course of chemical concentration for different exposure in various compartments. For parameterisation of the model, IVIVE and QSAR were applied. PBPK model was validated with the human kinetic study

having different dosing scenarios [56]. Integration of models like QSAR, PBPK, and IVIVE for emerging or known chemicals provides a framework that relies on less use of experimental data and can be helpful for risk assessment. Also, the emerging mechanistic based framework offers the potential of being applicable to multiple chemical exposure equivalents to the real exposure scenario. However, such a framework needs further improvement to account for uncertainties and variabilities at different exposure levels and organisms. But, with an increase in our level of understanding, we can be optimistic about developing mechanistic QSAR to predict different parameters and PBPK model for pharmacokinetics of different chemicals reducing the usage of *in vivo* studies.

4. Pharmacodynamics Model/Dynamic System analysis

4.1 Introduction

Pharmacodynamic (PD) has evolved from an empirical to quantitative outputs that characterize the effect of drug/chemical on response inside the body [57]. The interaction of a drug/chemical molecule with a receptor causes the initiation of a sequence of molecular events resulting in a pharmacologic response. The term PD refers to the relationship between drug/chemical concentrations at the site of action (receptor) and pharmacologic response, including the biochemical and physiologic effects that influence the interaction of drug/chemical with the receptor.

$$[Chemical] + [Target\ ] \Leftrightarrow [Drug - Target\ complex] \longrightarrow Response$$

There are five types of PD models: direct response model, biophase distribution indirect response, signal transduction and irreversible effect model (Figure 5). The first three types are extensively used while others have more limited applications. This part of the chapter focuses on all five-pharmacodynamics model. The choice of model for any drug/chemical depends on the action. For example, if PD response changes with concentration, then a direct response model is the preferred one. However, if there is a time delay between concentration and the response, an indirect model is used because of receptor-mediated effect or signalling cascades.

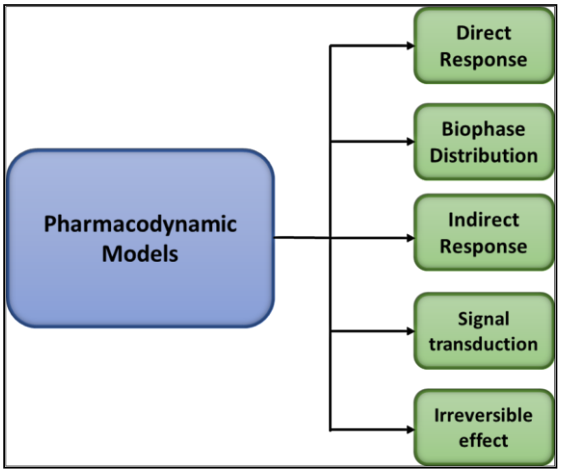


Figure 5: Types of Pharmacodynamic Model

4.2 Types of Pharmacodynamic Model

4.2.1 Direct Response Model

In the direct response model, drug/chemical directly induces the change in the PD response (Figure 6).

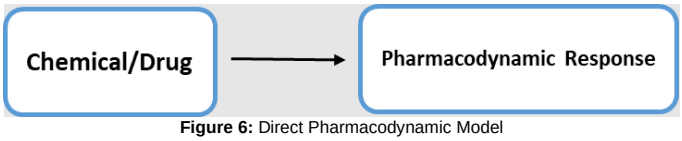


Figure 6: Direct Pharmacodynamic Model

The well-known modified Hill equations are used to describe the drug-receptor interactions empirically as follows:

a) Simple E_{max} model

This model was originally derived from the classical theory of drug-receptor interaction. It is based on the Hill equation which assumes that the effect of any chemical/drug is directly proportional to the receptor, also called linear transduction [58].

$$E = E_0 + \frac{E_{max} * C(t)}{EC_{50} + C(t)}$$

Where E_{max} is the maximum response, EC_{50} is the concentration at which 50% of E_{max} occurs and E_0 is the baseline response. C (t) is the effective chemical concentration i.e. concentration at the target site.

b) Sigmoid Emax model:

This model is a generalization of E_{max} model. It contains a curve fitting parameter γ also known as Hill factor describing the steepness of concentration and effect relationship.

$$E = \frac{E_{max} C(t)^{\gamma}}{EC_{50} + C(t)^{\gamma}}$$

E_{max}, EC_{50} and E_0 and γ represent the sigmodicity factor or Hill factor, γ=1 for simple E_{max} model and if γ>1 for a steeper curve, γ<1 for a smoother curve.

These models have been used to characterize the effect of a number of drugs/chemicals. For example, a pharmacodynamic estrus cycle model (PD-EC) has been developed for endocrine modulators that are weak E2 antagonists or weak E2 agonists. This PD-EC model of the hypothalamus-pituitary ovarian axis was used to examine quantitatively the response of endocrine modulators on reproduction, ovulation and tumour formation in rats [59].

4.2.2 Biophase distribution model

Biophase distribution model comes into picture when there is a delay in the effect after chemical/drug exposure. Sometimes, it led to the formation of hysteresis or a gap in response versus concentration plot. This model is extensively used for explaining the time lag in the response period. There are two types of biphase distribution models, one is the effect compartment, and another is extended catenary biophase model [60].

$$E = \frac{E_{max} . C_e^{n_H}}{EC_{50} - C_e^{n_H}}$$

The extended catenary biophase distribution model is most widely used and consists of two compartments, transfer effect and effect compartment (Equation). Here, Emax is the intrinsic activity, EC_{50} is the potency and n_H is the slope factor and Ce represent effect-site concentration. Brain extracellular fluid (ECF) concentration and time course of predicted effect-site concentration

of morphine was very well described by extended catenary biophase distribution [60]. They studied biophase distribution and P-glycoprotein interaction by morphine through electroencephalogram (EEG) using PK-PD modelling. The saturable biophase distribution model along with a three-compartment PK model was used to understand the respiratory depressant effect of norbuprenorphine in rats. The PK-PD simulation showed that IV administration of buprenorphine leads to concentration values well below the values that affect respiration due to the conversion of buprenorphine into norbuprenorphine [61].

4.2.3 Indirect response model

Drug/chemical does not directly affect the response. The indirect response models basically assume that the biological response is due to either inhibition or stimulation of the production or degradation (precursor) as a function of target chemical concentration (Figure 7).

$$\frac{dR}{dt} = k_{in}^{0} * f(t) - k_{out} * f(t) * R$$

Where, k_{in}^{0} zero-order constant for production of response, k_{out} first-order constant for loss of response, R_0 is the basal physiological concentration of response variable.
$f(t)$ is the function that describes the inhibition or stimulation of response variable synthesis or degradation.

$f(t) = S(t) = 1 + \frac{S_{max}C_t}{SC_{50}+C_t}$; Stimulatory function

$f(t) = I(t) = 1 - \frac{I_{max}C_i}{IC_{50}+C_i}$; Inhibitory function

S_{max}/ I_{max} is the maximum Stimulatory/inhibitory response, C_i is the concentration at the target site, SC_{50}/IC_{50} is the stimulatory/inhibitory concentration required to produce half-maximum response. There are four basic indirect response models that can be read in detail by going through reference [62].

Figure 7: Indirect Pharmacodynamic Model

4.2.4 Signal Transduction model

The signal transduction process comes into the picture for defining pharmacological effects of endogenous and exogenous compounds like hormones, drugs and certain chemicals. In this process, secondary messengers like cyclic AMP, phospholipases or receptors like cell-membrane receptors and nuclear receptors are involved to regulate the series of events leading to pharmacological ~~end-points~~endpoints. It leads to multiple processes in signal transduction, causing a delayed effect. It often involves action at the molecular or cellular level, where PD models face challenges for predicting response~~-~~ and time-profiles of the intermediate steps. The development of this model has been possible with the advancement in molecular biology with which we can analyse the receptors-mediated effect and gene expression. Detailed signal transduction models, such as transit compartment, gamma distribution or stochastic model, can be applied to simulate and predict delayed effects. In this section, we will discuss the transit compartment and more details about other models can be understood by going through the following reference [63]. Transit compartment models mostly involve a series of differential equations for describing the events between activated receptor (M_i) and response (R).

$$\frac{dR}{dt} = \frac{(M_i^{\gamma} - R)}{\tau}$$

Here, τ is transit time and γ is the power coefficient which increases or decreases the transduced signal. As per the receptor occupancy theory, the drug-receptor complex is directly proportional to the biological effector signal (E). Assuming receptor binding, the equation can be described as,

$$\frac{dE}{dt} = \frac{1}{\tau}\left(\frac{E_{max}C}{EC_{50} + C} - E\right)$$

Where E_{max} is a maximum induced signal, C is free chemical/drug concentration and EC_{50} is the concentration at which 50% of Emax occurs. This approach is often used for simulation and prediction of receptor and gene-mediated effect in corticosteroids and other chemicals [64]. For instance, the PD model was developed for systemic corticosteroids to analyse the effect on immune cells and the respective response. During *ex vivo* studies, linear transduction delay of drug activity was seen on immune cell proliferation and the same was modelled using signal transduction delay [65]. In general, corticosteroids mostly act on signal transduction pathways by acting on nuclear receptors (NF-Kb, nuclear factor-AT). Therefore, many PD models on corticosteroids consider the signal transduction model for explaining the effect.

4.2.5 Irreversible Effect model

Theoretically, the mathematical relationship between concentration and response assumes that increased occupancy of receptors leads to increased response, but this is not the case in all realistic scenarios. In certain cases, tolerance of the chemical/drug may occur eliciting irreversible effects. For example, antimicrobial drugs, anticancer, enzyme inhibitor chemicals often show such kind of effects and thus to explain their irreversible effect, the PD model was developed. The equation for the irreversible model is as follows:

$$\frac{dR}{dt} = -k\text{*C*R}$$

Where dR/dt is the irreversible response with time, R is for cell or receptor, k is second-order cell killing rate constant and C is the concentration of a chemical. This model is generally used to describe the cell-killing effect especially in the case of highly toxic chemicals or drugs [58].

4.3 Translation through PK/PD model

PD modelling alone is not enough to predict the response or effect of drug/chemicals in the human body. Different types of physiological processes like slow receptor binding, chemical-chemical interactions, disease progression, aging and enzyme kinetics add to the complexities of the PD prediction. Further, it is difficult to predict the response without knowing the concentration of chemicals in a particular site. Integrated Physiologically Based Pharmacokinetics (PBPK)/Pharmacodynamics (PD) modelling connects two different entities to provide a time course of response for a particular chemical (Figure 8). In this approach, the PK component allows for concentration vs time course of chemical in a different body organ and the PD component predicts the intensity of effect based on the concentration of the chemical at that site [66].

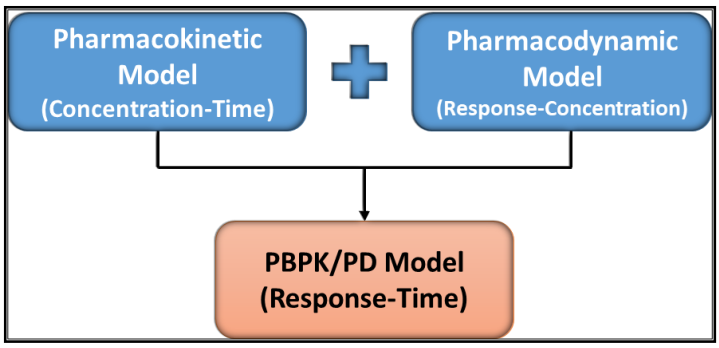


Figure 8: PBPK/PD model. PBPK model predicts the concentration in different compartments over time whereas PD model predicts effct of concentration on different organs which leads to response. PBPK/PD together predicts effect of particular chemicals inside living being with the time.

The mechanism-based PBPK/PD modelling helps in the prediction of dose-response relationship taking into account both intra- and inter-individual variability. It considers target site distribution of chemicals, binding and activation at site depending on various factors like physiochemical properties and biological properties [67]. –There are cases when there is an all-or-none type of effect as sometimes interaction of drugs with competitive mechanisms may produce an additive response (all effect) and response for non-competitive mechanisms may be antagonistic (none effect). In such situations, we use mechanistic pharmacodynamic models with PK models to explain this effect. Now, we are going to discuss some examples of the use of PK/PD models. An indirect response model was used to describe the action of coptisine and LPS on inflammatory factors; namely, a 2-compartment PK model was used for understanding kinetics of the chemical followed by the PD model [68]. In another case, the immunity response for the B cell population was characterised by a monoclonal antibody through an integrated model. The B cell population recovery during a chronic toxicology study was estimated by PK/PD. *In vivo* studies were done in cynomolgus monkey to further verify and observe PK and PD responses. Results showed that the PK and PD model for monoclonal antibodies were in line with experimental data [69]. Pharmacokinetics and toxicodynamic (PKTD) model was used for studying pralidoxime (drug) effects on paraxon-induced respiratory toxicity. The TD model was developed for describing enzymatic (cholinesterase) inhibition through diethylparaoxon and reactivation by pralidoxime. Further, *in vitro* model was used to estimate EC50 and *in vivo* for estimating other parameters. PK/TD model was further validated using *in vitro* and *in vivo* data [70]. Such models provided insights about the effects and also the concentration inside the body with time. The integration of the PK/PD model can be widely used for understanding the time-course and intensity of drug/chemical on the body. They are a one-step forward towards translation for better prediction inside the human body and further risk assessment. Some examples of EDs are mentioned. Sharma et al. developed a conceptual model for PBPK/PD for EDs mixture in order to integrate exposome-internal exposure-biological effect to the adverse outcome. The PK-PD interaction was pointed out as very important in the risk assessment process (Figure 9) [71]. Lohinavy et al. conducted a combined PK/PD study in male rats for studying mechanisms responsible for the high liver concentration of PCB 126 in humans. Using experimental data, they built a PBPK/PD model for understanding binding between PCB 126 and hepatic proteins and excretion of these proteins which cause the multi-drug resistance in humans. This study demonstrated the role of hepatic transporters for drugs in the disposition of EDs [72].

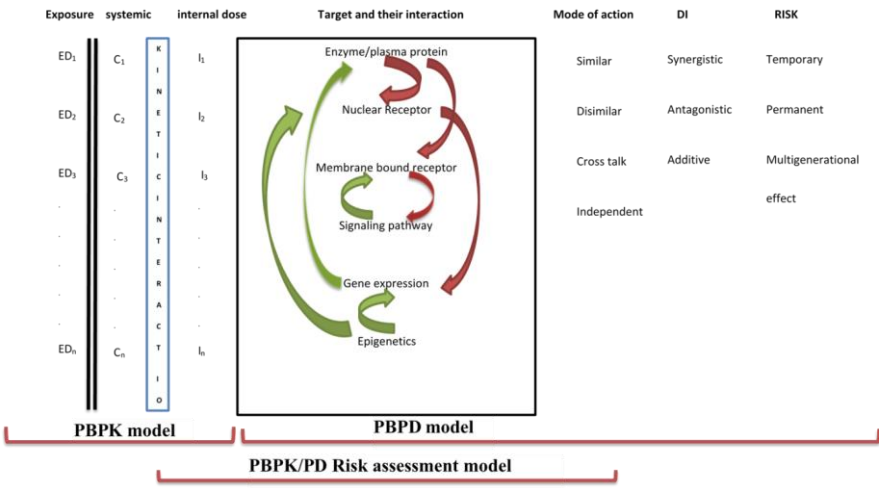


Figure 9: Conceptual model of PBPK/PD in assessing risk for chemical mixture (ED-endocrine disruptor exposure, C-concentration of ED in systemic circulation, I-concentration of ED in target organ or tissue, DI-dynamic interaction) ~~[35]~~ [71].

Formatted: Not Highlight
Formatted: Not Highlight
Formatted: Not Highlight
Formatted: Not Highlight

5. Quantitative in-vitro to in-vivo extrapolation (QIVIVE)

Advancement in the *in vitro* methodology and changes in OECD guidelines for reducing the usage of animals has led to an improvement in *in silico* techniques. There is also scientific evidence that animal models do not completely correlate with complex human anatomy and physiology.

QIVIVE is an approach used for the translation of an *in vitro* dose-response (DR) to *in-vivo* dose-response (DR). The reconstruction of *in-vivo* DR from the in-vitro studies involves the linear interpolation of transduction kinetics of the signaling pathway. It assumes that the *in-vitro* data reflecting DR after target cell exposure and the *in-vitro* derived dose-response model must have target cell exposure in input to be consistent. Such *in vivo* target cell (or by extension target organ) exposure, if not measured, is obtained by PBPK modelling (Figure 10. This can be done for animals or for humans, or both to help inter-species extrapolation.

Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic

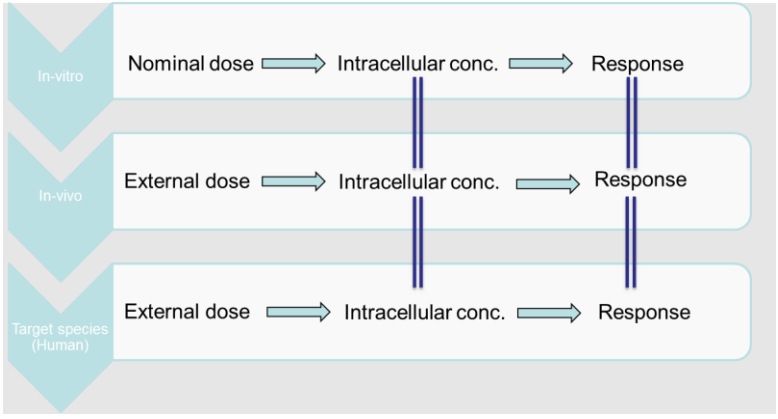


Figure 10: Describe the scheme for the QIVIVE approach where double lines represent equality assumptions and arrow represents the flow of information.

A PBPK model along with the QIVIVE has been used to determine the oral equivalent doses corresponding to in-vitro doses. If the determined oral equivalent doses are relevant to the environmental exposure levels, then the response was classified as an adverse effect [73]. DR curves obtained through QIVIVE are helpful in toxicological risk assessment and often needed to get point of departure for predicting safe exposure limits for humans. For instance, the PBPK model was used for estimating the time-concentration effect of different BPA isomers by utilizing *in vitro* data for validation. The human oral equivalent dose was estimated from EC_{50}/IC_{50} values using QIVIVE and *in vivo* relevant prioritization of chemicals [74]. In another example, the plasma concentration of three groups of toxicants -triazoles, glycol ethers, and phthalates- was predicted by incorporating PBK Indus chem fate QSAR model to QIVIVE. Such models are a key step towards correlating the toxic potency of compounds *in vitro* and *in vivo* [75]. Incorporation of QIVIVE to estimate *in vivo* parameters based on *in vitro* data is rapidly recognized as a critical component for risk with context to high throughput screening data [76].

6. Systems biology

6.1 Introduction

The term Systems Biology (SBs) is nearly 15 years old and it consists of different disciplines like engineering, biochemistry, genetics, pharmacology, bioinformatics, molecular biology, cell biology and other basic science disciplines [77]. Modern Systems biology provides a platform for integrating multiple components and interactions underlying cell, organ, and organism processes in health and disease [78]. It describes the functional interaction of biological components in a time-dependent fashion that uses genomics, metabolomics, and proteomics data [21,22]. Understanding the biomolecular mechanisms is of great interest to identify the toxicological effects at the advanced stage. Systems biology has long been of great interest in studying the adverse effects on human health, which basically involves linking perturbation (the result of chemical interactions with a biological target) on the normal biological network to adverse outcome response. In this part of the chapter, we focus on describing systems biology, different approaches of systems biology, models, examples and its context with EDSs.

Systems Biology includes a holistic approach for studying the biological system through computational and mathematical modelling [79]. Advancement in computational, genome sequencing and high throughput analysis (OMICS) has led to increased awareness among the scientific community. It is a missing link between genotype and phenotype taking into account multi-scale biological networks. Toxicogenomics is considered as a part of SBs that include molecular approaches to analyze genes and proteins for understanding toxicological patterns: proteomics, metabolomics, transcriptomics, genomics, and epigenomics, which are interconnected to different gene functions and expression. But, SBs are not just about genome scaling predictions, it is also about hypothesis-driven approaches which lead to experimental research for proving the hypothesis [80]. System-Level analysis of biological networks and dynamic properties is needed for an understanding of the properties of biological networks like adaptation, robustness which makes the biological cell to act in a controlled fashion [81]. Computational models play an important role in quantitative predictions, which can describe the perturbations in the biological systems. However, still, there is a lot more to explore and utilize the prediction power of these computational models for understanding the design principle of biological networks. There are three different approaches for systems biology: 1) Top-down systems biology, 2) Bottom-up systems biology and 3) the Middle-out approach.

6.2 Models for Systems Biology research

Systems biology involves extensive use of mathematical modelling for assessing the complex interaction of biological networks in a holistic approach. These models involve quantitative predictions and hypothesis-driven research, leading to the exploration of new biological behaviors and further experimental work to support hypotheses. Experimental data is used to develop and validate the SBs models. Chemicals/drugs affect human health in various ways like alteration in cellular uptake and efflux controlled by membrane transport proteins, the interaction of chemicals and metabolites with tissue macromolecules, immune response activation, metabolic transformation and much more [82]. Firstly, to understand these interactions at different levels of biological organization, molecular and cellular targets, different SBs models are required. *In silico* SBs involves gathering the experimental data for different parameters included in the mathematical model; it includes tools like metabolic control analysis (MCA), flux balance analysis (FBA), and elementary model analysis (EMA) to understand network-associated toxicity pathways and much more. These tools through different ordinary differential equations (ODEs) help in predicting and understanding how chemicals perturb the biological networks leading to risk for human health.

For *in silico* SBs models, different steps are involved.

The first step is constructing the model, which can be done by using different online software. ODEs are included and different kinetic parameters are used in the model. These parameters should define biological interactions and other biological behaviors.

The second step involves a steady-state analysis. Steady-state is reached when no changes occur in concentration or flux of chemical with time. This steady-state defines functions of modelled biological systems and is highly useful. The next step is MCA, which is often useful for understanding different processes in the biological network that determine the potential of toxicity of any chemical. MCA includes different coefficients like flux control coefficient (FCC) and elasticity coefficient. In simple terms, flux control coefficient (C_i^J) can be defined as a percentage change in steady-state metabolic flux (J) caused by a one percent change in enzyme activity (v).

$$C^J{}_{vi} = \frac{\left(\frac{\partial \ln J}{\partial p}\right)_{\text{systems steady state}}}{\left(\frac{\partial \ln v_i}{\partial p}\right)_{\text{metabolite,concnetration/all}}}$$

Here, p is specific for any enzyme which is i and $\partial \ln J$ is an effect on flux in the intact system; $\partial \ln v_i$ is an inhibitory effect on flux by the enzyme. For instance, if an inhibitor decreases the pathway flux by 30% at a concentration at which it inhibits the particular enzyme by 60% then the flux control coefficient would be 30/60 = 0.5. Through this equation, we can understand whether the enzyme is rate-limiting step if C=1 or not if C=0 and also other cases where enzyme may be controlling partial flux. When FCC is very low, in some cases it implies that a single enzyme will not ~~have an effect on~~influence the flux in a pathway. But it needs to be considered because the significance of FCC may vary based on the situation. More detail about FCC can be understood by going through the following reference [83]. The summation theorem is considered as the sum of metabolite concentration and flux control coefficient.

$$\sum_{i=1}^{n} C_i{}^J = 1$$

The sum has to be one for the flux control coefficient which means if the control coefficient of all the enzymes affecting metabolism in the cell is added it is equal to one [84]. If we raise the amount of any particular enzyme, flux control coefficient will decrease, as per the summation theorem, then FCC of some other enzymes will increase to keep the sum equal to 1.

There is another parameter called elasticity coefficient which is a local parameter of the enzyme measured by isolated substrate and enzyme. It is the measure of enzyme metabolite effect. It can be calculated by the following equation

$$€^{i}_{p}= \left(\frac{\partial\, In\, vi}{\partial\, In\, p}\right) other\ metabolic\ concentrations\ constant$$

Where v_i is the enzyme rate and p is parameter perturbing. A particular enzyme can have more than one elasticity. Metabolites which increase the reaction rate of an enzyme have positive value and metabolites which decrease the reaction rate of enzymes (inhibitors) have negative values for elasticity. There is no summation theory for elasticity.
Taken together, MCA can give a framework for quantifying the control coefficient to understand system functioning and can relate reaction kinetic properties (local) to other general properties of the whole intact pathway [85].

The next approach is Hierarchical Regulation analysis (HCA) which analyses that flux is getting controlled by metabolic activities or the gene expression [86]. Enzymes can increase the flux by either of these methods, gene expression, signal transduction and metabolically. The first two methods are quantified using the hierarchical regulation coefficient ($ρ_h$) and the third method is quantified by a metabolic regulation coefficient ($ρ_m$). At the steady-state sum ~~of all three~~ is equal to 1.

$$ρh + ρm = 1$$

ρh can be computed as a ratio of the percentage change in enzyme concentration by the flux change.

$$ρh = \frac{\log V_{max\,x1} - \log V_{max\,x\,2}}{\log J_{x1} - \log J_{x2}}$$

Here Vmax is the change in enzyme concertation at two respective conditions (x1 and x2), J is the flux flowing in the enzyme at two conditions (x1 and x2). The flux can be controlled by genetic change or the metabolite change. As per the above equation, the metabolic coefficient can be calculated using the regulation coefficient [87].

$$ρm = 1 - ρh$$

Metabolic Control Analysis (MCA) considers the dynamical representation of metabolism whereas, other approaches like Flux Balance Analysis (FBA), Metabolic Flux Analysis (MFA) and Elementary Flux Analysis (EFA) use steady-state stoichiometric models to analyze flux distribution. More information about the models available for flux analysis and equations can be understood by going through the reference [88,89].

After developing the model considering all the above approaches, the next step is model parametrization. This step includes setting the parametric values for simulation. Parameter values can be obtained through *in vitro*, *in vivo*, epidemiological or the cheminformatics model. This step also involves adjusting some of the parametric values so that a biologically meaningful steady state can be obtained. Uncertainties are also considered while model parameterization.

As mentioned above, we have considered all the steps needed to construct a systems biology model for studying toxicological pathways or analyzing toxicological risk. Here are some other examples of the SB model developed to understand how they can be used to evaluate toxicity [90]. For instance, an oxidative stress SBs model has been designed with the objective of understanding the complexity

of reactive oxygen species (ROS) management in the context of various aging-related disorders. Five designed principles for managing ROS networks integrating several biological components such as genes, mRNA, protein and metabolite cellular; and biological processes at the level of molecular, cellular and organism level. The detail of this model is available publicly at FAIRDOM Hub [91](http://doi.org/10.15490/fairdomhub.1.model.571.1). Another example is NF-κB on which different researchers have developed SBs models. The transcription factor nuclear factor kappa B (NF-κB) is required for many gene induction events with regard to inflammatory cytokines and pathogen driven substances [92]. Recently, a systems pharmacology model of NF-κB regulatory networks has been developed to evaluate quantitatively the therapeutic efficacy of NF-κB synthetic decoy oligodeoxynucleotides (ODN) on the dose and degradation rate. The kinetic model was based on ordinary differential equations for assessing population-level dynamics of a particular species in the network, and the stochastic model was based on master equations evaluating changes in the microstate of the NF-κB regulatory network. ODE in the kinetic model was solved by ODE solver in python. Transcriptor factor binding to DNA was considered diffusion-limited assuming uniform binding of NF-κB to all binding sites. Stochastic dynamics of NF-κB are considered by a master equation in the well-stirred limit. Kinetic Monte Carlo scheme was employed for solving these master equations for the NF-κB regulatory network to assess changes in genome binding sites and quantified temporal oscillation dynamics. Such a systems biology model provides a quantitative framework for translational research to find the therapeutic window of NF-κB ODN and overall risk assessment [93].

6.3 Systems Biology Model and Endocrine Disruptors

Not much research has been done on studying the effect of endocrine disruptors at the molecular level by building systems biology models. Recently, some limited examples of systems biology models to model the effect of EDs have been ~~started~~ developed[94,95]. One notable example is the effort by USEPA for evaluating the potential of thousands of EDs include integration of QSAR *in silico* methods and computational systems biology model approach using carbamate pesticide carbaryl as a case study [94]. It included in silico metabolite generation and biological pathway enrichment analysis. Metadrug^{Tm} was used for the qualitative prediction of the first pass and sequential metabolites. Canonical pathway map was built for predicted targets of carbaryl and estradiol metabolism for elucidating different molecular mechanisms. This study integrates QSAR, docking, and systems biology approach for risk assessment and screening of endocrine disruptor chemicals [94]. Dynamic systems biology model was developed for predicting ovarian sex steroid concentrations in female rats under normal estrous cycle and estradiol concentration for adult rats exposed to chemicals (BPA, Atrazine, etc.) by Quingot et al. [95]. The development of systems biology models for understanding mechanistic relationships and developing AOPs is highly helpful for improving knowledge about the mechanism of action and toxicity pathways.

6.4 The Future of Systems Biology

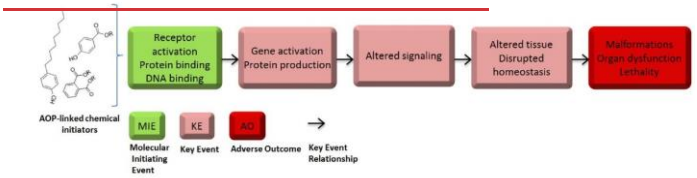
In earlier times, biologists faced many problems due to a lack of either mathematical or programming knowledge. However, now software packages are available to do mathematical tasks and programming. Different repositories and software are available through which research groups can exchange their models. The CellML project (https://www.cellml.org/), The Systems Biology Markup Language (http://sbml.org/Main_Page), Biomodel Database (http://www.ebi.ac.uk/biomodels-main/), JWS Online, PathCase-SB and many other open software platforms for modeling and analysis of data [96]. For instance, Systems biology workbench (SBW) is built on SBML and provides open-source software for systems biology research [96]. Such initiatives surely increase the knowledge of database available with us and provide a platform for further research. The transition from a molecular level to the systems level provides a path for understanding the complex biological pathways [97]. Now, SBs are moving forward to create the Virtual Physiological Human (VPH) for developing patient-specific models and apply them in personalized and predictive healthcare [98].

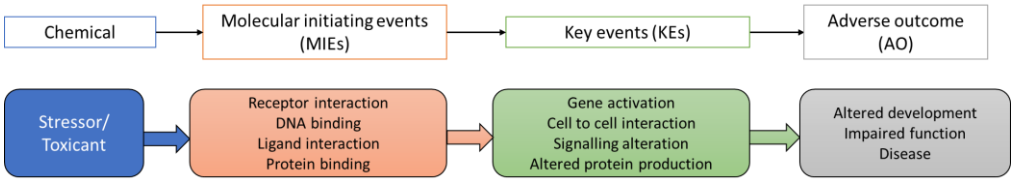
There are already some SBs models of separate organs like the cardiac structure model which is widely used by the pharmaceutical industries to understand the disease and drug-receptor interactions [99]. Extensive brain-related research and projects like ALLEN (https://portal.brain-map.org/), Human Brain Project (https://www.humanbrainproject.eu/en/), BRAIN Initiative (https://braininitiative.nih.gov/), MINDS (https://brainminds.jp/en/) all over the world will enable understanding about different neurons and synapses of the brain. Now, biology is going towards creating more models of the organs and toward modelling the complete human body. Readers can go through these links to find more details about such models like PHYSIOME project (http://physiomeproject.org/) and VPH project (http://www.vph-noe.eu/). Over the next 10 years, research will move forward to create predictive, personalized models for the diseases and chemical induced toxicity. Advanced technology allows the individuals to get their genome analysed and many parameters can be understood simply through blood analysis. Systems biology models will be able to simulate research and understanding about different pathological conditions in humans based on their specific genomic sequences and may be able to determine the future health of the individual. There are projects and different funding to explore the human genome at experimental and computational level by many organizations like "The National Human Genome Research Institute" (https://www.genome.gov/) and "Genomics England" (https://www.genomicsengland.co.uk/).

7. Adverse Outcome Pathways (AOPs)

7.1 Introduction

There has been a huge debate about AOPs and different researchers define it in their own terms. The main challenge lies in defining AOPs because some consider it as a part of systems biology while other researchers consider AOP as a separate entity. As per systems biology, AOPs are used for a detailed understanding of molecular interactions which causes toxicity [100]. In this Chapter, we are considering AOP as a separate entity. In general terms, an AOP describes a sequence of events commencing with initial interaction(s) of a stressor with a biomolecule within an organism that causes a perturbation in its biology (i.e., molecular initiating event, MIE), which can progress through a dependent series of intermediate key events (KEs) and culminate in an adverse outcome (AO), considered relevant to risk assessment or regulatory decision-making (Figure 11) [20,101,102]. An MIE is "a specialised type of key event that represents the initial point of chemical/stressor interaction at the molecular level within the organism that results in a perturbation that starts the AOP" [101,102]. A KE is "a change in a biological or physiological state that is both measurable and essential to the progression of a defined biological perturbation leading to a specific adverse outcome" [101,102]. A KE relationship is "A scientifically-based relationship that connects one key event to another, defines a causal and predictive relationship between the upstream and downstream event, and thereby facilitates inference or extrapolation of the state of the downstream key event from the known, measured, or predicted state of the upstream key event" [101,102].





~~**Figure 11:**~~ **Figure 11:** Schematics of AOP network describing series of key events linked to toxicants (endocrine disruptors) induced activation or inhibition of various biological targets (MIEs) to adverse outcome.

~~Schematics of AOP network describing the dose response linking the signal transduction pathway, as a series of key events due to chemically induced activation or inhibition of biological target (MIEs), to the adverse effects (https://aopwiki.org/).~~

7.2 AOPs Development Principle

OECD guideline[103] was published in 2013 for developing and assessing AOPs. The second edition was published in 2016. It describes all the steps involved to develop and register an AOPs on the international repertory AOP wiki (see below) [104]. There are four steps involved in the AOPs structure, which includes AOP description, KE description, KER description and assessment of AOP (Figure 7). AOP description is the initial steps for creating an AOP which is followed by different levels of biological organization in the KE and KER description [104]. Then, the last step involves assessment of the AOPs, which includes biological plausibility, evidence for KE and KER and quantitative understanding about KER (Figure 12) [105] (https://aopwiki.org/info_pages/2/info_linked_pages/1#J%20Overall%20Assessment%20of%20the%20AOP).

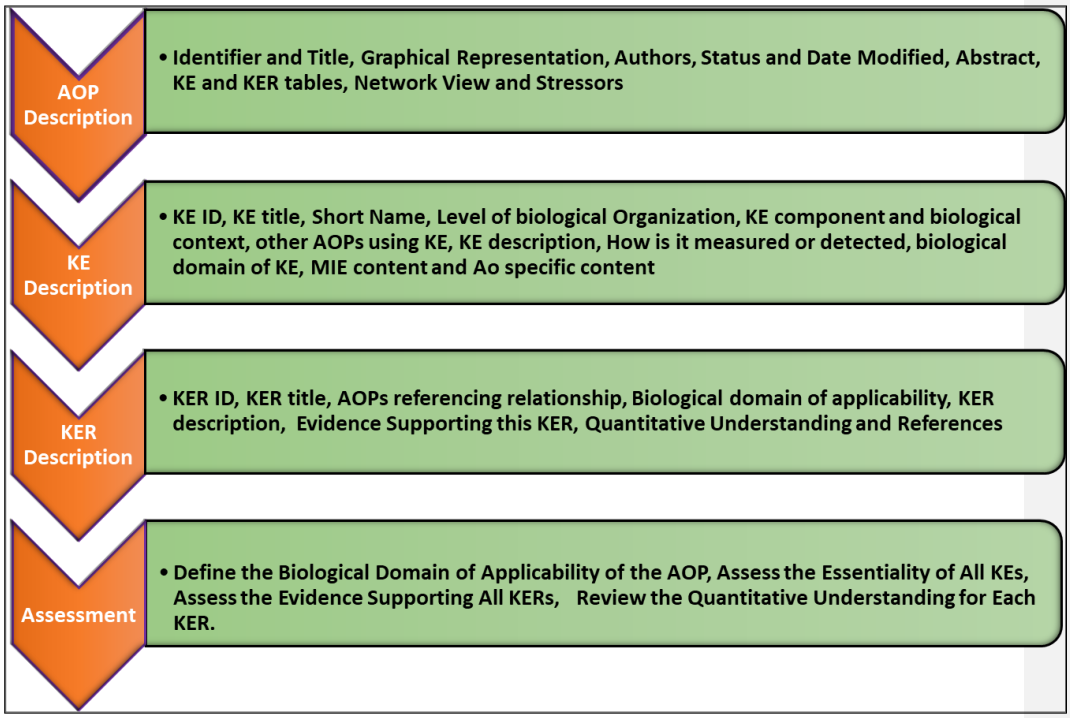


Figure 11: It describes the overall procedure for AOP development.

7.3 AOPs Knowledge and Models

There are different initiative by regulatory bodies and government authorities for developing AOPs. AOPs development program was started by OECD in 2012 [103] (https://www.oecd.org). It also includes the Extended Advisory Group on Molecular Screening and Toxicogenomic members (EAGMST) for AOPs development and internal reviews. There are several AOPs knowledge bases and tools available online to guide users about the mechanism and pathways by which chemicals induce adverse effects (Figure 13). The eAOP portal is the main AOP knowledge base. It is available publicly and easy to use. Search engine can be used by adding search keywords of AOP titles and it is easy to filter it based on creation date, update date, status and title in both ascending and descending order. This portal contains around 282 adverse outcome pathways and 2070 key events as per Nov 2019. More information about this portal can be seen online [106] (https://aopkb.oecd.org/search.ashx?qry=&sort=2). AOP-KB also called as Adverse Outcome Pathway Knowledge base projects contains different elements together in one platform like effectopedia, AOP wiki, AOP xplorer and intermediate effects DB (Figure 8). The first tool is AOP wiki which provides all the knowledge and research in a user friendly wiki interface. It is a collaborative effort by Society for the Advancement of Adverse Outcome Pathway (SAAOP) and OECD. It includes all the key events for AOPs and some online training courses are also available for increasing knowledge and participation in AOP process [105](https://aopwiki.org/). Online training course is available from different programs like national toxicology program (NTP) Human Toxicology Project Consortium for dissemination of AOP development and applications (https://humantoxicologyproject.org/about-pathways-2/aop-online-course/) [107]. Second tool is

Effectopedia, which is an open platform for knowing about AOPs. It is a modelling platform for development and utilization of AOPs. Trainings and videos are available online for learning. Through this engine, user can learn how to create visual AOP diagram, key events and key events relationship using OECD handbook, publishing AOP on Effectopedia database, making quantitative models for prediction through R or MATLAB [108](http://training.effectopedia.org/). Third tool is AOP xplorer through which users can visualize AOPs from AOP ontology. It is also possible to do data analysis using R workflow and packages and it is free software [109] (http://datasciburgoon.github.io/aopxplorer). Fourth tool is Intermediate effect DB contains chemicals related data obtained using no traditional methods and tell how the chemical cause MIEs and KEs. It is currently under development. All these tools are under AOP-KB project including third party software developers and this project is a collaborative effort from JRC, EPA and ERDC. Such kind of initiatives all over the globe are a good step for dissemination of knowledge and creating pathways for further improving the AOPs database and assessing human risk.

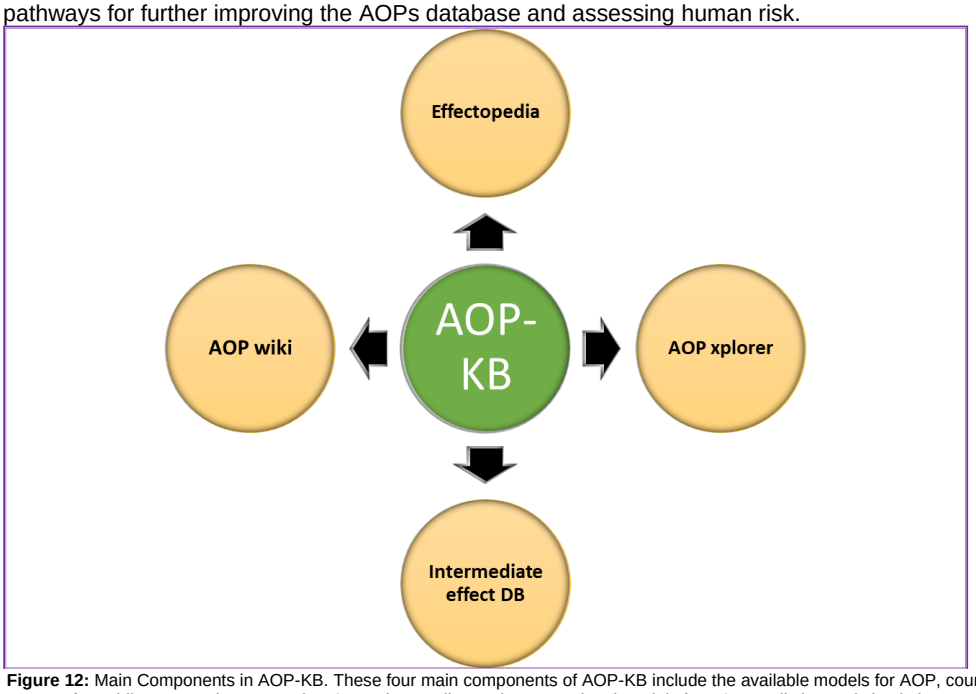


Figure 12: Main Components in AOP-KB. These four main components of AOP-KB include the available models for AOP, courses for guiding researchers towards AOP understanding and computational models for AOP prediction and simulation

7.4 AOPs and Endocrine Disruptors

Effects of ED on hormone nuclear receptors have been studied for decades [110]. These nuclear receptors if activated by some ligands control the transcription of genes which lead to cascade of events causing effects in the living beings [111]. OECD has provided a conceptual framework for testing and assessment of EDs (revised in 2012) [112] (http://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm). It is a detailed framework consisting of different levels which includes *in silico* predictions, *in vitro* and *in vivo* assays for specific endocrine mechanisms and pathways and data about adverse effects on endocrine relevant endpoints. For instance, the *in vivo* results for endocrine disruption from OECD testing can be compared and an

AOP conceptual framework for EDs can be further provided [113]. United States Environmental Protection Agency (USEPA) has the Endocrine Disruptor Screening Program (EDSP) for testing the different environmental chemicals for their effect on three hormonal pathways: androgen, estrogen and thyroid. The framework has been designed around modes of action and provides understanding about use of AOP concept in regulatory [114]. A number of the AOPs registered under AOP-KB are ED-relevant: there are around 40 AOPs for EDsS. including 6 estrogen AOPs, 9 thyroid AOPs, 4 androgen AOPs and 21 other endocrine AOPs [115].

8. Integrative Translational toxicology

Currently, there is a paucity of research that integrates all these above described methods and directly ties the results to a predictive adverse outcomes model (Figure 14). With advancement in computational toxicology, research is moving forward for translation of risk from exposure scenario to health risk assessment through *in silico* models. In this part of the chapter, we will review about combined approaches involving PK, PD, SBs, AOPs and much more. Right from screening of chemical parameters to impact on genetic changes inside human can be simulated through *in silico* tools. Now, translational toxicology involves integration of pharmacokinetic, pharmacodynamics, systems biology and AOPs. This integrative approach considers interaction with chemical and living beings at different levels of biological organization and computational simulation and modelling of the complete toxicological pathways for human risk assessment [100]. Compared to the traditional dose-response model integrative translational toxicology model implements a more complex structure, as shown in figure 14. In figure 14, Module 1 focuses on the pharmacokinetics describing relationship between the chemical exposures to the plasma concentrations. And this also includes the distribution of chemical to the target tissues (Plasma tissue kinetics; PTK) also called biological effective dose; Module 2 captures the interactions of this biological effective dose with a target receptor (proteins, genes or metabolites) and their intrinsic activity. Module 3 links this perturbation (intrinsic activity) to the signal transduction pathway linking whole biological network (in case of Systems biology models) or simple linear pathway model (in case of AOPs). It includes all *in silico* models right from screening of chemicals to impact on genetic changes inside human till risk assessment.

Different researchers all over the world are trying to link *in silico* models for prediction of the risk. Firstly, PBPK model when coupled with PD called as PK/PD model aim to link chemical concentration at the specific site to toxicological response [116]. This approach provides understanding about dose-toxicity profile. Integrative translational toxicology is like a hybrid in which models from PBPK, PD, systems biology, AOPs will be merged together to have quantitative understanding about how chemical affect human systems at cellular, molecular and organ level. For this, a platform for biological multiscale modelling and simulation is required for supporting integration at different physiological levels [117]. This extensive modelling framework will help in predicting toxicity of different chemicals for human risk assessment.

From a long time, authors have done integration of different computational tools. For instance, a framework for QSAR along with PBPK/PD has been developed to predict multiple chemicals and multiple route of exposure –[118,119]. It provides an integrative translational framework for risk assessment of chemicals through exposure by computational modelling. Linking of PBPK and SBs modelling can be explained by two examples: one is chemotactic signalling in E. coli and another one is enzymatic activation by nerve growth factor and extracellular growth factor receptor pathways. From this a simplified quantitative systems pharmacology model (QSP) has been developed to provide insight into drugs mechanism of action [120]. Now, with innovation in science and computational fields, scientists all over the world are trying to make an integrative platform which combines all the models from dose to risk. Multiscale modelling and simulation can make use of integrative translational integration of different software; PK-Sim was used for whole body

physiology and population variability and MoBi for cellular and molecular level. Multiscale model is constructed for pancreatic tumor and drug response is also modelled. Constructing a, virtual patient it is possible to investigate a prodrug, which undergoes activation through for example hepatic metabolization. In this approach, whole body PBPK model of the virtual prodrug and its metabolite is considered and coupled with the PD model and cellular model for signal transduction. It is a step forward towards replicative complete biological phenomenon for the drugs and endocrine disruptor chemicals (bisphenol A (BPA), phthalates and flame retardants). This model can be validated by the experimental data to provide further confidence [117]. Such integration of different models is highly helpful, less time consuming and cost effective.

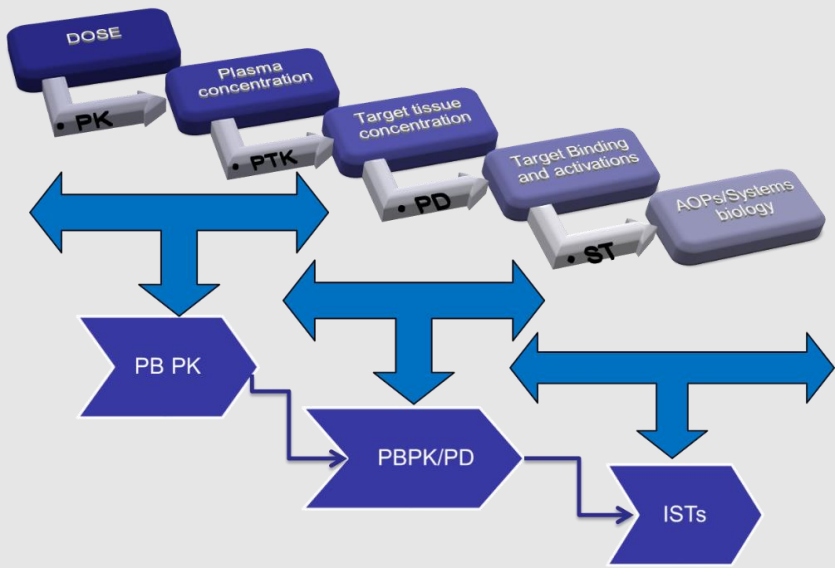


Figure 13: Schematic of integration of several approaches leading to the integrative systems toxicology model. PBPK comprising of both the PK (pharmacokinetics) and PTK (plasma-tissue kinetics) describing the time course of chemical concentration at plasma and tissue. PD is the pharmacodynamics describing the interaction of target tissue dose and biological ligand. Systems toxicology (ST) links the downstream pathway of biological network as a result of perturbed endogenous molecule.

8.1 Future of Integrative Translational Toxicology approach

In-silico techniques have been receiving an important attention as alternative methods to classical approach. NRC vision of toxicity testing in the 21st century is analysing biological responses through high throughput tests and modern methods. This vision proposes two main things: 1) Toxicity pathways through dose-response modelling and understanding of perturbations in pathway functions through computational systems biology model, 2) Extrapolation modelling from *in vitro* to *in vivo* and *in vivo* to human through pharmacokinetic models to predict concentration in different organs and under different conditions in humans [121]. Emerging high-throughput analysis, OMICS and tools such as PBPK, PD, SBs and AOPs offer an opportunity to understand the chemical fate

inside the body, the biological complexity and their multilevel connectivity. The successive use of the PBPK model in the field of toxicology is commendable since it has great advantage of predicting internal tissue dose of compounds or metabolites by utilizing the data derived from the in-vitro and in-silico tools such as QSAR, without any animal experiments. The PBPK also allows cross species extrapolation, cross-route, the dose interpolation, age and population specific without the need of experimental analysis. This brings a great advantage in the field of the environmental toxicology, by reducing the cost and the time of analysis to test a large amount of chemicals. Many biological adverse effects are the result of multiple signalling pathways. These signalling pathways involve nonlinear interactions consisting of many components, which requires more information for their specification than linear interactions do; thus, it may be hard to foresee what comes from nonlinear interactions. Systems biology suggests a solution to this problem – to reconstruct the biological behaviour in an *in silico* replica of the system. It is possible to reconstruct the biological emergence by translating the information about how components communicate into mathematical equations. By integrating the resulting system of mathematical equations in a computer, one should be able to simulate the biological system's behaviour. Basically, with the use of SBs modelling we could be able to solve the problems posed by complex biological systems. In parallel, AOPs has been developed as an approach to predict the adverse effects of chemicals. AOP approach presumes that the biological system's behaviours result from a linear sequence of biological interactions. This approach was developed to reduce the inherent biological complexity where the knowledge/data is lacking. Integration of wide range of *in silico* tools (QSAR, PBPK/PD, AOP, systems biology, etc.), and databases (OMICS, epidemiological and exposure data) can directly tie the results into a predictive adverse outcomes model. This integrative approach would lead to mechanistic understanding of adverse effects vs conventional empirical end points and animal-based testing. There are complex interaction going on inside human body and to analyse those interactions integrative approach is required [122]. The advancement in this approach also provides a framework for initiating personalized, preventive and precise healthcare system. These models can be further extended to assess the risks in population groups, based on genetic changes, age, race and sex. Such initiation will be helpful in future to explain why certain populations have more adverse effects upon exposure to certain toxicants [123].

Overall, collaboration among different fields right from biologist to mathematician, researcher to regulatory authorities is required to work for integrative translational approach. This approach will be a platform for toxicity testing and risk assessment. It will help in exploring mechanistic toxicity pathways for chemicals together with physiological perturbations in humans as well as in gathering data for personalized risk assessment. Also, in future it may be possible to predict the lifetime risk based on early exposure. Advancements in integrative translational toxicology is therefore expected bring a significant contribution to the future of risk assessment.

9. Acknowledgement

V. Kumar received funds from the Health Department of Catalonia Government trough "Pla Estratègic de Recerca i Innovació en salut" (PERIS 2016-2020). Deepika received funds from the Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie Neurosome Project grant agreement No.766251.

10. References

1. Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, et al. Research needs for the risk assessment of health and environmental effects of endocrine disrupters: A report of the U.S. EPA-sponsored workshop. Environ Health Perspect [Internet]. 1996 Aug;104(SUPPL. 4):715–40. Available from: https://www.jstor.org/stable/3432708?origin=crossref

2. Ohtake F, Takeyama K ichi, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, et al. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature [Internet]. 2003 May;423(6939):545–50. Available from: http://www.nature.com/articles/nature01606

3. Welshons W V., Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect [Internet]. 2003 Jun;111(8):994–1006. Available from: https://ehp.niehs.nih.gov/doi/10.1289/ehp.5494

4. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee DH, et al. Regulatory decisions on endocrine disrupting chemicals should be based on the principles of endocrinology. Reprod Toxicol [Internet]. 2013 Jul;38:1–15. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0890623813000191

5. Advancing the Next Generation of Chemical Evaluation [Internet]. Available from: https://www.epa.gov/chemical-research/toxicity-forecasting

6. Manganelli S, Roncaglioni A, Mansouri K, Judson RS, Benfenati E, Manganaro A, et al. Development, validation and integration of in silico models to identify androgen active chemicals. Chemosphere [Internet]. 2019 Apr;220:204–15. Available from: https://doi.org/10.1016/j.chemosphere.2018.12.131

7. Tollefsen KE, Scholz S, Cronin MT, Edwards SW, de Knecht J, Crofton K, et al. Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA). Regul Toxicol Pharmacol [Internet]. 2014 Dec;70(3):629–40. Available from: http://dx.doi.org/10.1016/j.yrtph.2014.09.009

8. Kneuer C, Charistou A, Craig P, Eleftheriadou D, Engel N, Kjaerstad M, et al. Applicability of in silico tools for the prediction of dermal absorption for pesticides. EFSA Support Publ [Internet]. 2018 Oct;15(10). Available from: http://doi.wiley.com/10.2903/sp.efsa.2018.EN-1493

9. Sturla SJ, Boobis AR, Fitzgerald RE, Hoeng J, Kavlock RJ, Schirmer K, et al. Systems toxicology: From basic research to risk assessment. Chem Res Toxicol. 2014;27(3):314–29.

10. Bloomingdale P, Housand C, Apgar JF, Millard BL, Mager DE, Burke JM, et al. Quantitative systems toxicology. Curr Opin Toxicol. 2017 Jun;4:79–87.

11. Jusko WJ. Moving from basic toward systems pharmacodynamic models. J Pharm Sci. 2013;102(9):2930–40.

12. Gabrielsson J, Weiner D. Non-compartmental Analysis. In: Reisfeld B, Mayeno AN, editors. Totowa, NJ: Humana Press; 2012. p. 377–89. (Methods in Molecular Biology; vol. 929).

13. Jusko WJ, Ko HC. Physiologic indirect response models characterize diverse types of pharmacodynamic effects. Clin Pharmacol Ther. 1994 Oct;56(4):406–19.

14. Timchalk C, Nolan RJ, Mendrala AL, Dittenber DA, Brzak KA, Mattsson JL. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci [Internet]. 2002 Mar 1;66(1):34–53. Available from: https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/66.1.34

15. Foxenberg RJ, Ellison CA, Knaak JB, Ma C, Olson JR. Cytochrome P450-specific human PBPK/PD models for the organophosphorus pesticides: Chlorpyrifos and parathion. Toxicology [Internet]. 2011 Jul;285(1–2):57–66. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0300483X11001417

16. Kuepfer L, Niederalt C, Wendl T, Schlender JF, Willmann S, Lippert J, et al. Applied Concepts in PBPK Modeling: How to Build a PBPK/PD Model. CPT Pharmacometrics Syst Pharmacol. 2016;5(10):516–31.

17. El-Masri H. Modeling for Regulatory Purposes (Risk and Safety Assessment). In: Reisfeld B, Mayeno AN, editors. Totowa, NJ: Humana Press; 2013. p. 297–303.

18. Bessems J, Coecke S, Gouliarmou V, Whelan M, Worth A. EURL ECVAM strategy for achieving 3Rs impact in the assessment of toxicokinetics and systemic toxicity. 2015;22.

19. Bell SM, Chang X, Wambaugh JF, Allen DG, Bartels M, Brouwer KLR, et al. In vitro to in vivo extrapolation for high throughput prioritization and decision making. Toxicol Vitr [Internet]. 2018;47:213–27. Available from: http://dx.doi.org/10.1016/j.tiv.2017.11.016

20. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, et al. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem. 2010 Mar;29(3):730–41.

21. Kitano H. Systems Biology: A Brief Overview. Science (80-) [Internet]. 2002 Mar 1;295(5560):1662–4. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1069492

22. Aderem A. Systems biology: Its practice and challenges. Cell. 2005;121(4):511–3.

23. Gim J, Kim HS, Kim J, Choi M, Kim JR, Chung YJ, et al. A system-level investigation into the cellular toxic

response mechanism mediated by AhR signal transduction pathway. Bioinformatics. 2010;26(17):2169–75.

24. Bhattacharya S, Shoda LKM, Zhang Q, Woods CG, Howell BA, Siler SQ, et al. Modeling drug- and chemical-induced hepatotoxicity with systems biology approaches. Front Physiol. 2012;3 DEC(December):1–18.

25. Deepika D, Sharma RP, Schuhmacher M, Kumar V. An integrative translational framework for chemical induced neurotoxicity – a systematic review. Crit Rev Toxicol [Internet]. 2020 Jun 12;1–15. Available from: https://doi.org/10.1080/10408444.2020.1763253

26. Fàbrega F, Nadal M, Schuhmacher M, Domingo JL, Kumar V. Influence of the uncertainty in the validation of PBPK models: A case-study for PFOS and PFOA. Regul Toxicol Pharmacol. 2016;77:230–9.

27. Nestorov I. Whole-body physiologically based pharmacokinetic models. Expert Opin Drug Metab Toxicol. 2007;3(2):235–49.

28. Andersen ME, Thomas RS, Gaido KW, Conolly RB. Dose-response modeling in reproductive toxicology in the systems biology era. Reprod Toxicol. 2005;19(3 SPEC. ISS.):327–37.

29. Bois FY. Physiologically based modelling and prediction of drug interactions. Basic Clin Pharmacol Toxicol. 2010 Mar;106(3):154–61.

30. Song G, Moreau M, Efremenko A, Lake BG, Wu H, Bruckner J V., et al. Evaluation of age-related pyrethroid pharmacokinetic differences in rats: Physiologically-based pharmacokinetic model development using in vitro data and in vitro to in vivo extrapolation. Toxicol Sci. 2019;169(2):365–79.

31. Bois FY, Paxman DG. An analysis of exposure rate effects for benzene using a physiologically based pharmacokinetic model. Regul Toxicol Pharmacol. 1992 Apr;15(PART 2):122–36.

32. Gerlowski LE, Jain RK. Physiologically based pharmacokinetic modeling: Principles and applications. J Pharm Sci. 1983 Oct;72(10):1103–27.

33. Cheng W, Ng CA. A Permeability-Limited Physiologically Based Pharmacokinetic (PBPK) Model for Perfluorooctanoic acid (PFOA) in Male Rats. Environ Sci Technol. 2017;51(17):9930–9.

34. Lee SK, Ou YC, Andersen ME, Yang RSH. A physiologically based pharmacokinetic model for lactational transfer of PCB 153 with or without PCB 126 in mice. Arch Toxicol [Internet]. 2007 Feb 5;81(2):101–11. Available from: http://link.springer.com/10.1007/s00204-006-0130-0

35. Lee SK. Comparison of Pharmacokinetic Interactions and Physiologically Based Pharmacokinetic Modeling of PCB 153 and PCB 126 in Nonpregnant Mice, Lactating Mice, and Suckling Pups. Toxicol Sci [Internet]. 2002 Jan 1;65(1):26–34. Available from: https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/65.1.26

36. Gaohua L, Wedagedera J, Small B, Almond L, Romero K, Hermann D, et al. Development of a Multicompartment Permeability-Limited Lung PBPK Model and Its Application in Predicting Pulmonary Pharmacokinetics of Antituberculosis Drugs. CPT Pharmacometrics Syst Pharmacol [Internet]. 2015 Oct;4(10):605–13. Available from: http://doi.wiley.com/10.1002/psp4.12034

37. Weijs L, Yang RSH, Das K, Covaci A, Blust R. 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 [Internet]. 2013 May 7;47(9):4365–74. Available from: https://pubs.acs.org/doi/10.1021/es400386a

38. Karrer C, Roiss T, von Goetz N, Skledar DG, Mašič LP, Hungerbühler K. Physiologically based pharmacokinetic (PBPK) modeling of the bisphenols BPA, BPS, BPF, and BPAF with new experimental metabolic parameters: Comparing the pharmacokinetic behavior of BPA with its substitutes. Environ Health Perspect [Internet]. 2018 Jul;126(7):077002. Available from: https://ehp.niehs.nih.gov/doi/10.1289/EHP2739

39. Adachi K, Suemizu H, Murayama N, Shimizu M, Yamazaki H. Human biofluid concentrations of mono(2-ethylhexyl)phthalate extrapolated from pharmacokinetics in chimeric mice with humanized liver administered with di(2-ethylhexyl)phthalate and physiologically based pharmacokinetic modeling. Environ Toxicol Pharmacol [Internet]. 2015 May;39(3):1067–73. Available from: http://dx.doi.org/10.1016/j.etap.2015.02.011

40. Sharma RP, Schuhmacher M, Kumar V. The development of a pregnancy PBPK Model for Bisphenol A and its evaluation with the available biomonitoring data. Sci Total Environ [Internet]. 2018 May;624:55–68. Available from: https://doi.org/10.1016/j.scitotenv.2017.12.023

41. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H. Anatomical, Physiological and Metabolic Changes with Gestational Age during Normal Pregnancy. Clin Pharmacokinet. 2012;51(6):365–96.

42. Sisson TR, Lund CJ, Whalen LE, Telek A. The blood volume of infants. I. The full-term infant in the first year of life. J Pediatr. 1959 Aug;55(2):163–79.

43. Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health. 1997;13(4):407–84.

44. Valentin J. Basic anatomical and physiological data for use in radiological protection: reference values. Ann ICRP. 2002 Sep;32(3–4):1–277.

45. Poulin P, Krishnan K. A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. Toxicol Appl Pharmacol. 1996 Jan;136(1):126–30.

46. Pelekis M, Poulin P, Krishnan K. An Approach for Incorporating Tissue Composition Data Into Physiologically Based Pharmacokinetic Models. Toxicol Ind Health. 1995 Sep;11(5):511–22.

47. Schmitt W. General approach for the calculation of tissue to plasma partition coefficients. Toxicol Vitr. 2008 Mar;22(2):457–67.

48. Peyret T, Poulin P, Krishnan K. A unified algorithm for predicting partition coefficients for PBPK modeling of drugs and environmental chemicals. Toxicol Appl Pharmacol. 2010 Dec;249(3):197–207.

49. Yun YE, Cotton CA, Edginton AN. Development of a decision tree to classify the most accurate tissue-specific tissue to plasma partition coefficient algorithm for a given compound. J Pharmacokinet Pharmacodyn. 2014 Feb;41(1):1–14.

50. Peyret T, Krishnan K. QSARs for PBPK modelling of environmental contaminants. SAR QSAR Environ Res [Internet]. 2011 Jan;22(1–2):129–69. Available from: http://www.tandfonline.com/doi/abs/10.1080/1062936X.2010.548351

51. Yang RSH, Thomas RS, Gustafson DL, Campain J, Benjamin SA, Verhaar HJM, et al. Approaches to developing alternative and predictive toxicology based on PBPK/PD and QSAR modeling. Environ Health Perspect. 1998;106(SUPPL. 6):1385–93.

52. Peyret T, Krishnan K. QSARs for PBPK modelling of environmental contaminants. SAR QSAR Environ Res. 2011;22(1–2):129–69.

53. Miller NA, Reddy MB, Heikkinen AT, Lukacova V, Parrott N. Physiologically Based Pharmacokinetic Modelling for First-In-Human Predictions: An Updated Model Building Strategy Illustrated with Challenging Industry Case Studies. Clin Pharmacokinet [Internet]. 2019 Jun 7;58(6):727–46. Available from: https://doi.org/10.1007/s40262-019-00741-9

54. Price K, Krishnan K. An integrated QSAR-PBPK modelling approach for predicting the inhalation toxicokinetics of mixtures of volatile organic chemicals in the rat. SAR QSAR Environ Res [Internet]. 2011 Jan;22(1–2):107–28. Available from: http://www.tandfonline.com/doi/abs/10.1080/1062936X.2010.548350

55. Jean J, Kar S, Leszczynski J. QSAR modeling of adipose/blood partition coefficients of Alcohols, PCBs, PBDEs, PCDDs and PAHs: A data gap filling approach. Environ Int. 2018 Dec;121(October):1193–203.

56. Sharma RP, Schuhmacher M, Kumar V. Development of a human physiologically based pharmacokinetic (PBPK) model for phthalate (DEHP) and its metabolites: A bottom up modeling approach. Toxicol Lett [Internet]. 2018 Oct;296(June):152–62. Available from: https://doi.org/10.1016/j.toxlet.2018.06.1217

57. Mager DE, Wyska E, Jusko WJ. Diversity of mechanism-based pharmacodynamic models. Drug Metab Dispos [Internet]. 2003 May 1;31(5):510–8. Available from: http://dmd.aspetjournals.org/lookup/doi/10.1124/dmd.31.5.510

58. Felmlee MA, Morris ME, Mager DE. Mechanism-Based Pharmacodynamic Modeling. In: Cam Design and Manufacturing Handbook [Internet]. 2012. p. 583–600. Available from: http://link.springer.com/10.1007/978-1-62703-050-2_21

59. Andersen ME, Clewell HJ, Gearhart J, Allen BC, Barton HA. PHARMACODYNAMIC MODEL OF THE RAT ESTRUS CYCLE IN RELATION TO ENDOCRINE DISRUPTORS. J Toxicol Environ Health [Internet]. 1997 Oct;52(3):189–209. Available from: http://www.tandfonline.com/doi/abs/10.1080/00984109708984060

60. Groenendaal D, Freijer J, De Mik D, Bouw MR, Danhof M, De Lange ECM. Influence of biophase distribution and P-glycoprotein interaction on pharmacokinetic-pharmacodynamic modelling of the effects of morphine on the EEG. Br J Pharmacol [Internet]. 2007 Jul;151(5):713–20. Available from: http://doi.wiley.com/10.1038/sj.bjp.0707258

61. Yassen A, Kan J, Olofsen E, Suidgeest E, Dahan A, Danhof M. Pharmacokinetic-pharmacodynamic modeling of the respiratory depressant effect of norbuprenorphine in rats. J Pharmacol Exp Ther [Internet]. 2007 May 1;321(2):598–607. Available from: http://jpet.aspetjournals.org/content/321/2/598.abstract

62. Dayneka NL, Garg V, Jusko WJ. Comparison of four basic models of indirect pharmacodynamic responses. J Pharmacokinet Biopharm [Internet]. 1993 Aug;21(4):457–78. Available from: https://www.ncbi.nlm.nih.gov/pubmed/8133465

63. Sun YN, Jusko WJ. Transit compartments versus gamma distribution function to model signal transduction processes in pharmacodynamics. J Pharm Sci [Internet]. 1998 Jun;87(6):732–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022354915505832

64. Mager DE, Jusko WJ. Pharmacodynamic modeling of time-dependent transduction systems. Clin Pharmacol Ther. 2001;70(3):210–6.

65. Hong Y, Mager DE, Blum RA, Jusko WJ. Population pharmacokinetic/pharmacodynamic modeling of systemic corticosteroid inhibition of whole blood lymphocytes: Modeling interoccasion pharmacodynamic variability. Pharm Res [Internet]. 2007 May 18;24(6):1088–97. Available from: http://link.springer.com/10.1007/s11095-006-9232-x

66. Derendorf H, Meibohm B. Modeling of Pharmacokinetic/Pharmacodynamic (PK/PD) Relationships: Concepts and Perspectives. Pharm Res [Internet]. 1999 Feb;16(2):176–85. Available from: https://doi.org/10.1023/A:1011907920641

67. Danhof M, de Lange ECM, Della Pasqua OE, Ploeger BA, Voskuyl RA. Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling in translational drug research. Trends Pharmacol Sci [Internet]. 2008 Apr;29(4):186–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165614708000497

68. Hu Y, Wang L, Xiang L, Wu J, Huang W, Xu C, et al. Pharmacokinetic-Pharmacodynamic Modeling for Coptisine Challenge of Inflammation in LPS-Stimulated Rats. Sci Rep [Internet]. 2019 Dec 5;9(1):1450. Available from: http://www.nature.com/articles/s41598-018-38164-4

69. Grimm HP, Schick E, Hainzl D, Justies N, Yu L, Klein C, et al. PKPD Assessment of the Anti-CD20 Antibody Obinutuzumab in Cynomolgus Monkey is Feasible Despite Marked Anti-drug Antibody Response in This Species. J Pharm Sci [Internet]. 2019 Nov 1;108(11):3729–36. Available from: https://doi.org/10.1016/j.xphs.2019.07.013

70. Hazra A, Pyszczynski N, DuBois DC, Almon RR, Jusko WJ. Modeling receptor/gene-mediated effects of corticosteroids on hepatic tyrosine aminotransferase dynamics in rats: Dual regulation by endogenous and exogenous corticosteroids. J Pharmacokinet Pharmacodyn [Internet]. 2007 Sep 21;34(5):643–67. Available from: https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/kfq152

71. Sharma RP, Schuhmacher M, Kumar V. Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. Environ Int. 2017 Feb;99:1–14.

72. Lohitnavy M, Lu Y, Lohitnavy O, Chubb LS, Hirono S, Yang RSH. A possible role of multidrug resistance - Associated protein 2 (Mrp2) in hepatic excretion of PCB126, an environmental contaminant: PBPK/PD modeling. Toxicol Sci. 2008 Jul;104(1):27–39.

73. Rouquié D, Heneweer M, Botham J, Ketelslegers H, Markell L, Pfister T, et al. Contribution of new technologies to characterization and prediction of adverse effects. Crit Rev Toxicol. 2015 Feb;45(2):172–83.

74. Punt A, Aartse A, Bovee TFH, Gerssen A, van Leeuwen SPJ, Hoogenboom RLAP, et al. Quantitative in vitro-to-in vivo extrapolation (QIVIVE) of estrogenic and anti-androgenic potencies of BPA and BADGE analogues. Arch Toxicol [Internet]. 2019 Jul 20;93(7):1941–53. Available from: https://doi.org/10.1007/s00204-019-02479-6

75. Fragki S, Piersma AH, Rorije E, Zeilmaker MJ. In vitro to in vivo extrapolation of effective dosimetry in developmental toxicity testing: Application of a generic PBK modelling approach. Toxicol Appl Pharmacol [Internet]. 2017 Oct;332:109–20. Available from: http://dx.doi.org/10.1016/j.taap.2017.07.021

76. Wetmore BA. Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. Toxicology [Internet]. 2015 Jun;332:94–101. Available from: http://dx.doi.org/10.1016/j.tox.2014.05.012

77. Janes KA, Chandran PL, Ford RM, Lazzara MJ, Papin JA, Peirce SM, et al. An engineering design approach to systems biology. Integr Biol (United Kingdom) [Internet]. 2017;9(7):574–83. Available from: http://dx.doi.org/10.1039/C7IB00014F

78. Arrell DK, Terzic a. Network systems biology for drug discovery. Clin Pharmacol Ther. 2010;88(1):120–5.

79. Nielsen J. Systems Biology of Metabolism. Annu Rev Biochem [Internet]. 2017 Jun 20;86(1):245–75. Available from: http://www.annualreviews.org/doi/10.1146/annurev-biochem-061516-044757

80. Costa PM, Fadeel B. Emerging systems biology approaches in nanotoxicology: Towards a mechanism-based understanding of nanomaterial hazard and risk. Toxicol Appl Pharmacol [Internet]. 2016;299:101–11. Available from: http://dx.doi.org/10.1016/j.taap.2015.12.014

81. Somvanshi PR, Venkatesh K V. A conceptual review on systems biology in health and diseases: From biological networks to modern therapeutics. Syst Synth Biol [Internet]. 2014 Mar 18;8(1):99–116. Available from: http://link.springer.com/10.1007/s11693-013-9125-3

82. Geenen S, Taylor PN, Snoep JL, Wilson ID, Kenna JG, Westerhoff H V. Systems biology tools for toxicology. Arch Toxicol [Internet]. 2012 Aug 9;86(8):1251–71. Available from: http://link.springer.com/10.1007/s00204-012-0857-8

83. Westerhoff H V., Kolodkin A, Conradie R, Wilkinson SJ, Bruggeman FJ, Krab K, et al. Systems biology towards life in silico: Mathematics of the control of living cells. J Math Biol [Internet]. 2009 Jan 16;58(1–2):7–34. Available from: http://link.springer.com/10.1007/s00285-008-0160-8

84. Saavedra E, Moreno-Sánchez R. Summation Theorem. In: Dubitzky W, Wolkenhauer O, Cho K-H, Yokota H, editors. Encyclopedia of Systems Biology [Internet]. New York, NY: Springer New York; 2013. p. 2028. Available from: https://doi.org/10.1007/978-1-4419-9863-7_1357

85. Hornberg JJ, Bruggeman FJ, Bakker BM, Westerhoff H V. Metabolic control analysis to identify optimal drug targets. In: Progress in Drug Research [Internet]. Basel: Birkhäuser Basel; 2007. p. 171–89. Available from: http://link.springer.com/10.1007/978-3-7643-7567-6_7

86. Kuile BH, Westerhoff H V. Transcriptome meets metabolome: Hierarchical and metabolic regulation of the glycolytic pathway. FEBS Lett [Internet]. 2001 Jul 6;500(3):169–71. Available from: http://doi.wiley.com/10.1016/S0014-5793%2801%2902613-8

87. Kuile BH, Westerhoff H V. Transcriptome meets metabolome: Hierarchical and metabolic regulation of the glycolytic pathway. FEBS Lett. 2001 Jul;500(3):169–71.

88. Rocha I, Maia P, Evangelista P, Vilaça P, Soares S, Pinto JP, et al. OptFlux: an open-source software platform for in silico metabolic engineering. BMC Syst Biol [Internet]. 2010 Dec 19;4(1):45. Available from: https://doi.org/10.1186/1752-0509-4-45

89. Trinh CT, Wlaschin A, Srienc F. Elementary mode analysis: A useful metabolic pathway analysis tool for characterizing cellular metabolism. Appl Microbiol Biotechnol [Internet]. 2009 Jan 15;81(5):813–26. Available from: http://link.springer.com/10.1007/s00253-008-1770-1

90. Hood L, Heath JR, Phelps ME, Lin B. Systems biology and new technologies enable predictive and preventative medicine. Science (80-) [Internet]. 2004 Oct 22;306(5696):640–3. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1104635

91. Detailed model of ROS management [Internet]. Available from: https://fairdomhub.org/models/571?version=1

92. Cheong R, Hoffmann A, Levchenko A. Understanding NF-κB signaling via mathematical modeling. Mol Syst Biol [Internet]. 2008 Jan 6;4(1):192. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1038/msb.2008.30

93. Wang Z, Potoyan DA, Wolynes PG. Modeling the therapeutic efficacy of NFκB synthetic decoy oligodeoxynucleotides (ODNs). BMC Syst Biol [Internet]. 2018 Dec 30;12(1):4. Available from: https://bmcsystbiol.biomedcentral.com/articles/10.1186/s12918-018-0525-6

94. Ruiz P, Sack A, Wampole M, Bobst S, Vracko M. Integration of in silico methods and computational systems biology to explore endocrine-disrupting chemical binding with nuclear hormone receptors. Chemosphere. 2017 Jul;178:99–109.

95. Quignot N, Bois FY. A Computational Model to Predict Rat Ovarian Steroid Secretion from In Vitro Experiments with Endocrine Disruptors. Porollo A, editor. PLoS One. 2013 Jan;8(1):e53891.

96. Alves R, Antunes F, Salvador A. Tools for kinetic modeling of biochemical networks. Nat Biotechnol [Internet]. 2006 Jun;24(6):667–72. Available from: http://www.nature.com/articles/nbt0606-667

97. Kitano H, Kitano H. Systems Biology : A Brief Overview. Science (80-). 2017;295(5560):1662–4.

98. Kohl P, Noble D. Systems biology and the virtual physiological human. Mol Syst Biol [Internet]. 2009 Jan 28;5(1):292. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1038/msb.2009.51

99. Noble D. Computational models of the heart and their use in assessing the actions of drugs. J Pharmacol Sci [Internet]. 2008;107(2):107–17. Available from: http://joi.jlc.jst.go.jp/JST.JSTAGE/jphs/CR0070042?from=CrossRef

100. Costa PM, Fadeel B. Emerging systems biology approaches in nanotoxicology: Towards a mechanism-based understanding of nanomaterial hazard and risk. Toxicol Appl Pharmacol. 2016;299:101–11.

101. OECD. "Users'' Handbook supplement to the Guidance Document for developing and assessing Adverse Outcome Pathways", OECD Series on Adverse Outcome Pathways, No. 1, OECD Publishing, Paris." 2018;(1).

102. OECD. "Users'' Handbook supplement to the Guidance Document for developing and assessing Adverse Outcome Pathways", OECD Series on Adverse Outcome Pathways, No. 1, OECD Publishing, Paris." 2016 May;(5):18.

103. OECD. Adverse Outcome Pathways, Molecular Screening and Toxicogenomics [Internet]. 2013. Available from: http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

104. OECD. Users' Handbook supplement to the Guidance Document for developing and assessing Adverse Outcome Pathways. Env/Jm/Mono(2016) 12. 2016;(OECD Series on Adverse Outcome Pathways No. 1):63.

105. Aopwiki [Internet]. Available from: https://aopwiki.org/info_pages/2/info_linked_pages/1#J Overall Assessment of the AOP

106. OECD. AOP knowledge base [Internet]. AOP knowledge base. 2017. Available from: http://aopkb.org

107. Willett C. Adverse Outcome Pathways [Internet]. Encyclopedia of Toxicology. 2014. p. 95–9. Available from: https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/comptox/ct-aop/aop.html

108. AOP Learning [Internet]. Available from: https://learning.aopkb.org/

109. AOPXplorer_ Visualize Your Bio Data [Internet]. Available from: http://datasciburgoon.github.io/aopxplorer

110. Solecki R, Kortenkamp A, Bergman Å, Chahoud I, Degen GH, Dietrich D, et al. Detailed Review Paper on the State of the Science on Novel In Vitro and In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors [Internet]. Archives of Toxicology. OECD; 2014. 1001–1006 p. (OECD Series on Testing and Assessment; vol. 91). Available from: http://link.springer.com/10.1007/s00204-016-1866-9

111. Benigni R, Battistelli CL, Bossa C, Giuliani A, Tcheremenskaia O. Endocrine Disruptors: Data-based survey of in vivo tests, predictive models and the Adverse Outcome Pathway. Regul Toxicol Pharmacol [Internet]. 2017 Jun;86:18–24. Available from: http://dx.doi.org/10.1016/j.yrtph.2017.02.013

112. OECD. OECD Work Related to Endocrine Disrupters - OECD [Internet]. 2019. Available from: https://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm

113. Benigni R, Battistelli CL, Bossa C, Giuliani A, Tcheremenskaia O. Endocrine Disruptors: Data-based survey of in vivo tests, predictive models and the Adverse Outcome Pathway. Regul Toxicol Pharmacol. 2017 Jun;86:18–24.

114. Browne P, Noyes PD, Casey WM, Dix DJ. Application of adverse outcome pathways to U.S. EPA's endocrine disruptor screening program. Environ Health Perspect [Internet]. 2017 Sep 22;125(9):096001. Available from: https://ehp.niehs.nih.gov/doi/10.1289/EHP1304

115. OECD. OECD work on Endocrine Disrupting Chemicals. 2018;1–26. Available from: http://www.oecd.org/chemicalsafety/testing/OECD%0AWork%0Aon%0AEndocrine%0ADisrupting%0AChemicals.pdf

116. Phillips JA, Bogdanffy MS. Editorial overview: Translational biomarker concepts and practices for toxicological assessment. Curr Opin Toxicol [Internet]. 2017 Jun;4:i–iii. Available from: http://dx.doi.org/10.1016/j.cotox.2017.08.003

117. Eissing T, Kuepfer L, Becker C, Block M, Coboeken K, Gaub T, et al. A computational systems biology software platform for multiscale modeling and simulation: Integrating whole-body physiology, disease biology, and molecular reaction networks. Front Physiol [Internet]. 2011;FEB(February):1–10. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2011.00004/abstract

118. Knaak JB, Dary CC, Zhang X, Gerlach RW, Tornero-Velez R, Chang DT, et al. Parameters for Pyrethroid Insecticide QSAR and PBPK/PD Models for Human Risk Assessment. In: Whitacre DM, editor. Reviews of Environmental Contamination and Toxicology [Internet]. New York, NY: Springer New York; 2012. p. 1–114. Available from: https://doi.org/10.1007/978-1-4614-3281-4_1

119. Knaak JB, Dary CC, Power F, Thompson CB, Blancato JN. Physicochemical and biological data for the development of predictive organophosphorus pesticide QSARs and PBPK/PD models for human risk assessment. Crit Rev Toxicol [Internet]. 2004 Jan 19;34(2):143–207. Available from: https://www.tandfonline.com/doi/full/10.1080/10408440490432250

120. Snowden TJ, van der Graaf PH, Tindall MJ. Model reduction in mathematical pharmacology: Integration, reduction and linking of PBPK and systems biology models. J Pharmacokinet Pharmacodyn [Internet]. 2018 Aug 26;45(4):537–55. Available from: https://doi.org/10.1007/s10928-018-9584-y

121. Andersen ME, Krewski D. Toxicity testing in the 21st century: Bringing the vision to life. Toxicol Sci [Internet]. 2009 Feb;107(2):324–30. Available from: https://academic.oup.com/toxsci/article/1683303/Toxicity

122. Bloomingdale P, Housand C, Apgar JF, Millard BL, Mager DE, Burke JM, et al. Quantitative systems toxicology. Curr Opin Toxicol [Internet]. 2017 Jun;4:79–87. Available from: https://doi.org/10.1016/j.cotox.2017.07.003

123. August GJ, Gewirtz A. Moving Toward a Precision-Based, Personalized Framework for Prevention Science: Introduction to the Special Issue. Prev Sci [Internet]. 2019 Jan 25;20(1):1–9. Available from: http://link.springer.com/10.1007/s11121-018-0955-9