

Development of Tools to Support the Application of Physiologically-Based Kinetic Modelling in Safety Assessment

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Abstract

In safety assessment, one alternative to animal testing, that is increasingly being recognised as a suitable method for obtaining data, is read-across. This is the process by which information from one (source) chemical is used to infer information for another similar (target) chemical. However, determining similarity between chemicals is difficult as they cannot be considered as absolutely similar, only similar with respect to a given property. The research undertaken involved the development of two resources to address this problem. Within this thesis a resource is presented, wherein available physiologically-based kinetic (PBK) models have been collated and used in the development of a second resource, a KNIME workflow that can help to identify similar chemicals that have an existing PBK model. This enables PBK model information from source chemical(s) to be used in a read-across approach to help develop new PBK models for target chemicals. Initially, a systematic review of available PBK models in literature was undertaken; extraction of relevant information from the papers identified was captured in a Microsoft Excel spreadsheet which consisted of over 7,500 PBK models. The chemical space coverage of the chemicals with PBK models was compared to that of six datasets for different types of chemicals (drugs, cosmetics, botanicals, industrial chemicals, pesticides and food additives) in terms of their key physicochemical properties (molecular weight, hydrogen bond acceptors/donors, Slog P, TPSA, and Lipinski rule violations). The PBK model dataset that had been created in Excel was used as a resource in the development of a KNIME workflow that helps to identify similar chemicals to a target chemical. This KNIME workflow was applied to six chemicals, one from each of the different datasets, to assess its applicability across various industries. Two complete case studies were then undertaken (using the drug atenolol and the pesticide flumioxazin) in which new PBK models were built for these two target chemicals, using data obtained from source chemicals that had been identified by the workflow as being similar. PBK models for the source chemicals were initially reproduced, before being adapted and used as templates for the target chemicals. The performance of the new PBK models was assessed by comparing the output of these to existing data. The results demonstrated that a read-across approach could be successfully applied for developing new PBK models for data-poor chemicals. Information acquired from these PBK models can be used to support safety assessment of chemicals, reducing reliance on animal testing.

Abbreviations

3Rs	Reduce, refine and replace animals
ad	Adipose
ADME	Absorption, distribution, metabolism or excretion
A_i	Amount
art	Arterial
AUC	Area under the curve
BBB	Blood brain barrier
bl	Blood
bo	Bone
br	Brain
CAS	Chemical Abstracts Service
C_i	Concentration
CL^* ; CL_{int}^* ; CL_{renal}^*	Clearance; Intrinsic clearance; renal clearance
CLP	Classification, labelling and packaging
C_{max}	Maximum concentration
co	Cardiac output
ECHA	European Chemicals Agency
EPAA	European Partnership for Alternatives to Animal Testing
EU	European Union
f_a	Fraction absorbed from intestinal tract
f_B	Bound fraction in blood
$F_{RS,oral}$; F	Oral bioavailability
$f_{u,p}^*$; f_{up}^* ; F_{ub}	Unbound fraction in plasma
gu	Gut
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
hep	Hepatic
HESI	Health and Environment Sciences Institute
ht	Heart
InChIKey	International Chemical Identifier Keys

IVIVE	<i>In vitro</i> -to- <i>in vivo</i> extrapolation
JRC	Joint Research Centre
k_a	Oral absorption rate constant
k_i	Kidney
K_{mI}	Michaelis constant
$K_{p,i}$	Tissue partition coefficient
KWAAS	KNIME workflow for assisting analogue selection
li	Liver
Log D	Logarithm of the distribution coefficient
log P	Logarithm of the octanol:water partition coefficient
lu	Lung
mu	Muscle
MW	Molecular weight
NAMs	New approach methodologies
NG	Next generation
ODEs	Ordinary differential equation
OECD	Organisation for Economic Cooperation and Development
PBBK	Physiologically-based biokinetic
PBK	Physiologically-based kinetic
PBPD	Physiologically-based pharmacodynamic
PBPK	Physiologically-based pharmacokinetic
PBPM	Physiologically-based biopharmaceutical model
PBTK	Physiologically-based toxicokinetic
PK	Pharmacokinetic
pKa; pKb	Acid dissociation constant; Base dissociation constant
PMD	Physiologically-based kinetic model dataset
pp	Slowly perfused tissues
PPB	Plasma protein binding
Q_i	Blood flow
(Q)SAR	(Quantitative) structure-activity relationship
R_{bp}^* ; R^* ; R_b^*	Blood-plasma concentration ratio

REACH	Registration, Evaluation Authorisation and restriction of Chemicals
rp	Rapidly perfused tissues
SCCS	Scientific Committee on Consumer Safety
sk	Skin
Slog P	Predicted logarithm of the octanol:water partition coefficient
SMILES	Simplified Input Line Entry String
sp	Spleen
T	Total
T_{\max}	Time taken to reach the maximum concentration
TPSA	Topological polar surface area
ur	Urine
US EPA	United States Environmental Protection Agency
ve	Venous
V_d	Volume of distribution
V_i	Volume
V_{\max}	Maximum velocity of metabolism

**The different terms here are those stated in the models that are quoted in the methods*

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Chapter 1 – Introduction

1.1 Safety assessment

Cosmetics, pharmaceuticals, food additives, and pesticides are just some of the things humans and animals are exposed to everyday that contain a variety of chemicals. The safety assessment of chemicals is a legal requirement and essential to ensure safe use for workers and consumers, as well as protection of domestic/farm animals and environmental species (Thompson et al., 2021). Safety assessment requires knowledge of exposure (external and internal) as well as intrinsic activity (toxicity). In terms of legislation, a particular requirement for plant protection agents and their product safety assessment is kinetic data and, whilst not formally required, incorporation of such data is widely recommended in other regulations, for example for classification, labelling and packaging (CLP) and the Registration, Evaluation Authorisation and restriction of Chemicals (REACH) (Pistollato et al., 2021). While for other sectors (e.g. cosmetics) the European Chemicals Agency (ECHA) and the European Union (EU) Commission have recommended the use of kinetic data for cosmetics and chemical safety (European Commission, 2008; ECHA, 2017; Scientific Committee on Consumer Safety, 2018).

Traditionally, animals have been used for safety assessment of chemicals. However, problems can arise when using animals, for example there are ethical implications as well as questionable human relevance. Cruelty free international (2022) reported that 192.1 million animals were used worldwide for scientific purposes in 2015. Traditional approaches to risk assessment are no longer considered sufficient to predict the potential risk associated with a given substance (European Commission, 2022). In 2016, animals were replaced by simulators and human-relevant models in US and Canadian medical schools after lobbying by the Physicians Committee for Responsible Medicine (2022). The OECD (2021a) have approved a new strategy which is shown to perform better than existing animal tests defining approaches on skin sensitisation involving the evaluation of results based on three different non-animal methods (OECD, 2021a; Macmillan and Chilton, 2019).

Product development and safety assessment regulation for cosmetics highlights the significance of alternative methods. The ban on the use of animals for testing finished cosmetic products has been in force since 2004, with the ban on ingredients testing effective

from 2009. Furthermore, the use of cosmetic ingredients or products that were tested on animals since the introduction of the ban have also been banned from being marketed in the EU since 2013 by the Cosmetics Regulation (Regulation (EC) No 1223/2009). The Directive 2010/63/EU sets out the aims for protecting animals in scientific research, where marketing authorisation holders are required to integrate the 3Rs and welfare standards for the treatment of animals in all aspects of development, manufacture and testing of animals.

US law also states that the reduction and replacement of animals should be undertaken as per the Frank R Lautenberg Chemical Safety for the 21st Century Act of Congress (Lautenberg, 2016). Although one of the most common approaches used as an alternative method is read-across (using information from a data-rich chemical to inform the prediction of toxicity to a similar data-poor chemical, see section 1.5 for more detail), quantitative structure-activity relationships (QSARs) are used as well. Thus, the extensive economic and ethical contribution of using *in silico* methods is being substantiated across industries (Madden et al., 2020).

1.1.1 Pharmacokinetic importance in safety assessment

Pharmacokinetic studies provide information on bioavailability, half-life, absorption, free drug concentration in plasma etc. The increase in development of tools for predicting pharmacokinetic profiles has resulted in a reduction in late-stage termination of drug candidates, as potential pharmacokinetic issues are now identified much earlier in the drug discovery process. This includes early detection of drug safety issues, such as potential drug-drug interactions, or non-linear kinetics (Walker, 2004). Guidance documents from ECHA (2017) and the Scientific Committee on Consumer Safety (2018) recommend making use of all available data (including kinetic data) to support decision-making in safety assessment. Incorporation of pharmacokinetic data in read-across predictions in safety assessment is also being increasingly used. Following a forum, organised by the European Partnership for Alternatives to Animal Testing (EPAA), Laroche et al. (2018) produced a report on finding synergies for reducing, refining and replacing (3Rs) testing on animals, detailing the need to use toxicokinetic data in read-across, see Section 1.5, in multiple industries (i.e. the pharmaceutical, cosmetics, food and agrochemical industries) and their associated regulatory organisations to produce a greater impact. Further, within the agrochemical industry, the use of pharmacokinetic information is a requirement for obtaining permission for pesticide use,

as a result of the revision of Directive 91/414/EC, to improve the risk assessment of non-pharmaceuticals (Loizou and Hogg, 2011).

1.1.2 *In-silico* methods for safety assessment

There is clear evidence that *in silico* methods (including QSAR, read-across, physiologically-based kinetic (PBK) modelling, chemical categories, and grouping) are encouraged to be used across many sectors to reduce and replace animal testing wherever possible. This requires the development of appropriate methodologies to provide the required information and fill gaps in knowledge; such methods have been in development for decades. Blaauboer et al. (2016) highlights the possibilities of applying *in silico* methods to the safety assessment of food, food ingredients, and mixtures. The authors proposed a stepwise roadmap highlighting the need to address gaps in current knowledge for safety evaluation including the applicability of data from alternative methods. Cronin et al. (2022) review different *in silico* approaches, for example PBK models, used for assessing exposure and hazard of cosmetic ingredients as well as approaches applied to modelling to predict hazard, for example read-across, and QSARs. Furthermore, the authors identify the need for acceptance of non-animal safety assessment for cosmetics and ingredients by manufacturers, consumers and regulatory authorities as essential for their use. However, a coordinated approach is needed to make greater impact, particularly on the regulatory community (Sewell et al. 2021).

In the EU, there have been multiple reports with subsequent implementation of a range of regulations to encourage the use of alternative methods. In June 2007, processes developed for the purpose of protecting humans and the environment from the adverse effects of chemicals came into effect through an EU regulation (2006) referred to as REACH. The REACH regulation promotes the use of *in silico* predictions (such as read-across approaches and QSAR) as alternatives to animal testing. The regulation requires that where results are derived from a QSAR model, scientific validity must be established and the model must be considered adequate for purpose, for example for classification or labelling. In addition, reliable documentation of the applied method must be provided, and the chemical must fall within the applicability domain of the *in silico* model. ECHA endorses the principle that dossiers being submitted for REACH should use results from alternative methods in place of new animal tests wherever possible. Further, ECHA's 4th report (2020) into the use of alternatives to testing on

animals for the REACH regulation highlighted that read-across remains the most commonly used alternative method.

1.2 New approach methodologies (NAMs)

Assessment of potential toxicity is needed to ensure products developed are safe for animals and humans. Predictive toxicology helps to avoid harm to animals that chemicals may elicit when used in testing to determine the effects of a chemical on the body. REACH regulation states animal testing should be used as a last resort. Therefore, new approach methodologies (NAMs) are needed to be developed to predict toxicity and understand the mechanisms by which this may occur. The application of *in silico* tools has expanded across multiple industries and regulatory sectors. Initially, these models and tools were developed for predicting chemical toxicity to environmental species (e.g. fish, aquatic invertebrates, algae and bees) (Balls et al., 2018). There is increasing recognition of the role of *in silico* tools in providing information for regulatory submissions to meet legislative requirements across multiple industries (e.g. cosmetics, pesticides and food) as well as the use of *in silico* methods in product development (Madden et al., 2020). In terms of drug development there is a history of using *in silico* models to maximise drug efficiency as well as reduce adverse effects and understand the underlying mechanisms. Only those drug candidates that are most likely to be successful are progressed through to the animal testing stages. *In silico* models can also be used to reduce the number of drugs subject to post-marketing withdrawal and for early screening of toxicity which is superior to *in vitro* and animals (Güneş et al., 2021). Hence, there is a wealth of knowledge of *in silico* model building that can be leveraged from such studies and applied to new areas.

NAMs provide data for safety assessment; they can be defined as any non-animal based approach that, used in combination or alone, can provide information for chemical safety assessment. Paini et al. (2019) highlighted the possibilities of applying NAMs and next generation (NG) safety assessment methods, to the development and use of PBK models, to address the lack of (kinetic) data for the majority of chemicals. Therefore, it is crucial to have alternative methods for providing these data. A European Commission report (2019) indicated that in 2017, 9.4 million animals were used for experiments, with an additional 14

million animals used for tissue and organ samples, breeding, and maintenance in Europe, despite the significant costs associated with testing on animals. According to the UK Home Office (2021) there has been a 15% decrease in the number of procedures involving animals in 2020 from the previous year, although the amount of procedures involving fish has increased. Acceptance and uptake of *in silico* tools is supported through the cross-disciplinary sharing of information and methods, allowing faster advancement of methodologies and development of tools. This application of tools, developed in one industry, to solve problems in another, can help with the acceptance of alternative methods being used for regulatory purposes.

1.2.1 Predicting intrinsic activity (toxicity)

There are two key components to predicting whether or not a chemical is likely to elicit a response in an organism: knowledge of the intrinsic activity (toxicity) of the chemical (or its derivatives) and the extent to which the organism is exposed. Note that “exposure” can be further subdivided into external exposure, the total dose received by ingestion, inhalation etc, and the internal exposure i.e. the amount that is taken up into various organs of the body (Thompson et al., 2021). Intrinsic activity (toxicity) may be predicted using a range of (quantitative) structure-activity relationship ((Q)SAR) models. These models rely on the relationship between properties of a chemical and/or its molecular structure and its activity. Chemicals with data available about their activity are used to develop models to predict activity for unknowns. The relationship of molecular features of a chemical and its activity may be defined by an SAR. When dealing with continuous data QSAR analysis is beneficial.

A wide range of predictive models are available, for example, models for toxicity, physicochemical properties, environmental fate and ecotoxicity. Some predictive software integrates QSAR models; one such software is EPISUITE (<http://www.epa.gov/opptintr/exposure/pubs/episuitedi.htm>; accessed July 2022). Other examples of software that predict toxicity from structural information include: VEGAHUB – this predicts a range of toxicity endpoints including skin sensitisation, mutagenicity, carcinogenicity and environmental endpoints (Roncaglioni et al, 2022; <https://www.vegahub.eu/>; accessed July 2022); Derek Nexus also predicts skin sensitisation, mutagenicity carcinogenicity as well as other endpoints such as teratogenicity, irritation etc

(<https://www.lhasalimited.org/products/derek-nexus.htm>; accessed July 2022) and; Toxtree which predicts skin and eye irritation/corrosion, skin sensitisation and others (<http://toxtree.sourceforge.net/>; accessed July 2022).

Using data from one (or more) chemicals to predict characteristics of other chemicals works on the premise that if chemicals are determined to be similar, the expectation is that they will elicit similar effects. However, there are inadequacies with this theory (e.g. presence or absence of other structural features may impact the response or alter activity) (Madden et al., 2020). Therefore, when making predictions, models should only be used when the chemical falls within the applicability domain of the model; any exceptions e.g. anomalous predictions need to be investigated separately as their mechanism of action may be different. Whilst predicting toxicity is clearly an essential component of safety assessment, this is not the focus of the current thesis. This thesis focuses on predicting the second key component referred to above i.e. exposure, specifically internal exposure.

1.2.2 Internal exposure and its prediction

To have complete knowledge of internal exposure at the organ-level, information regarding absorption, distribution, metabolism and excretion (ADME) is needed, in addition to the overall concentration-time profile for the chemical in the organ. Absorption is related to how a substance enters the body. There are many different routes of administration, for example orally through tablets, food or water; dermally through the skin; and intravenous injections. How much of a chemical is absorbed is determined by the dose and the regularity of doses, the rate of absorption (normally denoted as k_a) and the physicochemical properties of the chemical such as the logarithm of the octanol:water partition coefficient ($\log P$) and the ionisation constants (pK_a or pK_b), in addition to physiological parameters, for example pH and absorptive surface area. The lower gastro-intestinal (GI) tract is where most absorption takes place following oral dosing. Human intestinal absorption is an important parameter in modelling, being the percentage of the dose that reaches the portal vein after passing through the intestinal wall (Mostrag-Szlichtyng and Worth, 2010). Absorption through skin layers or across the surface of the alveoli in the lungs are important routes of uptake for dermally applied or inhaled chemicals respectively. Bioavailability (denoted as F) is the proportion of a chemical that reaches the systemic circulation in its unchanged form; it is directly

proportional to the amount of the chemical that is absorbed and inversely proportional to the amount that is metabolised before reaching the systemic circulation.

Distribution relates to where a chemical may go within the body (e.g. its site of action). The volume of distribution (V_d) is a hypothetical volume that relates the concentration in blood (or plasma) to the dose administered. Chemicals with a high V_d have a tendency to move out of the blood and into tissues; chemicals with a low V_d have a tendency to remain more within the blood compartment. Distribution is determined by the relative affinity of a substance for blood versus tissue components (e.g. the ability of a substance to bind with plasma proteins or tissue constituents) and its ability to permeate membranes. There are two sites of distribution of particular concern i.e. whether a chemical can cross the blood brain barrier (BBB) or the placenta (Mostrag-Szlichtyng and Worth, 2010). If a chemical can pass the BBB, neurotoxicity can occur; if it can cross the placenta teratogenic effects may result. Another consideration is the potential for the substance to accumulate in a tissue, for example in adipose. Plasma protein binding is an important factor for distribution as an unbound substance may more easily pass-through membranes, therefore, the substance may be more likely to be metabolised or excreted.

Often considered the most important component of ADME is metabolism (Doogue and Polasek, 2013) as it can significantly affect the toxicity of a chemical. A hazardous chemical may have no effect on the body, for example it may not reach the site of action, or it may be metabolised, whereas a non-hazardous chemical could become hazardous when metabolised by the body, through the formation of metabolites that are be considered to be toxic. Metabolism of a chemical can occur at many sites with most tissues having some metabolic capability, although the liver is the main site for metabolism. There are two types of metabolism, phase I metabolism which is the addition or exposure of a functional group on a molecule, and phase II metabolism which involves conjugation reactions that render the molecule more water soluble so that it can be readily excreted by the kidneys. Enzymes play a role within metabolism, where the enzymes of the CYP450 system account for approximately 75% of drug metabolism (Wang et al., 2015). There is a range of software for predicting metabolism. Some examples include, Meteor Nexus from Lhasa (<https://www.lhasalimited.org/products/meteor-nexus.htm>) which predicts metabolic fate,

Toxtree (<http://toxtree.sourceforge.net>) which is an open source software that applies a decision tree approach to predict toxic hazard, and the OECD Toolbox (<https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm>) which can predict a chemical's potential mechanism of action and identify chemicals with similar structural characteristics and mode of actions. Multiple industries (pharmaceutical, food and agricultural) are prioritising research on metabolism prediction (Wang et al., 2015). There is often an over prediction of potential metabolites by predictive software, however attempts are being made to solve these issues (Paini et al., 2019).

The final stage of the ADME process is excretion. This concerns the chemical or its metabolite(s) leaving the body normally through the kidneys, although clearance of a compound from the body can also occur through other means, such as through expired air, bile, faeces, sweat or breast milk.

Many datasets exist for ADME properties (for example those collated by Przybylak et al. (2018), however the quality of the data are highly variable. There are also multiple software packages available to predict ADME properties as reported by Madden and Thompson (2022), but often there is a lack of consistency between predictions. Patel et al. (2018) identified and assessed over 80 models to predict 31 different ADME endpoints. However, these authors reported significant difficulties in reproducing the published models. Many other methods for predicting ADME properties are available: Mostrag-Szlichtyng and Worth (2010) list available resources for ADME predictions and Wang et al. (2015) discuss the increase in predictive models for ADME properties.

Traditionally in safety assessments, correlating effects with external exposure (i.e. dose) has been employed, however the more relevant measure, the organ-level exposure (i.e. amount reaching the target site), is acknowledged to be linked more accurately to observed toxicity (WHO, 2010). Internal exposure predictions are needed to help determine the true effect a chemical may have on a body and the biological response. Predicting internal exposure with greater accuracy can help to reduce gaps in knowledge and the uncertainties associated with predicting biological effects.

1.3 PBK models

To provide predictions of chemical concentration at the organ-level, physiologically-based kinetic (PBK) models (synonymous with physiologically-based pharmacokinetic, toxicokinetic or biokinetic (PBPK, PBTK or PBBK) models) are developed and used in numerous industries. They include more detailed physiological processes than traditional PK modelling. Applications of PBK models are numerous and extend the original role of PBK modelling; traditionally PBK models were used in developing pharmaceuticals, utilised to predict first dose in man and drug-drug interactions (Perry et al., 2020). The influence of health status on kinetics, for example hepatic impairment, was also modelled using PBK models. Further, PBK models have been accepted for some regulatory purposes. These include for example, drug-drug interactions and extrapolation across different patient populations such as paediatric dosing and adjustments for those with impaired liver function. Many examples, detailing cases in which PBK models were used to impact drug development and regulatory decisions can be found in Shebley et al. (2018). However, there is still reluctance in the uptake of PBK models within public health agencies (Tan et al., 2018), as well as a reluctance to submit PBK models due to differing requirements across various agencies (Paini et al., 2017).

The use of PBK modelling in relation to environmental chemicals has increased significantly in recent years. Applications of PBK models for environmental chemicals include route-to-route extrapolation (e.g. extrapolating from one administration route to another, such as estimating properties for the inhalation route using data obtained from the oral route); determining the dose at target tissue following external exposure; dose extrapolation; inter- and intra-species extrapolation (accounting for species, population or genetic variability through adaptation of physiological and anatomical parameters); *in vitro*-to-*in vivo* extrapolation (IVIVE); ascertaining safe levels based on tissue dosimetry; estimating chemical exposure from biomonitoring or epidemiological data (using reverse dosimetry) and assessing potential for bioaccumulation (Thompson et al., 2021).

PBK models combine physiological and anatomical information (e.g. organ volume and blood flow) with chemical specific information (e.g. solubility, pKa, molecular weight, and log P) to predict the concentration-time profile in different organs or tissues. Physicochemical properties are also important in pharmacokinetics. For example, molecular weight can affect

the absorption of drug where the higher the molecular weight the lower the absorption. Similarly, aqueous solubility and log P also influence both absorption and distribution, influencing dissolution and membrane partitioning. The pKa of a drug also affects its distribution properties, as this influences the extent of ionisation – unionised molecules pass through biological membranes more readily. Protein binding is particularly influential as it is unbound chemicals that traverse membranes and, for example, enable the access to sites of metabolism or excretion. Hence, physicochemical properties affect a range of ADME properties which will directly impact PK predictions. Figure 1.1 outlines a generic schematic of a PBK model, where the body is split into different compartments (e.g. organs) which are connected by blood flow.

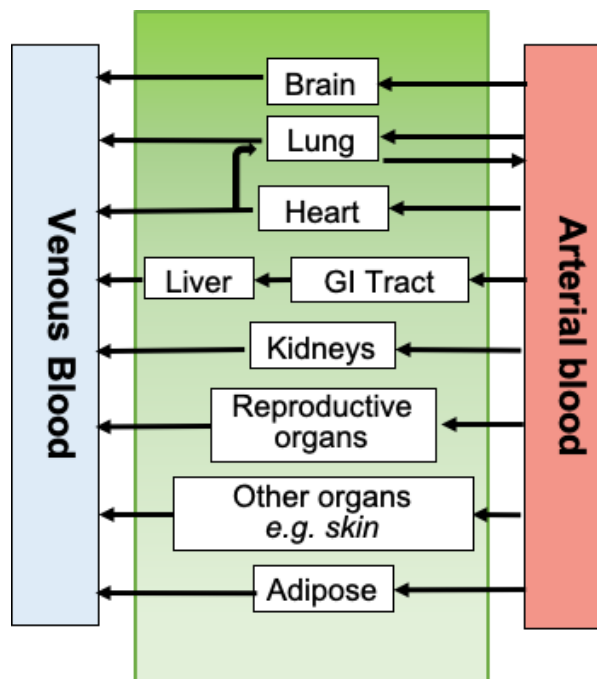


Figure 1.1 Schematic of a generic PBK model

Table 1.1 summarises key inputs required for building PBK models. PBK models can be data and resource intensive, requiring significant time to create because of the large number of parameters needed and difficulties in obtaining all of the data required to build the models.

Table 1.1 Key inputs required for PBK modelling

Inputs required for PBK modelling

Chemical-specific information	Physiological and anatomical information	Exposure Scenario
Tissue-partition coefficient	Organ blood flow	Dose
Blood-plasma concentration ratio	Organ volume	Frequency of dose
Oral absorption rate constant	Tissue composition (e.g. lipid content)	Route of administration
Fraction absorbed	Potential elimination routes potential	
Clearance (total, renal, intrinsic)		
Maximum velocity of metabolism		
Michaelis constant		
Oral bioavailability		
Fraction unbound in plasma		
Molecular weight		
Solubility		

In PBK modelling, the body is split into compartments, that represent either an organ, tissue or group of organs in the body. The level of complexity of a PBK model is dependent on the number of compartments incorporated into the model (Thompson and Beard, 2011). The rate of change in the concentration of the chemical in each compartment is represented using ordinary differential equations (ODEs). A fundamental unit of a PBK model is shown in Figure 1.2. Blood flows into an organ from the arterial blood and out of the organ to the venous blood, where the concentration of the chemical is given as a function of time. The sum of all these results in the overall PBK model. PBK models can assimilate new information as it becomes available to increase predictive capacity; these models provide an advantage over traditional one or two compartment kinetic models (Rowland et al., 2015).

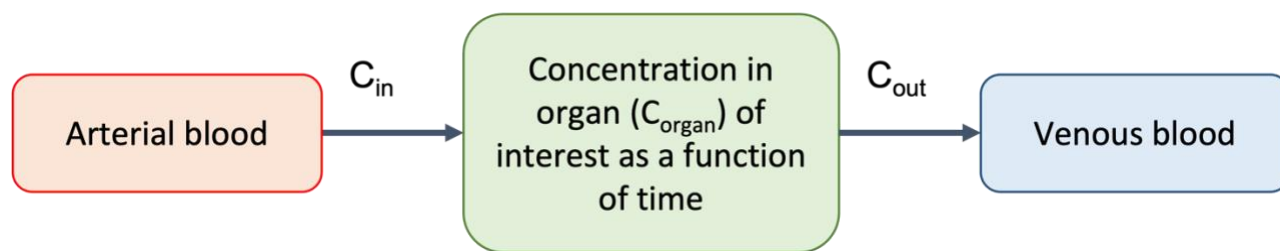


Figure 1.2 Fundamental unit of a PBK model

Key organs may be incorporated into a model as an individual compartment, or further divided into multiple compartments, known as sub-compartments. For example, the lung may be further divided into airway generations (e.g. tracheobronchial and alveolar) or into compartments representing the epithelium, the sub-epithelium and the epithelial lining fluid. Thus, this results in higher level, complex models. Although there are advantages to modelling ADME processes, the level of information required as input for complex models can be a hinderance (Sager et al., 2015). To create simpler models, organs may be grouped together, this is referred to as lumping and common lumping of organs includes all poorly perfused organs considered as one compartment and all highly-perfused organs considered as another (Thompson et al., 2021). Models consisting of very few compartments (for example comprising only blood, liver and gut) are commonly referred to as minimal PBK models.

The uncertainty of pharmacokinetics associated with extrapolation across species, populations, routes, between preclinical and clinical studies can be reduced with PBK modelling (Andersen et al., 1987, Punt et al., 2016, Gentry et al., 2017). It is important to investigate changes in species (or sub-species), life-stage, route of administration and sex separately as there are changes in the inputs required for the models. For example, the size of an organ or blood flow, as well as metabolic and excretion differences need to be accounted for when scaled to the relevant species or when comparing older populations to younger ones. The route of administration of a chemical effects how it is absorbed into the blood and how much of a chemical would be absorbed, e.g. topical dermal application of a drug would absorb differently to a drug that is taken as a tablet orally. An overview of how the key characteristics combine to create a PBK model and accurately describe the time course of a chemical is represented in Figure 1.3.

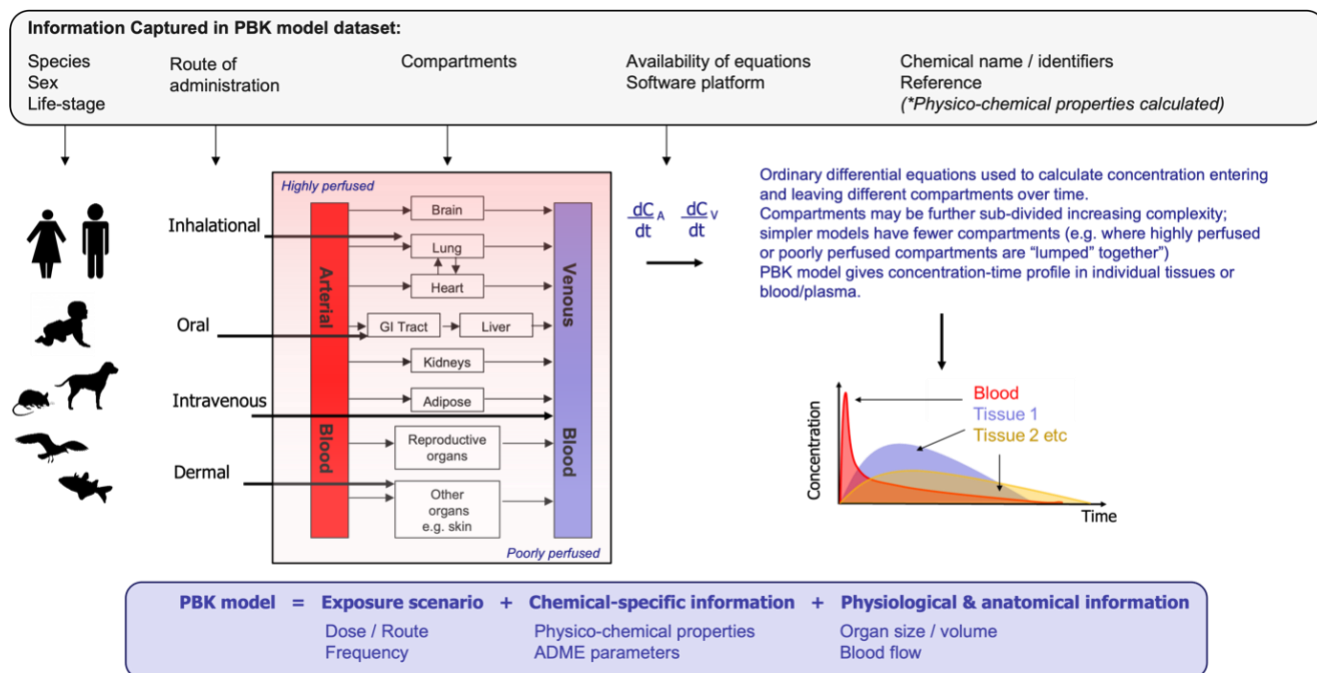


Figure 1.3 Key features that make up a PBK model (from Thompson et al. (2021))

There are multiple mathematical modelling and simulation tools available to assist in constructing and applying PBK models. Generic software for modelling, simulating and fitting data (all last accessed July 2022) includes: NONMEM (<https://www.iconplc.com/innovation/nonmem/>), and GNU MCSIM (<https://www.gnu.org/software/mcsim/mcsim.html>). Phoenix WinNonLin (https://www.certara.com/wp-content/uploads/Resources/Brochures/BR_PhoenixWinNonlin.pdf) is an industry standard software used for non-compartmental analysis and PBK modelling. Whereas MATLAB (<https://www.mathworks.com/products/matlab.html>) and Berkeley Madonna (<https://berkeley-madonna.myshopify.com/>) are software capable of constructing complex models. The open source software, PKSim (<http://www.systems-biology.com/products/PK-Sim.html>), is also a useful resource for PBK modelling that has a database with anatomical and physiological parameters for multiple species (e.g. human, mouse and dog), where the user can create different scenarios using interchangeable building blocks.

1.4 Validation

Information on the construction and validation of PBK models is well reported (USEPA, 2006, WHO, 2010, Kuepfer et al., 2016, Upton et al., 2016, Laroche et al., 2018, Madden et al., 2019). These publications report, the application of PBK models within different industries and the tools that are available to support building PBK models. The WHO (2010) report sets out the main principles for the characterisation and application of PBK models for safety assessment. These principles are built upon in the Organisation for Economic Cooperation and Development's (OECD) guidance document on the characterisation, validation and reporting of models for regulatory purposes, where they focus on the use of alternative approaches (*in silico* and *in vitro*) for parameterising PBK models (OECD, 2021b). PBK models developed for laboratory test species (such as rabbits, rats, dogs and mice), humans, species of ecological relevance (e.g. fish and birds) and farm animals are all applicable under the OECD guidance (Paini et al., 2021a). Furthermore, it is relevant for chemicals in a range of forms, for example biologicals, nanomaterials, metals and macromolecules. Chemicals used in medical devices are not included in the developed guidance.

OECD (2021b) recommends validating PBK models based on five characteristics these include: the biological basis of the model structure and parameters; theoretical basis of the model equations; reliability of input parameters; sensitivity of the model output to input parameters; and goodness-of-fit and predictivity of a given dose metric. Models can be validated by predictivity and fitting a model to data; however, this requires the availability of *in vivo* kinetic data for comparison to the PBK predictions. The model validation can be slowed by increasing complexity of the equations used within PBK models (Loizou and Hogg, 2011). Sensitivity and uncertainty analysis are commonly used to assess PBK models. Sensitivity analysis allows for uncertainty in model output to be attributed to one or more of the model input parameters (Patelli et al., 2010). Therefore, allowing for the confidence in the application of a PBK model to be evaluated. It is important for sensitivity analysis to be undertaken for validation of PBK models as there is lack of trust and understanding in PBK modelling that has led to a reluctance in uptake of regulatory use of these models (Paini et al., 2017, Paini et al., 2019).

Within a sensitive model, parameter uncertainties can have a stronger influence on the associated model predictions. Thus, identification of the relevance of input parameters is essential. Sensitivity analysis is split into three types, local sensitivity analysis, screening methods and global sensitivity analysis. The OECD guidelines recommend that local or global sensitivity analysis be undertaken on a PBK model to validate the model. For practical cases, local sensitivity analysis is commonly undertaken due to the lower computational costs (Saltelli, 2002). Global sensitivity analysis allows parameters that are linked to be assessed by investigating the entire range of variation of the input parameters. Screening methods (for example the Morris factorial sampling method (Morris, 1991)) have low computational cost and are meant for computationally expensive models, involving large numbers of parameters. In this method, input parameters are ranked according to their importance without quantifying how much more important one parameter is than another (Patelli et al., 2010).

For considerable interaction between estimated model parameters a global sensitivity analysis is desirable as all parameters at the same time point are analysed (Sobol, 2001, Saltelli et al., 2007, Patelli et al., 2010). Although, global sensitivity analysis can be computationally costly, such uncertainty estimations are required for modelling and simulation standards. One multidisciplinary software for quantifying uncertainty is COSSAN. COSSAN is a general purpose computational software for non-deterministic analysis (Patelli, 2016). This MATLAB-based software is flexible for the user to adapt to different purposes while being open source. Parameter uncertainty in PBK models can be analysed through construction and implementation of algorithms. Therefore, the validation of PBK models can be undertaken.

1.5 Read-across

There are many alternatives to animal testing to predict the effects of chemicals - a key method being read-across. This is an approach in which information from a chemical, rich in data (source chemical) is used to inform predictions for a chemical poor in data (target chemical), where the chemicals are considered similar (ECHA, 2017). In recent years there has been an increase in accessibility of resources to support read-across and *in silico* modelling, particularly for ADME data. For example, a review of resources to support read-across was

undertaken by Pawar et al. (2019). These authors identified over 900 databases, including 38 containing ADME-relevant data for *in silico* model development. Furthermore, a broad literature search by Sayre et al. (2020) resulted in a database of time-series concentration data being extracted. Read-across is reported as the most commonly used alternative method in safety assessment to reduce or replace animal testing (ECHA, 2020), with kinetic data being key within this approach (Schultz et al., 2015, ECHA, 2017). However, within the regulatory sector there is an increasing want for establishing best practices for conducting and evaluating read-across (Schultz et al., 2015).

There are two ways in which we can consider using PBK data for read across. Firstly, a PBK model for a source chemical can be read across (applied) to a target chemical to obtain PBK model data for the target. For example, liver concentrations for the target chemical are predicted over time, based on a new PBK model that was derived from information from the model for the source chemical. These liver concentration predictions can be confirmed to be in line with expected concentrations. The second way in which PBK-type data can be used, is to add the derived PK information to a read-across prediction for toxicity. For example, a maximum liver concentration is derived from a PBK model, and this is linked to a proposed toxic effect. If a source chemical has a particular functional group associated with liver toxicity and the target chemical has that same functional group (and is predicted to be similar in terms of pharmacokinetics) then the same liver toxicity may be expected. Thus, a read-across approach is supported by adding PBK information.

Read-across can be supported by using PBK models to derive relevant data. Uncertainties to do with ADME characteristics can be reduced using PBK models as they include the biokinetics of a chemical. As PBK models are so time and resource-intensive to generate, both PBK model development and safety assessment would benefit from the ability to use a read-across approach when developing PBK models (Laroche et al., 2018). Recently there have been publications (Lu et al., 2016a, Paini et al., 2021b) demonstrating the use of a read-across approach to obtain PK information from analogue chemicals. The data can be obtained in two ways: either from a PBK model for the target chemical, or from a PBK model for a source chemical that is identified as being similar to the target. In applying read-across to different chemicals or extrapolating to different species or routes of exposures, animal testing can be

reduced. However, to use an existing PBK model as a template for a target chemical, it is important to identify suitable similar chemicals with an existing PBK model. Guidance from the OECD (2021b) to increase the uptake of PBK models in the regulatory sector has been published outlining the use of read-across for PBK modelling.

1.6 Previous literature underpinning the thesis

The number of PBK models, and their applications, being published has increased considerably in the last 30 years (Tan et al., 2018). Previously, using papers from 1977 to 2014 a knowledgebase of available PBK models for 307 chemicals was created by Lu et al. (2016a). They applied a read across approach to build new PBK models, using information from existing PBK models from the knowledgebase. For building the ethylbenzene PBK model, information was drawn from PBK models for six chemicals identified as having similar structures. Information from the PBK model for gefitinib was used to predict information for seven chemicals with varying degrees of similarity to gefitinib. For these two case studies, similarity of chemicals was based on physicochemical properties. However, there is no consensus as to the best method to determine similarity between chemicals (Mellor et al., 2019). Ellison (2018) was one of the first to evaluate a PBK model where PK data were unavailable. This author used two approaches, involving structural and functional analogues, for PBK modelling of pharmaceuticals using models based on *in vivo* data. The approach of Ellison (2018) was extended successfully by Ellison and Wu (2020) where they applied the approach to PBK models built on *in vitro* and *in silico* data only (i.e. in the absence of *in vivo* PK data).

Previous resources collating literature PBK models have been created. One of which is the dataset, created by the US EPA, of available PBK models in the literature which is available on Figshare (Sayre et al., 2019). Information recorded in the dataset included species, gender, life-stage, route of administration, PubMed ID of the PBK models and the compartments modelled. Painsi et al. (2021b) outlined a framework for using a read-across approach to develop and evaluate PBK models to inform safety assessment, using information from data-rich chemicals to provide information for data-poor chemicals using chemicals in the PBK Knowledgebase. They undertook a case study, identifying estragole and safrole as appropriate source chemicals for the target chemical methyleugenol, as determined through

analysis of structural similarity. Thus, successfully demonstrating the principles of using information from existing PBK models to inform the development of new models for similar chemicals that may be applied in safety assessment.

Establishing the nature of the chemicals for which PBK models are available is important as similarity is commonly compared using structure and/or physicochemical properties – these key characteristics can be compared across datasets. As identified by Madden et al. (2020) the recognition of the application of a read-across approach and utilising existing data is an important tool in reducing animal testing. Thus, it is necessary to identify chemicals with available PBK models (and establish the nature of these chemicals) with the intention of assisting the application of this approach.

Therefore, one significant asset for researchers, industry and regulators, to reduce the number of animals used in chemical safety assessment and drug development, would be the availability of a resource, readily updatable and accessible, that contained information on the chemicals with available PBK models and the details of those models (Thompson et al., 2021). Another valuable asset to the 3Rs, would be an automated tool that can help to identify chemicals that can be considered as similar. This would enable predictions to be made for data-poor chemicals, using existing PBK models for data-rich chemicals. This could provide further mechanistic insight for the assessment of data-poor chemicals. A structured method to identify similar chemicals would help increase the uptake of using a read-across approach for PBK modelling. Furthermore, to help authenticate the use of such a tool for identifying similar chemicals, relevant case studies need to be undertaken. If successful, this could encourage the use of a read-across approaches (especially by regulators) to help towards the 3Rs.

1.7 Research aims

This thesis describes the creation of an extensive dataset of PBK models available in literature, and the development of an automated workflow that identifies chemicals from this dataset that are similar to a target chemical of interest. Case studies were undertaken to demonstrate the use of the workflow to identify existing PBK models for source chemicals that could be

used as templates for the development of PBK models for target chemicals; the performance of the developed models was also assessed. The ultimate goal is to develop methods that can reduce the number of animals used in safety assessment.

The use of an existing PBK model, for a data-rich chemical, to serve as a template on which to build a new PBK for a similar, data-poor chemical has been established (Lu et al., 2016a, Paini et al., 2021b). In order for this approach to be more accessible across a wider range of chemical types and more acceptable, particularly to regulators, there are three key steps. Firstly, existing PBK models, that may serve as templates, must be curated into a readily searchable resource. Secondly, an automated tool must be developed that assists in the identification of chemicals that can be considered as suitably similar to allow for read-across. Thirdly, case studies need to be undertaken in order to demonstrate the validity of the approach. These three steps form the basis of the research undertaken for this thesis: a systematic review of available PBK models and an analysis of their chemical space coverage; the development of a KNIME workflow to identify similar chemicals (analogues) for PBK modelling; and case studies demonstrating the application of the KNIME workflow for analogue selection, enabling read-across of PBK model-derived information. The research is described in the subsequent chapters as follows.

The aims for Chapter 2 were to:

(i) Develop a protocol for a systematic review of existing PBK models. This required the use of pilot studies, with iterative development and testing to optimise the protocol.

(ii) Undertake a pilot study to determine the most appropriate systematic review software to use in this study; SyRF and Covidence software were compared.

(iii) Conduct a systematic review of PBK models using the optimised protocol and the software determined as being most appropriate. Relevant information for the PBK models was captured in a flexible spreadsheet format, using both free text and controlled vocabulary to optimise searching. This spreadsheet is subsequently referred to as the PBK model dataset (PMD).

(iv) Ascertain the chemical space coverage (in terms of key physicochemical properties) of chemicals for which PBK models were available. The variation in these key physicochemical properties was compared across six specific types of chemicals.

The aims for Chapter 3 were to:

(i) Investigate a range of similarity assessment metrics (e.g. chemical fingerprints and physicochemical properties) to determine which method(s) resulted in the most appropriate selection of source chemicals (analogues) for the purposes of serving as a template in PBK model development for a target chemical.

(ii) Develop a KNIME workflow for assisting with analogue selection, employing the similarity metrics identified as being most appropriate; this utilises the PBK model dataset (PMD) from Chapter 2 as a source for identifying chemicals with existing PBK models

(iii) Test the KNIME Workflow for Assisting Analogue Selection (KWAAS) using six different types of chemicals (pesticides, botanicals, drugs, cosmetics, food additives and industrial chemicals) to demonstrate its utility across a range of industrial and regulatory sectors.

The aims for Chapter 4 were to:

(i) Demonstrate use of the KWAAS created in Chapter 3 by applying the approach to two case study target chemicals – atenolol and flumioxazin. Initially, source chemicals with PBK models available in the literature were identified, using the KWAAS. These models were reproduced for the source chemicals, and subsequently used as templates to enable new models to be derived for the two target chemicals using a read-across approach

(ii) Assess the performance of the newly-derived PBK models by comparing blood-concentration-time profiles with existing data from the literature. The model assessment was undertaken for each new PBK model created using global sensitivity analysis and comparison of fold error for key parameters obtained from the models.

The overall outcomes of this project and potential developments for the future are discussed in Chapter 5.

Chapter 2 – Systematic review of available PBK models and an analysis of their chemical space coverage

The research described in this chapter has been published in *Alternatives to Laboratory Animals* (Thompson et al, 2021).

DOI: <https://doi.org/10.1177/02611929211060264>

2.1 Introduction

As described in Chapter 1, PBK models (which describe the time-course of chemicals in different compartments of the body), are flexible and adaptable across multiple routes of administration, species and life-stages. As new data are made available, these can be incorporated into PBK models enabling them to better predict concentration-time profiles. Publications relating to, or referencing, PBK modelling has increased significantly in recent years, and the methods are increasingly becoming accepted by regulatory agencies (Sager et al., 2015). However, there can be a lack of confidence in the information provided by the models due to the limited availability of *in vivo* data that are used to build and validate the models (Lu et al., 2016a). One way in which the lack of data can be addressed is to use an existing PBK model, for a chemical that is “data-rich”, as a template to build a new model for a “data-poor” chemical. Making use of existing data in this way would be a significant asset for model development across a wide range of industrial and regulatory sectors. This approach requires knowledge of the existing PBK models and the nature of the chemicals for which such models are available.

The first requirement, therefore, is a curated dataset of as many PBK models as reasonably practicable along with information on their key model characteristics such as species, sex, life-stage, route of administration, compartments, availability of model equations and chemical identifiers. This can be achieved most efficiently by conducting a systematic review. A systematic review aims to bring together and summarise the available evidence on a pre-defined research question, with a rigorous and reproducible methodology (Hoffmann et al., 2017, Pollock and Berge, 2018). In addition, the minimisation of subjectivity is sought in the completion of a systematic review (Siddaway et al., 2019). The results of research are

systematically collated in a systematic review, this helps to reduce duplication of research, as well as ensuring the study is comprehensive and avoids bias (Siddaway et al., 2019).

Ideally systematic reviews should be updated regularly to incorporate new studies and ensure that conclusions remain valid (Pollock and Berge, 2018). Protocols, describing how the review will be conducted are a necessary part of the systematic review process. In order to increase transparency, of the process, to ensure that the method can be independently reproduced, and to help ensure selection bias is avoided the protocol should be published before beginning the review. Furthermore, this avoids research duplication by ensuring other researchers are aware that the review is being undertaken (Pollock and Berge, 2018, Siddaway et al., 2019). A detailed and transparent published protocol for the systematic review also ensures that the review can be readily updated as (in this case) more PBK models are published.

PBK models are used in multiple industries to provide predictions of the variation in chemical concentration over time, at the organ level. It is useful to know the chemical space coverage of existing PBK models as models are available for relatively few chemicals overall. Obtaining information on key properties of the chemicals for which models are available demonstrates how well (or otherwise) the chemical space of drugs, cosmetics, botanicals, pesticides etc is represented by the available models. Therefore, a chemical space analysis for chemicals with existing models is useful to understand this. However, one caveat is that there is no simple process by which a chemical can be designated as being a particular “type”, for example cosmetic ingredients may also be food additives; botanicals may have pharmaceutical properties etc.

In summary, the aims of this chapter were to:

(i) Develop a protocol for a systematic review of existing PBK models. This required the use of pilot studies, with iterative development and testing to optimise the protocol.

(ii) Undertake a pilot study to determine the most appropriate systematic review software to use in this study; SyRF and Covidence software were compared.

(iii) Conduct a systematic review of PBK models using the optimised protocol and the software determined as being most appropriate. Relevant information for the PBK models

was captured in a flexible spreadsheet format, using both free text and controlled vocabulary to optimise searching. This spreadsheet is subsequently referred to as the PBK model dataset (PMD).

(iv) Ascertain the chemical space coverage (in terms of key physicochemical properties) of chemicals for which PBK models were available. The variation in these key physicochemical properties was compared across six specific types of chemicals.

2.2 Method

2.2.1 Pilot studies

2.2.1.1 Systematic review application

A pilot study comparing two different systematic review applications, SyRF (<https://syrf.org.uk>) and Covidence (<https://www.covidence.org>), was undertaken to determine the most suitable software for completing the systematic review. SyRF is freely available and specifically designed for the purpose of conducting systematic reviews and meta-analysis of animal studies, whereas Covidence has more general application. The following characteristics were compared: upload speeds, the size of data files containing the abstracts of papers to be uploaded onto the systematic review software, how the review process was divided into different stages (e.g. automatically divided into abstract screening, full text screening, etc., or if these steps needed input from the user); and the availability of additional tools to expedite the overall review process (e.g. automatic removal of duplicates, machine learning assisted screening of abstracts etc).

The pilot study showed that Covidence automatically removes duplicates, reducing the time required to identify suitable abstracts and automatically divides each stage of the review into logical sections, whereas SyRF does not. However, SyRF incorporates a machine learning tool to aid the screening process, reducing the time taken overall. In SyRF there is the option to annotate studies and highlight additional information in the paper to streamline the extraction process (e.g. questions on the disease model induction, treatment outcome assessment etc). However, these questions would have to be answered for each paper in order to create a process for extraction but were not relevant to the type of data that needed to be captured for this particular review. Further, SyRF required smaller sizes of the files

containing the abstract information to be uploaded into the systematic review software. SyRF had issues with the abstract files being uploaded into the software when an author's name was incorrect in the EndNote library, e.g. 3rd or Jr. at the end of someone's name. These problems needed to be addressed before being able to be successfully uploaded to SyRF. To fix this the exported XML file had to be updated with all references containing the terms, ', 3rd', ', III', ', 2nd', ', II', ', Jr.' were removed. Therefore, it was determined that Covidence would be the most suitable software for the project.

2.2.1.2 Databases to search

A scoping study of potentially useful databases and search terms was undertaken to determine the most appropriate databases and terms for identifying published papers on PBK models. Some of these databases investigated were: Web of Science (<https://www.webofknowledge.com>), ProQuest (<https://www.proquest.com/central/fromDatabasesLayer?accountid=12118>), Sage Journals (<https://journals.sagepub.com>), Cochrane Library (<https://www.cochranelibrary.com/?cookiesEnabled>), PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Taylor and Francis Online (<https://www.tandfonline.com>). All databases for scientific papers available through the university library were assessed. Any database found to have less than 100 results and any that were a sub-section of a larger database were removed. Following this analysis Scopus (<https://www.scopus.com/>), PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) and Web of Science (<https://www.webofknowledge.com>) were identified as the most appropriate databases and used in the subsequent study.

2.2.1.3 Protocol

Multiple arrangements of strings of search terms were tested in order to determine the best outcome, in terms of relevant and comprehensive results, using the different search criteria. These terms included, PBK, PBPK, PBTK, PBBK, PBPD, and physiologically based, pharmacokinetic, toxicokinetic, biokinetic and "?" to replace a hyphen for any words that might be hyphenated. Initially plans within the project were to capture only rat and human models, noting other species for future reference. However, it was decided that including all

species would be best to capture all relevant PBK models. To begin, data captured from the papers included chemical name, species, administration route, sex, life-stage, software used for model development, compartments considered in the model and reference. After piloting it was decided that the availability of model equations, PubMed IDs and DOIs should also be included, as well as any additional information on the chemical, e.g. whether information related to metabolite or parent, and if metabolite data were present in the model. Further chemical identifiers i.e. Chemical Abstract Registry (CAS) numbers, Simplified Input Line Entry Strings (SMILES) and International Chemical Identifier Keys (InChiKey) from PubChem, as well as identifiers from the COSMOS databases were added to the data extraction spreadsheet.

2.2.2 Systematic Review

The systematic review used for this study was registered on PROSPERO (CRD42020171130, see Appendix 1 for the published protocol), the National Institute for Health Research's international prospective registration system, with the review question stipulated as: *“For which substances are physiologically-based kinetic (PBK) models available and which species, genders, life-stages and routes of administration have been investigated for these substances? This will include determining the chemical space coverage of the models and the availability of the associated model equations within the literature”* (Thompson et al., 2020). The review complies with the PRISMA reporting standards, the PRISMA checklist is given in Appendix 2.

The following method was used in order to complete the systematic review according to the published protocol.

Databases: A search of the following three databases was conducted – Scopus (<https://www.scopus.com/>), PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) and Web of Science (<https://www.webofknowledge.com>); in the timeframe of all available years. The search of these databases was completed in October 2020.

Search terms: (“pbpk” OR “pbk” OR “pbbk” OR “pbtk” OR “pbpd” OR “pbpm” OR “physiologically based”) AND (“pharmacokinetic” OR “toxicokinetic” OR “biokinetic” OR “pharmacodynamics” OR “biopharmaceutical”) were used to search abstracts, titles and keywords of papers within each database, across all years available.

A total of 14,803 papers were initially identified, however following automated removal of duplicates in Covidence, 6,771 remained. All abstracts were screened independently by two researchers using the same process specified by the protocol with all conflicts being resolved by discussion. The inclusion criteria encompassed PBK models for all routes of administration for chemical, biological and carrier systems, including cases where normal physiology was altered or interactions between administered chemicals were investigated. Models that could not be associated with a specific chemical (such as generic models applicable to large groups of chemicals) were excluded. Where an abstract was associated with a paper that had subsequently been retracted it was ensured that this model was excluded from the dataset.

Although standard practice in other systematic reviews, assessment of the quality of the reported models and risk of bias in reporting was considered unnecessary for this review. Judging the quality of a model is difficult for multiple reasons; a PBK model may not be of the highest quality but may be sufficient for some purposes depending on its use (e.g. a PBK model could be good enough for screening purposes but not for regulatory purposes). OECD (2021b) and WHO (2010) have published guidelines on assessing the robustness of PBK models. However, to undertake a systematic review on the quality of a PBK model would include confirming validity of the parameters used, comparing the output from a PBK model with literature values, undertaking local or global sensitivity analysis. Thus, it would be an enormous undertaking, taking a significant amount of time to assess the quality of every PBK model and there are currently no appropriate tools that exist that can aid in this process. Therefore, it would not be practicable to do. The purpose of the review was to document all available models, enabling interested researchers to rapidly identify potentially useful models to assist with future model development. The assessment of PBK model quality (aside from fundamental considerations relating to good modelling practice) needs to be considered in terms of fitness for a given purpose (OECD, 2021b), it is therefore context dependent and remains the decision of the model user.

Following the abstract screening, 3,120 abstracts were retained for full text screening. PBK model data were extracted from 1,649 of these papers, resulting in 7,541 individual models being captured. If oral and intravenous dosing were used for both male and female subjects for the same chemical this would be extracted as four individual models, hence there are

many more models than individual chemicals. Reasons for exclusion of papers during full text screening included: PBK model not being reported in the article; the article referenced a previously published model with no adaptations (information on the PBK model was extracted from the original publication); full article not being available in English or not being reasonably accessible. Inter-library loans were requested for the full text of all papers, as well as contacting the authors directly. However, not all responded, or they sent papers months later after the systematic review was in the process of publication.

2.2.3 Extraction of data from available PBK models

Data was manually extracted from these 1,649 papers, with information being acquired from text, tables, figures and supplementary information. The data were entered into a Microsoft Excel spreadsheet that captured details of the chemical: parent, with metabolites specified where appropriate, species (with a sub-category where relevant e.g. human ethnicity, or a subcategory for bovine of cattle, cow, etc.), sex, life-stage, route of administration, literature reference for the model (with DOI), compartments considered in the model, the software employed and the availability of PBK model equations within the article. Controlled vocabulary was used where possible to ensure consistency of data extraction and to enable the resulting spreadsheet to be readily filtered and searched for specific types of models, i.e. controlled vocabulary was used for species, sex, life-stage, route of administration, availability of equations and software used. The vocabulary was empirically derived, to enable the most efficient searching. For example, life stages can be reported in multiple ways – no. of weeks, months, years of age, young adult, adult, neonate, young child, juvenile etc. Therefore, for consistency this information was allocated to the more generic categories of pre-birth or pre-hatch; from birth or hatch up to adult; adult; pregnant; old age (if specified) as well as a generic category for health compromised (excluding old age) individuals. Some models were created for modified animals, i.e. animals with cells added to them which were not of the same species, or information from two species that are similar, i.e. mouse and rat, were combined to create the model. These differences in species have also been recorded.

All information extracted from the spreadsheets can be seen in Appendix 3. The controlled vocabulary options used for each column are listed below.

Species: aquatic, bird, bovine, cat, dog, gerbil, goat, guinea-pig, hamster, horse, human, non-human primate, mouse modified, mouse unmodified, oyster, polar bear, porcine, rabbit, rat, rodent combined, rodent unspecified, sheep.

Secondary species category or human ethnicity: bull, cattle, chicken, clam, cow, fish, minipig, monkey, oyster, pig, porpoise, scallop, swine, turkey, whale, African, African American, Caucasian, Chinese, Hispanic, Indian, Japanese, Korean, comparative ethnicity, human unspecified.

Sex: male, female, unspecified

Life-stage: pre-birth or pre-hatch, from birth or hatch up to adult, adult, pregnant, old aged (specified), health compromised (excluding old age), unspecified.

Administration route: buccal, dermal injection, dermal topical, gills, *in utero*, inhalation, intra-arterial, intramuscular, intraocular, intraperitoneal, intramammary, intratracheal, intravaginal, intravenous bolus injection, intravenous infusion, metabolism from parent, nasal, oral bolus, oral feed/water, unspecified.

Availability of equations in the paper: full equations in paper, combination of partial equations in paper and software files on request from author, partial equations in paper, equations in combination of primary and secondary references, equations in secondary references, equations on request from paper author, software files on request from paper author, model code in secondary references, not available, in supplementary material, model code available on Github, model code available on BioModels database, model code in appendix, model code in supplementary material.

Simulation software: ACSL/X/Xtreme/Libero/Xtreme Optium/Sim/TOX, ADAPT II/5, BASIC, Berkeley Madonna, C, C++, COPASI, Chrisis, CMATRIX, CSMP III, Crystal Ball, DARE-P(6), DESSolver, Ecolego, Fotran, G+, GastroPlus, Jacobian, KaleidaGraph, LSODA, Mathematica/2.2/9, MathCad/PLUS, Matlab, MLAB, MCSim, Microsoft Excel/Visual Basic, ModelMaker/4, MULTI-FORTE, Napp, NEXTSTEP, NONLIN, NONMEM, OpenFOAM, Pascal, Phoenix, PK-Sim/MoBi, PKQuest, Python, R, SAAM/II, SAS, SCIENTIST, ScoP, Simbiology, Simcyp, Simulink, SIMUSOLV, Sun/4, STELLA, Totalchrom, Phoenix WinNonlin, Multiple software used, not specified.

The free text columns are listed below with an explanation of the minimum information required to be inputted.

Chemical name: as stated in paper.

Additional information on chemical: Parent – stipulating if there is also information on metabolites in the spreadsheet; Co-administered/Interaction – indicates where co-administered with another chemical e.g. to check for interactions such as for drug-drug interaction studies; Co-exposure – co-exposure of the chemical with other chemicals; Mixture – mixture of more than one chemical; Inhibited – an inhibitor was present; Inhibitor – the inhibitor chemical; Pre-treatment – the chemical used for pre-treatment.

Reference: authors, year, journal, volume, pages.

PubMed ID: if available.

DOI.

A chemical may be identified using common names or chemical identifiers such as the Chemical Abstracts Service (CAS) registry number, a Simplified Molecular Input Line Entry System (SMILES) string or the International Chemical Identifier Key (InChiKey). Ideally multiple identifiers should be incorporated in the dataset to avoid ambiguity. Chemical identifier information was obtained by manually inputting the chemical name (as given in the publication) into PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and extracting the molecular weight, canonical SMILES, isomeric SMILES, InChiKey and CAS number. The CAS registry number from PubChem was used as input for the COSMOS database, version 2 (<https://cosmosdb.eu>). Where available, the CAS registry number and chemical name, as recorded in COSMOS, were extracted to confirm the identity of the chemical; the COSMOS ID was also extracted.

Model development can be performed using a range of software, the equations employed may be specified within the publication itself (or as part of the supplementary information accompanying the article).

2.2.4 Assessment of the chemical space coverage of the PBK model dataset in relation to other chemical datasets

In order to assess the nature of the chemicals in the PBK model dataset to be able to identify how many PBK models are available for the different “types” of chemicals, key physicochemical properties were generated and compared to those of chemicals appearing

in other datasets to assess their chemical space coverage. Relevant datasets have been compiled in-house at Liverpool John Moores University and comprise: botanicals (obtained from <https://www.efsa.europa.eu/en/data/compendium-botanicals>); pesticides (obtained from https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en); cosmetic ingredients (obtained from COSMOS db version 2 (<https://cosmosdb.eu>); food additives (obtained from <http://foodb.ca>); pharmaceuticals (obtained from www.drugbank.ca) and REACH chemicals (obtained from <https://echa.europa.eu/information-on-chemicals/pre-registered-substances>). The number of chemicals in the PBK model dataset that also appeared in each of the other datasets was ascertained and are shown in Table 2.1. Canonical SMILES for all chemicals in these datasets were generated using OpenBabel (v.3.0.0; http://openbabel.org/wiki/Main_Page). OpenBabel was used for ease and consistency across all datasets as each dataset could be inputted as one list and all results generated simultaneously. From the PBK model dataset 1,150 unique SMILES were identified with 1,187 unique InChiKeys (chemical isomers may have the same SMILES string but different InChiKeys). In order to determine how many chemicals with PBK models were present in each of the other six datasets the InChiKeys were compared using the “find duplicates” function in Excel. This was used to obtain the number of duplicates found when combining the InChiKeys of the PBK dataset with each of the other six datasets in turn.

The SMILES strings for all datasets were inputted into the RDKit (v. 2020.03.6; www.rdkit.org) Descriptor Node, accessed through KNIME software (v. 4.3.1; www.knime.com), in order to obtain the physicochemical properties for all chemicals. Properties included molecular weight, number of hydrogen bond donors/acceptors, predicted logarithm of the octanol:water partition coefficient (Slog P) and the topological polar surface area (TPSA); the number of Lipinski rule violations were calculated from this information. Whilst it is possible to generate thousands of physicochemical properties, here only a few readily calculable properties were selected, representing those most often used to broadly characterise chemicals in terms of size, polarity and partitioning behaviour. These simple properties were also used to determine Lipinski rule violations, frequently used to indicate potential poor oral absorption – a common route of administration for these models. The minimum, maximum, mean and median values and interquartile ranges of these properties were calculated using

Minitab version 19.2 for all datasets. Histograms were also generated using Microsoft Excel to enable a visual comparison of the property ranges between the different datasets.

2.3 Assessment of abstract screening and data extraction reliability

After screening all 6,771 abstracts in duplicate, 3,120 were taken forward to full text screening; of these, 1,362 papers were rejected at this stage. In addition, 109 papers could not be readily obtained or were not in English, therefore data were extracted from 1,649 papers, resulting in 7,541 models. The resultant spreadsheet comprises over 150,000 individual entries as for each model the species, sex, life-stage, route of administration, availability of equations, compartments, references and chemical identifiers were captured. It is expected that errors will arise when assessing the suitability of papers for inclusion and performing extensive manual processing, hence a quality assessment exercise was undertaken. Three aspects of the screening and data extraction processes were reviewed by a second investigator:

- (i) 5% of the papers that had been excluded at the full text screening phase were reviewed.
- (ii) 5% of PBK model data extracted from the papers (chemical information, species data (primary and secondary categories), sex, life-stage, route of administration, reference (DOI and PubMed ID if available), compartments investigated, availability of equations and simulation software were checked.
- (iii) 5% of the chemical identifier information from PubChem and COSMOS (chemical name, CAS registry number, molecular weight, canonical SMILES, isomeric SMILES, InChiKey, COSMOS ID) was obtained again from these sources and compared to the values in the spreadsheet.

The greatest source of “error” was determined to be the exclusion of papers that were considered as potentially relevant by a second investigator i.e., 6% of excluded papers. In terms of the systematic review this is not considered a highly substantial problem. PBK models are continually being published, hence there can never be a finalised set of models. For PBK model data, manually extracted from the papers, an error was detected in the information captured for 3.5 % of chemicals. This does not equate to 3.5 % of the total information being incorrect as this may indicate an error in only one (or possibly more) of the

13 columns that relate to the PBK model information. An error was detected in the data for 2.4% of the chemicals in relation to the identifier information. As above, this does not equate to 2.4% of the total information being incorrect, but that for 2.4% of chemicals an error was detected in one (or more) of the 7 columns associated with chemical identifier information.

2.4 Results and discussion

The results and discussion are combined in this section for clarity.

2.4.1 Systematic Review

The number of papers considered at each stage of the review process and the total number of models extracted in the PBK model spreadsheet is summarised in Figure 2.1. Of the 6,771 of papers initially identified, 3,120 remained after abstract screening, and data were extracted from 1,649 of these. There are more models than individual chemicals or papers, as if oral and intravenous dosing were used for both male and female subjects for the same chemical this would be extracted as four individual models, or a paper might have modelled more than one chemical.

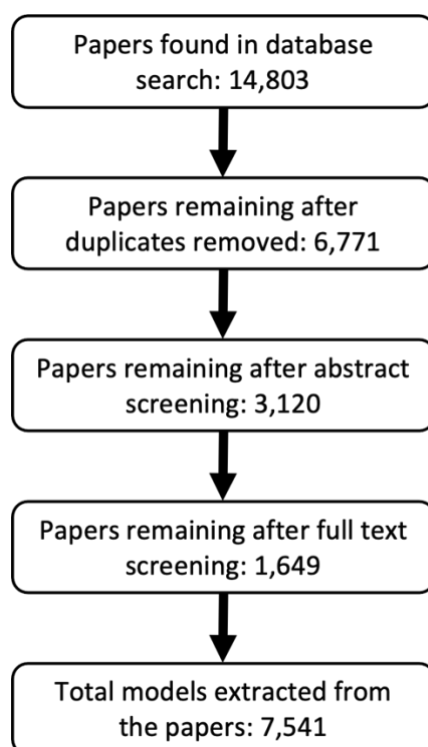


Figure 2.1 Summary of papers considered at each stage of the review process and total number of models extracted. Total number of models is greater than the total number of individual chemicals or papers, as if oral and intravenous dosing were

used for both male and female subjects for the same chemical this would be extracted as four individual models, or a paper might have modelled more than one chemical.

Any search strategy, even one that is well formulated, is liable to miss relevant information. The protocol outlined for this systematic review is set to capture as many papers as possible that have PBK models for a specific chemical. However, some PBK models (approximately 6%) may have been missed as was determined in the quality assessment exercise undertaken. The inclusion of PBK models for all chemical types, across all species, life-stages and routes of administration ensures as many PBK models as possible can be captured. Thus, the systematic review is relevant across all industry sectors.

2.4.2 Extraction of data from available PBK models

A total of 7,541 individual models were identified and extracted from the literature. For an individual chemical multiple models may be available in one paper. To enable more flexible searching, all models were categorised and recorded individually. For example, where use of either males or females was stipulated separately in the experimental protocol, these would be recorded as two separate models. Similarly, where more than one administration route had been given in the paper, each administration route would be recorded as a separate model. This would allow future searches to be conducted, not just by chemical name, but also by species or route of administration etc. Predefined lists of vocabulary were used to ensure consistency of the terms used when reporting the species (and secondary species or ethnicity category), life-stage, sex, route of administration, software, and availability of equations. Free text input was used for chemical name, additional chemical information, reference, DOI, PubMed ID, compartments and any additional notes.

Appendices 4 and 5 represent the spreadsheets curated from the systematic review, where Appendix 4 is a snapshot of the PBK dataset discussed in this Section (2.3.2), and Appendix 5 is the physicochemical properties associated with the chemicals in the PBK spreadsheet discussed in Section 2.4.3. A total of 1,889 chemical names are present in the PBK model dataset, some represent biological entities (such as monoclonal antibodies) or are not associated with a specific structure. Hence only 1,187 unique chemicals, with identifiable structures, were available, each of these is identified in the dataset by its unique InChiKey.

2.4.2.1 Species

All species (and sub-categories) captured within the PBK model dataset are listed in Section 2.2.3 and illustrated in Figure 2.2 that also summarises the number of models associated with each species. The largest number of models by species was 3,676 for human models, with models for goats and cats being the fewest, with only 4 models for each.

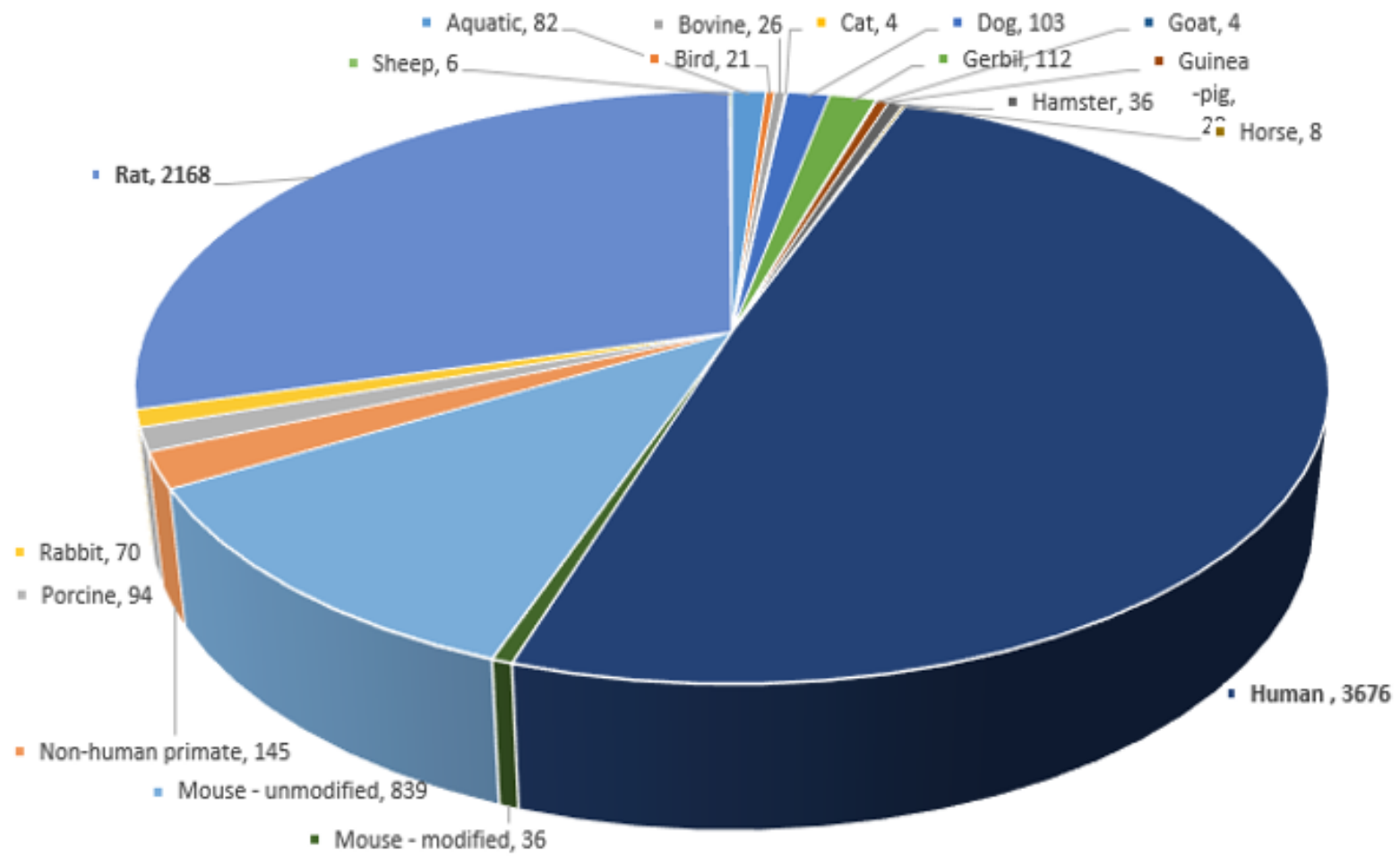


Figure 2.2 Proportion of models associated with different species from Thompson et al. (2021)

2.4.2.2 Life-stage

The majority of models reported in the dataset were either for a typical adult (1,666 models), or the life-stage was unspecified (4,746 models). There were fewer than 100 models for old aged, health compromised and pre-birth or pre-hatch. Although, there were 220 models for pregnancy and 730 models for the category from birth or hatch up to adult.

2.4.2.3 Route of administration

A pie chart of the proportion of models associated with different routes of administration is displayed in Figure 2.3. The three main administration routes were oral (1,903 models), inhalation (1,195 models), and metabolism from a parent chemical (2,138 models). However, there were 18 models with the route of administration unspecified within the papers.

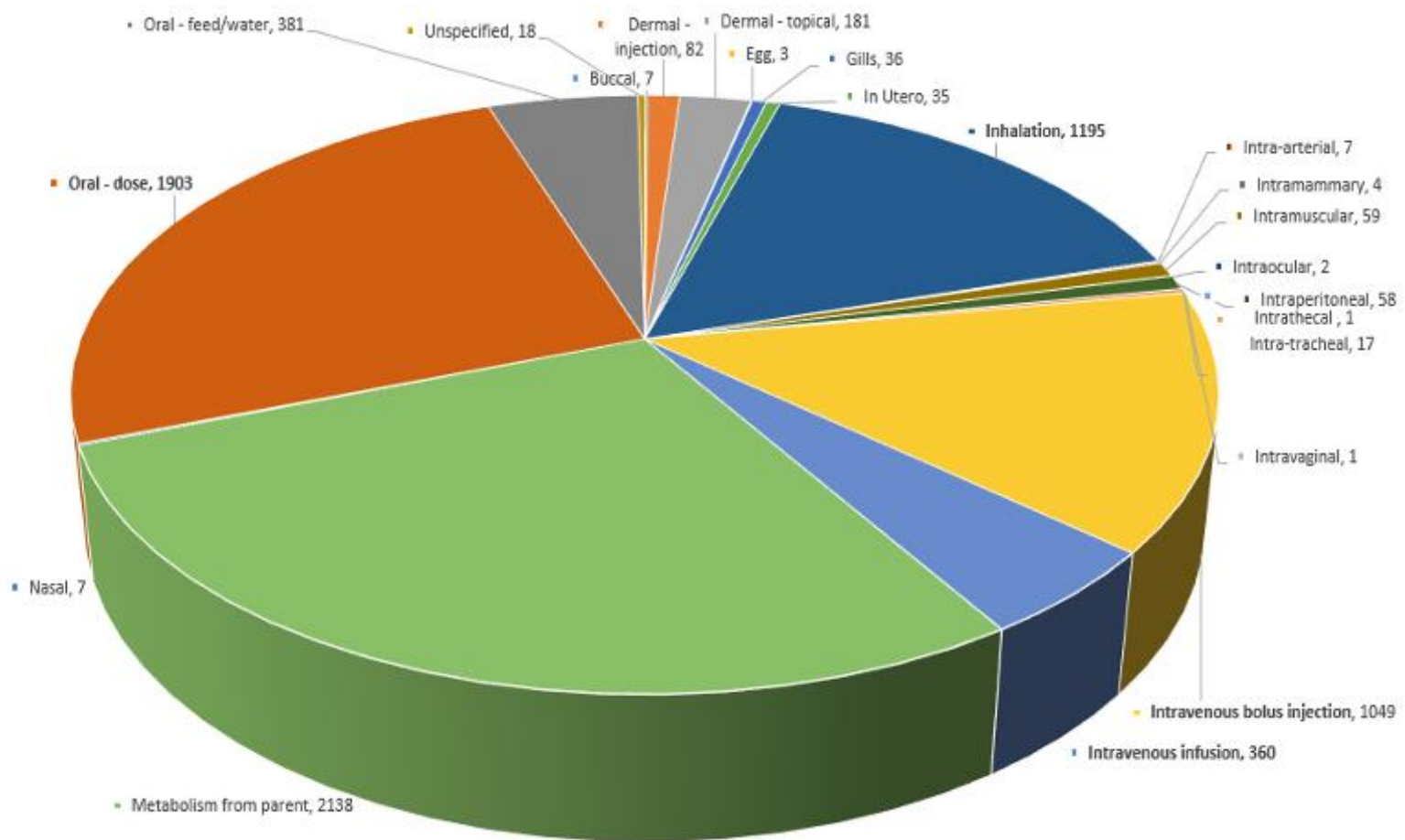


Figure 2.3 Proportion of models associated with different routes of administration from Thompson et al. (2021)

2.4.2.4 Modelling software and equation availability

The four main software used for solving the equations were SIMUSOLV, Symcyp, ACSL and Matlab, representing 10.3%, 9.0%, 11.0%, and 8.4% of software respectively. A quarter of models had full equations reported in the original paper, equally however, one quarter of reported models had no equations available. Equations found in secondary references were the case for just under one quarter of models, with the remaining quarter of models having equations or model code in supplementary material, available on request from the author, on a secondary online platform or a combination of these options.

Efforts to improve the reproducibility of mathematical models are increasing. Journals are increasingly making it a requirement that a precondition to publication is to make available implementations of mathematical models in online repositories, for example GitHub (<https://github.com>). Additionally, markup languages for representing models (e.g. Systems Biology Markup Language (SBML) and CellML (<https://www.cellml.org>)) is being specified and becoming more consistent in use for particular models (Hedley et al., 2001; Hucka et al., 2003). The Biomodels database (<https://www.ebi.ac.uk/biomodels/>) represent models in the SBML format.

2.4.2.5 Compartments

The main compartments included in the models were (arterial/venous) blood, poorly perfused or well perfused compartments, kidney, liver and lungs. A common feature was that models were often split into the individual organs, i.e. adipose, brain, intestines, gut, gastrointestinal tract, skin, bones, stomach, muscle, reproductive organs, etc. In addition, there were models created in more detail with a focus on a particular life-stage or administration route, i.e. models for pregnant animals included foetus and placenta; fish models included gills, etc.

Through undertaking a systematic review, information concerning over 7,500 PBK models from 1,649 papers was captured. The models encompassed 18 species (including rat, human, mouse, cow, guinea-pig) at various life-stages (e.g. juvenile, adult, pregnant, health-compromised) across 21 administration routes (e.g. oral, inhalation, *in utero*). The

information has been distilled into a Microsoft Excel spreadsheet that was constructed using controlled vocabulary to enable users to search using different criteria (e.g. to select models by species or routes of administration etc). It is anticipated that researchers or regulatory scientists can use this information to assist building or evaluation of new models, or as a resource from which to extract relevant pharmacokinetic or toxicokinetic data. As identified in the quality assessment exercise, some of the historic models were omitted, and it is also noted that it would not be possible to generate a finite list of all PBK models as this is such a dynamic area of research. The publication of new models has shown a rapidly increasing trajectory in recent years (Paini et al., 2017), the current dataset serves as a basis for continuing curation of existing models which will provide an increasingly rich source of information for modellers in future. The PBK modelling dataset (in the form of an Excel spreadsheet) has been added to the EURL ECVAM catalogue for alternatives (<https://data.jrc.ec.europa.eu/dataset/f98e9abf-8435-4578-acd6-3c35b5d1e50c>) and has been used as the underpinning knowledge for a PBK model browsing webtool, under development at the US Environmental Protection Agency.

2.4.3 Assessment of the chemical space coverage of the PBK model dataset in relation to other chemical datasets

Chemicals can be used for a variety of purposes, and it is often not feasible to allocate a chemical to unique “type” (e.g. there is a significant cross-over between chemicals used as food additives and as cosmetic ingredients, hence the same chemicals may appear in more than one dataset). It is therefore difficult to determine for which chemical “types” there are most PBK models. However, in the analysis undertaken here, to identify how many PBK models are available for the different “types” of chemicals, there are some trends discernible. Thus, to assess the extent to which the PMD was representative of the chemical space of other datasets. The results for the comparison of InChiKeys for chemicals in the PBK model dataset to those for the six comparative datasets are shown in Table 2.1. REACH chemicals were the largest dataset, and less than 1% of the chemicals had PBK models, whereas for the pesticide dataset (the smallest datasets analysed) just over 4% of the chemicals have an available PBK model. Across all six datasets only 4.23% of chemicals were chemicals present in the PBK model dataset.

Unsurprisingly, given that PBK modelling evolved in the area drug development, the greatest proportion of models correspond to chemicals in the DrugBank dataset. Pesticides are generally well studied and data-rich, therefore the second most common type of chemical with PBK models are the pesticides. For food additives and cosmetic ingredients, where there are often chemicals in common, similar proportions of chemicals have PBK models. Due to the size and generality of the REACH dataset it would be anticipated that relatively few chemicals would have existing PBK models. The results confirm the paucity of PBK models available in relation to different areas of chemical space and underline the importance of using existing PBK models to help fill data gaps.

Table 2.1 The number (and percentage) of chemicals with existing PBK models that are present in the six comparative datasets investigated (as reported in Thompson et al. (2021))

Dataset	Number of chemicals in dataset (with unique, identifiable structures)	Number of chemicals in dataset also present in PBK model dataset	Percentage of chemicals in dataset also present in PBK model dataset (%)
PBK model	1,186	N/A	N/A
Botanical	947	24	2.67
Pesticide	945	40	4.23
COSMOS	5,655	123	2.41
Food	2,674	88	3.37
DrugBank	6,716	382	5.80
REACH	75,663	620	0.85

In addition to the spreadsheet comprising the PBK models collated from the literature (Appendix 4), an additional spreadsheet (Appendix 5) comprises a list of the 1,144 chemicals in the PBK Model Dataset with unique SMILES and their associated key physicochemical properties. Table 2.2 shows the results of the statistical analysis of the key physicochemical properties (molecular weight, number of hydrogen bond donors and acceptors, predicted logarithm of the octanol:water partition coefficient (Slog P), topological polar surface area

and number of Lipinski rule violations for the chemicals in the PBK model dataset. The extreme values observed for molecular weight and log P are a result of the chemical vistarem, a magnetic resonance imaging contrast agent, with large hydrophilic chains. All datasets have chemicals with molecular weights with a mean between 200-393.65. The range of TPSA values for pesticides is significantly smaller than the PBK dataset and the other five datasets used for comparison.

Table 2.2 Statistical analysis of the physicochemical properties of the chemicals in the PBK model and the six comparative datasets

Variable ^a	Mean	Minimum	Q1	Median	Q3	Maximum	Range	IQR
PBK dataset, n=1,186								
MW	325.61	6.94	163.10	292.28	410.67	6496.20 ^b	6489.26	247.57
No. HBD	1.79	0.00	0.00	1.00	2.00	100.00	100.00	2.00
No. HBA	4.17	0.00	1.00	3.00	6.00	120.00	120.00	5.00
Slog P	2.25	-45.03 ^b	0.80	2.08	3.73	11.10	56.13	2.93
TPSA	73.07	0.00	23.47	56.92	93.00	2536.36	2536.36	69.53
No. Violations	0.37	0.00	0.00	0.00	0.00	4.00	4.00	0.00
Botanicals dataset, n=947								
MW	393.65	68.08	242.32	333.38	470.69	2749.89	2681.82	228.37
No. HBD	2.482	0.00	0.00	1.00	3.00	43.00	43.00	3.00
No. HBA	6.360	0.00	3.00	5.00	8.00	76.00	76.00	5.00
Slog P	2.31	-9.75	1.22	2.55	3.45	12.50	22.25	2.22
TPSA	95.30	0.00	40.16	65.56	118.22	1276.40	1276.40	78.06
No. Violations	0.59	0.00	0.00	0.00	1.00	4.00	4.00	1.00
Pesticides dataset, n=945								
MW	302.24	73.12	225.30	296.33	362.11	1199.38	1126.26	136.81
No. HBD	0.81	0.00	0.00	1.00	1.00	21.00	21.00	1.00
No. HBA	3.87	0.00	2.00	4.00	5.00	33.00	33.00	3.00
Slog P	3.17	-14.33	2.066	3.21	4.34	18.10	32.43	2.28
TPSA	56.71	0.00	29.54	50.41	69.22	535.59	535.59	39.68
No. Violations	0.21	0.00	0.00	0.00	0.00	3.00	3.00	0.00
COSMOS dataset, n=5,554								
MW	272.19	2.02	157.21	210.32	328.74	6179.37	6177.36	171.53
No. HBD	1.28	0.00	0.00	1.00	2.00	116.00	116.00	2.00
No. HBA	3.39	0.00	1.00	2.00	4.00	191.00	191.00	3.00

Slog P	2.22	-83.67	0.37	2.45	3.91	32.04	115.71	3.54
TPSA	61.93	0.00	23.73	38.69	78.66	3038.93	3038.93	54.93
No. Violations	0.33	0.00	0.00	0.00	0.00	4.00	4.00	0.00
Food dataset, n=2,679								
MW	200.47	4.00	132.06	164.25	214.22	2555.60	2551.60	82.16
No. HBD	0.84	0.00	0.00	0.00	1.00	37.00	37.00	1.00
No. HBA	2.78	0.00	1.00	2.00	3.00	49.00	49.00	2.00
Slog P	1.53	-21.07	0.94	2.08	2.98	25.56	46.63	2.04
TPSA	47.34	0.00	18.46	26.30	46.53	1109.38	1109.38	28.07
No. Violations	0.14	0.00	0.00	0.00	0.00	4.00	4.00	0.00
Drugbank dataset, n=6,716								
MW	348.44	6.94	231.26	323.40	414.59	6179.37	6172.43	183.33
No. HBD	2.74	0.00	1.00	2.00	4.00	116.00	116.00	3.00
No. HBA	5.29	0.00	3.00	4.00	6.00	191.00	191.00	3.00
Slog P	1.61	-83.67	0.050	1.91	3.49	24.57	108.24	3.44
TPSA	101.43	0.00	55.56	83.98	118.77	3038.93	3038.93	63.21
No. Violations	0.36	0.00	0.00	0.00	0.00	4.00	4.00	0.00
REACH dataset, n=75,662								
MW	314.1	2.02	180.1	251.3	384.4	10402.5	10400.5	204.3
No. HBD	1.11	0.00	0.00	1.00	2.00	141.00	141.00	2.00
No. HBA	3.98	0.0000	2.00	3.00	5.00	191.00	191.00	3.00
Slog P	2.31	-83.67	0.78	2.33	3.88	60.85	144.52	3.09
TPSA	69.12	0.00	26.30	49.69	86.74	4202.80	4202.80	60.44
No. Violations	0.37	0.00	0.00	0.00	0.00	4.00	4.00	0.00

**MW = molecular weight; No. HBD = number of hydrogen bond donors; No. HBA = number of hydrogen bond acceptors; Slog P = predicted logarithm of the octanol:water partition coefficient;*

TPSA = topological polar surface area; nViolations = number of violations of the Lipinski Rule of Five (Lipinski et al. 1997).

^b*The extreme values here are for vistarem refer to text*

Figure 2.4 (A-F) shows the comparison of these key physicochemical properties across the seven datasets. Similar trends in the spread of chemicals over six physicochemical properties can be observed for all datasets. While the pesticides dataset has the largest percentage of chemicals with a higher molecular weight of 601+ Da, the predominant trend across all datasets is that most chemicals are within the range of 100-600 Da. Cosmetic ingredients (COSMOS dataset) and industrial chemicals (REACH dataset) have the most diverse values for log P within. Pesticides and botanicals datasets have a greater percentage of chemicals in the log P ranges 3-4 (43% and 47% respectively), whereas the PBK dataset only has 28% of chemicals in this range. Chemicals with a higher number of hydrogen bond donors are observed within the botanical dataset.

Figure 2.4A shows the distribution of molecular weight across the seven datasets. As expected, the majority of chemicals fall within the range 100-600 Da but there are notable differences between the datasets. There are a relatively high number of chemicals in the PBK dataset with low molecular weight, these will include the volatile chemicals for which respiratory uptake has been extensively studied. Food additives and cosmetic ingredients (which have chemicals in common) show a relatively high proportion of low molecular weight chemicals. Chemicals that are designed to be biologically active, such as drugs and pesticides, tend to be developed in accordance with guidelines relating to preferred physicochemical properties. For example, the Lipinski Rule of Five stipulates that poor oral absorption of drugs is associated with those chemicals having molecular weight > 500 Da; log P > 5; >10 hydrogen bond acceptors or; > 5 hydrogen bond donors (Lipinski et al., 1997).

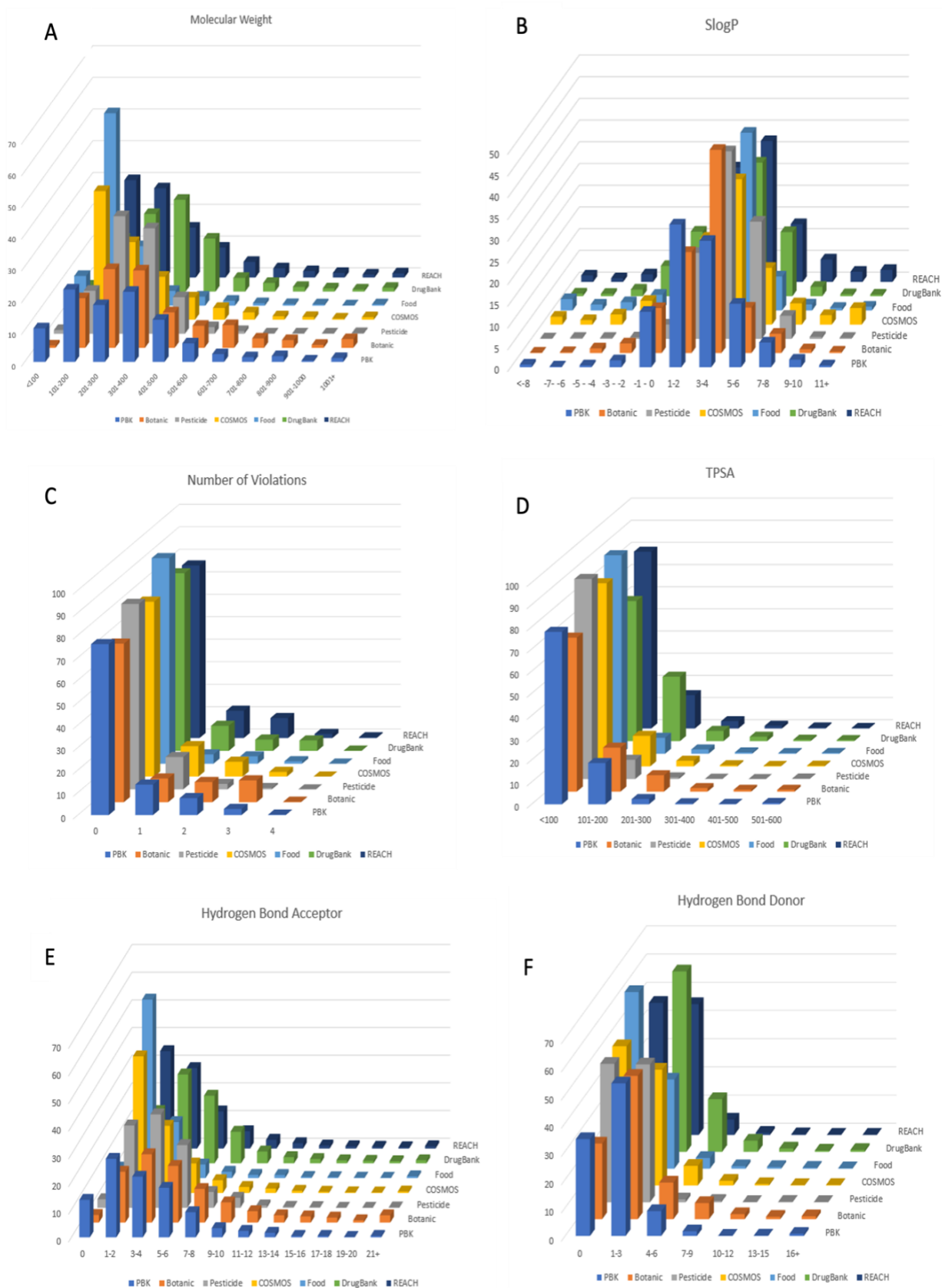


Figure 2.4(A-F). Comparison of the ranges of physicochemical properties across the seven datasets investigated (from Thompson et al. (2021)) *MW = molecular weight; No. HBD = number of hydrogen bond donors; No. HBA = number of hydrogen bond acceptors; Slog P = predicted logarithm of the octanol:water partition coefficient; TPSA = topological polar surface area; nViolations = number of violations of the Lipinski Rule of Five (Lipinski et al. 1997).

Other research has also suggested that TPSA >140 Å (Pistollato et al., 2021) is also unfavourable for oral absorption, consequently certain chemical types are designed to fall within narrower property ranges and such trends are evident in the property ranges here. A correlation between molecular weight and log P is often observed amongst groups of chemicals (although there are many exceptions to this), here a similar pattern to the range of values is generally observed for log P and molecular weight.

Pesticide and botanical datasets comprise fewer molecules capable of carrying a charge (associated with increased hydrophilicity) hence on average have higher log P values. This is significant as partitioning behaviour (often estimated using log P) is a key element in building PBK models. Whilst the extreme values for log P, calculated by the software used here, may be unrealistic (and therefore unsuitable for model building), when used for comparison they are still useful for demonstrating the trends in the data. The range in values for all of the physicochemical properties of pesticides, is narrower than for the other chemical types, indicating the more restrictive chemical properties required for these chemicals. Botanicals generally show a wider range of values for each of the physicochemical properties (in particular more chemicals show properties at the upper extremes of the ranges). A significant number of chemicals within this dataset are large and complex. Whilst in other datasets, molecules tend to be designed for a specific purpose (and those outwith given property ranges are filtered out) the same exclusions would not be applicable to this dataset.

Understanding the kinetic behaviour of a chemical within the body, particularly its concentration-time profile at a target site, is essential to accurately determine its potential effects. For the majority of chemicals there is a lack of data concerning toxicity and kinetics, however generating such information *de novo* would require excessive use of animals and is legally, ethically and financially constrained. Hence there is a need to leverage existing knowledge in order to obtain as much information as possible to assist decision making. Read-across is the most common method used by which information from data-rich chemicals is used to predict information for data-poor chemicals. Within this chapter, a comprehensive collation of existing PBK models that can be searched using multiple criteria is presented.

The PBK model dataset described in this chapter enables researchers to readily gain insight into available PBK models across multiple species, life-stages and routes of administration, such that the structure and parameterisation of PBK models for different chemicals is more accessible. This ensures maximum use of existing knowledge on PBK modelling and reduces the time and cost associated with developing new PBK models. The next chapter in this thesis identifies appropriate similarity metrics to assist in the identification of PBK models for analogues to use for read-across using the PBK model dataset curated in this chapter.

Chapter 3 – Development of a tool to identify similar chemicals for PBK modelling

3.1 Introduction

The dataset of PBK models created in Chapter 2 was utilised as a resource to create a tool that helps to determine which chemicals are similar to a target chemical. The ability to use a read-across approach (using data-rich chemicals to inform the development of models for chemicals that are data-poor) would help fill the current gaps in toxicokinetic data, especially for regulatory purposes. There are resources available on using and reporting read-across predictions. The European Chemicals Agency (ECHA) (2017) provide guidance on evaluating read-across predictions for regulatory submissions. Schultz et al. (2015) describe a workflow strategy on structuring and reporting read-across predictions for toxicity. However, to be able to use a read-across approach, identification of similar chemicals to a target is required by assessing the similarity of chemicals. Determining similarity of chemicals is a complex issue as there are multiple criteria for determining similarity, for example in terms of structural or physicochemical properties, mechanism of action or metabolite formation (Paini et al., 2021b). Similarity may be assessed using simple calculable properties, these options include:

- (1) Chemical fingerprints
- (2) Physicochemical properties
- (3) Absorption, distribution, metabolism, and excretion (ADME) properties

Chemical fingerprints are a method of identifying structurally similarity chemicals. The presence or absence of key structural features within a chemical is indicated by a '1' or '0' respectively (Madden et al., 2020). Figure 3.1 outlines the key concepts of fingerprints and calculating similarity. Similarity between chemical fingerprints are calculated using mathematical formulae, for example a Tanimoto score, to determine the degree of commonality in the presence of key structural features (bits). For example, a Tanimoto score between zero (dissimilar) and one (highly similar or identical) can be generated. A Tanimoto score of 0.6 is commonly used as a cut-off above which chemicals are considered to be similar, although this is an arbitrary value and other values may be suitable for different scenarios. Different structural features are used within different fingerprinting algorithms (e.g. MACCS

uses 166 structural features, PubChem uses 881 structural features) (Madden et al., 2020), thus resulting in different Tanimoto scores depending on the algorithm used. Mellor et al. (2019) demonstrated this issue of different scores and reported that there is no consensus as to which similarity metric is best.

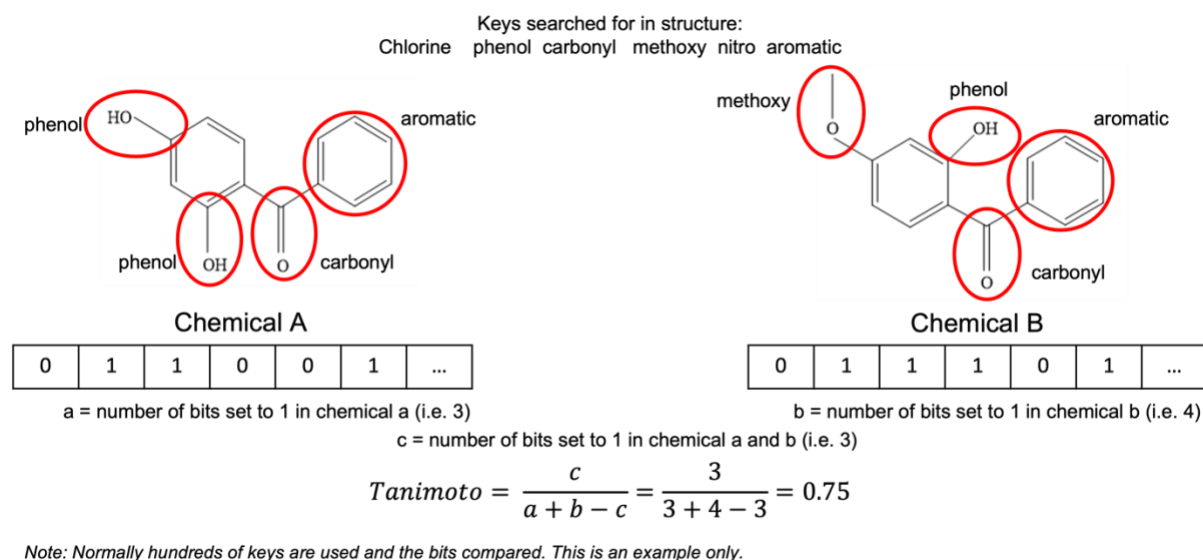


Figure 3.1 Key concepts of fingerprints and calculating similarity (figure adapted from Madden (2020))

Physicochemical properties include the logarithm of the octanol:water partition coefficient (log P), pKa (degree of ionisation), molecular weight, hydrogen bonding capacity (number of hydrogen bond donors and acceptors), solubility etc. Defining similarity of chemicals in terms of physicochemical properties alone is problematic as many diverse chemicals may have similar properties (expert judgement should also be used when determining similarity). However, this approach was successfully used by Lu et al. (2016b), wherein the authors adjusted chemical specific parameters of an existing PBK model to develop a model for a similar chemical. Chemical specific parameters can be derived from experimental measurements or predicted using software. Calculated values for certain parameters are more reliable than for others, e.g. calculated log P values are generally more reliable than calculated ADME properties (Madden et al., 2019). Resources for predicting fundamental physicochemical properties (e.g. log P, log D, pKa) include EPISUITE (<https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface>), ADMETlab 2.0 (<https://admetmesh.scbdd.com/service/evaluation/cal>) and Opera

(<https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.html>); EPISUITE also provides measured values where available.

There has been a significant increase in publications in regard to predicting ADME properties in recent years (Wang et al., 2015). ADME parameters include half-life, volume of distribution, clearance, absorption rate, etc. There are many tools that predict ADME properties and thirty-eight databases that include ADME information were identified by Pawar et al. (2019). However, there are often inconsistencies in measured values recorded in these databases. Table 3.1 outlines example resources for prediction and identifies some of the ADME properties that they can predict.

Table 3.1 Summary of resources that predict ADME properties and some example properties they can predict

Software	Website (all last accessed July 2022)	Example properties predicted
ADMETlab 2.0	https://admetmesh.scbdd.com/service/evaluation/cal	% Human Intestinal Absorption, Fraction Unbound, Half-Life, Total Clearance, Volume of Distribution, Bioavailability
admetSAR	http://lmmd.ecust.edu.cn/admetSar2/	% Human Intestinal Absorption, Fraction Unbound, Half-Life, Bioavailability
SwissADME	http://www.swissadme.ch/	Lipinski Rule Violations, % Human Intestinal Absorption, Bioavailability
pkCSM	http://biosig.unimelb.edu.au/pkcsM/	% Human Intestinal Absorption, Fraction Unbound, Volume of Distribution
Comptox	https://comptox.epa.gov/dashboard/	Fraction Unbound, Hepatic Clearance, Half-Life, Volume of Distribution

For more extensive examples of properties and the software used to generate them see Madden and Thompson (2022). There are recognised limitations with predicting ADME properties, for example, the models may not be applicable to certain chemicals, if similar chemicals were not incorporated in the training set. Although predicted ADME properties could have been used for assessing similarity in the research conducted here, there are arguments against using this approach. Firstly, there are many different methods for calculating ADME properties, often giving very different results. Secondly, estimates may be unreliable (particularly for certain chemicals) and not well represented by the training set. Moreover, in many cases the models are solely based on predicted physicochemical properties. Thus, in the present study, chemical similarity was assessed using chemical fingerprints and readily calculable physicochemical properties, rather than ADME properties.

PBK models require chemical specific (e.g. plasma protein binding fraction, molecular weight, solubility), anatomical (e.g. blood flow, organ volume), and physiological (e.g. glomerular filtration) information in order to build a model. Sources for PBK model inputs are available from multiple databases (see Madden et al. (2019) for resources). As discussed in Section 1.3, PBK models are time and resource intensive to build (Laroche et al., 2018). Therefore, it would be a great asset to be able to automate the process of identifying chemicals with existing PBK models that are similar to a target chemical to inform the development of new models. KNIME (<https://www.knime.com>) is an open-source software for data analysis. It allows for data to be transformed, blended, modelled and visualised through the workflows that a user creates. It is commonly used in chemoinformatic research and integrates machine learning and data-mining. Thus, this software was identified as being appropriate for creating a workflow to assist in selection of source chemicals, similar to a given target. This is subsequently referred to as the KNIME Workflow for Assisting Analogue Selection (KWAAS).

In summary, the aims of this chapter were to:

(i) Investigate a range of similarity assessment metrics (e.g. chemical fingerprints and physicochemical properties) to determine which method(s) resulted in the most appropriate selection of source chemicals (analogues) for the purposes of serving as a template in PBK model development for a target chemical.

(ii) Develop a KNIME workflow for assisting with analogue selection, employing the similarity metrics identified as being most appropriate; this utilises the PBK model dataset (PMD) from Chapter 2 as a source for identifying chemicals with existing PBK models

(iii) Test the KNIME Workflow for Assisting Analogue Selection (KWAAS) using six different types of chemicals (pesticides, botanicals, drugs, cosmetics, food additives and industrial chemicals) to demonstrate its utility across a range of industrial and regulatory sectors.

3.2 Methods

3.2.1 KNIME workflow for assisting analogue selection (KWAAS) development

3.2.1.1 *Building the KNIME workflow*

It was determined that there was a need for an automated process that gave the freedom of flexibility and adaptability when helping to identify similar chemicals. Thus, KNIME was determined to have these abilities that allow for an automated workflow to be created while giving the user the ability to adapt the workflow to their needs. After, several iterations of designing, testing and improving the workflow, the resulting KWAAS (fully outlined in 3.2.2) was created. To begin with, a method that calculated similarity using multiple fingerprints only and using expert judgement to analyse the results (generally considering a Tanimoto score of greater than 0.6 and chemical structure) was considered. However, this method could result in many chemicals requiring consideration. Thus, the inclusion of physicochemical properties to further refine results and reduce reliance on expert judgement in the early stages was considered preferable. This also helps to create a more reproducible and less subjective method of assessment. Initially, only molecular weight and log P were considered to be included in the workflow for refining the analogue selection. However, through discussion with project collaborators it was identified that log D and pKa would also be useful for refining results.

Another need, identified in the project discussions, was the ability to search the PMD for available models for the target as a first step in a universal workflow. The ability to search by more than just chemical name is essential, as became apparent during the systematic review (described in Chapter 2). There are often many different names for the same chemical or

minor differences in the spelling of the name, hence searching by name alone is not practical. A more reliable method of identifying unique chemicals is the use of identifiers, such as CAS registry numbers, SMILES strings or InChiKeys. There are known problems with CAS numbers, for example, multiple CAS numbers for the same chemical. Similarly, although SMILES strings are commonly used identifiers, there can be many SMILES representations for the same chemical. Therefore, there was a need to include an additional identifier that overcomes the problems associated with SMILES or CAS numbers. For this reason, the international chemical identifier (InChiKey) which is unique to a chemical was deemed most appropriate to include in the workflow when searching for specific chemicals within the PMD.

Additional features that were also deemed useful, were the ability to search the PMD for the target chemical or its analogues, refining the results by model characteristics (e.g. by sex, route of administration, species, etc). This would allow the user to identify the most appropriate models and the reference to the original source of the models. A node to allow a user to export results at any stage of the workflow was included to give the user the flexibility to use the nodes (methods of refining analogue selection) they identified as most appropriate for a given query. At every iteration in the design of the workflow, example chemicals were inputted to test its usability and ascertain the suitability of the results produced at each stage. Once the final format for the workflow had been established, six different chemicals (one from each dataset investigated in Chapter 2) was inputted to test the final workflow created.

3.2.1.2 Generating Identifiers and Properties

A spreadsheet of physicochemical properties was generated using RDKit as stated in Section 2.2.4. A user can generate these or other physicochemical properties using multiple resources, for example RDKit (www.rdkit.org), EPISUITE (<https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface>), ADMETlab 2.0 (<https://admetmesh.scbdd.com/service/evaluation/cal>) and Opera (<https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.html>). The predicted properties pKa and log D at pH 7.4 and 5.5 values using OPERA (<https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.html>) were generated. Log D is predicted at pH 5.5 and 7.4. OPERA automatically removes compounds for which a value cannot be calculated. For example unusually large compounds,

organometallics, mixtures and inorganic produced errors and were discarded by Opera. Thus, some chemicals in the PMD may not have pKa or log D values associated with them.

These spreadsheets of information were integrated into the KNIME workflow to use throughout the refinement and selection of appropriate chemicals using the selected nodes in KNIME. The physicochemical properties used within the KWAAS can be adapted by the user. Herein, molecular weight, log P, pKa and log D are included within the KWAAS, however a user could adapt the workflow to include Lipinski rule violations (or any other ADME properties they choose to predict and add to the spreadsheet). The chemicals for which Opera was unable to calculate values, e.g. pKa or pKb, have been left blank in the dataset and workflow and a user can use expertise whether to consider these chemicals as similar or not. The user can integrate these other properties into the KWAAS and use these to refine analogue selection, either instead of or in addition to the current properties included. The flexibility and adaptability of the workflow ensure that the user can use it however best suits their needs.

The addition of ADME properties (e.g. PPB/F_{ub}, Cl_{int}, absorption, tissue partitioning, etc.) were considered for inclusion in the similarity tool. However, which predicted ADME properties to include that are useful and relevant to a PBK model will depend on the user's requirements and the model being created. For example, a skin model will require skin absorption, whereas an intravenous model would not. It was determined that these properties may be best for the user to generate themselves, after having completed the workflow using fingerprints and physicochemical properties as the initial step. Once a list of potential analogues has been obtained these could be refined based on ADME properties, selected by the user as being most relevant.

3.2.2 Using the KWAAS

A workflow of the similarity tool created in KNIME is summarised in Figure 3.2. The figure outlines the process of using the KNIME workflow to search the database and determine similar analogue/s, where the number of analogues suggested as similar decrease as you refine using more properties. The KNIME workflow developed has three main stages listed below.

1. Search PBK model dataset (PMD) for an available model for the target chemical
2. Determine similar analogues to the target (i.e. potential source chemicals) based on structure and relevant properties, such as physicochemical properties
3. Refine the selection of source chemicals as necessary

A guide on how to use the similarity tool is in Appendix 6. The KNIME workflow can be downloaded from Github (<https://github.com/courtneythomp/KWAAS>).

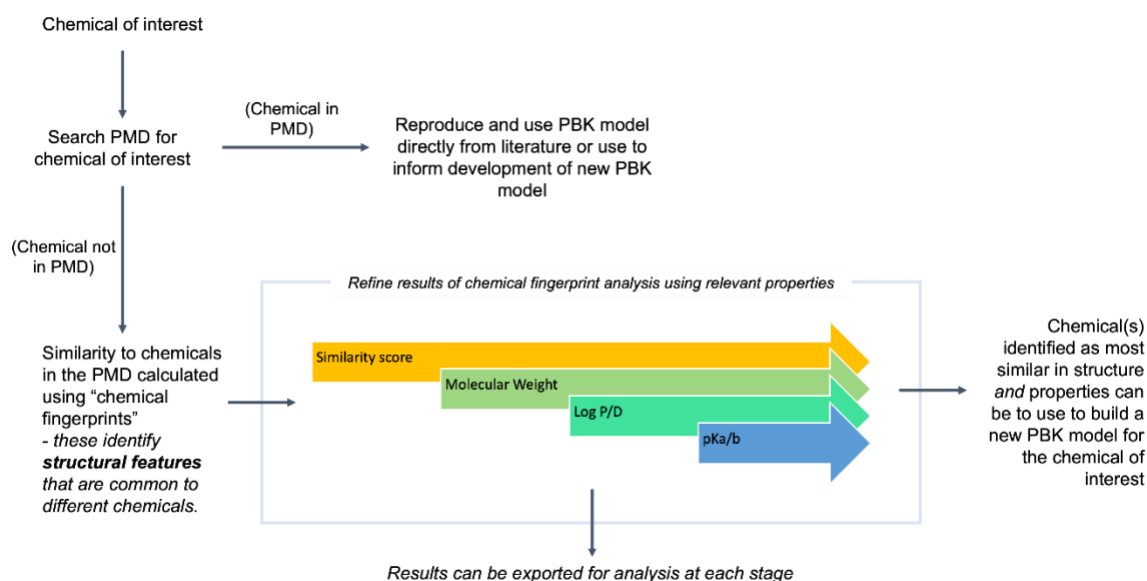


Figure 3.1 A summary of the KWAAS process, briefly outlining the process of using the KNIME workflow to search the database and determine similar analogue/s, where the number of analogues suggested as similar decrease as you refine by more properties shown in the coloured arrows.

3.2.2.1 Stage 1

To begin, the workflow checks the PBK dataset for available models for the target chemical. Chemical name, InChiKey or SMILES for the target chemical is required to search the PBK model dataset in the first stage of the workflow. InChiKeys are unique to the compound and express more information than SMILES; chemical isomers may have the same SMILES string but different InChiKeys. Chemicals can have many possible names and spellings, unless the same name and spelling is used as recorded in the PMD then no results may appear. Thus, in the first instance InChiKey is recommended for searching, however, SMILES can be used as can the chemical name. InChiKey and SMILES can be obtained from multiple webtools or websites, e.g. PubChem (<https://pubchem.ncbi.nlm.nih.gov>), ChemSpider

(<https://www.chemspider.com>). Chemical name, InChiKey or SMILES of the target chemical needs to be inputted and any available models matching the input will be given in an Excel file with all associated information from the dataset included.

3.2.2.2 Stage 2

If there were no models available in the PBK modelling dataset or if the models in the dataset were not relevant (in the opinion of the user) then the second stage can be used to identify similar chemicals from the dataset for which there are available PBK models.

Chemical fingerprints can be used to determine how similar one chemical is to another. A Tanimoto score can be generated, ranging from 0 (not similar) to 1 (very similar or identical). The workflow generates Tanimoto similarity scores for the source chemicals' similarity to the target chemical using nine different fingerprints. The results are ranked from most similar to least similar and the top five results identified for each type of the nine fingerprints are given (theoretically giving a maximum of 45 potential chemicals, although in many cases the same chemical will be ranked in the top five by multiple fingerprints). As each type of fingerprint can identify different analogues (as discussed in Section 3.1) nine different fingerprints are used to capture a wider range of chemicals that can be considered as similar chemicals. Analogues that are identified as similar by one type of fingerprint only are rejected, as this indicates the similarity is being assessed using unique criteria that does not represent more general similarity.

SMILES strings are used when determining the top five similar analogues by the nine different types of fingerprints. The chemical name can be inputted alongside the SMILES as a useful additional identifier. The fingerprint types used within the workflow include Morgan, FeatMorgan, AtomPair, Torsion, RDKit, Avalon, Layered, MACCS, and Pattern as these are readily available in RDKit. An Excel file of the results including chemical name, SMILES and structures of the analogue can be obtained at this stage. Refinements to the analogues can be made based on Tanimoto score and physicochemical properties. Once these 45 chemicals (different fingerprint algorithms may identify the same chemical/s as similar) identified as similar are obtained, the chemicals with a similarity score below 0.6 are removed. A minimum Tanimoto score of 0.6 (a commonly used cut off value within literature (Enoch et al., 2009))

is recommended for an analogue to be included for the next step in the workflow, however, this value can be adjusted by the user.

Physicochemical properties including, molecular weight, log P, pKa/pKb, and log D can all be used to further determine how similar one chemical is to another. The physicochemical properties (molecular weight, log P, TPSA, hydrogen bond donors/acceptors, and the number of Lipinski rule violations) from the PBK dataset in Chapter 2 are added to the table of similar chemicals. In this analysis molecular weight and log P were included into the workflow refinement options to identify the most similar chemicals; inclusion ranges can be set and adjusted by the user. A user can also select different physicochemical properties as discussed above.

For testing the workflow, the properties pKa and log D at pH 7.4 and 5.5 (predicted using OPERA) were included in refining the analogue selection. The user can input an appropriate range for log P; often ± 0.5 of the target chemicals' value is selected as it is commonly regarded as being the range of experimental error for log P. However, in order to not exclude too many chemicals (hence obtain too few or no candidates for source chemicals) the range can be expanded to ± 1 of the target chemicals' value. Log D (at pH of either 5.5 or 7.4) of ± 1 is recommended due to the problems associated with predicting log D compared to log P. Filtering by pKa/pKb (predicted by Opera) was the last step considered within the workflow during the testing phase. The user has the option to refine results by pKa/pKb by inputting an appropriate range. Values of ± 1 log of the target chemicals pKa are recommended to refine analogue selection. At every stage of the workflow there is the option to export the results to Excel. Figure 3.3 outlines the stages of refinement of the analogues. When using the KWAAS fingerprints, molecular weight and log P are recommended to be used initially as these are the most universal parameters. In addition, it is recommended that at every stage of the workflow the user looks at the structures of the chemicals identified as similar.

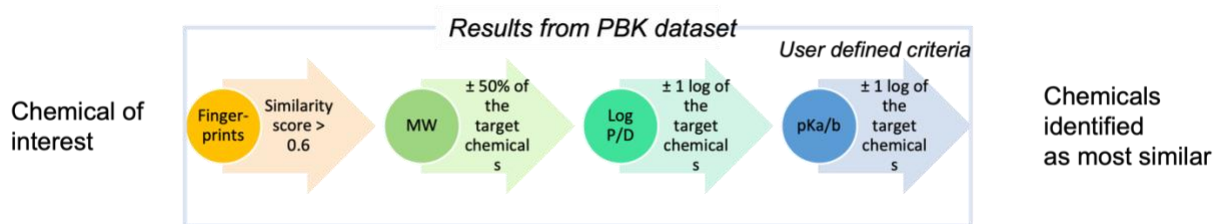


Figure 3.3 Stages of refinement and the recommended ranges to use at each stage of the KWAAS – user may adapt any of these as required. These are preliminary recommendations for using the KWAAS the user can add any other properties for selection that they can obtain and identify as being useful.

3.2.2.3 Stage 3

Finally, at the end of the analogue selection process, the PBK model dataset can be searched again, this time searching for all analogues that have been identified as being similar to the target. This will enable an Excel file to be generated containing information on all of the available PBK models associated with these analogues. All information associated with the PBK models, as recorded in the PBK modelling dataset, will be extracted. Further filtering of the available PBK models can be undertaken by the user with the option of filtering the PBK model dataset output by species, availability of equations, software, sex, life-stage, etc. The results at each step (i.e. after the application of each filtering node) can be exported to Excel and examined as described in Stage 2.

3.2.3 KWAAS testing

To test the tool's usability across different chemical types as relevant to different sectors, one chemical was chosen from each of the six datasets described in Chapter 2 (i.e. botanicals, pesticides, cosmetic ingredients, food additives, pharmaceuticals, and REACH chemicals). For each of the six chemical types, the chosen chemical's name was searched in PubChem to obtain its InChiKey; this was used for the initial search of the PBK dataset for available models. The availability of any PBK models for the selected chemicals themselves was recorded - Stage 1 of the KWAAS. The selected chemicals were then inputted into Stage 2 of the KWAAS i.e. to identify similar chemicals with existing PBK models. In this analysis all chemicals were progressed into Stage 2, even if at Stage 1 it was determined that PBK models were available for the chemical of interest itself. This was to determine how well the KWAAS performed in selecting analogues for the different types of chemicals.

Originally a range of 100 Da either side of the target chemicals molecular weight was considered for inclusion. However, this was determined to be insufficient when working with extreme values i.e., 50 or 4000 Da. Thus, it was determined that $\pm 50\%$ of the target chemical's molecular weight was ideal to account for chemicals that could have significantly large or small molecular weight. Inclusion ranges for log P, and log D (at pH 5.5) were selected to be within ± 1 log of the target.

Genistein was chosen at from a list of common botanical chemicals to represent botanicals. A molecular weight of 270.24 Da and log P of 2.58 for this chemical were obtained from the PBK dataset values. Bicyclopyrone was selected, a pesticide of interest by one of the industrial collaborators, hence was used for studying this type of chemical. A molecular weight of 399.4 Da was obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov>; accessed May 2022, log P of 1.45 obtained from ChemSpider (<https://www.chemspider.com>; accessed May 2022). Benzoic acid was used to represent the cosmetics as it is a common ingredient used within cosmetics. A molecular weight of 122.12 Da and log P of 1.3848 were obtained from the PBK dataset values. Methyleugenol was chosen to represent food additives, as this chemical had been part of the prior investigation by Paini et al. (2021b) and could be used to confirm the results from the KWAAS. A molecular weight of 178.23 Da and log P 2.43 were obtained from the PBK dataset values. Loxapine was chosen to represent pharmaceuticals. Molecular weight of 327.8 Da obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov>; accessed May 2022), log P of 3.6. A chemical of high concern (benzyl butyl phthalate) from the ECHA (<https://echa.europa.eu/chemicals-in-our-life/which-chemicals-are-of-concern/svhc>; accessed May 2022) was selected to represent industrial chemicals (REACH), this was selected as it is of high production value and therefore potentially of greater concern. Molecular weight of 312.4 Da was obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov>; accessed May 2022), log P of 4.82 was obtained from CompTox (<https://comptox.epa.gov/dashboard/>; accessed May 2022). Log D of 4.68 at pH 7.4 was obtained from ADMETlab 2.0 (<https://admetmesh.scbdd.com/service/evaluation/cal>; accessed May 2022).

3.3 Results

To test the tool's applicability across multiple types of chemicals, one chemical from each of the six datasets identified in Chapter 2.2.4 was selected. Initially, the PMD was searched for any available PBK models for each target chemical using its InChiKey.

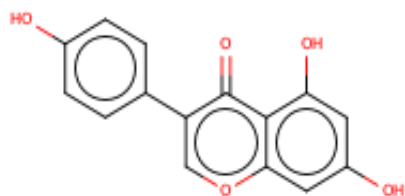
For botanicals, the chemical genistein was used, with a total of five PBK models for this chemical being found in the PBK model dataset. Stage 2 of the KWAAS was still used to identify similar chemicals to genistein. Table 3.2 summarises the target chemicals' property information and the source of this information, as well as the inclusion ranges used within the KWAAS process, and the number of chemicals identified as similar at each stage. Refining by molecular weight, log P and pKa made the similarity search too specific for genistein, resulting only in genistein itself being identified by the KWAAS. Thus, using fingerprints, molecular weight and log P alone within the KWAAS, a reasonable number of analogues (four) were identified, and this was determined a reasonable point at which to stop refining the selection. The four chemicals identified as similar and genistein's (the target's) structure are displayed in Figure 3.4, with the similarity scores calculated by different chemical fingerprint types also displayed (a discussion of the apparent similarities based on visual inspection are described in section 3.4).

Table 3.2 The target chemical, genistein, the inclusion range at each step of the refinement process, when applying the KWAAS, and the number of chemicals identified at each stage.

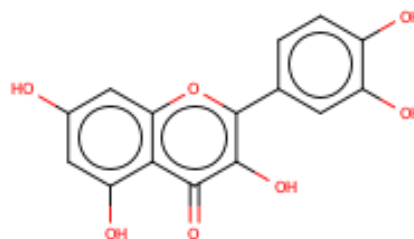
Features for searching and refining	Target - Genistein	Inclusion range for refining results	Number of chemicals identified at relevant KWAAS filter
Fingerprints			11*
Tanimoto score		> 0.6	9*
Molecular weight	270.24 Da <i>(from PBK dataset in Chapter 2)</i>	135.12 – 405.36 Da	7*
Log P	2.58	1.58 – 3.58	5*

	(from PBK dataset in Chapter 2)		
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*results include the target chemical, genistein.

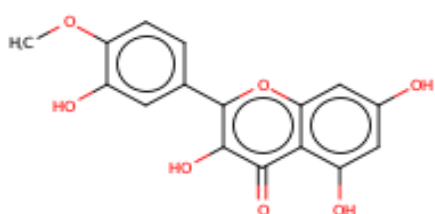


genistein (target)



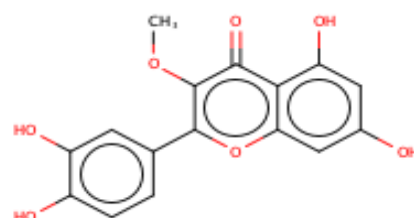
quercetin

0.618 (FeatMorgan), 0.65 (AtomPair),
0.676 (RDKit), 0.844 (Layered), 0.931
(MACCS), 0.813 (Pattern)



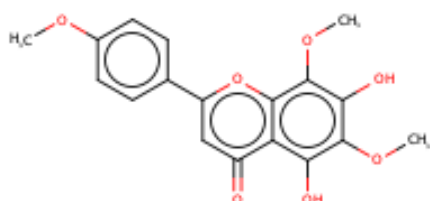
4'-o-methylquercetin

0.663 (RDKit), 0.797 (Layered), 0.871
(MACCS), 0.807 (Pattern)



3'-o-methylquercetin

0.688 (Avalon), 0.751 (Layered), 0.844
(MACCS), 0.807 (Pattern)



nevadensin

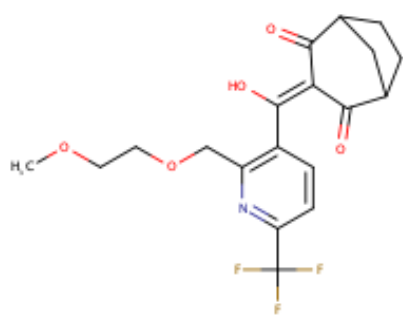
0.754 (Avalon), 0.716 (Layered), 0.818
(MACCS)

Figure 3.4 Structure of target chemical, genistein and the chemicals identified as similar after refining by log P

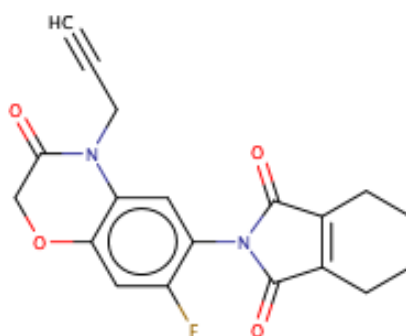
Bicyclopyrone had no available PBK models in the PMD. Initially, 42 unique chemicals were identified as similar to bicyclopyrone using fingerprints alone in the KWAAS, hence results were refined using MW and Log P. A summary of the chemical property information and the source of this information for the target chemical, as well as the inclusion ranges used within the KWAAS process, and the number of chemicals identified as similar at each stage are shown in Table 3.3. The three chemicals identified as similar and bicyclopyrone (target) structure are displayed in Figure 3.5, with the similarity scores calculated by different chemical fingerprint types also displayed.

Table 3.3 The target chemical, bicyclopyrone, the inclusion range at each step of the refinement process, when applying the KWAAS, and the number of chemicals identified at each stage.

Features for searching and refining	Target - Bicyclopyrone	Inclusion range for refining results	Number of chemicals identified at relative KWAAS filter
Fingerprints			42
Tanimoto score		> 0.6	15
Molecular weight	399.4 (PubChem (https://pubchem.ncbi.nlm.nih.gov ; accessed May 2022))	199.7 – 599.1 Da	12
Log P	1.45 (ChemSpider (https://www.chemspider.com ; accessed May 2022))	0.45 -2.45	3

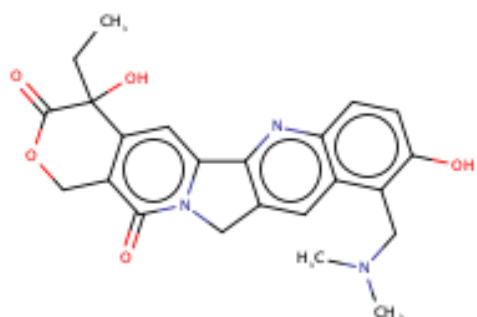


bicyclopyrone (target)



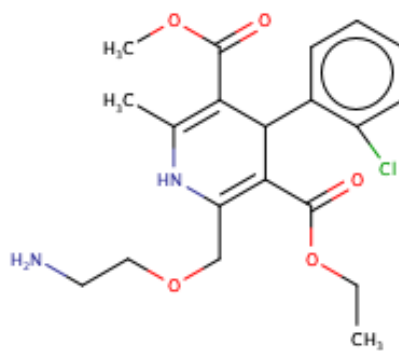
flumioxazin

0.706 (Layered)



topotecan

0.736 (RDKit)



amlodipine

0.702 (Layered)

Figure 3.5 Structure of target chemical, bicyclopyrone, and the chemicals identified as similar after refining by log P

Initially, four PBK models were identified in the PMD for benzoic acid before determining similarity. Table 3.4 summarises the target chemical's property information and the source of this information, as well as the inclusion ranges used within the KWAAS process, and the number of chemicals identified as similar at each stage. The five chemicals identified as similar and benzoic acid (target) structure are displayed in Figure 3.6, with the similarity scores calculated by different chemical fingerprint types also displayed.

Table 3.4 The target chemical, benzoic acid, the inclusion range at each step of the refinement process, when applying the KWAAS, and the number of chemicals identified at each stage.

Features for searching and refining	Target - Benzoic acid	Inclusion range for refining results	Number of chemicals identified at relative KWAAS filter
Fingerprints			12*
Tanimoto score		> 0.6	9*
Molecular weight	122.12 Da <i>(from PBK dataset in Chapter 2)</i>	61.06 – 183.18 Da	7*
Log P	1.38 <i>(from PBK dataset in Chapter 2)</i>	0.38 – 2.38	6*

*results include the target chemical, benzoic acid.

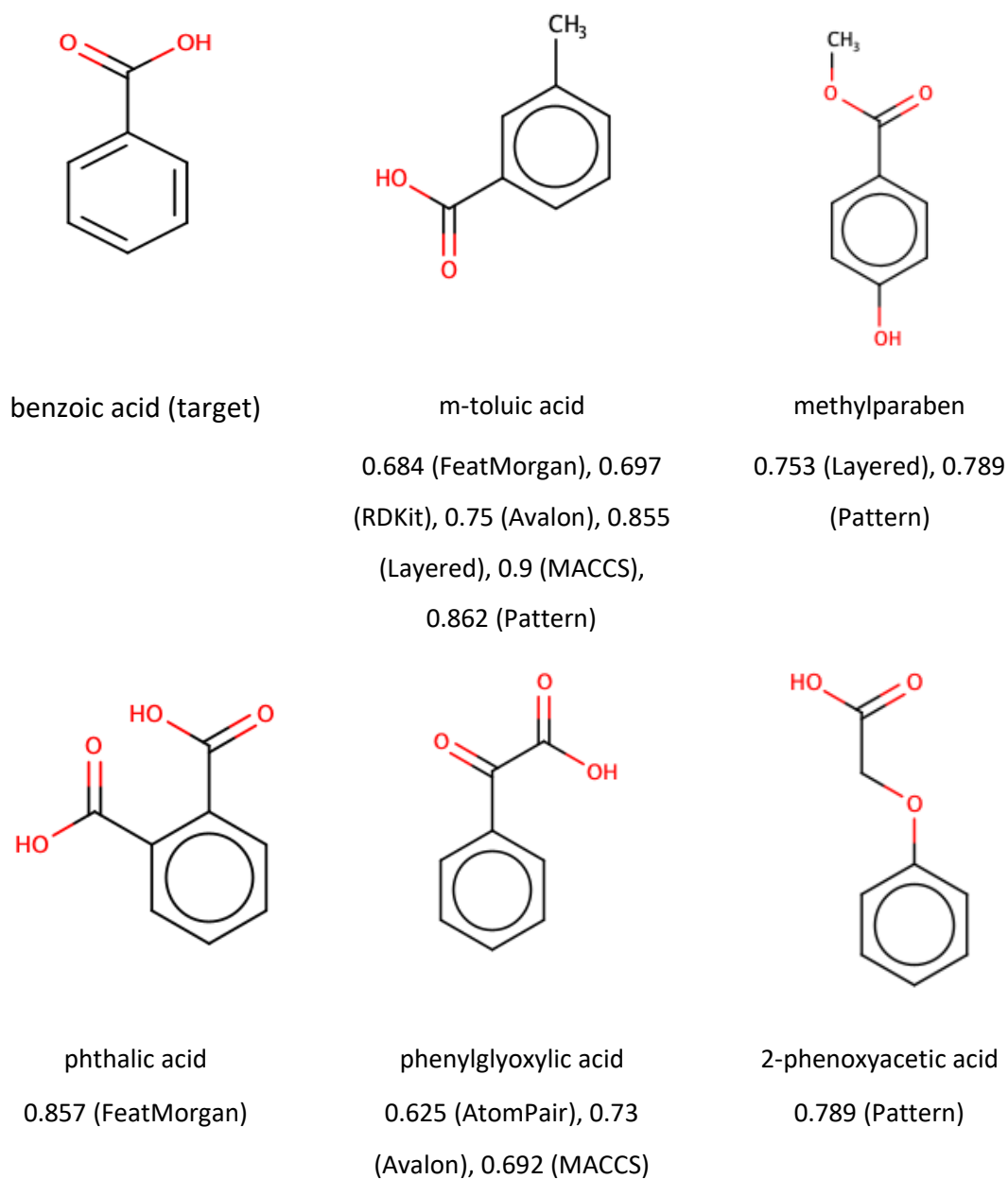


Figure 3.6 Structure of target chemical, benzoic acid, and the chemicals identified as similar after refining by log P

Using the chemical methyleugenol for the food additives sector, a rat and a human PBK model were available in the literature for safrole. A total of 11 chemicals, including methyleugenol, were initially identified as similar. Table 3.5 summarises the target chemical's property information and the source of this information, as well as the inclusion ranges used within the KWAAS process, and the number of chemicals identified as similar at each stage. The eight chemicals identified as similar and methyleugenol (target) structure are displayed in Figure 3.7, with the similarity scores calculated by different chemical fingerprint types also displayed. Note that some of chemicals identified as similar were metabolites of the target, here the PBK model of interest would be the one for the parent.

Table 3.5 The target chemical, methyleugenol, the inclusion range at each step of the refinement process, when applying the KWAAS, and the number of chemicals identified at each stage.

Features for searching and refining	Target - Methyleugenol	Inclusion range for refining results	Number of chemicals identified at relative KWAAS filter
Fingerprints			11*
Tanimoto score		> 0.6	11*
Molecular weight	178.23 Da <i>(from PBK dataset in Chapter 2)</i>	89.12 – 267.35 Da	10*
Log P	2.43 <i>(from PBK dataset in Chapter 2)</i>	1.43 – 3.43	9*

**results include the target chemical, methyleugenol.*

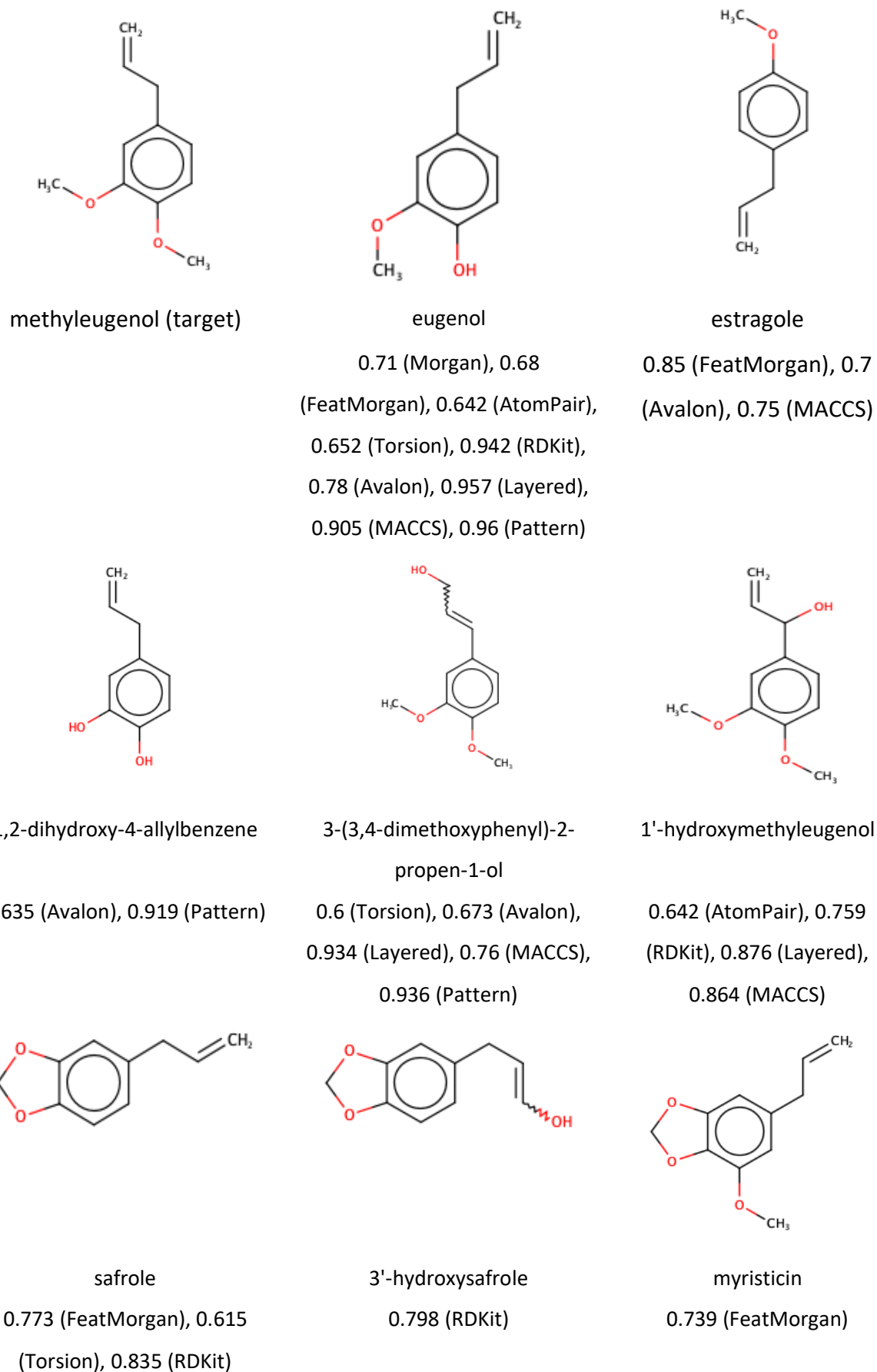
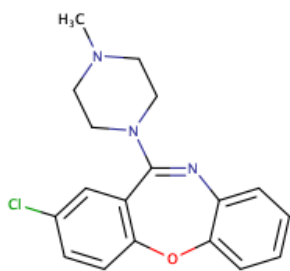


Figure 3.7 Structure of target chemical, methyleugenol, and the chemicals identified as similar after refining by log P

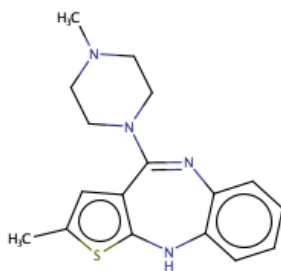
The pharmaceutical sector was represented using the chemical loxapine. A summary of the chemical property information and the source of this information for the target chemical, as well as the inclusion ranges used within the KWAAS process, and the number of chemicals identified as similar at each stage is given in Table 3.6. The five chemicals identified as similar and loxapine (target) structure are displayed in Figure 3.8, with the similarity scores calculated by different chemical fingerprint types also displayed.

Table 3.6 The target chemical, loxapine, the inclusion range at each step of refining the results from the KWAAS and the number of chemicals identified at each stage.

Features for searching and refining	Target - loxapine	Inclusion range for refining results	Number of chemicals identified at relative KWAAS filter
Fingerprints			18
Tanimoto score		> 0.6	13
Molecular weight	327.8 Da (PubChem (https://pubchem.ncbi.nlm.nih.gov ; accessed May 2022))	163.9 – 491.7 Da	7
Log P	3.6 (DrugBank (https://go.drugbank.com ; accessed May 2022))	2.6 – 4.6	5

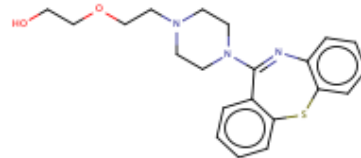


loxapine (target)



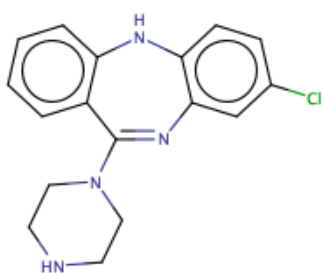
olanzapine

0.629 (AtomPair), 0.717
(Layered), 0.677 (MACCS),
0.771 (Pattern)



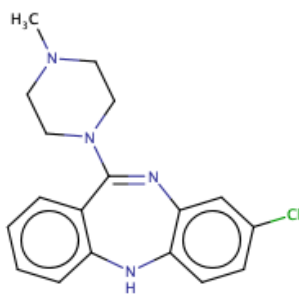
quetiapine

0.604 (Avalon), 0.762
(Layered), 0.801 (Pattern)



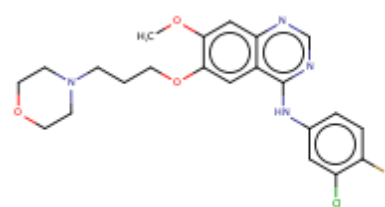
norclozapine

0.633 (Avalon), 0.805
(Layered), 0.712 (MACCS),
0.838 (Pattern)



clozapine

0.651 (FeatMorgan), 0.751
(AtomPair), 0.6 (Torsion),
0.674 (Avalon), 0.81
(Layered), 0.836 (MACCS),
0.861 (Pattern)



gefitinib

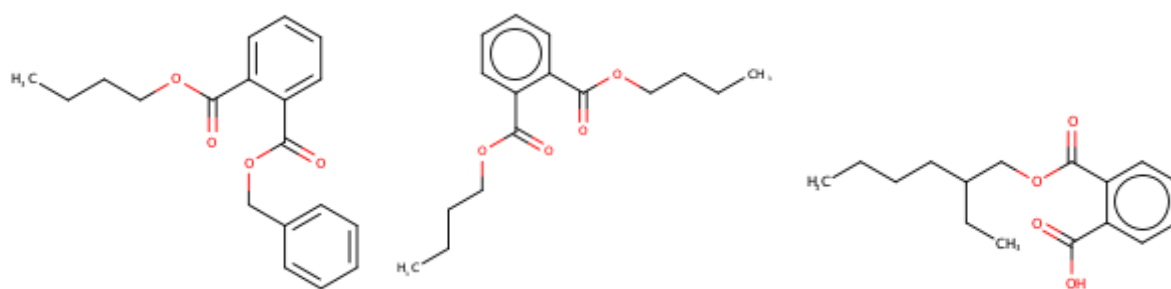
0.687 (MACCS)

Figure 3.8 Structure of target chemical, loxapine, and the chemicals identified as similar after refining by log P

Benzyl butyl phthalate was used to represent the industrial sector. A summary of the chemical property information and the source of this information for the target chemical, as well as the inclusion ranges used within the KWAAS process, and the number of chemicals identified as similar at each stage is given in Table 3.7. Filtering using log P resulted in no similar chemicals being identified, therefore log D was used as a filter in this case. The two chemicals identified as similar, and benzyl butyl phthalate (target) structure are displayed in Figure 3.9, with the similarity scores calculated by different chemical fingerprint types also displayed.

Table 3.7 The target chemical, benzyl butyl phthalate, the inclusion range at each step of the refinement process, when applying the KWAAS, and the number of chemicals identified at each stage.

Features for searching and refining	Target - Benzyl butyl phthalate	Inclusion range for refining results	Number of chemicals identified at relative KWAAS filter
Fingerprints			11
Tanimoto score		> 0.6	8
Molecular weight	312.4 Da (PubChem (https://pubchem.ncbi.nlm.nih.gov ; accessed May 2022))	156.2 – 468.6 Da	8
Log P	4.82 (CompTox (https://comptox.epa.gov/dashboard/ ; accessed May 2022))	3.82 – 5.82	0
Log D (pH 7.4)	4.68 (ADMETlab 2.0 (https://admetmesh.scbdd.com/service/evaluation/cal ; accessed May 2022))	3.68 – 5.68	1



benzyl butyl phthalate
(target)

di-n-butylphthalate

0.781 (Morgan), 0.826
(FeatMorgan), 0.676
(Torsion), 0.867 (RDKit),
0.953 (Avalon), 0.94
(Layered), 0.893 (MACCS)

mono(2-ethylhexyl)
phthalate

0.795 (RDKit), 0.6 (Avalon),
0.917 (Layered), 0.719
(MACCS), 0.89 (Pattern)

Figure 3.9 Structure of target chemical, benzyl butyl phthalate, and the chemicals identified as similar after refining by log D

3.4 Discussion

A KNIME workflow was developed to aid in the selection of similar chemicals (with existing PBK model(s) that could be used as a template to build new PBK models) through an iterative process of design and testing. The KWAAS was successfully run for six example chemicals, identifying where PBK models existed for the target itself in addition to finding similar chemicals with PBK models. Initial visual inspection of the analogues selected for five of the chemicals (genistein, benzoic acid, methyleugenol, loxapine and benzyl butyl phthalate) indicate one or more of these may be suitable starting points for developing new PBK models in a read-across approach. However, the chemicals identified for bicyclopurone were more diverse. Initially, assessing similarity by chemical fingerprints, molecular weight and log P is recommended as these properties are universal. Other properties can be used to refine the results by, and these can be added by the user. However, in this analysis for some chemicals the additional properties were too specific to a target chemical; hence if the target was already in the PMD, it was the only chemical identified as being “similar”. The results shown

in Section 3.3 demonstrate that the tool works satisfactorily, in that it can identify potential analogues and refine this selection using criteria deemed appropriate by the user. Although the KWAAS was tested here using molecular weight and log P, this may not be appropriate for all cases. Thus, not all chemicals identified may be suitable analogues, before taking the analysis further (e.g. developing a new PBK model) the user would need to use expert judgement to determine the suitability of the analogues.

Overall, the capabilities of the KWAAS described in this chapter have been tested using six different types of chemicals, thus demonstrating how analogue selection may be performed. Visual analysis indicates that reasonable source chemicals were identified for most chemicals following refinement. Each target chemical had at least ten chemicals identified as being as similar using fingerprints alone. There was a greater selection of chemicals with PBK models determined as being similar to methyleugenol (food additives) compared to genistein (botanicals). The structures of the chemicals identified as similar to genistein in Figure 3.4 are visually similar to each other compared to the structures of the chemicals identified as similar to bicyclopyrone in Figure 3.5 which are visually very different. Genistein has 3 benzene rings as do the four chemicals identified as similar, whereas bicyclopyrone has no key visual structures the same between it and the identified similar chemicals. The five chemicals identified as similar to benzoic acid (see Figure 3.6) were very similar in structure to benzoic acid, as were the suggested analogues for methyleugenol (Figure 3.7). However, when compared to the analogues found for loxapine (Figure 3.8) and benzyl butyl phthalate (Figure 3.9), they were less visually similar. This can be seen with loxapine having a chlorine attached to a benzene ring but two of the suggested analogues not having any chlorine attached to the structure.

The target chemical (bicyclopyrone) from the pesticide dataset resulted in the largest number of chemicals (42) initially identified as similar, this was expected as the pesticide dataset had one of the largest number of chemicals present in the PMD. However, the number of chemicals decreased significantly as the physicochemical property filters were included, this may be due to pesticides having a narrower range of values for physicochemical properties than other datasets. Although the three chemicals (flumioxazin, topotecan and amlodipine) identified as similar to bicyclopyrone after using the KWAAS visually look different (see Figure

3.5), they were highlighted as being similar by physicochemical properties (molecular weight within 50% of bicyclopyrone and log P within 1 log also) and Tanimoto score (all three analogues had a Tanimoto score greater than 0.7).

Not surprisingly, metabolites for chemicals are commonly highlighted as similar chemicals to given targets, particularly when using chemical fingerprints as the basis for similarity. This is because fingerprints identify key common features, and it is likely that many of these would be found in both parent and metabolite. There is a need for expert judgement when determining the most useful chemical with a PBK model to use as a template. Layered fingerprints was the only fingerprint type that had identified at least one chemical as similar after all refinements were included in the KWAAS. However, RDKit identified at least one chemical as similar after all refinements were included for all target chemicals except, loxapine. Torsion was the least successful fingerprint type to have identified similar chemicals after including all refinements, this could be as its designed to compare small molecule conformations.

The tool identifies analogues with published PBK models which can be used in two main ways. One method uses the exact equations of the model of the source chemical as a template to develop a new PBK model for a target chemical by changing parameter values to that of the target chemicals. This method will be undertaken in the next Chapter (Chapter 4). The second method, which is more qualitative, provides information to adapt a generic PBK model so that the key process appearing in the model for the source chemical are reflected in the model for the target chemical. Thus, rapidly providing a more accurate chemical-specific model that is suitably informative. Additionally, this method can be advantageous when there are difficulties in reproducing a PBK model, for example where insufficient model or parameter details are available for the published model. This tool can facilitate the inclusion of PBK model-derived data for a wide range of chemicals for which PBK models are currently lacking. This can help to fill the knowledge gaps within current toxicokinetic information for chemicals that are data poor. Using data from rationally selected analogues can help towards the integration of information from alternative methods into regulatory practice. This will assist in producing more accurate safety assessments of chemicals through providing supporting information, so increasing uptake of read-across for regulatory purposes. The KWAAS is an

option to use for those with KNIME expertise or expertise using similar software as the workflow can be altered and adapted readily, e.g. by adapting the Excel spreadsheet to include further physicochemical properties or altering ranges used for inclusion criteria when selecting analogues.

The automated approach taken within this chapter for determining similarity using structural similarity and physicochemical properties to use the information from the PBK models for source chemicals in a read-across approach to build new PBK models. Whereas previously Ellison (2018) evaluated a PBK model using *in vivo* data from structural and functional analogues, and Ellison and Wu (2020) evaluated PBK models built in the absence of *in vivo* PK data (on *in vitro* and *in silico* data only). This functional analogue approach focused on using significant expertise of chemical structure to determine similar analogues, and the functional analogue approach focused on comparing area under the curve (AUC) and maximum concentration (C_{max}) to determine the parameters most influencing predictions and then assessing similarity based on specific parameter values (bioavailability, volume of distribution and clearance). This approach requires a lot of research and expertise to determine similar analogues. Lu et al. (2016a) used a correlation matrix of multiple physicochemical properties to identify similar chemicals. This approach requires expertise in understanding the ranking of results to determine the most suitable analogue, and structural similarity was not assessed for determining similar analogues. However, there is no consensus among researchers regarding the best molecular descriptors and similarity calculations to use to determine similarity for read-across. Having a structured method, as developed here, can increase the uptake of using a read-across approach for PBK modelling.

This tool will be useful across all industries and could become increasingly significant if the dataset were to be populated with additional PBK models to use as templates. In future, when choosing the most appropriate PBK model, considering factors such as the reproducibility of the PBK model and the confidence in obtaining the correct input parameters could also be included into the KWAAS refinement criteria to help choose a suitable PBK model. Including ADME properties into the KWAAS to refine chemicals by would help identify chemicals that are similar for the purpose of the PBK model being created. For example, refining by skin absorption to ensure chemicals have similar kinetics for creating a model for a chemical that

may be to be administered (or exposed) via the skin. To further the analysis of the KWAAS and determine the suitability of the source chemicals proposed, two specific case studies are undertaken in Chapter 4. The KWAAS is used for two chemicals, atenolol and flumioxazin, for which new PBK models are built using available PBK models for chemicals identified by the KWAAS as being similar.

Chapter 4 – Application of the KNIME Workflow for Analogue Selection

4.1 Introduction

As referred to in Section 1.6 the number of papers published in recent years on PBK modelling has significantly increased (Paini et al., 2017, Lu et al., 2016a). The purpose of the PBK model dataset (PMD, described in Chapter 2) and the KNIME Workflow for Assisting Analogue Selection (KWAAS, described in Chapter 3) is to enable the process by which information from an existing PBK model for one chemical can be used to inform the development of a PBK model for another chemical that has been identified as being similar. This requires the existing model to be adequately reported and a suitable method to be employed for identifying similar chemicals.

Regarding chemical similarity, Lu et al. (2016) previously used a quantitative comparison of physicochemical properties to determine similarity when identifying which chemicals would be suitable as analogues for developing new PBK models. However, it is important to also capture similarity in terms of chemical structure. Hence, the need for a structured approach to determining similarity; this was achieved through the development of the KWAAS (outlined in Chapter 3) enabling a user to optimise analogue selection based on physicochemical properties, chemical fingerprints or other criteria subject to additional data (such as ADME properties) being supplied.

Obtaining the required input parameters for PBK models can be time-consuming and resource intensive (Punt et al., 2011). Physiological parameters (e.g. organ volume, blood flow rate), ADME properties (e.g. clearance, intestinal absorption) and physicochemical properties (e.g. log P, pKa, hydrogen bonding ability), are all required to build a PBK model (Paini et al., 2019). Efforts to predict ADME properties through *in silico* methods are increasing (see Madden et al. (2020) and Madden and Thompson (2022) for further resources), however experimental data are generally preferred.

There are multiple software that predict a range of physicochemical properties such as Opera (<https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.html>), ADMETlab (<http://admet.scbdd.com/calcpred/index/>), EPI SUITE (<https://www.epa.gov/tsca->

[screening-tools/epi-suitetm-estimation-program-interface](#)), etc. Variability in the output of a PBK model is expected where different values for these properties are used to generate the model. For example, higher log P values are associated with more of the chemical being absorbed by the body (leading to a higher concentration blood) until the log P reaches a value beyond which the amount of chemical absorbed decreases. Consequently, the value for log P that is incorporated in the model can have a significant effect on predicted internal concentrations.

The format for the reporting of PBK models in the literature varies considerably, as does the level of detail included in the reports of PBK models (Tan et al., 2020). Hence, when reproducing literature models this can lead to difficulties. However, the ability to reproduce these models accurately would enable wider application of the knowledge therein, which is especially important with the increasing number of models that can be found in the literature. This need to improve reporting standards for PBK models has led to the recently published OECD (2021b) guidance document on the characterisation, validation and reporting of PBK models. The guidance, which focuses on the use of alternative methods, incorporates a PBK model reporting template (shown in Table 4.1 below, as reported in OECD (2021b)) to ensure the level of detail required to reproduce a model is included.

Table 4.1 PBK Model Reporting Template (as given in OECD, 2021b)

PBK Model Reporting Template sections	Brief description of information to report for each section
A. Name of model	Provide a title of the model. The same should be reported in the checklist.
B. Model developer and contact details	Contact details of model developer.
C. Summary of model characterisation, development, validation, and regulatory applicability	Please capture main points in a brief summary regarding the development, validation and regulatory application.
D. Model characterisation (modelling workflow) Step 1 – Scope and purpose of the model (problem formulation) Step 2 – Model conceptualisation (model structure, mathematical representation) Step 3 – Model parameterisation (parameter estimation and analysis) Step 4 – Computer implementation (solving the equations) Step 5 – Model Performance Step 6 – Model Documentation	Follow the 6 steps of the modelling workflow chapter two. Report in detail the model structure, model biological plausibility, and parameters with assumptions and limitations, tables can be placed under section H. parameter tables. Under model performance report information on sensitivity analysis, predictive performance. Strategy on how the model validation was performed, e.g. using analogues or other sources or approaches should be reported in detail.
E. Identification of uncertainties model structure input parameters model output other uncertainties (e.g. model developed for different substance and/or purpose)	For each step of the modelling workflow uncertainties should be reported. Use the information provided in the guidance to report and assess (e.g. table in figure 3.3. to capture information on sensitivity and uncertainty for input parameters).
F. Model implementation details software (version no) availability of code software verification / qualification	Information on the model equation solver/software to run the equation should be reported here.
G. Peer engagement (input/review)	Report the extent of peer engagement and review in development of the model.
H. Parameter tables	All information relevant to model parameterisation should be included here: physiological anatomical, physicochemical and biochemical. Report values and units and the source of the parameters (e.g. in case of <i>in vitro</i> studies detailed experimental conditions and motivation for choice of experimental conditions in case of non-guideline studies, in case of <i>in silico</i> studies add information on models).
References and background information publications links to other resources	Main reference and publications linked to development and description of the model

The software used to create PBK models traditionally required significant expertise to use. However, as highlighted in Section 1.3 accessibility of PBK modelling software has increased as more generic software is now available e.g., QIVIVE (<http://www.qivivetools.wur.nl>),

PKSim (<https://www.open-systems-pharmacology.org>) etc. Although software that requires specific knowledge of computational languages is still widely used, e.g., R (<https://www.r-project.org>) and MATLAB (<https://www.mathworks.com/products/matlab.html>). MATLAB was chosen for use in this study, due to its greater functionality and transparency as determined in the pilot study undertaken in Section 4.2.

The work described in this chapter was based upon the use of the KWAAS, outlined in Chapter 3, to identify existing PBK models for analogues of two target chemicals, the drug atenolol and the herbicide flumioxazin. Atenolol is a beta blocker - a drug commonly used to reduce blood pressure. Flumioxazin is a pesticide that was highlighted for use as a case study by an industrial partner of the research funding body for this project. These chemicals were used as case studies as both chemicals have existing PBK models, hence these chemicals are considered as “pseudo-unknowns”. It is important at this stage to have data against which model results can be compared, to give confidence in the approach as a proof-of-principle. The importance of assessing the reliability of the models is summarised in Section 1.4. Assessment of a model’s predictivity is commonly performed by determining the fold error of the most common pharmacokinetic metrics (C_{max} , T_{max} and AUC) and undertaking a global sensitivity analysis to determine the input parameters that have the biggest impact on the output variables.

The aims of this chapter were to:

(i) Demonstrate use of the KWAAS created in Chapter 3 by applying the approach to two case study target chemicals - atenolol and flumioxazin. Initially, source chemicals with PBK models available in the literature were identified, using the KWAAS. These models were reproduced for the source chemicals, and subsequently used as templates to enable new models to be derived for the two target chemicals using a read-across approach

(ii) Assess the performance of the newly-derived PBK models by comparing blood-concentration-time profiles with existing data from the literature. The model assessment was undertaken for each new PBK model created using global sensitivity analysis and comparison of fold error for key parameters obtained from the models.

4.2 Pilot study on PBK modelling software

A published PBK model for atenolol from the literature (Peters, 2008) was reproduced using three software packages, MATLAB, PKSim, and QIVIVE, to compare the usefulness and accessibility of these software in order to determine which would be most appropriate to use for subsequent model development for this thesis. The analysis of each software considered ease of inputting parameters, usability of the software and accuracy of reproducing the literature model. In summary, less chemical and physiological information was needed to be inputted into PKSim and QIVIVE to obtain concentration-time graphs and no equations were required for input. Hence, these two software are considered to be more user friendly. SimBiology (MATLAB) required the most input, requiring information on all parameters to be inputted into the model including physicochemical and ADME properties, in addition to information on the ODEs. Therefore, MATLAB was found to require more expertise to use, however, the functionality of the software and adaptability is greatest, and it reproduced the atenolol model most accurately. Whilst QIVIVE was found to be most user friendly, in this analysis, model adaptability is restricted. For example, it was not possible to view or make any adaptations to the model equations or the compartments included in the model. Thus, MATLAB was deemed the most appropriate software to use for the analysis in this thesis as it allows for direct implementation of the model equations whereas the alternative software (PKSim and QIVIVE) considered start from pre-defined templates that can be adjusted.

4.3 Method

4.3.1 Identifying analogues with existing PBK models using the KNIME Workflow for Assisting Analogue Selection (KWAAS) for the target atenolol

The first case study was the drug atenolol. Initially, the PBK database was searched using the InChIKey for atenolol. Atenolol already has an available PBK model in the literature, hence the *in vivo* data in this literature could be used for assessing the predictions of any new models generated using the read-across approach. The chemical name and SMILES string of atenolol was inputted into the KNIME workflow to calculate similarity to other chemicals in the PMD using nine different fingerprints before being refined to find the most similar chemicals. Chemicals with similarity scores of 0.6 and above were included for further refinement (0.6 was selected as the cut-off for similarity as rationalised in Section 3.1).

Figure 4.1 shows the analogues selected by the KWAAS at every stage of the workflow. Using the nine fingerprints to identify similar chemicals resulted in eight potential analogues being identified. Atenolol has a molecular weight of 266 Da and log P of -0.11; hence when refining the results of the similarity analysis, chemicals with a molecular weight of 266 Da \pm 50% (i.e. 133-399 Da) and log P of -0.11 \pm 1 (i.e. -1.11-0.89) were sought. The KNIME workflow suggested six analogues based on chemical fingerprints and molecular weight; one of which was atenolol itself. The suggested analogues after refining the results by molecular weight included three other beta-blockers. Selection of an analogue PBK model to use for atenolol included filtering the resulting analogues from the spreadsheet using Stage 3 of the workflow as described in Chapter 3. It is important to note that this tool is to help fill gaps in kinetic data, it does not provide information on activity at the biological site of action. That is, the availability of full equations, human as species, as well as oral for route of administration were used to refine the results of available PBK models from the PBK model dataset.

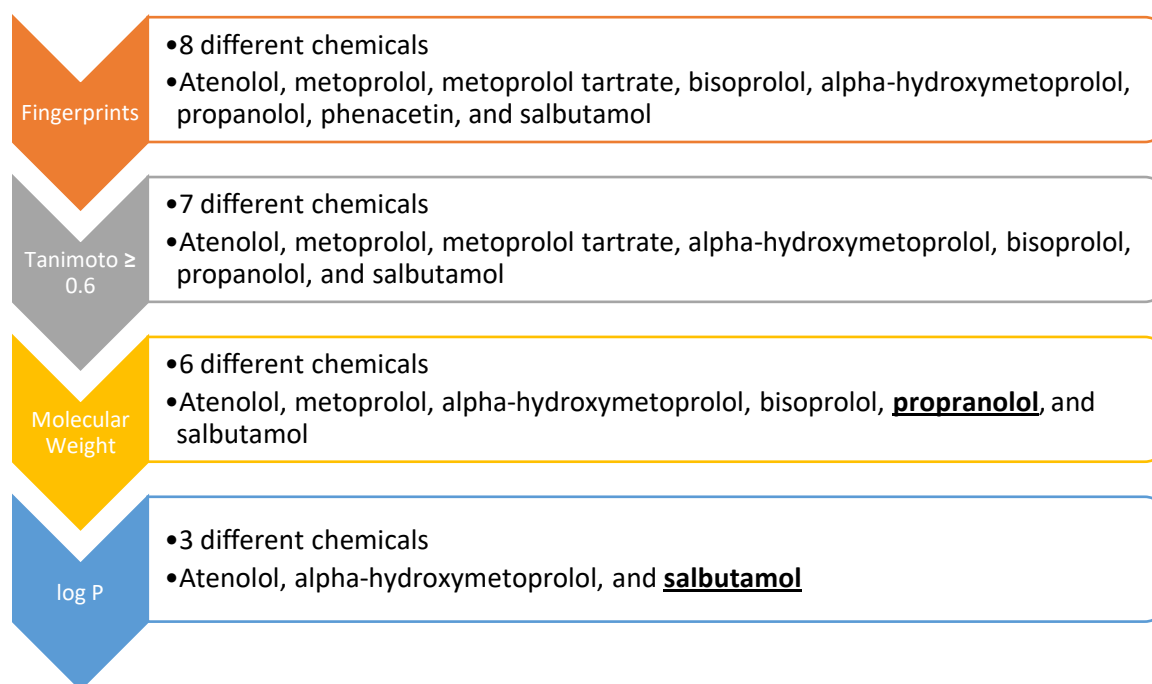


Figure 4.1 Results for identifying analogues for atenolol at each stage of using KWAAS. The arrow on left indicates the criteria used to refine the selection at each stage.

Analysis of the proposed analogues at different stages of the workflow were undertaken to assess the suitability of the analogues suggested at each stage. A PBK model for propranolol

was determined to be the most suitable after the refinement at the molecular weight stage (Stage 3 in Figure 4.1). When refining further and including log P as a similarity metric only one analogue was suggested, salbutamol, (the other two chemicals remaining at this stage were atenolol itself and a metabolite of the beta-blocker metoprolol). Thus, salbutamol was chosen as a second chemical to use as a template. Refinement of KWAAS results included only molecular weight and log P as the results produced at log P, were atenolol itself, one metabolite and one analogue suggestion. Thus, refinement has been undertaken as far as was reasonable, refining further would be too specific and only result in the target chemical itself being identified. The structures of the target chemical, atenolol, and the two source chemicals, propranolol and salbutamol, are displayed in Figure 4.2.

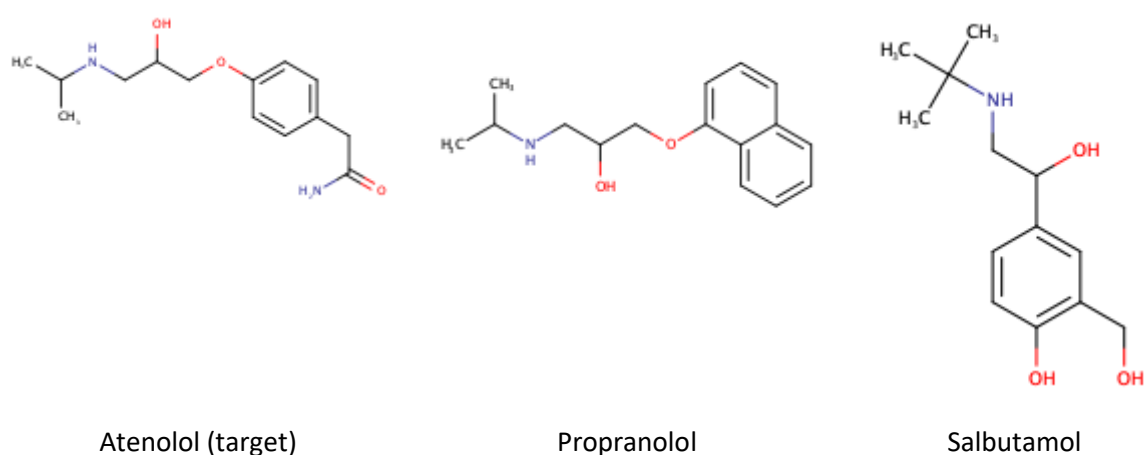


Figure 4.2 Structure of target chemical, atenolol, and the chemicals identified as similar at the molecular weight stage, propranolol, and at the log P stage, salbutamol

4.3.2 Propranolol PBK model

Kiriyama et al. (2008) describe a 13 compartment PBK model for propranolol, with a structure as shown in Figure 4.3. The compartments included in the model were: arterial (art), venous (ve), lung (lu), brain (br), heart (ht), liver (li), spleen (sp), gut (gu), kidney (ki), adipose (ad), muscle (mu), bone (bo), and skin (sk). The volume (V_i) and blood flow rates (Q_i) from Kiriyama (2008) are summarised in Table 4.2 and were used for both propranolol and atenolol simulations.

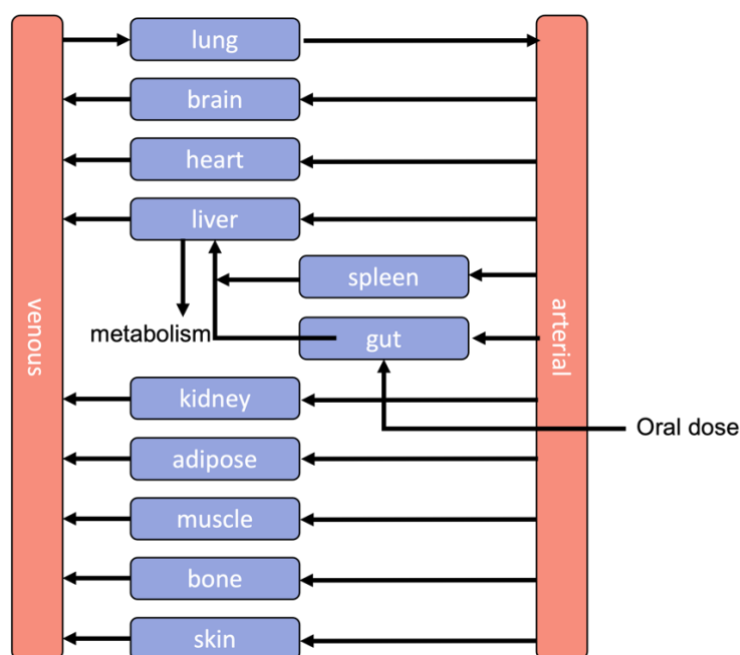


Figure 4.3 Schematic of the PBK model outlined in Kiriyaama (2008). Blood flows from the arterial compartment into the organs. These include brain, heart, liver, spleen, gut, kidney, adipose, muscle, bone and skin. Blood enters the venous compartment from the brain, heart, liver, spleen, gut, kidney, adipose, muscle, bone and skin. Blood from the venous enters the lung which then leaves the lung and enters the arterial compartment. Clearance is modelled from the liver, and the dose enters through the gut.

As tissue specific partition coefficients (K_p) require input of specific chemical properties (i.e. pK_a , $\log P$, pH and fraction of free drug in the plasma), they need to be calculated for each individual chemical. Rodgers and Rowland (2006) describe a method that calculates these using chemical properties and the volume fraction of various components of the tissue (i.e. volume fraction of water, lipids and phospholipids). The tissue partition coefficients for propranolol and those calculated for atenolol are given in Table 4.3. Propranolol's tissue partition coefficients are taken from Kiriyaama et al. (2008), with atenolol's being calculated using the method described in Rodgers and Rowland (2006). Elimination of the chemical from the body was assumed to be via metabolism in the liver (Kiriyaama et al., 2008).

Table 4.2 Organ blood flow rates and volumes from Kiriyama et al. (2008)

Compartment	Q (ml/min)	V (ml)
Arterial	5,270	1,720
Venous	5,270	3,440
Lung	5,270	320
Brain	110	360
Heart	260	210
Liver	930	2,310
Spleen	110	130
Gut	690	1,700
Kidney	70	460
Adipose	370	4,800
Muscle	1,460	25,530
Bone	640	2,620
Skin	310	12,000

The ODEs given by Equations 4.2-4.7 represent each organ included within the PBK model and were solved in MATLAB using the numerical regression solver for stiff differential equations - ode15s. These equations were reproduced from the original paper with the gut equation corrected to better model the dose entering the system. In the original paper, the reporting of the gut equation was incorrect where the dose and absorption of the drug in was multiplied by the stomach blood flow (highlighted in red in Equation 4.1). However, this was corrected in Equation 4.2 by adding the dose and absorption of the drug to the blood flow in the stomach after it was multiplied by the concentration in the stomach (highlighted in red in Equation 4.2). Once this model was accurately reproduced with the corrected equation, it was subsequently used as a template for atenolol.

Gut (absorption site) – incorrect equation from original paper

$$\frac{dC_{gu}}{dt} = \frac{1}{V_{gu}} \left(Q_{gu} \left(C_{art} + k_a \times D \times F_a \times e^{-k_a \times t} - \frac{C_{gu} \times R_{bp}}{K_{p,gu}} \right) \right)$$

Equation 4.1

Gut (absorption site) – corrected equation

$$\frac{dC_{gu}}{dt} = \frac{1}{V_{gu}} \left(k_a \times D \times F_a \times e^{-k_a \times t} + Q_{gu} \left(C_{art} - \frac{C_{gu} \times R_{bp}}{K_{p,gu}} \right) \right)$$

Equation 4.2

where F_a is the fraction absorbed from the intestinal tract, and D is the dose, that was orally administered.

Arterial blood

$$\frac{dC_{art}}{dt} = \frac{1}{V_{art}} \left(Q_T \left(\frac{C_{lu} \times R_{bp}}{K_{p,lu}} - C_{art} \right) \right)$$

Equation 4.3

where Q_T is the total blood flow through the body.

Venous blood

$$\frac{dC_{ve}}{dt} = \frac{1}{V_{ve}} \left(\sum Q_i \left(\frac{C_i \times R_{bp}}{K_{p,i}} \right) + (Q_{li} + Q_{sp} + Q_{gu}) \frac{C_{li} \times R_{bp}}{K_{p,li}} - Q_T C_{ve} \right)$$

Equation 4.4

where i represents the compartments brain, heart, kidney, adipose, muscle, bone, skin.

Lung

$$\frac{dC_{lu}}{dt} = \frac{1}{V_{lu}} \left(Q_T \left(C_{ve} - \frac{C_{lu} \times R_{bp}}{K_{p,lu}} \right) \right)$$

Equation 4.5

Liver

$$\frac{dC_{li}}{dt} = \frac{1}{V_{li}} \left(Q_{li} C_{art} + \sum Q_i \left(\frac{C_i \times R_{bp}}{K_{p,i}} \right) - (Q_{li} + Q_{sp} + Q_{gu}) \times \frac{C_{li} \times R_{bp}}{K_{p,li}} - \frac{C_{li} \times f_B \times R_{bp}}{K_{p,li}} \times \left(\frac{V_{max}}{K_{ml} + C_{li} \times f_B \left(\frac{R_{bp}}{K_{p,li}} \right)} + CL_{int} \right) \right)$$

Equation 4.6

where i represents the compartments spleen and gut.

Non-eliminating compartments,

$$\frac{dC_i}{dt} = \frac{1}{V_i} \left(Q_i \left(C_{art} - \frac{C_i \times R_{bp}}{K_{p,i}} \right) \right)$$

Equation 4.4

where i is compartments brain, heart, spleen, kidney, adipose, muscle, bone, skin.

Table 4.3 Tissue partition coefficients for each organ. Propranolol values from Kiriyama et al. (2008) and atenolol values calculated using the method outlined in Rodgers and Rowland (2006).

	Propranolol	Atenolol
Lung	6.46	5.2971
Brain	13.54	2.0133
Heart	4.38	3.6362
Liver	5.67	6.0850
Spleen	2.98	4.7545
Gut	8.22	3.8063
Kidney	5.18	6.4511
Adipose	0.18	0.5764
Muscle	3.20	3.1315
Bone	6.90	1.5818
Skin	7.22	2.3715

Additional parameter values used in the model for atenolol and propranolol are summarised in Table 4.4. Unbound fraction in blood and blood-plasma concentration ratio values for propranolol were taken from Obach (1997), with all other propranolol parameter values from Kiriyaama et al. (2008). Sources for the parameter values for atenolol are given in Table 4.4. In brief, parameters were obtained from the literature (Taylor and Turner, 1981, Vila et al., 1992), and from online databases: DrugBank (<https://go.drugbank.com>), Comptox (<https://comptox.epa.gov/dashboard>) and PubChem (<https://pubchem.ncbi.nlm.nih.gov>) - all accessed October 2021.

The doses used as input for the propranolol simulations were 10mg, 40mg, 80mg and 160mg. These were the same doses as used in the reports for the observed data which were used for comparison (to determine model accuracy). For atenolol the dose used was 100mg i.e. the same dose as used in the Peters (2008) publication.

Table 4.4 Parameter values used within the PBK model. Propranolol values noted from Kiriyaama et al. (2008) and Obach (1997). Atenolol values obtained from the literature and online databases.

Parameter	Propranolol	Atenolol	Source	Description
k_a	0.009 min ⁻¹	0.00633 min ⁻¹	Vilà et al. (1992)	Absorption rate constant
f_a	0.65	0.5	DrugBank	Fraction absorbed from intestinal tract
CL_{int}	9208 ml/min/human	301.6440 ml/min/human	Comptox	Intrinsic clearance
V_{max}	4572 µg/min/human	-	-	Maximum velocity of metabolism

K_{ml}	0.8921 μg/ml	-	-	Michaelis constant
f_B	0.13	0.8785	Comptox	Unbound fraction in blood
R_{bp}	0.89	1.07	Taylor and Turner (1981)	Blood-plasma concentration ratio
MW	259.34	266.34	PubChem	Molecular weight

4.3.3 Salbutamol PBK model

A nine compartment PBK model, with the structure as shown in Figure 4.4, (according to Boger and Fridén (2019)) for salbutamol was reproduced. This was then used as a template to develop a PBK model for atenolol from which atenolol concentrations in the blood were derived. Blood flow (Q_i), which is calculated from total body weight (70 kg), and organ volumes (V_i) are given in Table 4.5. Adipose (ad), gut (gu), liver (li), lung (lu), slowly perfused tissues (pp), rapidly perfused tissues (rp), spleen (sp), arterial (art) and venous (ve) were all included in the model. Blood flow for arterial and venous was assumed to be total cardiac output (co). Blood flows and organ volumes were the same for both compounds. Tissue-to-blood partition coefficients (K_p) for salbutamol and atenolol are both calculated using Rodgers and Rowland (2006) method for predicting K_p . The K_p values are summarised in Table 4.6.

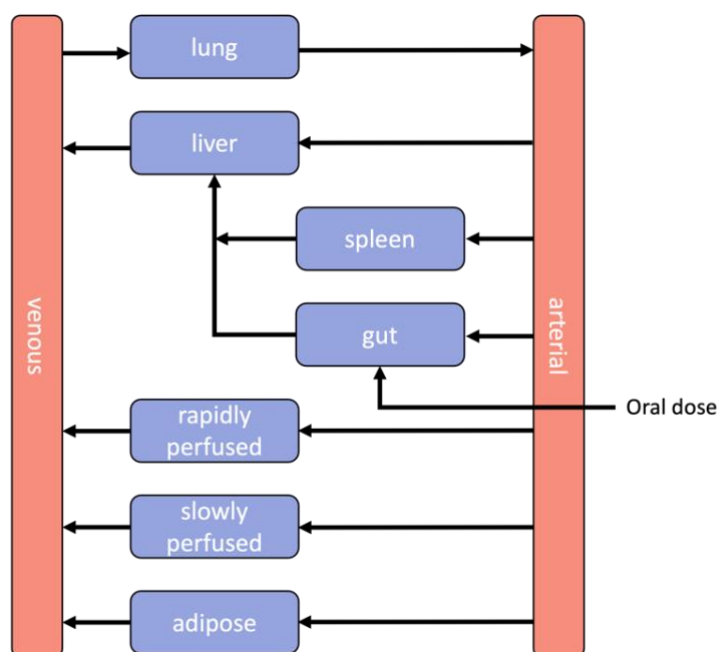


Figure 4.4 Schematic of the nine compartment PBK model for salbutamol as described in Boger and Fridén (2019), with the dose of the chemical entering through the gut. The nine compartments include lung, liver, spleen, gut, rapidly perfused organs, slowly perfused organs, adipose, arterial and venous.

Table 4.5 Organ blood flow rates and volumes from Boger and Friden (2019)

Compartment	Volume (L)	Blood flow (L/h)
Adipose	14.28	15.6
Gut	1.14	53.04
Liver	1.71	18.72
Lung	0.51	312.00 (Equal to Q_{co})
Slowly perfused	41.77	109.20
Rapidly perfused	1.94	109.20
Spleen	0.17	6.24
Arterial	1.71	
Venous	3.43	
Cardiac output		312.00

The Boger and Fridén model was created for both oral and inhalation administration routes, with the lung being split into 24 airway compartments, with further splitting of these airway generations into three separate compartments, the epithelial lining fluid, the epithelium and

the sub-epithelium. However, for simplicity the lung compartment was reduced to one equation in this analysis as the inhalation pathway was not relevant to this route of administration. The equations for all other compartments were reproduced exactly from the original paper with clearance only being modelled from the venous compartment in the original model. Equations 4.8-14 were coded and numerically integrated in MATLAB to simulate concentration-time graphs for salbutamol and atenolol.

Arterial

$$\frac{dC_{art}}{dt} = \frac{1}{V_{art}} \left(Q_{co} \left(\frac{RC_{lu}}{K_{p,lu}} - C_{art} \right) \right)$$

Equation 4.5

Venous

$$\frac{dC_{ve}}{dt} = \frac{1}{V_{ve}} \left(Q_{rp} \frac{RC_{rp}}{K_{p,rp}} + Q_{pp} \frac{RC_{pp}}{K_{p,pp}} + Q_{ad} \frac{RC_{ad}}{K_{p,ad}} + (Q_{li} + Q_{sp} + Q_{gu}) \frac{RC_{li}}{K_{p,li}} - Q_{co} C_{ve} - CLC_{ve} \right)$$

Equation 4.6

Liver

$$\frac{dC_{li}}{dt} = \frac{1}{V_i} \left(Q_{li} C_{art} + Q_{gu} \frac{RC_{gu}}{K_{p,gu}} + Q_{sp} \frac{RC_{sp}}{K_{p,sp}} - (Q_{li} + Q_{sp} + Q_{gu}) \frac{RC_{li}}{K_{p,li}} \right)$$

Equation 4.7

Gut

$$\frac{dC_{gu}}{dt} = \frac{1}{V_{gu}} \left(Q_{gu} \left(C_{art} - \frac{RC_{gu}}{K_{p,gu}} \right) + k_a A_{gu} \right)$$

Equation 4.8

where,

$$\frac{dA_{gu}}{dt} = -k_a A_{gu}$$

Equation 4.9

Other tissues

$$\frac{dC_i}{dt} = \frac{1}{V_i} \left(Q_i \left(C_{art} - \frac{RC_i}{K_{p,i}} \right) \right)$$

Equation 4.10

where i represents spleen, rapidly perfused, slowly perfused, and adipose.

Lung

$$\frac{dC_{lu}}{dt} = \frac{1}{V_{lu}} \left(Q_{lu} \left(C_{ve} - \frac{RC_{lu}}{K_{p,lu}} \right) \right)$$

Equation 4.11

Table 4.6 Tissue partition coefficients for each organ. Salbutamol values from Boger and Friden (2019) and atenolol values calculated using the method outlined in Rodgers and Rowland (2006).

	Salbutamol	Atenolol ($K_{p,u}$)
Liver	5.81	5.72
Spleen	3.80	4.47
Rapidly perfused (mean of brain, heart and kidney)	3.01	4.03
Slowly perfused (mean of bone and muscle)	2.27	2.36
Gut	3.63	3.58
Adipose	0.545	0.542
Lung	5.06	4.98

Table 4.7 summarises all parameters included in the model. Salbutamol values were obtained from Boger and Friden (2019). Atenolol values were gathered from literature (Taylor and Turner, 1981, Vila et al., 1992) and online databases (Comptox (<https://comptox.epa.gov/dashboard/>, accessed October 2021), admetSAR (<http://lmmd.ecust.edu.cn/admetSar2/>, accessed October 2021) and PubChem (<https://pubchem.ncbi.nlm.nih.gov>, accessed November 2021)).

Table 4.7 Parameter values used within the PBK model. Salbutamol values noted from Boger and Friden (2019). Atenolol values obtained from the literature and online databases.

Parameter	Salbutamol	Atenolol	Source	Description
R	0.96	1.07	Taylor and Turner (1981)	Blood-plasma ratio
CL	18.1452 (l/h)	18.0986 (l/h)	Comptox	Clearance
F _{RS,oral}	0.094 <i>(R-salbutamol oral)</i> 0.687 <i>(S-salbutamol oral)</i>	0.6286	admetSAR	Oral bioavailability
f _{u,p}	0.77	0.94 (<i>plasma</i>)	Comptox	Fraction unbound in plasma
k _a	0.63 h ⁻¹	0.38 h ⁻¹	Vilà et al. (1992)	Oral absorption rate constant
MW	239.3	266.34	PubChem	Molecular weight

An enantiomer is one of two stereoisomers of a chemical that are mirror images of each other. Salbutamol has an active enantiomer (R-salbutamol), however, as a mix of the enantiomers is being modelled, both need to be considered in an equal quantity. Clearance was calculated as a weighted average of each enantiomer by the below equation (Equation 4.15) from Boger and Friden (2019).

$$CL = \frac{F_R e^{-k_R}}{F_R e^{-k_R} + F_S e^{-k_S}} CL_R + \frac{F_S e^{-k_S}}{F_R e^{-k_R} + F_S e^{-k_S}} CL_S$$

Equation 4.12

where,

$$k_{R/S} = \frac{\ln(2)}{t_{\frac{1}{2}}}$$

Equation 4.13

where, $t_{1/2,R}=2.85\text{h}$ and $t_{1/2,S}=6.03\text{h}$ (Ward et al., 2000).

The dose (2mg) entering the body was calculated by including bioavailability as shown in Equation 4.17.

$$Dose = F_{RS} \frac{2 \text{ mg}}{MW} \times 10^6,$$

Equation 4.14

where,

$$F_{RS} = 0.5F_R + 0.5F_S.$$

Equation 4.15

4.3.4 Identifying analogues with existing PBK models using the KNIME Workflow for Assisting Analogue Selection (KWAAS) for the target flumioxazin

The second case study was for the herbicide flumioxazin. Initially, the PBK database was searched using the InChiKey for the flumioxazin. Flumioxazin already has an available PBK model in the literature, hence the *in vivo* data in this literature could be used for assessing the predictions of any new models generated using the read-across approach. The chemical name and SMILES string of flumioxazin was inputted into the KNIME workflow to calculate similarity to other chemicals in the PMD using nine different fingerprints before being refined to find the most similar chemicals. Chemicals with similarity scores of 0.6 and above were

included for further refinement (0.6 was selected as the cut-off for similarity as rationalised in Section 3.1).

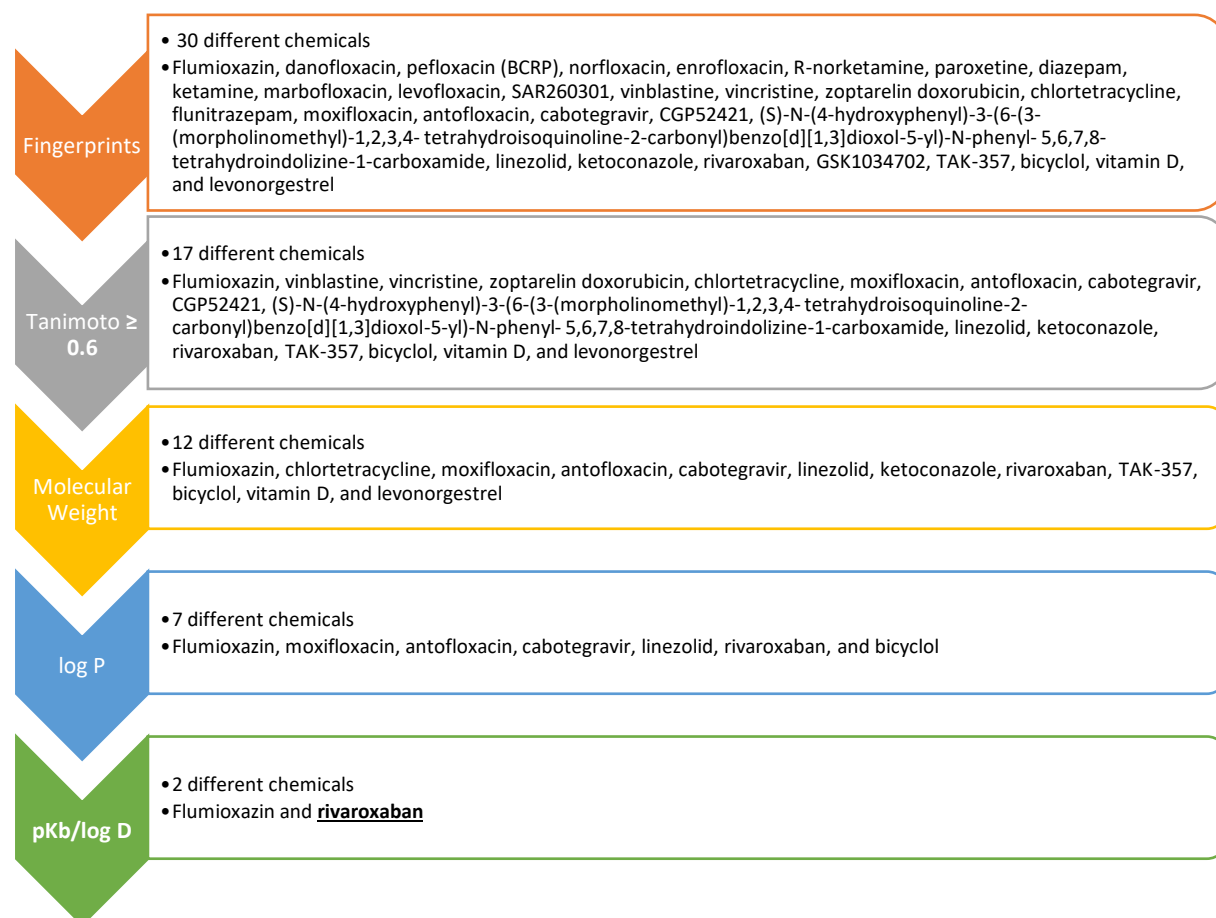


Figure 4.5 Results for identifying analogues for flumioxazin at each stage of using KWAAS. The arrow on left indicates the criteria used to refine the selection at each stage.

Figure 4.5 summarises the results at each stage of the workflow for flumioxazin. This chemical has a molecular weight of 354 and log P of 1.9281. These values were used for assessing similarity as well as including information on pKb (3.31) and log D (2.55) at pH 5.5. Ranges used for inclusion of analogues at each stage of the workflow were: molecular weight, 177-531; log P, 0.9281-2.9281; pKb, 2.31-4.31; log D, 1.55-3.55. 30 different chemicals were initially identified when fingerprints alone were considered for similarity, further refining of the analogues resulted in only one candidate analogue remaining after pKa, and log D were included in the similarity assessment. An existing PBK model for flumioxazin was also identified at every stage of the process; information from this model was used for comparison when assessing the accuracy of the model developed using the analogue as a template. It is

important to note that this tool is to help fill gaps in kinetic data, it does not provide information on activity at the biological site of action. As only one chemical, rivaroxaban, was determined to be similar after all stages of the KWAAS, it was selected to be used as a template for building a model for flumioxazin. The structures of the target chemical, flumioxazin, and the source chemicals, rivaroxaban, are displayed in Figure 4.6.

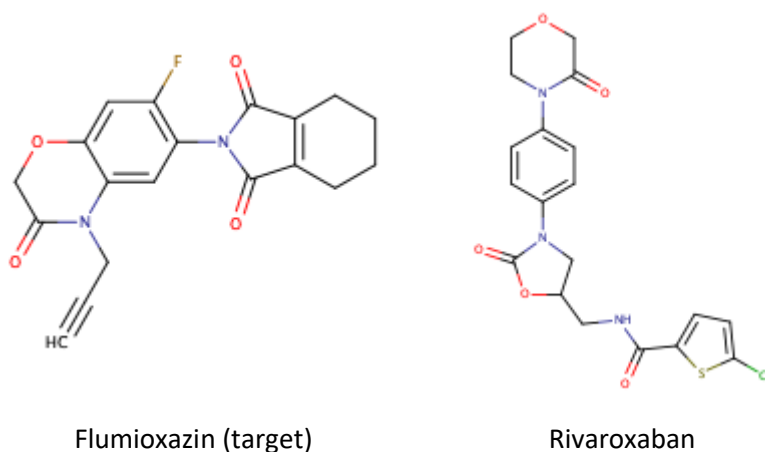


Figure 4.6 Structure of target chemical, flumioxazin, and the chemicals identified as similar using the KWAAS, rivaroxaban

4.3.5 Rivaroxaban PBK model

Yamazaki-Nishioka et al. (2019) describe a minimal PBK model consisting of a chemical receptor compartment, a metabolising compartment and a central compartment for oral administration of rivaroxaban. This model was reproduced directly, and subsequently adapted to be used as a template for flumioxazin. The minimal model incorporates the hepatic (hep), blood (bl), urine (ur) and gut (gu) compartments. Blood flow for hepatic and organ volumes are summarised in Table 4.8.

Table 4.8 Organ blood flow rates and volumes from Yamazaki-Nishioka et al. (2019)

Parameter	Abbreviation	Value
Hepatic blood flow	Q_{hep}	96600 (ml/h)
Hepatic volume	V_{hep}	1500 (ml)
Systemic circulation volume	V_{bl}	30800 (ml)
Urine volume	V_{ur}	62.5 (ml)

The minimal model is described by Equations 4.19-22. These were solved in MATLAB using the ODE solver - ode15s.

Gut

$$\frac{dA_{gu}}{dt} = -k_a A_{gu}$$

Equation 4.16

Hepatic

$$\frac{dC_{\text{hep}}}{dt} = \frac{1}{V_{\text{hep}}} \left(Q_{\text{hep}} C_{\text{bl}} - \frac{Q_{\text{hep}} C_{\text{hep}} R_b}{K_{\text{phep}}} + k_a A_{gu} - CL_{\text{int}} \frac{C_{\text{hep}}}{K_{\text{phep}}} f_{\text{up}} \right)$$

Equation 4.17

Blood

$$\frac{dC_{\text{bl}}}{dt} = \frac{1}{V_1} \left(-Q_{\text{hep}} C_{\text{bl}} + \frac{Q_{\text{hep}} C_{\text{hep}} R_b}{K_{\text{phep}}} - CL_{\text{renal}} C_{\text{bl}} \right)$$

Equation 4.18

Urinary

$$\frac{dC_{\text{ur}}}{dt} = \frac{1}{V_{\text{ur}}} (CL_{\text{renal}} C_{\text{bl}})$$

Equation 4.19

Parameters for rivaroxaban and flumioxazin are summarised in Table 4.9. Rivaroxaban values are taken from Yamazaki-Nishioka et al. (2019), while flumioxazin values are taken from online databases and ADME property predictors (Comptox (<https://comptox.epa.gov/dashboard/>), accessed November 2021), ADMETlab 2.0 (<https://admetmesh.scbdd.com/service/evaluation/cal>), accessed November 2021), Opera (<https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.html>), accessed November 2021). Liver to plasma concentration ratio was calculated using Rodgers and Rowlands (2006). However, no value for the blood-plasma concentration ratio, from online ADME property predictors or literature, could be found so the assumption that the ratio was equal to 1 was used (Mamada et al., 2021). The absorption rate constant was taken from Takaku et al. (2014).

Table 4.9 Parameter values used within the PBK model. Rivaroxaban values noted from Yamazaki-Nishioka et al. (2019). Flumioxazin values obtained from the literature and online databases.

Parameter	Rivaroxaban	Flumioxazin	Source	Description
log P	2.39	2.55	Opera	-
f _{up}	0.203	0.03	Comptox	Fraction unbound in plasma
R _b	0.893	1	Assumption	Blood-plasma concentration ratio
k _a	1.42 (h ⁻¹)	0.005 (h ⁻¹)	Takaku, Nagahori and Sogame (2014)	Absorption rate constant
CL _{renal}	2000 (ml/h)	4629.6 (ml/h)	ADMETlab 2.0	Renal clearance
CL _{int}	19000 (ml/h)	90072 (ml/h)	Comptox	Intrinsic hepatic clearance
K _{phep}	1.97	0.6855	Calculated using Rodgers and Rowlands (2006) method	Liver to plasma concentration ratio

4.3.6 Model assessment

4.3.6.1 Fold error calculation

For each chemical analogue model (original model reproduced from the literature) and template model (adapted literature model) the most common pharmacokinetic metrics, time taken to reach the maximum concentration (T_{max}), the maximum concentration (C_{max}), and the area under the curve (AUC), were calculated and compared with literature estimates to assess accuracy. The fold error was calculated for each taking a ratio between the predicted and literature values so that it was always greater than 1.

4.3.6.2 Comparison with a model for a chemical with a low similarity score

To demonstrate that using a PBK model for analogues identified as similar were more useful than, for example, using a randomly selected PBK model, a further analysis was undertaken wherein the PBK models for chemicals not identified as similar were investigated. The rivaroxaban PBK model described in Section 4.3.5 was used with the key chemical specific parameters for atenolol being inputted. Predictions using the rivaroxaban model were compared to the predictions from using propranolol and salbutamol PBK models as a template for atenolol. Further, the PBK model for salbutamol described in Section 4.3.3 was used as a low similarity analogue for flumioxazin. Key chemical specific parameters for flumioxazin were inputted into the salbutamol model with the predictions being compared to those obtained using the rivaroxaban PBK model as a template for flumioxazin.

4.3.6.3 Sensitivity analysis

When creating new models, it is important to understand which parameters require further investigation to ensure the most accurate data are used, thus reducing gaps in data. Discrepancies between models and data can be identified with sensitivity analysis where parameters that may need to be changed are identified. The general purpose software OpenCOSSAN (<https://cossan.co.uk>) was used to perform global sensitivity analysis on all three models developed above (i.e. two models for atenolol developed using models for propranolol and salbutamol as analogues and one model for flumioxazin developed using a model for rivaroxaban). OpenCOSSAN was used to perform a Sobol indices method for this global sensitivity analysis. The Sobol indices method determines the significance of each input parameter and their interactions to the overall model output variance. There is no assumption between model inputs and outputs, with the full range of each input parameter variation and interactions between parameters being evaluated. As explained in Section 1.4, the analysis was undertaken to establish the parameters with the greatest impact on predictions.

To complete global sensitivity analysis using OpenCOSSAN a probability distribution needs to be assigned to each parameter (e.g. normal, log normal, etc.), and sampling method (e.g. Monte Carlo, Latin Hypercube, Halton, etc.). A normal probability distribution is used to vary all parameters across this distribution using a Monte Carlo sampling method. A standard

deviation (σ) (see equation 4.23) of the normal distribution is chosen so that the variation is 10% of the mean (μ).

$$\sigma = 0.1\mu$$

Equation 4.23

The method assesses the influence of each input parameter on the output variables. Results from the global sensitivity analysis are illustrated through a Lowry plot, where parameters are ranked according to the magnitude at any given time of the total effects from left to right as a bar chart; the main effect of a parameter (black bar) and any interactions with other parameters (grey bar) together make up the total effect of the parameter which is represented by stacked bars; and the variance due to parameter interactions is represented by a blue ribbon across the plot (McNally et al., 2011). The total variance is represented by a red dashed line, where parameters seen as having significant contribution to the total variance are left of the red dashed line.

4.4 Results

4.4.1 Selecting the most appropriate analogue for read-across for the target atenolol

Kiriyama et al. (2008) outlined a PBK model for propranolol which was used as the first case study. The output from the Peters (2008) atenolol PBK model was used for comparison to assess the accuracy of using the chemical analogue model as a template for atenolol, changing only the chemical specific variables noted in Table 4.4 and the tissue partition coefficients. Both the Kiriyama et al. (2008) and the Peters (2008) model oral dosing in a human ensuring the most similar scenarios for comparison. Firstly, the original propranolol model from the Kiriyama et al. (2008) paper was reproduced. The model information including equations, parameters and variables were used as a template for atenolol, where chemical specific information was changed to be that of atenolol using predictive software or data from the literature. The atenolol model output and observed data from Peters (2008) was then used as validation to ensure the template model accurately predicts atenolol concentrations.

The salbutamol PBK model described in Boger and Friden (2019) was used as the second analogue chemical model to predict atenolol kinetics. The atenolol data from Peters (2008) was used for comparison to assess the accuracy of using the model for this analogue with key

variables changed to those for atenolol. Both models (Boger and Fridén (2019) and Peters (2008)) were developed for a human with oral dosing. The model for salbutamol was used as a template, using equations and parameters from Boger and Fridén (2019). The chemical inputs noted in Table 4.7 and tissue partition coefficients were adapted to those for atenolol and compared to the data from Peters (2008) to assess the accuracy of the model once it had been adapted for atenolol.

4.4.2 Propranolol PBK model

Simulations of propranolol at the doses 10mg, 40mg, 80mg and 160mg, to reproduce the PBK model in Kiriya et al. (2008) with comparisons to observed data for propranolol obtained from Kopitar et al. (1986) are shown in Figure 4.5. Simulations of propranolol with a dose of 80mg best fit the observed data. Overall, across all doses the model fits well, discrepancies in modelling lower doses to the observed data can largely be due to quantifying the chemical in the blood at such a low concentration.

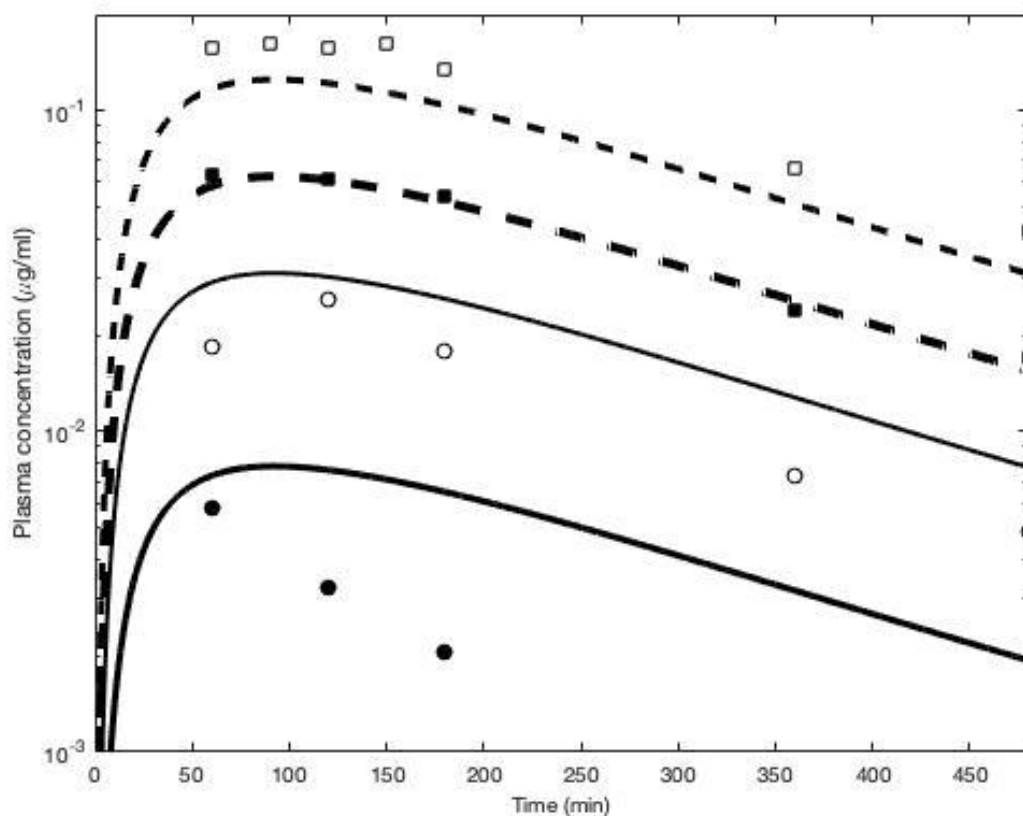


Figure 4.5 Simulations of propranolol at the doses 10mg (—), 40mg (—), 80mg (— · —) and 160mg (---) using the PBK model described in Kiriya et al. (2008). Individual data points of observed propranolol data from (Kopitar et al., 1986).

Key physicochemical information in the model was replaced with data for atenolol but organ volume and blood flow values remained the same, as did the model equations. Figure 4.6 shows the predicted atenolol simulation compared to observed and predicted atenolol data from Peters (2008), alongside propranolol predicted and observed data. The atenolol predictions using a low similarity scored chemical model, rivaroxaban, were also simulated on the same plot for comparison. The overall lineshape (the shape of the curve) of atenolol using the analogue chemical model (i.e. the model for propranolol) is similar to Peters (2008). However, the predicted concentration of atenolol decreases at a faster rate than that given in Peters (2008). The model for the less similar chemical rivaroxaban can be seen to significantly overpredict atenolol in comparison to the simulations using the analogue model.

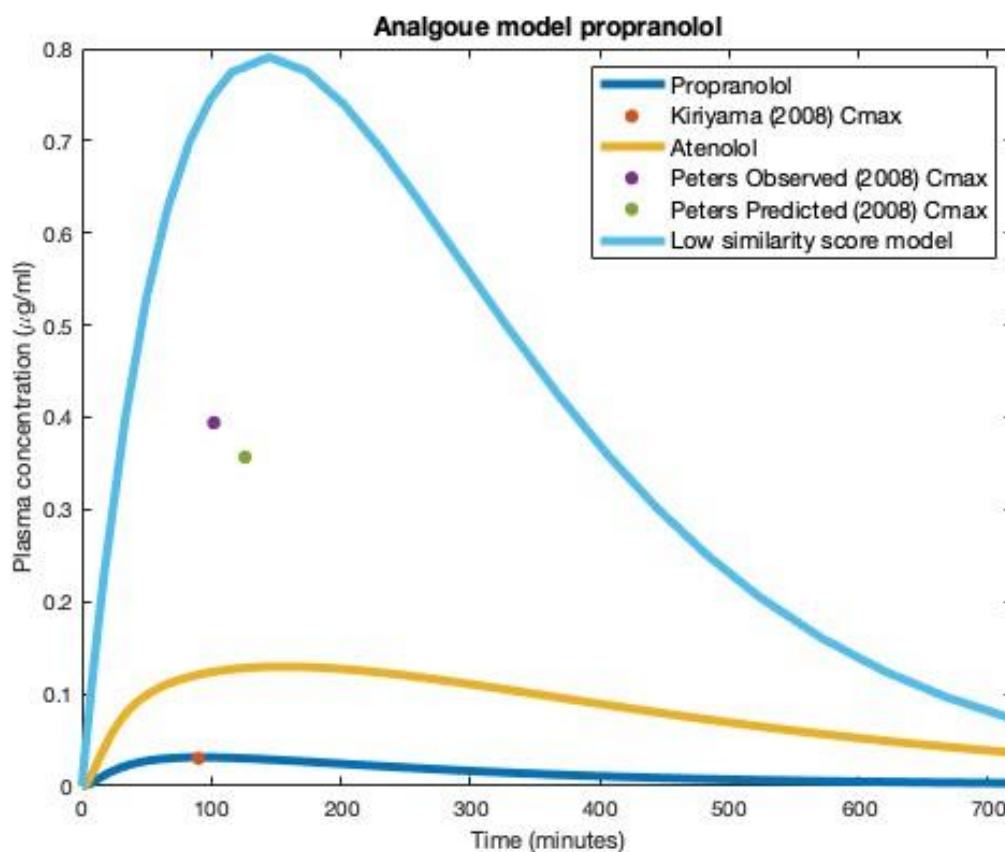


Figure 4.6 Predicted propranolol (40mg dose dark blue line) and atenolol (100mg dose using the propranolol model as a template represented by a yellow line and using the low similarity score model as a template represented by a light blue line) simulations compared to observed data from the literature (Kiriyaama et al, 2008 (C_{max} represented by a red dot), Peters, 2008 (observed C_{max} represented by a purple dot and predicted by a green dot)).

The propranolol model using the output at a dose of 40mg from the literature was reproduced, with the predicted C_{max} and T_{max} comparable with that observed in Kiriyaama et al. (2008). Predicted AUC was calculated to be within a 1.9-fold error of the literature. The resulting simulation using the model for propranolol as a template produced AUC and C_{max} values within 3-fold error of the values observed in the literature. However, the T_{max} was within a 1-fold error. Predicted C_{max} , T_{max} and AUC values for propranolol and atenolol are noted in Table 4.10 as are the literature values used for comparison.

Table 4.10 Comparisons of C_{max} , AUC and T_{max} of propranolol and atenolol to the literature (Kiryama et al, 2008, Peters, 2008). Fold errors of predictions are also shown..

	Propranolol	Kiryama, Honbo and Iga (2008)	Atenolol	Peters (2008)
C_{max} ($\mu\text{g/ml}$)	0.0312	0.03	0.1296	0.3942
Fold error	1.0		3.0	
AUC ($\mu\text{g}\cdot\text{min/ml}$)	11.3699	6.25	62.4018	195.6001
Fold error	1.8		3.1	
T_{max} (min)	92.39	90	155.9400	102
Fold error	1.0		1.5	

4.4.3 Salbutamol PBK model

Figure 4.7 shows the reproduced salbutamol PBK model compared to observed data from Boger and Fridén (2019) as well as the predicted atenolol simulations compared to observed and predicted data from Peters (2008). The predictions for atenolol using the low similarity scored chemical model (rivaroxaban) were also simulated on the same plot for comparison. This used the model equations, organ volumes and blood flow rates for salbutamol as a template for simulating atenolol, with the physicochemical information adapted to be the values for atenolol. The salbutamol simulation accurately represented the observed data, likewise, lineshape of the atenolol simulation was similar to the atenolol simulation in Peters (2008). The less similar rivaroxaban model can be seen to overpredict atenolol blood plasma concentrations in comparison to the simulations using the analogue model.

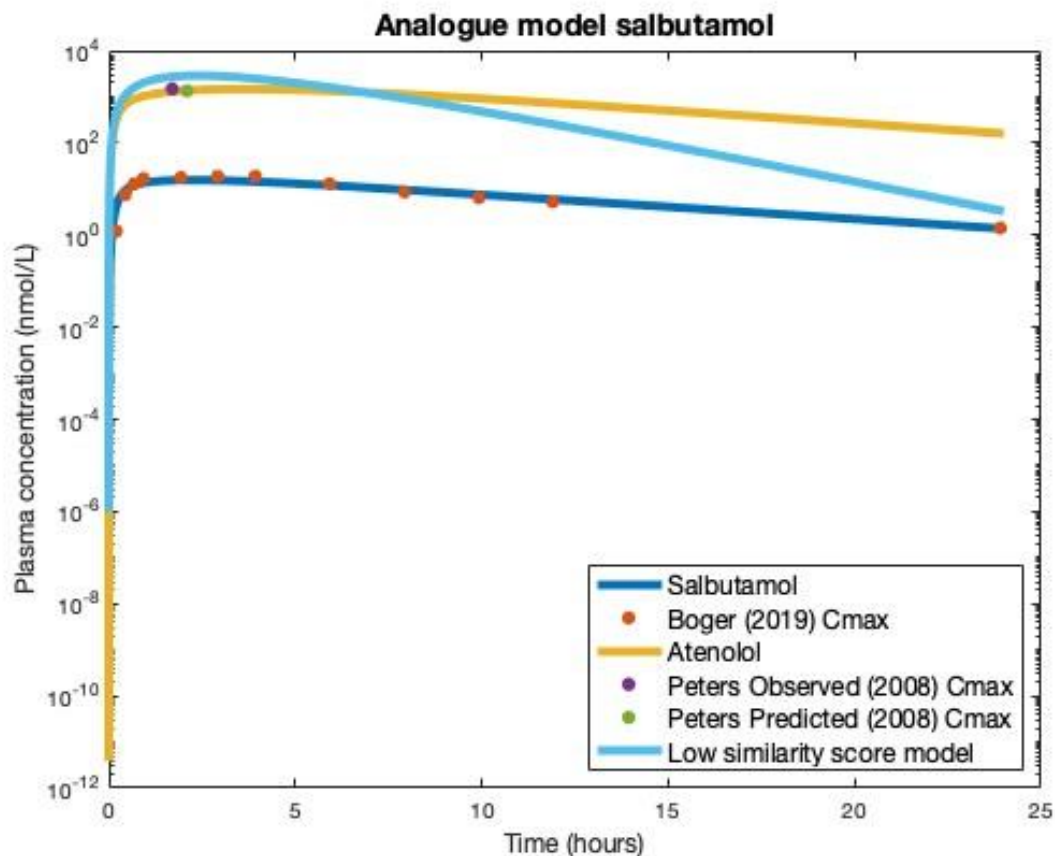


Figure 4.7 Predicted salbutamol (2mg blue line) and atenolol (100mg dose using the salbutamol model as a template represented by a yellow line and using the low similarity score model as a template represented by a light blue line) simulations compared to observed data from the literature (Boger and Fridén, 2019 (red data points), Peters, 2008 (purple and green data points)).

The Boger and Fridén salbutamol model was reproduced well for C_{max} and AUC. Using the analogue chemical model as a template, atenolol simulations were reproduced well (1-fold error) for C_{max} and T_{max} and AUC within a 2-fold error compared to the literature values. All C_{max} , T_{max} and AUC values predicted, and literature values are summarised in Table 4.11 with fold errors also given.

Table 4.11 Comparisons of simulated C_{max} , AUC and T_{max} for salbutamol and atenolol to the literature (Boger and Friden, 2019). Fold errors of predictions are also given.

	Salbutamol	Boger and Fridén (2019)	Atenolol	Peters (2008)
C_{max} (nmol/l)	16.1614	18.8464	1467.9	1480
Fold error	1.2		1.0	
AUC (nmol·h/l)	168.5676	181.0549	18152	12240
Fold error	1.1		1.5	
T_{max} (h)	2.4	3.9	3.7	1.7
Fold error	1.6		2.2	

4.4.4 Selecting the most appropriate analogue for read-across for the target flumioxazin

Yamazaki-Nishioka et al. (2018) report a PBK model for rivaroxaban which was used as a template to build a model to predict flumioxazin. The data from Takaku et al. (2014) was used for comparison to assess the accuracy of using the model for rivaroxaban as a template from which to build a model for flumioxazin. Both modelled oral dosing in a human however, Takaku et al. (2014) modelled data for a pregnant woman.

4.4.5 Rivaroxaban PBK model

The rivaroxaban PBK model from Yamazaki-Nishioka et al. (2019) was reproduced. Rivaroxaban simulations, in addition to the flumioxazin and low similarity model salbutamol simulations, compared to literature (Takaku et al., 2014) are shown in Figure 4.8. Flumioxazin concentration predictions do not reduce over time as would be expected when compared to the data in Takaku et al. (2014). This is possibly due to the effects of the clearance on the model, which was shown by the sensitivity analysis that was undertaken to have a significant effect. The low similarity score model, salbutamol, can be seen to significantly underpredict flumioxazin in comparison to the simulations using the analogue model.

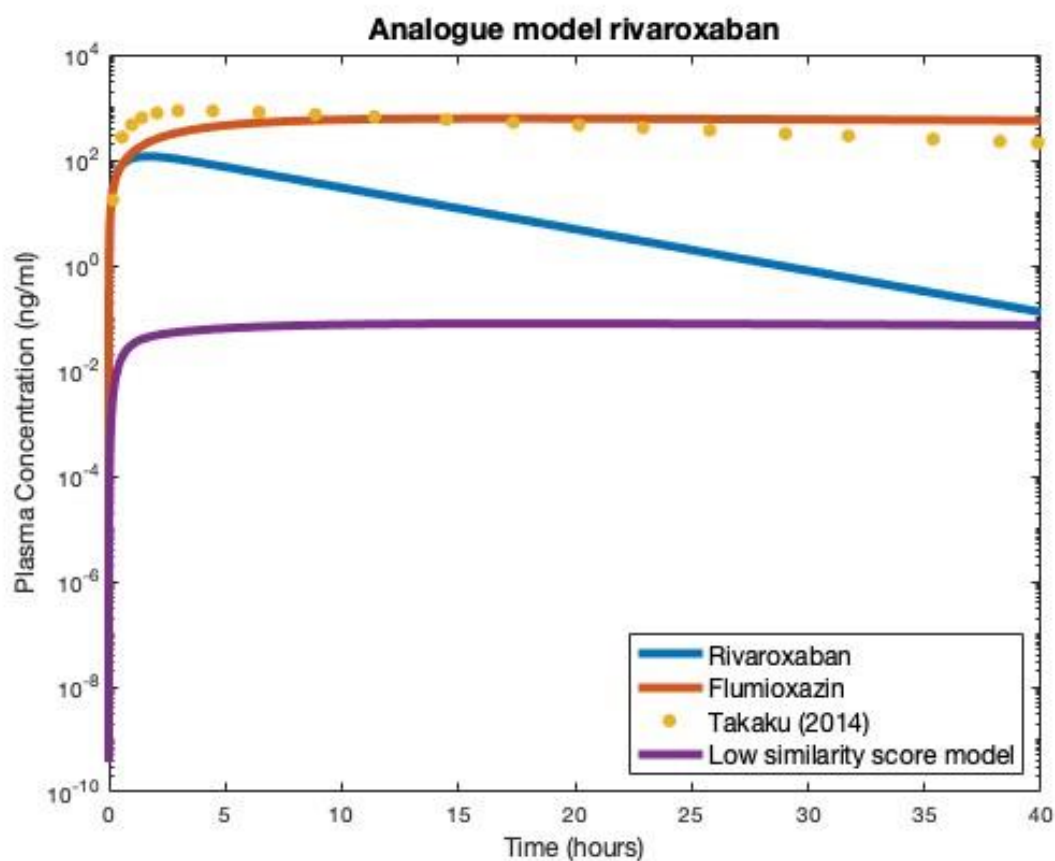


Figure 4.8 Predicted rivaroxaban (5mg dose blue line) and flumioxazin (1000mg dose using the rivaroxaban model as a template represented by a red line and using the low similarity score model as a template represented by a purple line) simulations compared to observed data from the literature (Takaku et al., 2014) represented by yellow dots.

Literature data for rivaroxaban for C_{max} and AUC, used to compare to simulation data, was taken from Yamazaki-Nishioka et al. (2019). Simulation of rivaroxaban is of a 5mg dose to accurately compare to the dose used in literature. The predicted and observed rivaroxaban AUC, and C_{max} , are compared in Table 4.12, as is flumioxazin predicted and observed data (Takaku et al., 2014). The model reproduced key metrics for rivaroxaban, furthermore, the flumioxazin metrics compared well to the literature. The line shape when using the analogue chemical model is not the same as Takaku's flumioxazin simulation. Some differences will be noted as the sex and life stage of model for the analogue chemical are different compared to the data in Takaku et al. (2014).

Table 4.12 Comparisons of C_{max} , AUC and T_{max} of rivaroxaban and flumioxazin to the literature (Yamazaki-Nishioka et al., 2019, Takaku et al., 2014). Fold errors of predictions are also given.

	Rivaroxaban	Yamazaki-Nishioka et al. (2018)	Flumioxazin	Takaku, Nagahori and Sogame (2014)
C_{max} (ng/ml)	117.0415	141	615.5223	856
Fold error	1.2		1.3	
AUC (ng·h/ml)	768.4955	816	21864	19351
Fold error	1.1		1.1	

4.4.6 Sensitivity Analysis

A global sensitivity analysis was performed to determine the parameters with the greatest impact on the predictions. Analysis was presented using a Lowry plot; comprising bars for each parameter associated with the main effect (black bar), interactions with other parameters (grey bar), the variance due to parameter interactions (blue ribbon), and the total variance (red dashed line). Parameters to the left of the red dashed line are those considered to have significant contribution to the total variance.

4.4.6.1 Source model: Propranolol

The results of using OpenCOSSAN with the Monte Carlo sampling method, when using the propranolol PBK model as a template to develop the atenolol model are summarised in Figure 4.9. The fraction absorbed from the intestinal tract (f_a) was highlighted as the most significant parameter within the model to contribute to total variance, with the total effect contributing to over 50% of the variance. In addition, the blood-plasma concentration ratio (R_{bp}), the fraction unbound in the plasma (f_{up}), the clearance (CL) and the fraction unbound in the blood (f_B) also had significant overall effects on the model. However, the rate of absorption (k_a) was deemed to only have a small contribution to the total variance of the model output. Thus, fraction absorbed is the key feature within the model.

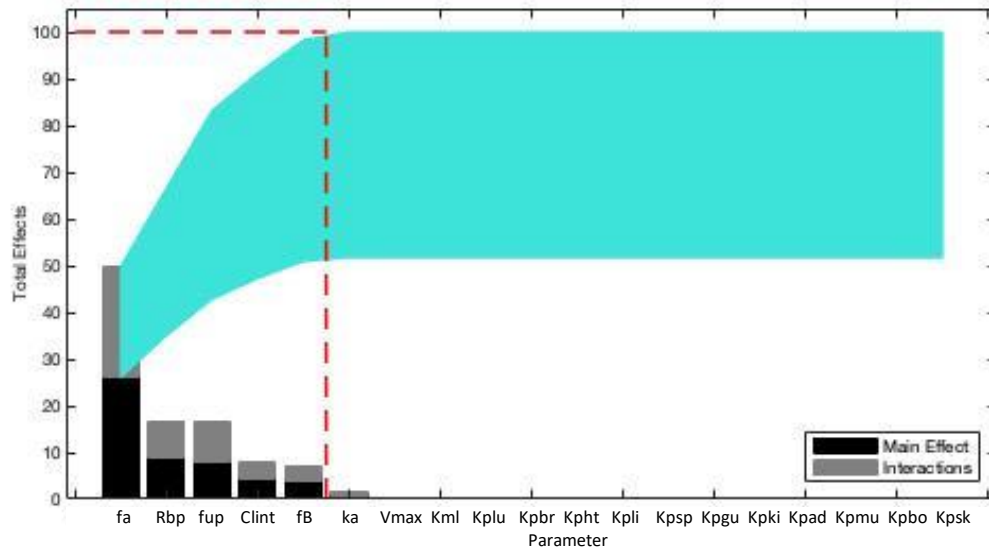


Figure 4.9 A Lowry plot showing the results of global sensitivity analysis when using propranolol as the template model for atenolol

4.4.6.2 Source model: Salbutamol

Global sensitivity analysis of the salbutamol model when used as a template for atenolol highlighted three parameters having significant contribution to the total variance, clearance (CL), blood-plasma ratio, (R), and slowly perfused tissue-to-blood partition coefficient (k_{ppp}). Figure 4.10 shows the Lowry plot for the global sensitivity analysis for the salbutamol template model for atenolol. The clearance was the most influential parameter on the predictions, with a total effect of 92%, with the slowly perfused tissue-to-blood partition coefficient having a 3% effect and blood-plasma ratio a 5% effect. The absorption and the gut tissue-to-blood partition coefficient (k_{pgu}) was highlighted as having a minimal effect.

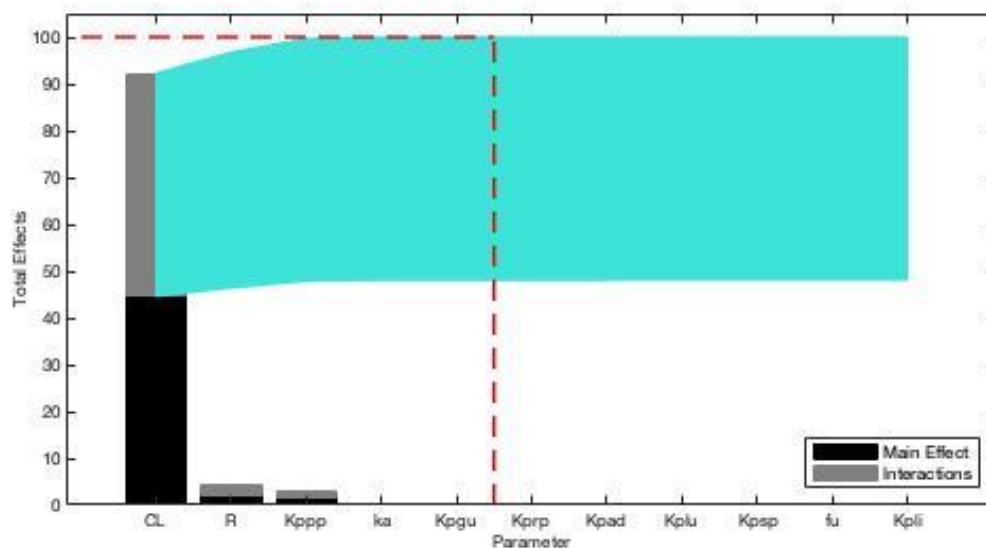


Figure 4.10 A Lowry plot showing the results of global sensitivity analysis when using salbutamol as the template model for atenolol

4.4.6.3 Source model: Rivaroxaban

All input parameters that were adapted from values for rivaroxaban in the source model, to those of flumioxazin (target) were analysed for uncertainty. The rate of absorption (k_a) was found to be the most sensitive and significantly contributed to the total variance, with a total effect of 55%. Renal (CL_{renal}) and intrinsic clearance (CL_{int}) contributed to the total variance, 20% and 8% respectively, as well as blood-plasma concentration ratio (R_b), 8%. A summary of the sensitivity analysis in a Lowry plot is shown in Figure 4.11. The fraction unbound in the plasma (f_{up}) had some effect, but this was not significant.

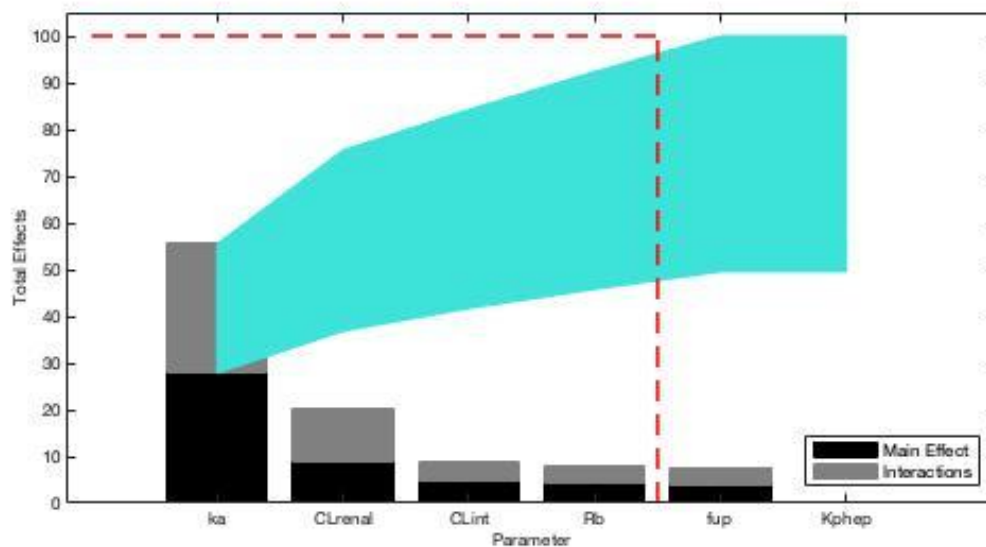


Figure 4.11 A Lowry plot showing the results of global sensitivity analysis when using rivaroxaban as a template model for flumioxazin

Sensitivity analysis helps to identify the discrepancies between the model and the data, hence identifying parameters that may need to be changed. By highlighting gaps in data, it can be used to help understand which parameters require further investigation to ensure the most accurate data are used and to reduce the gaps in data. Figures 4.9-11 outline the key parameters needed for improving the model to better fit the data, for example the template model for flumioxazin requires an accurate absorption rate value for the model to make accurate predictions. Whereas clearance highly impacted the output when using the salbutamol model as a template model for atenolol. In addition, these results from undertaking the sensitivity analysis can help with refining models when calibrating the model to better fit data.

4.5 Discussion

Developing PBK models for new chemicals using existing PBK models as a template would be a valuable asset for safety assessment and an advantage in terms of the 3Rs (Laroche et al., 2018). Hence, the development of a tool to identify chemical analogues with existing PBK models was developed and described in Chapter 3. To validate this KWAAS tool, case studies were undertaken for two chemicals, atenolol and flumioxazin. The KWAAS workflow initially identified five chemicals as being similar to atenolol, when only including fingerprints and

molecular weight as similarity metrics, reducing to two models when log P was also included - namely, models for the metabolite alpha-hydroxymetroprolol and salbutamol. The PBK models for these were reproduced and adapted using key parameters for atenolol. Comparisons to literature data were then used to assess the accuracy of each of these template models.

Although it was possible to reproduce the propranolol model in Kiriya et al. (2008), determining values for some of the parameters required for the model was less straightforward. The parameter f_a (the fraction absorbed from the intestinal tract) had multiple values reported in Kiriya et al. (2008); the values used in the model for particular simulations were not recorded in the paper. In addition, several other parameter values were only available from secondary references such as f_b and R_{bp} . Issues with reporting of equations incorrectly can further hinder reproducing models, as encountered when considering the equation for the gut in Kiriya et al. (2008). The reporting of the dose and absorption of the drug was multiplied by the stomach blood flow (highlighted in red in Equation 4.24), instead of being added to the blood flow in the stomach after it was multiplied by the concentration in the stomach (highlighted in red in Equation 4.25).

$$\frac{dC_{gu}}{dt} = \frac{1}{V_{gu}} \left(Q_{gu} \left(C_{art} + k_a \times D \times F_a \times e^{-k_a \times t} - \frac{C_{gu} \times R_{bp}}{K_{p,gu}} \right) \right)$$

Equation 4.24

$$\frac{dC_{gu}}{dt} = \frac{1}{V_{gu}} \left(k_a \times D \times F_a \times e^{-k_a \times t} + Q_{gu} \left(C_{art} - \frac{C_{gu} \times R_{bp}}{K_{p,gu}} \right) \right)$$

Equation 4.25

Additionally, the source terms in the venous equation in Kiriya et al. (2008) did not match with the schematic in the paper. The equations imply blood flow from the spleen and gut into the venous blood and liver, whereas the schematic shows blood flow from the spleen and gut into the liver only. Inconsistency in reporting of any parameters or equations will result in variation in the output, as occurred with the AUC values which could only be reproduced within a 2-fold error. Although the time course of the data points were captured and C_{max} and

T_{max} were reproduced reasonably well. Using the propranolol PBK model as a template for atenolol gave predictions for AUC and C_{max} within a 3-fold error of the literature values, however, T_{max} was better reproduced with a 1.5-fold error.

While the salbutamol model from Boger and Fridén (2019) could be reproduced, there were inconsistencies with how the model was reported. Clearance in the schematic appears to be from the liver, however, in the model equations clearance comes from the venous blood instead. Blood flow from the gut and spleen into the venous is modelled in the venous equation but did not match with the schematic, where blood flow is included from the gut and spleen into the liver. The body weight used within the model was not clear. After thoroughly searching through secondary and tertiary references it appears that body weight was assumed to be 70 kg. Also, in comparison to other literature values (e.g. 1650 ml/min, Peters, 2008) for liver blood flow rates the value used within the salbutamol model appear low. However, using the value reported in Boger and Fridén gave salbutamol predictions within a 1-fold error (i.e. the values were the same) of observed data. A 1-fold error was obtained when using salbutamol as a template model for atenolol, as well as reproducing the lineshape of that observed in Peters (2008) for atenolol. An improvement in results when using salbutamol, compared to using propranolol as a template model, was observed suggesting the further refinement of the similarity results from just fingerprints and molecular weight to also include log P in the workflow benefitted the predictions obtained using read-across for atenolol as the target chemical. This is due to the log P of a chemical being associated with the absorption level of a chemical in the body. Log P influences the transport of a compound around the body while also affecting clearance of a drug.

When comparing the chemical specific parameter values of the source models (propranolol and salbutamol) to the target (atenolol) large differences were found between some parameters for the source chemical and the target chemical. Clearance for propranolol was 9208 ml/min/human whereas atenolol was only 301.6440ml/min/human. Propranolol is highly metabolised by the liver; however, atenolol is poorly metabolised by the liver. Chemical specific parameter values for salbutamol and atenolol were similar, thus indicating that the chemical would have similar kinetics. Although propranolol may have more similarity structurally to atenolol, salbutamol was more similar based on key physicochemical

properties (molecular weight and log P) that can affect how a chemical may be distributed or cleared in the body. The importance of physicochemical properties on how a chemical will distribute around the body was highlighted through the predictions of atenolol after using the PBK model for each source chemical as a template. Thus, further indicating that the PBK model for salbutamol is a better template than propranolol for predicting atenolol.

When investigating the second chemical, the herbicide flumioxazin only one analogue chemical, with a PBK model, was identified as being similar by the KWAAS tool after every step of the workflow was undertaken. The rivaroxaban minimal PBK model was successfully reproduced with a 1-fold error. The rivaroxaban model was used as a template model and adapted for flumioxazin, resulting in predictions of C_{max} and AUC within a 1-fold error of the literature values, although, the flumioxazin model does not fit the data points very well. This indicates that the resulting model does not capture the time course of the data very well. However, discrepancies in the time course of the predicted model could be a result of using predicted $K_{p_{hep}}$ and the assumption of 1 for R_b . Peters (2008) propose a workflow for adapting parameters to improve the predicted curve to match the observed curve. Following this workflow, the suggestion of adding a multiplicative factor to the tissue partition coefficient values for flumioxazin to reduce or increase the tissue distribution coefficients, may better reproduce the time course of the data by making the predictions better fit the observed flumioxazin data in Takaku et al. (2014). Large differences were found between some parameters for the source chemical (rivaroxaban) and the target chemical (flumioxazin), the fraction unbound in plasma (f_{up}) and absorption rate constant (k_a). Rivaroxaban had a f_{up} of 0.203 and a k_a of 1.42 h^{-1} while flumioxazin had a f_{up} of 0.03 and a k_a of 0.005 h^{-1} . These differences in k_a parameter values could be due to rivaroxaban being highly bioavailable and is readily absorbed, whereas flumioxazin is not.

Comparisons to low similarity scored chemicals were undertaken to test the assumption of the framework, that similarity of the analogue chemical used as a template is essential for obtaining a good model for the target chemical. For both the atenolol and flumioxazin predictions it was found that the similar chemical (propranolol and salbutamol) better predicted the target chemical blood plasma concentrations than the less similar chemical (rivaroxaban). When comparing the less similar model to the propranolol template the less

similar model significantly overpredicted atenolol concentrations. Whereas the less similar model (salbutamol) predictions for flumioxazin significantly underpredicted flumioxazin concentrations. Thus, supporting the assumption that similarity of the analogue is essential for obtaining a good model for the target chemical. This assumption was also demonstrated by Lu et al. (2016a) who compared the results for analogues and dissimilar chemicals and likewise demonstrated that using a rationally selected similar analogue gave better predictions.

Historical papers can have limitations when reproducing models due in part to a lack of consistency in how models are reported. Some terms in equations were not consistent in the schematics, which may have been missed in the peer review process. Expertise is needed to identify these mistakes when reproducing these models, or ideally, these should be identified and corrected during the peer review process. However, this is difficult due to the complexity of many PBK models, and the level of detail required in reporting such models. Thus, using the reporting template in Table 4.1 would help to reduce these inconsistencies. The approach taken in this chapter, i.e. developing new models based on using existing models as templates for two target chemicals, atenolol and flumioxazin, provided evidence of the effectiveness of using the KWAAS outlined in Chapter 3 to identify chemical analogues. Further, the results provide evidence that the read-across approach for PBK models can be applied successfully as the model for salbutamol was able to predict well the literature values for atenolol concentrations. Similarly adapting the rivaroxaban model, enabled flumioxazin concentrations following acute dosing to be predicted within a 1-fold error. The PBK modelling undertaken in this chapter, demonstrates a potential contribution to the 3Rs in the area of safety assessment, as it demonstrates that the read-across approach, commonly applied to predictions of toxicity, is also applicable to obtaining predictions for kinetic data. Making optimal use of both existing toxicokinetic and toxicodynamic information increases the reliability of predictions for chemicals lacking data.

Chapter 5 – Discussion

5.1 Discussion

Safety assessment requires a lot of information about potential toxicity and potential for internal exposure. However, this information is not available for many chemicals. Therefore, predictive methods are needed as animal testing for all predictions of toxicity is not realistic or desirable. A common method used for predicting toxicity is read-across, which relies on using data from a source chemical considered similar to a target chemical. Within this thesis a tool was devised to assist with read-across, to enable prediction of PBK-type data. This was achieved through undertaking a systematic review of PBK models in literature where key information from the models was extracted, development of the KWAAS and demonstration of this tool with two case studies, atenolol and flumioxazin.

The systematic review undertaken in Chapter 2 provides a valuable resource for academia, regulators and industry. The published protocol detailing the specific methodology used to undertake the systematic review allows for it to be readily updatable as more information becomes available. Thus, ensuring a sustainable impact on the 3Rs by reducing the number of new animal tests undertaken as existing models and data can be used to aid PBK model development. The information collected in the systematic review can serve as a guide for the development and evaluation of new PBK models prior to publication. Organising the current state of knowledge concerning PBK modelling through curation of existing models provides a valuable resource for those working in the area to identify models for chemicals of interest, or analogues, that can be used to assist the development or evaluation of new PBK models for chemicals lacking such data. The PBK modelling dataset (PMD) created in Chapter 2 consists of over 7,500 PBK models from the literature, containing information on species, life-stage, sex, route of administration, availability of equations, and the software used. Originally only rat and human models were to be captured, however, the decision was taken to incorporate all species to create a resource with greater applicability across different sectors. Information on chemical identifiers, i.e. CAS number, COSMOS ID, and InChiKey, were captured, to ensure the chemicals associated with a model were correctly recorded. Overall, a total of 1,187 unique chemicals with existing models were identified.

Within industry, development and advancement of PBK models is often within a confined area of chemical space that is of particular relevance to the specific industrial sector. Thus, in addition to the creation of the PBK model dataset, Chapter 2 mapped the physicochemical property space of existing PBK models and compared this to other chemical types (i.e. food additives, cosmetic ingredients, drugs, REACH chemicals, botanicals and pesticides) to obtain an overall assessment of the chemical space coverage of the models across the various sectors. The PMD resource has been made available in its current form, as a tool to assist researchers in finding relevant PBK models. The PMD is already being used successfully by industry e.g. Syngenta (UK) and esqLabs (Germany) have used this resource to identify PBK models for potential source chemicals for targets of interest. The PMD is available on the Joint Research Centre European Union Reference Laboratory for Alternatives to Animal Testing Catalogue (<https://data.jrc.ec.europa.eu/collection/id-0088>) and forms the basis of a webtool for exploring existing PBK models, currently under development at US Environmental Protection Agency (US EPA). The underpinning knowledge was captured using an Excel spreadsheet and this has the capacity to be readily updated in future as newly published PBK models (or those that were previously inaccessible/erroneously omitted) become available. The intention is that this would become a living resource with potential for further development beyond the life-time of the current project.

The creation of the KWAAS outlined in Chapter 3 helps to identify similar chemicals with PBK models to a target chemical. This should be of benefit for the 3Rs by enabling existing data to be used more efficiently, facilitating the read-across of information from data-rich to data-poor chemicals. In the KWAAS, similarity is determined using fingerprints, initial results can be refined by properties (e.g. molecular weight and log P). However, the properties for refining the selection, demonstrated in Section 3.3, may not work for all cases. The flexibility of the KWAAS allows for the user to adapt the workflow to select a combination of similarity indices and/or physicochemical properties deemed most relevant to the user, as well as, set their own range limits to aid the selection of appropriate chemicals to use as templates for building new PBK models. However, there is a need for expert judgement to assess the suitability of the analogue before taking the analysis further and developing a PBK model using a suggested analogue. This supports and enhances the on-going work to address the issues surrounding the identification of similar chemicals for read-across purposes. The

methods described herein enables similarity in terms of toxicokinetic behaviour to be considered in addition to similarity in inherent activity alone when making a read-across prediction of effects. In using the KWAAS it is essential that the user decides for themselves the most appropriate filters to employ and applies their own expert judgement as to the suitability of any analogues proposed by the KWAAS.

Six example chemicals from different industries (botanicals, pesticides, cosmetics, food additives, pharmaceuticals, and industrial) were used to check the applicability of the KWAAS to identify similar chemicals to different target chemicals from these data sets. For some groups of chemicals more accurate results can be obtained than for others. Each target chemical had at least ten chemicals identified as being as similar using fingerprints alone. Visual inspection indicated that for most of the chemicals that one or more of these may be suitable starting points for developing new PBK models in a read-across approach. To demonstrate how analogue selection may be performed, physicochemical properties to refine the potential analogues were included. However, other properties can be used to refine the results by, which can be added by the user. It was found that additional properties were too specific for some chemicals analysed, although ultimately the results shown in Chapter 3 demonstrate that the tool works satisfactorily, identifying potential analogues and refine this selection using criteria deemed appropriate by the user.

Previously, a PBK model using *in vivo* data from structural and functional analogues and PBK models built in the absence of *in vivo* PK data (on *in vitro* and *in silico* data only) has been evaluated by Ellison (2018) and Ellison and Wu (2020) using an approach that requires expertise and thorough research to assess similarity based on parameter values (bioavailability, volume of distribution and clearance). A correlation matrix of multiple physicochemical properties was used by Lu et al. (2016a) to identify similar chemicals of which analogues were then chosen through expertise. The KWAAS described within Chapter 3 uses structural similarity and physicochemical properties to use the information from the PBK models for source chemicals in a read-across approach to build new PBK models. Although, there is no consensus among researchers for the best method to determine similarity, this approach extends the previous approaches taken through having a structured method that includes both structure and physicochemical properties to determine similarity. Additionally,

this method can be advantageous through facilitating the inclusion of PBK model-derived data for a wide range of chemicals for which PBK models are currently lacking which can help to fill the knowledge gaps within current toxicokinetic information for chemicals that are data poor. Thus, helping help towards the integration of information from alternative methods into regulatory practice, producing more accurate safety assessments of chemicals through providing supporting information.

The KWAAS has been used by industrial collaborators to identify similar chemicals to a target. The models for these source chemicals were investigated to identify any similarities in model structure and/or input parameters that could be used to adapt a generic model for the target chemicals. Further, key modes of actions from the source chemical PBK models and used to adapt a generic PBK model to create a new model for the target chemical. Thus, creating a new PBK model for a target chemical more rapidly and reducing reliance on new animal tests. This method can overcome some of the difficulties with reproducing literature models (e.g. missing data or parameters) as the complete model does not necessarily need to be reproduced. It may be sufficient to identify key features of the models and adapt a generic model, by incorporation of these feature, to make it chemical-specific. The case studies that were undertaken in collaboration with industrial collaborators confirmed the suitability of the approach and showed the influence of chemical selection and input parameters on model quality (Paini et al., 2021b; Thompson et al. 2022). The US EPA web-based tool, which is under development, is intended to be more simplistic to use, enabling those without KNIME or programming expertise, to simply search for PBK model information using an intuitive graphical user interface.

Read-across is one of the most important developments in recent years increasingly being recognised as providing a suitable alternative to animal testing in safety assessment. However, particularly amongst the regulatory sector there is apprehension as to the acceptability of predictions. To increase confidence in predictions to promote read-across acceptability, as much information as possible needs to be provided. This support in predictions in terms of the internal exposure can be achieved through including additional information and evidence from PBK models in read-across predictions. Case studies successfully using a read-across approach for PBK modelling were outlined in Chapter 4.

When attempting to directly reproduce existing models from the literature (for example as conducted in Chapter 4) the resulting models had discrepancies resulting in fold errors above one. Errors in reporting of equations and parameters in papers, significantly affect model output and can require significant expertise in PBK modelling to identify and resolve. This issue of irreproducibility of existing models is well reported (Tan et al., 2018, Painsi et al., 2017) however it is not a problem unique to PBK modelling. Such problems have also been reported for *in vivo*, *in vitro* and *in silico* models. The PBK models from the literature were used as templates for target chemicals in this study, however, predictions for atenolol using a propranolol PBK model as a template resulted in a 3-fold error. Whereas, when using a salbutamol PBK model as a template for atenolol C_{max} was accurately reproduced and a 1.5-fold error for AUC was achieved. While the rivaroxaban model could be used as a template for flumioxazin and reproduce the C_{max} and AUC accurately, lineshape was not accurately reproduced. Peters (2008) outlines workflows to adapt the lineshape of the curve to better fit the data through addition of a scaling factors for the tissue-partition coefficients. The case study chemicals in this analysis were selected as they had available literature data to use for comparison to evaluate the models. These provided examples of using a read-across approach for PBK modelling, thus, demonstrating the reliability and potential pitfalls of using this approach.

5.2 Strengths and limitations of approaches taken

Whilst strengths and limitations to the work have been considered in the individual chapters, some of the over-arching themes are highlighted here. The systematic review outlined in Chapter 2 summarises the literature on available PBK models for an existing chemical, this provides a valuable resource for researchers requiring PBK model information across multiple chemical sectors. The method undertaken to complete this review was made publicly available on PROSPERO. By having a publicly available protocol it allows for the review to be reproduced, particularly for when it is needed to be updated to capture new models that have been published since the protocol was undertaken. The methodology was transparent, and bias reduced as it was published prior to the systematic review being undertaken. To ensure the systematic review could be used to assist safety assessment by multiple sectors,

information was captured on all species, chemicals, life-stages, and routes of administration. However, even with this methodical search strategy some information was missed. The quality control exercise showed that approximately 6% of PBK models were incorrectly excluded. There are multiple reasons as to why this may have occurred. For example, there were issues relating to how the PBK models were reported (e.g. lack of clarity as to whether a previously existing model was used, or if the model was adapted sufficiently to be considered a new model). In such cases personal judgement was used, although this may vary between different researchers. Furthermore, without a clear timeframe or plan for updating the systematic review, it will not stay up-to-date and continue to be a living resource for capturing newly developed PBK models. Another potential criticism is that the quality of the PBK models themselves was not ascertained when the model information was extracted. This would have been excessively time-consuming and there is the additional issue that when assessing model quality, it is useful to know the intended use of the model. A model of “lower” quality may still provide useful information for prioritisation purposes. Notwithstanding, the development of a robust tool to evaluate the extracted models would allow for potential limitations associated with some PBK models, or significant flaws in models, to be identified prior to extraction.

Chapter 3 summarises the creation of a KNIME workflow to assist analogue selection, which is of benefit to those undertaking read-across. One limitation of this approach is the way in which similarity is determined. Similarity of chemicals can be determined in multiple ways considering different properties of the molecules, e.g. structural, physicochemical or mechanism of action. The KWAAS determines structural similarity, initially using fingerprints with further refinement using physicochemical properties. One important consideration here, is that different fingerprints use different structural keys (fragments of a molecule) to assess similarity, giving different results for the similarity assessment. Hence, in this study, nine types of fingerprints were used to capture as many potentially similar chemicals as possible, however, the use of other fingerprints methods may also be beneficial in identifying alternative chemicals. In addition, the tool is limited as it only considers the physicochemical properties: molecular weight, log P, log D and pKa. These properties influence how a chemical may behave in the body; however, they do not encompass all properties that may influence chemical behaviour.

The KWAAS outlined in Chapter 3 was assessed in Chapter 4 with regards to its ability to identify analogues that could potentially be used in a read-across approach for PBK modelling, using case studies. The methods described within Chapter 4 of using a read-across approach for PBK modelling, helps to provide additional information when predicting toxicity of chemicals that lack data. However, it is important to note that this tool is designed to help fill gaps in kinetic data, it does not provide information on activity at the biological site of action, e.g. potential toxic effects of a drug or industrial chemical. This is a limitation of this approach i.e. PBK modelling predicts chemical concentration-time profiles within the body but does not predict what the chemical does once it reach its sites of action. This is exemplified in the case study, whereby the PBK profile for salbutamol was predicted using a model for atenolol. These drugs show similarities in chemical structure; however, atenolol is an adrenergic receptor antagonist and salbutamol is an agonist at the target site. Further limitations arise from using predictive software for obtaining the values required for the target chemical (such as log P and pKa) to input into the newly built PBK models, where inaccurate predictions from the software could carry forward into the PBK model. Additionally, using existing PBK models in the literature relies heavily on all information required to build the source model being clearly reported in the original publication. The case studies undertaken highlighted that some information may be missing or unclear.

The ability to use information from an existing PBK model to build a PBK model for a similar chemical in a read-across type approach helps fill some of the gaps in knowledge. Read-across is one of many alternative methods being used to replace animals. Read-across is useful for predicting endpoints for new chemicals, however for this to be possible information on chemicals considered as similar must be available. PBK modelling has many uses: it can help to optimise dosing in specific groups of patients for drugs that are already considered to be safe; it can act as an additional barrier before compounds are taken forward into animal testing; it can replace some early-stage human toxicology testing and can help replace some animal tests in deciding which compounds to take forward to human tests. PBK modelling and the methods developed here are a step towards reducing or replacing animal testing, although, they do not completely solve the problem of replacing animal test with alternative methods.

5.3 Conclusions

To help reduce the number of animals used within safety assessment this thesis has described three key steps for using a read-across approach for PBK modelling. A dataset of existing PBK models was created with an analysis of their chemical space coverage; an automated KNIME workflow that assists identification of similar chemicals from this dataset to a target chemical was also developed. Lastly, case studies were undertaken for two chemicals to assess this workflow in identifying chemicals with existing PBK models that are similar to a target chemical to use as a template through using a read-across approach. The performance of these models developed were also assessed.

5.4 Future work

Although not investigated within this thesis, analogues identified through using the similarity tool could be used in a read-across approach to adapt a generic PBK model to include metabolic or clearance pathways specific to a chemical of interest. Preliminary investigations into this possibility were performed by industrial collaborators but a more formal analysis of the general applicability and usefulness of this approach could be undertaken. In developing or using models, uncertainty needs to be identified and communicated. In PBK modelling, uncertainty (or errors) may be present in the simulations of mathematical models themselves or may be present in the reporting of the models. In either case the reliability and reproducibility of the models is comprised. The Biomodels database (<https://www.ebi.ac.uk/biomodels/>) has been developed to address this issue. Users can attempt to reproduce mathematical models and/or key figures from the literature and record in the database whether or not the models were reproducible, or if any errors were encountered. Currently there are few PBK models reported in this database, but this may change as interest in this area expands. Assessment of more PBK models using the Biomodels approach would help to build confidence in using these models.

Reproducing literature models can be difficult as there is inconsistency in how PBK models are reported. This hinders the acceptance of PBK models used for read-across, particularly within the regulatory sector. To facilitate researchers in extracting relevant data, as well as,

reproducing published PBK models in the literature, a methodical approach to how PBK models are recorded is needed to be incorporated. Some proposed formats for consistent publishing of PBK models have previously been proposed by EMA (2018) and USFDA (2018). More recently Tan et al. (2020) proposed a reporting format for PBK models that was incorporated into the OECD (2021b) guidance. This guidance outlines a PBK checklist utilising the previous recommendations for PBK model reporting. Therefore, to assist other researchers and reduce the problems associated with reproducing existing models from literature, researchers are encouraged to use consistent reporting formats. The Health and Environment Sciences Institute (HESI) were involved in the development of the PBK model reporting template published by Tan et al. (2020). There are ongoing activities within this organisation to enhance reporting and increase acceptance of PBK models by public health agencies. Frameworks for the minimum data (e.g. *in vivo*, *in vitro*, *in silico*, ADME) required for different applications for PBK modelling is also being developed by HESI to help facilitate the consistent use of PBK models in safety assessment.

Moving forward, leveraging the information currently available in existing PBK models, could be achieved by incorporating available resources into free webtools, making such resources widely available. Furthermore, efforts to curate available models and continue developing a living repository of PBK models that can be used across multiple sectors, will help enhance the uptake and accessibility of these models. For example, updating, and making freely available, subsequent versions of the PMD could enable any models published from 2020 onwards, as well as previously excluded models, to be incorporated. This would enhance the coverage of the dataset. An updated and comprehensive dataset for information on published PBK models, that is applicable to multiple sectors, would be of great advantage for the 3Rs. Further enhancements to this dataset in future could be the inclusion of additional relevant data (i.e. V_{max} , K_{ml} , clearance etc) from current PBK models or alternative sources in the literature, that could benefit future modellers when developing PBK models. Ideally, all resources necessary for model building and evaluation would be available within a single resource.

Future developments of the KWAAS would include incorporating ADME properties to the KWAAS and selecting analogues based on these properties to help better identify similar

chemicals. Specifically, incorporating information on metabolism and absorption, would be useful for determining the most similar chemicals, however, prediction of these properties is not always accurate using currently available methods. One property that is notably difficult to predict is clearance (Madden et al., 2019), improved models to predict this property and inclusion of accurate clearance values into the tool would be a great asset. Including ADME properties into the KWAAS to assist selection of analogues similar in respect of such properties would help to identify suitable PBK models. For example, refining by skin absorption to ensure chemicals have similar kinetics for creating a model for a chemical that is administered through the skin. Furthermore, other improvements to the KWAAS could be the ability to filter potential analogues based on the presence or absence of specific structural features associated with known routes of metabolism. For example, filtering results based on the presence or absence of aromatic amine function. (These can be metabolised to electrophilic nitrenium ions which in turn can react with nucleophilic sites within DNA, leading to mutagenicity (Madden et al., 2020) such knowledge may be useful when linking pharmacokinetic to pharmacodynamic activity in future.

Moreover, considering factors such as reproducibility of the PBK model and the confidence in obtaining correct input parameters when choosing the most appropriate PBK model, could be included in the KWAAS as a refinement criterion to help choose a suitable PBK model. Undertaking more case studies from multiple sectors (e.g. botanicals, cosmetics, industrial compounds) using the PBK model dataset to create new PBK models is needed to further highlight its usability and adaptability. The ability to identify analogues based on whether or not PK data are available to utilise in creating the new PBK model would also be of great benefit. When used correctly PBK modelling and the information within is not only a more ethical method for predicting toxicity but also a more feasible method than using animals.

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Appendices

Appendix 1 – PROSPERO systematic review protocol

This protocol has been published on PROSPERO (Thompson et al, 2020).

URL: <https://www.crd.york.ac.uk/prospero/>

Citation

Courtney Thompson, Judith Madden, Peter Penson. Systematic review to determine the chemical space of existing physiologically-based kinetic (PBK) models. PROSPERO 2020 CRD42020171130 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020171130

Review question

For which substances are physiologically-based kinetic (PBK) models available and which species, genders, life-stages and routes of administration have been investigated for these substances? This will include determining the chemical space coverage of the models and the availability of the associated model equations within the literature.

Context and rationale

In a PBK model, an organism is represented as a series of compartments (key organs, or subcompartments within organs/tissues) that are linked via blood. Information on the substances administered to the organism (e.g. solubility, partitioning behaviour) along with physiological information (e.g. blood flow rates, organ volumes etc) are used as inputs for the model. A series of differential equations are then applied to predict the overall time-course of the substance within the individual compartments. This approach can be used to: predict the internal dose of a substance, at relevant sites within the body, from external doses; extrapolate dose-response relationships from in vitro to in vivo scenarios and; estimate chemical exposure from biomonitoring data. They are highly adaptable, enabling predictions to be extrapolated across species or populations within a species, but they are also resource intensive to generate ab initio. It is essential to have a reliable, updateable resource that enables researchers to readily identify for which substances PBK models are currently available, for which species, gender, life-stage and route of administration as well as the availability of the equations used in the models and their chemical space coverage. The existing models can be used to inform the development of new PBK models (reducing reliance on animal testing) which has implications in safety assessment, not only of new active pharmaceutical ingredients, but also agrochemicals, consumer products, etc. Hence, information on all existing PBK models will be gathered using systematic review and the chemical space covered by the models will be assessed.

Searches [1 change]

Search all years with the search terms “pbpk OR pbk OR pbbk OR pbtb OR pbpd OR pbbm OR “physiologically based” AND (pharmacokinetic OR toxicokinetic OR biokinetic OR pharmacodynamics OR biopharmaceutical). Additional references identified through ad hoc searching, personal communications, etc. Sites searched:

PubMed

Scopus

Web of Science

Study designs to be included [1 change]

Inclusion criteria:

Any procedure where a substance has been administered to an animal for the purpose of determining the kinetics of that substance (or determining the effect of that substance on the kinetics of another co-administered or endogenous substance) using a physiologically-based kinetic modelling approach. This includes simulated models that have been validated, or robustly evaluated, using experimental data. Any software platform used to generate the physiologically-based model (commercial or publicly available) is

Appendix 2 – PRISMA Checklist

This research has been published in Alternatives to Laboratory Animals (Thompson et al, 2021).

DOI: <https://doi.org/10.1177/02611929211060264>



A Systematic Review of Published Physiologically-based Kinetic Models and an Assessment of Their Chemical Space Coverage

CV Thompson, JW Firman, MR Goldsmith, CM Grulke, Y-M Tan, A Paini, PE Penson, RR Sayre, S Webb and JC Madden

PRISMA Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction and Methods
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses*.	Criteria in methods and Prospero ¹ NB*
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Methods: "Systematic Review"
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Methods: "Extraction of data from available PBK models" and section: "Assessment of screening and data extraction reliability"
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect*.	Methods NB*
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.*	Methods NB*
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	NB*
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	NB*
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	NB*
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data	NB*

Appendix 3 – Data captured in PBK model dataset

This research has been published in Alternatives to Laboratory Animals (Thompson et al, 2021).

DOI: <https://doi.org/10.1177/02611929211060264>

This is a snapshot of the descriptions for each column heading in the PBK model dataset and the controlled vocab that can be selected or if input under a column heading will be free text. Chemical descriptors and information on the model are collected, i.e. species, sex, route of administration, availability of model equations.

1	A	B	C	D	E	F
2	Chemical Name	Additional Information on Chemical	Species Primary Category	Human Ethnicity or	Sex	Lifestage
3	Free Text	Free Text	Controlled Vocabulary	Controlled Vocabulary	Controlled Vocabulary	Controlled Vocabulary
4						
5		<i>Parent - stipulated if there is also information on metabolites in the spreadsheet</i>	Aquatic	Bull	Male	Pre-birth or pre-hatch
6		<i>Coadministered/Interaction - indicates where co-administered with another substance</i>	Bird	Cattle	Female	From birth or hatch up to adult
7		<i>- to check for interactions such as for drug-drug interaction studies.</i>	Bovine	Chicken	Unspecified	Adult (typical)
8		<i>Pre-treatment - the substance used for pre-treatment</i>	Cat	Clam		Pregnant
9		<i>Inhibitor - the inhibitor substance</i>	Dog	Cow		Old aged (specified)
10		<i>Inhibited - an inhibitor was present</i>	Gerbil	Fish		Health compromised (excluding old age)
11		<i>Mixture - mixture of more than one chemical</i>	Goat	Minipig		Unspecified
12		<i>Coeposure - coeposure of the chemical with other chemicals</i>	Guinea-pig	Monkey		
13			Hamster	Oyster		
14			Horse	Pig		
15			Human	Porpoise		
16			Non-human primate	Scallop		
17			Mouse - modified	Swine		
18			Mouse - unmodified	Turkey		
19			Oyster	Whale		
20			Polar bear	African		
21			Porcine	African American		
22			Rabbit	Asian		
23			Rat	Caucasian		
24			Rodent - combined	Chinese		
25			Rodent - unspecified	Hispanic		
26			Sheep	Indian		
27				Japanese		
28				Korean		
29				Comparative Ethnicity		
30				Human - Unspecified		
31						
32				<i>Comparative Ethnicity - if comparisons between ethnic groups is specified</i>		
33				<i>Human-unspecified is used if no details are given</i>		
34						
35			<i>Mouse - modified: is used when part of one species is translated into a mouse e.g. human liver cells translated into a mouse</i>			<i>From birth or hatch up to adult is used for all birth to</i>
36			<i>Rodent - combined: is used when a rodent model is specified, but the model includes a combination of both mouse and rat data</i>			
37			<i>Rodent - unspecified: a rodent model is mentioned but the type of rodent is not specified</i>			
38						
39						
40						
41						
42						
43						
44						

Appendix 4 – PBK model dataset

This research has been published in Alternatives to Laboratory Animals (Thompson et al, 2021).

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This is a snapshot of the data in the PBK model dataset. The dataset consists of over 7,500 rows with information collected under 13 different column headings. The dataset can also be found in the JRC catalogue (<https://data.jrc.ec.europa.eu/dataset/f98e9abf-8435-4578-acd6-3c35b5d1e50c#contributors>).

Chemical Name	Additional Information on Chemical	Species Primary Category	Human Ethnicity or Life Stage	Sex	Lifespan	Administration Route	Reference	PubMed ID if available	DOI	Availability of Equations in	Simulator
methylmercury		Aquatic	Fish	Unspecified	Unspecified	Oral - feed/water	Wang and Wang, 2015, Environ	26214340	10.1021/acs.est.5b02033	Full equations in paper	SAAM II
BDE-126		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-154		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-183		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-184		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-188		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-201		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-202		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-204		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-197		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-206		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-207		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
BDE-208		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
1,1,2,2-tetrachloroethane		Aquatic	Fish	Unspecified	Unspecified	Gills	Nichols et al., 1991, Toxicol Appl	1949008	10.1016/001-0084(91)	Equations in secondary references	Not Specific
1,1,2,2-tetrachloroethane		Aquatic	Fish	Unspecified	Unspecified	Dermal - topical	Nichols et al., 1996, Fundam App	8789789	10.1006/taap.1996.0001	Equations in combination of primary	ACSL (Adva)
1,1,2,2-tetrachloroethane	mixture	Aquatic	Fish	Unspecified	Unspecified	Gills	Lien et al., 2001, Aquat Toxicol, 5	11090804	10.1016/s164-454x(01)	Partial equations in paper	ACSL (Adva)
2,2,3,3,4,4',5,5',6,6'-decabromodiphenyl ether		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Wan et al., 2013, Environ Sci Tec	23387833	10.1021/es304926f	Equations in combination of primary	Not Specific
2,2,5,5'-tetrachlorobiphenyl		Aquatic	Fish	Unspecified	Unspecified	Oral - feed/water	Nichols et al., 2004, Toxicol Sci, 7	14657515	10.1093/toxic/kfh033	Full equations in paper	ACSL (Adva)
2,3,7,8-tetrachlorodibenzo-p-dioxin		Aquatic	Fish	Unspecified	Unspecified	Gills	Parthasarathy and Li, 2014, Environ T	24375488	10.1002/etc.2904	Partial equations in paper	acsl
arsenic		Aquatic	Fish	Unspecified	Unspecified	Gills	Liao et al., 2005, Environ Pollut, 1	15734542	10.1016/j.envpol.2004.	Full equations in paper	MATLAB
arsenic		Aquatic	Fish	Unspecified	Unspecified	Gills	Tsai and Liao, 2006, Environ Tox	16463258	10.1002/tx.20150	Full equations in paper	MATLAB
arsenic		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Ling et al., 2005, Integr Environ J	16637146	10.1897/iam.2004.4	Full equations in paper	Crystal Ball
benzo[a]pyrene		Aquatic	Scallop	Unspecified	Unspecified	Gills	Liu et al., 2014, Environ Toxicol P	24561533	10.1016/j.etap.2014.0	Equations in combination of primary	MATLAB
bisphenol A		Aquatic	Fish	Unspecified	Unspecified	Gills	Paery et al., 2014, Environ Sci Te	24295030	10.1021/es404301q	In supplementary material	Not Specific
ibuprofen		Aquatic	Fish	Unspecified	Unspecified	Gills	Paery et al., 2014, Environ Sci Te	24295030	10.1021/es404301q	In supplementary material	Not Specific
endosulfan alpha		Aquatic	Fish	Unspecified	Unspecified	Gills	Paery et al., 2014, Environ Sci Te	24295030	10.1021/es404301q	In supplementary material	Not Specific
endosulfan beta		Aquatic	Fish	Unspecified	Unspecified	Gills	Paery et al., 2014, Environ Sci Te	24295030	10.1021/es404301q	In supplementary material	Not Specific
ethinyl-estradiol		Aquatic	Fish	Unspecified	Unspecified	Gills	Paery et al., 2014, Environ Sci Te	24295030	10.1021/es404301q	In supplementary material	Not Specific
Rotenone		Aquatic	Fish	Unspecified	Unspecified	Intramuscular	Yang et al., 2013, J Vet Pharmac	22712485	10.1111/j.1365-2885.2	Full equations in paper	ACSL (Adva)
Rotenone		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Yang et al., 2013, J Vet Pharmac	22712485	10.1111/j.1365-2885.2	Full equations in paper	ACSL (Adva)
hexachloroethane		Aquatic	Fish	Unspecified	Unspecified	Gills	Nichols et al., 1991, Toxicol Appl	1949008	10.1016/001-0084(91)	Equations in secondary references	Not Specific
hexachloroethane		Aquatic	Fish	Unspecified	Unspecified	Dermal - topical	Nichols et al., 1996, Fundam App	8789789	10.1006/taap.1996.0001	Equations in combination of primary	ACSL (Adva)
hexachloroethane	mixture	Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Lien et al., 2001, Aquat Toxicol, 5	11090804	10.1016/s164-454x(01)	Partial equations in paper	ACSL (Adva)
oxytetracycline		Aquatic	Fish	Unspecified	Unspecified	Oral - dose	Brookshank, Hamdar and Law, 2	9312751	N/A	Not available	Not Specific
p,p'-dichlorodiphenylchloroethane	metabolite from parent: p,p'-dichlorodiphenyl	Aquatic	Porpoise	Male	Unspecified	Metabolism from parent	Weijts et al., 2013, Environ Sci Te	23560461	10.1021/es400386a	In supplementary material	Multiple sof
p,p'-dichlorodiphenylchloroethane	metabolite from parent: p,p'-dichlorodiphenyl	Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2013, Environ Sci Te	23560461	10.1021/es400386a	In supplementary material	Multiple sof
p,p'-dichlorodiphenylchloroethylene	metabolite from parent: p,p'-dichlorodiphenyl	Aquatic	Porpoise	Male	Unspecified	Metabolism from parent	Weijts et al., 2013, Environ Sci Te	23560461	10.1021/es400386a	In supplementary material	Multiple sof
p,p'-dichlorodiphenylchloroethylene	metabolite from parent: p,p'-dichlorodiphenyl	Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2013, Environ Sci Te	23560461	10.1021/es400386a	In supplementary material	Multiple sof
p,p'-dichlorodiphenylchloroethylene	parent	Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2013, Environ Sci Te	23560461	10.1021/es400386a	In supplementary material	Multiple sof
parasetamol		Aquatic	Fish	Unspecified	Unspecified	Gills	Abbas and Hayton, 1997, Toxicol	9221837	10.1006/taap.1997.831	Full equations in paper	CSMP III (C)
PBDE 100		Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2012, Environ Pollut,	22325441	10.1016/j.envpol.2011.	Equations in secondary references	Berkeley Mi
PBDE 153		Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2012, Environ Pollut,	22325441	10.1016/j.envpol.2011.	Equations in secondary references	Berkeley Mi
PBDE 47		Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2012, Environ Pollut,	22325441	10.1016/j.envpol.2011.	Equations in secondary references	Berkeley Mi
PBDE 99		Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2012, Environ Pollut,	22325441	10.1016/j.envpol.2011.	Equations in secondary references	Berkeley Mi
PCB 101		Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2011, Toxicol Appl P	21851832	10.1016/j.taap.2011.0	Equations in secondary references	Berkeley Mi
PCB 118		Aquatic	Porpoise	Male	Unspecified	Oral - feed/water	Weijts et al., 2011, Toxicol Appl P	21851832	10.1016/j.taap.2011.0	Equations in secondary references	Berkeley Mi

Appendix 5 – PBK model dataset physicochemical properties

This research has been published in Alternatives to Laboratory Animals (Thompson et al, 2021).

DOI: <https://doi.org/10.1177/02611929211060264>

This is a snapshot of the data in the PBK model dataset. Physicochemical properties associated with the chemicals from the PBK dataset.

Information on molecular weight, Slog P, TPSA, hydrogen bond donors and acceptors, and the number of Lipinski rule violations.

Name	SMILES	MWt	NumHBD	NumHBA	SlogP	TPSA	No Lipinski Viol.
{1}-S-verapamil	<chem>COC1CC(CCC1)CCN(CCC1)CC(C1)OCC</chem>	454.611	0	6	5.09308	63.95	1
[R]-warfarin	<chem>CC(=O)CC1C(=O)OC2C(C1O)C=CC2</chem>	308.333	1	4	3.6096	67.51	0
[2H8]-toluene	<chem>Cc1ccccc1</chem>	92.141	0	0	1.99502	0	0
5-aminobenzotriazole	<chem>Nn1nc2c1ccccc2</chem>	134.142	1	4	0.1451	56.73	0
1-chloro-1,1-difluoroethane	<chem>CC(Cl)FF</chem>	100.495	0	0	1.8379	0	0
1-hydroxypyrene	<chem>Oc1ccc2c3c1ccc1c3c(cc2)ccc1</chem>	218.255	1	1	4.2896	20.23	0
1-hydroxypyrene glucuronide	<chem>OC1(O)(OC(C1O)(=O)O)Oc1ccc2c3c1ccc</chem>	394.379	4	6	1.855	116.45	0
1-methoxy-2-propylacetate	<chem>COCC(OC=O)C</chem>	132.159	0	3	0.5844	35.53	0
1-methyluric acid	<chem>O=C1[NH]c2c([NH]1)(=O)n(c([O])[NH]2)C</chem>	182.139	3	4	-1.7568	103.51	0
1-methylxanthine	<chem>Cn1c([O])[NH]c2c(c1=O)[NH]cn2</chem>	166.14	2	4	-1.0501	83.54	0
1-naphthol	<chem>Oc1ccc2c1ccccc2</chem>	144.173	1	1	2.5454	20.23	0
1-phenylimidazole	<chem>c1ccc([n]1)nc1c</chem>	144.177	0	2	1.8723	17.82	0
1,1-dichloro-2,2-bis(p-chloro	<chem>ClC(Cl)C(Cl)C(Cl)C1C=CC(C1)Cl</chem>	318.03	0	0	6.1879	0	1
1,1-dichloro-2,2-trifluoro	<chem>ClC(Cl)F(F)F</chem>	152.93	0	0	2.3524	0	0
1,1-dichloroethylene	<chem>ClC=CCl</chem>	96.944	0	0	1.9352	0	0
1,1-difluoroethane	<chem>CC(F)F</chem>	66.05	0	0	1.2714	0	0
1,1,1-trichloro-2,2-bis(p-chl	<chem>ClC(Cl)C(Cl)C(Cl)C1C=CC(C1)Cl</chem>	354.491	0	0	6.4955	0	1
1,1,1-trichloroethane	<chem>CC(Cl)(Cl)Cl</chem>	133.405	0	0	2.3765	0	0
1,1,1,2-tetrachloroethane	<chem>ClC(Cl)(Cl)Cl</chem>	167.85	0	0	2.5954	0	0
1,1,1,2,3,3,3-heptafluoropro	<chem>FC(F)(F)F(F)F(F)F</chem>	170.027	0	0	2.4491	0	0
1,1,1,3,3-pentafluoropropan	<chem>FC(CF)F(F)F</chem>	134.047	0	0	2.2039	0	0
1,1,1,3,3,3-hexafluoropropan	<chem>FC(CF)F(F)F(F)F</chem>	152.037	0	0	2.5011	0	0
1,1,2-trichloro-1,2,2-trifluo	<chem>FC(Cl)(Cl)F(F)F</chem>	187.375	0	0	2.9189	0	0
1,1,2-trichloroethane	<chem>ClCC(Cl)Cl</chem>	133.405	0	0	2.0289	0	0
1,1,2-trichloroethylene	<chem>ClC=CCl</chem>	131.389	0	0	2.5017	0	0
1,1,2,2-tetrachloroethane	<chem>ClC(Cl)(Cl)Cl</chem>	167.85	0	0	2.5938	0	0
1,1,2,2-tetrachloroethylene	<chem>ClC(Cl)(Cl)Cl</chem>	165.834	0	0	3.0682	0	0
1,2-cyclohexanediol	<chem>OC1CCCCC1O</chem>	116.16	2	2	0.2822	40.46	0
1,2-dichlorobenzene	<chem>Clc1ccccc1</chem>	147.004	0	0	2.9934	0	0
1,2-dichloroethane	<chem>ClCCCl</chem>	98.96	0	0	1.464	0	0
1,2-dichlorovinylcysteine	<chem>OC=O(CN(C=CC)C)CS</chem>	216.089	3	3	1.2355	49.33	0
1,2-dihydroxy-4-allylbenzene	<chem>C=CC1C=CC(C=C1)O</chem>	150.177	2	2	1.8263	40.46	0
1,2-epoxy-3-butene	<chem>C=CC1CO1</chem>	70.091	0	1	0.5712	12.53	0
1,2-trans-dichloroethylene	<chem>ClC=CCl</chem>	96.944	0	0	1.9352	0	0
1,2,3,4,6,7,8-heptachlorodit	<chem>Clc1c(Cl)cc2c(c1)Oc1(O2)c(Cl)c(c1)Cl</chem>	425.309	0	2	8.1584	18.46	1
1,2,3,4,6,7,8-heptachlorodit	<chem>Clc1cc2c(c1)Cl)Oc1c2c(Cl)c(c1)Cl)Cl</chem>	409.31	0	1	8.1598	13.14	1
1,2,3,4,7,8-hexachlorodiben	<chem>Clc1cc2Oc3c(Oc2cc1)C)c(c3)Cl)Cl</chem>	390.864	0	2	7.505	18.46	1
1,2,3,4,7,8-hexachlorodiben	<chem>Clc1cc2c(c1)Oc1c2c(Cl)c(c1)Cl)Cl</chem>	374.865	0	1	7.5064	13.14	1
1,2,3,4,7,8,9-heptachlorodit	<chem>Clc1cc2Oc3c(c2c1)Cl)Cl)c(c3)Cl)Cl</chem>	409.31	0	1	8.1598	13.14	1
1,2,3,6,7,8-hexachlorodiben	<chem>Clc1c(Cl)cc2c(c1)Oc1(O2)c(Cl)c(c1)Cl</chem>	390.864	0	2	7.505	18.46	1
1,2,3,6,7,8-hexachlorodiben	<chem>Clc1cc2c(c1)Cl)Oc1c2c(Cl)c(c1)Cl)Cl</chem>	374.865	0	1	7.5064	13.14	1
1,2,3,7,8-pentachlorodibenz	<chem>Clc1cc2Oc3c(Oc2cc1)C)c(c3)Cl)Cl</chem>	356.419	0	2	6.8516	18.46	1
1,2,3,7,8-pentachlorodibenz	<chem>Clc1cc2c(c1)Oc1c2c(Cl)c(c1)Cl)Cl</chem>	340.42	0	1	6.853	13.14	1

Appendix 6 – KNIME workflow for assisting analogue selection (KWAAS) for PBK modelling

Information from a PBK model for a chemical rich in data (source chemical) can be used to create a PBK model for a similar chemical lacking data (target chemical). This document explains how to use the automated tool created to help determine similar analogues with available PBK models. A flow chart of the steps taken to use the tool and determine the most similar chemicals from the PBK dataset is shown in Figure A7.1. These models can be used either as a template to develop a new PBK model for a target chemical, or provide information to adapt a generic PBK model, rapidly providing a more accurate chemical-specific model. Thus, this will assist more accurate safety assessment of chemicals by providing kinetic information for chemicals of interest.

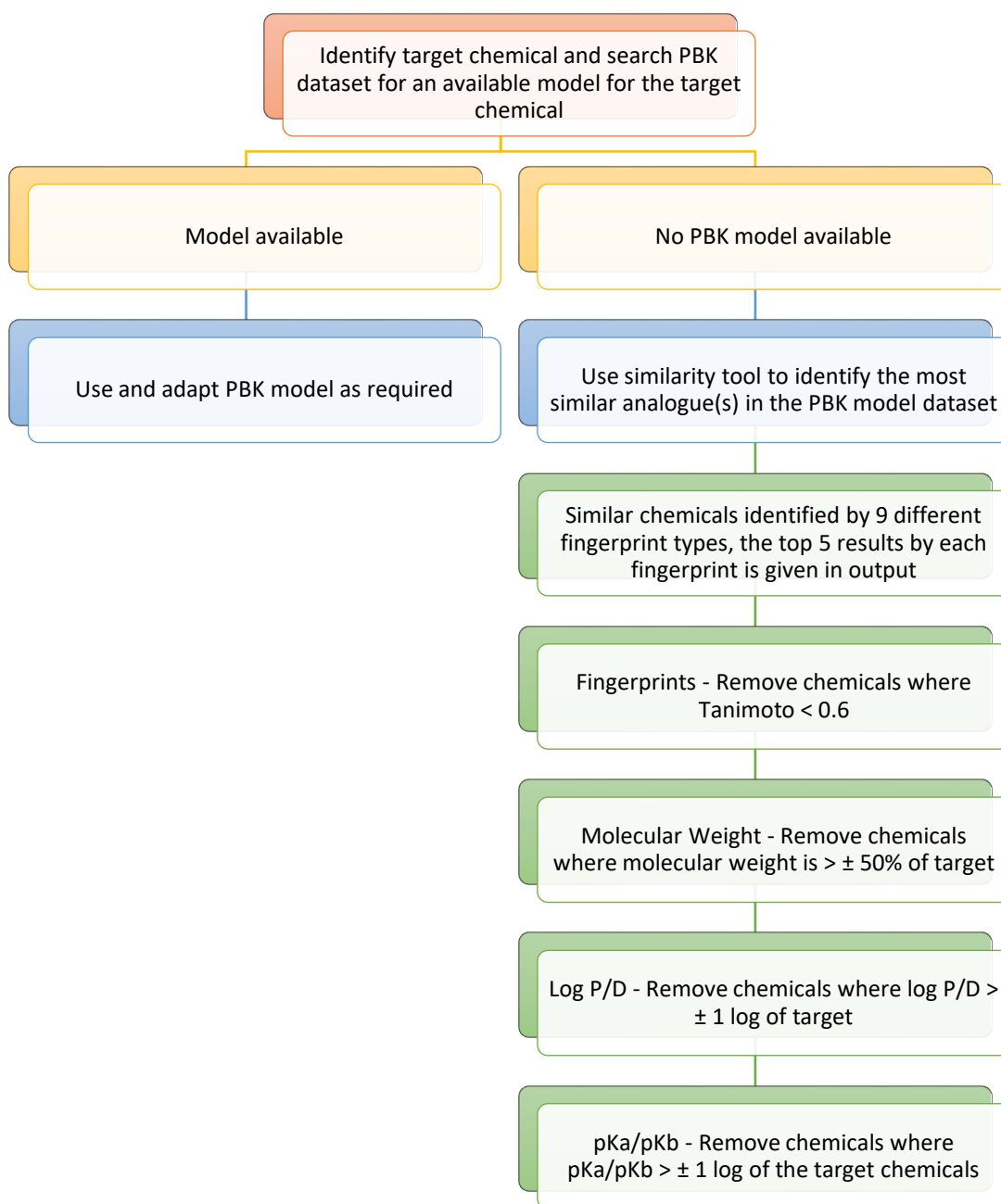


Figure A7.1 Flowchart of the steps taken to determine the most similar chemicals in the PBK dataset to a target chemical.

Step-by-step guide on using the KWAAS

Instructions on how and where to download KNIME can be found at the below link,

<https://www.knime.com/downloads>.

Key information for using KNIME:

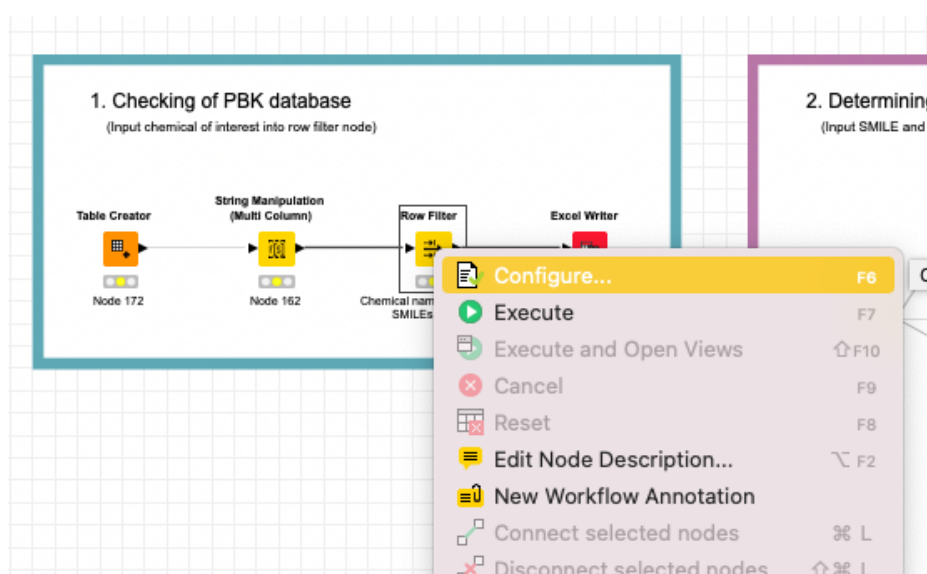
- Red light under node – node needs to be configured
- Yellow light under node – node is configured, and needs to be executed

- Green light under a node – node executed
- Right click any node to get a list of options of commands, including configure, execute and an option to view results.

Stage 1 – Checking the PBK model dataset for an existing model for a target

To check the PBK model dataset to determine if there are any PBK models currently available within the dataset for the target chemical of interest.

Right click and select configure on the ‘row filter’ node.

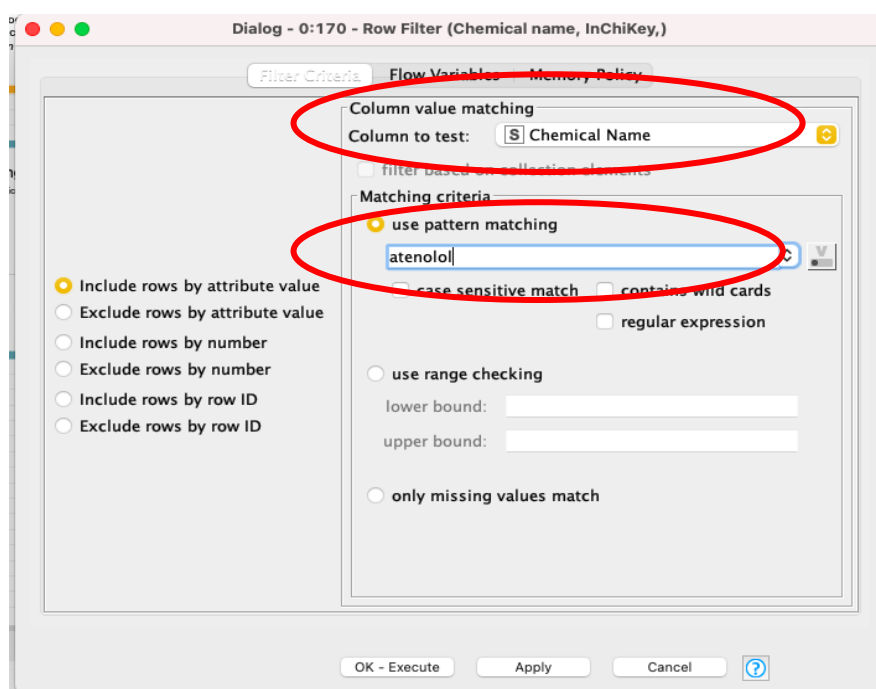


Choose a column option to search the dataset, either by Canonical SMILES, InChiKey or chemical name. Input the canonical SMILES, InChiKey or chemical name under ‘use pattern matching’ and click OK, then execute the node.

Example: Flumioxazin

Select InChiKey and input the InChiKey for flumioxazin (i.e. FOUWCSDKDDHKQP-UHFFFAOYSA-N)

Output should include 2 models for atenolol (1 model for a human and 1 model for a rat).

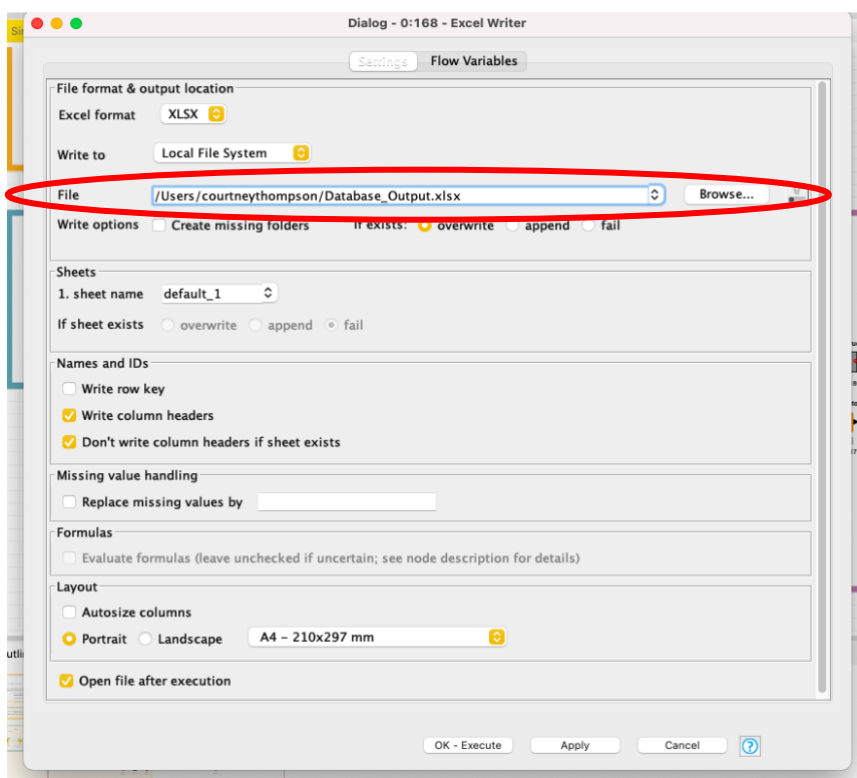


Enter the term
"Chemical Name",
"Canonical SMILES"
or "InChiKey" here to
select which column
is to be searched

Enter the name of
the chemical, its
SMILES string or
InChiKey, according
to the column type
selected above



To obtain the results in an Excel spreadsheet, right click
and select configure on the 'Excel writer' node



Under 'file' select the location where you want to save your excel file before executing the node.

Stage 2 – Finding similar analogues to a target chemical

Similar chemicals to a target chemical may be identified using chemical fingerprints and/or physicochemical properties. Nine chemical fingerprints, Morgan, FeatMorgan, AtomPair, Torsion, RDKit, Avalon, Layered, MACCS, and Pattern are all used to calculate similarity between chemicals in which a score is given. Excel output can be obtained at every step of the workflow (follow steps in stage 1 on how to configure the Excel node).

Configure the first table creator by inputting the Canonical SMILE and chemical name in the first row for the target chemical.

2. Determining similar analogues
(Input SMILE and chemical name into row 0 of the first table node)

Table Creator

Node 1

Molecule Type Cast
Node 2

RDKit From Molecule
Node 4

Counter Generation
Node 43

Node 1

Dialog - 0:1 - Table Creator

Table Creator Settings Flow Variables Memory Policy

Input line:

Row0 [SMILE] [Chemical Name] atenolol

Row1

Row2 CC(=O)CC... (R)-warfarin

Row3 Cc1ccccc1 [2H8]-tolu...

Row4 Nn1nnc2c... 1-aminobe...

Row5 CC(Cl)(F)F 1-chloro-1...

Row6 Oc1ccc2c3... 1-hydroxy...

Row7 OC1C(O)C... 1-hydroxy...

Row8 COCC(O)C... 1-methoxy...

Row9 O=c1[nH]c... 1-methylur...

Row10 Cn1c(=O)[... 1-methylx...

Row11 Oc1cccc2c... 1-naphthol

Row12 c1ccc(cc1)... 1-phenyli...

Row13 CC(=C)C1c... 1,1-dichlo...

Row14 CC(Cl)(F)F... 1,1-dichlo...

Row15 CC(=C)Cl 1,1-dichlo...

Row16 CC(F)F 1,1-difluor...

Row17 CC(Cl)cc... 1,1,1-trich...

Row18 CC(Cl)(Cl)C 1,1,1-trich...

Row19 CCCC(Cl)(Cl)C 1,1,1,2-te...

Row20 FC(Cl)(F)F... 1,1,1,2,3-...

Row21 FC(Cl)(F)F... 1,1,1,3,3-...

Row22 FC(Cl)(F)F... 1,1,1,3,3-...

The output table has 1145 rows and 2 columns. Highlight output table

OK Apply Cancel ?

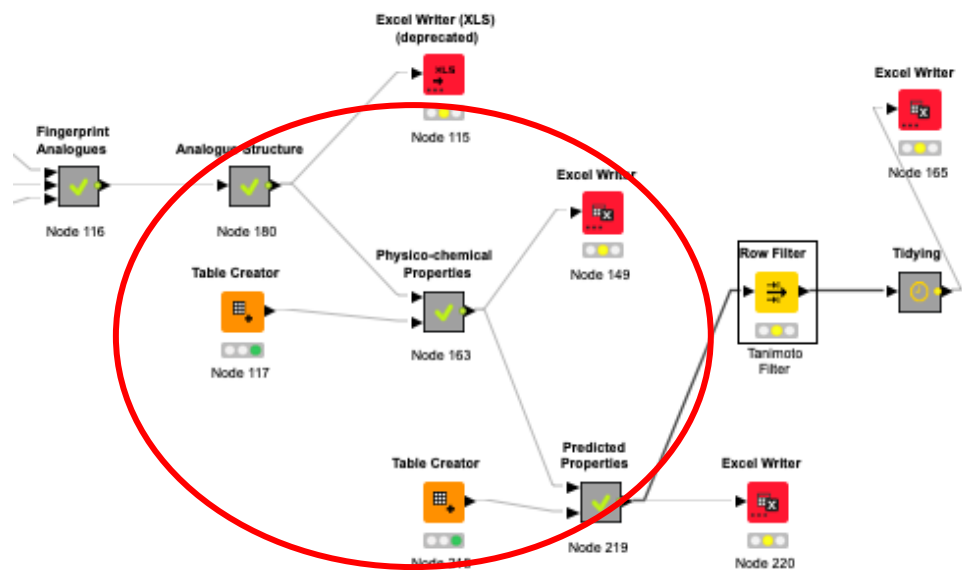
Enter the chemical name and SMILE in Row 0 of the first table.

Example: Flumioxazin

Input under SMILES column: C#CCN1C(=O)COC2=CC(=C(C=C21)N3C(=O)C4=C(C3=O)CCCC4)F

Input under chemical name column: flumioxazin

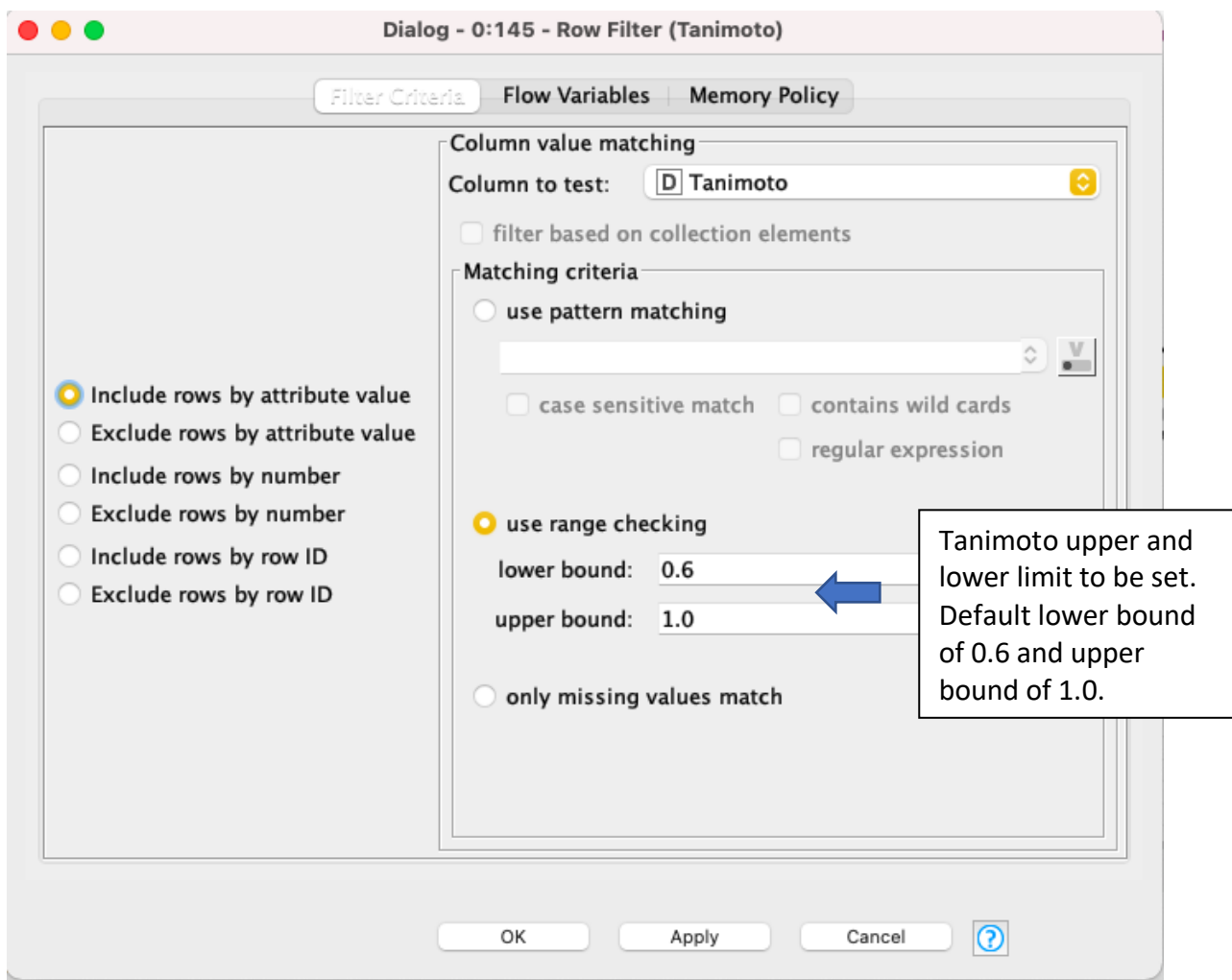
Execute the metanode 'fingerprint analogues' to obtain the analogues determined as being similar according to chemical fingerprints. The next three metanodes in the sequence, will provide the structures, physicochemical properties (molecular weight, log P, hydrogen bond donors and acceptors, TPSA (topological polar surface area), and the number of Lipinski rule violations) and predicted properties (pKa, pKb, log D at pH 5.5, log D at pH 7.4, and CL_{int}) for each suggested analogue when executed.



All nodes up until the first 'row filter' node can be executed without configuration and excel output obtained at each stage. A Tanimoto index for similarity is generated giving a score between 0 and 1, where 0 is not similar and 1 indicates an identical chemical. The row filter node named 'Tanimoto filter' is set at default to include all analogues of a with a Tanimoto index of 0.6 or higher. However, this Tanimoto value can be set by the user.

Example: Flumioxazin

After filtering by Tanimoto > 0.6, there should be 25 rows of results consisting of 17 chemicals.



Refining analogue selection by Molecular Weight

Once a set of suitable analogues has been identified these can be further refined. The 'MW filter' row filter node sets upper and lower boundaries to include analogues that fall within them limits.

It is recommended to set a lower bound of 50% less than the target chemical's molecular weight and an upper bound of 50% greater than the target chemical's molecular weight (although the range can be set to any value of the user's choice).

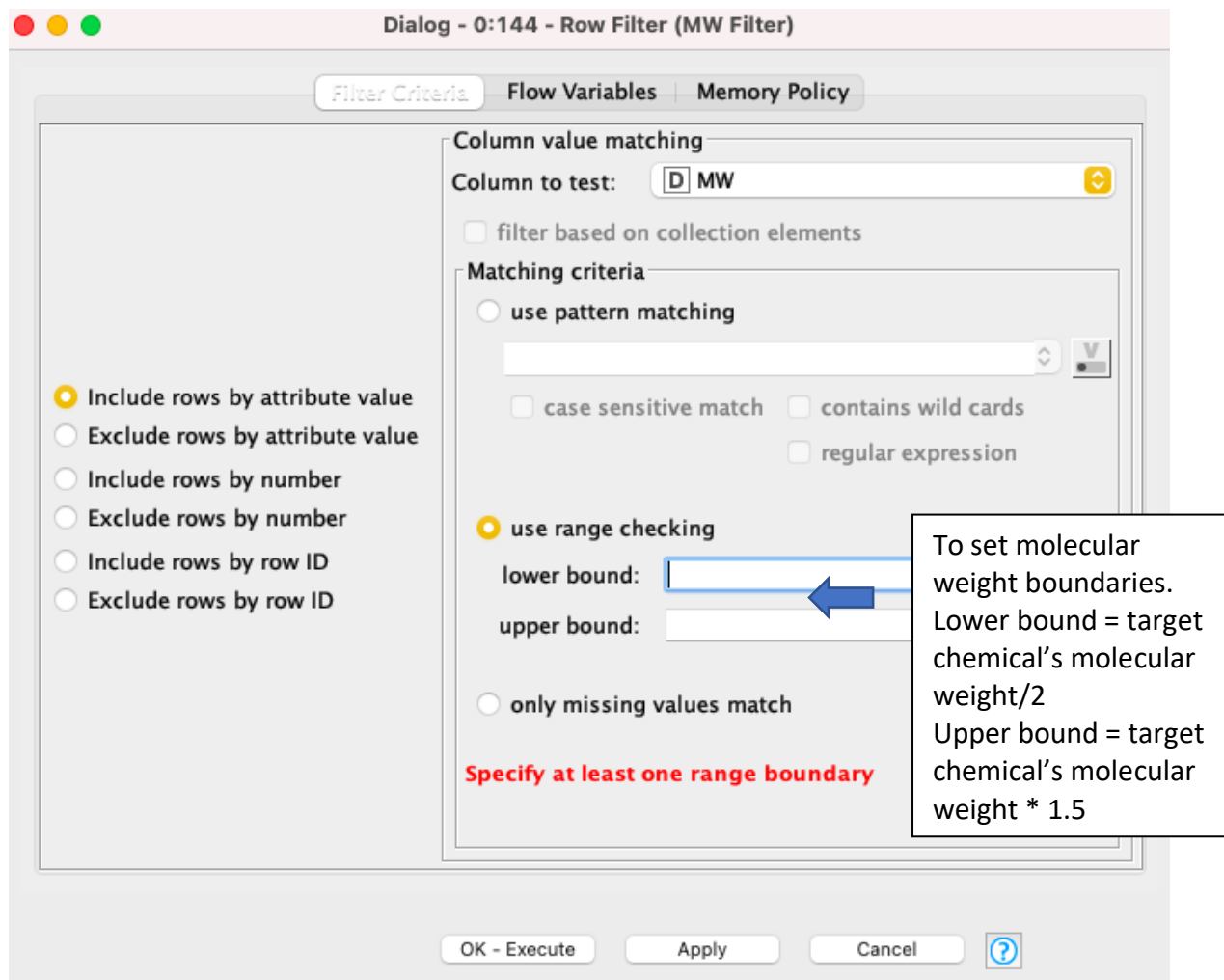
Example: Flumioxazin

Molecular weight for flumioxazin: 354.3 Da.

Lower bound input: 177.15

Upper bound input: 531.45

After filtering there should be 20 rows of results consisting of 12 chemicals.



Refining analogue selection by log P/log D

Results can be refined further to find the most similar chemicals. The 'log P filter' and 'log D filter' row filter node sets upper and lower boundaries to include analogues that fall within those limits.

A log D at pH 5.5 or pH 7.4 needs to be chosen before inputting boundaries to obtain results within the specified range. It is recommended to set a lower bound of 1 log less than the target chemical's log P/log D and an upper bound of 1 log greater than the target chemical's log P/log D (although the range can be set to any value of the user's choice).

Example: Flumioxazin

If choose to filter by log P. Log P for flumioxazin: 1.9281

Lower bound input: 0.9281

Upper bound input: 2.9281

After filtering there should be 15 rows of results consisting of 7 chemicals.

If choose to filter by log D. Log D (at pH 5.5) for flumioxazin: 2.55

Lower bound input: 1.55

Upper bound input: 3.55

After filtering there should be 13 rows of results consisting of 5 chemicals.

Choose the term 'log D (5.5)' or 'log D (7.4)' here to select which column of log D is to be searched, pH 5.5 or pH 7.4, respectively.

To set log P/log D boundaries.
Lower bound = target chemical's log P/log D - 1
Upper bound = target chemical's log P/log D + 1

OK - Execute Apply Cancel ?

Refining analogue selection by pKa/pKb

Results can be refined further to find the most similar chemicals. The 'pKa/pKb filter' row filter node sets upper and lower boundaries to include analogues that fall within them limits.

It is recommended to set a lower bound of 1 log less than the target chemical's pKa/pKb and an upper bound of 1 log greater than the target chemical's pKa/pKb (although the range can be set to any value of the user's choice).

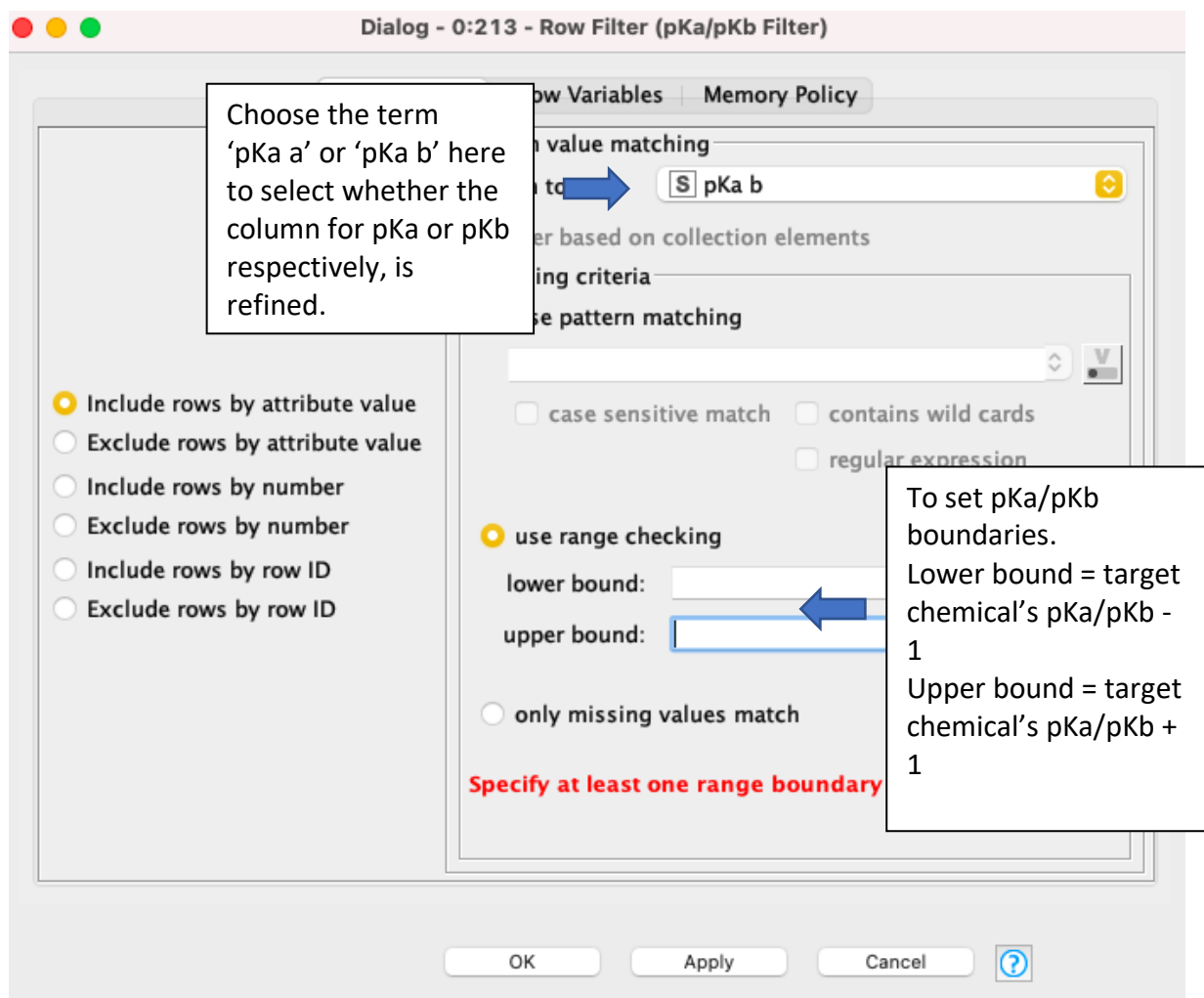
Example: Flumioxazin

pKb for flumioxazin: 3.31

Lower bound input: 2.31

Upper bound input: 4.31

After filtering there should be 10 rows of results consisting of 2 chemicals.

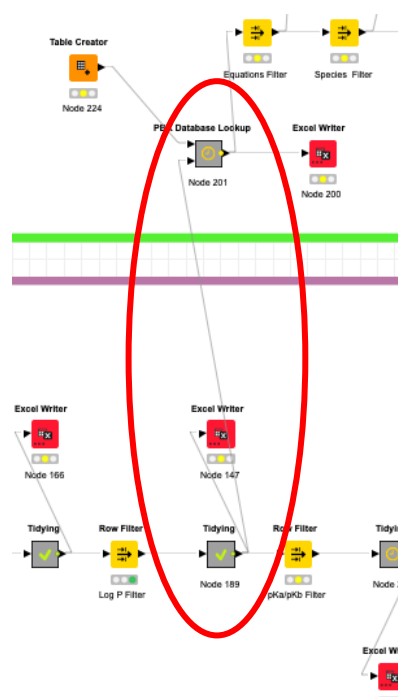


Generating the Excel output after employing these filters will result in an Excel file containing the analogues in the PBK model dataset that are considered most similar to the target.

Similarity after all these steps has been determined in terms of the Tanimoto score for nine chemical fingerprints and molecular weight, log P, pKa and log D falling within set boundaries.

Stage 3 – Refining determined similar models from the dataset

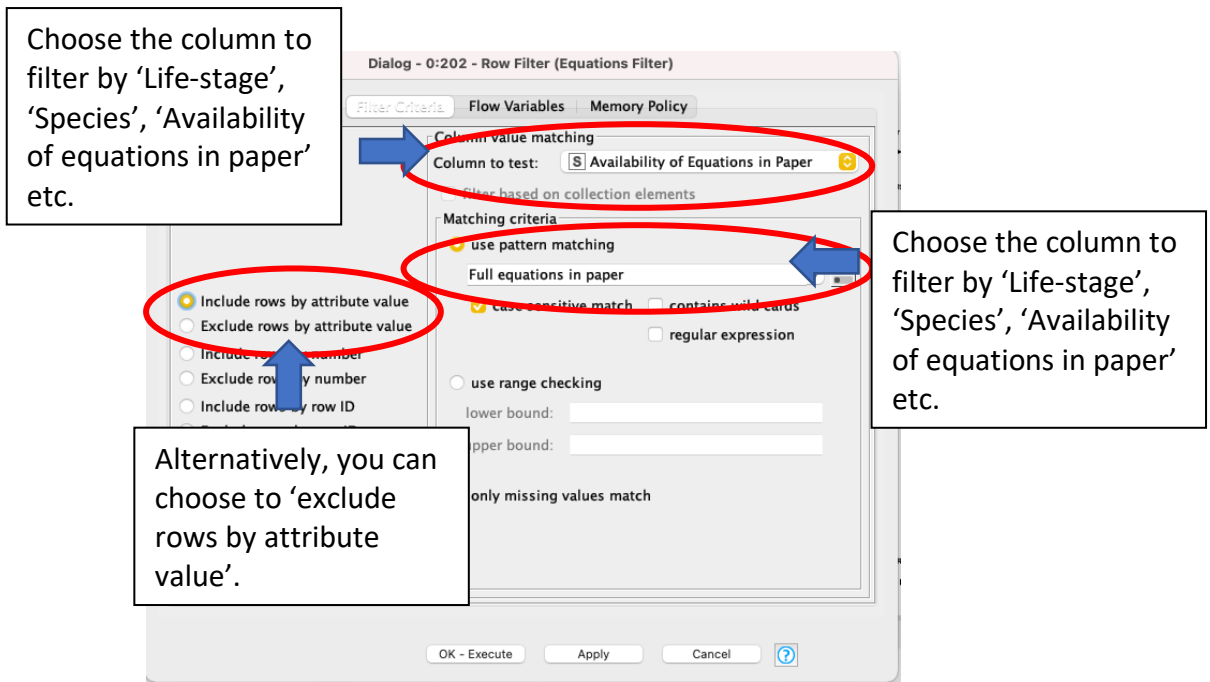
The PBK model dataset can be searched again, this time searching for all analogues that have been identified as being similar to the target and refining to show the analogue models most specific to the users wants. Excel output can be obtained at each step within filtering (again follow the steps in stage 1 on how to configure the Excel node). Output can be exported at any stage to look at the models available for the identified chemicals to see if the chemicals PBK models being determined as similar are useful to the user.



Connect the last row filter node used within determining similarity (stage 2) to the 'PBK model dataset lookup' metanode (in stage 3). This can be done by clicking the arrow coming out of the last node used and dragging it to the bottom input arrow of the 'PBK model dataset lookup' metanode. Executing this metanode will result in all available models associated with the analogues determined as being similar at the last filter stage.

Each row filter connected to the 'PBK model dataset lookup' metanode can be set and executed as desired. Currently it is set to filter in the order available equations, species, software, life-stage, route of administration. Filtering the PBK dataset results allows the user to refine the available models in respect to the purpose of the search, e.g. refine the species by human, and life-stage by pregnant to obtain only pregnancy human PBK models.

When configuring a row filter node, first select a 'column to test'. Then under 'use pattern matching' select the statement you would like to only include from the drop-down option.



Alternatively, you can choose to exclude by a specific species, sex, life-stage, software or availability of equations. This can be achieved through choosing to 'exclude rows by attribute value' and following the same steps on how to include a specific row.