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Using Read-Across to build Physiologically-Based Kinetic models: Part 2. Case studies for atenolol and flumioxazin

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ABSTRACT

Read-across, wherein information from a data-rich chemical is used to make a prediction for a similar chemical that lacks the relevant data, is increasingly being accepted as an alternative to animal testing. Identifying chemicals that can be considered as similar (analogues) is crucial to the process. Two resources have been developed previously to address the issue of analogue selection and facilitate physiologically-based kinetic (PBK) model development, using read-across. Chemical-specific PBK models, available in the literature, were collated to form a PBK model dataset (PMD) of over 7,500 models. A KNIME workflow was created to accompany this PMD that can aid the selection of appropriate chemical analogues from chemicals within this dataset (i.e. chemicals that are similar to a target of interest and are known to have an existing PBK model). Information from the PBK model for the source chemical can then be used in a read-across approach to inform the development of a new PBK model for the target. The application of these resources is tested here using two case studies (i) for the drug atenolol and (ii) for the plant protection product, flumioxazin. New PBK models were constructed for these two target chemicals using data obtained from source chemicals, identified by the workflow as being similar (analogues). In each case, the published PBK model for the source chemical was initially reproduced, as accurately as possible, before being adapted and used as a template for the target chemical. The performance of the new PBK models was assessed by comparing simulation outputs to existing data on key kinetic properties for the targets. The results demonstrate that a read-across approach can be successfully applied to develop new PBK models for data-poor chemicals, thus enabling their deployment during early-stage risk assessment. This assists prediction of internal exposure whilst reducing reliance on animal testing.

1. Introduction

Demonstrating the safety of chemicals is essential to protect the health of individuals who are exposed (e.g., operators or consumers). However, for many chemicals there is a lack of data on which to base safety assessment decisions. These decisions require information on both hazard (effect or potency) and exposure (both internal and external) to determine overall risk to health. Physiologically-based kinetic (PBK) models can simulate concentration–time profiles of chemicals in the blood and individual internal organs providing a dose metric that is more realistically associated with the potential to elicit an effect. PBK models can be both time- and resource-intensive to build due to the large number of parameters required and the difficulties in obtaining these [1]. Data required to build a PBK model *de novo* include: physiological parameters (e.g., organ volumes, blood flow rates); absorption, distribution, metabolism and excretion (ADME) properties (e.g., intrinsic clearance and intestinal absorption); and physico-chemical properties (e.g., octanol:water partition coefficients and pKa) [2]. Due to these demands, PBK models are available for relatively few chemicals.

Read-across is an approach wherein information from a data-rich (source) chemical is used to fill in knowledge gaps for a data-poor (target) chemical [3,4]. It has previously been shown that a PBK model for one chemical can be used to inform the development of a PBK model for another chemical in a read-across approach, providing that a PBK model is available for a "similar" chemical [5,6]. Thompson et al. (2021) have previously published a PBK modelling dataset (PMD),

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comprising information on existing PBK models [7]. These authors have also developed a structured framework for identifying similar (source) chemicals to a target of interest [8]. The framework for identifying similar chemicals uses a KNIME workflow to assist analogue selection, subsequently referred to herein as the KWAAS. Within this framework, similar chemicals (analogues) can be identified using similarity in terms of chemical structure, physico-chemical or ADME properties. Although it is preferable to use experimental data when populating a PBK model with chemical-specific information, there are an increasing number of resources for estimating values for both physico-chemical and ADME properties *in silico* when experimental data are not available. Resources for obtaining or predicting physico-chemical and ADME properties, have been reviewed recently [9,10]. Experimental or predicted properties can be incorporated into the KWAAS and used to refine the selection of analogues.

The KWAAS provides a structured approach to determining similarity using physico-chemical properties, chemical fingerprints or other criteria, such as ADME properties, as selected and optimised by the user [8]. Herein we provide evidence of the practical applications of the approach by describing two case studies where read-across was used in the development of new PBK models for two target chemicals - atenolol (a commonly used drug) and flumioxazin (a plant protection product). Existing PBK models are available for both atenolol and flumioxazin; however, these chemicals were chosen so that the parameters obtained from the newly-developed PBK models could be compared to existing models to validate the approach. Hence atenolol and flumioxazin represent "pseudo-unknowns" rather than true unknowns. The KWAAS (as described by Thompson et al. [6]) was used firstly to identify existing PBK models for the two targets and then to identify PBK models for analogues. The existing models for the analogues were reproduced, as accurately as possible before being adapted to use as "templates" to build PBK models for the target chemicals. The performance of the newly-derived PBK models was assessed by comparing blood concentration–time profiles with existing data from the literature. Model assessment was undertaken for each new PBK model created using global sensitivity analysis and comparison of fold error for key metrics – maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), and the area under the concentration–time curve (AUC).

2. Methods

2.1. Identifying analogues, with an existing PBK model, from the PBK modelling dataset (PMD) using the KWAAS

This paper is Part 2 of a study into using a read-across approach to build PBK models for chemicals lacking data. For details on how to obtain and use the KWAAS, along with information on how analogues are selected and the types of output available, please refer to the linked publication [8]. Here, we report the *application* of the KWAAS to two case studies and an assessment of the quality of the new models developed for the two target chemicals, atenolol and flumioxazin. Initially, the InChiKeys for the two target chemicals were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov) and used to search the PMD for existing models for the two chemicals. These targets had been selected as they were known to have existing PBK models, hence the *in vivo* data from these models could be used to assess the predictions from the newly developed models generated using the read-across approach. The chemical name and SMILES string of the two chemicals were inputted into the KNIME workflow to calculate structural similarity to other chemicals in the PMD, using nine different fingerprints, before being refined to identify those chemicals that were also similar in terms of their physico-chemical properties. In this case, chemicals with similarity scores of 0.6 and above were included for further refinement. A Tanimoto score of 0.6 or above has been proposed previously as a suitable cut-off value for identifying similar chemicals [11]. Analogue selection was refined based on molecular weight being within ± 50 % of the target chemical's molecular weight and log P or log D values being within ± 1. The purpose of selecting property ranges within finite values is to identify a suitable number of analogues that are considered sufficiently similar by these criteria. The values can be set at any range the user selects, this is in part determined by the number of analogues identified at each stage (i.e. if too many analogues are suggested a narrower range can be used; if too few are identified the cut-off values can be adjusted to encompass a wider range). Further explanation of selection criteria is given in Thompson et al [8].

2.1.1. Atenolol

The analogues for atenolol, selected by the KWAAS at each stage in the workflow, are shown in Fig. 1. 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 ± 50 % (i.e., 133–399 Da) and log P of −0.11 +/- 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. After refining the results of the chemical similarity analysis by incorporating molecular weight, three chemicals (other than atenolol) were identified. The final selection of the analogue PBK model to use for atenolol, involved filtering the results of initial analogue identification, based on availability of full equations, humans being the subjects used, and the drug being administered via the oral route. This corresponds to Stage 3 of the previously described KWAAS.

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 refinement at the molecular weight stage. 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 to explore the effects of using the KWAAS at different levels of refinement.

2.1.2. Flumioxazin

Fig. 2 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 the 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; and 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 pKb, and log D were included in the similarity assessment. An existing PBK model for flumioxazin was also identified. Information from this model was used for comparison when assessing the accuracy of the model developed using the analogue as a template. 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.

2.2. Selecting the most appropriate analogue for read-across

2.2.1. Atenolol

The propranolol PBK model developed by Kiriyama et al [12] was used as a template for atenolol. The output from an atenolol PBK model by Peters [13] was used for comparison and to assess the accuracy of using the propranolol model as a template. To ensure that comparisons can be made between the PBK model used for read-across and the observed data from the literature, the most similar dosing scenarios were selected for comparison. Both the Kiriyama et al. [12] and the

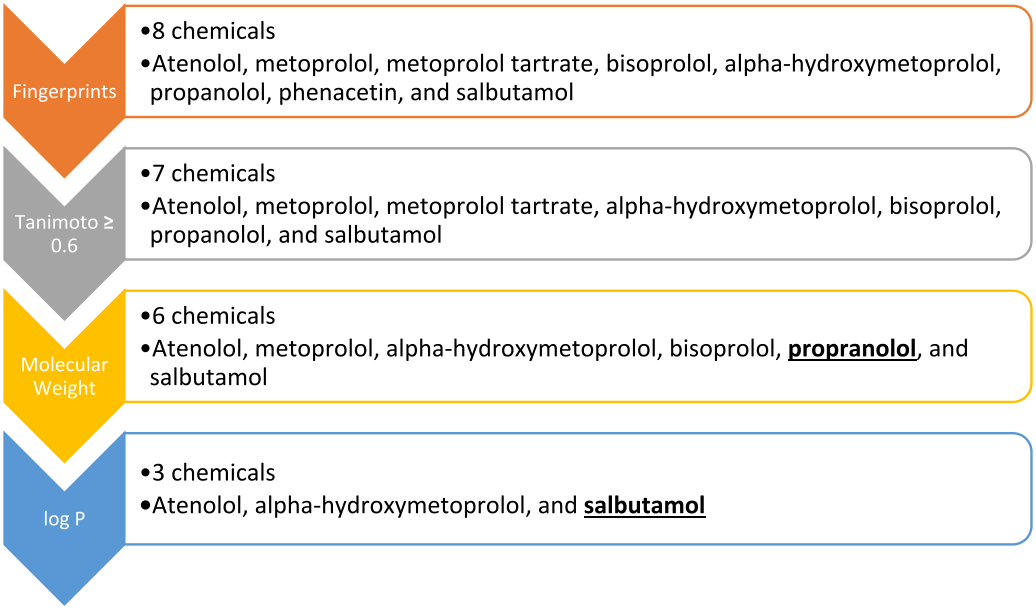

Fig. 1. Results for identifying analogues for atenolol at each stage of using the KWAAS. The arrow on left indicates the criteria used to refine the selection at each stage.

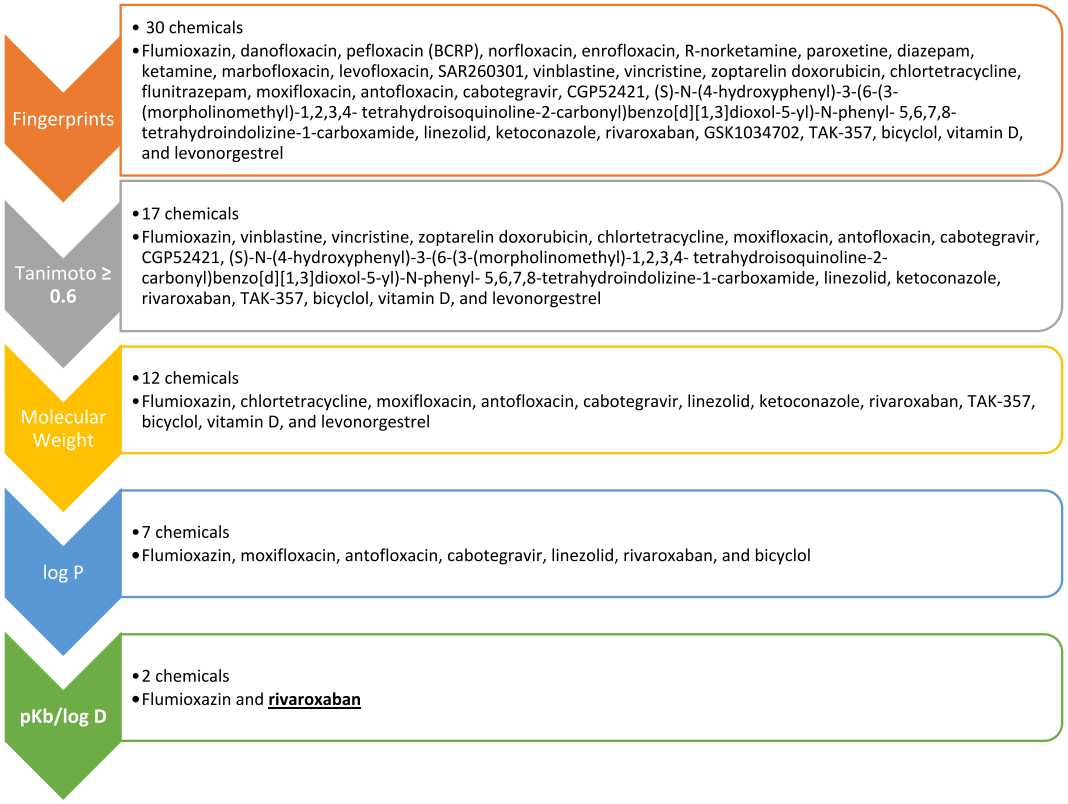

Fig. 2. Results for identifying analogues for flumioxazin at each stage of using KWAAS. The arrow on left indicates the criteria used to refine the selection at each stage.

Peters [13] models were developed for oral dosing in human. The original propranolol model from the Kiriyama et al. paper [12] was reproduced, and the 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) [13] were used to validate the template model. To assess the suitability of analogues at different stages of the workflow, a salbutamol PBK model described by Boger & Fridén [14] was used as a second analogue chemical model to predict atenolol concentrations. As with the propranolol analogue PBK model, the atenolol data from Peters [13] was used for comparison to assess the accuracy of the template model. Both the Boger & Fridén [14] and Peters [13] models were designed for oral dosing in humans. The model for salbutamol was used as a template,

adapting the chemical inputs to be for atenolol.

2.2.2. Flumioxazin

The PBK model for rivaroxaban by Yamazaki-Nishioka et al. [15] was used as a template for flumioxazin. The data from a PBK model for flumioxazin itself, published by Takaku et al. [16] was used for comparison to assess the accuracy of the new read-across PBK model for flumioxazin (based on rivaroxaban). Both models simulate oral dosing in humans; however, Takaku et al. [16] modelled data for a pregnant woman.

2.3. PBK models

Source chemical PBK models from the literature were firstly reproduced as accurately as possible for the source chemical itself, before being adapted for the target chemical. The ordinary differential equations for each model were solved in MATLAB using the numerical regression solver for stiff differential equations (ode15s) to simulate concentration–time plots. All PBK model details can be found in the Supplementary material.

2.3.1. Propranolol PBK model

The PBK model for propranolol described by Kiriyama et al [12] was used in the analysis. The model has 13 compartments: arterial, venous, lung, brain, heart, liver, spleen, gut, kidney, adipose, muscle, bone, and skin. Elimination of the chemical from the body is assumed to be via metabolism in the liver [12]. The equation for gut was adjusted to simulate the dose entering the system and all other parameters remained as described in the original paper. The Supplementary material includes full details of the propranolol PBK model and the modifications used for atenolol. The doses used for the propranolol simulations were 10, 40, 80, and 160 mg. These were the same doses used within the reports for comparing model simulations with observed data. For atenolol, the dose used was 100 mg, i.e., the same dose as used in the Peters publication [13]. The dose entering the body (2 mg) was calculated by including bioavailability (equations in Supplementary material).

2.3.2. Salbutamol PBK model

Boger and Fridén [14] outlined a nine-compartment model consisting of lung, liver, spleen, gut, rapidly perfused organs, slowly perfused organs, and adipose. The organs are linked by arterial and venous blood. The model allows 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 (epithelial lining fluid, epithelium, and sub-epithelium). However, for simplicity the lung compartment was reduced to one equation in this analysis as the inhalation route of administration was not relevant here. The equations for all other compartments were reproduced exactly from the original paper. The Supplementary material includes full details of the salbutamol PBK model and the model adaptations for atenolol. Salbutamol has an active enantiomer (R-salbutamol); however, as it is a mix of the enantiomers being modelled, both were considered in combination. Thus, clearance was calculated as a weighted average of each enantiomer.

2.3.3. Rivaroxaban PBK model

A minimal PBK model consisting of hepatic, blood, urine, and gut compartments for oral administration of rivaroxaban was described by Yamazaki-Nishioka et al. [15]. When adapting the model for flumioxazin, no blood-plasma concentration ratio value could be found (neither from online ADME property predictors nor from literature). Flumioxazin is a neutral, lipophilic compound so it was assumed that the ratio was equal to 1 [17]. The supplementary material provides the full details of the rivaroxaban PBK model, and the model adaptations used for flumioxazin.

2.4. Model assessment

2.4.1. Fold error calculation

For each source model (i.e. the original model reproduced from the literature) and the model as adapted for the target (i.e. the literature model for the source chemical with adaptations to make it relevant for target chemical) the most common pharmacokinetic metrics, i.e., 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 in each case by taking a ratio between the predicted and literature values so that it was always greater than 1.

2.4.2. Sensitivity analysis

The general-purpose software OpenCOSSAN (https://cossan.co.uk; accessed October 2023) was used to perform global sensitivity analysis of all three models developed (i.e., two models for the target atenolol, based on propranolol and salbutamol, and one model for the target flumioxazin based on rivaroxaban). Sobol indices were calculated to determine which parameters had the most significant impact on model AUC. The Sobol indices method determines the significance of each input parameter and the contribution of their interactions to variance in model output. The full range of each input parameter variation and interactions between parameters are evaluated. The method involves the generation of random parameter vectors based on assigned parameter probability distributions (e.g., normal, log normal, etc.) and a sampling method (e.g., Monte Carlo, Latin Hypercube, Halton, etc.). Normal probability distributions and a Monte Carlo sampling method were used. Results from the global sensitivity analysis are illustrated with 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 and any interactions with other parameters together make up the total effect and the variance due to parameter interactions is represented by a ribbon across the plot [18].

3. Results

3.1. Comparison of data from existing and newly-generated PBK models

3.1.1. Propranolol PBK model

Simulations of propranolol at doses 10, 40, 80 and 160 mg were applied to reproduce the PBK model from Kiriyama et al [12] with comparisons to observed data for propranolol obtained from Kopitar et al. [19]. The comparisons are shown in Fig. 3. Simulations of propranolol with a dose of 80 mg best fit the observed data. The approach was able to successfully reproduce the original model, as shown in Fig. 3 (c.f. results from Kiriyama et al. [12]). Overall, the Kiriyama model fits well across all doses. Discrepancies between model-simulated outputs and the observed data at lower doses could be a result of the difficulties in quantifying the chemical in the blood at such low concentrations.

Key physico-chemical information in the model was replaced with data for atenolol but organ volumes, blood flow rates, and model equations remained the same. Fig. 4 shows the predicted atenolol simulation compared to observed and predicted atenolol data from Peters [11], alongside propranolol predicted and observed data. The overall line shape of atenolol using the analogue chemical model (i.e., the model for propranolol) is similar to Peters (see Fig. 3a from Peters [11]). The peak concentration of atenolol can be seen approximately 2 h after administration of the dose with a gradual decline in concentration as the chemical is cleared.

The propranolol model using the output at a dose of 40 mg from the literature was reproduced, with the predicted C_{max} and T_{max} comparable with that observed in Kiriyama et al [10]. Predicted AUC was calculated to be within a 1.8-fold error of the Kiriyama et al simulations. The resulting read-across model for atenolol produced AUC and C_{max} values within 3-fold error of the observed values from Peters [13]. A fold error

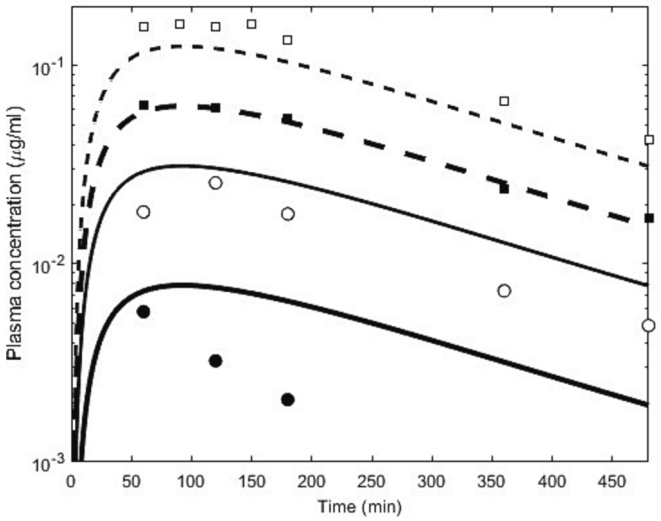

Fig. 3. Simulations of propranolol at doses 10 (-), 40 (-), 80 (– – –) and 160 mg (– – –) using the PBK model described by Kiriyama et al. [12]. Individual data points of observed propranolol data from Kopitar et al. [19] at doses 10 (●), 40 (○), 80 (■) and 160 mg (□).

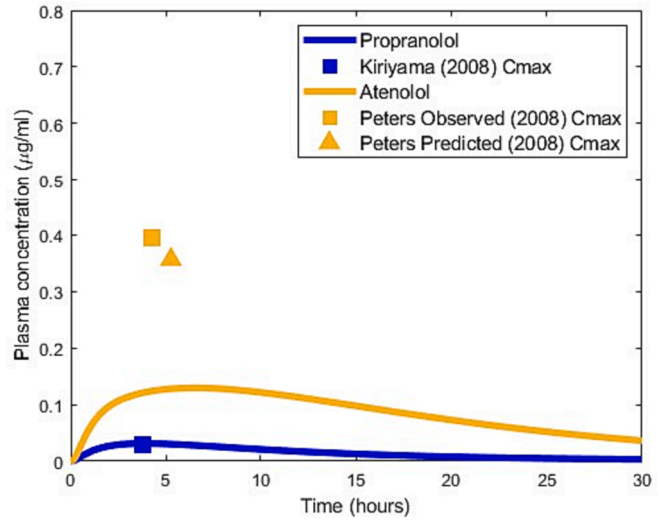

Fig. 4. Predicted propranolol (40 mg dose, blue line) and atenolol (100 mg dose, yellow line) simulations compared to observed data from the literature (propranolol observed data from Kiriyama et al. [12] (C_{max} represented by a blue square); and atentolol observed data from Peters [13] (observed C_{max} represented by a yellow square and predicted by a yellow triangle)).

of 3 in the context of PBK model development and validation is not considered unreasonable [18,19]. 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 1 as are the literature values used for comparison.

3.1.2. Salbutamol PBK model

Fig. 5 shows the reproduced salbutamol PBK model compared to observed data from Boger & Fridén [14] as well as the predicted atenolol simulations compared to observed and predicted data from Peters [13]. 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, and likewise the line shape of the atenolol simulation was similar to the atenolol data and simulation of Peters [13].

Table 1
Comparisons of C_{max}, AUC, and T_{max} of propranolol and atenolol to the literature [12,13]. Fold errors of predictions are also shown.

	Propranolol	Kiriyama et al. [12]	Atenolol	Peters [13]
C_{max} (μg/ml)	0.0312	0.03	0.1296	0.3942
Fold error	**1.0**		**3.0**	
AUC (μg·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**	

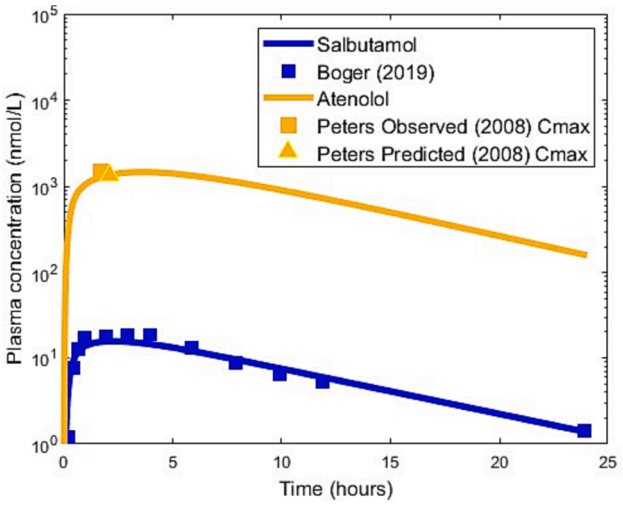

Fig. 5. Predicted salbutamol (2 mg dose, blue line) and atenolol (100 mg dose, yellow line) simulations compared to observed data from the literature (Boger & Fridén, (blue squares); and Peters (yellow square and triangle)).

The Boger & Fridén salbutamol model was reproduced well, resulting in comparable values for C_{max} and AUC. Using the analogue chemical (salbutamol) 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 observed data. All C_{max}, T_{max} and AUC values predicted, and literature values are summarised in Table 2 with fold errors also given.

3.1.3. Rivaroxaban PBK model

The rivaroxaban PBK model from Yamazaki-Nishioka et al. [15] was reproduced see Table 3. Rivaroxaban and flumioxazin simulations are shown in Fig. 6, as well as flumioxazin data [16]). Flumioxazin concentration predictions do not reduce over time as would be expected when compared to the data reported by Takaku et al. [16].

The pharmacokinetic metrics C_{max} and AUC (no T_{max} values were

Table 2
Comparisons of simulated C_{max}, AUC, and T_{max} for salbutamol and atenolol to the literature [14,13]. Fold errors of predictions are also given.

	Salbutamol	Boger & Fridén [14]	Atenolol	Peters [13]
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	18,152	12,240
Fold error	**1.1**		**1.5**	
T_{max} (h)	2.4	3.9	3.7	1.7
Fold error	1.6		**2.2**	

Table 3
Comparisons of C_{max}, and AUC of rivaroxaban and flumioxazin to the literature [15,16]. Fold errors of predictions are also given.

	Rivaroxaban	Yamazaki-Nishioka et al. [15]	Flumioxazin	Takaku et al. [16]
C_{max} (ng/ml)	117.0415	141	615.5223	856
Fold error	**1.2**		**1.3**	
AUC (ng·h/ml)	768.4955	816	21,864	19,351
Fold error	**1.1**		**1.1**	

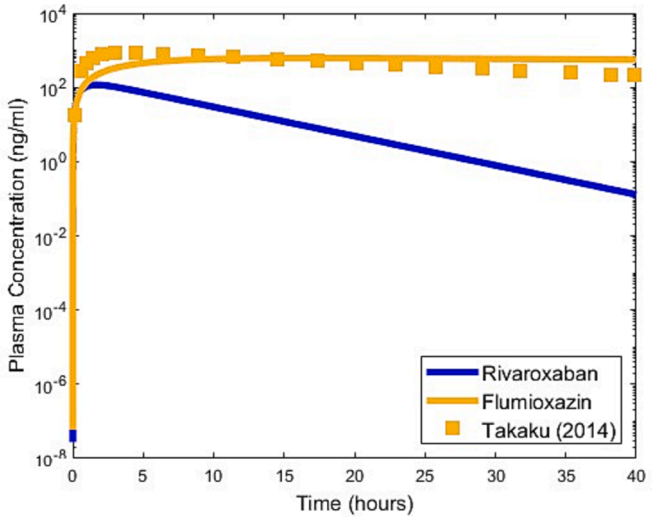

Fig. 6. Predicted rivaroxaban (5 mg dose, blue line) and flumioxazin (1,000 mg dose, yellow line) simulations compared to observed data for flumioxazin from the literature (Takaku et al. [16] represented by yellow squares.

available for comparison from Yamazaki-Nishioka et al. [15] for rivaroxaban) were predicted using a reproduced version of the rivaroxaban PBK model, following an oral dose of 5 mg. Results were compared to measured data from Yamazaki-Nishioka [15]. Predicted and observed PK metrics are compared in Table 3 for both rivaroxaban and flumioxazin. The model reproduced key metrics for rivaroxaban when compared to simulations in Yamazaki-Nishioka et al [15] 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 are expected since the sex and life stage used in developing the model for the analogue chemical are different to those used in the comparator data reported in Takaku et al. [16].

3.2. Sensitivity analysis

Global sensitivity analysis (GSA) results are visually presented in Figs. 7-8 using Lowry plots. The Lowry plot consists of bars for each parameter and its associated main effect (black bar), interactions with other parameters (grey bar), and the cumulative frequency of variance due to interactions (blue ribbon). The upper bound of the ribbon represents the cumulative sum of the total effects and is indicated by a red dashed line. Parameters to the left of the red dashed line are those considered to have a significant contribution to the total variance.

3.2.1. Source model: Propranolol

Results of the GSA when using the propranolol PBK model as a template to develop the atenolol model are summarised in Fig. 7. The fraction absorbed from the intestinal tract (f_a) was highlighted as the most sensitive parameter, contributing most to model output, with the total effect contributing to over 50 % of the variance. In addition, the blood-plasma concentration ratio (R_{bp}), fraction unbound in the plasma (f_{up}), intrinsic clearance (CL_{int}) and 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, the fraction absorbed is considered to be a key feature within the model.

3.2.2. Source model: Salbutamol

Global sensitivity analysis of the salbutamol model when used as a template for atenolol highlighted three parameters that significantly contributed to the total variance: clearance (CL), blood-plasma ratio, (R), and slowly perfused tissue-to-blood partition coefficient (k_{ppp}). Fig. 8 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 model, accounting for 45 % of the variance due to main effects and up to 92 % including interactions. The slowly perfused tissue-to-blood partition coefficient and blood-plasma ratio contributed a further 3 % and 5 % total effect on variance, respectively. The absorption (k_a) and the gut tissue-to-blood partition coefficient (k_{pgu}) were deemed to be relatively less sensitive.

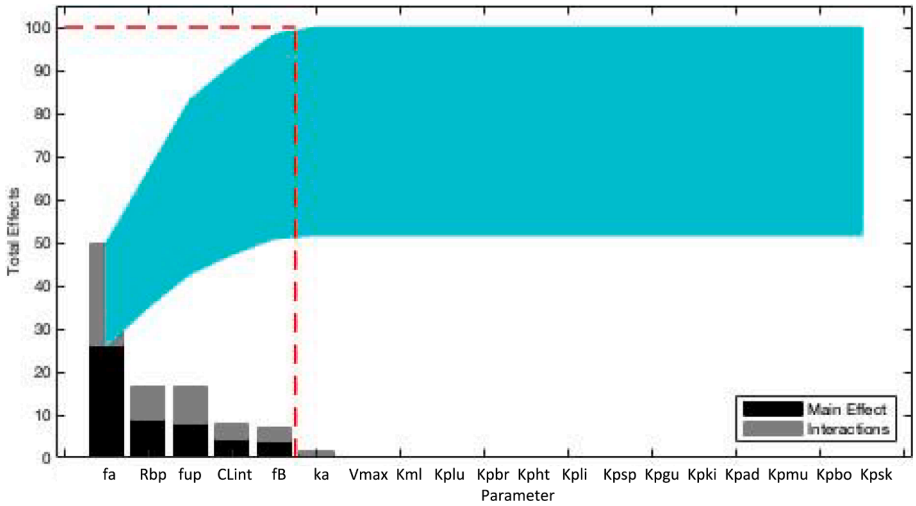

Fig. 7. A Lowry plot showing the results of global sensitivity analysis when using propranolol as the template model for atenolol.

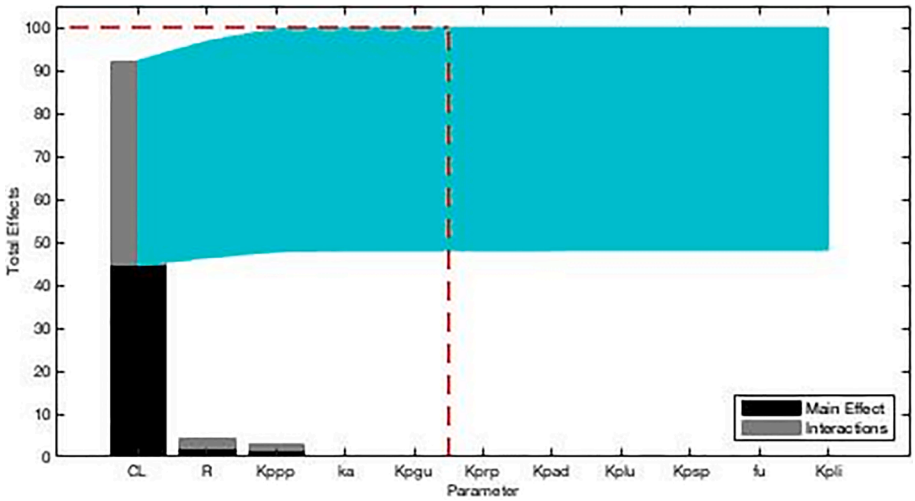

Fig. 8. A Lowry plot showing the results of global sensitivity analysis when using salbutamol as the template model for atenolol.

3.2.3. *Source model: Rivaroxaban*

All input parameters that were adapted during model building (i.e. from the values for rivaroxaban in the source model, to the values for flumioxazin (the target) were analysed for uncertainty. These were adapted by using data from literature or predictive software (e.g. Opera https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera; accessed October 2023) and ADMETlab (2.0 https://admetmesh.scbdd.com/service/evaluation/index; accessed October 2023). 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 Fig. 9. The fraction unbound in the plasma (f_{up}) had some effect, but this was not statistically significant.

Sensitivity analysis can help to identify reasons for discrepancies between data derived from the model and the literature data, by identifying the parameters that have the most influence on the model. Highlighting these parameters as a priority for further investigation helps to improve the accuracy of the model as it is iteratively refined. Figs. 7-9 identify these most influential parameters as determined in the two exemplar case studies here. For the propranolol template model (used to derive a model for atenolol) influential parameters were fraction absorbed in gut, intrinsic clearance and factors relating to partitioning in blood. For the salbutamol template (used to derive a model for atenolol) key parameters again related to clearance and partitioning in blood as well as partitioning between poorly-perfused tissues and blood. In the case of the rivaroxaban model (used to derive a model for flumioxazin) key parameters were rate of absorption, clearance and partitioning in blood. Parameters relating to absorption and clearance are often not only defining parameters for overall internal exposure but also are recognised (particularly in the case of clearance) to be highly variable when measured experimentally. To improve model accuracy, it is essential that these parameters are further investigated. For example, additional searches within the literature may be conducted to obtain corroboratory information to increase confidence in the values obtained for these key parameters. Alternatively, sensitivity analysis can help to identify which *in vitro* assays may yield the most valuable information for increasing the accuracy of the model.

4. Discussion

One asset for safety assessment of chemicals would be the ability to use existing PBK models as templates to inform the development of new PBK models, for chemicals lacking this information. This would also be of benefit to the reduction, refinement, and replacement of animal testing (3Rs). The previous paper by Thompson et al. [8] described the development of a KWAAS to identify similar chemicals with available

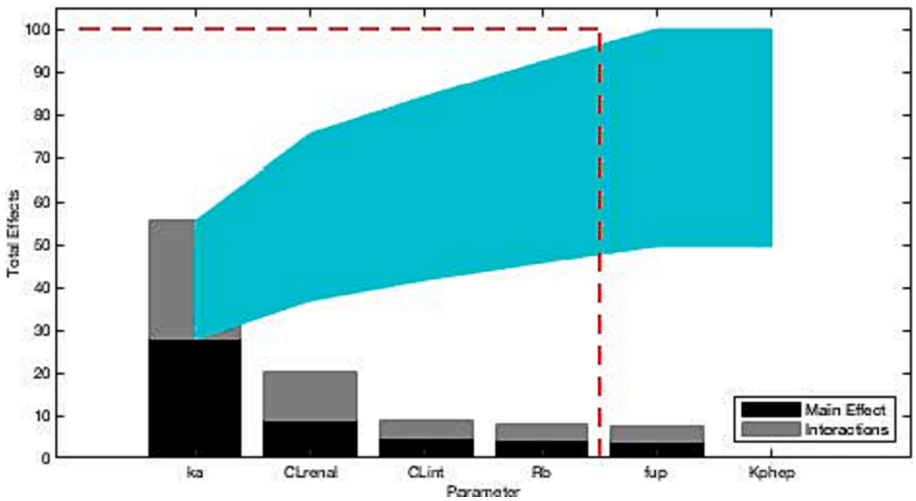

Fig. 9. A Lowry plot showing the results of global sensitivity analysis when using rivaroxaban as a template model for flumioxazin.

PBK models to a target chemical. Validation of the KWAAS was undertaken in this paper, where case studies for two chemicals, atenolol and flumioxazin, were undertaken. It was demonstrated that, for the two chemicals selected, the KWAAS identified suitable analogues. The PBK models for these analogues were used, successfully, to inform the development of PBK models for the target chemicals, with key pharmacokinetic metrics (C_{max}, T_{max} and AUC) falling within an acceptable range of published values.

In attempting to reproduce PBK models from the literature, several problems were identified which highlight some of the potential pitfalls in the approach. For the PBK model reported in Kiriyama et al. [12] and used to develop a model for atenolol, there were multiple values quoted for the fraction absorbed from the intestinal tract (f_a) with no clarity as to the actual value used. Additional parameter values for fraction unbound in the blood (f_B) and blood-plasma concentration ratio (R_{bp}) were only available in secondary references. Furthermore, there were also inconsistencies in the reporting of equations compared to the representative PBK model schematic. The schematic indicated blood from the spleen and gut flows directly into the venous compartments, while the liver equation describes blood flow from the spleen and gut into the venous and liver compartments. There were also discrepancies in the calculations for the stomach compartment. Inconsistency in reporting of any parameters or equations will result in discrepancies in the model output. This may explain why the AUC could only be reproduced within a 2-fold error, although lineshape of the curves were similar and C_{max} and T_{max} were reproduced reasonably well.

The second chemical with a PBK model used as a template for atenolol was salbutamol. The Boger & Fridén model [14] for salbutamol was reproduced successfully, as judged by comparison of model output to literature values; however, there were difficulties regarding how the model was reported. There was a lack of clarity regarding blood flow and organ volumes, as calculated using body weight, and discrepancies between the PBK model schematic and the equations employed. Notwithstanding, accurate simulations of atenolol concentration dynamics (including lineshape) were successfully produced by using the salbutamol model as a template, as demonstrated by comparison with previously published *in vivo* data. Using the source chemical salbutamol as a template in a read-across approach for building a PBK model for atenolol gave more accurate predictions than using the propranolol PBK model as a template. This suggests that, in this instance, further refinement of analogue selection was of benefit in model building.

For the second case study chemical, flumioxazin, only one chemical (rivaroxaban) was identified as being sufficiently similar following analogue selection and refinement using the KWAAS. The minimal PBK model for rivaroxaban was reproduced successfully and used as a template for flumioxazin. Values for C_{max} and AUC were predicted reasonably well as compared to literature values but the lineshape was dissimilar. The predicted liver-to-plasma concentration ratio (K_{phep}) and blood-plasma concentration ratio (R_b) assumption of 1 could have affected the lineshape of the curve. A workflow for adapting parameters to improve predicted lineshape and better replicate observed values has been proposed by Peters [13]. A possibility in this case would be to adapt the tissue distribution coefficients by adding a multiplicative factor to the tissue partition coefficient values. Further, when considering the differences in rivaroxaban and flumioxazin parameters there were large differences in the fraction unbound in plasma (f_{up}) and the absorption rate constant (k_a) (see Supplementary material). 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}. The differences in these kinetic parameters, between the source and target chemical, may explain why the rivaroxaban model does not replicate the correct lineshape for flumioxazin, although C_{max} and AUC were reasonably well-predicted.

The issues outlined above highlight some of the limitations of the present study. These include the use of only two case study chemicals, potential bias in selecting analogues and problems created by inconsistent or inadequate reporting of key parameter values needed for PBK modelling. It would be valuable for further case studies to be undertaken by users of the PMD and KWAAS to identify how these resources could be improved. One possibility would be to include additional properties of chemicals in the KWAAS, for example physico-chemical or ADME properties generated or obtained from resources that have been assessed for reliability. This may help to increase consistency in model development to some extent. Analogue selection should incorporate expert judgement of those working within a given chemical space with full justification given for selection.

5. Conclusions

The read-across approach outlined in this paper, wherein new models for the target chemicals atenolol and flumioxazin were developed using PBK models for source chemicals, provides evidence of the effectiveness of using the KWAAS described by Thompson et al. [8] to identify chemical analogues. This paper demonstrates a potential contribution to the 3Rs in the area of safety assessment through the use of PBK modelling and read-across. Two case studies were carried out within this paper; however, application of the KWAAS to chemicals relevant to other sectors (e.g., botanicals, cosmetics, or industrial compounds) would be beneficial.

To enable a successful read-across approach, all the information that is necessary to build the source PBK model needs to be clearly reported to allow for the adaptation and development of the new PBK model. However, when using software to predict values such as log P or pKa for example, any inconsistencies from these predictions will potentially be brought forward into the PBK model. The addition of ADME properties into the KWAAS, e.g., absorption or metabolism properties, may help with the selection and refinement of suitable PBK models for read-across. For example when developing a model for chemicals administered via the dermal route information regarding skin uptake may help in model selection e.g. the ability to refine by skin absorption parameters would allow for PBK models with similar kinetics to be identified.

Discrepancies in reporting of PBK models leads to difficulties when attempting to reproduce PBK models from the literature. This problem has been highlighted previously [20]. The recently published OECD guidance on the characterisation, validation, and reporting of PBK models [21] should help to improve reporting if it is embraced by the PBK modelling community. Hence, use of this template is highly recommended – other minimum reporting standards have also been recommended by the European Medicines Agency [22], the US Food and Drug Administration [23] and a consortium of pharmaceutical industries [24]. Other initiatives, such as the Biomodels database (https://www.ebi.ac.uk/biomodels/; accessed October 2023), help to facilitate model reproducibility (or allow reporting of any problems identified in terms of reproducibility) and increase confidence in the application and re-use of existing models. As PBK models are resource-intensive to generate, applications that enable re-use or re-purposing of the models is an important step in capitalising on the rich information they hold.

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CRediT authorship contribution statement

Courtney V. Thompson: Methodology, Investigation, Data curation, Writing – original draft, Visualization, Software. **Steven D. Webb:** Methodology, Writing – review & editing, Visualization, Formal analysis, Supervision. **Joseph A. Leedale:** Writing – review & editing, Formal analysis. **Peter E. Penson:** Supervision, Writing – review & editing. **Alicia Paini:** Conceptualization, Writing – review & editing. **David Ebbrell:** Methodology. **Judith C Madden:** Conceptualization,

Writing – review & editing, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comtox.2023.100293.

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