OpenRiskNet

RISK ASSESSMENT E-INFRASTRUCTURE

Case Study

Reverse dosimetry and PBPK prediction [**RevK**]

SUMMARY

This case-study demonstrates and documents the use of a web interface to physiologically-based pharmacokinetic models for forward and reverse dosimetry calculations. Forward calculations compute internal concentrations from given exposure doses. Reverse calculations compute exposure doses from internal concentrations or measured biomarker levels (*e.g.*, urine concentration data). The result of those calculations can be used in risk assessments to help with *in vitro* to *in vivo* extrapolations or interspecies extrapolations.

Three tools have been developed for this case-study at NTUA and have been integrated into the OpenRiskNet infrastructure through the Jaqpot web-based computational platform. More specifically, the popular high-throughput toxicokinetic (*httk)* R package and the *PKSim* software tool for whole-body physiologically based pharmacokinetic modeling were integrated, but we also developed infrastructure for developing and deploying user-defined model.

For each of these three web tools, simulations are performed and results are presented for reference chemicals or drugs, namely Imazalil for the *httk* model, Diazepam and Chlorpyrifos for showcasing the In-house R PBPK workflow and Theophylline for the *PKSim* model. The exposure scenarios chosen are in the range of corresponding environmental or therapeutic levels.

Finally, a brief overview describing how to access custom-made PBPK models and run simulations through the Jaqpot Graphical User Interface (GUI) is presented in the Annex.

DESCRIPTION

Implementation team

CS leader	Team
Frederic Bois / Celine Brochot (INERIS)	Haralambos Sarimveis, Periklis Tsiros, Pantelis Karatzas, Philip Doganis (NTUA) Cleo Tebby (INERIS)

- The code and web implementation was developed by NTUA
- The case-studies' simulations were run by INERIS and NTUA
- Code and technical service documentation was provided by NTUA. INERIS documented the users' operations and results of the case-study demonstrations.

Case Study objective

The objective of this case study is to demonstrate and document the capabilities of the OpenRiskNet-developed web-services for Physiologically Based PharmacoKinetic (PBPK) modeling with illustration of both forward and reverse dosimetry predictions.
PBPK models offer a methodology for predicting the internal distribution and exposure of a compound in an organism. Their nature is mechanistic; they consist of compartments representing real organs and tissues, whose number varies based on the target substance, species, administration route and available information. A common approach is to incorporate in the model the main body tissues, i.e. brain, heart, kidney, skin, spleen, liver, lung, gut, bone, adipose and muscle (Jones et al., 2013). Nevertheless, the dimensionality of a PBPK model can be reduced using lumping methods (Pilari et al. 2010, Nestorov et al., 1998) . In most cases, PBPK models are utilised for describing the kinetics of a substance in the whole body of a species, thus such models are more formally called "whole body physiologically-based pharmacokinetic" (WBPBPK) models. However, there are models developed to describe in detail the kinetics of a specific organ or body area, which is divided into separate subcompartments. This modeling approach is called "partial" PBPK models (Sturm, 2007).
PBPK models have inherent advantages due to their mechanistic nature. Firstly, they enable predictions of concentration/mass profiles of individual organs and not just plasma. In addition, their relation with physiology and modularity facilitate the integration of literature information, making predictions prior to *in vivo* experiments possible (Nestorov, 2003). Lastly, their biggest advantage is the ability to perform inter-species (e.g. from rat to human) or intra-species (e.g. from adults to children) extrapolation through scaling methods.

Risk assessment framework

The application frameworks are, for example: REACH risk assessments; SEVESO II directive on safety around industrial plants; Internal chemical, cosmetic, or pharmaceutical company assessments of workers' safety, or consumer's safety. All those require integration and extrapolation of *in vitro* and/or *in vivo* data on animals to assess human risks.

DEVELOPMENT

Databases and tools

We use open source software able to implement PBPK models within the Jaqpot platform (Chomenidis et al., 2017): *httk* package (Pearce et al., 2017), *PKSim* (Willmann et al., 2003), and an in-house R client for custom PBPK modelling. The *Jaqpot* biokinetic services are used to publish the PBPK models as web services. Service clients are developed in the R language. Databases of parameter values are provided by the *httk R* package, and the *PKSim* model.

Technical implementation

Implementation of the chosen PBPK model as web services:

PBPK models for a specific class of chemicals and animal species can be selected by the user from a particular PBPK modelling environment (*e.g.*, *httk* in *R*, *PKSim*).

The chosen PBPK model is exposed as a web service using the Jaqpot modelling platform. This is possible through the Jaqpot Protocol of Data Interchange (JPDI) which allows to dynamically and seamlessly incorporate practically any algorithmic implementation into Jaqpot. The protocol specifies the form of data exchange between Jaqpot services and third party algorithm web service implementations. The Jaqpot framework already provides wrappers for the R language and the Python language. Integration with R is made possible through the OpenCPU system, which defines an HTTP API for embedded scientific computing based on R, although this approach could easily be generalized to other computational back-ends (Ooms, 2014). OpenCPU acts as a wrapper to R that is readily able to expose R functions as RESTful HTTP resources. The OpenCPU server takes advantage of multi-processing in the Apache2 web server to handle concurrency. This implementation uses forks of the R process to serve concurrent requests immediately with little performance overhead. By doing so it enables access to those functions on simple HTTP calls converting R from a standalone application to a web service.

Demonstration of PBPK models that have been exposed as web services:

The three simulation tools (*httk*, *PKSim* and user-specified) are demonstrated with Imazalil, Theophylline, Diazepam and Chlorpyrifos in rainbow trout respectively.

For Imazalil and Theophylline, we start by identifying relevant human exposures (*e.g.* from ExpoCast, or published literature) to be used in forward dosimetry. For Diazepam and Chlorpyrifos, reverse dosimetry is examined; we identify (*e.g.* from the US NHANES database, or the scientific literature) typical blood or urine concentrations found in humans to be used as input to the exposure dose reconstruction.

Each model is parameterized using user-specified or pre-programmed tabulated physiological data. For forward dosimetry predictions, each model is run with the given exposure scenario to predict internal concentrations after 24 hours, while for reverse dosimetry, the model is run forward iteratively with user set exposures so as to match the input biomarkers (that is: manually invert the model). The external exposure level leading to data-matching biomarker level is recorded as final estimate.

OUTCOMES

In this section, several implementations of this case study are described:

The first implementation uses *httk* and Imazalil. We describe all the steps required to develop the models as web services through the Jaqpot API or the Jaqpot GUI.

The second is a generic OpenRiskNet framework , which can be used with custom-made PBPK models. Two examples are provided, a PBPK model for Diazepam in humans, and a generic (i.e. not substance-specific) PBPK model in fish. In the case of diazepam, the tools were used to analyse biomonitoring data regarding diazepam blood levels in drivers. In the case of the fish PBTK model, exposure levels which lead to in vitro effects on biomarkers in liver were estimated.

The last implementation is the integration of a PBPK model for Theophylline, originally developed in the PKSim software. We describe all the steps required to develop the model as a web service through the Jaqpot API.

We are providing all the steps required to perform dosimetry through the Jaqpot GUI using the custom-made model as examples.

The results of this case-study demonstrate that the OpenRiskNet framework can be used as a central e-platform for the biokinetics community, where the users can publish, share, search and use PBPK models.

Integration of *httk* in OpenRiskNet Infrastructure through the Jaqpot modelling platform

High-Throughput toxicokinetics *(httk*) is an R package for simulation and analysis of chemical toxicokinetics. httk has been integrated in the Jaqpot platform and is accessible from the OpenRiskNet infrastructure. The goal of this tutorial is to demonstrate how to obtain toxicokinetics predictions from the httk package using Jaqpot services and functionalities through both API and GUI.

The following httk parameters are supported:

1. “chem.name”, which is the chemical name of the compound under study
2. “species”, which is the species of interest (either "Rat", "Rabbit", "Dog", "Mouse", or default "Human")
3. “days”, the length of the simulation in days,
4. “dose”, the amount of the single dose specified (mg/kg/day) with the default value being NULL.

We use the example of administering Imazalil to a human (BW = 70 kg) also initializing other basic parameters based on the multiple compartment model introduced in Kilford et al. (2008). The compartments used in this model are the gutlumen, gut, liver, kidneys, veins, arteries, lungs, and the rest of the body. The extra compartments include the amounts or concentrations metabolized by the liver and excreted by the kidneys through the tubules.

Accessing *httk* through Jaqpot API

The following steps should be followed using the Swagger JaqPot API at https://api-jaqpot.prod.openrisknet.org/jaqpot/swagger/:

1) Produce a JaqPot resource that implements the httk model as a web service

a. Use the **POST/biokinetics/httk/createmodel** method.

Insert the following information in the parameter fields:

Title and description: Any title and description is fine

Parameter string:
{"chem.name":["imazalil"],"species":["Human"],"days":[10],"dose":[10]}

Press the ‘Try it out!’ button. In the field response body a json string will appear. There the user can find the task ID.

b. Use the **GET /task/{id}** method. Copy the task ID in the relevant field and press the ‘Try it out!’ button. If the status at the end of the

response body is not COMPLETED wait for a few seconds and try again. The model has been created and its complete URI is shown in the response body. For the next step store the model id. This is the id appearing after model/ in “result” in the response body.

The raw model together with the initial parameters of the multiple compartment model are returned.

2) Use the model for obtaining drug concentration-time profiles

a. Use the **POST biokinetics/httk/model/{id}** method in the biokinetics section. Submit the model id obtained before and then press the button ‘Try it out!’. A task ID is created.

b. Use the **GET /task/{id}** method. Copy the task ID in the relevant field and press the ‘Try it out!’ button. The dataset with drug concentration –time data has been created. For the next step store the dataset id. This is the id found after dataset/ in the “result” section of the response body.

c. Use the **GET /dataset/{id}** method: copy the dataset id in the relevant field and press ‘Try it out!’. In the Response Body, drug concentration values are shown along with corresponding time points (in days), and AUC (area under the curve) of the plasma concentration values.

These are the first lines of the result giving concentrations in uM:

"Substance","Agutlumen","Atubules","Cplasma","Cart","Ckidney","Crest","Cliver","Cgut","Ametabolized","Cven","time","Clung","AUC"

"1","2355.4749","0","0","0","0","0","0","0","0","0","0","0","0"

"2","1365.8025","0.1105","13.1333","9.2261","59.1467","2.8637","277.1605","258.6781","0","9.5873","0.0104","9.3056","0.0598"

"3","791.9496","0.5618","20.3668","14.7784","106.6832","11.1376","356.6631","205.9325","0","14.8678","0.0208","14.501","0.2427"

"4","459.2054","1.1426","21.3648","15.6043","115.3854","19.338","318.4675","156.002","0","15.5963","0.0312","15.2265","0.4629"

"5","266.2664","1.7291","20.5252","15.0134","111.6516","25.5365","259.9901","120.0944","0","14.9834","0.0417","14.6313","0.6817"

"6","154.3925","2.2885","19.4394","14.2189","105.7722","29.7367","210.7735","96.3627","0","14.1908","0.0521","13.8573","0.8898"

"7","89.5232","2.8195","18.5569","13.5674","100.7951","32.4253","175.7869","81.3744","0","13.5466","0.0625","13.2273","1.0874"

"8","51.9093","3.3285","17.938","13.1087","97.2449","34.0891","152.7805","72.164","0","13.0947","0.0729","12.7851","1.2773"

"9","30.0992","3.8225","17.5331","12.8081","94.9023","35.0962","138.2902","66.602","0","12.7992","0.0833","12.4959","1.4619"

"10","17.4528","4.3068","17.2782","12.6186","93.42","35.6962","129.3984","63.2808","0","12.6131","0.0938","12.3137","1.6431"

Other examples:

{"chem.name":["bisphenol A"],"species":["Rat"],"days":[10],"dose":[10]}

{"chem.name":["imazalil"],"species":["Rat"],"days":[10],"dose":[10]}

{"chem.name":["Acetochlor"],"species":["Rat"],"days":[10],"dose":[10]}

Acessing *httk* through Jaqpot Graphical User Interface

A user interface for the specific httk services has been developed and is integrated into the overall Jaqpot GUI (https://ui-jaqpot.prod.openrisknet.org/). After login, the user can use the navigation bar of Jaqpot UI and select the httk application (Figure 1).

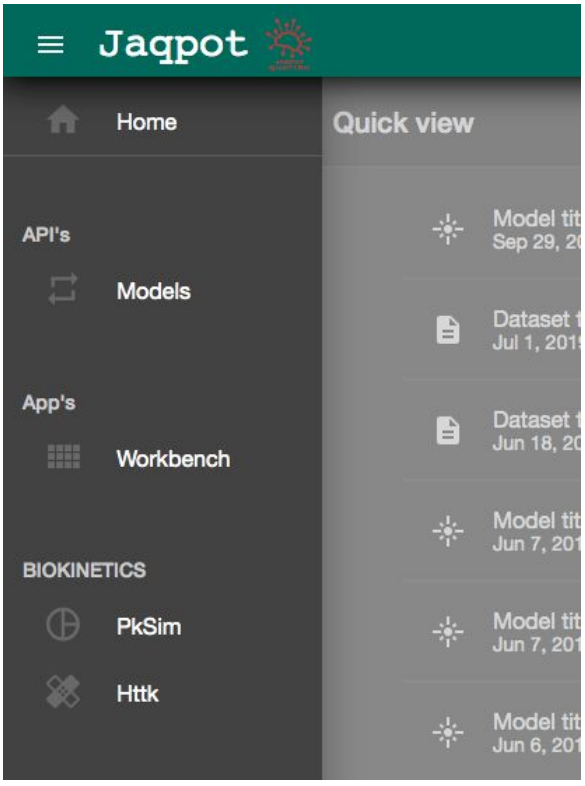


Figure 1. Jaqpot's Navigation bar.

The user is subsequently directed to the landing page of the *httk* Jaqpot web service. First the user is requested to complete the simulation information, which comprises 7 steps (Figure 2). Specifically, these steps are:

1. Select species; human and rat compose the available choices.
2. Select the chemical. This is done through an autocomplete system. After pressing a letter, all available chemicals that start with this letter are loaded. The user can continue the letter filling process until the available choices are narrowed down to the compound of interest.
3. Set the dose administered
4. Set the duration of the simulation
5. Provide a title for the simulation for archiving purposes
6. Provide a short description for the simulation
7. Create the model and obtain the predictions

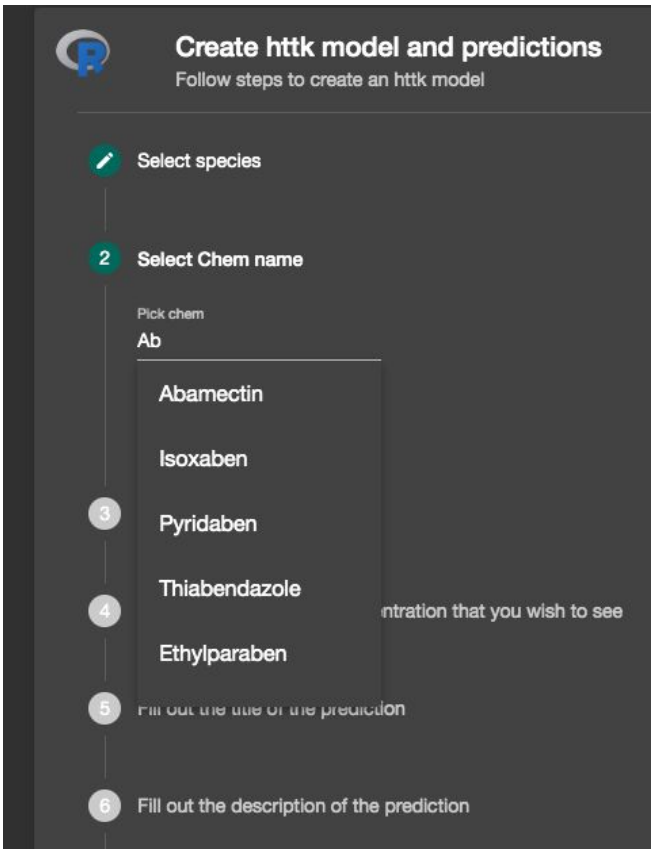
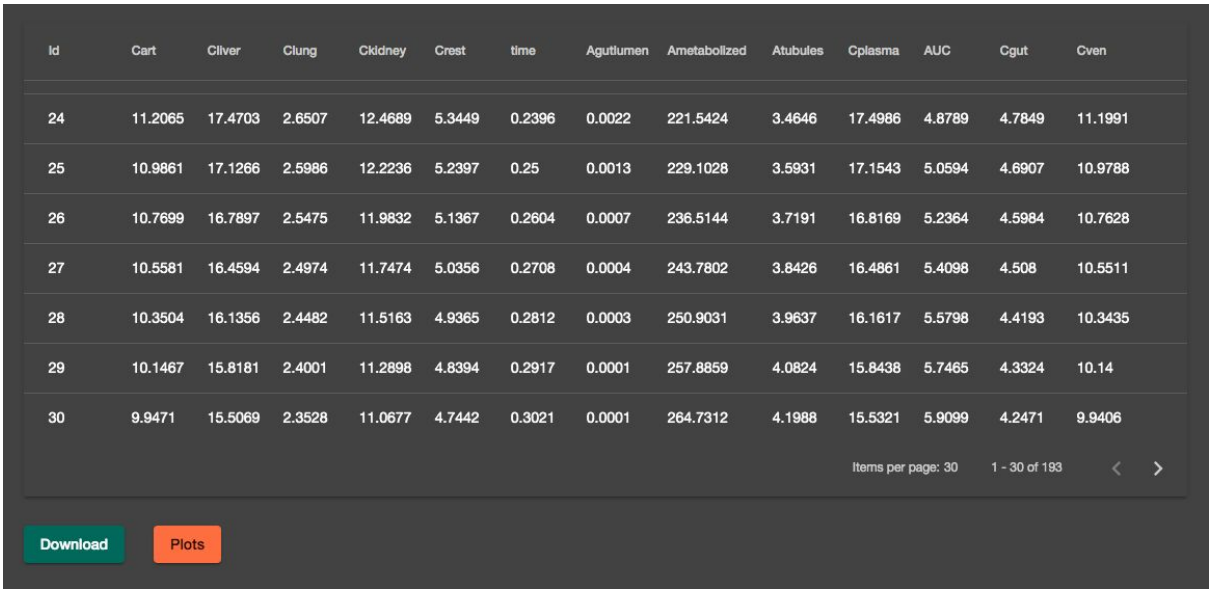


Figure 2. Steps of the httk model creation process.



Id	Cart	Cliver	Clung	Ckidney	Crest	time	Agutlumen	Ametabolized	Atubules	Cplasma	AUC	Cgut	Cven
24	11.2065	17.4703	2.6507	12.4689	5.3449	0.2396	0.0022	221.5424	3.4646	17.4986	4.8789	4.7849	11.1991
25	10.9861	17.1266	2.5986	12.2236	5.2397	0.25	0.0013	229.1028	3.5931	17.1543	5.0594	4.6907	10.9788
26	10.7699	16.7897	2.5475	11.9832	5.1367	0.2604	0.0007	236.5144	3.7191	16.8169	5.2364	4.5984	10.7628
27	10.5581	16.4594	2.4974	11.7474	5.0356	0.2708	0.0004	243.7802	3.8426	16.4861	5.4098	4.508	10.5511
28	10.3504	16.1356	2.4482	11.5163	4.9365	0.2812	0.0003	250.9031	3.9637	16.1617	5.5798	4.4193	10.3435
29	10.1467	15.8181	2.4001	11.2898	4.8394	0.2917	0.0001	257.8859	4.0824	15.8438	5.7465	4.3324	10.14
30	9.9471	15.5069	2.3528	11.0677	4.7442	0.3021	0.0001	264.7312	4.1988	15.5321	5.9099	4.2471	9.9406

Items per page: 30 1 - 30 of 193

Download Plots

Figure 3. Tabular format of model predictions.

The results are presented in a tabular format (Figure 3). For a better understanding of the process kinetics, the user is given the ability to visualise the results by plotting the concentration-time profiles. This is realised by pressing the “Plots” button, which opens a window that requests the user to set the x and y-axis by selecting the compartments of interest through a drag-and-drop gesture (Figure 4). Figure 5 presents the plots resulting after selecting the compartments of Figure 4, namely the concentration of the gut, liver and rest-of-the-body compartments.

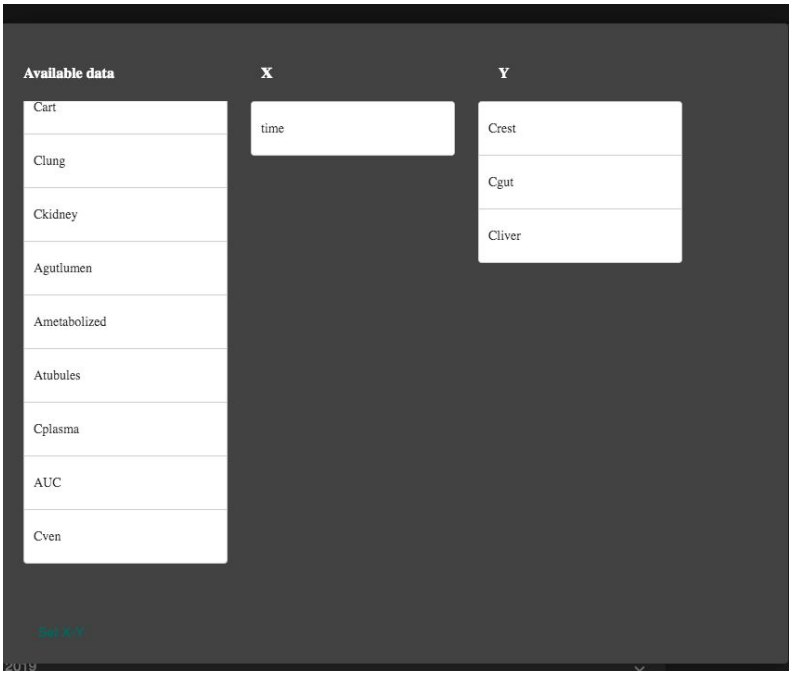


Figure 4. Pop-up window for setting up plots.

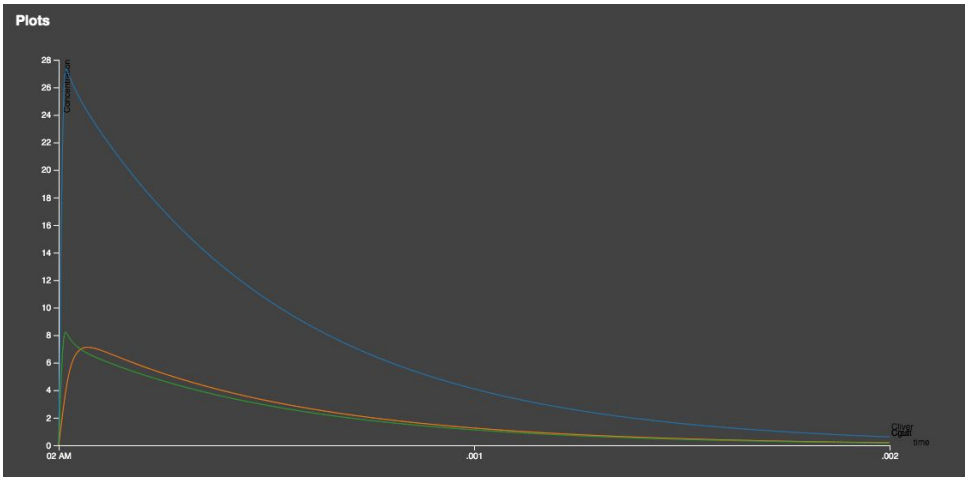
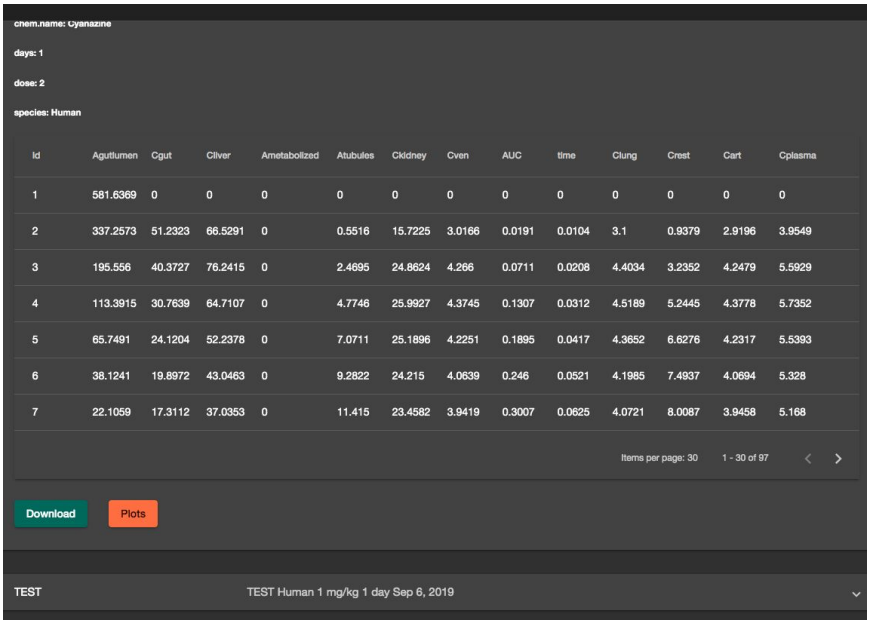


Figure 5. Plots generated in the httk web service GUI.

All information generated during the in-silico experiment is archived for accessing it at a later point in time, so that recreation of the same model, and thus needless spent of computational resources, is avoided. If a user wishes to revisit archived model predictions, pressing the “Previous predictions” makes all previous predictions and models available.



Id	Agutlumen	Cgut	Cliver	Ametabolized	Atubules	Ckidney	Cven	AUC	time	Clung	Crest	Cart	Cplasma
1	581.6369	0	0	0	0	0	0	0	0	0	0	0	0
2	337.2573	51.2323	66.5291	0	0.5516	15.7225	3.0166	0.0191	0.0104	3.1	0.9379	2.9196	3.9549
3	195.556	40.3727	76.2415	0	2.4695	24.8624	4.266	0.0711	0.0208	4.4034	3.2352	4.2479	5.5929
4	113.3915	30.7639	64.7107	0	4.7746	25.9927	4.3745	0.1307	0.0312	4.5189	5.2445	4.3778	5.7352
5	65.7491	24.1204	52.2378	0	7.0711	25.1896	4.2251	0.1895	0.0417	4.3652	6.6276	4.2317	5.5393
6	38.1241	19.8972	43.0463	0	9.2822	24.215	4.0639	0.246	0.0521	4.1985	7.4937	4.0694	5.328
7	22.1059	17.3112	37.0353	0	11.415	23.4582	3.9419	0.3007	0.0625	4.0721	8.0087	3.9458	5.168

Figure 6. “Archive/history” page

Integration of custom made PBPK models in the Jaqpot modelling platform

Besides the integration of httk and PKSim, we offer the option to the users to create, upload and share a custom-made PBPK model through the Jaqpot infrastructure. A user-friendly R client has been developed that allows model creators to expose their PBPK models as ready-to-use web services on the Jaqpot platform. The R client utilizes the deSolve package (R language) for solving differential equations. The deployment process is realised through a function with which the user needs to specify a series of components and it has been designed in a way that offers extended flexibility on the models to be uploaded (Figure 7). Specifically, the first component, 'user.input', should be a list containing the names of the input features, which the end-user needs to fill in. 'predicted.feats' is again a list consisting of the names of the predicted features that will be the final output on the Jaqpot GUI. The next element, 'create.params', is a function that receives as input the input of the end-user and transforms it according to the needs of the model. Following that, 'create.inits' and 'create.events' receive as input the output of create.params and create the initial conditions of the ODEs and a dataframe containing events that force changes on the state variables of the ODE system, respectively. The ability to use a custom function inside the ode function ('ode.func') is provided by 'custom.func', while 'ode.func' is a function that is forwarded to the 'ode' function of the R package 'deSolve' and contains the ODEs. The function is requested in the following format: ode.func(integration.time, initial.values, parameters, custom.func). Finally, the user can select a specific solver from the ones provided in 'deSolve' package through the 'method' argument and can also pass additional arguments (e.g. 'rtol' for changing the relative tolerance of the solution) down to the solver through the three dots R argument ('...').

```
> deploy.pbpk(user.input, predicted.feats, create.params, create.inits, create.events,
+                         custom.func, PBPK.model, method = "lsodes")
Base path of jaqpot *e.g.: https://api.jaqpot.org/ : https://api.jaqpot.org/
Please choose authentication method ([1]=login / [2]=Provide Api Key): 1
Username: PeriklisTs
Title of the model: Toy Example()
Short description of the model:This is a test model
[1] "Model created. The id is:  3XtlC0Z2N98MTeywSDES . Please visit the application to further document your model."
>
```

Figure 7. In-house R client for custom PBPK modelling-Call of the deployment function.

Once the deployment procedure is completed, a user can access the model through the Jaqpot platform and run simulations, e.g. generate forward and reverse dosimetry scenarios, provided that she/he has access to a model through the organization she/he is part of.

The model environment comprises 4 tabs: 'Overview', 'Features', 'Predict/Validate' and 'Discussion'. The 'Overview' tab provides a coarse description of the PBPK model, as well as specific directions which refer to the model, e.g. how to fill in the input section (Figure 8). The 'Features' tab informs the users about the dependent and independent features; each feature comes with description and units (Figure 9).

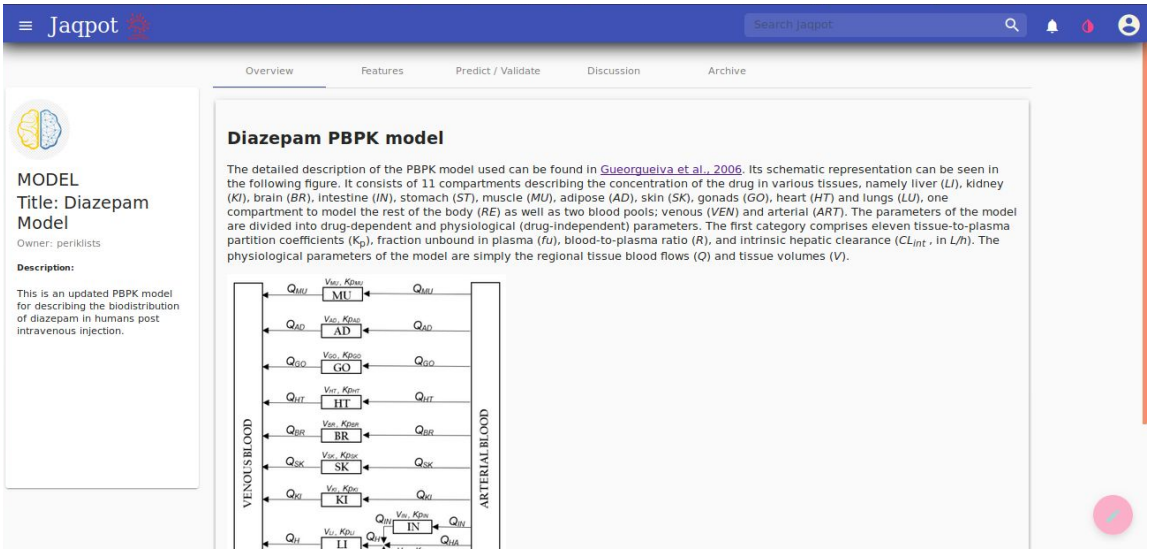


Figure 8: 'Overview' tab of the Diazepam model.

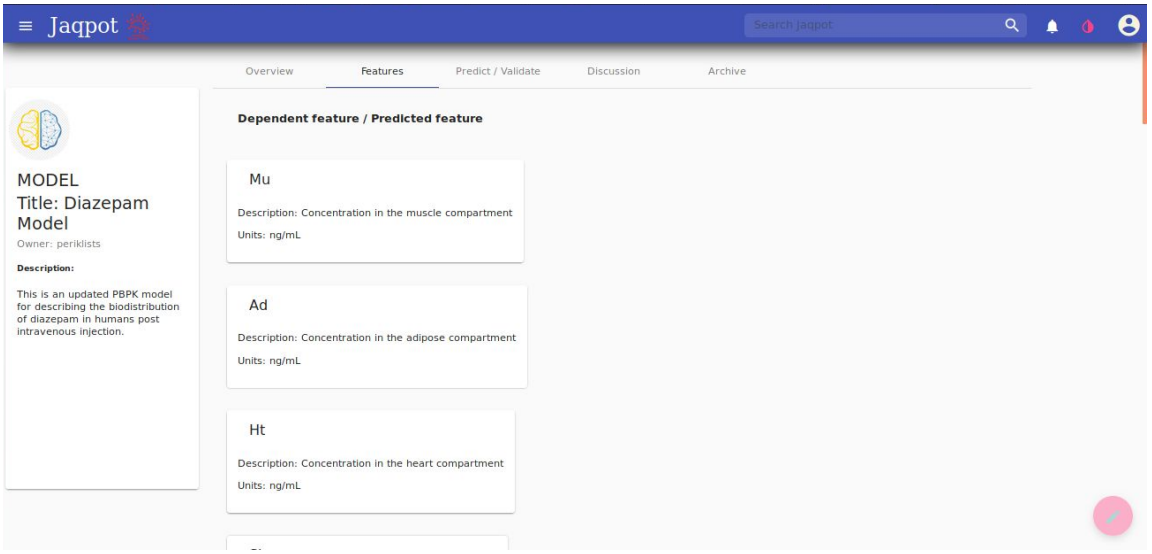


Figure 9. 'Features' tab of the Diazepam model.

The 'Predict/Validate' tab is the core of the model environment, where the user can provide an instance of the independent features and acquire model predictions, which, in the case of PBPK models consist mainly or concentration or mass - time profiles (Figure 10). The user can provide input in two ways: the first one is through uploading a csv file containing the respective information and the second one is through filling in the input directly in Jaqpot's Graphical User Interface (GUI). Finally, the user can add comments and remarks or ask a question regarding the model under the 'Discussion' tab (FIgure 11).

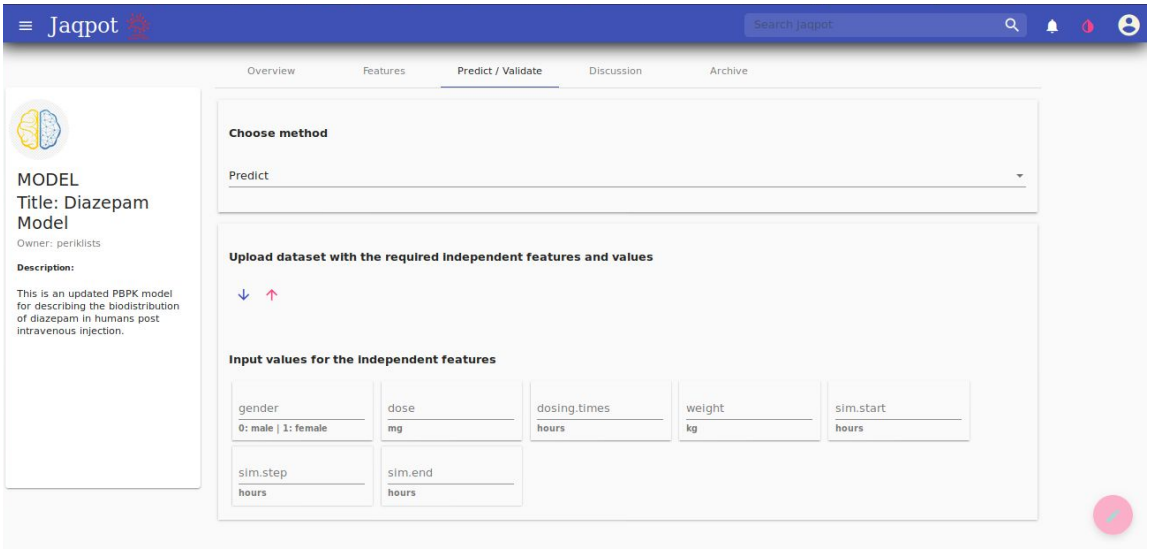


Figure 10. 'Predict/Validate' tab of the Diazepam model.

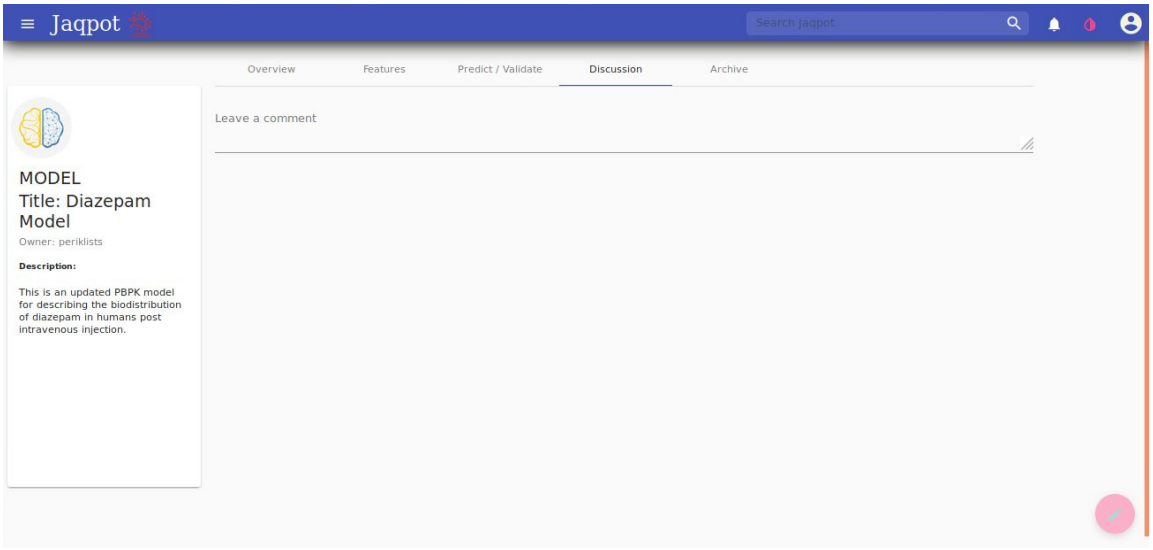


Figure 11. 'Discussion' tab of the Diazepam model.

More details on how to access and use PBPK models through the Jaqpot API are provided in the Appendix. Two examples of custom PBPK models that have been deployed as web services and can be accessed through the Jaqpot platform are described here. The first one is the PBPK model for diazepam in humans described in (Gueorguieva et al., 2006), which describes the biokinetics of diazepam in humans. The second example is a generic PBTK model for fish described in (Grech et al., 2019). The two models can be accessed in https://ui-jaqpot.prod.openrisknet.org/model/qof7CZIajxBHb6fU7SJz and https://ui-jaqpot.prod.openrisknet.org/model/V92BiEXxeoP35R4Nyp2y respectively.

PBPK model for diazepam in humans

The detailed description of the PBPK model used can be found in Gueorgueiva et al. (2006). Its schematic representation can be seen in Figure 12. It refers to intravenous injection of diazepam in healthy adults and consists of 11 compartments describing the concentration of the drug in various tissues, namely liver (*LI*), kidney (*KI*), brain (*BR*), intestine (*IN*), stomach (*ST*), muscle (*MU*), adipose (*AD*), skin (*SK*), gonads (*GO*), heart (*HT*) and lungs (*LU*), one compartment to model the rest of the body (*RE*) as well as two blood pools; venous (*VEN*) and arterial (*ART*). The parameters of the model are divided into drug-dependent and physiological (drug-independent) parameters. The first category comprises eleven tissue-to-plasma partition coefficients (K_p), fraction unbound in plasma (*fu*), blood-to-plasma ratio (*R*), and intrinsic hepatic clearance (CL_{int} , in *L/h*). The physiological parameters of the model are simply the regional tissue blood flows (*Q*) and tissue volumes (*V*).

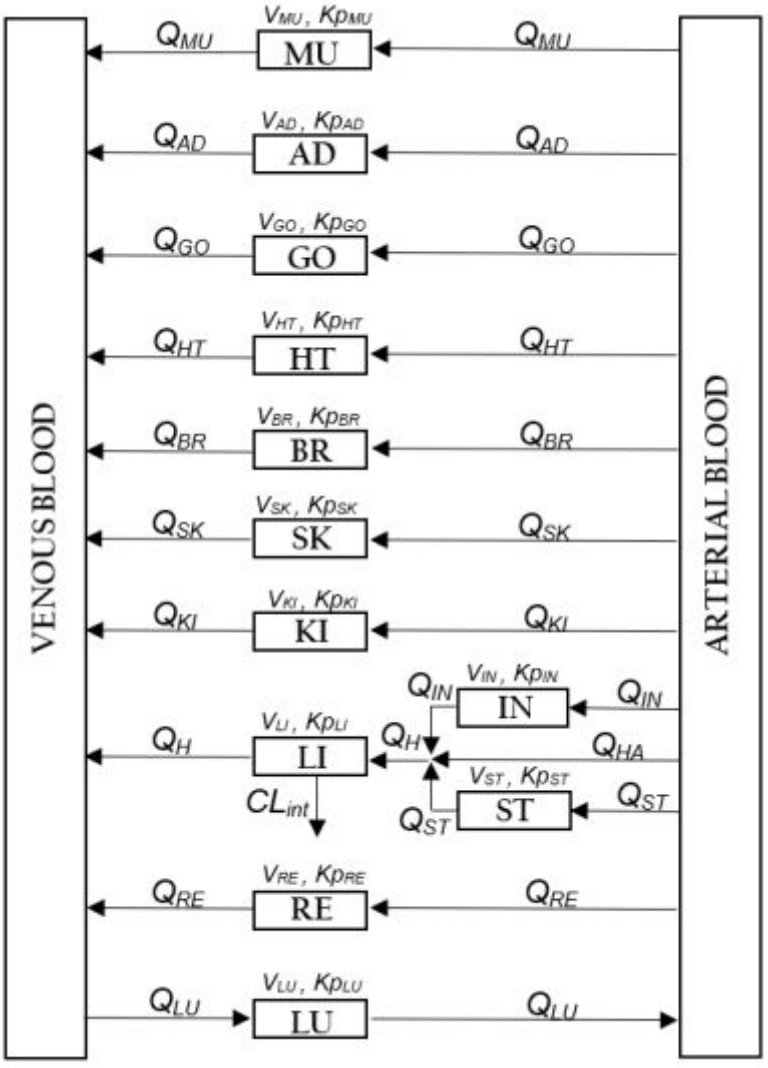


Figure 12. Schematic presentation of the diazepam structural model.

The values of the parameters are the product of research work performed by partners at NTUA and INERIS, involving the recalibration of the model under a different statistical model, published (this work has been published by OpenRiskNet partners (Tsiros et al., 2019)). It has been made available on Jaqpot at https://ui-jaqpot.prod.openrisknet.org/model/qof7CZIajxBHb6fU7SJz .

Generic PBTK model for four species of fish

Description of the model

The fish PBTK model developed by Grech et al. (2019) (Figure 13) was uploaded to Jaqpot and me available at https://ui-jaqpot.prod.openrisknet.org/model/V92BiEXxeoP35R4Nyp2y. This model describes the kinetics of xenobiotics in four species of fish, rainbow trout, fathead minnow, zebrafish and three-spined stickleback, according to a single general structure. The model comprises 12 compartments: arterial and venous blood, gills, gastrointestinal tract, skin, kidney, fat, liver, gonads, brain, poorly perfused tissues, and richly-perfused tissues. All the organs/tissues are modelled as well-mixed compartments with a blood flow-limited distribution.

Both gastro-intestinal (in case of food ingestion) and branchial absorption are modelled. Chemical binding to plasma proteins is considered by introducing an unbound fraction of the chemical in plasma. Metabolism is modelled in the liver or in the plasma. Excretion can occur via urine, expired water, and faeces, as unabsorbed fraction or by biliary excretion. Compounds excreted by the gills and urine are released in the water and can be reabsorbed in static water conditions.

The PBTK model includes a growth model based on the dynamic energy budget (DEB) theory (Kooijman, 2010). Two growth models were implemented. The first is a standard DEB model (Kooijman, 2010) and can be represented by a von Bertalanffy growth curve. This model was used for zebrafish and stickleback, in accordance with the Add-my-pet database (www.bio.vu.nl/thb/deb/deblab/add_my_pet). The second growth model is described by a DEB model with type M acceleration (Kooijman and Lika, 2014) and is characterised by an up-curving of length-at-time since birth at constant food (Kooijman, 2014). This model was used for rainbow trout and fathead minnow.

In case the tissue:blood partition coefficients are unknown, they can be automatically estimated by QSAR modelling. However, the user has to ensure that the chemical studied in the applicability domain of the QSAR models that are implemented (non polar, non ionizable, and log Kow between 2 and 6).

An extensive literature search was performed to identify experimental data informing the physiological PBTK model's parameters for the four species, distinguishing male and female when data was available.

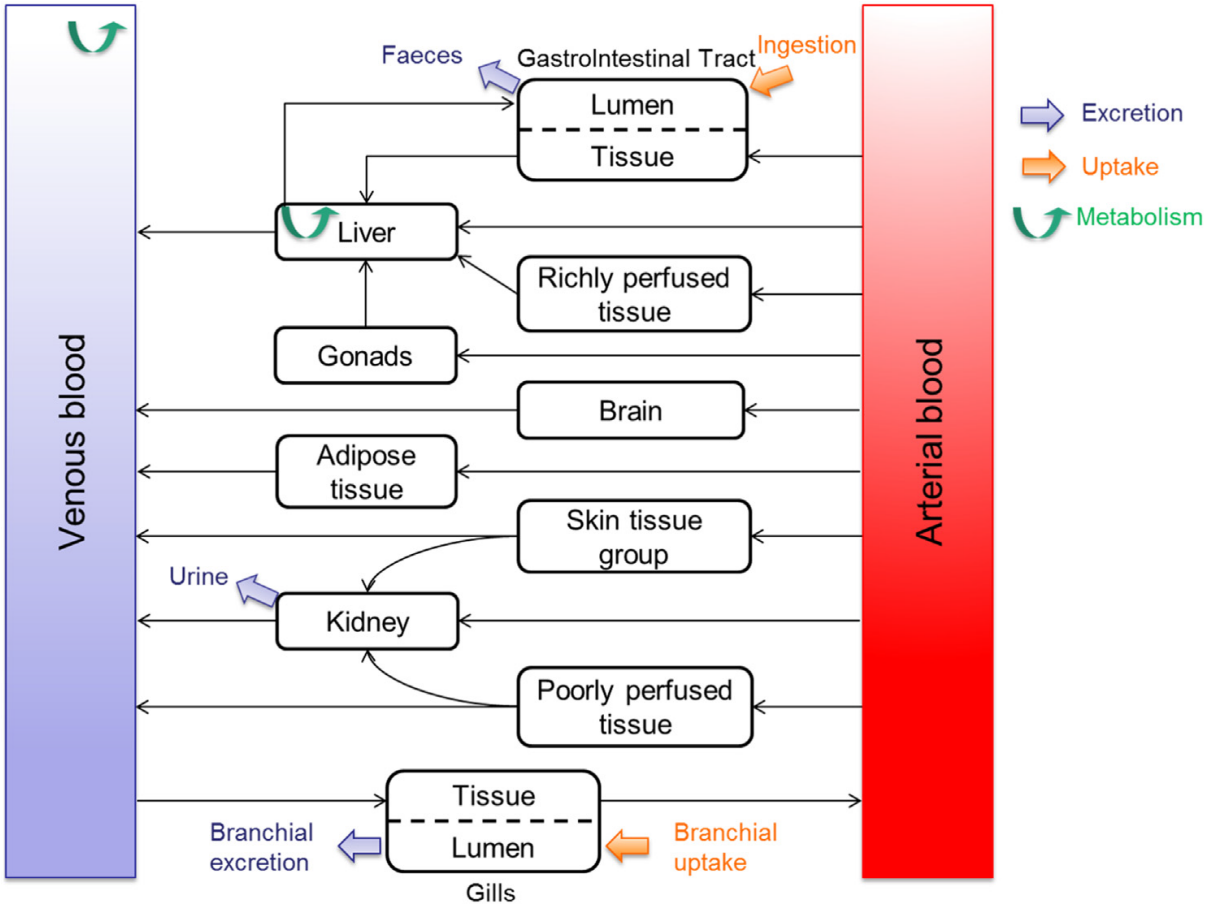


Figure 13. PBTK model structure for fish.

Implementation in Jaqpot

Because this PBTK model is generic, the user defines as many as 40 parameters which relate to:

- Chemical-specific absorption: Ku, frac_abs (oral), PC_bw (if NA estimated using log_Kow),
- Chemical-specific excretion: Ke_bile, K_BG, Ke_urine, Ke_feces, PC_bw, unbound_fraction
- Chemical-specific distribution: PC_l, PC_k, PC_s, PC_f, PC_p, PC_r, PC_gi, PC_go, PC_b, Ratio_blood_plasma
- Chemical-specific metabolism: rate_plasma, Cl_liver, Km, Vmax
- Exposure and experimental setup: WaterQuantity, IngestQuantity, IVQuantity, V_water, Ke_water, period, frac_renewed, time_first_exposure, time_last_exposure, Bw_i, Temperature, f_cst, Gender, Species

- Simulations to be carried out: sim.start, sim.end, sim.step

The output of the simulations is a table of internal concentrations (C_art, C_plasma, C_liver, C_kidney, C_brain, C_fat, C_gonads, C_GIT, C_rp, C_pp, C_skin, C_tot), Body weight (BW), fish length, and total amount administered to/absorbed by the fish, as a function of time.

Integration of PKsim in OpenRiskNet Infrastructure through the Jaqpot modelling platform

This subsection presents a step-by-step demonstration on how to create a PBPK model using PKSim (Willmann et al., 2003) and expose it as a web service through the Jaqpot modelling platform.

Software requirements: The user should download and install in his/her PC the Open Systems Pharmacology Suite:
https://github.com/Open-Systems-Pharmacology/Suite/releases/tag/v7.1.0

and the gene expression database:

https://github.com/Open-Systems-Pharmacology/Suite/releases/download/v7.1.0/GENEDB_human.mdb

In PKSim select Utilities, then Options, then “Application” and for Human you give the path where the database is stored.

All other necessary files that should be downloaded are available in the google drive folder used for the OpenRiskNet/OpenTox Euro 2017 Biokinetics Workshop, where this example was presented:

https://drive.google.com/drive/folders/1wGmqNYI8GnDL_orrE2JqPQAMauHStbPj

Assuming that the PBPK model has been saved in xml format (as explained in Powerpoint presentation), the following steps should be followed in Swagger JaqPot API documentation http://jaqpot.org:8080/jaqpot/swagger/ :

1) Create a JaqPot dataset containing the physiological parameters of the individual on which the PBPK model was developed. We assume that these parameters (age, height, weight) are included in a csv file testPK.csv
    a. Use the **POST /dataset/createDummyDataset** method: Choose the ***testPK.csv*** file and give any title and description to the produced dataset. In the end of the response body a dataset id is generated.
    b. Use the **GET /dataset/{id}** method: Copy the dataset id in the relevant field and press Try it out!. In the Request URL the full dataset URI of the produced dataset is shown. Store the URI of the dataset.
    Example: http://jaqpot.org:8080/jaqpot/services/dataset/0JRSR55QrpHpMi (the dataset can be accessed through Swagger using its dataset ID, **0JRSR55QrpHpMi**).

2) Produce a JaqPot resource that implements the PBPK model as a web service
    a. Use the **POST /biokinetics/train** method. Insert the following information in the parameter fields:
        - *File*: Choose the PBPK xml file ***sim_individual_1_XML.xml***
        - Dataset-uri: Copy the full dataset URI
        - Title and description: Any title and description is fine
        - Algorithm-uri: http://jaqpot.org:8080/jaqpot/services/algorithm/pk-sim (more information on this algorithm can be obtained over Swagger using the **GET /algorithm/{id}** method http://jaqpot.org:8080/jaqpot/swagger/#!/algorithm/getAlgorithm with the ID of the algorithm, **pk-sim**)
        - *Parameters*: {"ageUnit":["years"], "individual":[1],"heightUnit":["m"], "weightUnit":["kg"],"drug":["Theophylline"]}

      After pressing the Try it out! button, a task ID is generated In the end of the response body
    b. Use the **GET /task/{id}** method. Copy the task ID in the relevant field and press the Try it out! Button. If the status in the end of the response body is not COMPLETED wait for a few seconds and try again. The model has been created and its complete URI is shown in the response body. You can now share the model with the rest of the world. For the next step store the model id. This is the id after model/ in “result” in the response body
3) Use the model for obtaining drug concentration-time profiles
    a. Use the **POST /model/{id}** method. In the dataset_uri you can use the same dataset URI that was used in the first step. Alternatively, you can create an alternative dataset for a different individual. In the id field copy the id of the produced model. After pressing the Try it out! button a task ID is created.
    b. Use the **GET /task/{id}** method. Copy the task ID in the relevant field and press the Try it out! Button. The dataset with drug concentration –time data has been created. For the next step store the dataset id. This is the id after dataset/ in “result” in the response body
    c. Use the **GET /dataset/{id}** method: Copy the dataset id in the relevant field and press Try it out!. In the Response Body, drug concentration values are shown along with corresponding time points.

Reverse dosimetry

Workflow for reverse dosimetry

Reverse dosimetry of diazepam in humans was performed by selecting a relevant internal concentration from the literature. The diazepam PBPK model (at https://ui-jaqpot.prod.openrisknet.org/model/qof7CZIajxBHb6fU7SJz) was then run using forward dosimetry (i.e. predicting the internal concentration consequently to a given exposure scenario) iteratively in order to estimate the exposure dose which produces the selected internal concentration (Figure 14).

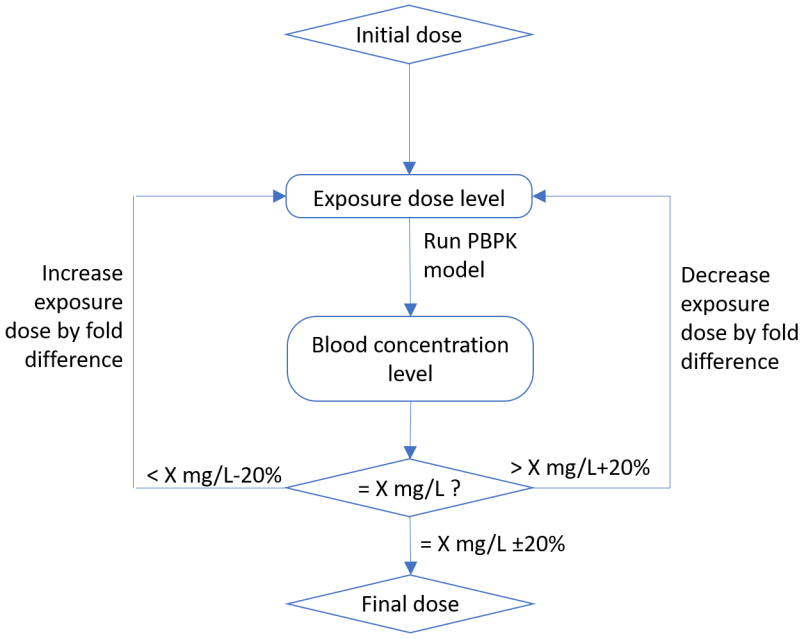


Figure 14. Workflow for reverse dosimetry of diazepam. X is the target internal concentration.

Diazepam

No biomonitoring of diazepam in the general population was identified in the literature. Summary data on diazepam concentrations in drivers apprehended for driving under the influence of drugs in Sweden was available in the literature (Jones et al., 2012). A median value of 0.20 mg/L diazepam in blood was estimated in 1,000 blood samples where diazepam and its metabolite nordiazepam were both present.

The diazepam model simulates intravenous injections. The exposure scenario was assumed to be daily injections. The initial daily dose in the reverse dosimetry is set to 5mg (equivalent to one pill per day).

Chlorpyrifos

Reverse dosimetry with the fish PBTK model (at https://ui-jaqpot.prod.openrisknet.org/model/V92BiEXxeoP35R4Nyp2y) was performed

with the same procedure in the case of continuous waterborne exposure to chlorpyrifos in rainbow trout (Grech et al., 2019). PBPK parameter values are listed in Table 1.

Table 1. User-defined parameter values used for the chlorpyrifos PBTK in rainbow trout.

Parameter	Unit	Value	Source
Log K_{ow}	-	4.96	
V_{max}	µg/d/g liver	3375.38	Lavado and Schlenk, 2011
K_m	µg/mL	50.31	Lavado and Schlenk, 2011
Cl_liver	mL.d-1	NA	
Rate_plasma		0	
Unbound_fraction	-	0.05	Weelling et al., 1992
Ke urine	d^{-1}	0	Weelling et al., 1992
Ke bile	d^{-1}	0	Weelling et al., 1992
Ke feces	d^{-1}	0.83	Nichols et al., 2004
K_{BG}	d^{-1}	1e12	
Ku	d^{-1}	0	
Frac_abs		0	
Ratio blood to plasma	-	1	
Temperature*	°C	17	
Ke water*	d^{-1}	0	
WaterQuantity*	µg	9e09	
V_water*	mL	1e12	
IngestQuantity*	µg	0	
IVQuantity*	µg	0	
Period*	d	NA	
Time_first_dose*	d	NA	
Time_last_dose*	d	NA	
Frac_renewed*		0	
BW_i*	g	100	
f_cst*		0.3	
Species*		RT	
Gender*		0	

*Study dependent

Table 2. QSAR-estimated partition coefficients

Partition coefficient	Value
blood:water	1119
liver:blood	8.56
gonads:blood	17.93
brain:blood	5.95
fat:blood	8.15
skin:blood	3.75
GIT:blood	6.03
kidney:blood	13.54
RPT:blood	5.52
PPT:blood	2.04

No biomonitoring data on internal chlorpyrifos concentrations in fish was available. Reverse dosimetry was performed in order to estimate the chlorpyrifos concentration level in water that would elicit the EC50 level for the EROD biomarker on rainbow trout liver cells (RTL-W1) in vitro which was estimated to be 0.022 mg/L (Babín et al., 2005) The initial water concentration in the reverse dosimetry procedure was set to 9 µg/L, which is the lower range of LC50 in rainbow trout (Wheelock et al., 2005) determined by Phipps and Holcombe (Phipps and Holcombe, 1985). The higher range of LC50 values is 45 µg/L for 96hr acute toxicity (Kikuchi et al., 1996).

Implementation in Jaqpot

Diazepam

For the diazepam PBPK, the parameters for repeated exposure can be entered in the fields (Figure 15).

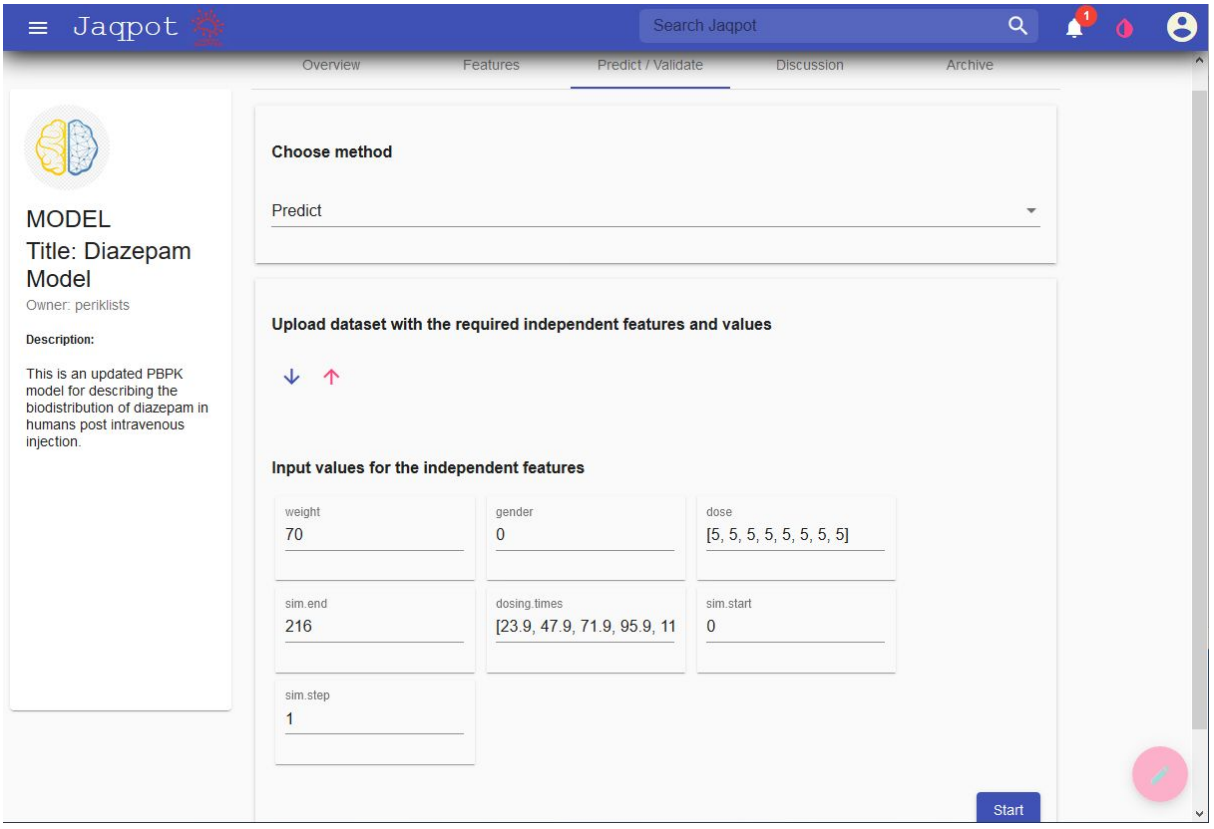


Figure 15. Parameters for the diazepam PBPK with repeated exposure.

Chlorpyrifos

For the fish PBTK model, the parameters for waterborne exposure to chlorpyrifos can also be entered either as a csv file or in the parameter input fields (Figure 16 and Figure 17).

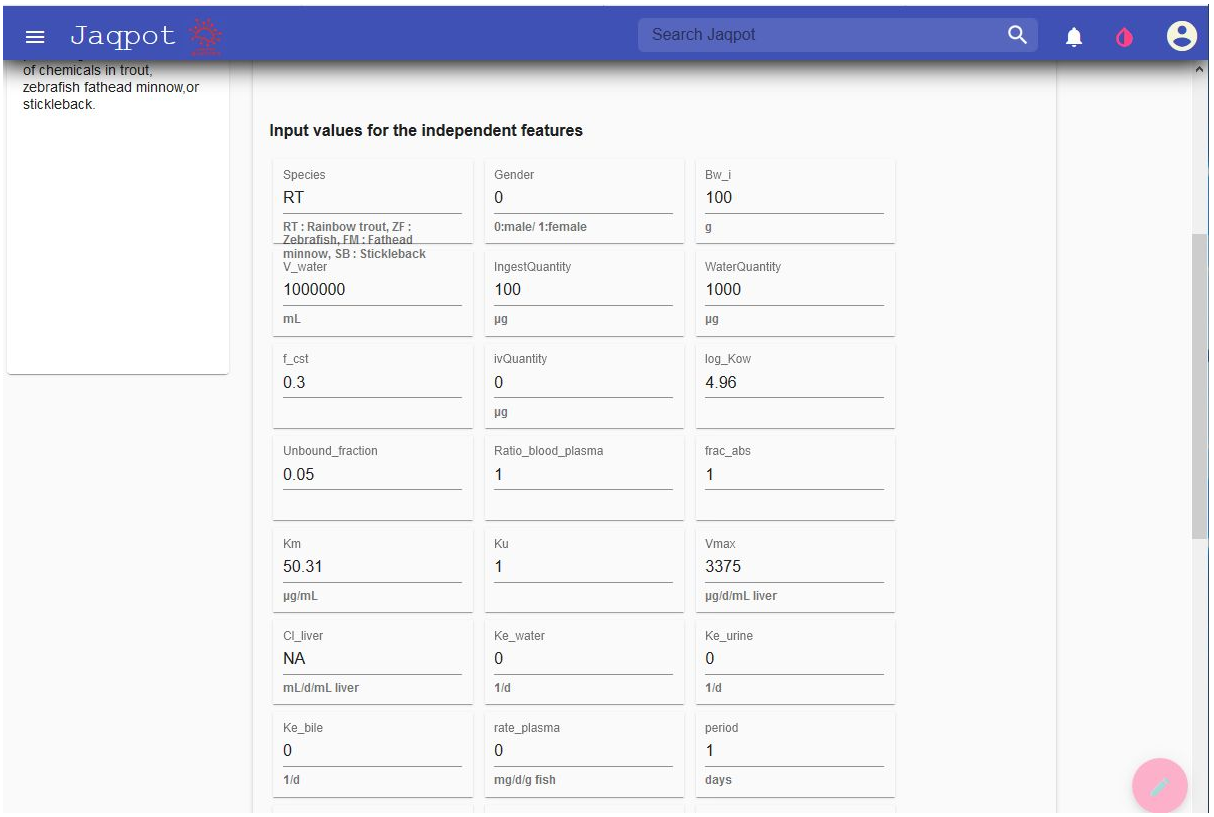


Figure 16. Parameters for the fish PBTK for chlorpyrifos.

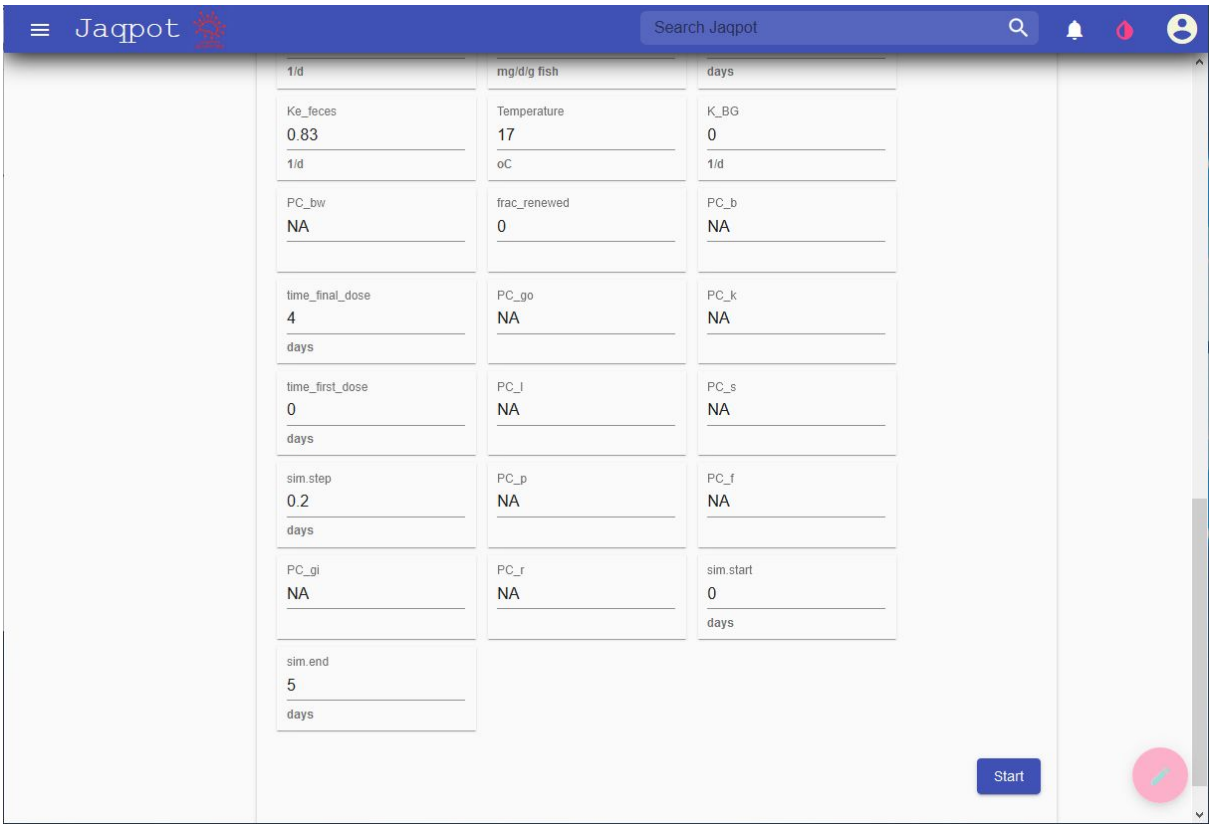


Figure 17. Parameters for the fish PBTK (continued).

Results

Diazepam

Diazepam levels in blood were predicted in a 70kg male subject first with a 5mg IV daily dose (Figure 18 and Figure 19). the level increased sharply, due to the simulated injection, then decreased sharply and followed a slow, approximately linear, decrease one hour after the dose and until the next dose. Steady state was obtained after 7 doses, when the peak and lowest concentration levels were the same every day.

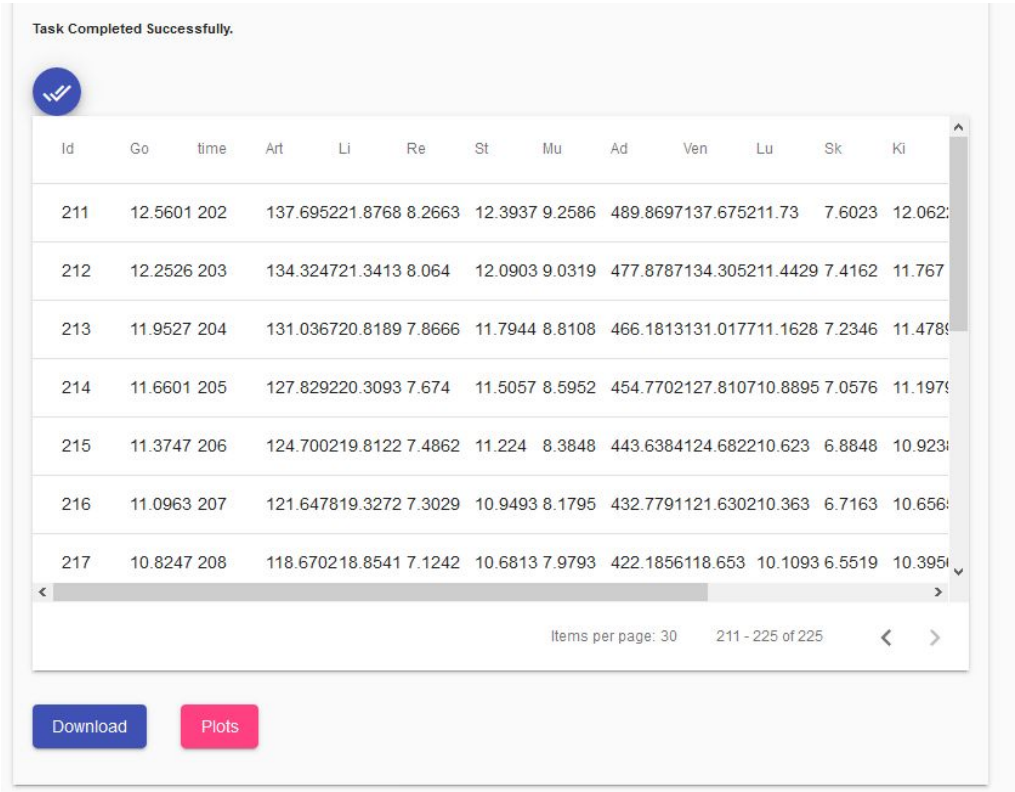


Figure 18. Output at last simulation times for repeated exposure to diazepam - mg daily IV doses in 70 kg male subjects.

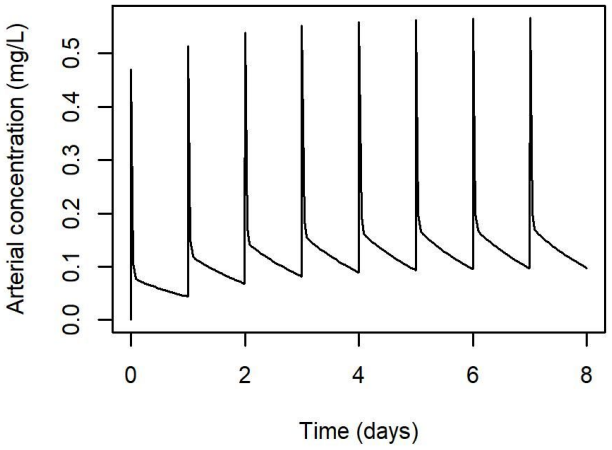


Figure 19. Predicted diazepam concentrations in blood resulting from 5mg daily IV doses in 70 kg male subjects.

The strong peak in concentration could be due to the simulated mode of administration (IV). Since diazepam would most often be administered orally, the time point selected for

comparison with the biomonitoring data in drivers was 4 hours after the dose, well after the peak concentration.

4 hours after the 8th dose, the concentration in blood was 0.159 mg/L, which is very close to the median in drivers (0.20 mg/L). The simulated administered dose was increased to 6.26 mg according to the workflow. The resulting blood concentration 4 hours after the 8th dose was 0.20 mg/L (Figure 20).

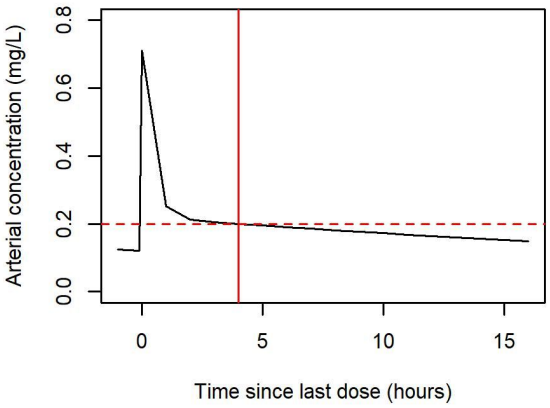


Figure 20. Predicted diazepam concentrations in blood resulting from 6.26 mg daily IV doses in 70 kg male subjects (focus on period after 8th dose).

Chlorpyrifos

Exposure of rainbow trout to a constant exposure chlorpyrifos was simulated for juvenile fish (body weight set to 100 g) at 17°C in order to reproduce the experimental conditions reported by (Phipps and Holcombe, 1985). Internal concentrations reach steady state after about 60 days when fish are exposed to 9 µg/L (Figure 21A). After 96 hours of simulated exposure to chlorpyrifos, as expected the lethality LC50 resulted in liver concentrations which where higher than the EC50 for the EROD biomarker. Indeed, the liver concentration reached 21 mg/L, which is 1000-fold higher than the 0.022 mg/L in vitro EC50 for EROD inhibition. Consequently, to perform reverse dosimetry, the exposure concentration was set to 0.0093 µg/L which resulted in a liver concentration of 0.021 after 96 hours (Figure 21B), which was within 20% of the target internal concentration.

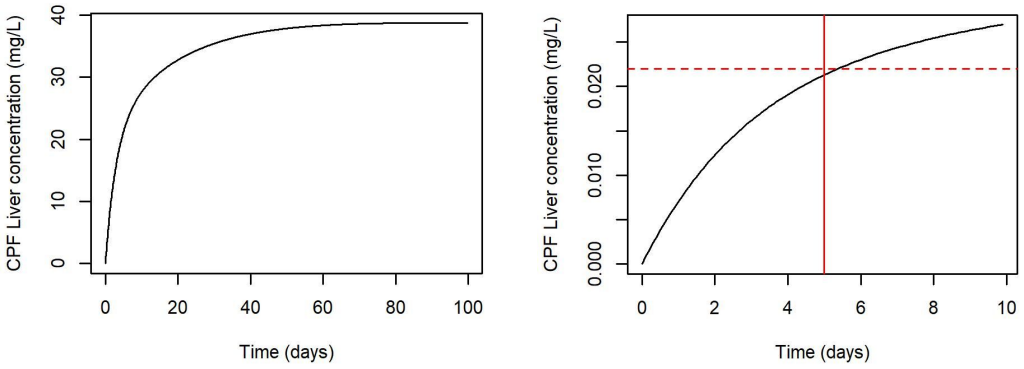


Figure 21. Predicted concentrations of chlorpyrifos in rainbow trout liver exposed to 9 µg/L (A) or 0.0093 µg/L (B) chlorpyrifos at 17°C.

In both diazepam and chlorpyrifos examples, reverse dosimetry was straightforward due to almost linear kinetics in the dose range considered.

REFERENCES

Babín, M.M. and Tarazona, J.V., *In vitro toxicity of selected pesticides on RTG-2 and RTL-W1 fish cell lines.* Environmental Pollution, 2005. **135**(2): p. 267-274. https://doi.org/10.1016/j.envpol.2004.11.001

Chomenidis, C., Drakakis, G., Tsiliki, G., Anagnostopoulou, E., Valsamis, A., Doganis, P., Sopasakis, P., Sarimveis, H., *Jaqpot Quattro: A Novel Computational Web Platform for Modeling and Analysis in Nanoinformatics.* Journal of Chemical Information and Modeling, 2017. **57**: p. 2161-2172. https://doi.org/10.1021/acs.jcim.7b00223

Grech, A., Tebby, C, Brochot, C., Bois, F.Y., Bado-Nilles, A., Dorne, J.L., Quignot, N., Beaudouin, R., *Generic physiologically-based toxicokinetic modelling for fish: Integration of environmental factors and species variability.* Science of the Total Environment, 2019. **651**: p. 516-531. https://doi.org/10.1007/s10928-019-09630-x

Gueorguieva, I., Aarons, L., Rowland, M., *Diazepam Pharamacokinetics from Preclinical to Phase I Using a Bayesian Population Physiologically Based Pharmacokinetic Model with Informative Prior Distributions in Winbugs*. Journal of Pharmacokinetics and Pharmacodynamics, 2006. **33**(5): p. 571–594.

Jones, A. and Holmgren, A., *Concentrations of Diazepam and Nordiazepam in 1000 Blood Samples From Apprehended Drivers —Therapeutic Use or Abuse of Anxiolytics?* Journal of Pharmacy Practice, 2012. **26**(3): p. 198-203. https://doi.org/10.1177/0897190012451910

Jones, H.M. and Rowland-Yeo, K., *Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development*. CPT Pharmacometrics Syst Pharmacol, 2013. **2**:e63. doi: https://doi.org/10.1038/psp.2013.41

Kikuchi, M., Miyagaki, T. and Wakabayashi, M., *Evaluation of Pesticides Used in Golf Links by Acute Toxicity Test on Rainbow Trout.* NIPPON SUISAN GAKKAISHI, 1996. **62**(3): p. 414-419. https://doi.org/10.2331/suisan.62.414

Kilford, P. J., Gertz, M., Houston, J. B. and Galetin, A., *Hepatocellular binding of drugs: correction for unbound fraction in hepatocyte incubations using microsomal binding or drug lipophilicity data*. Drug Metabolism and Disposition, 2008. **36**(7): p. 1194-7. https://doi.org/10.1124/dmd.108.020834

Kooijman, S.A., *Notation of dynamic energy budget theory for metabolic organisation*. 2010: Cambridge University Press.

Kooijman, S.A.L.M., *Metabolic acceleration in animal ontogeny: An evolutionary perspective.* Journal of Sea Research, 2014. **94**: p. 128-137. https://doi.org/10.1016/j.seares.2014.06.005

Kooijman, S.A.L.M. and Lika, K., *Resource allocation to reproduction in animals*. Biological Reviews, 2014. **89**(4): p. 849-859. https://doi.org/10.1111/brv.12082

Lavado, R. and Schlenk, D., *Microsomal biotransformation of chlorpyrifos, parathion and fenthion in rainbow trout (Oncorhynchus mykiss) and coho salmon (Oncorhynchus kisutch): Mechanistic insights into interspecific differences in toxicity.* Aquatic Toxicology, 2011. **101**(1): p. 57-63. https://doi.org/10.1016/j.aquatox.2010.09.002

Nestorov, I., *Whole body pharmacokinetic models*. Clin Pharmacokinet, 2003. **42**(10): p. 883-908. https://doi.org/10.2165/00003088-200342100-00002

Nestorov, I.A., Aarons, L.J., Arundel, P.A., Rowland, M., *Lumping of whole-body physiologically based pharmacokinetic models*. J Pharmacokinet Biopharm, 1998. **26**(1): p. 21–46. doi: https://doi.org/10.1023/A:1023272707390

Nichols, J.W., Fitzsimmons, P.N., Whiteman, F.W., Dawson, T.D., Babeu, L., Juenemann, J., *A physiologically based toxicokinetic model for dietary uptake of hydrophobic organic*

compounds by fish - I. Feeding studies with 2,2 ',5,5 '-tetrachlorobiphenyl. Toxicological Sciences, 2004. **77**(2): p. 206-218. https://doi.org/10.1093/toxsci/kfh033

Ooms, J. *The OpenCPU System : Towards a Universal Interface for Scientific Computing through Separation of Concerns*. *arXiv,* 2014. p. 1–23.

Pearce, R., Setzer, R., Strope, C., Sipes, N., Wambaugh, J., *httk: R Package for High-Throughput Toxicokinetics*. Journal of Statistical Software, *2017.* **79**(4): p. 1 - 26. doi:http://dx.doi.org/10.18637/jss.v079.i04

Phipps, G.L. and Holcombe, G.W., *A method for aquatic multiple species toxicant testing: Acute toxicity of 10 chemicals to 5 vertebrates and 2 invertebrates.* Environmental Pollution Series A, Ecological and Biological, 1985. **38**(2): p. 141-157. https://doi.org/10.1016/0143-1471(85)90073-X

Pilari, S., Huisinga, W., *Lumping of physiologically-based pharmacokinetic models and a mechanistic derivation of classical compartmental models*. J Pharmacokinet Pharmacodyn, 2010. **37**(4): p. 365–405. doi: https://doi.org/10.1007/s10928-010-9165-1

Sturm, R.A., *Computer Model for the Clearance of Insoluble Particles from the Tracheobronchial Tree of the Human Lung*. Comput Biol Med, 2007. **37**(5): p. 680–690. doi: https://doi.org/10.1016/j.compbiomed.2006.06.004

Tsiros, P., Bois, F. Y., Dokoumetzidis, A., Tsiliki, G., Sarimveis, H., *Population pharmacokinetic reanalysis of a Diazepam PBPK model: a comparison of Stan and GNU MCSim.* Journal of Pharmacokinetics and Pharmacodynamics, 2019. 46(2): p. 173–192. https://doi.org/10.1016/0143-1471(85)90073-X

Weelling, W. and De Vries, J., *Bioconcentration kinetics of the organophosphorus insecticide chlorpyrifos in guppies (Poecilla reticulata).* Ecotoxicology and Environmental Safety, 1992. **23**: p. 64–75. https://doi.org/10.1016/0147-6513(92)90022-U

Wheelock, C.E., et al., *Individual variability in esterase activity and CYP1A levels in Chinook salmon (Oncorhynchus tshawyacha) exposed to esfenvalerate and chlorpyrifos.* Aquatic Toxicology, 2005. **74**(2): p. 172-192. https://doi.org/10.1016/j.aquatox.2005.05.009

Willmann S, Lippert J, Sevestre M, Solodenko J, Fois F, Schmitt W. *PK-Sim®: a physiologically based pharmacokinetic 'whole-body' model*. BIOSILICO, 2003. **1**(4): p. 121–4. http://dx.doi.org/10.1016/S1478-5382(03)02342-4.

APPENDIX

USER GUIDANCE ON PBPK MODELS IN JAQPOT

HOW TO ACCESS A CUSTOM PBPK MODEL

An important module introduced in Jaqpot 5 is custom PBPK models that have been deployed through a developed R client. A Jaqpot user can run simulations using these models, e.g. generate forward and reverse dosimetry scenarios, provided that he has access to a model through the organization she/he is part of.

After logging into Jaqpot, the user is directed to Jaqpot's Home page, from where he can go to the models section by clicking the 'Models' tab on the left banner of the screen (Figure A1).

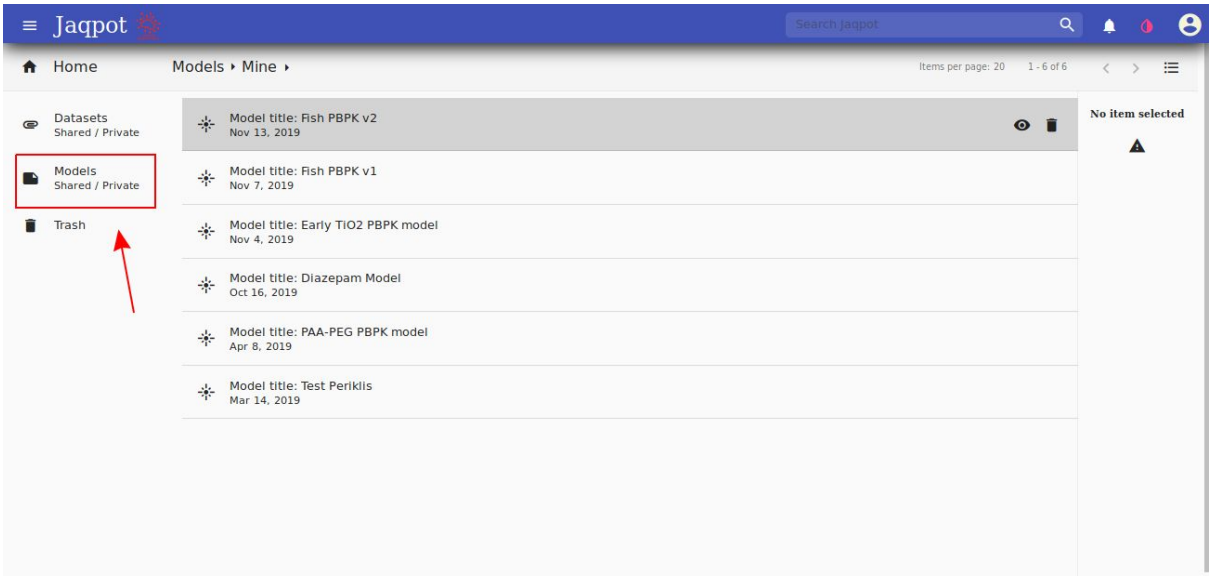


Figure A1. 'Models' Tab.

The initial screen includes models deployed by the user, private and shared ones. In order to access models that have been shared to the user via organisations, the user should click on the arrow right next to the 'Mine' tab on the top of the screen and then click the 'Shared' button (Figure A2).

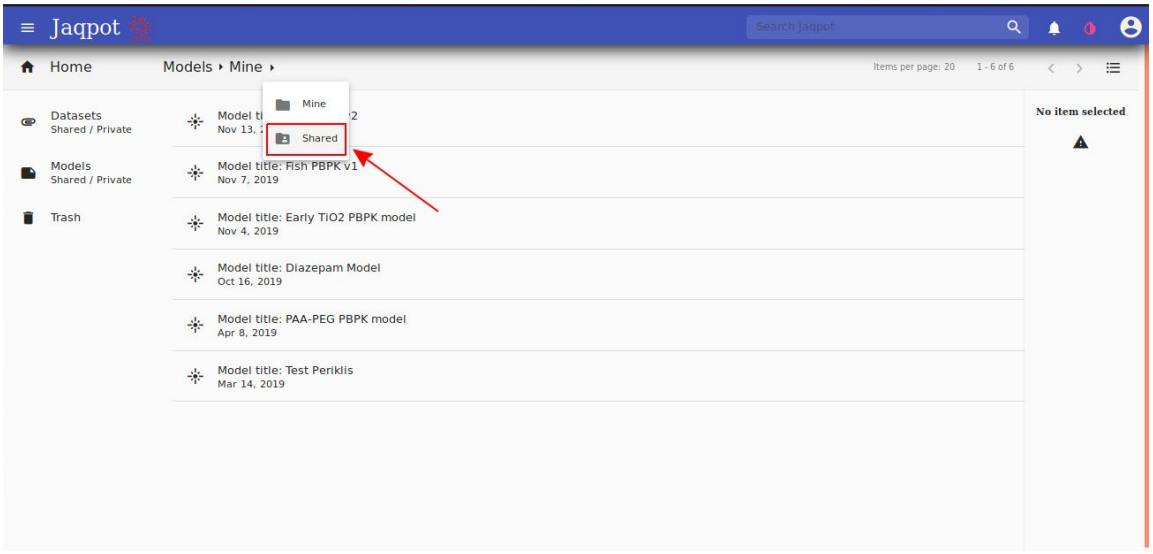


Figure A2. Selecting Share models.

Following that, the user can access a specific model by clicking on the 'View' button, which is located on the far right end of the model's row (Figure A3).

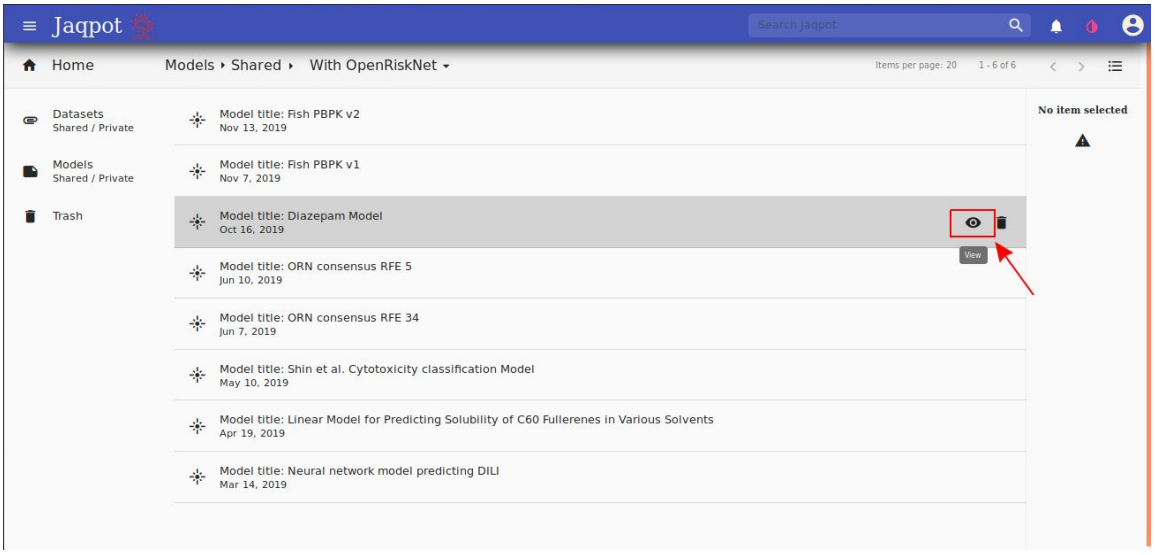


Figure A3. Clicking on the 'View' tab.

NAVIGATING A MODEL PAGE

The model environment comprises 4 tabs: 'Overview', 'Features', 'Predict/Validate' and 'Discussion'. The 'Overview' tab provides a coarse description of the PBPK model, as well as specific directions which refer to the model, e.g. how to fill in the input section (Figure A4).

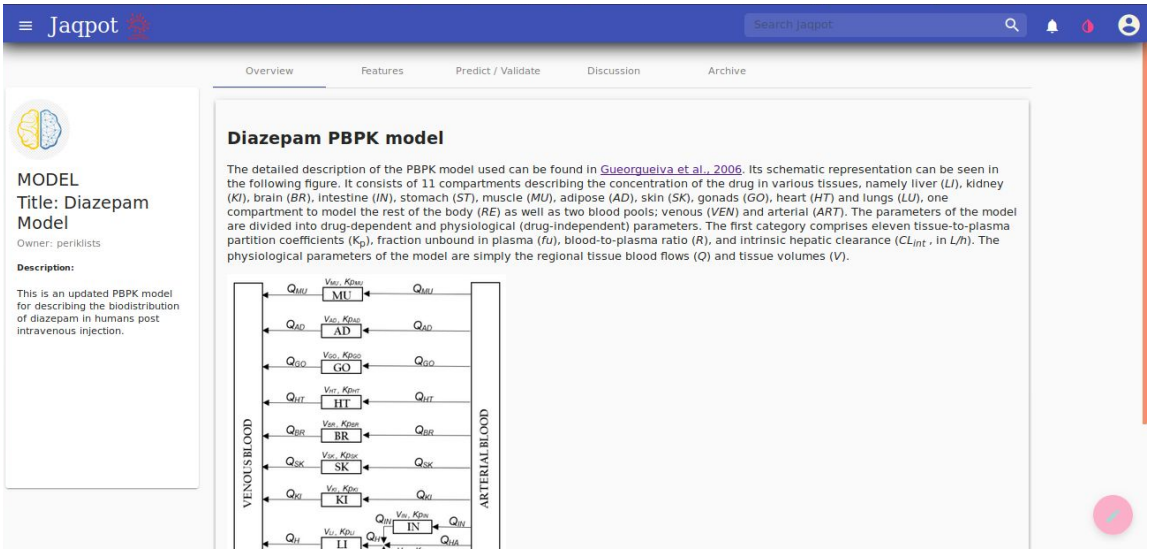


Figure A4. 'Overview' tab of the Diazepam model.

The 'Features' tab informs the users about the dependent and independent features; each feature comes with description and units (Figure A5).

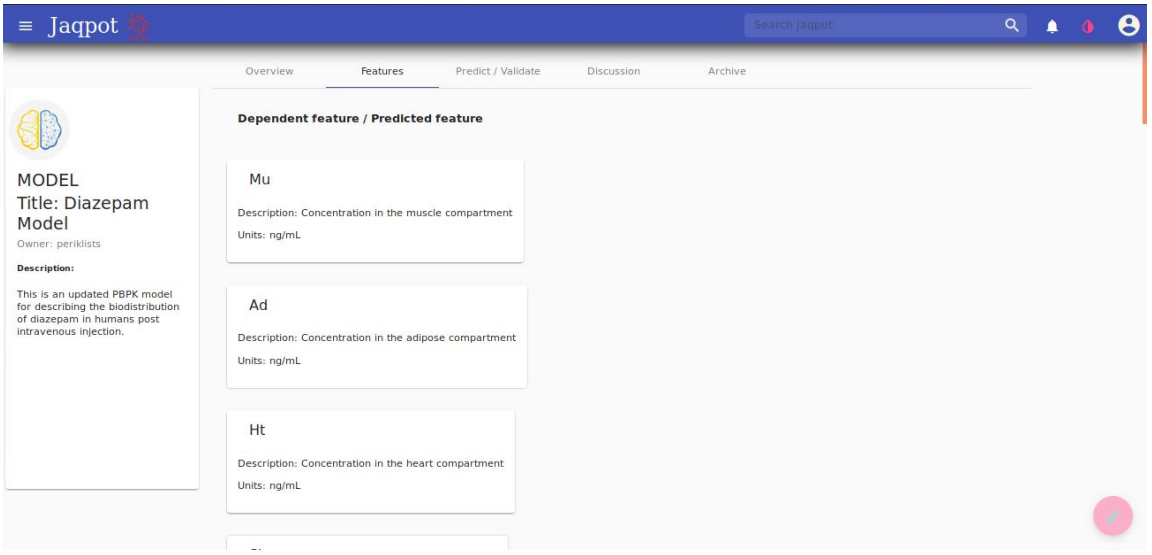


Figure A5. 'Features' tab of the Diazepam model.

The 'Predict/Validate' tab is the core of the model environment. Here, the user can provide an instance of the independent features and acquire the model predictions, which, in the case of PBPK models consist mainly or concentration or mass- time profiles. The user can provide the input in two ways: the first one is through uploading a csv file containing the respective information and the second one is through filling in the input directly in

Jaqpot's Graphical User Interface (GUI). In case the input consists of many features, it is strongly recommended that the user follows the first method, i.e. download the csv template (Figure A6), fill in the values (Figure A7), upload the complete csv (Figure A8), select 'None' in the pop up window asking for a dataset ID (FIgure A9) and then start the prediction process (Figure A10).

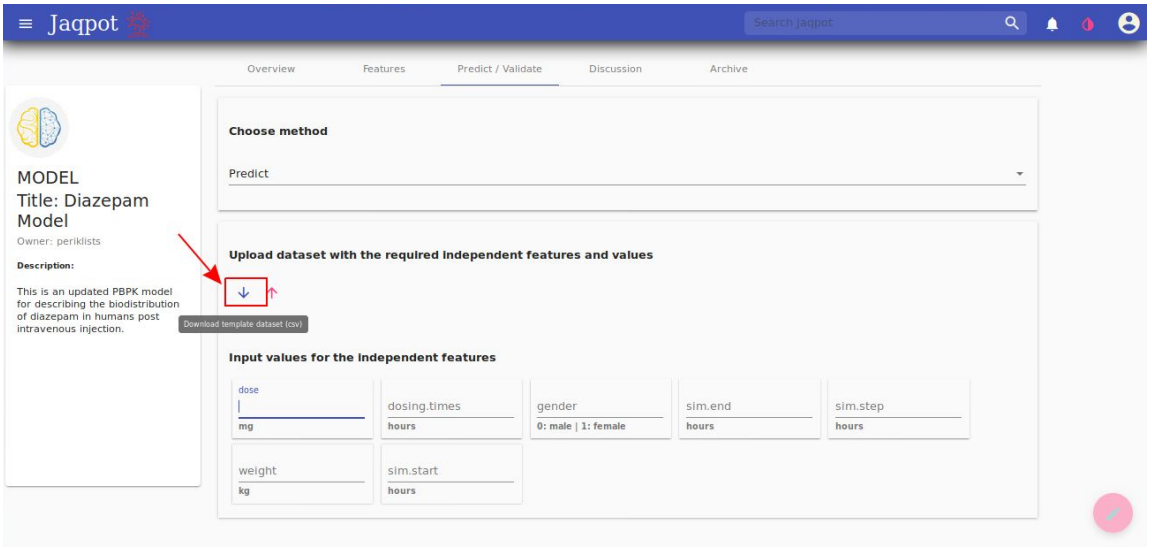


Figure A6. How to download a dataset template in csv format.

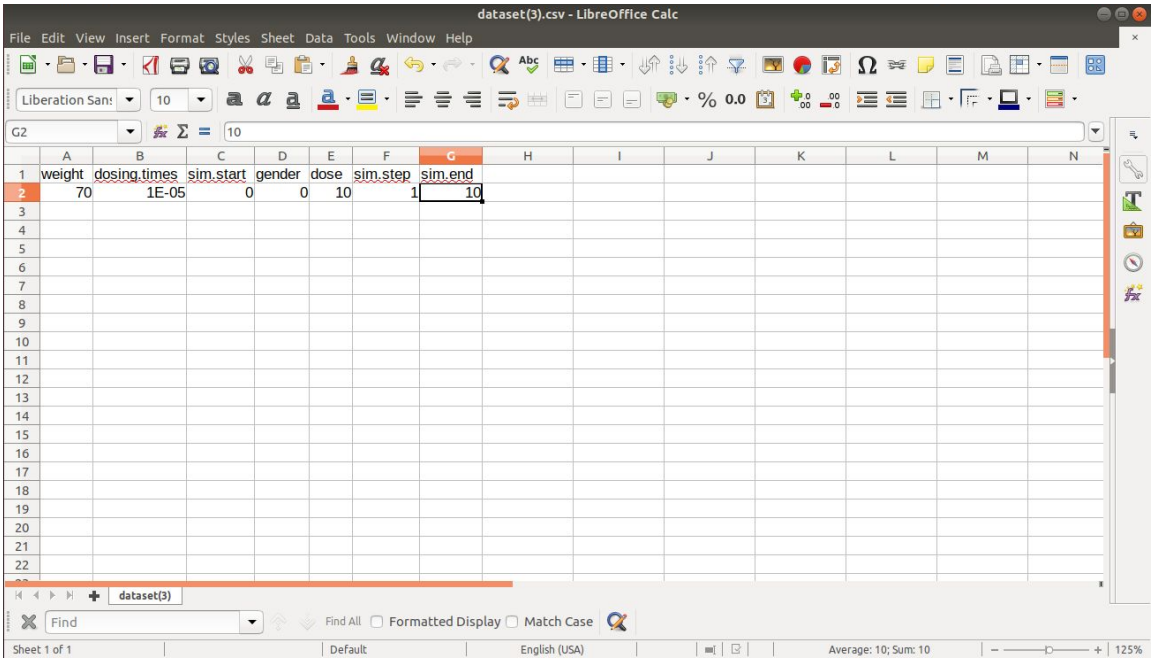


Figure A7. Complete the csv with appropriate values.

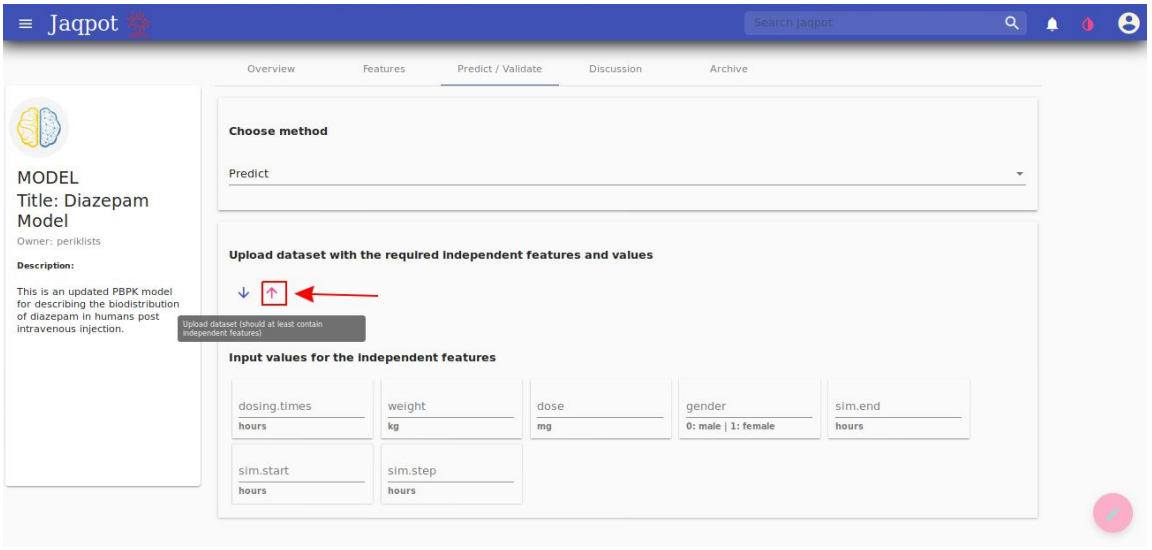


Figure A8. Upload the complete dataset.

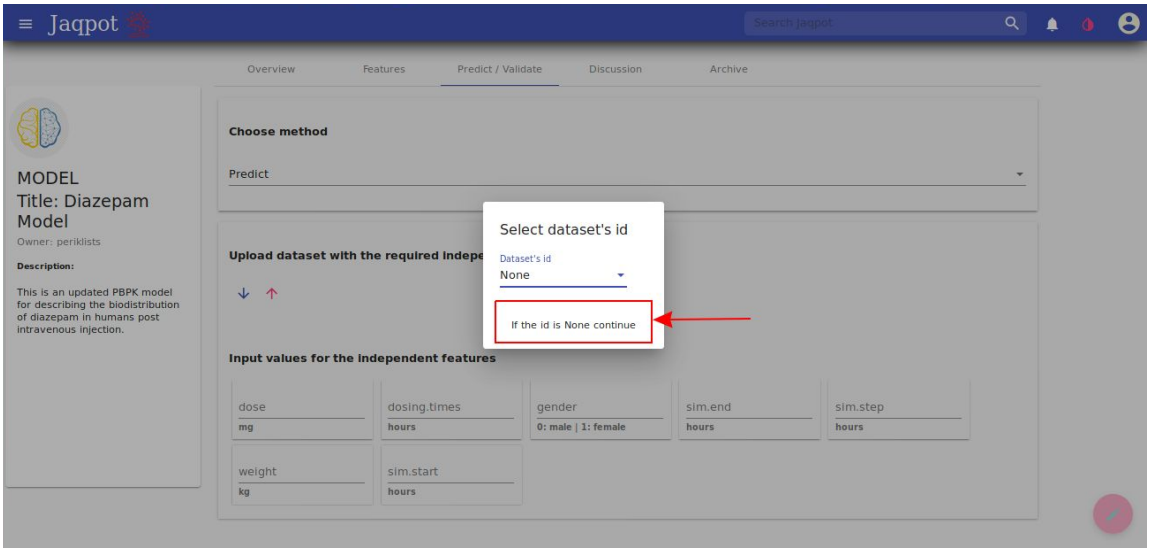


Figure A9. Select ‘None’ in the dataset id and then click on ‘continue’.

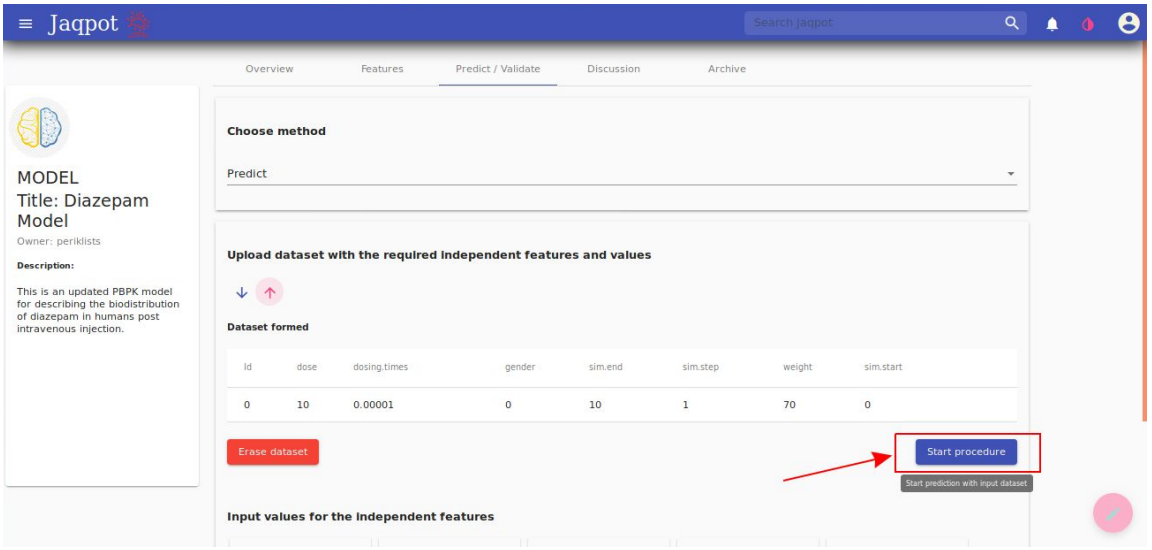


Figure A10. The upload process unlocks the 'start procedure' button.

As it is clear in Figure A10, once the csv is uploaded the user can review the filled values and then press the 'start procedure' button to initiate the prediction, or click on 'Erase dataset' if a mistake is spotted.

It has to be noted that if a model supports vectorized input (e.g. a vector of multiple doses), the user can only provide this kind of input only through the GUI in the following format: [value1, value2, ...] (Figure A11). In this case, the 'Start' button on the bottom right end of the screen appears only after all values have been filled in, so NULL values are not feasible.

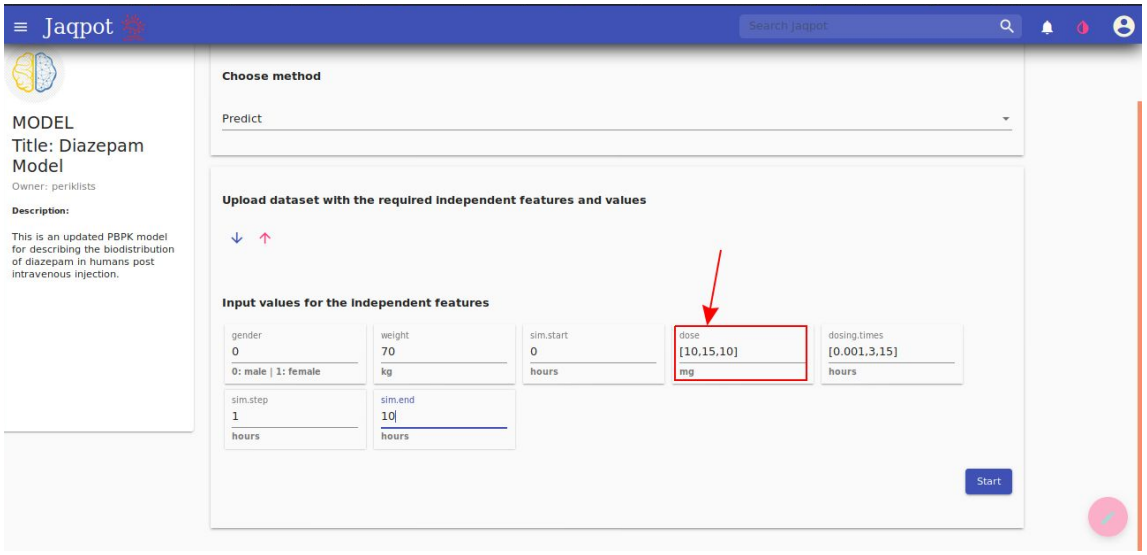


Figure A11. Example of a vector input on the GUI.

When the prediction process is initiated, a small log is generated on the screen and, if no error occurs, the user can proceed to the results by clicking on the double arrow icon on the bottom of the screen (Figure A12).

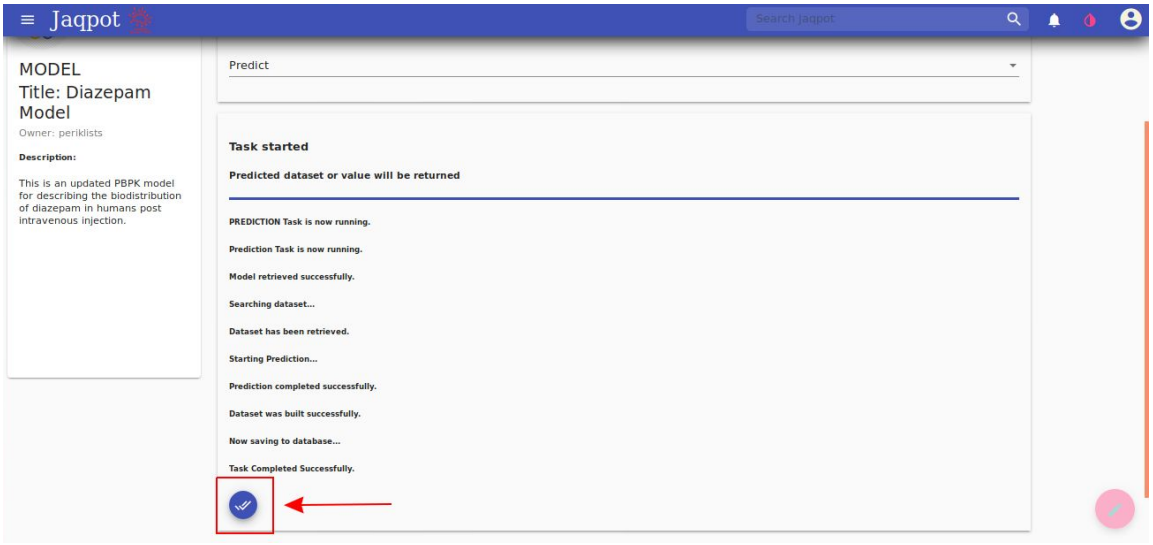


Figure 12. Click on the 'View prediction' icon to obtain the predictions.

The results are given on a tabular format on the GUI and can be downloaded for further processing by clicking on the download button (Figure A13).

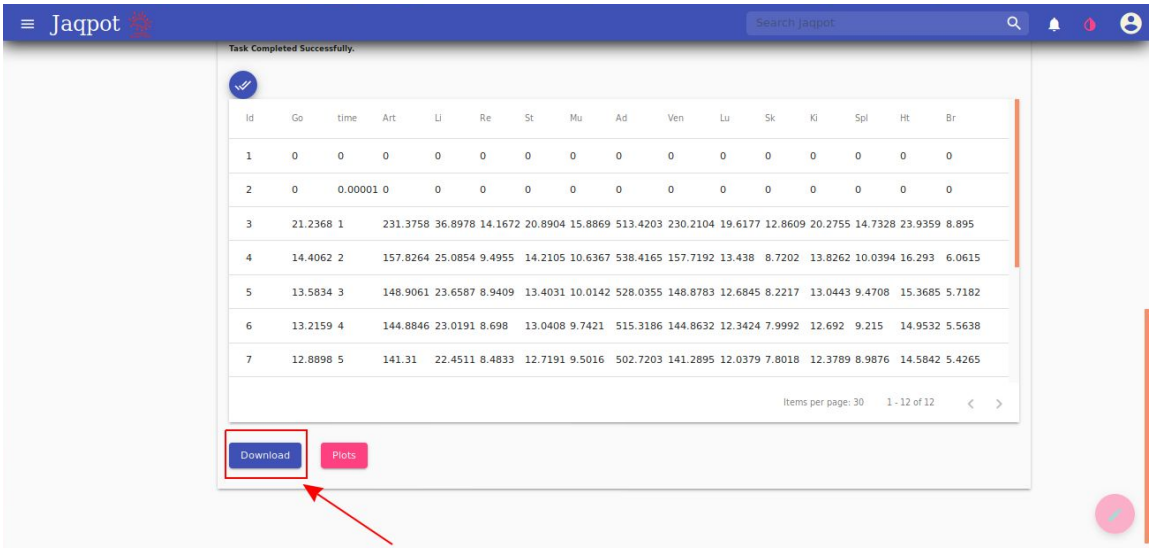


Figure A13. The 'Download' button allows downloading the results in a csv format.

The 'Plots' button which is positioned right next to the 'Download' button allows the user to produce plots by selecting the desired dependent features using the drag-and-drop technique (Figure A14). The desired plot then appears under the predictions (Figure A15).

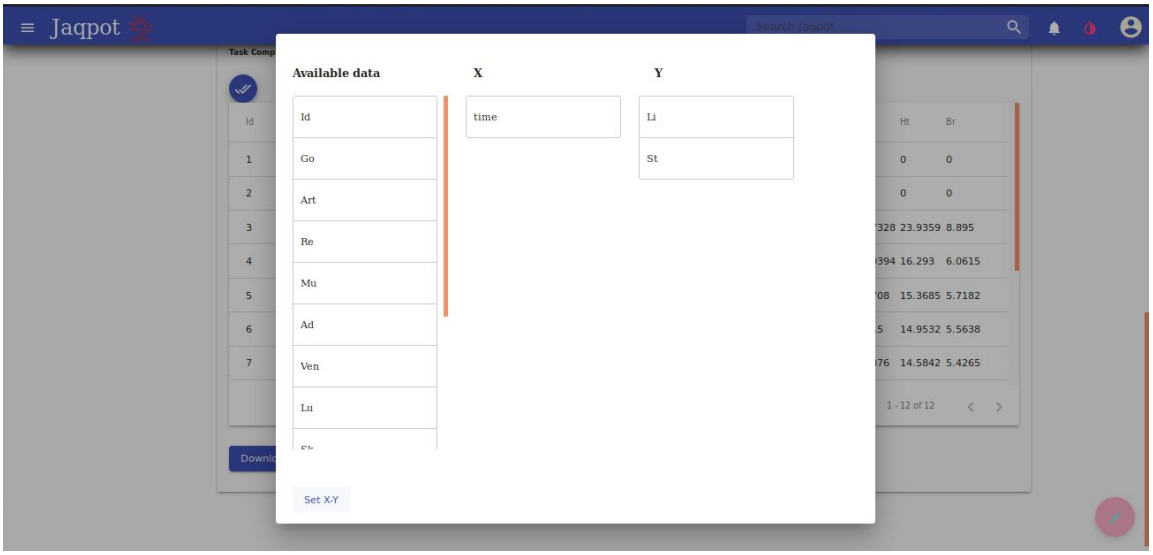


Figure A14. A plot can be generated by dragging and dropping the desired dependent features on the x and y-axis respectively.

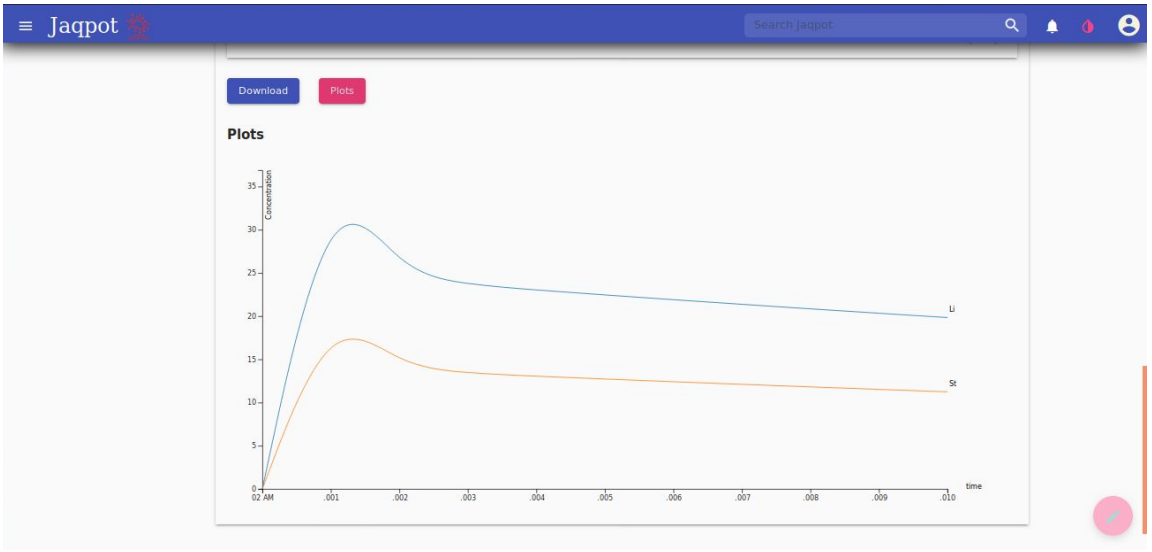


Figure A15. Plot that shows the concentration of diazepam in the liver and stomach compartment.

Finally, the user can add comments and remarks or ask a question regarding the model under the 'Discussion' tab (Figure A16).

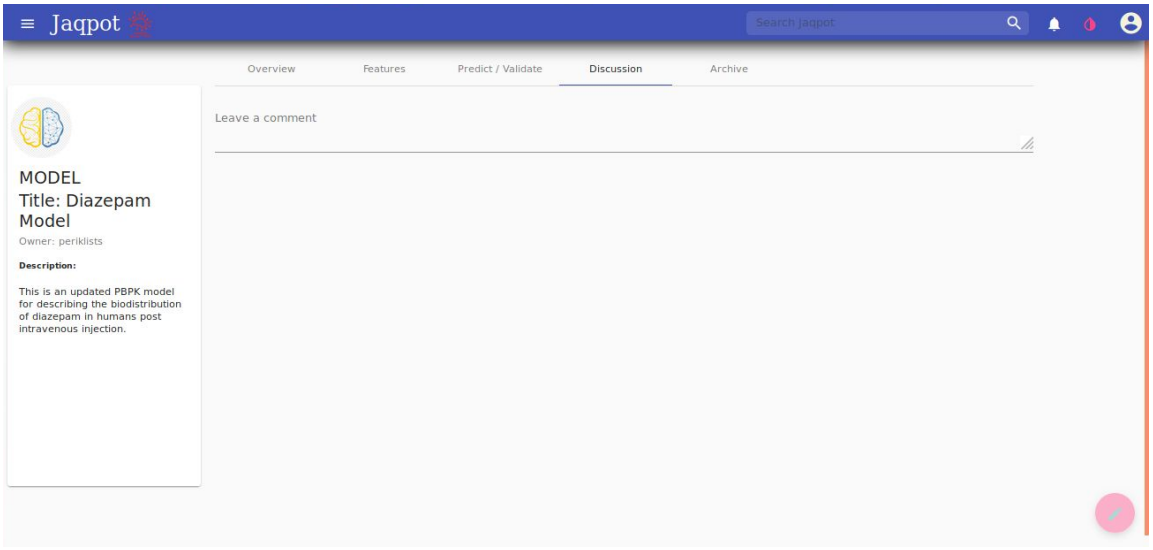


Figure A16. 'Discussion' tab of the Diazepam model.