U.S. Environmental Protection Agency
Office of Research and Development
Center for Computation Toxicology & Exposure
Chemical Characterization & Exposure Division
Computational Exposure and Toxicokinetics Branch

Quality Assurance Project Plan

Title: Pharmacokinetic Parameter Estimation Using R Package "invivoPKfit"

QA Category: ☐ A ☒ B

ORD National Program Project/Task ID: Click here to enter text.

QAPP was Developed: ☒ Intramurally ☐ Extramurally: Click here to enter text.

QAPP Accessibility: QAPPs will be made internally accessible via the ORD QAPP intranet site upon final approval *unless the following statement is selected*.
☐ I do NOT want this QAPP internally shared and accessible on the ORD intranet site.

Project Type(s) (check all that apply):
☐ Environmental Measurements ☐ Environmental Technology ☐ Decision Support Tool ☒ Existing Data ☒ Informatics ☐Geospatial ☐ Method Development ☐ Model Application ☒ Model Development
☒ Software and Data Management ☐Remote Sensing ☐ Technical Assessment
☐ Other

Approvals

Prepared by: John Wambaugh	Digitally signed by JOHN WAMBAUGH Date: 2020.10.23 15:19:31 -04'00'	
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Part A. Project Management
A1. Description of the EPA Quality System

Disclaimer

The EPA does not consider this internal planning document an official Agency dissemination of information under the Agency's Information Quality Guidelines, because it is not being used to formulate or support a regulation or guidance; or to represent a final Agency decision or position. This planning document describes the overall quality assurance approach that will be used during the research study. Mention of trade names or commercial products in this planning document does not constitute endorsement or recommendation for use.

The EPA Quality System

The EPA requires that all data collected for the characterization of environmental processes and conditions are of the appropriate type and quality for their intended use. This is accomplished through an Agency-wide quality system for environmental data. Components of the EPA quality system can be found at http://www.epa.gov/quality. EPA policy is based on the national consensus standard ANSI/ASQ E4-2004 Quality Systems for Environmental Data and Technology Programs: Requirements with Guidance for Use.

This QAPP provides information concerning refinement, evaluation, and support of a suite of models and data for predicting the toxicokinetics of chemicals using *in vitro* data as well as *in vitro-in vivo* extrapolation of high throughput screening data.

A2. Distribution List

Name	Affiliation	Title/Role	Email
Wambaugh, John	CCTE	Technical Lead	wambaugh.john@epa.gov
Noel, James	CCTE	Quality Assurance Manager (QAM)	noel.james@epa.gov
Vacant	CCTE/ORISE	CvTdb Curator and Programmer	
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Setzer, Woodrow	CCTE	Principal Investigator / Statistics	setzer.woodrow@epa.gov
Lau, Christopher	CPHEA	Principal Investigator/ Pharmacokinetics	lau.christopher@epa.gov

A3. Project/Task Organization and Responsibilities

This Quality Assurance Project Plan (QAPP) was developed with reference to Guidance for Quality Assurance Project Plans for Modeling EPA QA/G-5M (U.S. Environmental Protection Agency, 2002).

This QAPP covers a project that supports R package "invivoPKfit". This open source package allows statistical estimation of pharmacokinetic parameters describing chemical absorption, distribution, metabolism, and excretion by the body. The following list provides the project participants along with their respective responsibilities.

John Wambaugh is a principal investigator in the CCTE. He is responsible for designing the structure of the invitroPKfit package; overseeing the development of new code and writing technical manuscripts.

James Noel is the Quality Assurance Manager (QAM) in the CCTE and will receive copies of all QA and QC related information. They will review the QAPP and any pertinent QA related documents to ensure the quality of the research is upheld. The QAM will perform, at their or the technical lead's discretion, an audit of the process.

Caroline Ring is a principal investigator in the CCTE. Caroline helped create the R package invivoPKfit and provides secondary examination of the proper function of the parameter estimation.

EPA is currently recruiting an ORISE masters-level trainee who will help coordinate the toxicokinetic Concentration vs. Time database (CvTdb). The individual will apply invivoPKfit to the data in the CvTdb to obtain estimates of key parameters such as volume of distribution and half-life.

Woodrow Setzer is an emeritus scientist in CCTE and an expert in statistical methods for parameter estimation.

Christopher Lau is a principal investigator in the Center for Public Health and Environmental Assessment (CPHEA) and leads an *in vivo* laboratory for pharmacokinetic studies.

A4. Problem Background

The study of toxicokinetics (TK) and/or pharmacokinetics (PK) allows understanding of the body's absorption, distribution, metabolism, and excretion of

chemicals or pharmaceuticals, respectively. TK is a critical component in understanding toxicological responses by linking chemical exposure to internal tissue concentrations. TK/PK studies typical consist of controlled dosing of live subjects to a chemical, followed by serial measurement of chemical concentration in different matrices (for example, serum or urine). Sacrificial animal studies may kill, dissect, and analyze specific tissues at sequential times. These *concentration time-course data* are referred to variously as "concentration vs. time" or "Cvt" data. TK/PK involves mathematically describing TK/PK data such that extrapolation is possible.

Scientific studies of TK/PK typically include both the acquisition of new in vivo data and the statistical analysis of that data. Here we are focused on only the former. The goal of the project is to develop standardized TK/PK statistical analysis methods to estimate key parameters such as the chemical elimination half-life from the body. To accomplish this, Caroline Ring, John Wambaugh, and Woody Setzer developed a generic statistical approach (Wambaugh et al., 2018) to estimate relevant TK parameters that is designed to be applicable to a wide-range of data, including the data contained in the EPA's Cvt database (Sayre, Wambaugh, & Grulke, 2019). This tool should reduce the variability in TK/PK study results (Wetmore et al., 2015; Wetmore et al., 2012) by making the statistical methods standard, though experimental issues remain. The methods have been described in the open source software package "invivoPKfit" for the open source R statistical language.

Broadly, there are three types of TK/PK analysis. The first, "noncompartmental" analysis refers to graphically describing the slope and area of the concentration time-course data. A pre-existing R package "PK" (Jaki & Wolfsegger, 2011) was used to accomplish these estimates. The second approach "empirical compartmental" analysis (O'Flaherty, 1981) is the primary focus of invivoPKfit and involves describing the body as being composed of one or more homogenous (that is, well-mixed) compartments that could correspond, for example, to the plasma or deeper tissues but are not considered to have direct physiological analogs. Compartmental models allow predictions of key tissues under potentially novel exposure scenarios but cannot be used to extrapolate across species. Finally, a third type of compartmental modeling focuses on "physiologically-based toxicokinetics (PBTK)" (Reddy, Yang, Andersen, & Clewell III, 2005) in which the compartments and flows between them all have direct physiologic analogs. PBTK can be used to extrapolate between species by varying physiology. The application of invivoPKfit to PBTK models is a longer-range goal.

invivoPKfit provides a statistically rigorous, reproducible, and open-source methodology for obtaining key PK/TK information to inform chemical risk assessments. The package is not static, it is updated in conjunction with ORD research projects which it supports and new versions are cleared as supplemental

information when those projects are completed. The most recent version is available at:

https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit

A5. Task Description and Approximate Schedule

The objective of this project is to further develop, refine, and evaluate PK parameter estimation methods and the R package "invivoPKfit" in support of the Chemical Safety for Sustainability (CSS) program's Rapid Exposure and Dosimetry (RED) project. Contributing products are listed below:

Product 1

Estimation of empirical compartment model PK parameters for all chemicals in the EPA's Cvt Database. (**FY19**)

Product 2

Estimation of empirical compartment model parameters for perfluorobutane sulfonate (PFBS). (**FY20**)

Product 3

Classification of all chemicals in the EPA's Cvt Database with respect to restrictive- or non-restrictive metabolic clearance using information theoretic measures of parsimony. (**FY21)**

Product 4

Estimation of tissue partition coefficients for a generic PBTK model for all chemicals in the EPA's Cvt Database. (**FY22**)

Product 5

Enhance vetting by the scientific community with release of invivoPKfit through R's public software repository CRAN (the Comprehensive R Archive Network, https://cran.r-project.org). (**FY23**)

Product 6

Publish peer-reviewed scientific article describing the use of the invivoPKfit package. (**FY24**)

A6. Quality Objectives and Criteria for Model Inputs/Outputs

All information related to a project will be stored in a directory named:

L:\Lab\NCCT_ExpoCast\ExpoCast[YEAR]\invivoPKfit

The data will be stored in standard machine-readable format (typically a comma separated value, CSV, file) that is easily read by different programming languages and can be easily disseminated to others via required EPA systems (for example, ScienceHub). All new methods will ultimately be distributed as a new version of the invivoPKfit R package.

https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit

A7. Specialized Training Requirements/Certification

Specialized expertise and qualifications needed to participate in this project include proficiency with statistical methods of parameter estimation and scientific computing in modern computer programming languages. Team members must be knowledgeable in at least one of the following fields: biomedical engineering, mathematical physiology, applied mathematics, applied statistics, physics, mathematics, chemistry, chemical engineering, or computer science. Team members should be fluent programmer in a modern programming language (for example, R, Python, Julia). This research project involves data science, mathematical modeling, toxicokinetics, and analysis.

Team, members must become comfortable with the R programming language, toxicokinetic (TK) modeling, and statistics. Team members need to be comfortable with the use of version control software (that is, Git).

For trainees working on the project, a basic knowledge of programming, mathematics, and statistics will be required. Additional training will be provided, if needed. Reference to this training will be documented in an electronic lab notebook that is easily accessible to all with the initials of the trainer and trainee and date of completion. If expert workshops or meetings are required to advance the working knowledge of individuals involved in the projects, the name of these workshops, the attendees, and their dates of attendance will also be placed into the electronic lab notebook. If certificates were issued for these workshops, they will be placed into a separate binder containing relevant lab documents, or, if

digital, will be placed into a folder labeled "Certificates of Training" that will be located on the Principal Investigator's computer desktop.

A8. Documentation and Records

When a new individual becomes involved in the project, this QAPP will be sent to her or him by John Wambaugh. The individual will be encouraged to ask questions should any part of this document be unclear, and upon reading their name(s) and dates of completion will be entered into an open lab notebook or electronic record of training. Each project contributor should be given access to L:/Lab/NCCT_ExpoCast. Each project contributor should be given a Confluence and Bitbucket account with access to the "invivoPKfit" git repository.

Part B. Assessment and Oversight

B1. Assessment and Response Actions

At the discretion of the researchers as well as the QAM a mid-project technical system audit may be requested by the project QAM to assess the quality assurance process of the project. Any findings, research best practices, or improvements will be compiled in a report and filed with other QA documents. Any corrective actions and responses will be documented and filed with the Assessment Report. These findings and corrective measures will be in the office of the Principal Investigator.

B2. Reports to Management

The director and the QAM will be apprised of progress on a semi-annual basis and at the end of the study, or as and when the data will be presented during work-in-progress meetings or at scientific meetings. The types of progress reports may include brief (i.e., 1-5 page) progress reports, presentations, scientific posters, meeting abstracts, and/or manuscript drafts. Dr. Wambaugh will be responsible for preparing and distributing the reports.

Part C. Estimation Method Development

C1. Parameter Estimation Methodology

For compartmental models the parameters are estimated by maximizing a likelihood function that assumes that the data are log-normally distributed around the logarithm-transformed concentrations predicted by the TK model (Gaddum, 1945). Each data source and chemical is assigned its own standard deviation (σ)

for the measurement error, such that a chemical with data from two sources would have two separate standard deviations estimated (Altman & Bland, 1983). Optimization proceeds for each data source separately and as well as for all data sources together.

The R package optimx (https://CRAN.R-project.org/package=optimx, (Nash, 2014; Nash & Varadhan, 2011)) is used to perform a bounded optimization of the likelihood function using the method "L-BFGS-B" that implements the bounded (i.e., limits on lower and upper values) approach (Byrd, Lu, Nocedal, & Zhu, 1995).

All observations within a factor of two of the limit of quantitation are treated as "censored" (Cox & Hinkley, 1979): Any prediction below twice the limit of quantitation added the cumulative distribution from zero to the limit to the likelihood. If the limit of quantitation is unknown, it is assumed to be 45% of the lowest observed value.

Confidence intervals on the estimated parameters are calculated using the Hessian of the likelihood function to estimate standard deviations of the log-scale parameter values (Bartlett, 1953a, 1953b). Standard deviations *sd* around the optimized parameters are reported on the log scale – a 95% confidence interval may be calculated as $\left[e^{\ln x - 1.97*sd}, e^{\ln x + 1.97*sd}\right]$.

Compartmental model analysis (O'Flaherty, 1981; Riviere, 2011) allows data from both *intravenous* (*iv*) and oral exposures to be jointly analyzed, when data from both routes was available. As in Figure 1, for orally dosed animals the dose was modeled as first entering the gut, from which a fraction F_{bio} of the total dose was being absorbed with rate k_{gutabs} for the orally dosed animals, while the iv animals were modeled with the total dose appearing in the central compartment at time zero. If no oral dosing data were available, only quantities that can be estimated from *iv* dosing were estimated (for example, Vd, k_{elim}) while absorption rate and bioavailability were not estimated.

For non-compartmental analysis (Riviere, 2011) all concentration data was normalized by dose. A "batch" analysis was conducted allowing for different studies with the same route of exposure to be analyzed jointly, however the oral and *iv* routes still had to be analyzed separately. Vd and k_{elim} (see Figure 1) can be determined from the *iv* data, while F_{bio} was determined as the ratio of the oral time-integrated area under the plasma concentration curve (AUC) to the *iv* AUC.

C2. PK/TK Models

Any PK/TK models used should be fully described, implemented within invivoPKfit or compatible packaged (for example, "httk"), and evaluated. The empirical one- and two-compartment models (Figure 1) are currently implemented within

invivoPKfit. For new models, the system of equations should be provided in written form, along with tables defining all parameter meanings, values, and units. Additionally, a model diagram should be provided to schematically represent the system.

Models may be implemented in any computer coding language that is compatible with the rest of the invivoPKfit functionality. Most likely this will be some combination of R, C, and MCsim, but as long as it is documented, reproducible, and works the language does not matter.

Many systems of equations permit analytic solutions. If these are available (as with the one- and two-compartment models) these should be used since numerical solution of systems of equations is time-intensive. Using analytic solutions greatly improves the speed of parameter estimation.

For optimization of the likelihood function it is often advantageous to re-parameterize the system. For instance, if there are two rates k_1 and k_2, it may be much faster to estimate k_1 and R_{k2tok1} and then calculate $k_2=R_{k2tok1}* k_1$. If R_{k2tok1} is constrained so that it is always greater than 1, then k_2 will always be faster than k_1. Any transformations should be hard coded. Quadrature (see below) will be needed to propagate error through re-parameterized parameters.

All models should be evaluated. Measured data should be regressed upon predictions and relevant statistics (RMSE, R2, etc) should be calculated and recorded as part of the model documentation process.

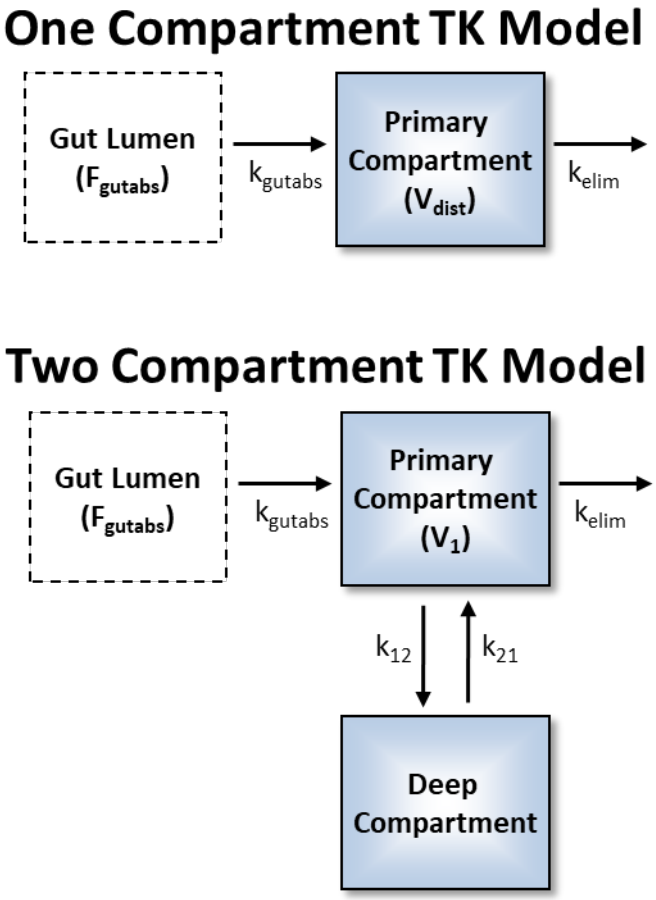

Figure 1: Both the empirical 1- and 2-compartment models are available with invivoPKfit.

C3. Model Selection

"I remember my old friend Johnny von Neumann used to say, "With four parameters I can fit an elephant, and with five I can make him wiggle his trunk."
Enrico Fermi (Dyson, 2004)

Model parsimony is an important consideration for regulatory applications (Chiu et al., 2007). The aim is to achieve a balance between describing the data well and using as simple a model as needed. Information criteria provide a means for comparing models. invivoPKfit calculates the Akaike Information Criterion (AIC) (Akaike, 1974). The magnitude of the AIC's does not matter, only the difference in values ΔAIC. For two models the AICs are only comparable if the exact same data are used. In that case, the model with the smaller AIC is more likely, roughly according to $e^{\Delta AIC}$ (Burnham & Anderson, 2003).

Whenever model selection is used, code should be built into the invivoPKfit package to calculate the information criterion that is used. A script should be created and stored that calculates the value of the criterion for each model examined and computes the relative likelihood of the selected model.

C4. Error Propagation with Quadrature

The standard deviation for parameters that are composites of multiple estimated parameters (for example, V_d) should be propagated with quadrature. From (Harrison, 2011):

If you have two parameters X and Y with errors ΔX and ΔY then for functions of those parameters, $Z = F(X,Y)$, the error is given by

$$\Delta Z^2 = \left(\frac{\partial F(X,Y)}{\partial X}\Delta X\right)^2 + \left(\frac{\partial F(X,Y)}{\partial Y}\Delta Y\right)^2.$$

Sometimes this is trivial (adding, multiplication, division), but sometimes properly propagating the error is tricky. Any function used to propagate error should be hard coded into the invivoPKfit package for reproducibility.

C5. Statistical Significance Testing

DO NOT MAKE COMPARISONS OF STATISTICAL SIGNIFICANCE SIMPLY BASED UPON THE OVERLAP OF THE CONFIDENCE INTERVALS. Instead, use the mean and standard deviation (hence the need for proper error propagation) to compare two populations using Welch's modified two-sample *t*-test (Cressie & Whitford, 1986). This can be easily done with R package BSDA (https://CRAN.R-project.org/package=BSDA, (Kitchens, 2002)).

The method used should be documented and a script should be stored allowing reproduction of any significance testing.

C6. Random Number Seeds

If any software used either internally or in running parameter estimation makes use of artificial random number sequence generation, it is incredibly important to use the command "set.seed(SEED)" within any associated script. The value of SEED is immaterial but must be properly recorded to allow reproduction of

"random" sequences. This should be used for all manuscript figures, analyses, and tests.

C7. Review, Verification, and Validation

Built-in plotting functions allow inspection of the predicted concentration vs. time curves based upon the estimated parameters. This provides the user with a check that check that invivoPKfit is fitting HTTK models to *in vivo* PK data sets correctly.

Graphical comparison between the available data and the model predictions should be presented in two ways: First, a concentration vs. time- prediction should be given, as in Figure 2. Concentration vs. time plots provide a qualitative evaluation of the model predictions in a way that makes the kinetics of the model clear. Each experimental condition (both with data and for extrapolation) should be replicated so that it may quickly be discerned how the model behaves over time and how similar are the conditions to which the model has been extrapolated. Second, an observed vs. predicted plot should be provided as in Figure 3, typically with the results for a linear regression of the observed on the predicted values along with summary statistics such as RMSE, R^2, etc. This second type of plot allows quantitative evaluation of the model predictions. The dotted line in Figure 3 indicates the "identity" line along which perfect predictions will lie. In an observed vs. predicted plot, uncertainty may be directly characterized along with biases, and overall ability to explain the data. For example, in Figure 3, the data for kidney are slightly underpredicted. Both Figure 2 and Figure 3 allow assure that the experimental data sets have been converted to the same units and that the model makes predictions in those units.

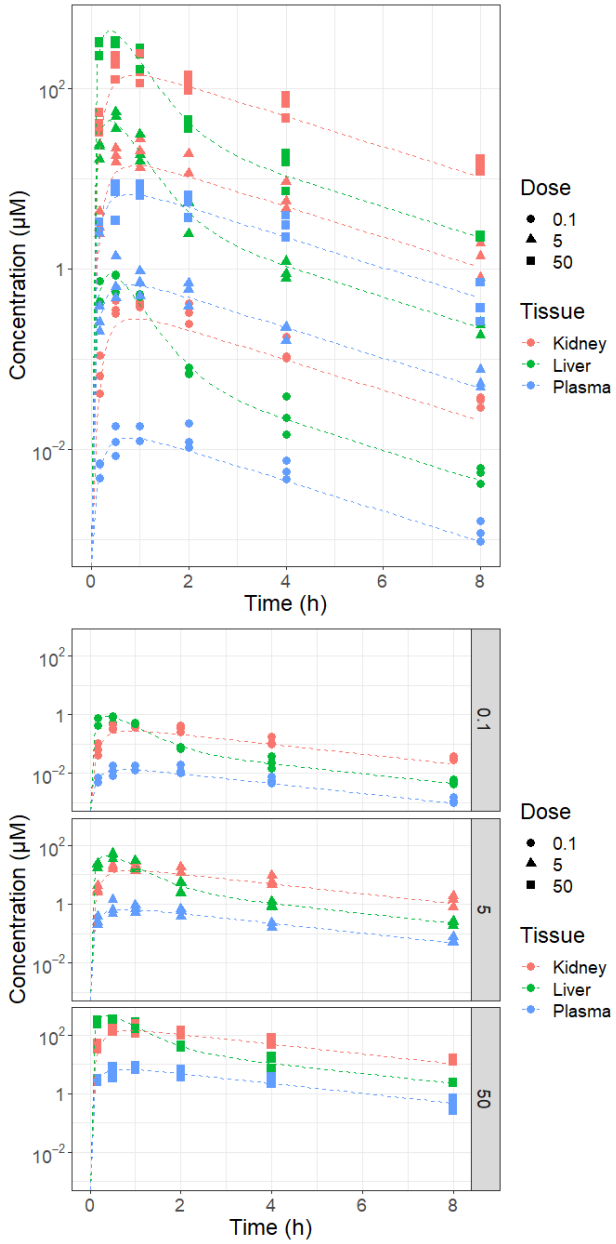

Figure 2: Graphs of simulated concentration vs. time data (Figure from Tan et al. (2020)).

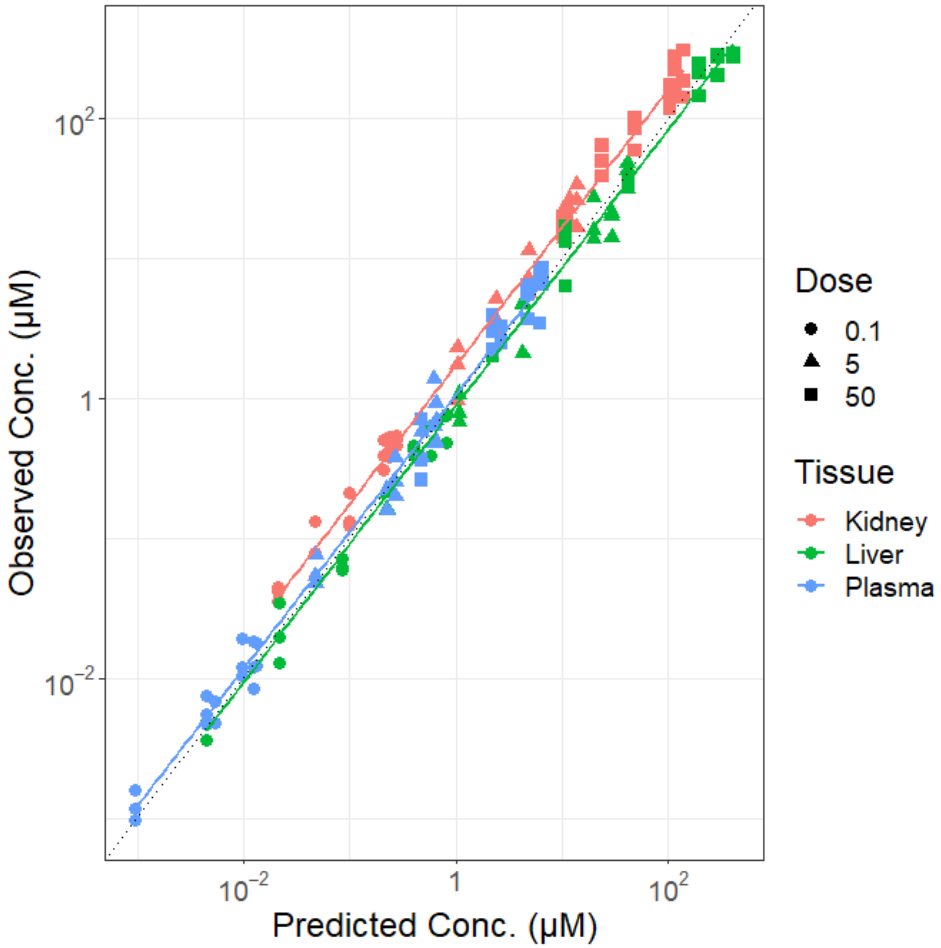

Figure 3: Observation vs. prediction plot for quantitative analysis of predictive ability (Figure from Tan et al. (2020)).

Methods are considered to be fully reviewed, verified, and validated upon EPA clearance of a peer-reviewed manuscript in which the methods are included (potentially as supplemental material). Along with the manuscript, a new version of the R package "invivoPKfit" (https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit) will be publicly released. The package will also be archived with EPA's Science Inventory.

Code checking is partially accomplished through the peer-reviewed publication process, but is also assured through the use of the "tests" directory in the R package (see https://r-pkgs.org/tests.html). Briefly, scripts using the package code are included along with saved output corresponding to correct operation. When the package is "built" for distribution, each script is run and its new output is compared to the saved output. Discrepancies are flagged, allowing problems to be identified.

Part D. R Package Maintenance

D1. Method Coding

R packages are assigned three version numbers: [MAJOR].[MINOR].[PATCH]. While public releases of invivoPKfit are numbered sequentially (for example, 1.1.0, 1.2.0, 1.3.0, …) within ORD there may be many different versions. Different versions of the package are managed collaboratively using the "git" version control software as implemented with Bitbucket. Internal versions should always have major version number 99. Each project should have a unique minor number. Versions will be renumbered with the next sequential public number when it is cleared for release.

Internal packages can be built and shared within ORD and with Material Transfer Agreement partners. Use "R CMD build [PATH]" to get a distributable version.

All functions and data sets should be documents using roxygen2 (https://CRAN.R-project.org/package=roxygen2). Data files are documented in data.R file in /R directory. Need to add a \describe{ \item{colname}{}} with an item for each column. If all code is properly documented using roxygen2, then documentation files can be created by going into the "invivoPKfit" package directory (setwd) within R and running "roxygenize()". Documentation of parameter descriptions can be reused with @inherit, @inheritParams, and @inheritSections.

Programmers are encouraged to include the author tag in the code they create and to add themselves to the authors list if modifying pre-existing code. CRAN allows the recognition of different levels of contribution – the two most likely to be relevant are "aut" – "Full authors who have made substantial contributions to the package and should show up in the package citation" – and "ctb" – "Authors who have made smaller contributions (such as code patches etc.) but should not show up in the package citation". If you have created a new model you are a full author.

D2. Using the R "tests" Directory Functionality

"Testing is a vital part of package development. It ensures that your code does what you want it to do" (http://r-pkgs.had.co.nz/tests.html) R includes automated testing functionality that runs whenever you build ("R CMD build") a package. Test scripts are stored in the "invivoPKfit/tests" directory along with saved output.

Within the "invivoPKfit/tests" folder:

1) The code in each ".R" file should run

2) The outputs in the ".Rout.save" files should match (unless you changed something that should impact it)

To create a new ".Rout.save" file use "R CMD BATCH - myTest.R myTest.Rout.save" from the command prompt.

If you calculate a RMSE you can always use a statement like "mymodel.RMSE <= X" so that if the RMSE improves no warning is thrown.

When you add a new feature, add something to **/tests** *to test it*

D3. Uploading to GitHub

New versions of the package are cleared as supplemental material for peer-reviewed scientific manuscripts. Once cleared, the package is made public by uploading to GitHub. GitHub is a public source for exchanging computer programming code.

https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit

Part E. Manuscript Documentation

Each manuscript should have an archival directory named:

L:/Lab/NCCT_ExpoCast/ExpoCast[YEAR]/[PROJECTNAME]

A final working data processing script should be stored along with the raw data on the L:/ drive in a directory called:

L:/Lab/NCCT_ExpoCast/ExpoCast[YEAR]/[PROJECTNAME]/rawdata/

This data processing code should take raw data and convert it to the form used by the paper to make figures. A final working script to make all figures associated with the paper and any necessary processed data should be stored in

L:/Lab/NCCT_ExpoCast/ExpoCast[YEAR]/[PROJECTNAME]/figures/

Multiple copies of manuscript milestones should be stored:

Milestone	Sub-Directory
Initial drafts, figures, scripts, and other material	EarlyDrafts
Version submitted to tech review and edits	TechReview

Version submitted to clearance review and edits	ClearanceReview
Version submitted to journal	Submission
Any revisions submitted to journal and edits	Revision[#]
Any submissions to new journal	Submission[#]

Part F. References

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