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<b>14. ABSTRACT</b> The objective of the project is to develop a software system to process general pharmacologic, toxicological, or other biomedical research data that exhibit a non-monotonic dose-response relationship - for which the current parametric models fail. The software will analyze dose-response relationships using both monotonic and non-monotonic data. This will have significant effects on the prevention and treatment of injuries and diseases of soldiers and civilians. Military agencies, government agencies, and private sponsors will be benefited from new and improved approaches to analyze the general pharmacologic, toxicological, or other biomedical research data that exhibit a non-monotonic dose-response relationship for which the existing parametric models fail. We understand that the difference between monotonic and non-monotonic doses and responses can impact patient response and recovery. If existing and proposed methods for the study of the relationship between dose and response can be effectively implemented and compared, it will enable us to uncover important dose-response features (e.g., drug resistance or virus mutation) in response to viral infection or treatment evaluation based on experimental/clinical trial data.					
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## **1. Introduction**

The drug dose–response relationship describes the change in effect on an organism caused by a given amount of an agent or differing levels of exposure. It is critical for the selection of doses and dosing regimen which is often the bottleneck and failure point for the model-informed drug discovery and development. Model-based dose determination and optimization frequently rely on the well-characterized and accurate dose-response curves. The objective of this research project is to continue development of a software using innovative approach and new models to analyze dose-response data and accurately describe the dose-response relationship at any amount of an agent or level of exposure. In Phase I, we have developed a prototype for proof of concept to demonstrate the feasibility of developing a software based on the innovative approach and new models. The new approach and new models are effective, responsive, efficient, and successfully detect monotonic and non-monotonic dose-response relationship using the collected test data. Phase II will pursue the completion of the development and produce a Commercial Off-The-Shelf (COTS) software product.

## **2. Keywords**

- Pharmacokinetic/pharmacodynamics (PK/PD) modeling and simulation
- Dose-response relationship
- Non-monotonic dose-response
- Non-parametric
- IC50
- ED50
- LD50
- Four parameters logistic model and Five (5) parameters logistic model

## **3. Accomplishments**

The Phase I project is very successful. We have accomplished all tasks proposed in Phase I on time within budget. During Phase I, we have performed all necessary researches and collected sufficient data to validate our approach and successfully conduct proof-of-concept to analyze dose-response data to detect monotonic and non-monotonic dose-response relationship. We have designed the algorithms and initiated the simulation studies to test non-monotonic relationship. We have successfully developed PKPDBiois software prototype and the web portal to demonstrate the software features and services we will offer. The Phase I project has technically validated and approved our concept of PKPDBios software, and has setup a solid foundation for the success of Phase II.

### **3.1 What were the major goals of the project?**

The objective of the project is to develop a software system to process general pharmacologic, toxicological, or other biomedical research data that exhibit a non-monotonic dose-response relationship - for which the current parametric models fail. Our software will analyze dose-response relationships using both monotonic and non-monotonic data. This will have significant effects on the prevention and treatment of injuries and diseases of soldiers and civilians. Military agencies, government agencies, and private sponsors will be benefited from new and improved approaches to analyze the general pharmacologic, toxicological, or other biomedical research data that exhibit a non-monotonic dose-response relationship for which the existing parametric models fail. We understand that the difference between monotonic and non-monotonic doses and responses can impact patient response and recovery. If existing and proposed methods for the study of the relationship between dose and response can be effectively implemented and compared, it will enable us to uncover important dose-response features (e.g., drug resistance or virus mutation) in response to viral infection or treatment evaluation based on experimental/clinical trial data.

### 3.2 What was accomplished under these goals?

We have accomplished all tasks proposed in Phase I successfully. The followings list the specific tasks completed in Phase I.

#### 3.2.1 PKPDBios Prototype and Web Portal Development

We have developed PKPDBios software prototype and the web portal <http://www.gridsite1.com> to demonstrate features and services to be offered. Users are able to load input dose-response data file to the prototype. Data are processed for verification and validation. The verified and validated dataset can be saved to database. The basic descriptive statistics (mean, count, deviation, distribution, range, outlier, etc.) of the dose-response dataset can be displayed and reviewed.

#### 3.2.2 Designing Algorithm for Testing Non/monotonic Curve

The algorithm for testing non/monotonic curve is designed as follow. Let  $0 \leq r \leq s - 2 \leq n - 2$  be integers and  $a, b$  be constants. For each pair of  $(r, s)$ , define the estimators of  $a$  and  $b$  by  $\hat{a} = \hat{a}(r, s)$  and  $\hat{b} = \hat{b}(r, s)$ , as the arguments of the following objective function:

$$\operatorname{argmin}_{(a,b)} \sum_{i=r+1}^s \{Y_i - (a + bx_i)\}^2.$$

- Let  $T_m = \max\{-\hat{b}(r, s)Q(r, s) : 0 \leq r \leq s - m \leq n - m\}$ , where  $m$  satisfies  $2 \leq m \leq n$
- A large  $T_m$  indicates that the null hypothesis,  $g(x)$  being monotonic, should be rejected.
- To obtain the  $t_m$  on the basis of  $T_m$ , data are fitted with the nonparametric model  $Y = g(x) + \varepsilon$

- Obtain an estimator of  $\varepsilon$  by a consistent estimator of  $g(x)$ , such as the local linear estimator
- Assuming that a constant function is the most difficult nondecreasing form to be tested,  $Y = \varepsilon$  is used to obtain the  $t_m$
- Specifically, using the estimated  $\hat{\varepsilon}$ , resample and obtain a new dataset  $Y = \hat{\varepsilon}$ , by which to obtain  $t_m$
- Repeatedly sampling  $B$  times resulted in a set  $t_m$  of size  $B$ . By taking the  $100(1 - \alpha)$ th ordered  $t_m$ s as the critical value for  $T_m$
- When  $T_m$  obtained from the real data is greater than this  $t_m$ , we claim that the function is non-monotonic at the  $(1 - \alpha)$  level

### 3.2.3 Comprehensive Literature Review

In the literature, various qualitative methodologies have been applied by coupling the statistical strength and the biological plausibility. After intensively reviewing several scientific reports, review articles and over 300 related articles, we have identified about 250 publications related to NMDRs (Non Monotonic Drug Response) in the PubMed database. Of these publications, more than 60 experimental studies described one or more NMDR profiles. Out of these studies, some were epidemiological studies, some *in vitro* studies, and some *in vivo* studies. From these studies, more than 160 dose-effect relationships were claimed by the authors as NMDR. These NMDR profiles were reported for both animal sexes, for various physiological and behavioral effects, and for several organs or systems. Affected organs and systems included the central nervous system, hypothalamic-pituitary axis, mammary glands, adrenal glands, the cardiovascular system, and the male and female reproductive systems. Furthermore, in a scientific report of 290 pages for substances in the area of food safety, the authors critically reviewed the scientific peer-reviewed literature from 2002 to 2016, and identified 202 *in vivo* datasets (from 49 studies), 311 *in vitro* datasets (from 91 studies) and 9 epidemiological/human datasets (from 2 studies) related to NMDR. For 49 % of datasets, a significant better fit was shown with a nonmonotone model than with a monotone model. Of the datasets, 18 % had at least two dose groups underlying the NMDR shape, and the dose-response curves of 39% datasets consisted of at least two directions. Unfortunately, most of the publications did not explicitly state which statistical criteria were used for deciding that a given DR dataset shows evidence for an NMDR. It appeared that most of the authors used a combination of visual inspection and statistical significance testing among dose groups. In [References](#) we list the literature we have comprehensively reviewed.

### 3.2.4 Existing Software Review

We have identified the following software/packages which can fit monotonic dose-response curves: [BIOASSAY](#), [BMDS](#), [Dr-fit](#), [GraphPad](#), [PROAST](#), [Prism](#), [Sigmaplot](#), [TraceDrawer](#), [UNISTAT](#), R packages: [DoseFinding](#), [drfit](#), [Grofit](#), [MCPmod](#), [drc](#) [dr4pl](#). Di Veroli et al. (2015) proposed a modified model (called multiphasic Hill model) and automated fitting procedure to describe dose-

response curves with multiphasic features. The resulting model was declared to enable interpreting each phase of the dose-response as an independent dose-dependent process. However, their strategy and fitting procedure have their own limitations or even deficiencies as well. First, their strategy can generally not handle non-monotonic case we face. Second, with the appearance of multiphasic paths, how many phases should be chosen? Third, given the number of multi-phases, the convergence problem of the algorithm/fitting is of great concern since the model may not fit the observations well.

### 3.2.5 Designing Simulation Studies

We have designed simulation studies to check the performance of the algorithm for testing non/monotonic curve as described in section a). Specifically, we have considered situations under which the relationship between the dosage level and the response is monotone (i.e., the null hypothesis is true) and the relationship is non-monotone (i.e., the alternative hypothesis is true). Under the null hypothesis, the dose-response curve satisfies

$$f_1(x) = 1 - \frac{1}{\left\{1 + \left(\frac{x}{IC_{50}}\right)^d\right\}} \text{ for } x \in (0,2),$$

Where  $IC_{50} = 0.5$  and  $d=0.2, 0.5, 1, \text{ or } 2$ . Under the alternative hypothesis, the dose-response curve satisfies

$$f_2(x) = 1 - \frac{1}{\left\{1 + \left(\frac{x}{IC_{50}}\right)^d\right\}} - \exp\{-250(x - 0.75)^2\} \text{ for } x \in (0,2).$$

The residual error  $\varepsilon$  follows a normal distribution  $N(0, \sigma^2)$  with  $\sigma = 0.1$  or  $0.5$ . We have also considered different sample sizes varying from 20 to 100. For each simulation setting, we generate 1000 replicates.

### 3.2.6 Collecting Real Datasets in Dose Response Curve Studies

We have made all efforts to collect datasets of real dose-response studies from various resources such as from R repository, NIH/ National Institute of Environmental Health Sciences database, and Cancer Cell Line Encyclopedia. So far we have collected around 110 datasets, for which we have done a deep investigation. Viewing their scatterplots, we can easily observe very diverse patterns. Some patterns are evidently non-monotonic. We have further fit these datasets by using the four-parameter logistic (FPL) models, which are the most popularly, flexibly and frequently used in fitting monotonic dose-response curves. We have also fitted these datasets by using the proposed method. We target the following two aims: i) whether can the curve be properly fitted by a FPL model fitting is unnecessarily inevitable even the dose-response curve is monotonic? Comparing the curves based on the FPL model and the proposed method, we can conclude that the FPL model works properly if two curves close each other, and inappropriate otherwise. ii) When curves are non-monotonic, FPL models may fail to fit the data. In this scenario, the proposed

method can still fit the data and give a dose-response curve, which can be further used to obtain other important parameters such as  $IC_{50}$ ,  $EC_{50}$ ,  $ED_{50}$ ,  $LD_{50}$ ,  $K_m$ ,  $V_{max}$ , and AUC. In fitting FPL models, we need to choose initial values since this is essentially a nonlinear least square fitting. We may encounter convergence problems if the initial values are arbitrarily selected to far away from the true values. We also need to monitor whether we gain convergence of the objective function locally or globally. Implementing the proposed method, we also need to carefully decide how many observations for fitting to balance the bias and variance since the method is a locally data-driven one. We attach the partial results in the [Appendix B](#), in which the dots, green lines (if available) and blue lines stand for the observations, the estimated dose-response curves based on the FPL models and the proposed method, respectively. The small rectangles (if available) and filled triangles represent the associated  $IC_{50}$  values. Among these 110 datasets, we successfully fit 33 datasets by using FPL models, but failed for the remaining datasets. When FPL fitting is successful, the two curves generally close each other. In last two weeks, we have downloaded and organized 2,000 datasets, maintained by the Cancer Cell Line Encyclopedia (CCLE), which contain 10,000 datasets with various pharmacologic profiles for 24 anticancer drugs across 504 CCLE lines. We didn't have time to test and fit these 2000 datasets though we have organized them and they are ready to be used. We will explore and fit these 2000 datasets in Feb and March, and download the remaining 8000 datasets, which will be cleanup, explored and fitted in Phase II to further illustrate the applicability and advantages of the proposed method.

### 3.2.7 Initiating Algorithm for Estimating Non-monotonic Dose Response Curve

Suppose the relationship between the response  $Y$ (continuous case) and dose concentration  $x$  are related in the form

$$Y = g(x) + \varepsilon,$$

where  $\varepsilon$  is the measurement error with mean zero and finite variance,  $g(\cdot)$  is a mathematical function, but *no restrictions* are imposed on the form of  $g(\cdot)$ ; i.e, we don't assume that it is linear, nonlinear or any closed-form. We estimate  $g(\cdot)$  based on the observations  $(x_1, y_1), \dots, (x_n, y_n)$ , denoted as  $\hat{g}(\cdot)$ , by appropriate statistical techniques, for example the local polynomial estimator and the estimator based on B-splines.

### 3.2.8 Implementing Algorithm for Testing Non/monotonic Curve

We have implemented the algorithm for testing non/monotonic curve in C language. As is described in section 1.1, the algorithm involves the calculation of  $Q(r, s)$  for each pair  $(r, s)$  such that  $0 \leq r \leq s - m \leq n - m$ . Additionally, resampling method is required to determine the critical value for the test statistic. Therefore, we implement the algorithm in a C program which is considerably faster than some other languages. The input arguments of the program include sample size  $n$ , an  $n \times 1$  vector of dosage level, an  $n \times 1$  vector of responses, and the number of resampling samples. The output arguments include p-value of test, non-parametrically estimated dose-response curve. Additionally, the program also includes the fit of several parametric models such as the four-parameter logistic regression model and the four-parameter Weibull model.

### **3.2.9 Conducting simulation studies**

We have conducted simulation studies to check the performance of the test described in section 2. Particularly, we are interested in whether the described test can successfully detect non-monotonicity with a high power under the alternative hypothesis while controlling the false positive rate under the null hypothesis when the dose-response curve is monotone. Simulation results based 1000 replicates demonstrate that the test can successfully detect non-monotonicity when the true dose response curve is  $f_2(x)$  and the false positive rate is low when the true curve is  $f_1(x)$ .

### **3.2.10 Software Design and Implementation**

We have performed full life cycle of software development process including requirement analysis, system design, development and testing to develop PKPDBios software tool. We use AGILE software development methodology and take iterative and incremental approach to efficiently develop the software. We have selected most suitable system architect, technologies and programming languages to implement PDPKBios and developed use cases. Special consideration has been given for performance issues because of computation intensive requirement. The software has capability for users to load and securely manage their data. The software is designed to be a web based application and can be deployed in both Cloud and on-premises environments. The PKPDBios test web portal, <http://www.gridsite1.com> has been developed to demonstrate the software features.

### **3.3 What opportunities for training and professional development has the project provided?**

Nothing to Report.

### **3.4 How were the results disseminated to communities of interest?**

Nothing to Report.

### **3.5 What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to Report.

## **4. Impact**

### **4.1 What was the impact on the development of the principal discipline(s) of the project?**

The product will benefit various agencies, research community and industry that are concerned with development of new drug and new treatment, or study of safety in presence of resistance mutations, for instance, FDA, clinical research hospitals, pharmaceutical companies, medical schools. Eventually they will greatly benefit public health and biomedical research.

### **4.2 What was the impact on other disciplines?**

Nothing to Report.

### 4.3 What was the impact on technology transfer?

Nothing to Report.

### 4.4 What was the impact on society beyond science and technology?

Nothing to Report.

## 5. Changes/Problems

### 5.1 Changes in approach and reasons for change

Beside the aims we ever planned in the first version of the proposal, we have also identified two additional topics with the progress of Phase I, and proposed associated methods to handle these problems. Specifically, (i) when the response is binary

In dose-response studies, the response is sometimes binary (for example, dead or alive) instead continuous. In this case, all models we mentioned cannot be used to study the relationship between the response and dose concentration. A national option is to use logistic regression; i.e., model the probability of response against the dose

$$\text{logit}\{P(y = 1|x)\} = \beta x,$$

where  $\text{logit}(t) = e^t/(1 + e^t)$ . Although this model has shown success in many situations and is easy to implement, it has some limitations. One serious limitation is the linear assumption on the log odds ratio, which may not be true. Alternatively, extending from single index models, we shall develop smoothing techniques to estimate the nonlinear factors in generalized logistic regression:

$$\text{logit}\{P(y = 1|x)\} = g(x),$$

where  $g(x)$  is an unknown function. We approximate the unknown function  $g(x)$  by a local linear or spline first, then estimate associated coefficient, and the curve by using local smoothing method.

(ii) Comparison of two treatment effects

In comparison of two treatment effects based on dose response curves, one may use various typical tests such as t-test, Wilcox test, or even ANOVA. However, these tests generally impose implicit assumptions like normality. When these assumptions violated, the results based on these tests may not be reliable or even misleading. To develop a flexible method with least assumptions is greatly on demand. In this project, we propose a solution as follows.

We estimate the dose response curves using the proposed data-driven method. Let  $\hat{\eta}_1(t)$  and  $\hat{\eta}_2(t)$  be the estimates of the curves of groups 1 and 2. The standard error of the difference between the estimates is defined as

$$\text{se}_{\text{diff}}(t) = \sqrt{\text{se}_1^2(t) + \text{se}_2^2(t)}$$

where  $se_1(t)$  and  $se_2(t)$  are the standard errors of the estimated population curves  $\hat{\eta}_1(t)$  and  $\hat{\eta}_2(t)$ , respectively. A reference band with two standard errors wide and centered on the average of the two estimated curves is used as a rough check for difference between the two groups. If the two curves represented by  $\hat{\eta}_1(t)$  and  $\hat{\eta}_2(t)$  are enclosed in the reference band, we conclude that the two treatments do not have not significantly different outcomes.

## **5.2 Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to Report.

## **5.3 Changes that had a significant impact on expenditures**

Nothing to Report.

## **5.4 Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to Report.

## **5.5 Significant changes in use or care of human subjects**

Nothing to Report.

## **5.6 Significant changes in use or care of vertebrate animals**

Nothing to Report.

## **5.7 Significant changes in use of biohazards and/or select agents**

Nothing to Report.

## **6. Products**

### **6.1 Publications, conference papers, and presentations**

Nothing to Report.

### **6.2 Website(s) or other Internet site(s)**

The PKPDBios software web portal has been developed. The web site address is <http://www.gridsite1.com>.

### **6.3 Technologies or techniques**

Non-monotone dose response relationship can be detected and simulated to accurately calculate optimal dosage. Regulatory agencies, researchers and industry involved in drug research can use PKPDBios software to identify dose response reactions in classes of drugs. Research institutions, hospitals and other direct point patient caregivers can use PKPDBios software to target specific problems in patients such as individualizing dosage regimen of an individual patient for precision medicine. Pharmaceutical and biotech companies can use PKPDBios software to make fast and well-informed decisions about their drug development programs and clinical trial designs, and ultimately speed up the new drug development and save cost. It can be used from the preclinical phase through all clinical phases of drug development. Optimal use of PKPDBios

software will lead to fewer failed compounds, fewer study failures and smaller number of studies needed for registration.

## 6.4 Inventions, patent applications, and/or licenses

Nothing to Report.

## 6.5 Other Products

Software and models. The following brochure image illustrate the features of PKPDBios software COTS product.

**TConnex**  
Connecting Technologies

# Software for Pharmacodynamics and Bioassay Studies

## Description

The software tool provides a novel approach applicable to fit general pharmacologic, toxicology, or other biomedical data, that may exhibit a non-monotonic dose-response relationship for which the current parametric models fail.

The software explores dose-response relationships using both monotonic and non-monotonic models, and estimates the associated dose-response curves, which can further be used to obtain various important parameters such as IC50, EC50, ED50, LD50, Km, Vmax, and AUC. The tool can be used for hormesis dose response curves. In toxicology, hormesis is a special dose response feature characterized by low dose stimulation and high dose inhibition. Furthermore, the tool can model U-shaped dose response relationships frequently observed in toxicology and epidemiology studies.

## Major Software Features

- Test whether dose-response curve is monotonic or not.
- Verify whether a specific dose-response model (like linear, quadratic, orthogonal polynomials, exponential, linear in log-dose) is appropriate when the curve is monotonic.
- Assist to find initial values for fitting sigmoidal models.
- IC50 calculation for high-throughput inhibition assays.
- Km or Vmax calculation for high-throughput enzyme kinetics assays.
- EC50 calculation for high-throughput ligand-binding assays.
- High-throughput non-monotonic-response assays.
- Half-life calculation for high-throughput activity decay assays.
- Identify potential outliers and assess the impact of outliers on the analysis.
- Interfaces for integrating to database and web-servers.
- Command line for calling by scripts.
- Save fitting curves as commonly used format such as JPEG, PDF, TIFF files.
- Java application supports most systems.
- Customization to meet customer's needs.

## 7. Participants & Other Collaborating Organizations

### 7.1 What individuals have worked on the project?

Name	Hua Liang
Project Role	PI
Researcher Identifier	ORCID ID: 0000-0001-8772-5984
Nearest person month worked	6
Contribution to Project	Propose methods, design algorithms and simulation studies, review the related literature, download datasets of real dose-response studies, prepare report, and assist/direct software development
Funding Support	STTR: Developing Software for Pharmacodynamics and Bioassay Studies( Contract No: W81XWH-17-C-0153 )

Name	Larry Liu
Project Role	Project Coordinator
Researcher Identifier	N/A
Nearest person month worked	4.5
Contribution to Project	Project management and coordination
Funding Support	STTR: Developing Software for Pharmacodynamics and Bioassay Studies( Contract No: W81XWH-17-C-0153 )

Name	George Lu
Project Role	Software Development
Researcher Identifier	N/A
Nearest person month worked	6
Contribution to Project	Software prototype and web portal development.
Funding Support	STTR: Developing Software for Pharmacodynamics and Bioassay Studies( Contract No: W81XWH-17-C-0153 )

Name	Guoqing Diao
Project Role	Statistician
Researcher Identifier	0000-0001-7304-9591
Nearest person month worked	6
Contribution to Project	Working closely with the PI, Dr. Diao has performed work in the area of literature review of existing methods for testing monotonicity test and design of simulation studies. Dr. Diao has implemented the monotonicity test in C language and performed simulation studies and analysis of a real data set. Dr. Diao has also worked on some technical aspects of the Phase II proposal.
Funding Support	STTR: Developing Software for Pharmacodynamics and Bioassay Studies( Contract No: W81XWH-17-C-0153 )

Name	Nancy Liu
Project Role	Project Coordinator
Researcher Identifier	N/A
Nearest person month worked	1.5
Contribution to Project	Project management and coordination
Funding Support	STTR: Developing Software for Pharmacodynamics and Bioassay Studies( Contract No: W81XWH-17-C-0153 )

Name	Peter King
Project Role	Database Architect
Researcher Identifier	N/A
Nearest person month worked	1
Contribution to Project	Database Architecture Design and Configuration
Funding Support	STTR: Developing Software for Pharmacodynamics and Bioassay Studies( Contract No: W81XWH-17-C-0153 )

Name	Kong Li
Project Role	System Architect
Researcher Identifier	N/A
Nearest person month worked	1
Contribution to Project	System Architecture Design and Configuration
Funding Support	STTR: Developing Software for Pharmacodynamics and Bioassay Studies( Contract No: W81XWH-17-C-0153 )

**7.2 Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report.

**7.3 What other organizations were involved as partners?**

Nothing to Report.

**8. Special Reporting Requirements**

Nothing to Report.

**9. Appendices**

**9.1 PKPDBios Prototype and Web Portal Development**

We have developed PKPDBios software prototype and the pilot web portal <http://www.gridsite1.com> to demonstrate features and services to be offered. Users are able to load input dose-response data file to the prototype. Data is processed for verification and validation to ensure the data quality. The verified and validated dataset can be saved to database.

The basic descriptive statistics (mean, count, deviation, distribution, range, outlier, etc.) of the dose-response dataset can be displayed and reviewed. The following screen shots, Figure 1 and Figure 2, illustrate the PKPDBios software data processing capability:

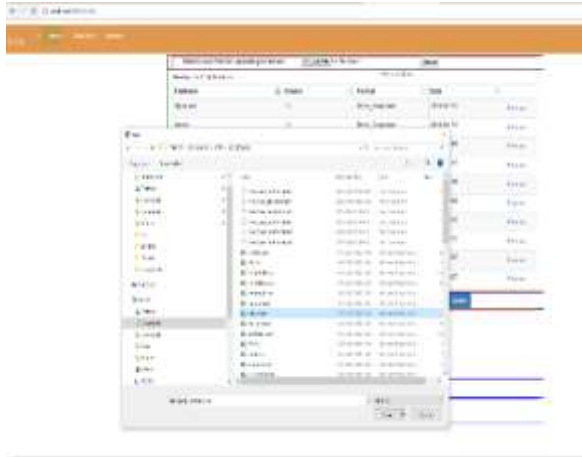


Figure1: Data file loading

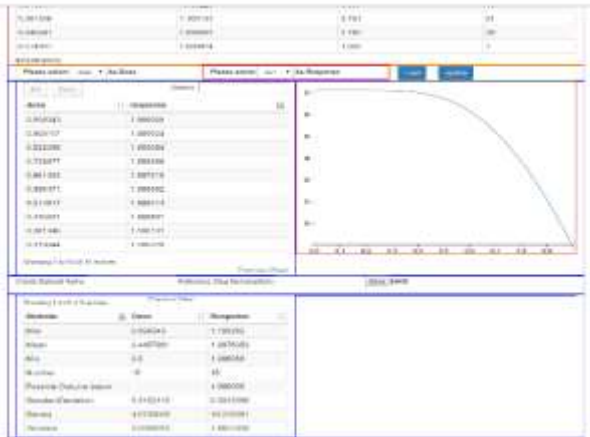


Figure 2: Processing data file, saving dataset to database and review basic statistics and curve

The following screen shot, Figure 3, displays the mockup simulation result of input dataset using multiple algorithms.

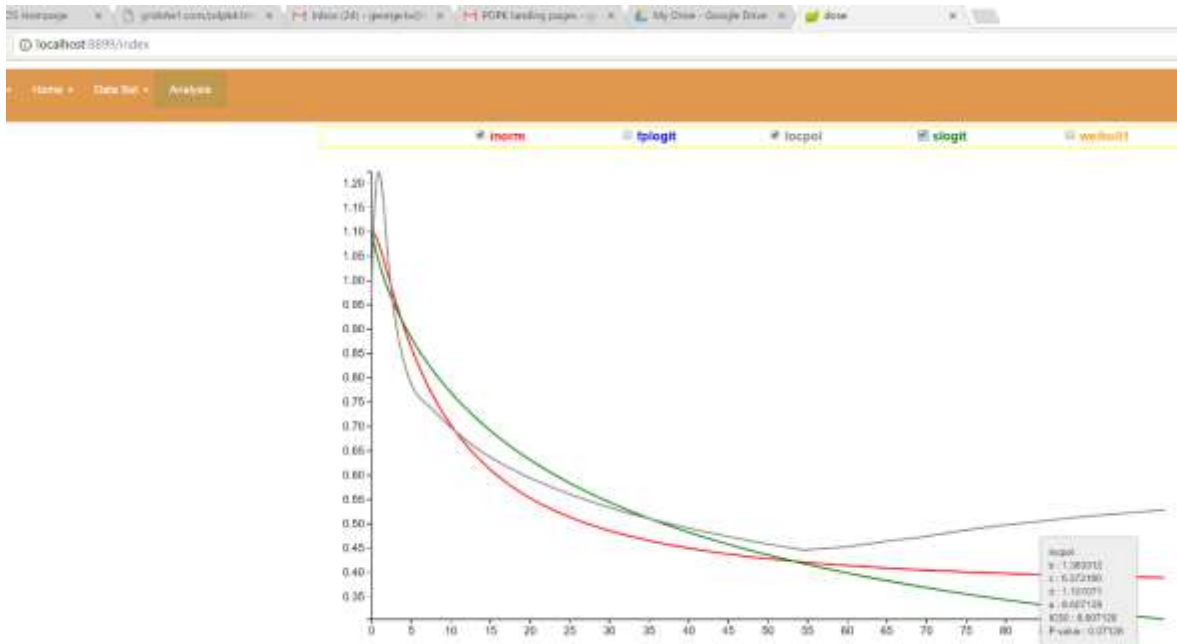


Figure 3: Processing, simulating and displaying result

The following screen shot, Figure 4, displays the PKPDBios web portal <http://www.gridsite1.com>.

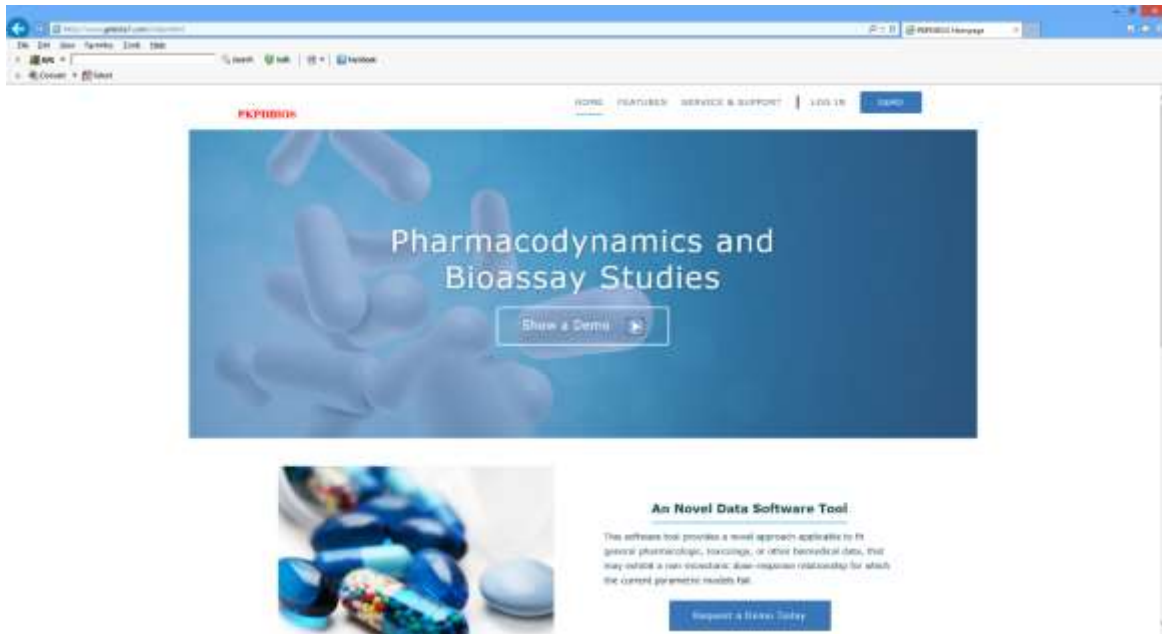


Figure 4. PKPDBios software pilot web portal <http://www.gridsite1.com>

## 9.2 The followings illustrates four simulation cases

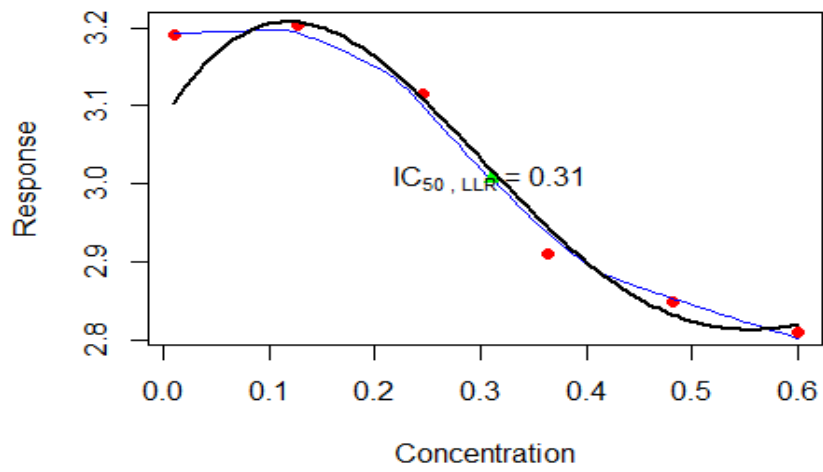
### Case 1

The truth:

$$\sqrt{2\pi} \left[ \frac{2}{\sqrt{2\pi} * .3^2} \exp\left(\frac{x^2}{.3^2}\right) \frac{3}{\sqrt{2\pi} * .7^2} + \exp\left\{\frac{(x-1)^2}{.3^2}\right\} \right]$$

---

### Case 1



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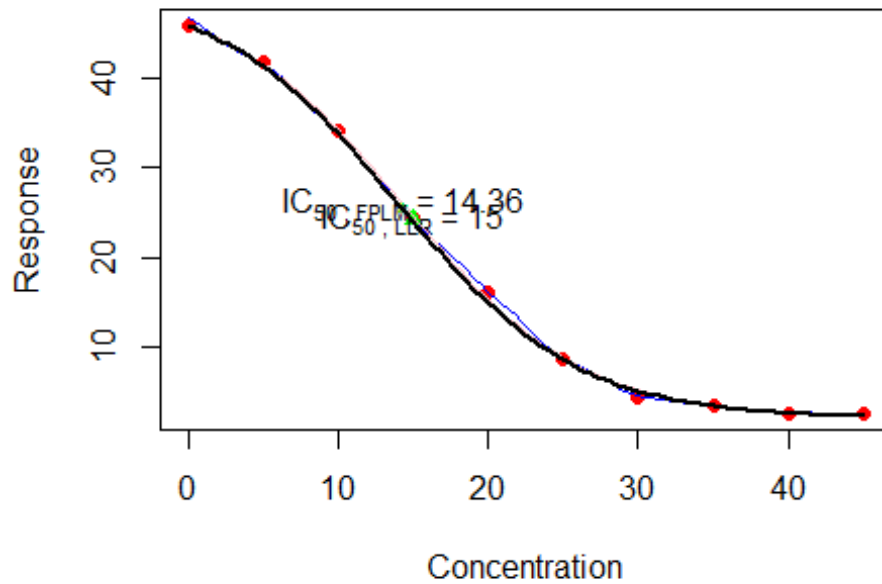
Case 2

The truth:

$$y(x) = \phi_1 + \frac{\phi_2 - \phi_1}{1 + \exp\{(x - \phi_3)/\phi_4\}}$$

---

Case 2




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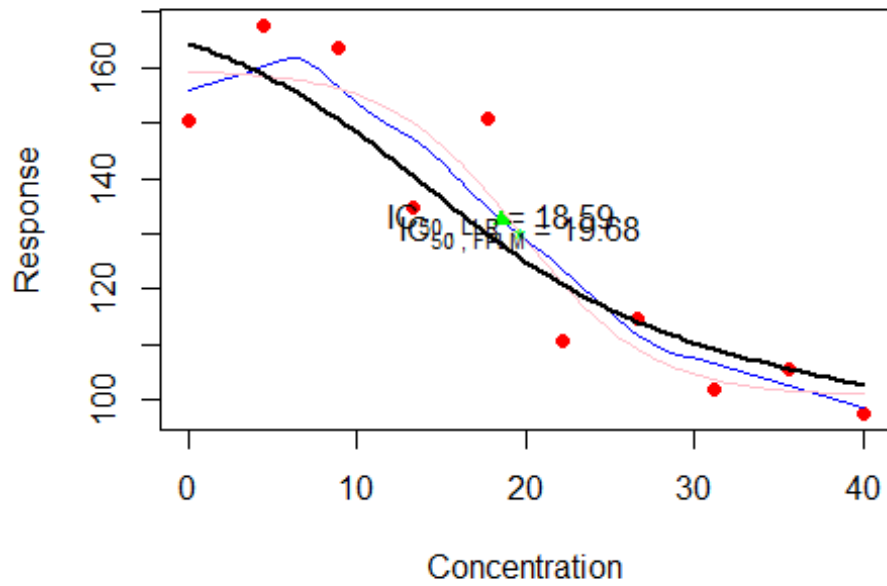
Case 3

The truth: biphasic four-parameter logistic function

$$y(x) = \left\{ \phi_1 + \frac{\phi_2 - \phi_1}{1 + \exp\{(x - \phi_3)/\phi_4\}} \right\} + \frac{3\phi_2 - \phi_1}{1 + \exp\{(x - 6\phi_3)/(10\phi_4)\}}$$

---

Case 3




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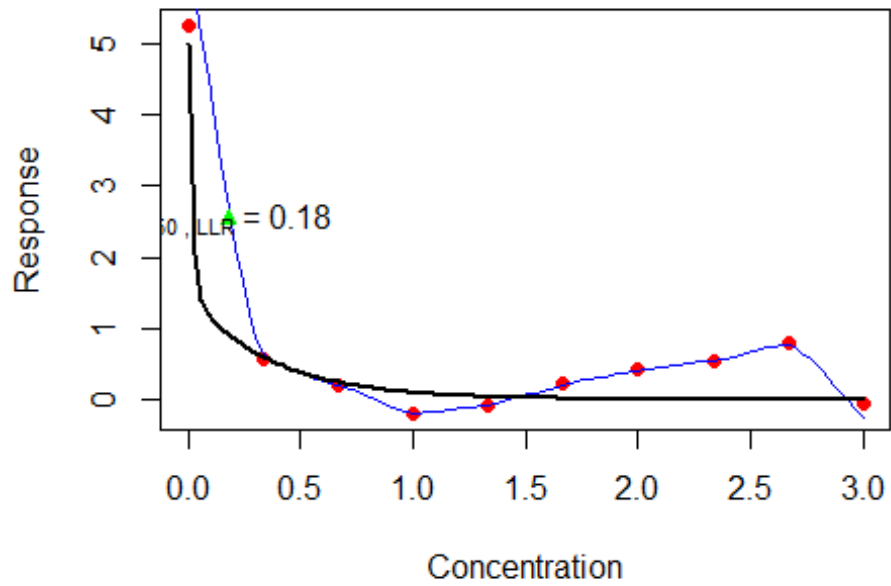
Case 4

The truth:

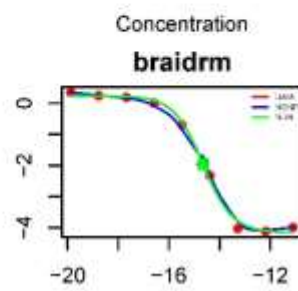
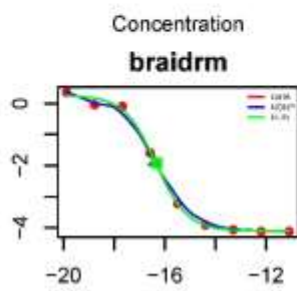
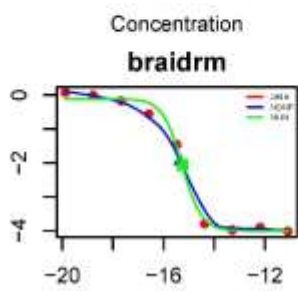
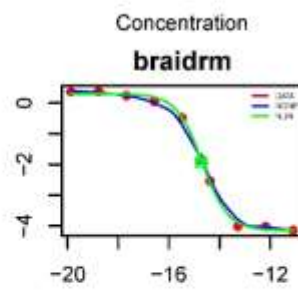
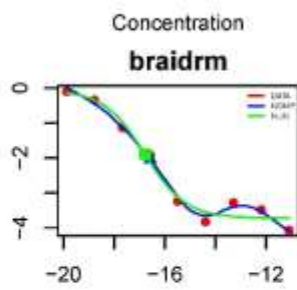
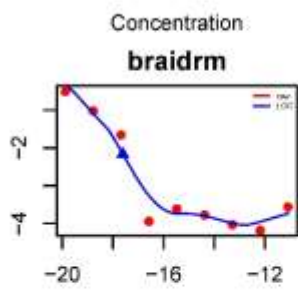
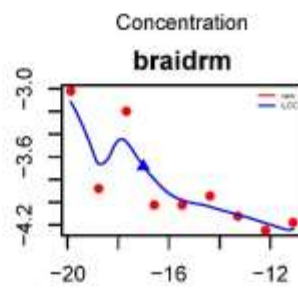
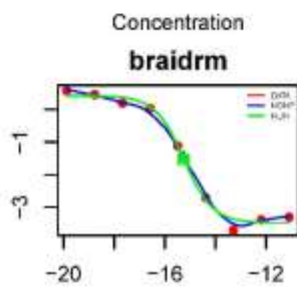
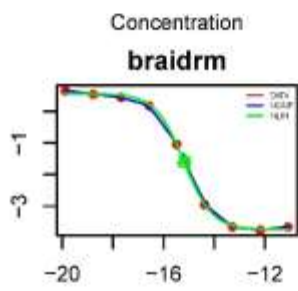
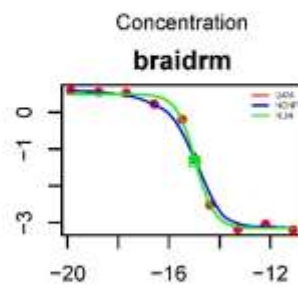
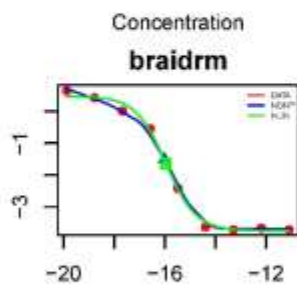
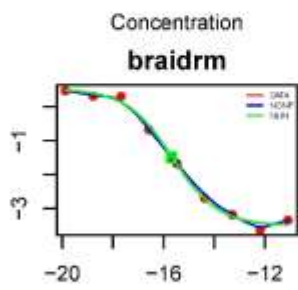
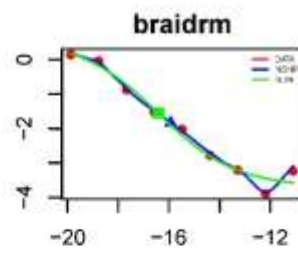
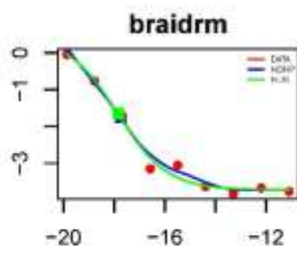
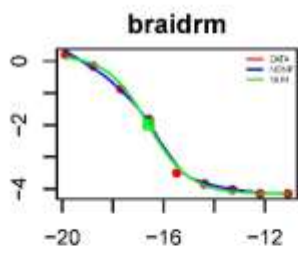
$$y(x) = \phi_1 \exp\{-\exp(\phi_2)x\} + \phi_3 \exp\{-\exp(\phi_4)x\}.$$

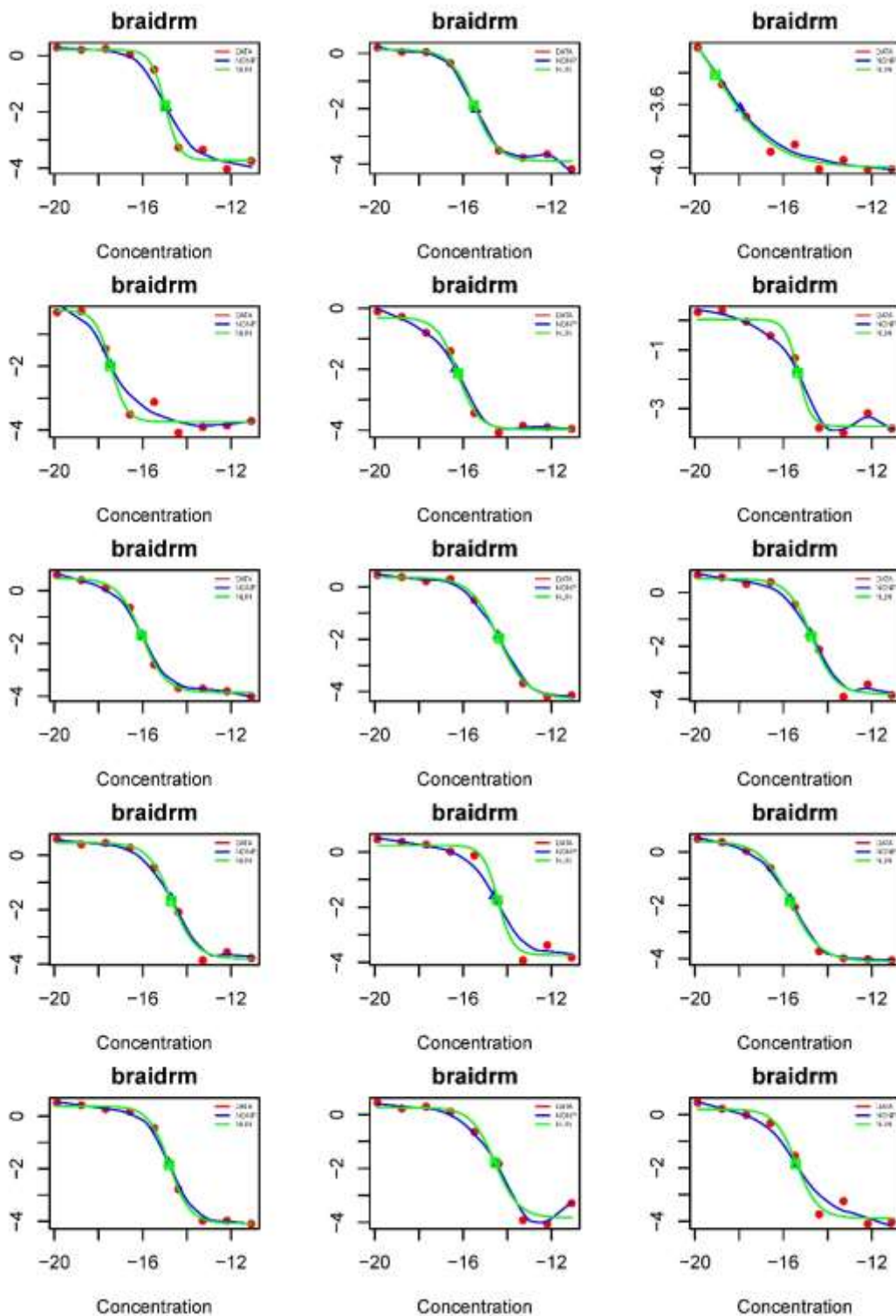
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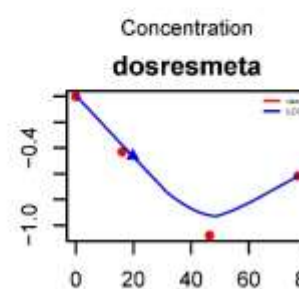
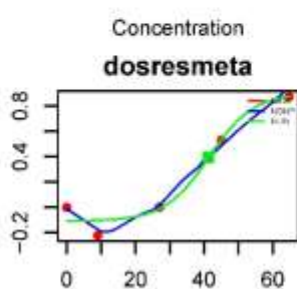
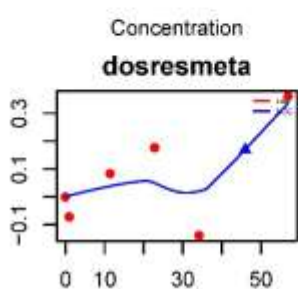
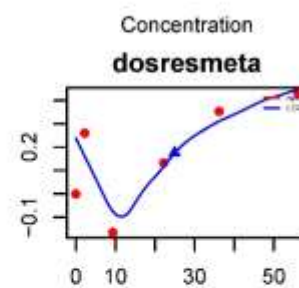
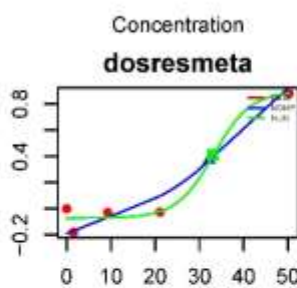
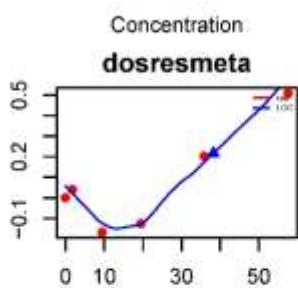
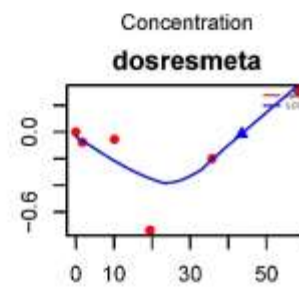
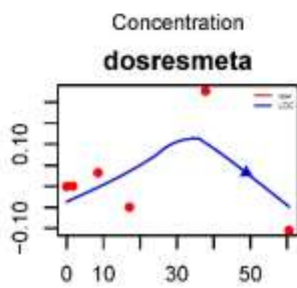
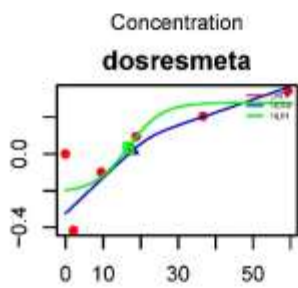
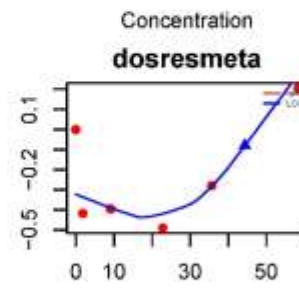
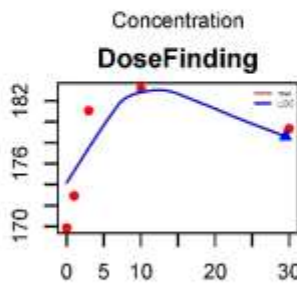
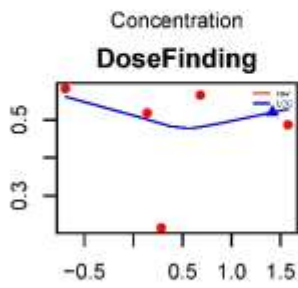
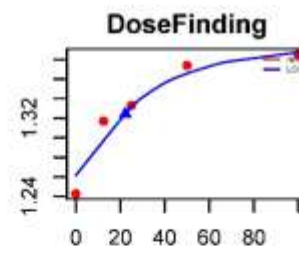
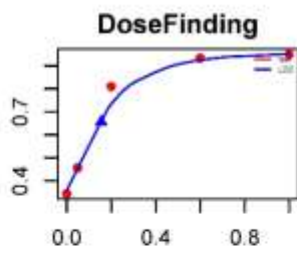
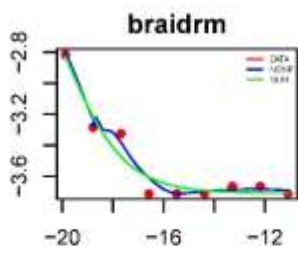
Case 4

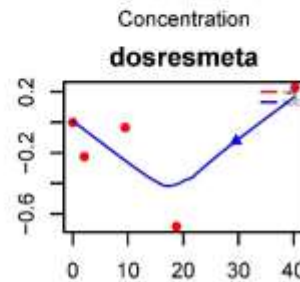
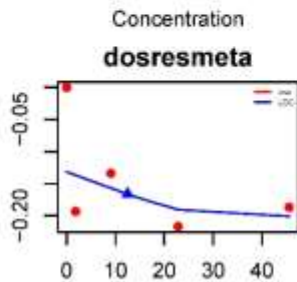
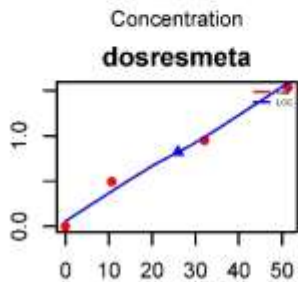
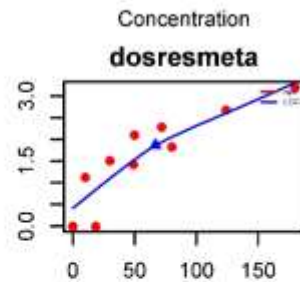
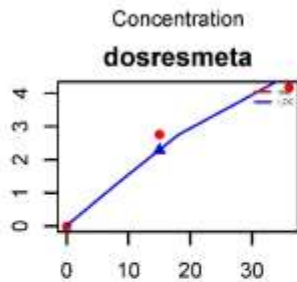
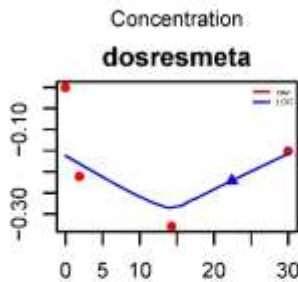
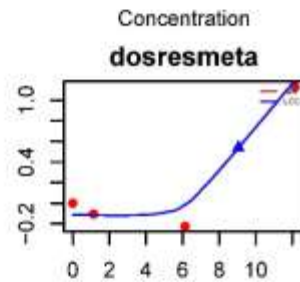
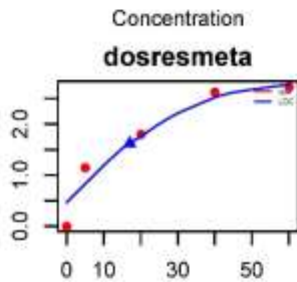
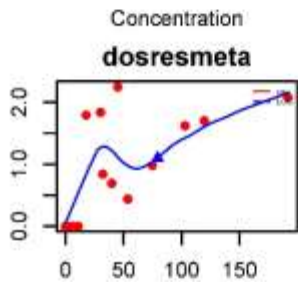
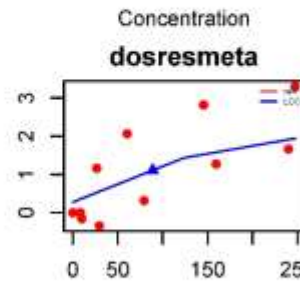
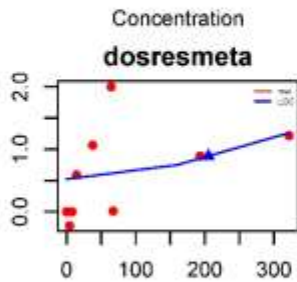
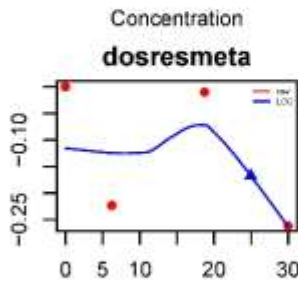
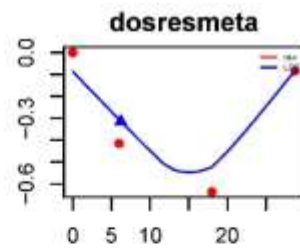
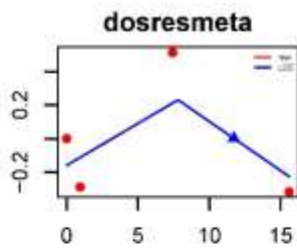
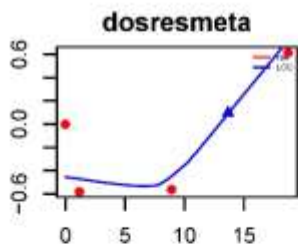


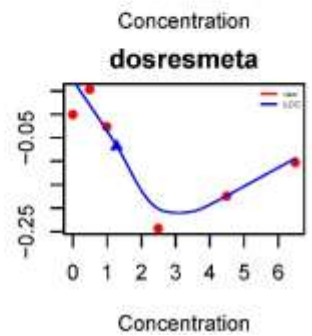
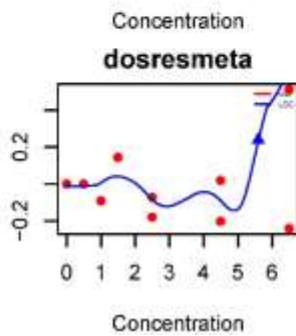
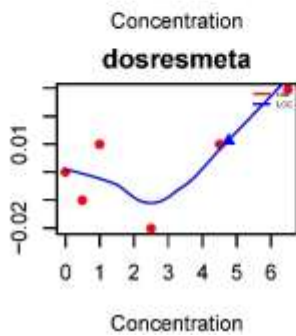
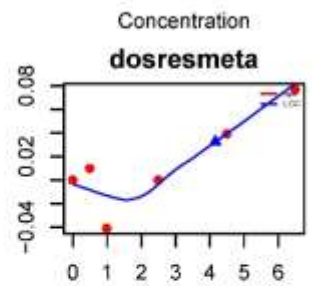
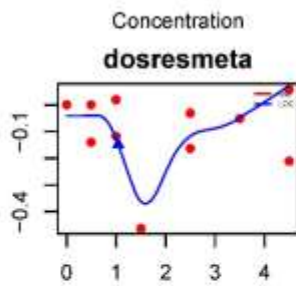
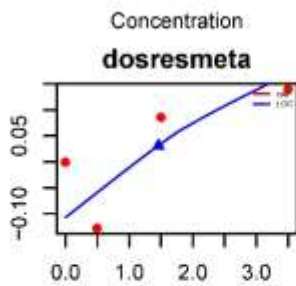
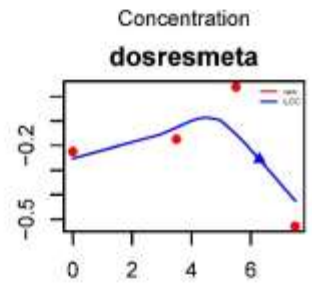
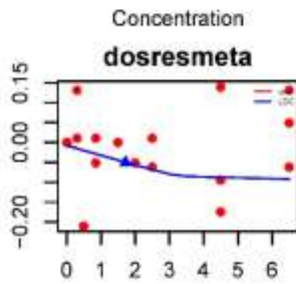
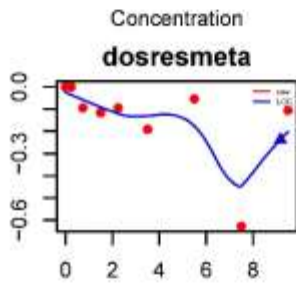
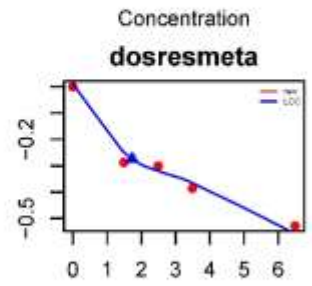
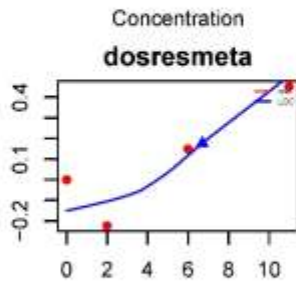
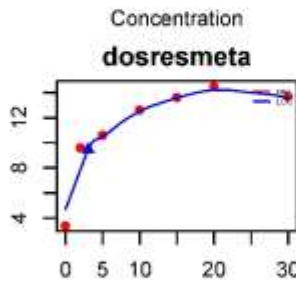
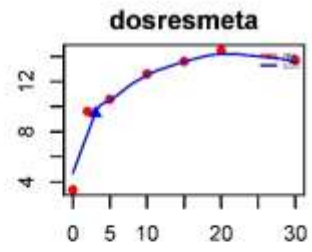
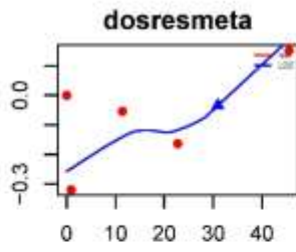
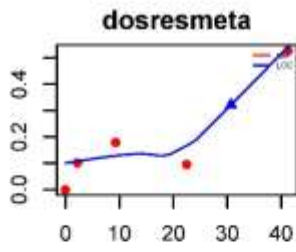
**9.3 The following images illustrate dose-response relationships**

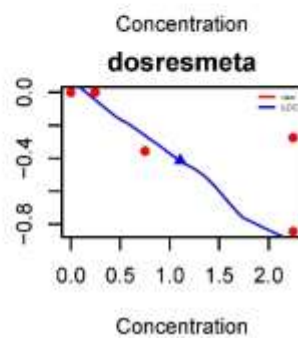
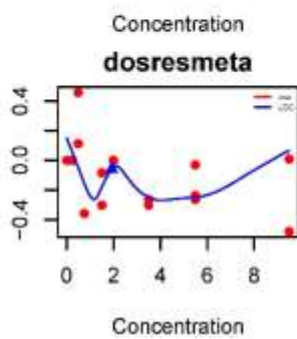
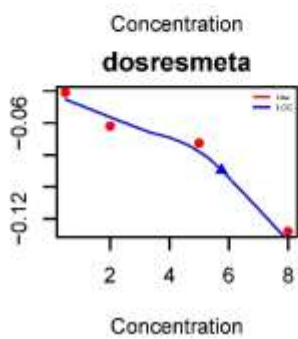
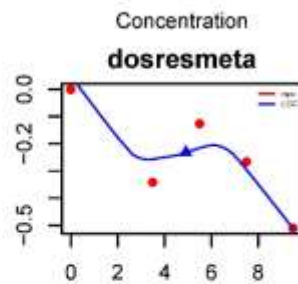
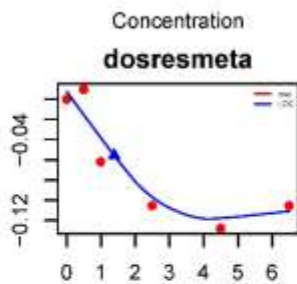
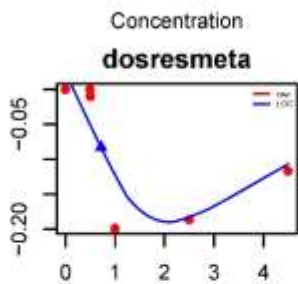
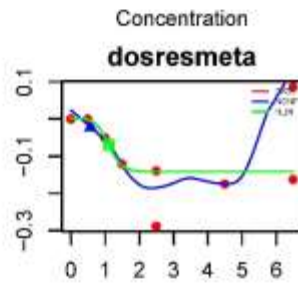
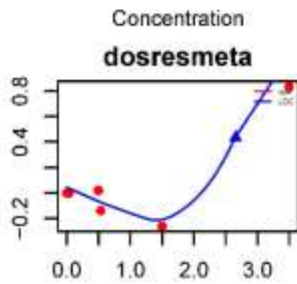
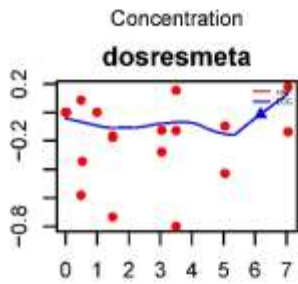
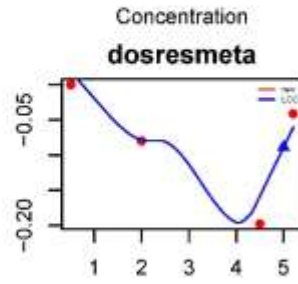
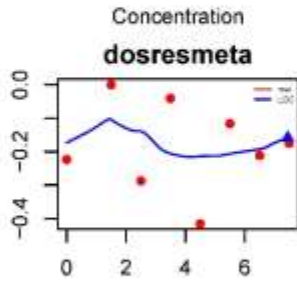
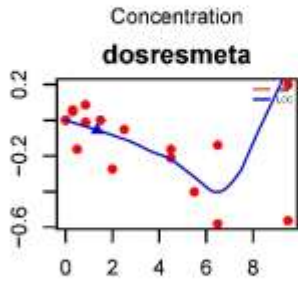
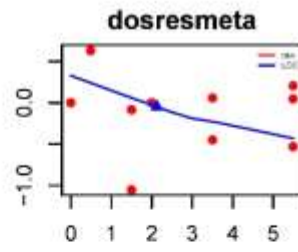
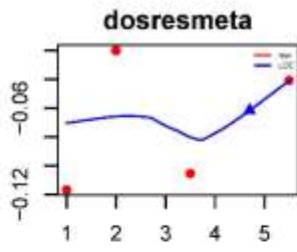
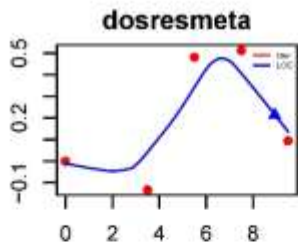




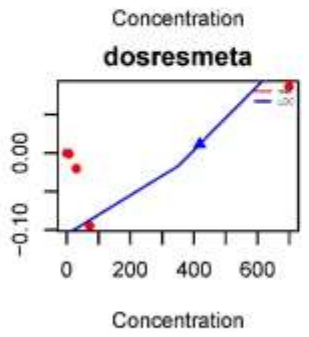
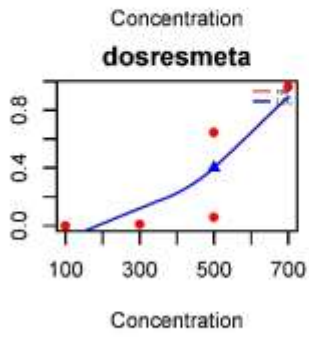
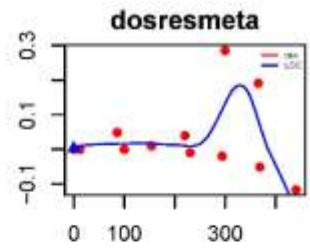
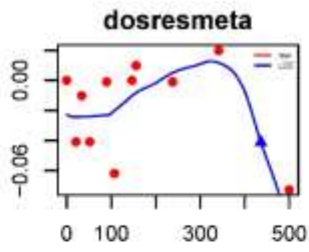
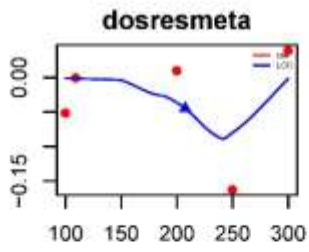












## References

- [1] Beausoleil C, Ormsby JN, Gies A, Hass U, Heindel JJ, Holmer ML, Nielsen PJ, Munn S, Schoenfelder G. Low dose effects and non-monotonic dose responses for endocrine active chemicals: science to practice workshop: workshop summary. *Chemosphere* 2013; 93(6):847-856.
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