



log₁₀(Agonist) – Sigmoidal 4P – Normalized 0–100% response

The objective of this case study is to investigate how the biological response changes as agonist concentration increases and to quantify that response using nonlinear curve fitting in **Isalos Analytics Platform**. In this case study, the **log₁₀(Agonist) – Sigmoidal 4P – Normalized 0–100% response** model is used to estimate the midpoint potency and curve steepness of a normalized agonist response, using a four-parameter logistic framework in which the response is constrained between 0% and 100%.

The dataset used in this case study is a published agonist dose-response dataset describing acetylcholine-induced contraction of **guinea pig ileum tissue** under control conditions, that is, in the absence of atropine. It contains log₁₀ acetylcholine concentration values and the corresponding normalized response values, expressed as the percentage of maximum response reported in the study. In this model, the independent variable (X) is the **log₁₀ of agonist concentration**, and the dependent variable (Y) is the **normalized response (%)**.

The **log₁₀(Agonist) – Sigmoidal 4P – Normalized 0–100% response** model describes a monophasic stimulatory sigmoidal relationship in which the response increases from the lower plateau toward the upper plateau as agonist concentration increases. The equation used in this analysis is:

$$Y = \frac{100}{1 + 10^{(\log EC50 - X) \times HillSlope}}$$

which produces the familiar sigmoidal dose-response curve on a logarithmic concentration scale. In this model, log *EC*50 represents the log₁₀ concentration of agonist that produces a response halfway between the lower and upper plateaus, and **HillSlope** describes the steepness of the curve.

The purpose of this analysis is to determine whether the observed data are consistent with a standard sigmoidal agonist response and to convert the measured response values into biologically meaningful quantitative outputs. The main results obtained from the fit are log *EC*50, the corresponding *EC*50, and **HillSlope**, where *EC*50 represents the agonist concentration required to produce 50% of the normalized maximal response and **HillSlope** reflects how sharply the response changes across the concentration range. These results are useful because they allow direct comparison between agonists, cell systems, treatments, receptor variants, or assay conditions, supporting interpretation of potency and response behavior.

Isalos version used: 2.0.2

Scientific Article <https://www.researchsquare.com/article/rs-5363679/v1>

Step 1: Import data from file

Right-click on the input spreadsheet panel on the left and choose **Import from File**. Then browse to the file containing the XY dataset for this case study and load the sheet in which each row corresponds to one log10 agonist concentration value and one measured normalized response value.

	Col1	Col2	Col3	Col4	Col5	Col6	Col7	Col8
User Header	User Row ID							
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								

- Show Spreadsheet Toolbar
- Import from File
- Import from Spreadsheet
- Import from Multiple Spreadsheets
- Adjust Spreadsheet Precision
- Export Spreadsheet Data
- Clear Spreadsheet

The data will appear on the left spreadsheet.

The screenshot shows the Isalos Analytics Platform interface. At the top, there is a navigation bar with 'File', 'Edit', 'Data Transformation', 'Analytics', 'Statistics', and 'DOE' menus. Below this is a toolbar with icons for file operations. The main area is divided into two panels. On the left is a spreadsheet with the following data:

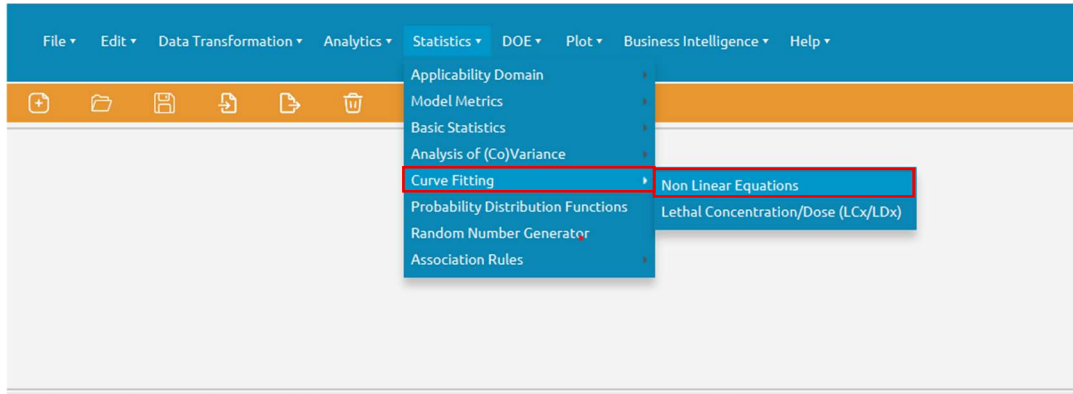
User Header	Col1	Col2 (D)	Col3 (I)	Col4	Col5	Col6
	User Row ID	log10 of agonist concentration	normalized response (%)			
1		-9.0	0			
2		-8.5	17			
3		-8.0	31			
4		-7.5	79			
5		-7.0	100			
6		-6.5	100			
7		-6.0	100			
8		-5.5	100			
9		-5.0	100			
10		-4.5	100			
11						
12						
13						
14						
--						

On the right is a 'Curve Fitting - Non Linear E' panel with the following settings:

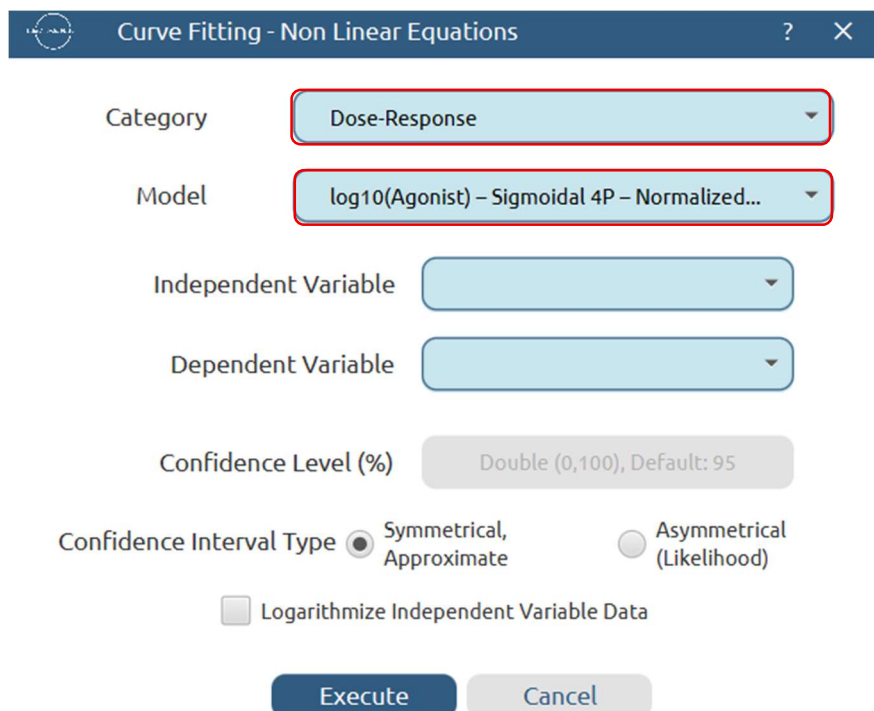
- Category: Dose-Response
- Model: log10(Agonist) - Sigmoidal 4P
- Independent Variable: Col2 - log10 of a
- Dependent Variable: Col3 - normaliz
- Confidence Level (%):
- Confidence Interval Type: Symmetrical, Approximate
- Logarithmize Independent Variable
- Show Chart

Step 2: Select the desired statistical analysis model

From the toolbar, open the **Statistics** drop-down list and navigate through Statistics > Curve Fitting > Non Linear Equations.

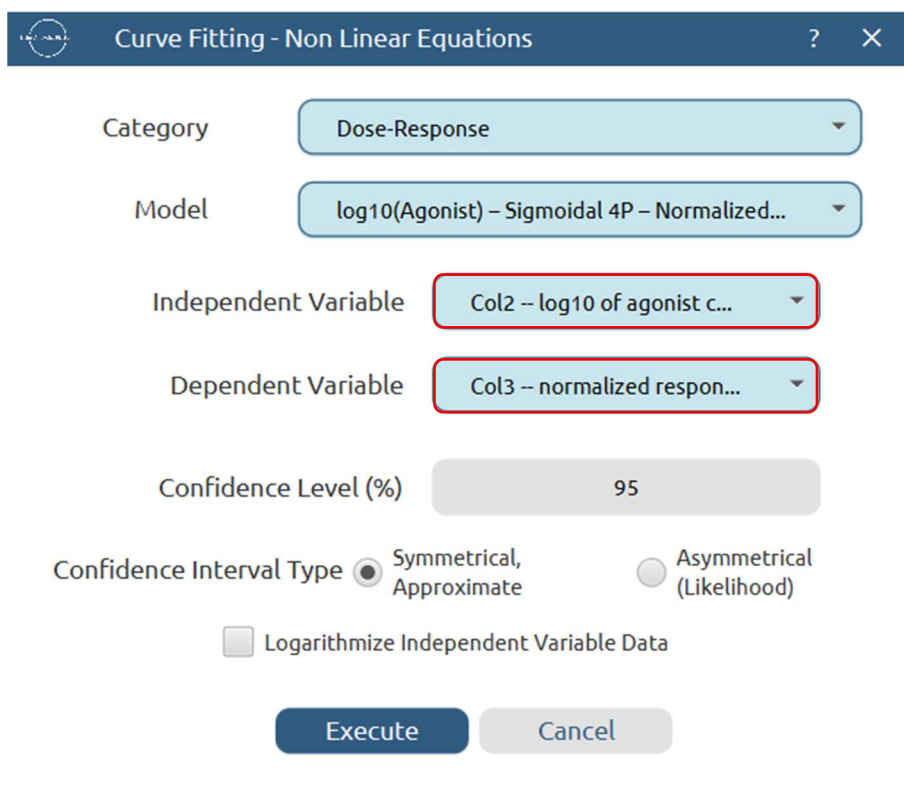


In the category list, select the **Dose-Response** category and then choose **log₁₀(Agonist) – Sigmoidal 4P – Normalized 0–100% response** from the model menu.

A screenshot of the 'Curve Fitting - Non Linear Equations' dialog box. The 'Category' dropdown is set to 'Dose-Response' and the 'Model' dropdown is set to 'log10(Agonist) – Sigmoidal 4P – Normalized...'. Both dropdowns are highlighted with red boxes. Below these are fields for 'Independent Variable' and 'Dependent Variable', both currently empty. The 'Confidence Level (%)' is set to 'Double (0,100), Default: 95'. Under 'Confidence Interval Type', 'Symmetrical, Approximate' is selected with a radio button. There is an unchecked checkbox for 'Logarithmize Independent Variable Data'. At the bottom are 'Execute' and 'Cancel' buttons.

Step 3: Configure variables and confidence intervals

Set the column containing the log₁₀ agonist concentration values as the independent variable (X), and set the column containing the normalized response values as the dependent variable (Y). Set the confidence level to **95%** and choose **Symmetrical Approximate** as the confidence interval type.



The screenshot shows a dialog box titled "Curve Fitting - Non Linear Equations". It contains the following settings:

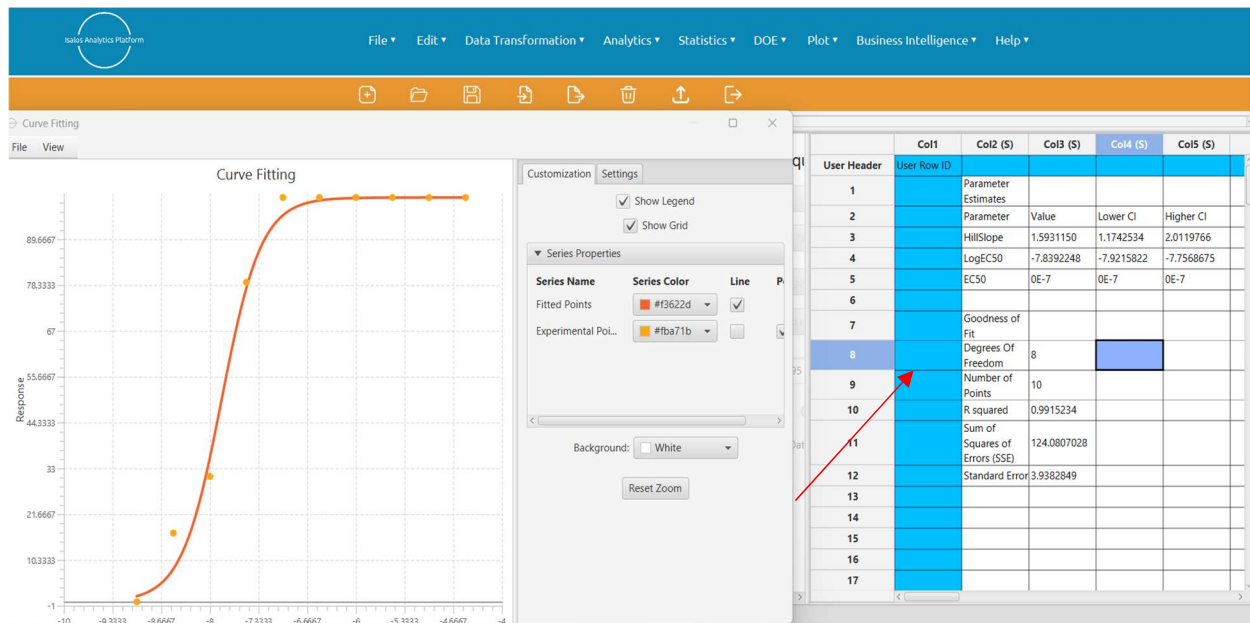
- Category:** Dose-Response
- Model:** log₁₀(Agonist) - Sigmoidal 4P - Normalized...
- Independent Variable:** Col2 -- log₁₀ of agonist c...
- Dependent Variable:** Col3 -- normalized respon...
- Confidence Level (%):** 95
- Confidence Interval Type:** Symmetrical, Approximate (selected), Asymmetrical (Likelihood)
- Logarithmize Independent Variable Data
- Buttons:** Execute, Cancel

Step 4: Analyze the output and fitted curve

Once the analysis is completed, Isalos presents the fitted sigmoidal curve together with the experimental data points, allowing direct visual assessment of how well the model captures the observed agonist response pattern. The results page presents the estimated model parameters with their confidence limits, together with goodness-of-fit statistics and the corresponding fitted plot.

In the **log₁₀(Agonist) – Sigmoidal 4P – Normalized 0–100% response** model, the main fitted parameters are **log EC₅₀**, the corresponding **EC₅₀**, and **HillSlope**, and the graphical output should display an increasing sigmoidal curve in which the normalized response rises from low values toward the upper plateau as agonist concentration increases.

The **Goodness of Fit** section of the table summarizes key fitting statistics such as the **number of data points used, degrees of freedom, residual sum of squares,** and the **standard error** of the regression. These outputs should be interpreted together with the fitted plot in order to evaluate how well the model describes the data and how reliable the estimated parameters are.



The fitted results indicate that acetylcholine acts as a potent agonist in this guinea pig ileum preparation and produces a steep concentration-dependent increase in response. A **HillSlope** value of approximately **1.59** suggests a relatively sharp transition around the midpoint of the curve, meaning that small changes in agonist concentration near the EC50 produce substantial changes in normalized response.

The fitted **LogEC50** value of about **-7.84** indicates that half-maximal response is achieved at a low agonist concentration, consistent with high agonist potency in this tissue preparation. The narrow confidence intervals and the high R^2 value further suggest that the standard sigmoidal agonist model provides a good description of the observed data.

References:

- (1) Wilfred, O., Urangikor, I.M., Itiri, E., Samuel, V., Wilfred, O.M., Ogbonna, V.N. and Ezealisiobi, E.E., 2024. Concentration-response Curve for Acetylcholine (Ach) in the Absence and Presence of Atropine and Measurement of the Equilibrium Dissociation Constant (Kb) Using Schild Analysis.