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NLM Citation: Campbell RM, Dymshitz J, Eastwood BJ, et al. Data Standardization for Results Management. 2012 May 1 [Updated 2017 Oct 20]. In: Markossian S, Grossman A, Arkin M, et al., editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004-.
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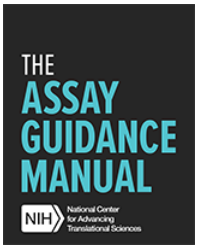

Data Standardization for Results Management

Robert M. Campbell,[1] Julia Dymshitz,[2] Brian J. Eastwood,[3] Renee Emkey,[4] David P. Greenen,[5] Julia M. Heerding,[6] Dwayne Johnson,[7,*] Thomas H. Large,[8] Thomas Littlejohn,[9] Chahrzad Montrose,[10] Suzanne E. Nutter,[11] Barry D. Sawyer,[12] Sandra K. Sigmund,[13] Martin Smith,[14] Jeffrey R. Weidner,[15,†] and Richard W. Zink[16]

Created: May 1, 2012; Updated: October 20, 2017.

Abstract

Raw data collected in screening assays should be appropriately analyzed to derive activity expressed as potency or efficacy values of tested compounds (IC_{50} or EC_{50} values). In this chapter the authors discuss standardized approaches to processing radioligand binding, enzyme and functional assays used in HTS and lead optimization. Detailed account is given in processing normalizing raw data and curve fitting. A glossary is also included defining the terms used for consistency in processing raw data in a standardized manner.

Introduction

Definitions of Result Levels

Raw data: Individual measurements as produced by the instruments used in the experiment.

Normalized well level: Individual data values that have been transformed to provide a consistent, biologically relevant context. This is often done using vehicle and maximally efficacious compound controls. (Inhibition, Stimulation, etc; in most cases Inhibition and Stimulation are expressed as a % of the dynamic range of the assay)

Aggregate: median (preferred) or mean normalized well level data when replicates exist in a single run (Inhibition, Stimulation, etc.). This level provides for a consistent determination of n as it applies to *in vitro* results.

Derived data: Results calculated from groups normalized or aggregate well level data based upon the fit of this data to a mathematical model. (IC_x, Relative IC_x, EC_x, K_i, K_b, etc.)

Author Affiliations: 1 Eli Lilly & Company, Indianapolis, IN. 2 Eli Lilly & Company, Earlwood Manor, UK. 3 Eli Lilly & Company, Earlwood Manor, UK. 4 Amgen Inc., Boston, MA. 5 Eli Lilly & Company, Indianapolis, IN. 6 Eli Lilly & Company, Indianapolis, IN. 7 Eli Lilly & Company, Indianapolis, IN. 8 Sunovian Pharmaceuticals Inc., Boston, MA. 9 Eli Lilly & Company, Indianapolis, IN. 10 Eli Lilly & Company, Indianapolis, IN. 11 Eli Lilly & Company, Indianapolis, IN. 12 Eli Lilly & Company, Indianapolis, IN. 13 Eli Lilly & Company, Indianapolis, IN. 14 Eli Lilly & Company, Indianapolis, IN. 15 AbbVie, Chicago, IL. 16 Eli Lilly & Company, Indianapolis, IN.

* editor

† editor

Summarized data: Statistical summarization of results across multiple runs. (geometric mean ICx, Relative IC_x, K_i, K_b or average Inhibition, Stimulation, etc.)

Absolute IC_{50}, Relative IC_{50} or Relative EC_{50}

For assays described in this chapter, absolute IC_{50} (abs IC_{50}), relative IC_{50} (rel IC_{50}) and relative EC_{50} (rel EC_{50}) are predominantly used to derive a value that can be used to compare results within and across runs in the same assay, as well as between different assays. Abs IC_{50} and rel IC_{50} are used when different assumptions are applied; the selection of either is at the discretion of the scientist but should be applied consistently and not changed for a defined assay.

For consistency, rel IC_{50} is used for inhibition assays while rel EC_{50} is used for stimulation assays, even though there is no fundamental difference between them. Because of their relative simplistic composition, biochemical *in vitro* assays can be easily labeled as either "Stim" or "Inh", while every biochemical whole cell assay can be either as "Stim" or "Inh" depending on multiple factors. Therefore, the guideline for defining whole cell biochemical assays is to use the label that better reflects the perceived pharmacology, regardless of the direction (increasing or decreasing with test substance concentration) of the raw signal. How an assay is defined can also drive which result type label to use. For instance, if an assay categorized by cell cycle modulation is attempting to inhibit the cell cycle, the rel IC_{50} should be used.

Guidelines for Curve Fitting

- Three or four parameter logistic curve fits are acceptable.
- Under appropriate conditions, the top may be fixed to 100 (maximum or compound control level) and the bottom may be fixed to 0 (minimum or vehicle control level).
- It is recommended that the Hill Coefficient not be preset to any fixed number, unless supported by a statistician.
- Cubic spline curve fits *are not recommended*, unless supported by a statistician.
- The Fitting Error of the IC_{50}/EC_{50} should not exceed 100%, unless supported by a statistician. (*It should be noted that this "standard error" is a measure of "goodness of fit" of the data to the curve fitting equation and not the "standard error" of aggregate data values*).

Normalizing Data using a Positive Control Curve

In some cases (such as where there is a non-linear standard curve for the analyte), it is preferable to use a reference curve to define the dynamic range of the assay. In those cases, the fitted top of the reference curve is substituted for the max while the fitted bottom of the reference curve is substituted for the min in normalization calculations. This may be particularly useful in agonist assays where the use of a reference agonist curve is strongly recommended. It is still preferable to define the dynamic range on each plate so that individual plate drift is assessed and single plates can pass/fail. Additionally, the upper and lower asymptote of the reference curve should be established by the data in order to use them for dynamic range determination.

Application of a Standard Curve

Use of a standard curve is required wherever possible when the raw data is not a linear function of the biological response. For example, optical densities, fluorescence units and luminescence units often cannot be directly used for calculations of activity as they are often non-linear functions of the concentration of the relevant biological product. A standard curve is used to convert the raw data to concentration of biological substance. The calculated concentrations are then used to calculate the *Normalized Result*, as discussed in most thoroughly in the Immunoassay chapter. The standard curve data should be generated with an appropriate number of points

and concentration range, fit by an appropriate concentration-response model so that bias and precision are within acceptable limits. All raw data within the scope of the assay can be converted to the biological response.

Data Types and Associated Rules for Radioligand Binding Assays: Inhibition Mode

Normalized Results

For radioligand binding methods, the use of Inhibition is recommended to quantify the ability of individual concentrations of a substance to inhibit the total specific binding of radioligand. The use of % bound *for normalization is discouraged*, but it's recommended that biologists calculate and track changes to % bound as a measure of assay performance.

Calculation:

$$\text{Inhibition} \quad (\%) = 100 - \left[\frac{(\text{Measured Binding} \quad - \text{Min})}{(\text{Max} - \text{Min})}\right] x100$$

Max = maximum binding

Min = non-specific binding

Derived Results: Absolute IC_{50} and Relative IC_{50}

Absolute IC_{50} = the molar concentration of a substance that reduces the specific binding of a radioligand to 50% of the maximum specific binding.

Relative IC_{50} = the molar concentration of a substance that reduces the specific binding of a radioligand to 50% of the range of the binding curve (Top – Bottom) for that particular substance.

Notes:

- For incomplete curves, the response data should span above 50% for an IC_{50} to be used for the determination of a K_i.
- The Top and Bottom parameters should be within +/- 20% of the Top and Bottom dynamic range control values.

Derived Results: K_i

The equilibrium dissociation constant of a test compound (Ki) should be calculated using the standard Cheng-Prusoff equation:

$$K_i = \frac{IC_{50}}{1 + \frac{[R]}{K_d}}$$

[R] = concentration of radioligand used in the assay

K_d = the equilibrium dissociation constant of the radioligand in the assay

Notes:

- K_i carries the same prefix as the IC_{50} from which it is derived.
- For competitive binding mechanisms, a K_i is recommended to be reported for radioligand binding assays, from IC_{50} values generated using 3 or 4-parameter curve fitting methods.
- For uncompetitive or complex (ill-defined) binding mechanisms, an IC_{50} is preferred, because one of the main assumptions for the use of the Cheng-Prusoff equation is based on a competitive, bimolecular interaction.

Data Types and Associated Rules for Enzymatic Assays: Inhibition Mode

Normalized Results

Inhibition with a Unit of Measurement (UOM) of % based on complete enzyme inhibition (dynamic range of the assay)

Calculation:

$$\text{Inhibition}\quad (\%) = 100 - \left[\frac{(\text{Activity of Enzyme with Test Cmpd \& Substrate} - \text{Min})}{(\text{Max} - \text{Min})}\right] x100$$

Max = observed enzyme activity measured in the presence of enzyme, substrate(s) and cofactors utilized in the method.

Min = observed enzyme activity measured in the presence of substrate(s) and cofactors utilized in the method, and (a) in the absence of enzyme, or (b) in the presence of a fully inhibited enzyme.

Derived Results: Absolute IC_{50}, Relative IC_{50}

Relative IC_{50} = the molar concentration at which 50% of maximal inhibition for that substance is observed.

Absolute IC_{50} = the molar concentration of a substance that reduces the enzymatic activity to 50% of the total enzymatic activity.

Data Types and Associated Rules for *In Vitro* Functional Assays

Antagonists

Normalized Results

Inhibition with a UOM of % should be calculated for responses to individual concentrations of test substances.

Calculation:

$$\text{Inhibition}\quad (\%) = 100 - \left[\frac{(\text{Response in presence of Test Cmpd \& Ref Agonist} - \text{Min})}{(\text{Max} - \text{Min})}\right] x100$$

Max = response in presence of some concentration of a reference agonist challenge dose.

Min = (a) response in the presence of diluents and in the absence of test substance and agonist; or (b) response in the presence of maximally effective antagonist and challenge dose of agonist.

Derived Result: Rel IC_{50}

Relative IC_{50} = the molar concentration of a substance (antagonist) that reduces the efficacy of the reference agonist or the constitutive activity of the biological target by 50% of the antagonist curve (Top-Bottom) for that particular test substance.

Derived Result: K_b

Calculation of K_b by Schild analysis isn't standard practice due to throughput and cost disadvantages. Consequently, the Cheng-Prusoff equation is typically used to reduce the data and subsequently assigned the label of K_b.

Calculation: Use standard Cheng-Prusoff equation for functional assays.

$$Kb = \frac{IC_{50}}{1 + \frac{[A]}{EC_{50}}}$$

[A] = the concentration of the reference agonist that is being inhibited

EC_{50} = the Relative EC_{50} of the reference agonist determined in the same run of the assay.

If the slope of the curve for the reference agonist deviates significantly from 1, the use of the modified Cheng-Prusoff equation is recommended.

Other Derived Results:

Schild K_b

Schild K_b is measure of affinity for a competitive antagonist that is calculated using the ratios of equi-active concentrations of a full agonist (most typically EC_{50} concentrations are used) in the absence and presence of one or more concentrations of the antagonist. Schild K_b offers a true evaluation of a test compound's ability to mechanistically perform as an antagonist. This process exposes toxic effects and compound precipitation as false positive activity, and therefore, should be used when time and cost are not limitations.

E_{min}

The maximum activity of an antagonist test substance relative to a reference agonist. This is obtained by first generating a fitted top from a %Inhibition curve and then converting that to the corresponding %Stimulation of the reference agonist curve. The E_{min} value for antagonist mode should equal the relative efficacy for agonist mode for competitive inhibitors. In order to make use of E_{min}, the selected agonist concentration (i.e. EC_{80}) should produce an activity above the expected E_{min} value (Figure 1).

Notes:

- K_b carries the same prefix as the IC_{50} from which it is derived.
- The use of Abs IC_{50} is discouraged.
- Because partial antagonists exist, a full response curve with defined Top & Bottom can be achieved even if the %Inh doesn't exceed 50%.
- A concentration response curve for the reference agonist should be determined in each experimental run if a K_b is to be determined. The frequency within the run depends on assay variability. A statistician should be consulted concerning this frequency during the assay validation process.

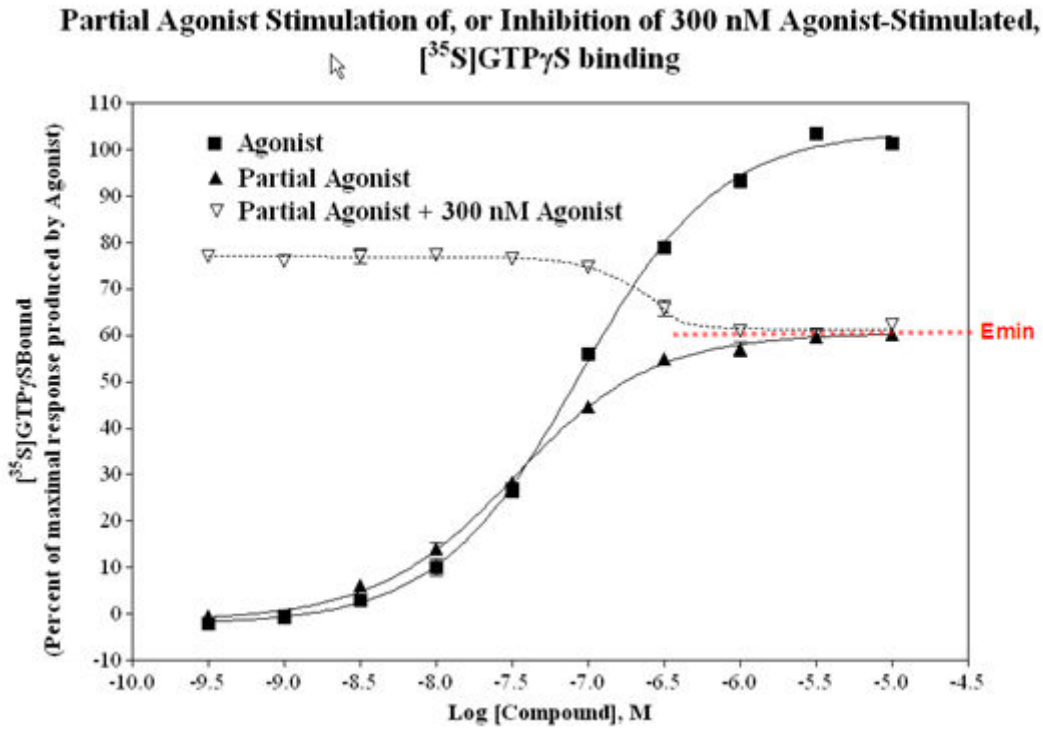

Figure 1: Partial agonist stimulation or inhibition of 300 nM agonist-stimulated [^{35}S]GTPγS binding

Agonists

Normalized Data

Stimulation with a UOM of % should be calculated for responses to individual concentrations of test substances.

Calculation:

$$\text{Stim (\%)} = \left[\frac{(\text{Response in presence of Test Cmpd} - \text{Min})}{(\text{Max} - \text{Min})}\right] x100$$

Min = the fitted Bottom of a 4 parameter logistic curve fitting equation applied to data generated from the positive control (reference agonisit).

Max = (a) the maximum activity of a positive control agonist determined by the fitted Top of a 4 parameter logistic curve fitting equation applied to a concentration response curve from the positive control; or (b) the maximum activity of a positive control in Max wells, which should represent the empirically-derived saturating concentration of the positive control.

Derived Results: Relative EC_{50} and Relative Efficacy

Relative EC_{50}= the molar concentration of a substance that produces 50% of that test substance's maximum stimulation.

Relative Efficacy = the maximum activity of a test substance relative a reference agonist. The UOM for Relative efficacy is %.

Calculation:

$$\text{Rel Eff (\%)} = \left[\frac{\text{Fitted Top of Test Cmpd}}{\text{Fitted Top of Reference Agonist}} \right] x100$$

Other Derived Results:

Fold Activity and Fold Activity Max

The fold activity (or fold activity max) result is useful when comparing *test compounds evaluated across multiple functional assays* because varying levels of efficacy can be observed amongst the different or same reference agonists.

The intended use of this calculation is to provide additional information to reduce or define differences between assays, so that differences between compound activities can be further quantified. For example, a compound run in an assay normalized to a *reference agonist with low efficacy* would appear to be more efficacious when compared to another compound run in a separate assay normalized to a *reference agonist with high efficacy.* Comparing folds activities, which looks at the magnitude of compound-induced activity relative to baseline, enables a scientist to make a conclusion that is not influenced by differences in reference agonist responsiveness. Also, the fold activity result of a control compound can be useful to quality control chart, tracking changes in assay responsiveness over time.

Calculation:

$$\text{Fold Act \& Fold Act Max} = \frac{\text{Raw data response in presence of Test Cmpd}}{\text{Min}}$$

Min = Raw basal activity of constitutive receptor.

Relative AUC

Relative AUC (Area Under the Curve) is defined as the ratio of the area under the fitted concentration-response curve for the test compound to the area under the fitted concentration-response curve for the reference compound. Specifically, areas are calculated as the area under the curve that lies above the horizontal line y = 0%. The area calculation corresponds to the shaded region in the figure below, where the contribution to the area as one move along the concentration axis is proportional to the log of the concentration distance covered, **not** the linear concentration distance covered. One should calculate the area using an exact formula when it is available, as is the case for the 4PL and 3PL models. Otherwise, one may use an approximation method, such as the trapezoid rule. In either case, for the calculated value of relative AUC to be meaningful, the areas for both the test and reference compounds should be computed in the same concentration range. Likewise, the comparison between two relative AUCs is only meaningful when each is computed in the same concentration range. If the same concentration range was not used for assaying the test and reference compounds, the equations for the fitted curves may be used for extrapolation in order to compute the components of the relative AUC over the same concentration range.

Rel AUC is useful with functional assays in which compounds are measured with varying efficacies (agonists and partial agonists) and potencies. Because Rel AUC measures the area of activity, *both efficacy and potency data are essentially combined, generating a value that provides an overall assessment of activity and selectivity between tested compounds.* However, Rel AUC should not be a substitute but rather a supplement to individual efficacy and potency data during the analysis process. Figure 2 illustrates the "area of activity" that is used in the calculation as Rel AUC.

Calculation:

$$\text{Rel AUC} = \left[\frac{\text{AUC of Test compound}}{\text{AUC of Reference compound}} \right]$$

Notes:

- A four-parameter curve fit should be used for the Ref Agonist.
- The maximum and minimum asymptotes should be defined by the data for the Ref Agonist
- Calculation of Rel Eff assumes that both the test compound and positive control each have a defined Top asymptote.

Orphan Receptors – Stimulation Mode

Assays exist for which there are no identified positive control or reference agonist compounds. An example of this situation is an assay that utilizes an "orphan" target as a bio-entity. An "orphan" target is a bio-entity that has a primary sequence suggesting it is a member of one of the super families of biological targets; however, *no ligand for this "receptor" has been identified*. Generally, it is the aim of the research effort to identify ligands for this "orphan" so that a protocol for a validated assay can be developed. Until at least enough data is gathered to identify a ligand for these types of bio-entities, assays utilizing them will be considered "validated" at only the hit to lead level. During this period, responses to individual concentrations of test substances can be normalized by one of the following formulae, which either make use of a known nonspecific activator or simply use basal activity of the constitutive receptor.

Orphan Receptors Normalized to Nonspecific Activator

Stimulation with a UOM of % should be calculated for responses to individual concentrations of test substances.

$$\text{Stim (\%)} = \left[\frac{(\text{Response in presence of Test Cmpd} - \text{Min})}{(\text{Max} - \text{Min})} \right] x100$$

Max = fully activated by nonspecific activator.

Min= constitutive basal activity of the receptor (no activation)

Orphan Receptors Normalized to Constitutive Receptor

Responses to individual concentrations of test substances that increase the measured activity of the orphan target are normalized to the basal level of activity of the target measured in the absence of the test substance. These responses can be expressed as either a percent of the basal activity or as a fold of the basal activity using one of the following

Calculation:

$$\text{Fold Act \& Fold Act Max} = \frac{\text{Raw data response in presence of Test Cmpd}}{\text{Min}}$$

Min = Raw basal activity of the constitutively active receptor

Notes:

- Results from this equation can generate percents much greater than 100.
- Expression of Fold Act or Fold Act Max should only be determined until either a nonspecific activator or ligand is identified; and should only be used to rank order compounds tested in the same assay.

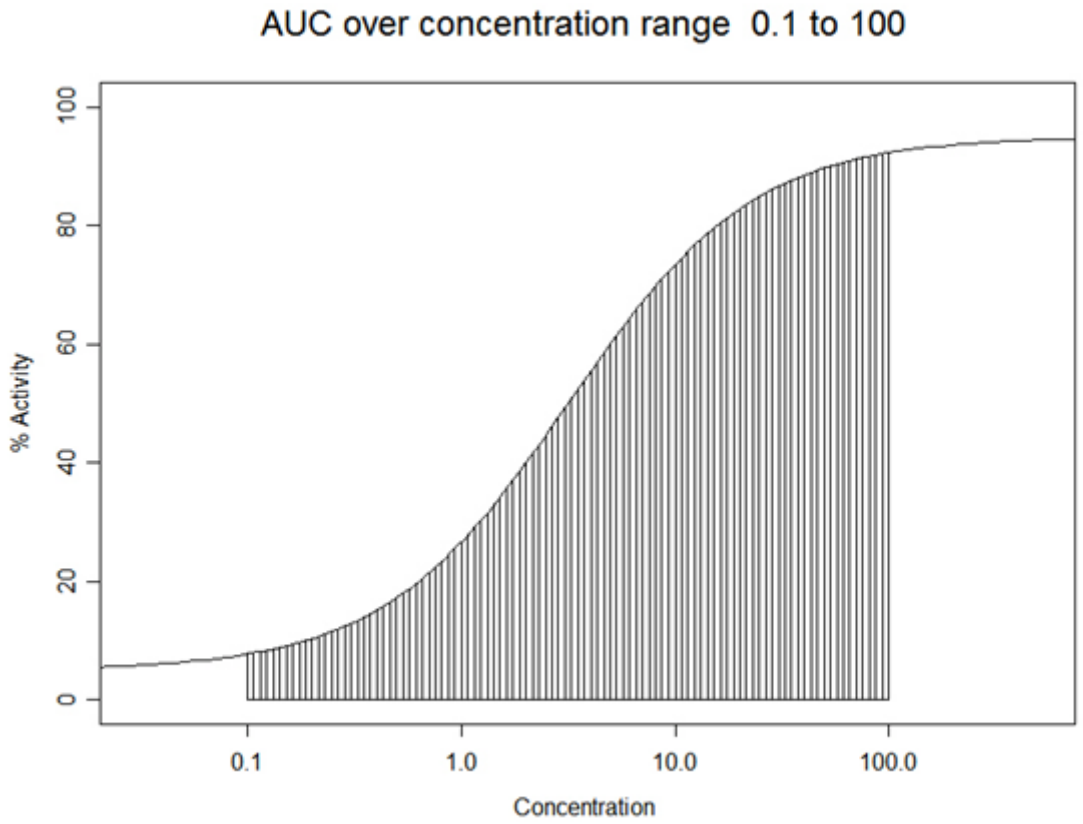

Figure 2: The "area of activity" that is used in the calculation as Rel AUC.

- The calculated Fold Act or Fold Act Max value is expected to be greater than 1 for an agonist. If the calculated value is less than 1, the test compound could be an inverse agonist.

Potentiators

Potentiation assays measure the ability of an *test substance to augment the response produced by a relatively low concentration of an active substance in some biological system*. Currently, these assays are run in *one of two modes*. The following paragraphs address the most frequently used mode.

The first mode involves the addition of one or more concentrations of a test substance in the presence of a fixed concentration of the known active substance called the "Reference Agonist". In this mode, potentiation is the response produced by the combination of substances minus the response produced by the specific concentration of Reference Agonist alone. *But, how does one normalize this response?*

It is recognized that potentiation assays might be executed when no known potentiator exists. However, no potentiation assay should be run without the existence of a known Reference Agonist. Therefore, the response to the specific concentration of the Reference Agonist plus the test substance (potentiation) should be normalized to the fitted Top of a concentration response curve of the Reference Agonist, determined at least once in every run of the assay. The frequency of the determination of the concentration response curve of the Reference Agonist for the purpose of normalizing other responses in any potentiation assay would be depend upon other factors such as plate variability and run-to-run reproducibility.

Normalized Data

Stimulation with a UOM of % should be calculated for responses to the Reference Agonist.

Potentiation with a UOM of % should be calculated for responses to individual concentrations of test substances.

Calculation:

$$\text{Pot (\%)} = \left[\frac{(\text{Response in presence of Test Cmpd \& Challenge Dose - Min})}{(\text{Max - Min})} \right] x100$$

Min = Response in the presence of challenge dose EC_{10} of reference agonist

Max = Response in the presence of full agonist dose

Notes:

- This provides for a Potentiation equal to 0% when the response to the combination of test substance and Reference Agonist is equal to the response to the Reference Agonist alone (e.g. a test substance that is not a potentiator).

Derived Data

Relative EC_{50}= the molar concentration of a substance that produces 50% of that test substance's maximum stimulation.

Relative Potentiator Efficacy: There is little if any discussion in the scientific literature addressing a standard term or calculation of efficacy of a potentiator. It is suggested that this result type be termed Relative Potentiator Efficacy (or Rel Pot Eff) to distinguish it from the Relative Efficacy of an agonist. It is equal to the fitted Top of the potentiation curve minus the normalized response to the specific concentration of Reference Agonist alone divided by 100 minus the normalized response to the specific concentration of Reference Agonist alone. Figure 3 illustrates the above decisions.

Inverse Agonists

According to multiple models of drug-receptor interaction, receptors have been demonstrated to exist in equilibrium between two states. These two states are R*, the active form of the receptor, and R, the inactive form.

Agonists exhibit higher affinity for the active form of the receptor. When an agonist binds to a receptor, it stabilizes the active form of the receptor, shifts the equilibrium toward the active state and produces a response in the biological system under investigation. Substances that produce this effect possess positive intrinsic activity.

Antagonists exhibit equal affinity for both forms of the receptor. When an antagonist binds to a receptor, it stabilizes the initial equilibrium between the active and inactive forms of the receptor. Therefore, no observable change in the activity of the biological system occurs. Substances of this type possess zero intrinsic activity.

Inverse agonists exhibit higher affinity for the inactive state of the receptor. When an inverse agonist binds to a receptor, it stabilizes the inactive form of the receptor, shifts the equilibrium toward that state and produces an opposite response in the biological system. These substances possess negative intrinsic activity.

Receptors have been demonstrated to exist in a constitutively active state both *in vitro* and *in vivo*. *In vitro*, the constitutive activity observed in assays utilizing transfected cell lines is generally attributed to the over expression of the receptor at levels hundreds to thousands of times higher than occur in vivo. Under these conditions, the total number of receptors in the active state is sufficiently high to produce a measurable response even when no exogenous substance has been added to the system. *The addition of an inverse agonist to the system*

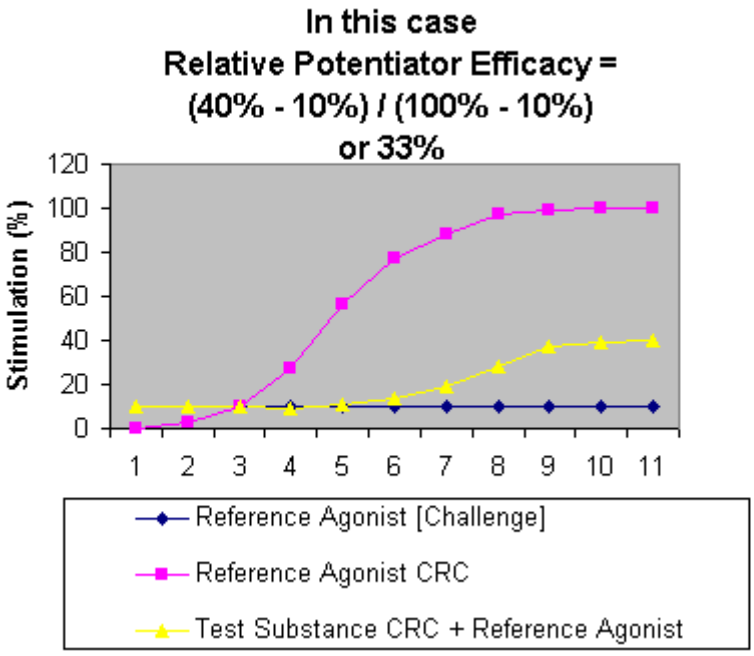

Figure 3: Relative Potentiator Efficacy example plot.

produces a decrease in the measured response. The magnitude of the decrease is related to the amount of negative intrinsic efficacy of the inverse agonist.

The possibility for confusion exists when one desires to quantify results for potential drug candidates that are inverse agonists. Some of the questions that arise are:

1. Because the measured response is a decreased activity produced by an inverse agonist, is the normalized result type Inhibition or Stimulation?
2. What is the algorithm for normalized results?
3. What is the algorithm for fitting concentration response curves?
4. Is the result type describing potency of a test substance a Relative EC_{50} or a Relative IC_{50} or another measure?
5. How is the result type describing potency of a potentiator differentiated from the potency result type for an agonist?
6. Is Relative Efficacy a negative number?

There are no absolute answers to these questions provided by the current literature; however, there is a consistent theme.

1. The most frequently used normalized result type is Inhibition with a unit of measure of % activity.
2. The dynamic range for inverse agonists is the difference between activity in the absence of, or fully inhibited, biological target and the constitutive activity. *Use of the "absence" method is preferable in early development of inverse agonist assays because it eliminates the dependency on a pre-existent known inverse agonist to compare responses of test substances to*. However, as with other functional assays, as soon as an appropriate inverse agonist has been found, it should be utilized as a positive control in the assay for the purpose of calculating relative efficacies.

Assays Normalizing Data to an Inverse Agonist Control

Normalized Data

Inhibition with a UOM of % should be calculated for responses to individual concentrations of test substances.

Calculation:

$$\text{Inh (\%)} = \left[\frac{(\text{Response in presence of Test Cmpd - Min})}{(\text{Max - Min})}\right] x100$$

Min = Response activity in presence of constitutively active receptor alone

Max = Response activity in presence of positive control and receptor

Derived Data

Relative EC_{50} Inverse = the molar concentration of a substance that produces 50% of the range of inverse agonist curve (Top – Bottom) for that particular test substance.

Rel Efficacy Inverse = 100 x (Fitted Top of the test substance expressed as %/Fitted Top of the Positive Control Reference Inverse Agonist expressed as %)

Calculation:

$$\text{Rel Eff Inv (\%)} = \left[\frac{\text{Fitted Top of Test Cmpd}}{\text{Fitted Top of Reference Inverse Agonist}}\right] x100$$

Notes:

- Because inverse agonist response curve profiles look similar to profiles generated by toxic compounds, it's advised that a confirmation assay be used to provide more evidence that a given test compound is an inverse agonist.
- Hill Coefficient and Rel Efficacy Inverse values are positive.
- The calculated Fold Act or Fold Act Max value is expected to be greater than 1 for an agonist. If the calculated value is less than 1, the test compound could be an inverse agonist.

Assays Normalizing Data to No Receptor Control (Orphan Receptor)

Normalized Data

Inhibition with a UOM of % should be calculated for responses to individual concentrations of test substances.

Calculation:

$$\text{Inh (\%)} = \left[\frac{(\text{Response in presence of Test Cmpd \& Receptor - Min})}{(\text{Max - Min})}\right] x100$$

Min = Response activity in the presence of the constitutively active receptor alone

Max = Response activity in the absence of the receptor

Derived Data

Relative EC_{50} Inverse = the molar concentration of a substance that produces 50% of the range of inverse agonist curve (Top – Bottom) for that particular test substance.

Notes:

- Because inverse agonist response curve profiles look similar to profiles generated by toxic compounds, it's advised that a confirmation assay be used to provide more evidence that a given test compound is an inverse agonist.
- Hill Coefficient and Rel Efficacy Inv values are positive.
- The calculated Fold Act or Fold Act Max value is expected to be greater than 1 for an agonist. If the calculated value is less than 1, the test compound could be an inverse agonist.

Assays Normalizing Data to Reference Agonist:

Normalized Data

Stimulation with a UOM of % should be calculated for responses to individual concentrations of test substances.

Calculation:

$$\text{Stim (\%)} = \left[\frac{(\text{Response in presence of Test Cmpd} - \text{Min})}{(\text{Max} - \text{Min})}\right] x100$$

Min = the fitted Bottom of a 4 parameter logistic curve fitting equation applied to data generated from the Reference Agonist

Max = the maximum activity of a Reference Agonist determined by the fitted Top of a 4 parameter logistic curve fitting equation applied to a concentration response curve from the positive control.

Notes:

- % Stimulation values will be negative for inverse agonist test compounds.

Derived Data

Relative EC_{50} Inverse = the molar concentration of a substance that produces 50% of that test substance's inverse agonism.

Relative Efficacy = the maximum activity of a test substance relative to a Reference Agonist. The UOM for Relative efficacy is %.

Calculation:

$$\text{Rel Eff Inv (\%)} = \left[\frac{\text{Fitted Bottom of Test Cmpd}}{\text{Fitted Top of Reference Agonist}}\right] x100$$

Notes:

- Rel Efficacy and Hill Coeff values for inverse agonists will be negative.
- Calculation of Rel Eff assumes the test compound has a defined Bottom asymptote and Reference Agonist have a defined Top asymptote.

- Because inverse agonist response curve profiles look similar to profiles generated by toxic compounds, it's advised that a confirmation assay be used to provide more evidence that a given test compound is an inverse agonist.
- The calculated Fold Act or Fold Act Max value is expected to be greater than 1 for an agonist. If the calculated value is less than 1, the test compound could be an inverse agonist.

Reference

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Glossary

Abs IC_{50}

Absolute IC50; the molar concentration of a substance that inhibits 50% of the dynamic range of the assay. In contrast to Rel IC_{50}, Abs IC_{50} is not the inflection point of the curve. It's determined to be the concentration at which 50% inhibition is realized.

Bottom

The lower asymptote of a logarithmically derived curve. The Bottom value can be determined with real values or predicted using the logarithm applied to the result data set.

CRC

Concentration-response curve mode. The mode to describe an assay performed with multiple concentrations of a given test substance, which might then render a logarithmically-derived graph curve.

E_{min}

The maximum activity of an antagonist test substance relative to a reference agonist. This is obtained by first generating a fitted top from a %Inhibition curve and then converting that to the corresponding %Stimulation of the reference agonist curve. The E-min value for antagonist mode should equal the relative efficacy for agonist mode for competitive inhibitors.

Fold Activity

The ratio of biological activity in the presence of an exogenous substance to that in its absence. It is the test compound's observed response (raw data value) divided by the median of the same plate's Min wells. This result type is used exclusively with single point assays. If the value is greater than 1, the test compound is likely an agonist. If the calculated value is less than 1, the test compound could be an inverse agonist.

Fold Activity Max

The maximum observed Fold Activity in a concentration response curve whether it was excluded or not. It is the test compound's observed response (raw data value) divided by the median of the same plate's Min wells. If the value is greater than 1, the test compound is likely an agonist. If the calculated value is less than 1, the test compound could be an inverse agonist.

Fold Activity Max (FA)

The maximum observed Fold Activity in a concentration response curve whether it was excluded or not. The (FA) indicates that his result type is summarized. Because activity can be detected at different test substance concentrations, the summarized value must be viewed with this knowledge.

Hill Coeff

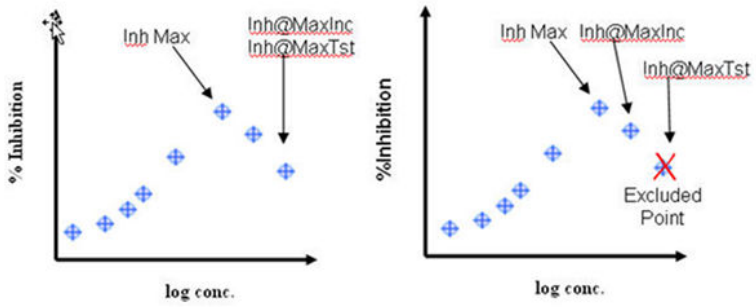
Derived slope a three or four parameter logistic curve fit. Should not be fixed to any given value without consultation with a statistician. It should not be a negative value except for inverse agonist assays.

Inh

Activity determined for a single point inhibition assay. Unit of Measure is always %.

Inh @ Max Inc

Inhibition observed at the highest included (i.e. not excluded) concentration of a substance tested in a concentration response mode method version regardless of whether it was included in the parametric fit to produce derived results. (See Illustration below)



Inh @ Max Tst

Inhibition observed at the maximum concentration of a substance tested in a concentration response mode method version regardless of whether it was included in the parametric fit to produce derived results. (See Illustration below)

Inh Max

Maximum inhibition produced by any concentration that was included for the application of a curve fit algorithm (See Illustration below).

Inh Max (FA)

Maximum inhibition produced by any concentration that was included for the application of a curve fit algorithm. This result type differs from Inh Max by allowing summarization to occur; the FA is defined as 'for averaging'. Because this result type could yield an average value from multiple test substance concentrations, the value should be used with this knowledge and therefore with caution.

K_i

Result from the Cheng-Prusoff equation or from a slightly modified derivation. This label is used primarily with binding assays (see standard texts for formula) and represents the affinity of a compound for a receptor. Documentation of the formula and any changes to the Cheng-Prusoff should be noted in the assay protocol.

K_b

Result from the Cheng-Prusoff equation or from a slightly modified derivation. This label is used primarily with functional antagonist assays (see standard texts l for formula) and represents the affinity of a compound for a receptor. This label doesn't represent results mechanistically determined via the Schild analysis; rather the label Schild Kb is used in those calculations.

Pot

Potentiation result type for single point mode. Many potentiation assays involve the addition of one or more concentrations of a test substance in the presence of a fixed concentration of the known active substance called the Reference Agonist. In this mode, potentiation is the response produced by the combination of substances minus the response produced by the specific concentration of Reference Agonist alone.

Pot @ Max Inc

Potentiation observed at the highest included concentration of a substance from an analysis of a concentration response curve.

Pot @ Max Tst

Potentiation observed at the maximum concentration of a single substance tested in a concentration response mode method version regardless of whether it was included in the parametric fit to produce derived results.

Pot Max

The maximum potentiation observed for a substance in a single run of a potentiation concentration response mode method regardless of whether it was included in the parametric fit to produce derived results.

Rel AUC

Defined as the ratio of the area under the fitted concentration-response curve for the test compound to the area under the fitted concentration-response curve for the reference compound.

Rel EC_{50}

Relative EC50; the molar concentration of a substance that stimulates 50% of the curve (Top – Bottom) for that particular substance. It can also be described as the concentration at which the inflection point is determined, whether it's from a three- or four-parameter logistic fit.

Rel EC_{50} Inv

The Relative EC_{50} of an inverse agonist.

Rel Eff

The maximum activity of a test substance relative to a standard positive control agonist. The result is expressed as percent from the following formula: 100 x Fitted Top of the test substance divided by the Fitted Top of an Agonist control. The agonist control should have a four parameter curve fit with defined lower and upper asymptotes but can have the Bottom fixed to zero in certain cases. The test compounds should have a four parameter curve fit but can have a three parameter fit with the bottom fixed to zero if the data warrants it.

Rel Eff Inv

The maximum activity of a test substance relative to a standard positive control inverse agonist. The result is expressed as percent from the following formula: 100 x Fitted Top of the test substance divided by the Fitted Top of the Inverse Agonist control. The inverse agonist control should have a four parameter curve fit with defined

lower and upper asymptotes but can have the Bottom fixed to zero in certain cases. The test compounds should have a four parameter curve fit but can have a three parameter fit with the bottom fixed to zero if the data warrants it.

Rel IC_{50}

Relative IC_{50}; the molar concentration of a substance that inhibits 50% of the curve (Top – Bottom) for that particular substance. It can also be described as the concentration at which the inflection point is determined, whether it's from a three- or four-parameter logistic fit.

Rel Pot Eff

The fitted top of the potentiation curve minus the normalized response to the specific concentration of Reference Agonist alone divided by 100 minus the normalized response to the specific concentration of Reference Agonist alone.

Stim

Activity determined for a single point stimulation assay. Unit of Measure is always %.

Stim @ Max Inc

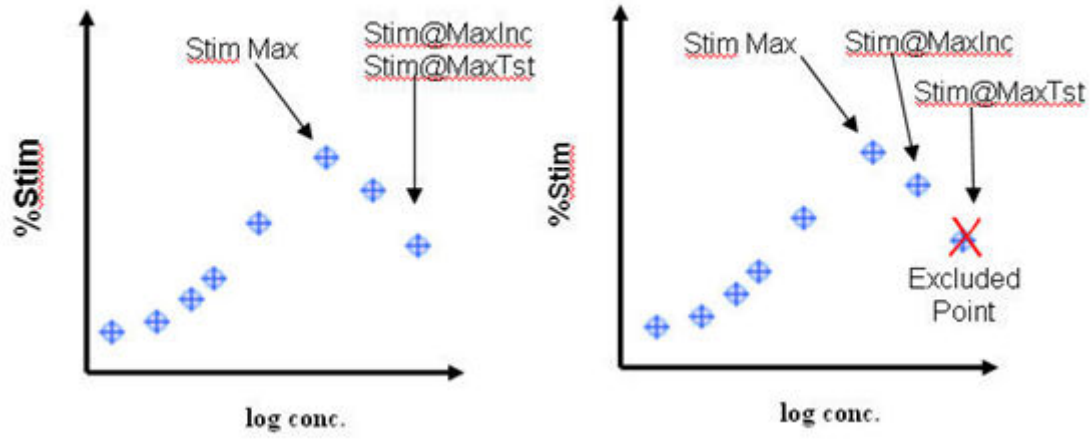
Stimulation observed at the highest included (i.e. not excluded) concentration of a substance tested in a concentration response mode method version regardless of whether it was included in the parametric fit to produce derived results. (See illustration below)

Stim @ Max Tst

Stimulation observed at the maximum concentration of a substance tested in a concentration response mode method version regardless of whether it was included in the parametric fit to produce derived results. (See illustration below)

Stim Max

Maximum stimulation produced by any concentration that was included for the application of a curve fit algorithm (See illustration below)



Stim Max (FA)

Maximum stimulation produced by any concentration that was included for the application of a curve fit algorithm. This result type differs from Stim Max by allowing summarization to occur; the FA is defined as 'for

averaging'. Because this result type could yield an average value from multiple test substance concentrations, the value should be used with this knowledge and therefore with caution.

Schild K_b

A measure of affinity for a competitive antagonist that is calculated using the ratios of equi-active concentrations of a full agonist (most typically EC_{50} concentrations are used) in the absence and presence of one or more concentrations of the antagonist. See pp. 335-339, Pharmacologic Analysis of Drug-Receptor Interaction, 3rd Ed. by Terry Kenakin.

SP

Single point mode. Assay performed with once concentration of test substance. Common result types used include Inh and Stim. Result values should always include the concentration of the test substance used to determine the activity.

Stephenson's K_p

A measure of affinity for a partial agonist that is calculated through the comparison of equi-active concentrations of a full agonist in the absence and presence of a single concentration of the partial agonist. See pp. 284-286, Pharmacologic Analysis of Drug-Receptor Interaction, 3rd Ed. by Terry Kenakin.

Top

The upper asymptote of a logarithmically derived curve. The Top value can be determined with real values or predicted using the logarithm applied to the result data set.

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