NaPDI Repository Data Entry SOP: In vitro Enzyme Inhibition Studies

Version 1

Creation Date: March 2017

Author: Jessica Tay-Sontheimer

NOTE: This version is provided as supplemental information for a manuscript. Please check the github repository for this project as any more recent version on github supercedes this version:

https://github.com/dbmi-pitt/NaPDI-SOPs

Contents

1 BACKGROUND

1.1 SCOPE

The purpose of this Standard Operating Procedure (SOP) is to describe how to enter *in vitro* enzyme inhibition results into the NaPDI repository. Natural Products (NPs) are expected to be evaluated as causative agents of inhibition (*Precipitants*). The victim drugs (*Objects*) are probe substrates of known enzymes.

Most of the information entered in the repository will come directly from the study report; avoid interpretations of the authors' conclusions. However, several text fields are provided throughout the admin site to allow the addition of relevant comments that may pertain to the experimental study design and conditions, the study results, and/or the mechanism of inhibition. This additional information should be reviewed with the principal investigators during the validation process as it will be used to enrich the users experience and understanding of the results.

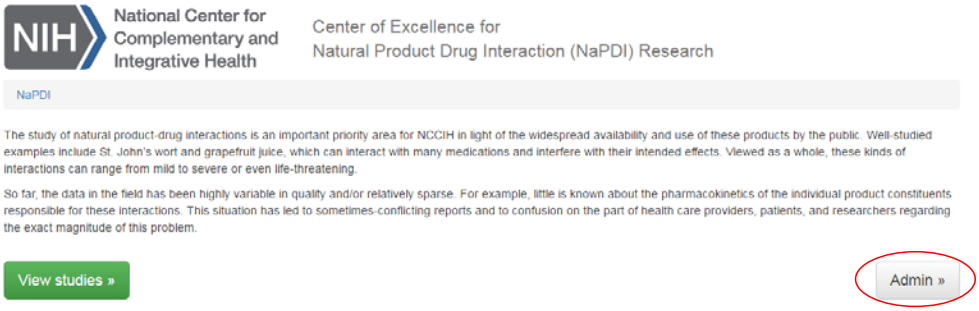
1.2 DEFINITIONS

Add user-centered definitions (alphabetically)

2 CREATING A STUDY

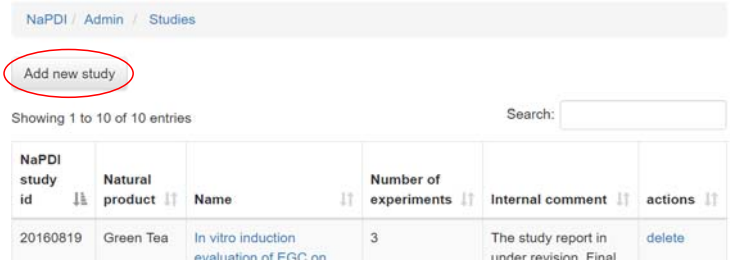
Use the following steps to create a new study.

2.1 Navigate to the Admin page of the NaPDI Repository



2.2 Using the admin page, click on "Studies"

2.3 then, click on “Add new study”



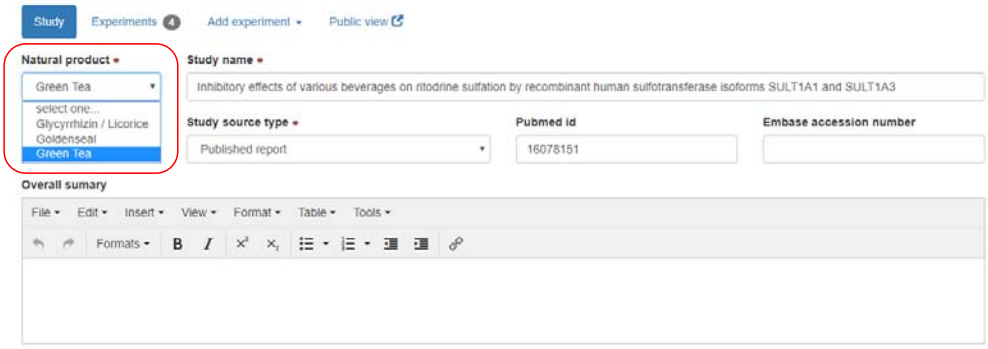
3 STUDY PAGE

A study can only accept data from one Natural Product and one species. For example, *in vitro* data with Licorice *Glycyrrhiza glabra* L., *Glycyrrhiza uralensis* Fish have to be reported in two different studies, one for each Licorice species.

3.1 Select the **Natural Product** tested in the *in vitro* study from the drop down list provided (select one; required):

- Licorice
- Goldenseal
- Green Tea

3.2 From the Study Report, enter the **Study Name** and **NaPDI Study ID** (required, as presented in Study Report).



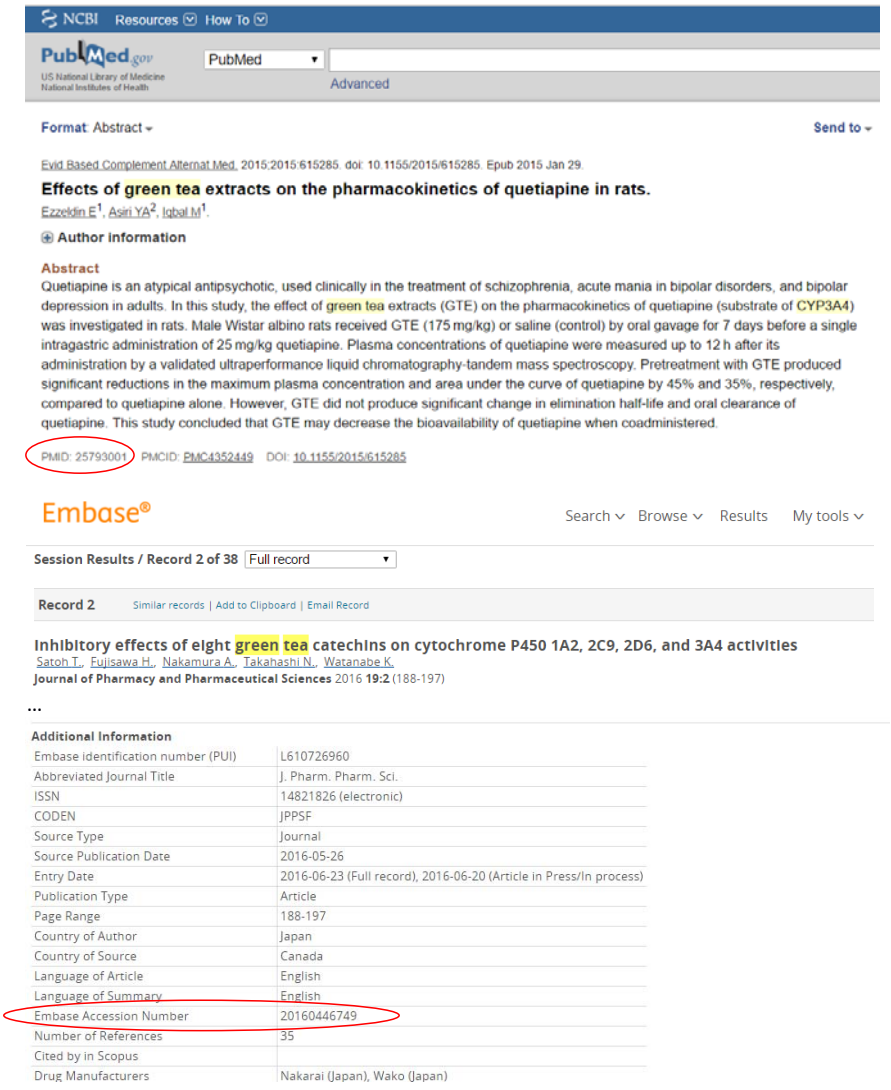
If a entries originate from a published paper, used the Pubmed ID or Embase PUI as the NaPDI Study ID (e.g., "PMID:23268924")

3.3 Select the **study source type** or source from which the study was obtained (required).

- Published report
- Manuscript prepared or submitted for peer-reviewed publication
- Unpublished data submitted through a NaPDI form

3.4 When a study has been published, enter the **PubMed ID** and/or **Embase Accession** number(s) (optional).

Tip: If the PubMed ID or Embase Accession number(s) cannot be located in the Study Report, they can be found under the abstract in PubMed or in the "Additional Information" section when the article's full record is viewed in Embase.



NCBI Resources How To

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed

Advanced

Format: Abstract

Send to

Evid Based Complement Alternat Med. 2015;2015:615285. doi: 10.1155/2015/615285. Epub 2015 Jan 29.

Effects of green tea extracts on the pharmacokinetics of quetiapine in rats.

Ezzeldin E[1], Asiri YA[2], Iqbal M[1].

Author information

Abstract

Quetiapine is an atypical antipsychotic, used clinically in the treatment of schizophrenia, acute mania in bipolar disorders, and bipolar depression in adults. In this study, the effect of green tea extracts (GTE) on the pharmacokinetics of quetiapine (substrate of CYP3A4) was investigated in rats. Male Wistar albino rats received GTE (175 mg/kg) or saline (control) by oral gavage for 7 days before a single intragastric administration of 25 mg/kg quetiapine. Plasma concentrations of quetiapine were measured up to 12 h after its administration by a validated ultraperformance liquid chromatography-tandem mass spectroscopy. Pretreatment with GTE produced significant reductions in the maximum plasma concentration and area under the curve of quetiapine by 45% and 35%, respectively, compared to quetiapine alone. However, GTE did not produce significant change in elimination half-life and oral clearance of quetiapine. This study concluded that GTE may decrease the bioavailability of quetiapine when coadministered.

PMID: 25793001 PMCID: PMC4352449 DOI: 10.1155/2015/615285

Embase®

Search Browse Results My tools

Session Results / Record 2 of 38 Full record

Record 2 Similar records | Add to Clipboard | Email Record

Inhibitory effects of eight green tea catechins on cytochrome P450 1A2, 2C9, 2D6, and 3A4 activities

Satoh T., Fujisawa H., Nakamura A., Takahashi N., Watanabe K.

Journal of Pharmacy and Pharmaceutical Sciences 2016 **19:2** (188-197)

...

Additional Information

Embase identification number (PUI)	L610726960
Abbreviated Journal Title	J. Pharm. Pharm. Sci.
ISSN	14821826 (electronic)
CODEN	JPPSF
Source Type	Journal
Source Publication Date	2016-05-26
Entry Date	2016-06-23 (Full record), 2016-06-20 (Article in Press/In process)
Publication Type	Article
Page Range	188-197
Country of Author	Japan
Country of Source	Canada
Language of Article	English
Language of Summary	English
Embase Accession Number	20160446749
Number of References	35
Cited by in Scopus	
Drug Manufacturers	Nakarai (Japan), Wako (Japan)

3.4 **Overall summary**: this summary should provide a concise overall conclusion of the *in vitro* study and also discuss the possible mechanism(s) involved (optional).

Comment [RDB1]: How many words? Studies can consist of numerous experiments. Please provide some example statements so that the scope of this comment field is more clear.

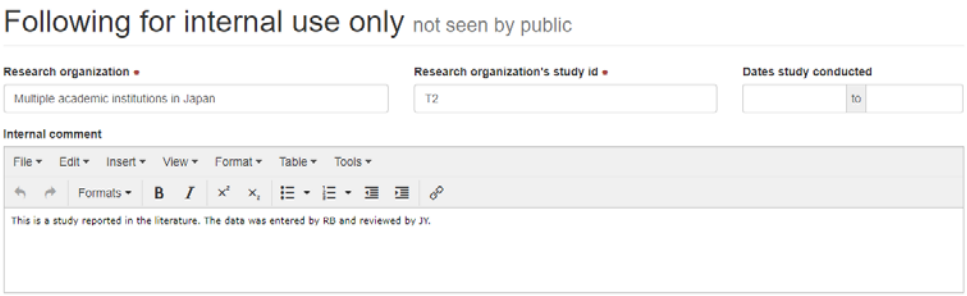
If entries are from a published paper, copy and paste the abstract into the Overall summary box.

3.5 The **Following for internal use only** section is designated for internal notes and will not be displayed to users.

3.5.1 Enter the **Research organization** name (where the study was performed) and their **study ID number** in the internal use section displayed below (required).

3.5.2 From the Study Report, enter the **dates the study was conducted** (optional). If only months are provided, select the first and last days of the month for the starting and ending date, respectively. For example, March to April, 2017 will be entered as 03/01/2017 to 04/30/2017.

3.5.3 Enter **internal comments** associated with the study that are intended for internal use only (optional).



3.6 Select the status of the current study entry

- Draft – selected when the curator is in the process of entering the data or checking the data
- Pending review – selected when the study had been fully entered by the curator and needs to be reviewed and validated by a second editor
- Published – selected after validation and is ready for public display

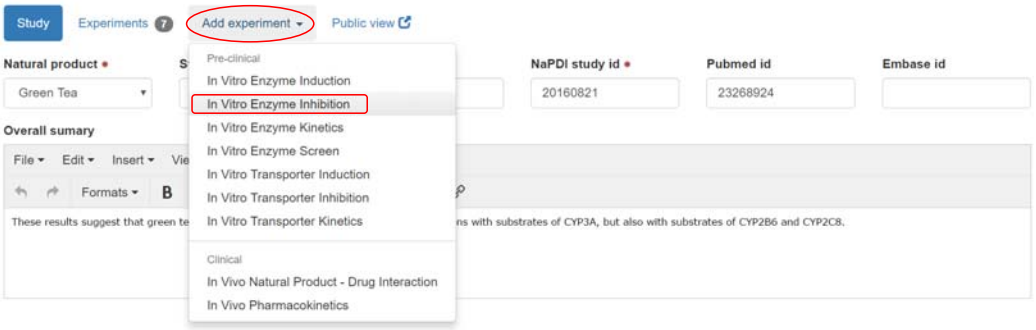
Comment [JS2]: Tell chris that a non-unique ID triggers an error.

Comment [RDB3]: Please refer to a specific reference that these ids will come from.

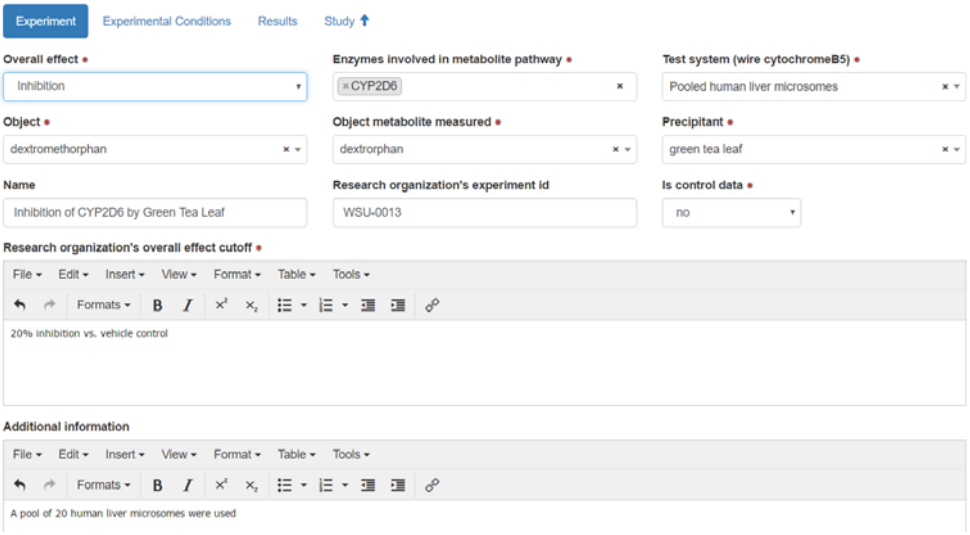
4 EXPERIMENT

After a study has been created, use the following steps to add a new experiment. Add a new experiment for determinations of parameters having a distinct set of experimental conditions from previous experiments (i.e., different object concentrations, different inhibitor concentrations, etc.).

4.1 Click on **add experiment**, then select **In Vitro Enzyme Inhibition** from the drop-down menu.



4.2 Select the **Overall effect**: "Inhibition", or "Negligible Inhibition" of the metabolism of the *Object,* based on study findings stated in the Study Report (select one; required). Use the authors' conclusions to make this determination. For example, if inhibition was observed, but the authors concluded no effect because it was not statistically significant, then "Negligible Inhibition" should be selected. Conversely, if 19% inhibition was observed with a 20% cut-off and the authors concluded weak inhibition, then "Inhibition" should be selected.



4.3 Select the **Enzyme(s) involved in the metabolite pathway** as stated in the Study Report (select many; required). Multiple selections can be made; therefore, select all enzymes responsible for the formation of the metabolite (if specific formation of metabolite is measured) or parent disappearance (if parent disappearance is measured).

Notes:

(1) When an enzyme studied is not listed in the drop-down menu, add the enzyme or state the enzyme in the **Additional information** section (see below).

(2) When variant enzymes are studied, select the variant enzyme and specify the variant in the **Additional information** text box (see below).

4.4 Select the *in vitro* **test system** used (select one; required).

Notes:

(1) For **recombinant expression systems** only, appropriately select **Cytochrome b5** conditions.

Choose:

- "Yes, co-expressed" if Cytochrome b5 was used and co-expressed in the recombinant system
- "Yes, supplemented" if Cytochrome b5 was used and was added to the incubation
- "No" if Cytochrome b5 was not used
- "Not available" if conditions regarding Cytochrome b5 are not provided in the Study Report

(2) If a study used a few donors of human liver microsomes, but each donor was evaluated individually, and results were presented as the mean value from all the donors, select

"pooled" source and add a comment stating that experiments were conducted in HLMs from individual donors in the **Additional information** section.

4.5 Select the **Object** and **Object metabolite** measured from the compound lists (select one; required). If the compound is not listed, add the compound. Enter only the study vehicle control (i.e. not the positive or negative control) as the object. Enter the details regarding the positive/negative control in the Additional Information section under this tab.

4.6 Select the **Precipitant** from the compound list (select one; required). If the compound is not listed, add the compound.

4.7 Enter an experiment **Name**, if provided (optional). Use title case, where the first word and all major words are capitalized (i.e., "Inhibition of CYP2D6 by Green Tea Leaf", NOT "Inhibition of CYP2D6 by green tea leaf".) Experiment names are used as sub-headings in the public view; therefore, names that describe the enzyme(s) involved in the pathway of the metabolite and the precipitant used in the study are best suited for this purpose.

4.8 If provided, enter the **research organization's experiment identification number** for this experiment only (optional).

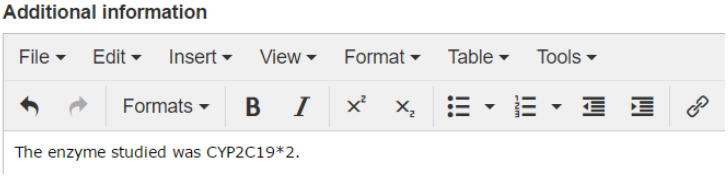
4.9 If this data corresponds to the control experiment for the study, choose "yes" from the **Is control data** drop-down menu (select one; required). Otherwise, choose "no". The "Is control data" function allows experiments to be linked within the repository. It only appears on the admin side and not in the public view.

4.10 Enter the **Research organization's overall effect cutoff** (required) described in the Study Report (for example, "20% inhibition versus control"). Enter multiple cut-offs if more than one is provided in the Study Report. If the authors did not provide this, attempts should be made to obtain this information. Enter "not applicable" when the cut-off is not applicable.
For published reports, enter "not available" or "N/A" when this information is not available or not applicable.

4.11 Enter **Additional information** important to the overall study, but where the details were not included in the fields above (optional). Enter only the study vehicle control (i.e. not the positive or negative control) as the object. Enter the details regarding the positive/negative control in the Additional Information section under this tab.

For example:

- variant enzymes, other enzymes or test systems not listed in the drop-down menu



- variations on the precipitant selected

Goldenseal flower bud methanol extract was used.

- results of other experiments used to determine the enzyme(s) involved in the formation of the metabolite

The enzymes responsible for the formation of the metabolite were identified through phenotyping studies.

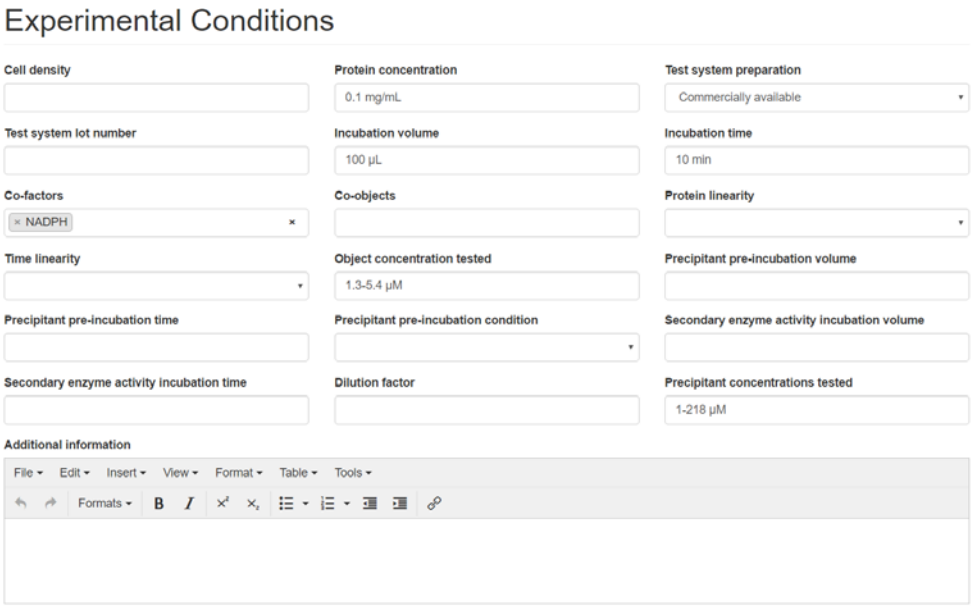
Comment [RDB4]: It will be REALLY helpful if folks can save as they complete each section, or just anytime at all. In fact, these days, people are used to data being saved as they type it in. Rather than 'submit' or 'save; one could 'validate' or 'finalize submission' or similar.

5 EXPERIMENTAL CONDITIONS

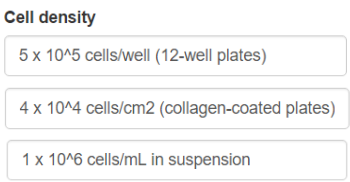
Enter experimental details that are provided in the Study Report.

Note that in many cases, some of the provided fields may be left blank.

> When entering conditions from published literature that refer to experimental conditions described in a reference, check the reference for conditions that are not clearly stated in the article. For example, if an article states that CYP1A2 substrates and concentrations used in Vivid CYP screening assays were used as described in Cheng et al. and the authors do not describe any further details, check Cheng et al. for experimental conditions and enter those stated therein. Also, make a comment in the additional information section regarding which parameters were extracted from the reference citation (*e.g.*, Object and object concentrations tested were extracted from Cheng et al., 2017).



5.1 Cell density: Enter the cell density in scientific notation. Specify the units and the type of culture plates or dishes used (optional). Examples are shown below:



5.2 **Protein concentration:** Enter the total protein concentration and specify the units as they are presented in the Study Report (optional). If a protein amount and volume used are provided, calculate the concentration by dividing the amount by the volume (*e.g.*, 0.1 mg/0.5 mL = 0.2 mg/mL).

Comment [RDB5]: Is this conversion required?

Protein concentration

0.2 mg/mL

5.3 **Test system preparation:** Select one of the following from the drop-down menu (select one; optional):

- **In-house preparation** – select this when the *in vitro* test system used was prepared in house
- **Commercially available** – select this when the *in vitro* test system used originally provided by a commercial vendor

5.4 **Test system lot number**: If provided, enter the test system lot number for those that were provided by a commercial vendor (optional).

Test system lot number

06103045

5.5 **Incubation volume**: Enter the total volume used in the incubation, specify the units as they are presented in the Study Report (optional). If volumes were listed in steps, the total incubation volume may be calculated by adding the volumes used in each step (*e.g.*, 100 µL buffer + 10 µL NADPH + 40 µL compound tested + 50 µL precipitant used = 200 µL total volume).

Incubation volume

200 µL

5.6 **Incubation time**: Enter the duration of the incubation, specify the units as they are presented in the Study Report (optional). This duration implies the presence of all necessary components of the incubation (*i.e.*, the enzyme, the object, the precipitant, and if used, co-factors). Specify pH for experiments using conditions other than pH 7.4

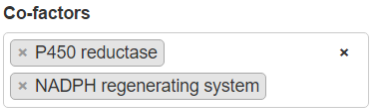
Incubation time

5 min

6 Incubation time

30 min at pH 6.8

5.7 **Co-factors**: Select co-factors used in the incubation from the drop-down list provided. Multiple co-factors may be selected as needed (select many; optional).



5.8 **Co-substrates**: Select co-substrates used to study enzyme activity from the drop-down list provided (select many; optional).

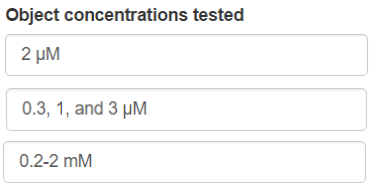
5.9 **Protein linearity**: Select one of the following from the drop-down menu (select one; optional):

- **Available** – select this when the linearity of product formation or substrate depletion with protein concentration is tested
- **Not available** - select this when no indication of testing linearity of product formation or substrate depletion with protein concentration is provided

5.10 **Time linearity**: Select one of the following from the drop-down menu (select one; optional):

- **Available** – select this when the linearity of product formation or substrate depletion with incubation time is tested
- **Not available** - select this when no indication of testing linearity of product formation or substrate depletion with incubation time is provided

5.11 **Object concentrations tested**: Enter the object concentration(s) used in the incubation, specify the units as they are presented in the Study Report (optional). Enter only the study vehicle control (i.e. not the positive or negative control) as the object. Enter the details regarding the positive/negative control in the Additional Information section under this tab. A single concentration, multiple concentrations or a range of concentrations may be entered, see below for examples. If possible, avoid entering "0" as a starting concentration, but rather, use the lowest concentration provided as the starting concentration (*i.e.*, do not enter 0-2 mM, but rather enter 0.2-2 mM).



5.12 **Precipitant concentrations tested:** Enter the precipitant concentration(s) used in the incubation, specify the units as they are presented in the Study Report (optional). A single concentration, multiple concentrations or a range of concentrations may be entered.

Precipitant concentrations tested

5 nM

2, 10, and 50 µM

1-10 µM

****

The following fields are to be used for mechanism-based or time-dependent inhibition studies only:

5.13 Precipitant pre-incubation volume (optional): Enter the total volume used for the primary incubation (inactivation of the enzyme), specify the units as they are presented in the Study Report (*e.g.*, 100 µL).

5.14 Precipitant pre-incubation time (optional)**:** Enter the duration used for the primary incubation (inactivation of the enzyme), specify the units as they are presented in the Study Report (*e.g.*, 10 min).

5.15 Precipitant pre-incubation condition (select one; optional)**:**

- **NADPH with precipitant** – select this when both NADPH and the precipitant are present in the primary incubation (inactivation of the enzyme)
- **NADPH with no precipitant** – select this when NADPH but not the precipitant is present in the primary incubation (inactivation of the enzyme)
- **No NADPH with precipitant** – select this when the precipitant but not NADPH is present in the primary incubation (inactivation of the enzyme)
- **No NADPH with no precipitant** – select this when neither NADPH nor the precipitant is present in the primary incubation (inactivation of the enzyme)

5.16 Secondary enzyme activity incubation volume (optional)**:** Enter the total volume used for the secondary incubation (measurement of enzyme activity), specify the units as they are presented in the Study Report (*e.g.*, 200 µL).

5.17 Secondary enzyme activity incubation time (optional)**:** Enter the duration used for the secondary incubation (measurement of enzyme activity), specify the units as they are presented in the Study Report (*e.g.*, 30 min).

5.18 Dilution factor (optional)**:** Enter the dilution factor used going from the primary to the secondary incubation (*e.g.*, 1:10).

****

5.19 Additional information (optional)**:** As needed, add any other information that is important to the experimental conditions, but that were not detailed in the fields above. If reporting a vehicle control, enter the details regarding the positive/negative control in this section. In the public view, this section will appear before any of the other experimental details entered.

Examples of additional information might include issues limiting the experimental design (*e.g.*, solubility), deviations from physiological pH (*e.g.*, studies were conducted at pH 6.0), details regarding substrate cocktail assays, etc.

Additional information

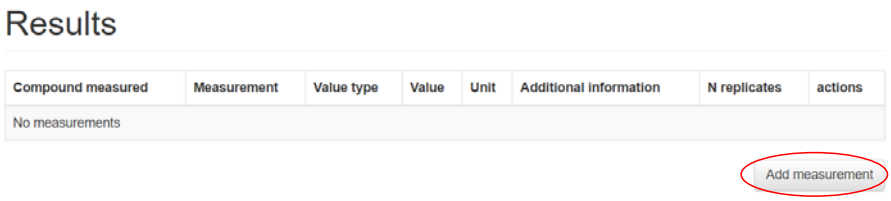
File Edit Insert View Format Table Tools

Formats

Due to solubility issues, the highest tested concentration of green tea leaf was 218 µg/mL.

6 RESULTS

6.1 Use the **Add measurement** function to add a new measurement to the table of results.



The object compound (or metabolite) name selected in the experiment page will automatically be populated in the **Compound measured** field.

6.2 Select a **Measurement type** (select one; required) from the drop-down list, the associated **Unit** (select one; required) and the **Value Type** (select one; required) based on the available data in the Study Report. Use separate entries for each type of measurement. Available measurement types include:

Measurement type	Selection criteria
% Inhibition$_{pre\text{-}incubation}$	The percent inhibition is reported during the pre-incubation phase of a mechanism-based or time-dependent inhibition assay.
% Inhibition$_{co\text{-}incubation}$	The percent inhibition is reported during the co-incubation phase of a mechanism-based or time-dependent inhibition assay.
% Inhibition	Percent inhibition is reported.
Ki total	Ki corresponding to the total (bound and unbound) precipitant is reported. For published literature: If apparent Ki is reported without information regarding protein binding, select Ki total and make a note in the additional information section.
Ki unbound	Ki corresponding to the unbound precipitant is reported.
$IC_{50\ pre\text{-}incubation}$	The IC_{50} value reported during the pre-incubation phase of a mechanism-based or time-dependent inhibition assay.
$IC_{50\ co\text{-}incubation}$	The IC_{50} value reported during the co-incubation phase of a mechanism-based or time-dependent inhibition assay.
IC_{50}	IC_{50} value is reported.
IC_{50} fold-shift	The IC_{50} fold-shift (fold-change) is reported.
K_{inact}	For mechanism-based or time-dependent inhibition, the K_{inact} is reported.

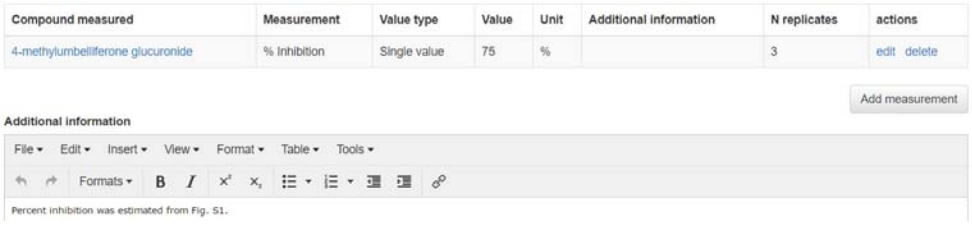
K_I	For mechanism-based or time-dependent inhibition, the K_I is reported.
K_{inact}/K_I	For mechanism-based or time-dependent inhibition, the K_{inact}/K_I is reported or this value can be calculated from the K_{inact} and K_I values provided.

According to the selections, appropriate fields will appear, including the following:

6.2.1 **Value** (select one; required) – based on the Study Report choose mean, median, or single value (mean or median is not specified) for the parameter to be entered. Before the value field, select ">", "≥", "<", or "≤" when provided in the Study Report; "=" is the default if no selection is made.

Comment [JS6]: Chris has not yet implemented this

For data from published articles: If the **percent inhibition** value is not explicitly stated in the article it can often be estimated from presented figures. Estimate the percent inhibition and in the additional information box located directly below the results, state that the value entered was estimated and specify the information source.



6.2.2 **Variability** (required) – enter the standard error of the mean (**SEM**), percent coefficient of variation (**%CV**), standard deviation (**SD**), 90% or 95% confidence interval (**90% CI**, **95% CI**) or **range** associated with the value.

6.2.3 Enter the total number of **(N) replicates** (required) used in the study (e.g., enter 1 when only one test was conducted, 3 when a total of 3 replicates were used, etc.).

Comment [JS7]: Ask chris to add a range as an input option for N replicates and to make this field optional as this is not always provided in published literature

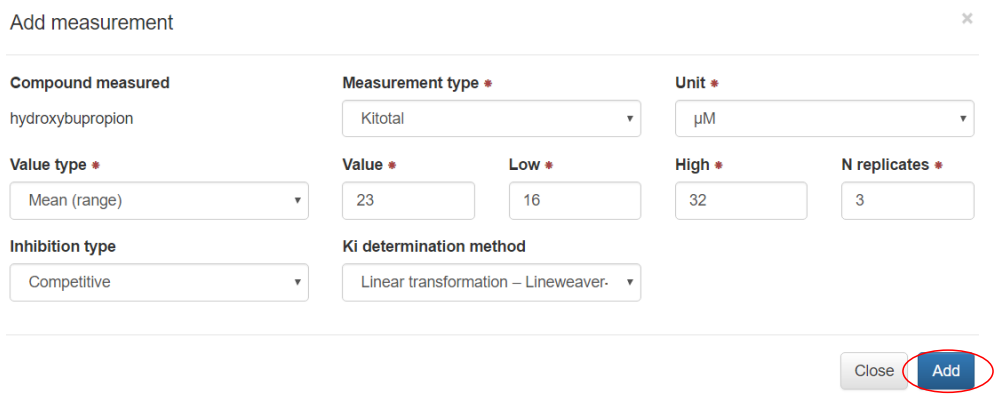
6.2.4 When statistical tests are conducted, select a significance level of the **P value** tested or **not significant** (optional) when the null hypothesis is true. If the authors give a P value that is not available in the drop-down menu, chose the level of significance that is true (e.g., if P = 0.0009 in the Study report, choose P < 0.001).

6.2.5 **Ki determination**: according to the Study Report, select the method used to determine Ki from the following options:

- Nonlinear least-squares regression
- Linear transformation – Eadie-Hofstee plot

- Linear transformation – Lineweaver-Burk plot
- Linear transformation – Dixon plot
- Graphic Read
- Not Available

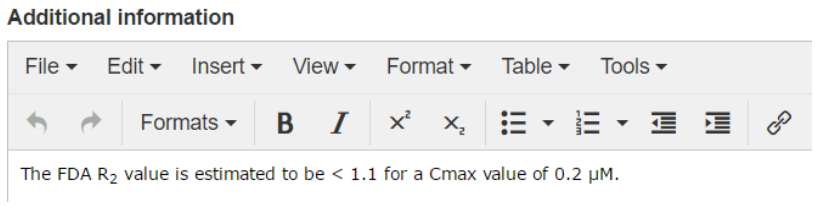
6.3 When all PK measurements have been entered for that entry, click **Add**.

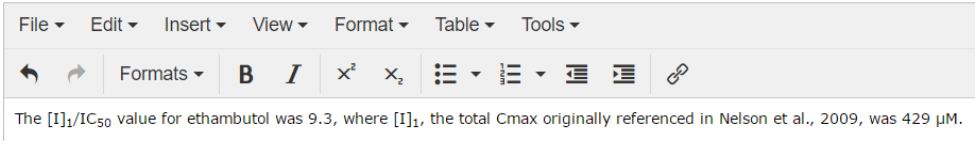


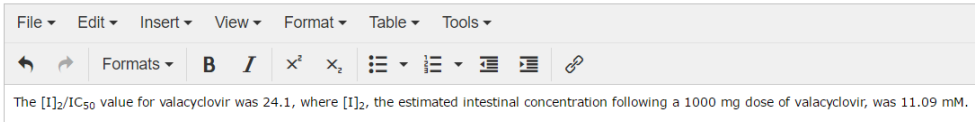
6.4 **Additional Information:** as needed, add any other information that is important to the result, but that were not detailed in the results table. If reporting a vehicle control, enter the details regarding the positive/negative control in this section.

For example,

In vivo DDI predictions ($[I]/IC_{50}$ or $[I]/K_i$ ratios, R_2 values, etc.) provided in the Study Report. Indicate the C_{max} and dosing information provided, otherwise, cite the original references (First Author, Year).

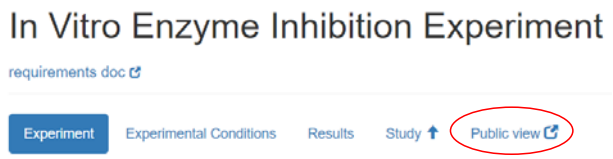






Click Submit to save the entries.

After submitting the study entry, it can be viewed as it will appear to the public by clicking on the "Public View" function near the top of the page.



Note regarding units: For consistency use the following abbreviations for the specified units below. If a unit is not listed below, use the units specified in the Study Report.

Unit	Abbreviation
hour(s)	h
minute(s)	min
second(s)	s
day(s)	day(s)
liter	L
per unit	/unit (*e.g.*, /min)
micro	µ
fold	-fold (*e.g.*, 3.2-fold)
exponents	^ (*e.g.*, 10^-6)
less than, less than or equal to	< , ≤
greater than, greater than or equal to	> , ≥
plus or minus	±

- Use molar concentration rather than moles per liter (i.e., use µM rather than µmol/L). In the case of natural products, the use if grams per liter (i.e., µg/mL) may be necessary.
- Do not convert gram concentrations (*e.g.*, µg/mL) to molar concentrations (*e.g.*, µM), even if the molecular weight of the compound is provided.
- If the units provided for a given field are different from the units in its corresponding drop-down menu, convert the units provided in the study report to the units provided in the drop-down menu. If this is not possible (for example, µg/mL cannot be converted to µM for natural product

Comment [RDB8]: Please clarify. Does this mean don't use molar units per L? Are there any conversions that the computer can do to make it simpler for the annotator and likely more accurate and consistent overall?

mixtures because there is not a molecular weight available for the conversion), add the new unit to the drop-down menu.