NaPDI Repository Data Entry SOP: Metabolomic Studies

Version 1

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# Background

* 1. Scope

The purpose of this Standard Operating Procedure (SOP) is to describe how to enter *in vitro* enzyme induction results into the NaPDI repository. Natural Products (NPs) are expected to be evaluated as causative agents of induction (*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 induction. 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. Definitions

Add user-centered definitions (alphabetically)

# Creating a study

Use the following steps to create a new study.

* 1. After logging in, navigate to the “Studies” page

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* 1. then, click on “Add new study”

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# Study Page

A study can only accept data from one Natural Product and one species or from a compound. 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. There is also the potential for studies to have the focus of a single compound that may exist in multiple species of an Natural Product such as *licoricidin*, a compound found in both *Glycyrrhiza glabra* L*.*, *Glycyrrhiza uralensi*. A characterization of material

* 1. Select **Natural Product** from the dropdown **Subject of study**
  2. Select the **Natural Product** with binomial tested in the study from the drop down list provided (select one; required):
* Cannabis (Cannabis sativa)
* Goldenseal (Hydrastis canadensis)
* Green Tea (Camellia sinensis)
* Kratom (Mitragyna speciosa)
* Licorice (Glycyrrhiza glabra)
* Licorice (Glycyrrhiza uralensis)
* Licorice (Glycyrrhiza inflata)
  1. From the Study Report, enter the **Study Name** and **NaPDI Study ID** (required, as presented in Study Report).

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

* 1. 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
  1. When a study has been published, enter the **PubMed ID** and/or **Embase** **Accession** number(s) (optional)

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**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.

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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).

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

* 1. The **Following for internal use only** section is designated for internal notes and will not be displayed to users.
     1. Enter the **Research organization** name (where the study was performed) and their **study ID number** in the internal use section displayed below (required).
     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. **Of**
     3. Enter **internal comments** associated with the study that are intended for internal use only (optional).

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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

# Experiment

*After* a study has been created, use the following steps to add a new experiment.

* 1. Click on **add experiment**, then select **Characterization of Material** from the drop-down menu.

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* 1. Enter an experiment **Name**, if provided. Use title case, where the first word and all major words are capitalized (i.e., “Induction of CYP3A4 by Green Tea Leaf”, NOT “Inhibition of CYP3A4 by green tea leaf”.) Experiment names are used as sub-headings in the public view.
  2. If provided, enter the **research organization’s** **experiment identification number** for this experiment only (optional).
  3. 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, if the vehicle itself is not specified in the study. Enter the details regarding the positive/negative control in the Additional Information section under this tab.

4.10.1 If the vehicle is specified, enter the vehicle experiment data as a separate experiment, if possible

For example:

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

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* variations on the precipitant selected

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# 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).

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   1. **Material Preparation – Mass of sample material:** Enter the mass of the sample material (optional).

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* 1. **Material Preparation – Volume of extraction vessel:** Enter the volume of the extraction vessel (optional).



* 1. **Material Preparation – Solvent used**: Enter the solvent that was used (optional).



* 1. **Material Preparation – Volume of solvent**: Enter the volume of the solvent that was used (optional).



* 1. **Material Preparation – Temperature of storage**: Enter the temperature the sample was stored at (optional).



* 1. **Material Preparation – Additional information**: As needed, add any other information that is important to the experimental conditions regarding material preparation, but that were not detailed in the fields above. In the public view, this section will appear before any of the other experimental details entered.

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* 1. **NMR Analysis – NMR instrument used:**  Enter the instrument that was used for NMR analysis (optional).



* 1. **NMR Analysis – Nucleus:**  Select one of the following from the dropdown menu (select one; optional).
     + **1H (proton)**
     + **13C (carbon)**
     + **15N (nitrogen)**
  2. **NMR Analysis – Field strength:**  Enter the field strength of the NMR analysis (optional).

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* 1. **NMR Analysis – Solvent used:**  Select one of the following from the dropdown menu (select one; optional).
     + **Methanol-d4 (CD3OD)**
     + **Chloroform-d (CDCI3)**
     + **Acetone-d6 ((CD3)3)**
     + **Dimethyl sulfoxide-d6 ((CD3)2SO)**
     + **Pyridine-d5 (C5D5N)**
  2. **NMR Analysis – Sample concentration:**  Enter the concentration for the sample used in the NMR analysis (optional).

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* 1. **NMR Analysis – Additional information**: As needed, add any other information that is important to the experimental conditions regarding material preparation, but that were not detailed in the fields above. In the public view, this section will appear before any of the other experimental details entered.

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* 1. **Mass Spectrometry – Instrument used:** Enter the instrument used in the mass spectrometry analysis (optional).

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* 1. **Mass Spectrometry – Sample concentration:** Enter the sample concentration of the mass spectrometry analysis (optional).

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* 1. **Mass Spectrometry – Tandem spectra:** Enter the tandem mass spectra (optional).

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* 1. **Mass Spectrometry - Ionization:** Enter the ionization of the mass spectrometry analysis (optional).

****

* 1. **Mass Spectrometry – Ionization mode:** Select one of the following from the dropdown menu (select one; optional).
     + **Positive**
     + **Negative**
     + **Positive/Negative switching**
  2. **Mass Spectrometry – LC Instrument:** Enter the liquid chromatograph used in the mass spectrometry analysis (optional).

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* 1. **Mass Spectrometry – Solvent system:** Enter the solvent system used in the mass spectrometry analysis (optional).

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* 1. **Mass Spectrometry – Gradient conditions:** Enter the gradient conditions used in the mass spectrometry analysis (optional).

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* 1. **Mass Spectrometry – Flow rate:** Enter the flow rate used in the mass spectrometry analysis (optional).

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* 1. **Mass Spectrometry – Column used:** Enter the column used in the mass spectrometry analysis (optional).

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* 1. **Mass Spectrometry – Additional information:** As needed, add any other information that is important to the experimental conditions regarding material preparation, but that were not detailed in the fields above. In the public view, this section will appear before any of the other experimental details entered.

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* 1. **Metabolite Quantification – Method of quantification:** Enter the method of quantification of the metabolite (optional).

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* 1. **Metabolite Quantification – Solvent Used:** Enter the solvent used for the metabolite quantification (optional).

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* 1. **Metabolite Quantification – Number of calibration points:** Enter the number of calibration points for the metabolite quantification (optional).

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* 1. **Metabolite Quantification – Sample concentration range:** Enter the concentration range of the sample used in metabolite quantification (optional).

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* 1. **Metabolite Quantification – Curve fitting method:** Enter the curve fitting method used in metabolite quantification (optional).

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* 1. **Metabolite Quantification – Weighting method:** Enter the weighting method used in metabolite quantification (optional).



* 1. **Metabolite Quantification – Methods:** As needed, add any other information that is important to the experimental conditions regarding material preparation, but that were not detailed in the fields above. In the public view, this section will appear before any of the other experimental details entered.

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* 1. **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. 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), etc.

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# PCA Methods

Enter details regarding how the principal component analysis was completed.

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* 1. **Missing value**: Too many missing values can cause difficulties in analysis. Enter the percentage of missing values for features that were removed (optional).



* 1. **Filtering**: The purpose of the data filtering is to identify and remove variables that are unlikely to be of use when modeling the data. Enter the method that was used to filter out features (optional).

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* 1. **Normalization**: The sample normalization allows general-purpose adjustment for differences among your sample. Enter the normalization method used (optional).



* 1. **Data transformation**: Enter the data transformation method used (optional).

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* 1. **Data scaling**: Enter the data scaling method used (optional).



# Results

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

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The precipitant compound name selected in the experiment page will automatically be populated in the **Compound measured** field.

* 1. 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 avaliable data in the Study Report. Use separate entries for each type of measurement.

Note: a common strategy in induction studies is to use cells from at least 3 individual livers in order to capture interindividual variability. In these cases, enter the most potent result. Other results may be entered in the Additional Information section (see below).

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

* + 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.
    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.
    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.).
    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 avaliable 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).
  1. When all PK measurements have been entered for that entry, click **Add**.

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* 1. **Additional Information:** as needed, add any other information that is important to the result, but that were not detailed in the results table.

For example,results not entered in the measurements table.

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* 1. Use the **Add PCA data by XLSX** function to add measurements and PCA plot data at once. The **PCA Upload Tutorial** button will walk you through this process as well.

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* 1. Upload a file that contains a mapping of **Natural product samples**, this must contain the code used to identify the product, manufacturer, lot number, product name, form, and size. Also select the **Natural product** these samples originate from.

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* 1. The program will try to match and fill in the required fields but will prompt you to confirm before proceeding.

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* 1. Select the features file to accompany the PCA data by either uploading it or entering the URL where this information can be accessed. You must also select the **Analysis type** (select one; required).
* **NMR**
* **MS**

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* 1. If a **Certification of Analysis** experiment exists for a sample that was uploaded in step **7.6** you will be presented the option to link this metabolomics experiment with that **CoA**.

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* 1. If you have a file containing the **compound concentrations** you may upload it. This is useful if you want to see the compound concentration in the context of the PCA plot and it a part of the NaPDI recommended approach (optional).

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* 1. After processing your **compound concentration** file you will have to match the compound listed in your file with a compound that exists in our system. Compounds that we think may match will be listed, however, you can click the "Search" button to view all available compounds or "Add" to create a new one. You will also have to specify the unit of measurement, measurement type, and value type for the concentrations. At the bottom of the form select the **unit of measure**, **measurement type**, and **value type** of the the concentrations.

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* 1. Upload your **Principal Component Analysis (PCA)** file.

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* 1. After processing your PCA file you will have to specify the weights in percentage for each principal component. The total of which can not add up to more than 100.

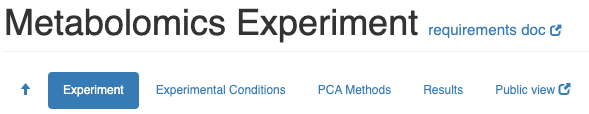
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Click to save the entries.

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