# A multi-faceted enzyme lab:
# Looking at the effect of an organophosphate insecticide on acetylcholinesterase activity in the bean beetle

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## Why use this system?

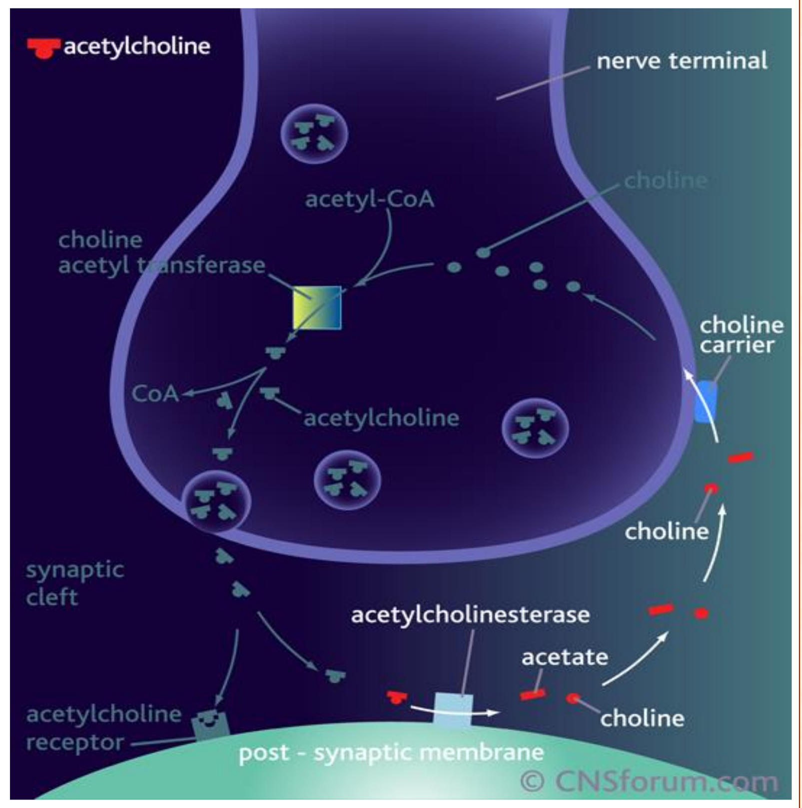
- Bean beetles are common pests
- Bean beetles are easy to maintain in the laboratory
- Organophosphate insecticides are commonly used to control this pest organism
- Ties in several course topics: Introduction to enzymes, the role of inhibitors in enzyme activity, cell-cell signaling, and potential ecological consequences

## Materials

- Bean beetles, *Callosobruchus maculatus*
- Incubator with light and temperature control
- Insecticide malaoxon
- ATCI as the substrate
- DTNB as the indicator
- Spectrophotometer, vortexer, centrifuge
- Water bath, autoclave, micropipetters
- Other standard laboratory equipment

## What should students know?

- An introduction to the life cycle of the insects
- Basic structure of proteins
- Basics about enzymes
- The basics of competitive and non-competitive inhibition
- Basics of synaptic signaling
- Function of acetylcholinesterase

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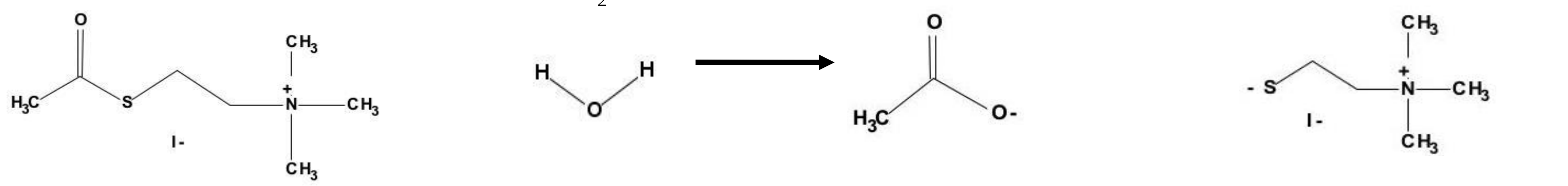
## Enzyme Assay

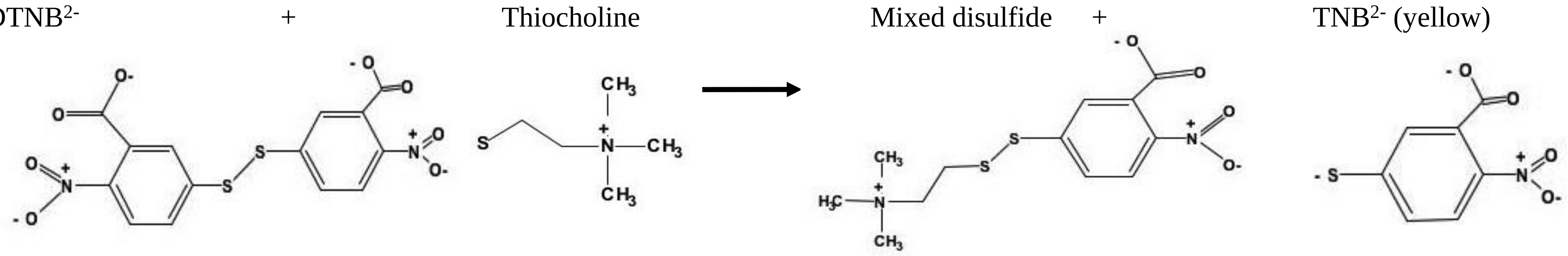
Based on the work done by Ellman et al. (1961), Ffrench-Constant and Bonning (1989), Spencer et al. (1998), and Gbaye et al. (2012).
In the presence of the enzyme AChE, the compound ATCI (acetylthiocholine iodide) is hydrolyzed to produce acetate and thiocholine.

ATCI + $H_2O$ AChE Acetate + Thiocholine Iodide + $2H^+$



The thiol (R-SH) group of thiocholine can react with the indicator compound DTNB (5-5'-dithio-bis-2-nitrobenzoic acid) to form $TNB^-$ (5-thio-2-nitrobenzoate), which ionizes to the $TNB^{2-}$ in water at mildly alkaline pH; this is yellow and can be detected spectrophotometrically.

$DTNB^{2-}$ + Thiocholine Mixed disulfide + $TNB^{2-}$ (yellow)



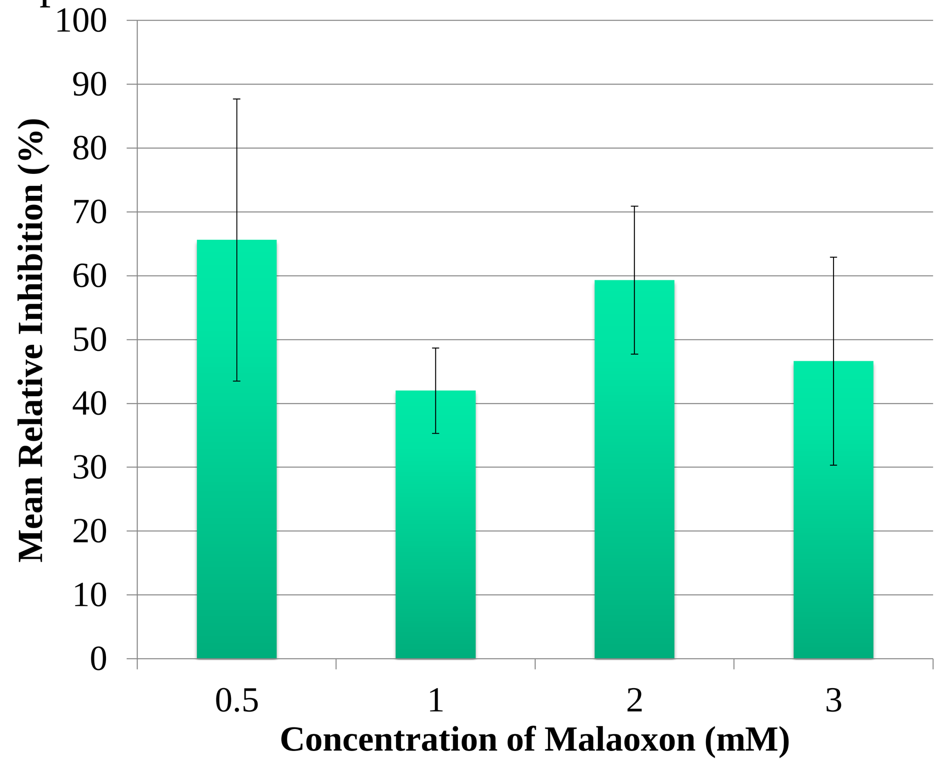
## Sample Student Results

**Table 1:** Relative inhibition of activity of AChE (acetylcholinesterase) caused by the insecticide malaoxon. AChE was extracted from bean beetles grown on black-eyed peas. Relative inhibition calculated as:

$$100 \times \frac{\text{Absorbance without malaoxon} - \text{Absorbance with malaoxon}}{\text{Absorbance without malaoxon}}$$

| Beetle # | Gender | Relative inhibition (%) |
|---|---|---|
| 1 | F | 49.6 |
| 2 | M | 30.2 |
| 3 | M | 34.2 |
| 4 | M | 17.7 |
| 5 | M | 25 |
| 6 | F | 41.2 |
| 7 | F | 12.5 |
| 8 | M | 3.42 |
| Mean | | 26.7 |
| SD | | 15.3 |

**Figure 2:** The effect of different concentrations of the insecticide malaoxon on the AChE enzyme activity. Columns represent mean relative inhibition caused by the insecticide, and error bars represent standard deviations. Sample size was 4.




## Comments

- This lab exercise is easily doable with first semester college students
- It can be done in regular 3-hour laboratories
- The topic can be supplemented with readings such as:
  - Rachel Carson's "Silent Spring"
  - London et al. (2005) linking organophosphate insecticides and suicide
  - Sadeghi Hashjin et al. (2013) linking malathion with anxiety in rodents

## Current Research

- To develop longer-term student projects to determine whether food source affects sensitivity of the AChE to insecticide
- To determine whether the beetles can be frozen at -20°C or -80°C for future studies without significant changes in enzyme activity
- To determine whether incubation time can be optimized

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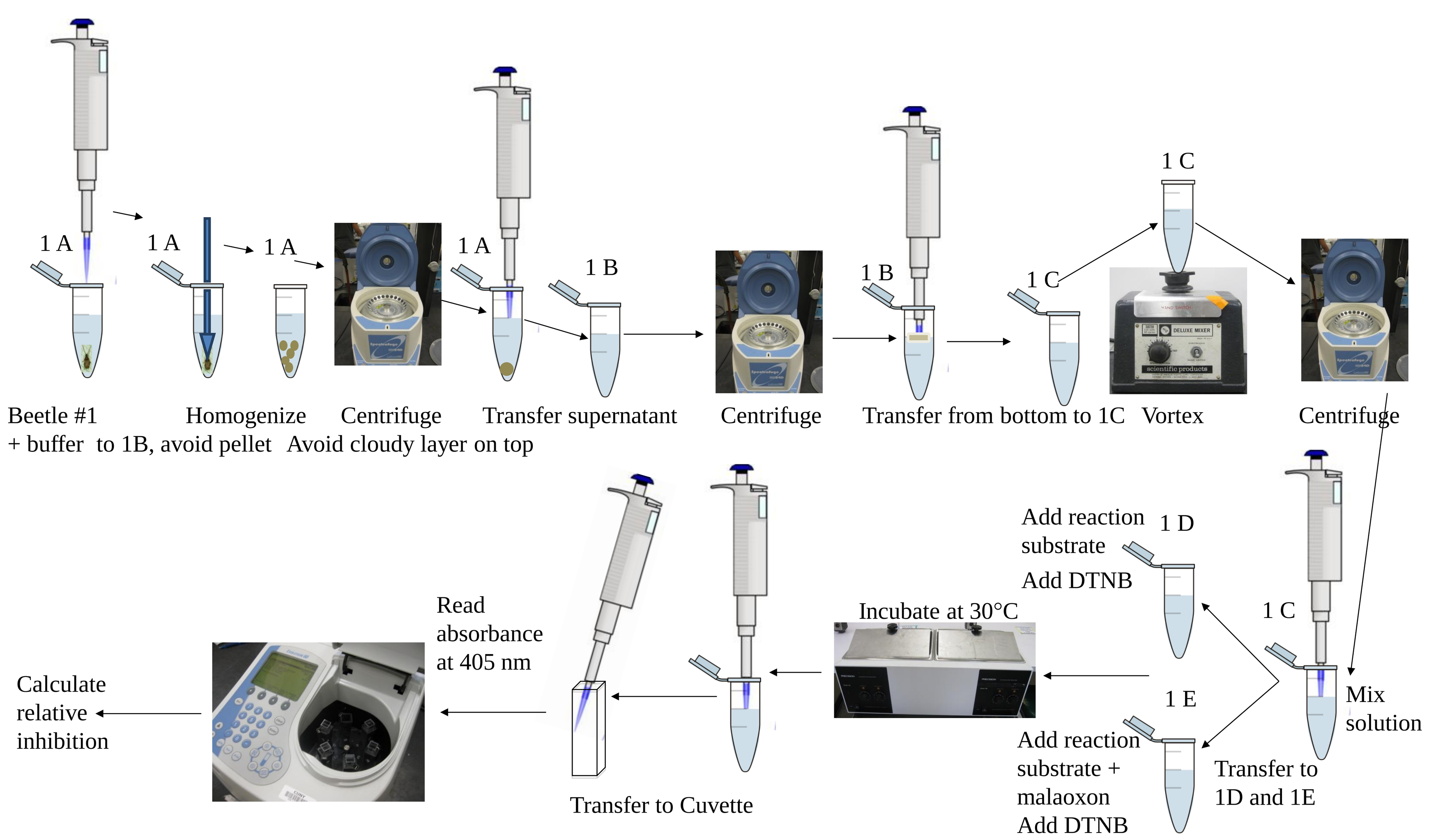
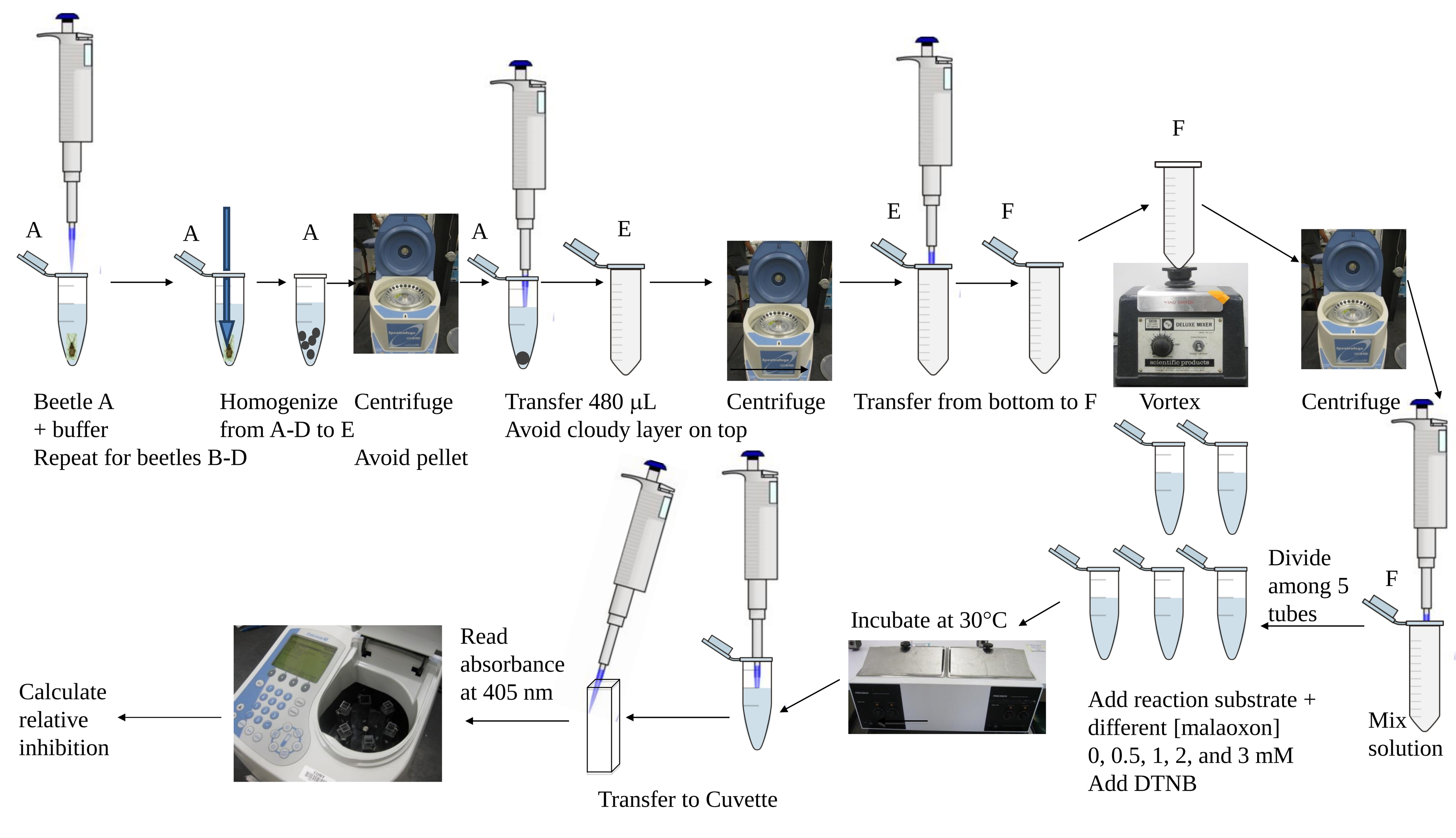
**Disclaimer:** Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessary reflect the views of the National Science Foundation

## Basic Procedure




## Procedure for Comparing Different Concentrations of Malaoxon

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