TOXICOLOGY EXPLORER

ACTIVITY AT A GLANCE

Goal:
It is the objective of this primer to, through the basics of toxicology testing; bring real world scientific research into the high school.

Learning Objectives:
Testing the toxicology of common items using immersion of crickets teaches lab techniques, scientific method along with various other skills such as:
- Serial dilution and associated math
- Lab safety
- Data gathering
- Statistical analysis
- Reporting

Teaching Time:
60-90 minutes (4 class periods)

National Science Education Standards Addressed:
Science as Inquiry – Students develop scientific inquiry skills in posing scientific questions and predictions, taking ownership of their research, and using varied tools and evidence to characterize the insect samples that they chose to study.

Science and Technology – The students integrate field work with taxonomic classification in the lab to determine insect Order using a computerized key

Life Science – In this activity, students use field work, classification, and basic content on symbiosis to learn about evolution and ecology of insects, the interdependence of microbes and animals, and the organization of living systems including insect body structure, life cycle, and associations with microbes.
The science of toxicology is defined as the study of the adverse effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects (official definition crafted by the Society for Toxicology and adopted by the National Library of Medicine).

Paracelsus is considered as the “father of toxicology”, in part for his recognition of the observation quoted above. This quote focuses attention on a key process in toxicology, which is determination of the amount (e.g., dose) of a substance that causes harm. That determination reveals the dose-response (or concentration-response) relationship between the amount (dose or concentration) of a substance to which an organism (or ecosystem) is exposed and any specific adverse effect (response) that may result from the exposure. The amount and nature of a potentially toxic substance are not the sole criteria that determine toxicity, however. A substance must first gain access to the site wherein damage can be caused. The route of administration is critically important to understanding toxic effects of substances.

Toxicity is usually determined by evaluating effects under carefully controlled conditions in test subjects (whether molecules, cells, organisms or ecosystems) that are as uniform as possible. When determining toxicity in an organism, issues such as age, gender, nutritional status, day-night period, season and prior exposure to the same or confounding substances must all be considered and kept as similar as possible between test organisms receiving the toxic substance and the untreated or control organism that does not.

Toxicity can be defined in many ways, but is probably most widely recognized by the term lethal dose (LD). The LD$_{50}$ is the dose of a substance that causes half (50%) of the exposed organisms to die within a specified period following exposure. The uniform Material Safety Data Sheet (MSDS) for common table salt notes that the oral LD$_{50}$ of this substance, in the male rat, is 3,000 milligrams (mg)/kilogram (Kg) of body mass. For this test, rats often have a mass of about 300 grams (g), which means that eating about 1 g of salt will kill half the rats that do so (within a 24 hour period of observation). An LC$_{50}$ by analogy is the concentration of a substance that kills half the organisms exposed. Dose will be used from this point forward, unless there is a specific need to refer to concentration of a substance.

Determination of an LD$_{50}$ for a substance usually requires testing several (5 or more) doses, each in groups of several (usually six or more) organisms. This is costly and requires many test subjects. The value of an LD$_{50}$ is that it provides a precise number by which to compare toxicity of different substances. Despite the fact that the LD$_{50}$ is a very limited evaluation of toxicity, determination of the LD$_{50}$ can teach many basic concepts of toxicity testing and will be used for this exercise.

The EC$_{50}$ is the effective concentration or the concentration that causes the desired effect in 50% of the animals tested.


**MATERIALS**

Per activity station for four students:

- Calibrated pipetter (e.g., Pipetman) for measuring milliliter or microliter quantities of chemicals that are fluids
- Balance for measuring milligram or sub-milligram quantities of chemicals that are solids
- Uncontaminated glassware for making serial dilutions and storing chemical stock solutions
- Large supply of 60 mm Petri dishes to check for Knockdown in each group (such as, 6 organisms/group x 5 groups = 30 containers)
- Sturdy box or tray for storage and transfer of treatment dishes.
- Counter space covered with clean, absorbent, disposable material, such as paper towel
- Safe storage location for all stock solutions, solvents, and all experimental containers (fume hood, if possible)
- Disposable vinyl (or latex) gloves
- Protective eyewear and lab coat (as advised and needed)
- Spring water (tap water that has been aged in a open container for at least a week is often just as safe)
- Ethanol and disposal jar for disposing of living crickets
- Capped or covered containers for storage of stock solutions (approximately 100-500 ml volume)
- Scientific calculator
- Paper towels
- Adhesive labels or colored labeling tape for labeling test dishes and containers with stock solutions
- Permanent marking pen (sharpies)
- Experimental organisms (crickets) with appropriate maintenance or culture container (an old plastic aquarium is fine)
- Supplies for handling, feeding, and care of organisms (see cricket care guide)
- Thermometer (to document temperature of all experiments)
- Test chemicals, along with MSDS sheets and/or International Chemical Safety Card with pertinent technical information about density, solubility, formula weight, handling instructions, hazards, safety, storage, etc.
- Bound notebook and pen for record keeping
- Dissecting or compound microscope with light source
- Recording/monitoring devices (e.g., camera, camcorder, video camera, etc.), if desired for documentation of behavioral effects (optional)
- Timer
Crickets can be purchased at almost any fish and tackle shop. Many pet supply stores also have them for sale. The cricket colony may be set up in a covered aquarium with guinea pig bedding or any simple enclosed covered box may be used. Students may be asked to bring in household chemicals from home or the teacher may simply purchase a few for testing. Some “green” pesticides efficacy could also be tested.

Pre-laboratory solution preparation:
1. Determine substance to be used:
   a. Undiluted will be 100% (50 mL)
   b. Use serial dilution procedure to make test solutions

   ![Diagram of solution preparation]

   
   $50 \text{ ml } 100\% + 25 \text{ ml } H_2O \rightarrow 25 \text{ ml } H_2O \rightarrow 5 \text{ ml } H_2O \rightarrow 25 \text{ ml } H_2O$
   
   Take 25 ml out

   $25 \text{ ml } 100\% \rightarrow 25 \text{ ml } 50\% \rightarrow 25 \text{ ml } 25\% \rightarrow 25 \text{ ml } 12.5\% \rightarrow 25 \text{ ml } 6.25\%$

2. Make enough solution for each station or you may wish to have students do this themselves. In a 90 minute class they would probably have time to do this but in a 60 minute block you may wish to do the initial preliminary solutions and set them up before the class begins or the previous day.

3. Place 10 60 mm Petri Dishes at each station. As long as the students work from least concentrated to most concentrated the same dishes may be used.

4. At the end of a period it is advised that you have the students rinse the Petri dishes and blot them dry for the next class.

5. Make copies of student group worksheets.

6. Copy and distribute Pre-test

Lab Preparation:
Each activity station should include one full set of materials. Place tubes with solutions at each station. Additional tubes (or beakers) for drainage of test chemicals to recover the solution when removing the test specimen should also be labeled to prevent contamination of each subsequent test group.
ACTIVITY PROCEDURE

Review the activity with your class and encourage them to formulate a hypothesis before starting the activity. Hand out student activity sheets and mini-reports. Students should work in groups with one collecting data, one timing immersion and one or more doing the immersion. Stress that they follow the protocol outlined on the student activity sheet. Note that student teams are encouraged to briefly review their observations with you before destroying specimens.

Follow up option: Groups of students can be asked to make short presentations of their preliminary data. This can be done in a single class period or for a few minutes each period over several of the lab periods. The wrap up day should include an amalgamation of all data to get a more statistically strong set of results.
Hypothesis: Formulate a hypothesis about the toxic effects of the chemical that you are testing.

MATERIALS

- Uncontaminated glassware for making serial dilutions and storing chemical stock solutions
- Large supply of 60 mm Petri dishes to check for Knockdown in each group (such as, 6 organisms/group x 5 groups = 30 containers)
- Sturdy box or tray for storage and transfer of treatment dishes.
- Counter space covered with clean, absorbent, disposable material, such as paper towel
- Safe storage location for all stock solutions, solvents, and all experimental containers (fume hood, if possible)
- Disposable vinyl (or latex) gloves
- Protective eyewear and lab coat (as advised and needed)
- Spring water (tap water that has been aged in a open container for at least a week is often just as safe)
- Ethanol and disposal jar for disposing of living crickets
- Capped or covered containers for storage of stock solutions (approximately 100-500 ml volume)
- Scientific calculator
- Paper towels
- Experimental organisms (crickets) with appropriate maintenance or culture container (an old plastic aquarium is fine)
- Supplies for handling, feeding, and care of organisms (see cricket care guide)
- Thermometer (to document temperature of all experiments)
- Test chemicals, along with MSDS sheets and/or International Chemical Safety Card with pertinent technical information about density, solubility, formula weight, handling instructions, hazards, safety, storage, etc.
- Bound notebook and pen for record keeping
- Dissecting or compound microscope with light source
- Recording/monitoring devices (e.g., camera, camcorder, video camera, etc.), if desired for documentation of behavioral effects (optional)
- Timer
- Adhesive labels or colored labeling tape for labeling test dishes and containers with stock solutions
- Permanent marking pen (sharpies)
INTRODUCTION
In this activity, you will:
1. Measure the contact toxicity of some common substance.
2. Collect data from each set of test specimens
3. Knockdown is defined as any 5 second or more inability to right itself when flipped on its back.
4. Mortality of any group is the number of specimens that do not survive the 30 second exposure.

NOTE: Remember to remove any dead crickets from the colony each day so that the colony health can be protected. Do not mix treated crickets in with the untreated colony!! 😊

Immersion study instructions:

1. Be sure to wear gloves, goggles and lab coat
2. Obtain cricket specimens (10 per each solution to be tested – approximately 60) Crickets should be placed in a bucket with a small piece of cardboard or foil to prevent cricket escapees.
3. Begin testing with the control solution (water) and work upward from least concentrated to most concentrated.
   a. Place a cricket into the solution and hold him under with index finger to ensure full immersion
   b. Sustain immersion for 30 seconds. Use timers (any watch with a second hand will do).
   c. Quickly pour liquid into second labeled tube (carefully because this tube will now become the tube used for the next specimen) capturing the cricket gently with your index finger, middle finger and thumb.
   d. Place cricket into 60 mm Petri dish and cover.
   e. Flip Petri dish containing cricket over and observe for knockdown. [Remember that knockdown is defined as inability to flip back to his feet for 5 or more seconds in this immersion study]
   f. Tally the number of crickets in which knockdown is observed.
   g. Tally the number of crickets in which mortality is observed.
   h. Place all crickets into alcohol waste jar after observations are complete.
   i. Blot Petri dishes dry.
4. Repeat this procedure with each subsequent concentration working upward from 6.25% to 100%.
   a. It is important to note that you be as consistent as possible with your handling of the crickets in and out of solution.
   b. Varying the time by even 2 or 3 seconds increases exposure time and will adversely affect the results.
   c. Any cricket damaged by handling should not be used in the immersion results as this could also affect the results.
5. At any concentration level in which there is a 100% mortality rate cease testing. [For example if 50% concentration elicits 100% mortality it is assumed that 100% will do so as well.]

See optional activity if you desire a more thorough toxicity test.
Data Table:

<table>
<thead>
<tr>
<th>Dosage group (%)</th>
<th>Knockdown Tally</th>
<th>Mortality Tally</th>
<th>Knockdown %</th>
<th>Mortality %</th>
</tr>
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<tbody>
<tr>
<td>100</td>
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<td>25</td>
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<td>12.5</td>
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<tr>
<td>6.25</td>
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<tr>
<td>water</td>
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</tbody>
</table>

- Students should be advised that the search for an EC50 for their particular chemical may take many concentrations and various exposure times.

Day 2 – Day ?
- The work truly begins
- Students may test any number of parameters for as long as they wish.
  - Exposure time
  - Concentrations
  - Combinations of chemicals
  - Infinite possibilities as long as controls are established for each group
- Remember the same control group can be used for a number of test concentrations as long as no other parameters are changed.

Discussion and Wrap up Day
- Discuss results and how students should display results
  - [http://www.twingroves.district96.k12.il.us/ScienceInternet/ChartsGraphs.html](http://www.twingroves.district96.k12.il.us/ScienceInternet/ChartsGraphs.html) may help
- Give Post – test
- Dispose of any remaining crickets in ethanol (unless you plan to keep the colony).
Appendix I – Mississippi Science Standards Correlation

Lesson Objectives:

- Correctly identify and use basic laboratory equipment used in toxicology study. Balances (or scales), pipetters, graduated cylinder... (1a)
- Observe proper safety techniques such as gloves, goggles, aprons (or lab coats) and correct handling and disposal of all chemicals according to MSDS guidelines. (1b)
- Design a toxicology study using chosen chemicals and either crickets, meal worms or some other invertebrate that tests dose response and sublethal effects. (1c)
- Present the toxicology of your chosen substance it a PowerPoint presentation or on a science board to the entire class. (1d)
- Investigate via the World Wide Web the toxicology of various substance including the fire ant venom alkaloids and possible therapeutic uses of what would otherwise be considered toxins. (1c)
- Record observations of test and control invertebrates to monitor for an observable behavioral effect (6a)
COMPETENCIES and Suggested Teaching Objectives:

1. Utilize critical thinking and scientific problem solving in designing and performing biological research and experimentation. (L, P, E)
   a. Demonstrate the proper use and care for scientific equipment used in biology.
   b. Observe and practice safe procedures in the classroom and laboratory.
   c. Apply the components of scientific processes and methods in the classroom and laboratory investigations.
   d. Communicate results of scientific investigations in oral, written, and graphic form.

6. Examine the behavior of organisms. (L)
   a. Analyze the behavioral responses of an organism to internal and external stimuli.
Appendix II - Care and Feeding of Acheta domesticus (the house cricket)

Introduction
House crickets are commonly encountered in all parts of the southeast except Florida where they occur in only two contexts: bait for fish and food for pets. This is because they do not survive very well in the wild in peninsular Florida for some reason. The house crickets that are sold in bait and pet stores are reared in large commercial cricket factories or by local entrepreneurs.

Distribution
The house cricket is probably native to southwestern Asia, but has been widely distributed by man. In the United States it occurs wherever it is sold, but it survives in feral populations only in the eastern United States (except peninsular Florida), and southern California. Why it fails to survive in peninsular Florida is not known.

Life Cycle
House crickets take two to three months to complete their life cycle when reared at 80 to 90°F. They have no special over wintering stage, but survive cold weather in the northern States and Canada in and around buildings and in dumps, where heat from fermentation may sustain them. Eggs are deposited in whatever damp substrate is provided — for example, sand or peat moss. Juveniles resemble the adults except for being smaller and wingless.

Identification
The house cricket is a 16 to 21 mm long, light yellowish-brown cricket, with wings that cover the abdomen. It has three dark transverse bands on the top of the head and between the eyes. All house crickets have long hind wings when they become adult, but they sometimes shed them later. Female crickets have an egg-laying tube (ovipositor) extending from their rear end that is inserted in the soil for egg-laying.
Habitat
House crickets are usually found where they have recently escaped or been released — for example, on the shores where people fish.

Song
As in most other crickets, male house crickets make a calling song by rubbing a scraper on the inner edge of the left wing against the teeth of a file that is beneath the right wing. The calling song is a series of short chirps. Each chirp consists of two or three pulses which correspond to two or three wing closures. Wing openings are silent.

Rearing
House crickets, as well as various native ground-dwelling crickets, are easy to rear in small numbers in the home or schoolroom.

Cages
Use wide-mouth glass jars, plastic containers or five-gallon cans. Treat the smooth inner surface with mineral oil, vegetable shortening, vaseline, varnish and furniture polish or floor wax. Cover with wire screen or muslin. A simple wooden frame may be built and covered with ordinary window screening about three feet square and two feet high. Use galvanized sheet metal for the box bottom, extending eight inches up all four sides to prevent spiders and ants from entering the box. Crickets are natural food for spiders, ants, centipedes, lizards, etc. You may also use a simple old aquarium with a vented top as a cage for crickets.

Cage Preparation
Place four inches of clean, dust free, damp sand (good brick sand) in the cage bottom. You may also use commercial rodent bedding available from any pet store. Any old aquarium with a plastic top is a sufficient cage for maintaining the colony. Cover sand with six inches of coarse wood shavings to protect small crickets from being eaten by adults. Some use a small plastic container of moist sand placed on top of dry sand in the cage for an egg-laying site.

Feeding
Crickets are easy to raise. House crickets eat most edible foods such as stale bread, poultry mash, cornmeal, powdered dog food, etc. Slices of apple, banana or pieces of lettuce or cabbage are a treat. Place food in a shallow container, discarding unused portions for cleanliness and freedom from mold. A feeder can be any open vessel pressed into the sand. Dog food provides a simple, well-balanced diet sufficient in protein, and also poultry laying mash. A water supply is very important. Place cotton or similar material in the water container to keep small nymphs from drowning. (Cotton will "wick" water from a small bottle by capillary action.) Bury the bottle in the sand with the wick out to keep smaller crickets from falling in and drowning.

Other Factors
Provide resting or hiding places using paper containers with punched holes, excelsior or folded corrugated cardboard. Old egg cartons are excellent for this purpose. Crickets need warm temperatures of at least 80°F. Nymphs held at 80°F require up to 60 to 65 days to mature, while those held at 90°F require only 30 to 35 days to complete development. Purchase a cheap thermostat and heater such as used for small chick brooders. Keep the cricket container in subdued light since, in nature; crickets are most active at night. Artificial light is acceptable, but direct sunlight should be avoided.
Management
Generally house crickets do no harm. However, if crickets kept for pet food or fish bait escape into your home and annoy you with their calls, you can eliminate them by setting out baits that are sold for cockroach or earwig control.

Deceased Crickets
Dead crickets in the colony must be removed as the others will eat them and this may cause harm to the overall health of the colony.

For more info on rearing crickets:
http://www.ca.uky.edu/entomology/entfacts/ef007.asp
http://www.triciaswaterdragon.com/crickets.htm#Keeping
http://www.anapsid.org/crickets.html
1. Entry of a chemical into the body through a surface such as the skin, digestive tract, or respiratory tract.
   A. resorption
   B. adsorption
   C. absorption
   D. exposure

2. Use of a living organism to estimate the amount of a chemical in a test sample
   A. biosensor
   B. bioassay
   C. bioinformatics
   D. control group

3. A chemical that taints or corrupts soil, water, food, or air, thus making it impure
   A. contaminant
   B. toxin
   C. precipitate
   D. dose

4. The total amount of a chemical given to an organism at one specific time
   A. dosage
   B. total
   C. titer
   D. dose

5. Any observable or measurable biological response of an organism to chemical exposure
   A. affect
   B. effect
   C. reflex
   D. behavioral change

6. The frequency of deaths in a group of organisms exposed to a chemical
   A. mortality
   B. lethality
   C. morbidity
   D. LC50

7. Describing a state in which an organism is beginning to die or is near death
   A. paralysis
   B. mortality
   C. moribund
   D. dose response

8. The disappearance of toxicity effects in an organism and return to normal function and behavior
   A. recovery
   B. vitality
   C. morbidity
   D. NOEL
9. Naturally occurring chemical compounds containing basic nitrogen atoms
   A. analyte
   B. amyloid
   C. altoid
   D. alkaloid

10. Group of organisms that has not been exposed to the test chemical but which has, in every other way, been subjected to conditions and procedures that are identical to those in groups exposed to the test chemical
   A. test group
   B. control group
   C. behavioral group
   D. pre-exposure group

11. The rate of administration of a chemical or drug to an organism
   A. dose
   B. dosage
   C. titer
   D. LC50

12. The concentration that produces a level of effect equal to fifty percent of the maximum effect
   A. LC50
   B. LD50
   C. EC50
   D. PC50

13. Occurring in an abnormal situation in the living body and often introduced from outside
   A. foreign
   B. toxin
   C. hazard
   D. risk

14. A liquid that is capable of dissolving other chemicals
   A. solute
   B. hydrophilic
   C. hydrophobic
   D. solvent

15. A biological effect caused by chemical exposure at a concentration below that which causes death
   A. sublethal effect
   B. trans lethal effect
   C. LC50
   D. EC50

16. The study of the adverse effects of chemicals on living organisms
   A. morphology
   B. controlled study
   C. toxicology
   D. biology
17. Any chemical that does not occur in the normal biochemical pathways of an organism
   A. xenobiotic
   B. toxin
   C. alkaloid
   D. amyloidal

18. The capacity of a chemical to produce harmful effects or the degree to which something is able to produce illness or damage to an exposed organism
   A. risk
   B. hazard potential
   C. EC50
   D. toxicity

19. The minimum amount of a chemical that just causes an observable effect in a group of organisms
   A. EC50
   B. LC50
   C. dosage
   D. threshold concentration

20. Refers to chemicals that are soluble in water
   A. hygroscopic
   B. hydrophilic
   C. hydrophobic
   D. saturated