Toxicology Explorer

A Student Primer on Toxicology

"O for a Muse of Fire that would ascend the brightest heaven of invention…"

Shakespeare, Henry V, Prologue

Solenopsis invicta
Photo courtesy of Alex Wild/myrmecos.net

Toxicology of Substances in *Acheta domesticus* (the house cricket)

http://buzz.ifas.ufl.edu/487a.htm

THE UNIVERSITY OF MISSISSIPPI
MEDICAL CENTER

MILLSAPS COLLEGE

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

MBL Biological Discovery in Woods Hole

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DISCLAIMER

The user assumes all responsibilities for the safe and proper handling and application of chemicals listed and described herein. UMC assumes no liability relating to the use and effects of these listed chemicals, or any others. Before handling any chemical, users should obtain a **Material Safety Data Sheet** *(MSDS)* and/or International Chemical Safety Card for each chemical from: http://hazard.com/msds/

These sheets should be made available to all users in the laboratory. Before performing experiments, users should carefully follow warnings and instructions on all labeling of consumer products. Mention herein of any product brand names in no way represents a favorable or unfavorable endorsement of these products.

Much of this primer is based on *Biological Smoke Detectors* and the work of:

Charles D. Drewes
EEOB Department, 339 Science II
Iowa State University
Ames, IA 50011
http://www.eeob.iastate.edu/faculty/DrewesC/htdocs

BACKGROUND

It is important that humans have a way of measuring the toxicity of the food we eat, the water we drink, and even the air we breathe. **Toxicity** of substances is an important measure to determine as many substances that are beneficial at one dose are potentially lethal at another. Physicians, pharmacists, product engineers, and chemical engineers must understand **toxicology** as an integral part of their jobs. Environmental engineers need to understand toxicology in order to gauge the amount of **exposure** to toxins that not only can be tolerated in short term exposure but also the dangers of long term exposure.

Many organisms can be used as **biosensor**s that detect contaminants in food or water. The first such use was the "canary in the coal mine." When the level of gas became so toxic that it caused the canary to become unconscious the miners knew that it was time to get out of the mine. A canary in a cage, as used by miners to warn of gas could be considered a biosensor. Many of today's biosensor applications are similar, in that they use organisms which respond to toxic substances at a much lower level than us to warn us of their presence. Such devices can be used both in environmental monitoring and in water treatment facilities.

In a much more modern example GloFish® fluorescent zebra fish were originally bred to help detect environmental pollutants. By adding a natural fluorescence gene to the fish, which only expresses in the presence of certain pollutants, scientists could quickly and easily determine waterway contamination.

OBJECTIVES

Toxicology is the study of the adverse effects of chemicals on organisms. Though formal training in toxicology may not begin until professional school, student interest and inquiry in this area may begin much earlier. A motivated high school student, for example, may study toxicity effects for a biology research project or science fair. This student faces an array of important questions. What scientific problem will be studied? Can simple yet meaningful experiments be done? What organism, chemical, materials, methods, and safety precautions will be used? How will experiments be designed and how will results be interpreted

When undertaking a toxicology project, a student may quickly discover that general biology texts, school and public libraries, or the World Wide Web provide little practical guidance or tutorial assistance. Faced with this lack of guidance, the result for many students is frustration and haphazard results due to flawed research design. However, student research in toxicology need not be an unguided or haphazard experience.

It is not the aim of this guide to promote toxicology experimentation at the high school level. Rather, it is to provide young, ambitious, and careful students (who have already decided to attempt a toxicology project) with a toxicology primer that contains useful suggestions and practical guidance that enhance the quality, meaningfulness, and safety of their experiments. Hopefully, such information should be valuable to biology teachers and other research mentors, at all educational levels.

Ideas in this booklet derive from the HHMI funded study "Muse of Fire". A partnership between the University of Mississippi Medical Center and the Marine Biological Laboratory at Woods Hole developed to look at several aspects of the **red**

imported fire ant including toxicology of the **alkaloid solenopsin** venom components.

For many reasons it is not recommended that students undertake any toxicology project that includes hazardous compounds or vertebrate animals. It is also imperative that this project only be undertaken with the cooperation of a qualified mentor or teacher. Safety must be maximized and health risk minimized in all toxicology studies.

Nevertheless, given a little creative and careful thinking, It is possible to design many novel, safe, and scientifically valuable investigations can in toxicology for novice researchers, even when faced with limited resources. Above all, research students should remember the double-K.I.S.S. guidelines:

first: **K**eep **I**t **S**cientifically **S**ound!
second: **K**eep **I**t **S**imple and **S**afe!

PURPOSE OF INVERTEBRATE TOXICITY TESTING

Toxicity testing involves the discovery and analysis of chemical effects on organisms. Extensive toxicity testing, using many species, is needed to understand the full spectrum of biological effects of chemicals and to decrease the health risks that chemical effects pose for humans and ecosystems. Toxicity testing is crucial for a wide variety of chemicals that are present in drugs, cosmetics, pesticides, food additives, cleansers, solvents, and industrial wastes. Invertebrates are used in toxicity testing for one of two reasons:

1. Some invertebrate species may have considerable relevance to the environment. For example, earthworms are ecologically beneficial to soil ecosystems and zooplanktons are key links in aquatic food chains. Some invertebrates may also have environmental relevance, not because they are beneficial, but because they are pests. In either case, it is important to know if and how the presence of a

chemical in the environment might affect any of these organisms. Laboratory testing under controlled experimental conditions is an important approach to understanding and predicting possible effects of chemicals in ecosystems.

2. Invertebrates may provide useful insights to understanding chemical effects on human health. This is because invertebrates share some of the same biochemical and physiological processes that exist in nearly all animals, including humans. For example, many nerve cell functions are common to worms, insects, fish, rats, humans, etc. Therefore, invertebrate toxicity testing may be a useful tool for understanding and detecting biological effects of chemicals at molecular, cellular, or behavioral levels in many organisms.

LETHAL AND SUBLETHAL EFFECTS

Toxicity testing in the past 20 years has moved away from **lethality** (or **LC50**) studies and toward studies of **sublethal effects**. This is because sublethal effects occur at concentrations below those for lethality and thus are more sensitive indicators of toxicity.

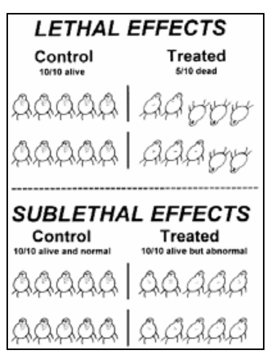

Fig. 1. Comparison of lethal and sublethal treatment effects. Treatment with high chemical concentrations may be lethal in some or all treated organisms. Lethal effects are easy to recognize and tabulate. Treatment with lower concentrations may produce sublethal effects which are sometimes difficult to discern but, nevertheless, important from a behavioral or ecological standpoint. (http://www.eeob.iastate.edu/faculty/DrewesC/htdocs)

One hurdle for a student toxicology project is deciding what sublethal effects to study. This is a challenge because such effects seldom have been described in invertebrates and there are few standard methods for measuring them. Thus, it is advisable to begin by carefully observing an organism's normal behavior prior to any toxicity testing. Then, during preliminary testing, look for obvious effects, such as changes in an organism's color, posture, spontaneous movements, or perhaps changes in its reaction to stimuli such as light, touch, or body inversion. Some effects may only be seen under magnification, such as rhythmic movements of the organism's heart or respiratory system. To systematically study behavioral effects, students may need to design and build simple devices for handling, observing, or testing organisms. In addition, they may need to develop criteria for scoring or measuring effects. From such observation and testing, students will likely gain new insights about the biology and behavior of normal as well as treated organisms.

JIMINY CRICKET! AN IDEA FOR TOXICOLOGY TESTING

Acheta domesticus (the brown cricket) is an invertebrate that can be used with various techniques including controlled spraying, topical application and full immersion. They are relatively cheap and the colony can be self sustaining if proper care is taken (see the care guide in the appendix section). The sex of each cricket is also easy to determine so that differences in toxicology tolerance between sexes could be studied as well. Also, they are relatively easy to handle and easily disposed of in a plain alcohol dish that can be discarded with the regular trash.

[It should be noted that freshwater organisms, such as aquatic oligochaetes are also good choices for toxicity testing because: (1) they are important parts of aquatic ecosystems and food chains, (2) they are exposed to many chemicals that contaminate water and sediments, and (3) certain freshwater species, such as *Lumbriculus variegatus* (the blackworm or mudworm), have been used previously for studying toxicity effects. *Lumbriculus* is cheap (commercial or field sources), easily cultured in the lab (asexual reproduction), and simple to handle (Drewes, 1996b). Most important, there are interesting aspects of this worm's biology that may be useful indicators of toxicity (Rogge and Drewes, 1993; Drewes, 1997; Lesiuk and Drewes, 1999).] ← see *Biological Smoke Detectors* for more information

SUBLETHAL CHEMICAL EFFECTS (In *ACHETA DOMESTICUS*)

The sublethal effect observed in the preliminary study was called knockdown. Knockdown will be defined here as the inability of the cricket to right himself when placed on his feet in a closed 60 mm Petri dish and the dish is flipped over putting the cricket on his back. Any normal cricket will flip to his feet immediately. An affected cricket will be slower or display **ataxia** and the knockdown crickets will display full **paralysis** and thus be unable to flip to their feet. Observations of this sublethal effect can be taken as immediate and at any increment of time the investigator chooses (minutes or hours after exposure).

These are only suggestions for sublethal effects. Many other effects (physiological, biochemical, and behavioral) likely occur which may also be amenable to study, but there has been very little research study or publication of any such effects. This should be viewed as a great opportunity and source of motivation for students to make novel and significant contributions using such toxicity assays.

SELECTING THE CHEMICAL(S)

When selecting a chemical for toxicity testing, consider its relative safety in handling, availability, and relevance to real-world ecological or medical situations. A few chemicals that meet these criteria are listed below, along with brief descriptions of their use and relevance. Most are available in either pure-form or diluted commercial formulations. A source for pure-form chemicals is Sigma Chemical Company (P.O. Box

14508, St. Louis, MO 63178). Sigma will also fax ***Material Safety Data Sheets (MSDS)*** for specific chemicals, upon request.

When using any chemical, carefully consult and comply with all information given on the MSDS Sheet and International Chemical Safety Card for each chemical. This information is available at the following World Wide Web sites:

- http://hazard.com/msds/ or
- http://www.cdc.gov/niosh/ipcs/icstart.html

To see a sample MSDS Sheet and a sample International Chemical Safety Card for a compound such as d-limonene (one chemical listed below in the LIST OF POSSIBLE TEST CHEMICALS), see the following sites:

- http://hazard.com/msds/f/bwn/bwnws.html
- http://www.cdc.gov/niosh/ipcsneng/neng0918.html

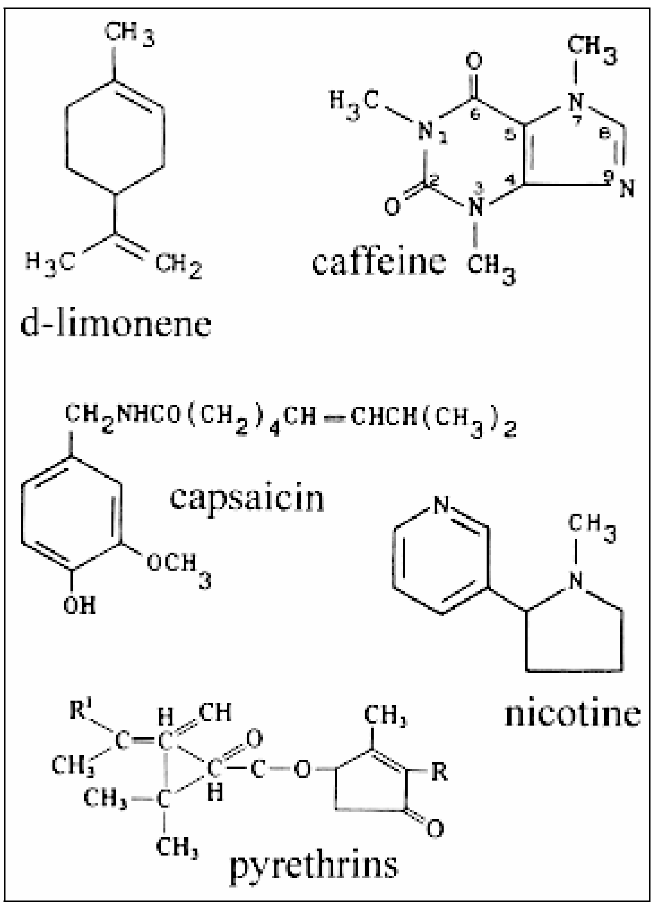

Fig. 4. Chemical structures of some compounds that cause significant and interesting sublethal effects (http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/Toxweb3.htm)

LIST OF POSSIBLE TEST CHEMICALS

It should be noted that **hydrophilic** substances (soluble in water) are suitable for immersion studies as immersion in any alcohol base solution is sufficiently toxic to cause **mortality** in the cricket.

- **boric acid:** An inorganic salt (H_3BO_3).An insecticidal powder used indoors for cockroach and ant control. Occurs in nature as the mineral, sessolite. Used for weatherproofing wood and fireproofing fabrics. Used externally on humans as an antiseptic, eye ointment, and antibacterial agent. Used extensively in industry for cements, glass, leather products, carpet, soaps, cosmetics, dyeing, printing, painting, and photography. If ingested by humans, may cause many toxic effects: vomiting, cramps, skin lesions, circulatory collapse, speeding up of the heart, and convulsions. Known to cause reproductive and developmental toxicity effects in mammals. A good candidate chemical for toxicity effects on worm regeneration. Very soluble in water.
- **caffeine:** An alkaloid that occurs naturally in tea and coffee leaves and cola nuts. Known to stimulate many nervous system functions, heart rate, respiration, and urine flow in mammals. Present in caffeinated soft drinks. Active ingredient in many over-the-counter anti-sleep drugs. Very soluble in water. (cf., Lesiuk and Drewes, 1999).
- **capsaicin:** Main active ingredient in red pepper, or chili pepper (genus Capsicum). Known to affect nervous system functions and development of sensory neurons. Creates stinging, burning sensation on skin or mucus membrane. Used in some cat/dog repellents. Nearly insoluble in water. Freely soluble in ethanol. Example of a commercial source is red pepper powder.
- **carbonic acid:** Dissolved CO_2 in water = carbonated water = seltzer water. Toxic to aquatic invertebrates, such as worms. Sometimes used by microscopists to narcotize invertebrates prior to chemical preservation.
- **chlorinated water:** Chlorinated water contains chlorine, a purifying agent for drinking water. Power plant effluents produce high chlorine levels in marine and fresh waters. Chlorine has short-term stability in water (hours or days). Chlorinated water also contains varying amounts of chloramine, formed by the reaction of ammonia with chlorinated water. Chloramine also has disinfectant and sanitizing properties but has longer stability in water than chlorine. Chlorine and chloramine in water are extremely toxic to aquatic organisms, including invertebrates and fish. Data regarding

the chlorine concentration (and concentrations of other constituents) in municipal water supplies are normally available to the public from water treatment personnel.

- **CMA (calcium magnesium acetate):** Used for de-icing highways. Believed to be less toxic to aquatic life than NaCl. Effects on many aquatic organisms are unknown. Commercial formulation of CMA is Chevron Ice-B-Gon Deicer. Water soluble.
- **ginseng:** Extracts from roots of ginseng plants (genus Panax) contain ginsenosides (types of saponins). Used in oriental medicine as a tonic. Claimed to enhance circulation, heart contraction and revitalization. Believed to reduce stress and fatigue in humans. Very water soluble. Commercial source: Panax Ginseng Extract, available in oriental food stores, consists of a water extract from red ginseng roots that is nicely packaged as ten separate 10-ml vials intended for full-strength human consumption or dilution in other drinks. This commercial extract, when diluted to 1/50th full strength, appears to be a potent disrupter of locomotor reflexes in Lumbriculus. Ginsenosides, obtained from water extracts from actual ginseng roots, have potent effects on Lumbriculus blood vessel pulsation rates (S. Wong, personal communication).
- **limonene:** A naturally occurring substance in lemon, orange, caraway and dill. Constitutes about 98% of orange peel oil by weight. Used as an insecticide and insect repellent. Widely used for control of fleas, lice, mites and ticks. Virtually non-toxic to warm-blooded animals, but can cause skin sensitivity and irritation. Pleasant lemon-like odor. Practically insoluble in water but miscible with ethanol. Example of commercial source: Natures Answer Flea and Tick Dip contains 78.2% d-limonene and the label recommends diluting the product at a ratio of 3 parts product to 256 parts water (= 0.9%) and then applying directly to the pet. Major effects, including neural and behavioral toxicity, rapidly occur in Lumbriculus at 0.009%, or less. This is ≤1/100th of the recommended concentration for pets and serial dilutions can be made from this concentration. (see Karr et al., 1990)
- **nicotine:** A highly toxic alkaloid. Principal active ingredient in tobacco products and a controlled substance. Formerly used extensively as an insecticide for home, farm, and orchard. Nicotine in liquid form is readily absorbed through the skin (example = nicotine patch). Effects occur at many sites within the central and peripheral nervous systems of vertebrates and invertebrates. Mimics the action of the neurotransmitter, acetylcholine. Symptoms of toxicity in humans include salivation, abdominal cramping, headache, loss of coordination, and respiratory failure. Very water

soluble. An aqueous extract, made by soaking the tobacco contents of one cigarette in 100 ml of water, will provide a potent stock solution from which serial dilutions can be made. Short-term treatment with these solutions will have major effects on Lumbriculus locomotion and blood pulsations (Lesiuk and Drewes, 1999). CAUTION: The aqueous extracts from even one cigar or cigarette may cause serious adverse effects in humans if ingestion or prolonged contact with the skin occurs.

- **pyrethrum:** An extract from flowers of a chrysanthemum grown in Africa and South America that contains several closely related insecticidal compounds (= pyrethrins). Dried and crushed flower heads were used as a louse powder in the Napoleonic Wars. Pyrethrins act on insects and other invertebrates with phenomenal speed, causing temporary paralysis (knock-down) but not always death. Formulated as household insecticidal sprays and dusts for use on vegetables. Considered generally safe to humans and domestic animals. Not very toxic if ingested by humans because pyrethrins are hydrolyzed in the gastrointestinal tract. Skin contact may cause dermatitis. Synthetic pyrethrin-like compounds (= pyrethroids) are used in many commercial insecticide formulations because they may be more stable and more active than natural pyrethrins. Pyrethroids are potent neurotoxins that modify function of voltage-gated sodium channels in neuronal membranes and induce repetitive firing of action potentials. Practically insoluble in water but very soluble in ethanol. Example of commercial source: Scratchex Power Dip For Dogs and Cats, designed to kill fleas and ticks on contact. Scratchex contains 0.54% pyrethrins. The label recommends diluting 1 part from the bottle with 64 parts of water (= 0.0084%) before application to pets. Major effects on Lumbriculus rapidly occur at 0.000084%, or less. This is ≤1/100th of the recommended concentration for pets.
- **household chemicals:** Many household chemicals contain toxins that are suitable for testing and the advantage to using a substance like Windex® is that it is cheap and readily available. For a list you could consult http://www.oregontoxics.org/alternatives.html.

SAFETY

Obtain and study Material Safety Data Sheets *(MSDS)* and/or International Chemical Safety Cards for all solvents and test chemicals that you will use in testing. MSDS sheets and International Chemical Safety Cards are readily available from the University of Vermont which maintains a huge electronic data base relating to chemical safety. The address is: http://hazard.com/msds/.

Learn and follow all safety measures for the laboratory facility in which you will be carrying out your study. Learn and follow all written safety precautions for the chemicals you are using. Handle all volatile or toxic materials in a fume hood. Wear a lab coat, protective vinyl (or latex) gloves and use protective eyewear when opening or handling any chemical storage containers, stock solutions, pipettes, or exposure containers.

Clearly label the contents and concentrations of all chemical solutions in containers. Properly dispose of all used solutions, surplus solutions, or chemically-exposed materials such as pipette tips or filter paper. Use absorbent towel to thoroughly remove any drips or spills of solutions to which humans may come in contact. Thoroughly and carefully scrub and clean all glassware or plastic ware that was exposed to chemicals. Use ethanol and then water rinses to clean containers that held water-insoluble chemicals.

It is very important not to leave any chemical residues on glassware, thus the emphasis on careful cleaning. Soap residues could have a detrimental effect on the observed experimental outcome.

EXPOSURE METHODS

A simple way to expose crickets to water-soluble chemicals is by immersion. Crickets are placed in individual containers (such as test tubes) along with a small volume (about 30-50 ml) of test solution of known chemical concentration. The crickets are fully immersed in the solution by using a glass stirring rod and held in that position for 30 seconds. (exposure time could be varied if the student desires – another way to study the toxic response – possibilities abound!) The chemical is thus absorbed through the skin (termed **contact**

exposure). Always use just one cricket per container, since a dead, decaying cricket may be toxic to others.

Another way is to spray a known concentration on the cricket and observe for knockdown. However, the immersion technique seems to have greater efficacy and more predictability.

All toxicity tests should include a **control group** in which the cricket is immersed in pure water (or sprayed) for the same amount of time as the test subjects

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PRELIMINARY EXPERIMENTS AND CONCENTRATION RANGE-FINDING

Sublethal effects of some chemicals may occur within a narrow range of concentrations. High concentrations may rapidly kill organisms while lower ones may cause no effect. Since concentration ranges for sublethal effects differ among chemicals, an important step in toxicity testing for a chemical is to determine its **threshold concentration**, **NOEL**, and **dose-response** relationship. This requires preliminary range-finding experiments which are time-consuming but lead to more meaningful results during final stages of toxicity testing.

To make a stock solution, dissolve a known amount of pure chemical (liquid or solid) in a small, known volume of water. Typically, a few milligrams or milliliters of the chemical are dissolved in 100-1000 ml of solvent. The concentration should be expressed as*:* mg of chemical per liter of solvent if the chemical is a solid. This is the same as **parts per million**. If the chemical is a liquid, then concentrations will be in milliliters of chemical per liter of solvent. This concentrated stock solution is used to make a series of weaker stock solutions by **serial dilution**. Each concentration step may be several times weaker than the preceding one, such as 25, 5, and 1 ppm.

Sometimes the exact amount of chemical may be unknown because it is present in an unpurified, crude form. In this case, the volume or weight of crude material should still be measured and recorded in making a stock solution. Then, dilutions of stock solution are used for range-finding experiments, with concentrations expressed as percentages of the original stock solution.

FINAL STAGES OF TOXICITY TESTING

Preliminary experiments should provide an indication of concentration range and duration of exposure for final stages of testing. The following are essential considerations in this testing.

1. Concentrations in treated groups**:** Try to use at least 2-4 concentrations which based on preliminary testing, will likely cause sublethal effects. Also, try to use at least one slightly lower concentration that causes no effects. A minimum of 5-6 crickets should be used for each concentration, although 8-10 provide even more statistical power. Select female crickets of similar size for all groups. Use a separate container for each.
2. Controls**:** In addition to groups of treated crickets, it is essential to have a control group (see Glossary). The purpose of the control group is to verify that effects in exposed groups are, in fact, due to the chemical itself rather than to some other aspect of the procedure. Therefore, the number of organisms, handling procedures, temperature, lighting, testing methods, use of solvents to distribute chemicals, exposure times, etc. should all be identical to those used in treated groups. If control conditions cause effects, then these must be subtracted from effects in treated groups in order to obtain true measure of the chemical's effects.
3. Effects**.** Results from preliminary experiments often provide clues regarding expected types of sublethal effects and expected timing for appearance and disappearance of effects.
4. Exposure duration and frequency of testing**.** One strategy for toxicity testing is to make a single set of short-term observations or tests of organisms after exposure to the chemical for a fixed time period, such

as 24 hours. This minimizes handling of organisms and provides a standardized basis for comparing results between different researchers and laboratories.

5. Reversibility and rescue**.** If chemical effects on an organism are truly sublethal, then the crickets should survive if exposure is promptly stopped. But survival does not always mean full or immediate recovery from effects. Study the persistency or reversibility of toxicity effects (**recovery**) by simply placing organisms into chemical-free conditions and continuing observations and testing. Effects may disappear in minutes, hours, or days. Mortality usually occurs within 24 hour if attributable to the immersion.

Another testing strategy is to perform a series of repeated tests and measurements that better describe the sequence and time-table of symptoms and effects caused by a chemical. This may be especially important for chemicals that rapidly cause neurotoxicity effects that, in turn, lead to other effects. So, if crickets are not observed or tested frequently, important effects may be missed.. There are no standard procedures for doing this and the experimenter should exercise his/her own judgment based on results from preliminary experiments.

Knockdown effects should be determined as quickly as possible after exposure. Otherwise it may be difficult to get consistent data.

Special care should be taken to avoid cross-contamination of containers or implements that are used to handle treated crickets or fluids. Repeated observations and testing may be done at any desired interval, but the frequency of testing should be the same in all groups, including a control.

OTHER ORGANISMS, OTHER IDEAS

In environmental toxicology the selection of an invertebrate test organism and test chemical are often closely inter-related. Chemicals that are relevant to terrestrial/soil ecosystems, for example, might be tested using commonly available invertebrates such as earthworms, pillbugs, insect larvae, or nematodes. Tests with chemicals that are relevant to freshwater ecosystems might utilize aquatic invertebrates such as water fleas, ostracods, copepods, hydra, planaria, snails, or amphipods (scud). The effect that is tested might have special ecological relevance to predator avoidance, food acquisition, ability to react to stimuli, or ability to locomote. Behavioral effects could be quantified using some defined scoring system, or effects could be analyzed using videotape playback.

Another approach to environmental toxicity is collection and testing of soil or water samples from real-world sites where contamination is suspected. Water samples from a site may be used in laboratory toxicity tests and effects may be compared to those in control groups as well as to groups treated with concentrations of a pure chemical which is the suspected contaminant in the water samples. Such experiments utilize invertebrates as true "**bioassay**" organisms. In cases of soil samples, organisms could be exposed to water extractions (leachates) derived from soil samples.

For toxicity testing relating to human health concerns, attempts should be made to match the kinds of effects that might be expected in humans (say, neurotoxicity effects or developmental effects) with organisms in which similar effects might be present and readily testable.

For any student interested in science fairs, environmental impact of any substance should be researched and discussed in some detail as this is a key area of concern.

OBTAINING BACKGROUND INFORMATION

If possible, locate general texts in toxicology which may contain more helpful or specialized information. Recommended reference books include: Kamrin (1988), National Research Council (1991), Viccellio (1993), Ware (1996), Hodgson and Levi (1997), Ottoboni (1997). Additional reference books that are likely to have key technical information are: *CRC Handbook of Chemistry and Physics* and *Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals.*

The most reliable and up-to-date information about toxicity effects of chemicals on invertebrates and other organisms is found in primary references: namely, original articles that are published in scientific journals. References to such journal articles can be located in many college, university, or medical libraries using several different electronic data bases for scientific literature. Three of the most useful for toxicology purposes are: AGRICOLA, MEDLINE, AND BIOLOGICAL AND AGRICULTURAL INDEX (BIAG).

A very limited amount of credible and relevant information about effects of specific toxicants may be available on the World Wide Web. Considerable caution should be exercised in evaluating any web-derived information relating to chemicals or chemical effects.

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APPENDICES

Appendix I - Materials List (typical equipment and supplies)

1. Calibrated pipetter (e.g., Pipetman) for measuring milliliter or microliter quantities of chemicals that are fluids
2. Balance for measuring milligram or sub-milligram quantities of chemicals that are solids
3. Uncontaminated glassware for making serial dilutions and storing chemical stock solutions
4. Large supply of 60 mm Petri dishes to check for Knockdown in each group (such as, 6 organisms/group x 5 groups = 30 containers)
5. Sturdy box or tray for storage and transfer of treatment dishes.
6. Counter space covered with clean, absorbent, disposable material, such as paper towel
7. Safe storage location for all stock solutions, solvents, and all experimental containers (fume hood, if possible)
8. Disposable vinyl (or latex) gloves
9. Protective eyewear and lab coat (as advised and needed)
10. Spring water (tap water that has been aged in a open container for at least a week is often just as safe)
11. Ethanol and disposal jar for disposing of living crickets
12. Capped or covered containers for storage of stock solutions (approximately 100-500 ml volume)
13. Scientific calculator
14. Paper towels
15. Adhesive labels or colored labeling tape for labeling test dishes and containers with stock solutions
16. Permanent marking pen (sharpies)
17. Experimental organisms (crickets) with appropriate maintenance or culture container (an old plastic aquarium is fine)
18. Supplies for handling, feeding, and care of organisms (see cricket care guide)
19. Thermometer (to document temperature of all experiments)
20. 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.

21. Bound notebook and pen for record keeping
22. Dissecting or compound microscope with light source
23. Recording/monitoring devices (e.g., camera, camcorder, video camera, etc.), if desired for documentation of behavioral effects (optional)
24. Timer

Appendix II - Glossary of Terms

1. **absorption** - Entry of a chemical into the body through a surface such as the skin, digestive tract, or respiratory tract.
2. **acute toxicity -** Adverse effects of a chemical on an organism after brief *exposure* to a relatively large amount of the chemical. Often, acute effects occur a few minutes or hours after exposure begins. [compare to *chronic toxicity*]
3. **alkaloid** - are naturally occurring chemical compounds containing basic nitrogen atoms. The name derives from the word alkaline and was used to describe any nitrogen-containing base. Alkaloids are produced by a large variety of organisms, including bacteria. fungi, plants and animals and are part of the group of natural products. Examples are the local anesthetic and stimulant cocaine, the stimulant caffeine, nicotine, the analgesic morphine, or the anitmalarial drug quinine. Some alkaloids have a bitter taste.
4. **analyte** - is a substance or chemical constituent that is determined in an analytical procedure, such as a titration. For instance, in an immunoassay, the analyte may be the ligand or the binder, while in blood glucose testing, the analyte is glucose. In medicine, *analyte* often refers to the type of test being run on a patient, as the test is usually determining a chemical substance in the human body.
5. **ataxia** - Inability to produce coordinated movements or locomotion due to neurotoxicity effects or neurological disorder.
6. **behavioral toxicology -** Study of the disruptive effects of chemicals on the behavior of organisms.
7. **bioassay** - *Strict definition*: Use of a living organism to estimate the amount of a chemical in a test sample. In toxicology, this is done by comparing the toxicity effects produced by a test sample, which contains an unknown amount of a chemical, to the toxicity effects produced by known amounts of the chemical. *Loose definition*: The

use of an organism to investigate or test for toxicity effects of chemicals.

8. **biosensor** - is a device for the detection of an analyte that combines a biological component with a physicochemical detector component.
9. **chronic toxicity** - Adverse effects of a chemical on an organism as a result of long-term exposure to a relatively small amount of the chemical. Often, chronic effects become evident only many days or weeks of repeated or continuous exposure. [compare to *acute toxicity*]
10. **contaminant** - A chemical that taints or corrupts soil, water, food, or air, thus making it impure. [compare to *toxicant* and *toxin* and *pesticide.*
11. **control group** - 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.
12. **contact exposure** - Exposure of an organism to a chemical by direct contact with a surface of the body, such as skin.
13. **dose** - The total amount of a chemical given to an organism at one specific time. [compare to *dosage*]
14. **dosage** - The rate of administration of a chemical or drug to an organism. A stated dosage includes the dose, dose frequency, and total period of time that a chemical is administered to the organism. [compare to *dose*].
15. **dose-response relationship** - A quantitative relationship between the amount of chemical given to (or taken in) by organisms in a group and the measured effect of the chemical in the organisms, as determined by some type of toxicity test. In a dose-response graph, the amount of chemical is shown on the x-axis.
16. **EC50** - In a dose-response relationship showing sublethal effects, the EC50 is the concentration that produces a level of effect = 50% of the maximum effect. For example, the EC50 may be the concentration that causes a particular behavioral effect in 50% of the organisms that are tested. [compare to *LC50*]
17. **effect** - Any observable or measurable biological response of an organism to chemical *exposure*. The measured effect in a toxicity test may be lethality -- that is, death caused by chemical exposure -- or the measured effect may be sublethal, such as a change in an organism□s behavior, physiology, and/or biochemistry.
18. **environmental toxicology** - A subdivision of toxicology that deals with the presence, movement, chemical fate, and biological effects of chemical contaminants within air, land, or water

environments, especially in relation to individual organisms, populations of organisms, food chains, or habitats.

19. **exposure** - Contact of an organism with a chemical. [see *chronic toxicity* and *acute toxicity*]
20. **foreign** - a**:** occurring in an abnormal situation in the living body and often introduced from outside <a *foreign* body lodged in the esophagus> **b:** not recognized by the immune system as part of the self <*foreign* proteins>
21. **hazard** - A danger or threat that a chemical poses in terms of some toxicity effect(s) [compare to *risk*]
22. **LC50** - In a dose-response relationship, the LC50 is the concentration of chemical that is expected to produce death in 50% of the organisms that are exposed to that concentration. [compare to *EC50*]
23. **lethality** - Death of an organism caused by chemical effects.
24. **lipid soluble/lipophilic** - Refers to chemicals that tend to be soluble in lipids but not water. Lipophilic substances tend to easily cross cell membranes and enter the body. [compare to *hydrophilic*
25. **mode of action** - Refers to the biological/biochemical mechanism (or mechanisms) by which a toxicant is known to (or is believed to) exert its effects on an organism.
26. **mortality** - The frequency of deaths in a group of organisms exposed to a chemical. [compare to *moribund*]
27. **moribund** - Describing a state in which an organism is beginning to die or is near death. [Compare to *mortality*]
28. **MSDS** -Material Safety Data Sheet. [Source for MSDS:http://hazard.com/msds/]
29. **neurotoxicology** - Study of the adverse effects of chemicals on the structure or function of the nervous system; neurotoxicity effects often cause behavioral effects. [see *behavioral toxicology*]
30. **neurotransmitter** - A chemical (such as acetylcholine) that is released by a nerve cell at a chemically transmitting synapse.
31. **no observed effect level (NOEL)** - In a dose-response relationship, the NOEL is the highest concentration of a chemical that causes no observable effect in a group of organisms. [compare to *threshold concentration/dose*]
32. **non-target organism** - An organism that is exposed to, but is not the intended target for, an applied pesticide
33. **paralysis** - Inability to move the body or body parts due to effects of disease or toxicity.
34. **parts per million (ppm)** - A unit of chemical concentration. The concentration of a chemical is 1 ppm if one weight unit of chemical (for example, 1 milligram) is dissolved in one million weight units of water (1,000,000 milligrams of water = 1 liter). Very low

concentrations of chemicals may be expressed in parts per billion or high concentrations in parts per thousand.

35. **pesticide** - A chemical used to kill organisms that are considered pests. [see *non-target organism*]
36. **poison** - Synonym = *toxicant*. Any chemical that causes harmful biological effects.
37. **recovery** - The disappearance of toxicity effects in an organism and return to normal function and behavior. If this occurs, it often occurs at some point in time after sublethal exposure to a chemical has ended.
38. **red imported fire ants** - (*Solenopsis invicta*), or simply RIFA, is one of over 280 members of the widespread genus *Solenopsis*. Colonies were accidentally introduced into the United States in the 1930s through the seaport of Mobile, Alabama. Cargo ships from Brazil docking at Mobile unloaded goods infested with the ants; they have since spread from Alabama to the coastal plain and piedmont of almost all of the south-eastern states, as well as into California.
39. **risk** - The probability that adverse effects will occur if an organism is exposed to a chemical under a specific conditions.
40. **serial dilution** - Creation of a series of separate solutions with concentrations that differ in a regular, step-wise fashion, such as a series of concentrations that decrease by a factor of five: 50, 10, 2, 0.4 ppm. Serial dilutions may be used for both range-finding and final stages of toxicity testing.
41. **solenopsin** - ($C_{17}H_{35}N$) an alkaloid which inhibits angiogenesis, in addition to contributing to the known and often despised toxic effect of fire ant venom. Solenopsin has also been shown to have cytotoxic, hemolytic, necrotic, insecticidal, antibacterial, antifungal, and anti-HIV properties. Originally synthesized in 1998, several groups have designed novel and creative methods of synthesizing enantiopure solenopsin and other alkaloidal components of ant venom. Though the use of ant venom in a remedial sense has often been marginalized to homeopathic therapies, basic science research is just beginning to
42. **solvent** - A liquid that is capable of dissolving other chemicals.
43. **sublethal concentration** - A concentration of chemical that does not kill an organism.
44. **sublethal effect** - A biological effect caused by chemical exposure at a concentration below that which causes death.
45. **threshold concentration/dose** - In reference to a dose-response relationship, the threshold dose/threshold concentration is the minimum amount of a chemical that just causes an observable

effect in a group of organisms. [compare to ***no observed effect level***]

46. **toxic** - Synonym = harmful or poisonous.
47. **toxicant** - Synonym = poison. Any chemical that causes harmful biological effects.
48. **toxicity** - The capacity of a chemical to produce harmful effects. orthe degree to which something is able to produce illness or damage to an exposed organism. Toxicity can refer to the effect on a whole organism, such as a human or a bacterium or a plant, or to a substructure, such as a cell (cytotoxicity) or an organ (organotoxicity such as the liver (hepatotoxicity).
49. **toxicity test** - A controlled test in which the effects of a toxicant are studied on living cells, tissues, or organisms.
50. **toxin** - A toxicant produced by a living organism. [compare to toxicant and contaminant]
51. **toxicology** - The study of the adverse effects of chemicals on living organisms.
52. **voltage-gated channel** - A membrane channel protein, usually in nerve and muscle, that opens (or closes) in response to membrane depolarization. Voltage-gated channels generate electrical impulses (= action potentials).
53. **water soluble/hydrophilic** - Refers to chemicals that are soluble in water but not in lipids. [compare to *lipid soluble*]
54. **xenobiotic -** Any chemical that does not occur in the normal biochemical pathways of an organism, a xenobiotic compound is a compound that is foreign to the organism.

Appendix III - Care and Feeding of *Acheta domesticus* (the brown cricket)

http://creatures.ifas.ufl.edu/misc/crickets/adomest.html

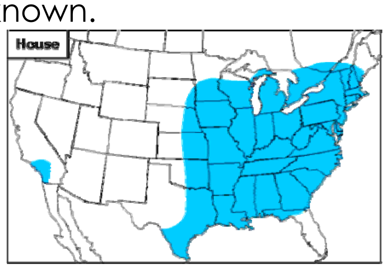
www.dragon-seekers.com/images/crickets.jpg

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.


female house cricket, hindwings intact

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.wnyherp.org/care-sheets/general/cricket-care.php
http://www.anapsid.org/crickets.html

Appendix IV – 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)

BIOLOGY II

CONTENT STRANDS:

Life Science (L) Earth and Space Science (E) Physical Science (P)

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 V – Pretest / Post test

Muse of Fire - Toxicology Explorer Student ID #

____

____ 1. Entry of a chemical into the body through a surface such as the skin, digestive
tract, or respiratory tract.

A. resorption
B. adsorbtion
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

Appendix VI – Quickguide – Step by step instructions

Day 1

- Establish cricket colony. (See appendix III)
- You may purchase crickets at any bait store.
- Give pretest prior to any other toxicology discussion in order to gauge the progress of student before and after this training.
- Give pre-lab text material to students or provide links to materials for students to review

http://www.wikihow.com/Raise-Your-Own-Crickets

Day 2

- Begin discussing the toxicology primer.
- Choose a chemical or chemicals to study. You may wish to study household product and a fairly complete list with harmful effects can be found at http://householdproducts.nlm.nih.gov/
- If you use a liquid product such as Windex ®, you will need to ensure that students know how to dilute the product by percentages. A serial dilution could be set up as follows.

➔ ➔ ➔ ➔

50 ml
100% Windex
Take 25 ml out

25 ml 100 %

25 ml H_2O
+
25 ml 100%

50 ml 50%

25 ml H_2O
+
25 ml 50%

50 ml 25%

25 ml H_2O
+
25 ml 25%

50 ml 12.5%

25 ml H_2O
+
25 ml 25%

50 ml 6.25%

- For dry chemicals milligrams per liter = parts per million or you could use appropriate molar concentrations. Just select those preliminary concentrations based on prior research or you may choose the "shot in the dark" method. Start with an extremely high concentration and dilute it as above. Just take care to label all solutions carefully and follow the MSDS guidelines for storage and disposal. (see http://www.msds.com/)
- Discuss research plan and how to keep a lab notebook and properly recorddata http://www.swarthmore.edu/NatSci/cpurrin1/notebookadvice.htm

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!! ☺

Day 3

- Begin preliminary immersion tests. Your design will determine the concentrations and immersion times but in our preliminary study of Windex ® we used 30 second immersion at each of the above concentrations.
- Our submersion response table for 30 sec. immersion in Windex is below. Each group had 5 crickets. This was an extremely preliminary result attempting to establish tolerated threshold concentrations of the substance for *Acheta domesticus*
- Water was used as the control.

Dosage group (% Windex)	**Knockdown (%)**	**Mortality (%)**
100	100	100
50	100	100
25	60	60
12.5	40	40
6.25	10	10
5	10	20
2.5	0	0
water	0	0

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

Day 4 – 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 may help
- Give Post – test
- Dispose of any remaining crickets in ethanol (unless you plan to keep the colony).