

Biological Smoke Detectors

*A Toxicology Primer
for Student Inquiry*

DISCLAIMER:

The user assumes all responsibilities for the safe and proper handling and application of chemicals listed and described herein. The author (CDD) 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 the author's favorable or unfavorable endorsement of these products.

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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.

At the outset, I want to strongly emphasize that *my aim is not to popularize and promote toxicology experimentation at pre-college levels*. 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 my research experience and mentoring activities in ecotoxicology and neurotoxicology, mostly involving aquatic and terrestrial oligochaete worms. So, I admit to a strong “worm bias” and the content of this article reflects that bias. However, I think most toxicologists would agree that these organisms offer good potential for toxicity studies in both science education and professional research. Many other invertebrate species, of course, may be equally or more useful, depending on one’s objectives and interests. I hope the ideas here will be of general use, as a conceptual template, regardless of species. Since the vocabulary of toxicology may be alien to students, I have included a glossary of common toxicological terms.

For economic, ethical, and pedagogical reasons I do not recommend that any pre-college student undertake a toxicology project that uses vertebrate animals. Also, I do not recommend that students use hazardous chemicals or attempt a toxicology project unless they have approval and supervision from a qualified teacher/mentor. Maximizing safety and minimizing human health risks are paramount concerns for all toxicology investigations! Nevertheless, given a little creative and careful thinking, I believe that many novel, safe, and scientifically valuable investigations can be done in toxicology by novice researchers, even when faced with limited resources. Above all, research students should remember the “double-K.I.S.S.” guidelines:

*first: **Keep It Scientifically Sound!***

*second: **Keep It Simple and Safe!***

“BIOLOGICAL SMOKE DETECTORS”

Recently, I met with several middle school students and their teacher to discuss their proposed student research project in environmental toxicology. One of these students asked me why anyone would study effects of a toxicant on invertebrate organisms, such as freshwater oligochaete worms. I tried to explain and justify using the old analogy of a “canary in the coal

mine.” However, based on the group’s quizzical reaction, I realized that the analogy was alien and from a by-gone era. Obviously, I needed an updated analogy to emphasize how *living organisms are used as “early warning systems,” or sentinels, for detecting harmful substances in the environment and for studying their biological effects.* One warning device that comes to my mind is a “smoke detector”... thus the title, “Biological Smoke Detectors.” When a smoke detector goes off it doesn’t necessarily mean there’s a fire or that anyone will be harmed, but it does indicate that there is a potential problem that requires close and immediate inspection. The same is true for *toxicity tests* that signal adverse biological effects on living organisms.



Fig. 1. The canary was once used as sensitive bio-indicator for the presence of poisonous gas in underground mine shafts. Today, the term “canary in a coal mine” is an antiquated metaphor used in reference to many types of “early warning systems.”

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 zooplankton 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* (see Glossary). This is because sublethal effects occur at concentrations below those for lethality and thus are more sensitive indicators of toxicity.

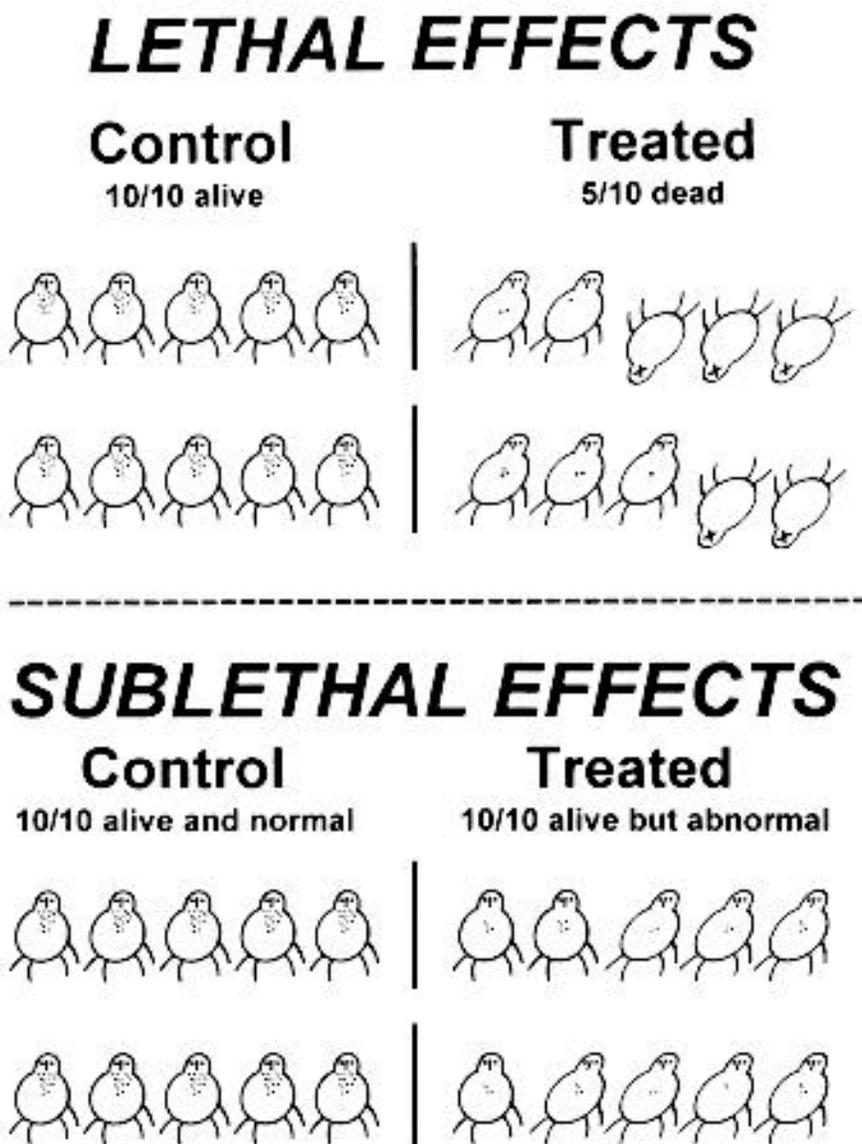


Fig. 2. Comparison of lethal and sublethal treatment effects. Treatment with high chemical concentrations may be lethal in some or all treated organisms. Lethal effects are easy to recognize and tabulate. Treatment with lower concentrations may produce sublethal effects which are sometimes difficult to discern but, nevertheless, important from a behavioral or ecological standpoint.

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, or 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.

SOME WORMY IDEAS FOR TOXICITY TESTING

Freshwater organisms, such as aquatic oligochaetes are 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).

[Note that the genus *Lumbriculus* is very different from the mud-dwelling genus *Tubifex*. *Lumbriculus* displays unique locomotor behaviors, such as helical swimming and reversal (Drewes, 1999; Drewes and Cain, 1999). *Tubifex* are much less acrobatic and display neither of these behaviors.]

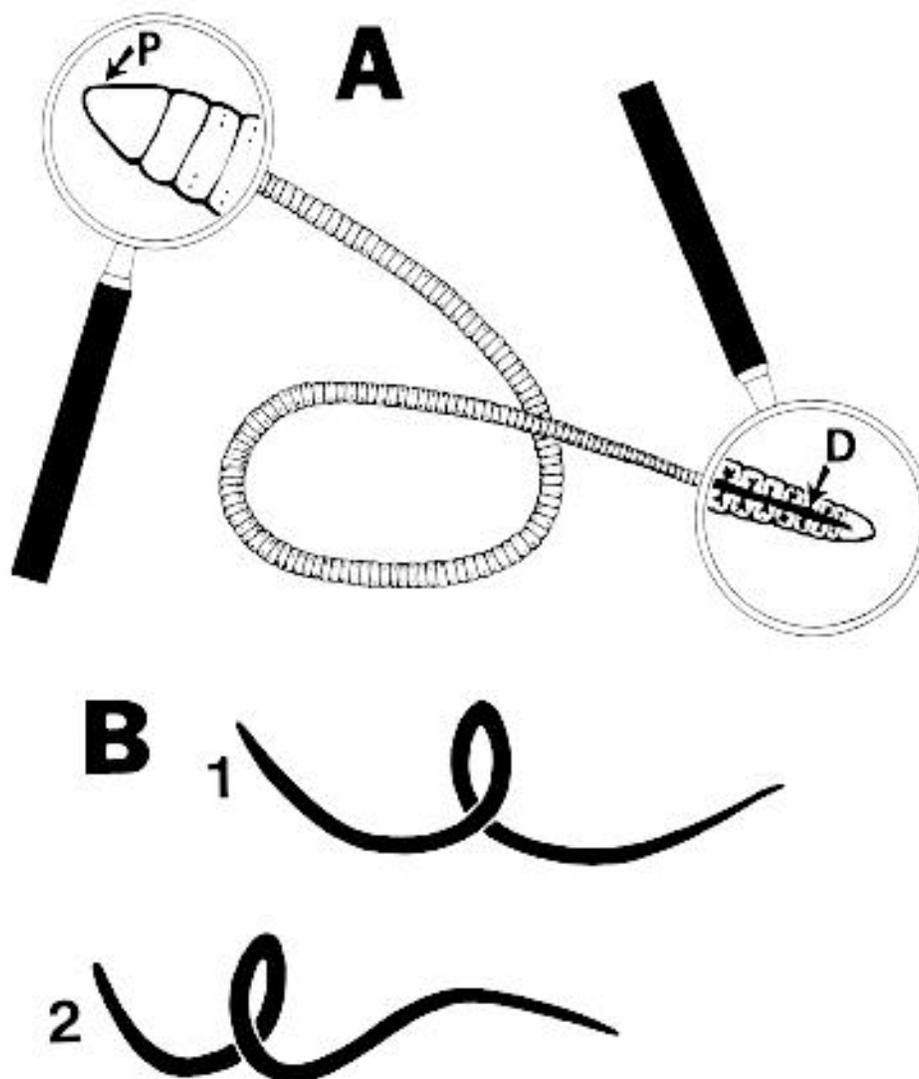


Fig. 3. The segmented oligochaete, *Lumbriculus variegatus*, is widely distributed throughout the U. S. and easily cultured in the laboratory. Several behavioral and physiological features of this worm may be sensitive indicators of sublethal toxicity effects. A) Diagram of an untreated, whole worm showing prostomium (P) and dorsal blood vessel (D). B) Two freeze-frame images of the cork-screw shape of a worm's body during helical swimming -- a normal response to tail touch when the worm is in open water. Note that the first wave (B1) has a counter-clockwise, or left-handed helical orientation. The second wave, occurring about 1/10 sec later, has a clockwise, or right-handed, orientation. [For details, see Drewes, 1999; Drewes and Cain, 1999].

SUBLETHAL CHEMICAL EFFECTS IN *LUMBRICULUS*

Possible sublethal effects in blackworms include changes in *body shape or behaviors* such as swelling, coiling, rigidity, convulsions, limpness, paralysis, ataxia, hyperactivity, constrictions, or segment autotomy (body fragmentation). Some chemicals may cause changes in *body color* due to circulatory effects such as blood pooling or blood loss in different body regions, especially

the tail end. Also, there may be important and interesting effects on other body functions that are only evident with more detailed inspection and testing of treated and normal worms.

One function that may be useful and relevant to both ecological and medical toxicity testing is the pulsation rate of the worm's dorsal blood vessel (Fig. 3A). Just as in humans, pulsation rates in worms may speed up or slow down as a result of toxicant exposure. Lesiuk and Drewes (1999) describe methods for measuring pulsations rates in the dorsal blood vessel before, during, and after exposure to common pharmacological agents such as nicotine and caffeine.

Other functions that may be potentially affected by toxicants include locomotor behaviors such as swimming (Fig. 3B), crawling, and body reversal -- all behaviors that are easily evoked and readily measured (Drewes, 1999; Drewes and Cain, 1999). These functions have special environmental relevance because they relate to the worm's ability to move about within its environment and escape from predators.

Another biological process that is easily studied and measured is regeneration of head and tail segments (Drewes, 1996a). Regeneration of lost segments is a key developmental process that has great adaptive significance to worms. It is a means for restorative growth following loss of segments, which frequently occurs in nature as a result of predatory attack or spontaneous fragmentation; the latter is a normal mechanism for asexual reproduction in these worms.

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/>
<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>

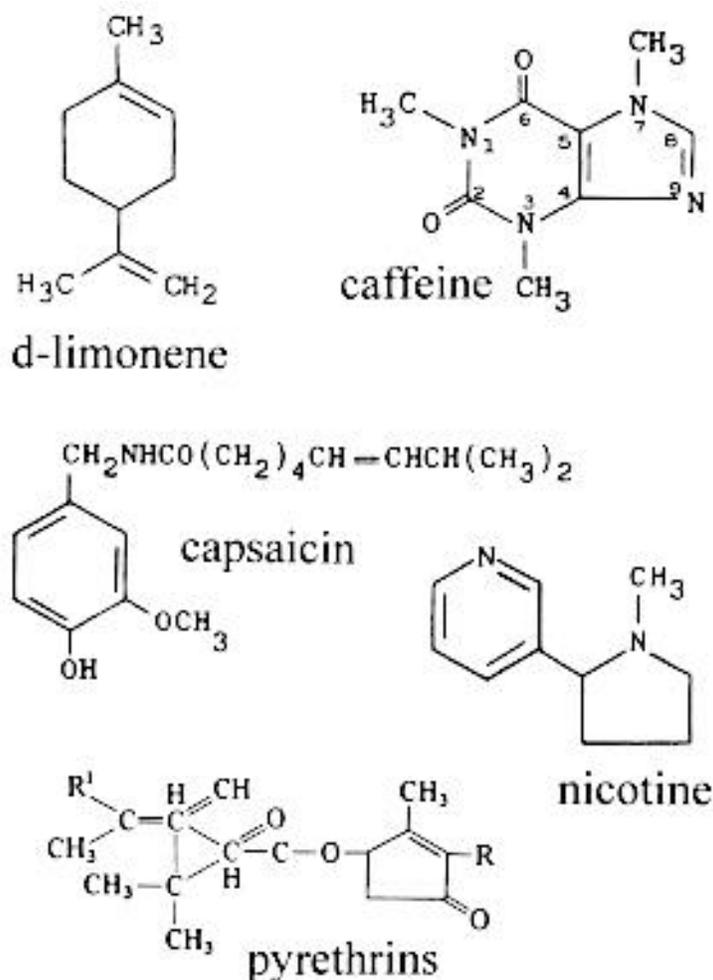


Fig. 4. Chemical structures of some compounds that cause significant and interesting sublethal effects on *Lumbriculus variegatus*.

LIST OF POSSIBLE TEST CHEMICALS

boric acid: An inorganic salt (H₃BO₃). An insecticidal powder used indoors for cockroach and ant control. Occurs in nature as the mineral, sassolite. 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 CO₂ 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.

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 plasticware 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.

Finally, *it is important to begin experiments without any traces of soap residue on glassware*. Soap residues are especially toxic to many aquatic invertebrates.

EXPOSURE METHODS

A simple way to expose worms to water-soluble chemicals is by immersion. Worms are placed in individual containers along with a small volume (about 20-30 ml) of test solution of known chemical concentration. The chemical is thus absorbed through the skin (termed contact exposure). Always use just one worm per container, since a dead, decaying worm may be toxic to others.

For water-insoluble (and non-volatile) chemicals, there is a simple and reliable alternative to exposure by immersion. This involves placing the worm in direct contact with wet filter paper that has been uniformly pre-treated with the insoluble test chemical. Pre-treatment is done by placing a dry filter paper disk in the bottom of the glass exposure container. The disk should fit snugly and flatly at the bottom of the container. Then, prepare stock solutions as described in section “E” below. Each solution should contain a known amount of the water-insoluble chemical dissolved in a known volume of suitable solvent, such as ethanol or isopropyl alcohol.

Using a calibrated, hand-held pipette, transfer just enough of the desired stock solution to completely saturate the filter paper disk. Allow the solvent to evaporate completely in a fume hood. This leaves behind a known and nearly uniform residue of the test chemical on the paper (assuming that the test chemical is not volatile). Next, add a known volume of spring water into the container so that the paper is immersed in shallow water. Use enough water volume so that the worm could be easily drawn up into a disposable pipet if later transfer is needed. For example, 5 ml of water is adequate for a 6 cm diameter plastic petri dish. Next, add a worm.

All toxicity tests should include a control group in which the paper in test containers is initially wetted with an identical volume of solvent (but no chemical in it). Once the solvent evaporates, water and a worm are added, just as in treated groups.

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* (see Glossary). 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 or other suitable solvent (such as, ethanol or isopropyl alcohol for non-water soluble chemical). 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*” (see Glossary). 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* (see Glossary). 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.

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 worms should be used for each concentration, although 8-10 provide even more statistical power. Select worms of similar size for all groups. Use a separate container for each worm.

Controls. In addition to groups of treated worms, *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.

Effects. Results from preliminary experiments often provide clues regarding expected types of sublethal effects and expected timing for appearance and disappearance of effects.

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 or 48 hours. This minimizes handling of organisms and provides a standardized basis for comparing results between different researchers and laboratories.

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 worms 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.

Ideally, effects should be determined while worms are still in their original exposure container. However, this may not always be practical or desirable, especially if filter paper is used in the container, because it may obscure viewing or interfere with testing. In such instances, a worm may be very carefully removed from the test solution with a disposable pipet so that it may be briefly examined or tested while in another container without the chemical. Before doing this, however, worms should be briefly and quickly rinsed in spring water and then transferred to the new container for viewing and/or testing.

After testing, the worm should be replaced into the original test solution if further exposure is desired. Use a disposable plastic pipette for transferring worms. Special care should be taken to avoid cross-contamination of containers or implements that are used to handle treated worms 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.

Reversibility and rescue. If chemical effects on an organism are truly “sublethal,” then organisms 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.

TYPICAL EQUIPMENT AND SUPPLIES

Here is a list of materials and equipment that may be used for toxicity testing in freshwater or terrestrial oligochaetes. Check with instructor/mentor for approval or modification of the list.

Materials List

- 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 identical covered dishes or jars (such as clean, dry baby food jars with covers; jars should thoroughly cleaned but must not contain any soap residues since these will kill many aquatic invertebrates); containers must not be prone to spills and should be easy to handle; one organism recommended per container (such as, 6 organisms/group x 5 groups = 30 containers)
- 5) Filter paper discs that easily fit into the bottom of the exposure dish or jar (some trimming may be necessary)
- 6) Sturdy box or tray for storage and transfer of treatment dishes or jars
- 7) Counter space covered with clean, absorbent, disposable material, such as paper towel
- 8) Safe storage location for all stock solutions, solvents, and all experimental containers (fume hood, if possible)
- 9) Disposable vinyl (or latex) gloves
- 10) Protective eyewear and lab coat (as advised and needed)
- 11) Spring water (Note: Freshwater organisms survive and perform best in spring water. Examples of brand names of “worm-friendly” spring water are *Evian*, *Poland Spring*, *Naya*, and many others). Aqueous stock solutions of chemicals and serial dilutions of stock solutions all should be made with this water and stored in separate, clearly labeled containers. Chlorinated

water, directly from the tap, is highly toxic to freshwater invertebrates. However, tap water that has been aged in a open container for at least a week is often just as safe to worms as spring water.)

- 12) Ethanol, or other solvent (needed only if chemical is not water-soluble)
- 13) Capped or covered containers for storage of stock solutions (approximately 100-500 ml volume)
- 14) Scientific calculator
- 15) Paper towels
- 16) Adhesive labels or colored labeling tape for labeling test dishes and containers with stock solutions
- 17) Permanent marking pen
- 18) Experimental organisms with appropriate maintenance or culture containers (large dishes, aquaria. etc.)
- 19) Supplies for handling, feeding, and care of organisms (such as air pump for aquatic species)
- 20) Thermometer (to document temperature of all experiments)
- 21) Test chemical, 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.
- 22) Bound notebook and pen for record keeping
- 23) Dissecting or compound microscope with light source
- 24) Recording/monitoring devices (e.g., camera, camcorder, video camera, etc.), if desired for documentation of behavioral effects (optional)
- 25) Blackworms (*Lumbriculus variegatus*); commercial sources may be found at:
http://www.zool.iastate.edu/~c_drewes/WORMSO5.htm

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 a true “*bioassay*” organisms (see Glossary). 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.

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.

[NOTE: Upon request, I will gladly send copies of detailed background information about *Lumbriculus* biology, or reprints of any research papers below, to students or teachers.]

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GLOSSARY OF TOXICOLOGICAL TERMS

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

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*]

ataxia. Inability to produce coordinated movements or locomotion due to neurotoxicity effects or neurological disorder.

behavioral toxicology. Study of the disruptive effects of chemicals on the behavior of organisms.

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.

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*]

contaminant. A chemical that taints or corrupts soil, water, food, or air, thus making it impure. [compare to *toxicant* and *toxin* and *pesticide*].

control group. A 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.

contact exposure. Exposure of an organism to a chemical by direct contact with a surface of the body, such as skin.

dose. The total amount of a chemical given to an organism at one specific time. [compare to *dosage*]

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*].

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.

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*]

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.

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.

exposure. Contact of an organism with a chemical. [see *chronic toxicity* and *acute toxicity*]

hazard. A danger or threat that a chemical poses in terms of some toxicity effect(s) [compare to *risk*]

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*]

lethality. Death of an organism caused by chemical effects.

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*]

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.

mortality. The frequency of deaths in a group of organisms exposed to a chemical. [compare to *moribund*]

moribund. Describing a state in which an organism is beginning to die or is near death. [Compare to *mortality*]

MSDS. Material Safety Data Sheet. [Source for MSDS: <http://hazard.com/msds/>]

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*]

neurotransmitter. A chemical (such as acetylcholine) that is released by a nerve cell at a chemically transmitting synapse.

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*]

non-target organism. An organism that is exposed to, but is not the intended target for, an applied pesticide.

paralysis. Inability to move the body or body parts due to effects of disease or toxicity.

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.

pesticide. A chemical used to kill organisms that are considered pests. [see *non-target organism*]

poison. Synonym = *toxicant*. Any chemical that causes harmful biological effects.

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.

risk. The probability that adverse effects will occur if an organism is exposed to a chemical under a specific conditions.

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.

solvent. A liquid that is capable of dissolving other chemicals.

sublethal concentration. A concentration of chemical that does not kill an organism.

sublethal effect. A biological effect caused by chemical exposure at a concentration below that which causes death.

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*]

toxic. Synonym = harmful or poisonous.

toxicant. Synonym = *poison*. Any chemical that causes harmful biological effects.

toxicity. The capacity of a chemical to produce harmful effects.

toxicity test. A controlled test in which the effects of a toxicant are studied on living cells, tissues, or organisms.

toxin. A toxicant produced by a living organism. [compare to *toxicant* and *contaminant*]

toxicology. The study of the adverse effects of chemicals on living organisms.

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).

water soluble/hydrophilic. Refers to chemicals that are soluble in water but not in lipids. [compare to *lipid soluble*]

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.