August 31, 2005

Lecture 3: Nature of Toxicity (Measuring Toxicity, Expressing Toxicity, Factors Influencing Toxicity)

I. What Is Toxicity

A. Two variables are most important in determining the likelihood that exposure to a toxicant will result in an adverse response: dose (amount of exposure) and time (frequency and duration of exposure).

B. Based on these variables, the following definition has been proposed: Toxicity "is the accumulation of injury over short or long periods of times that renders an organism incapable of functioning within the limits of adaptation or other forms of recovery." (Rozman et al. 2001, Dose, time, and other factors influencing toxicity. p. 7 in Handbook of Pesticide Toxicology, vol. 1, R. Krieger (ed.). Academic Press)

1. Note that this definition focuses on the organism, but toxicity adversely affecting many members of a population will eventually result in effects at higher levels of organization.

II. Measuring Toxicity

A. We have lofty goals of protecting ecosystems, but measuring hazards at this level and scale are not easily done. Rather, we tend to measure hazards at lower levels, and than attempt to extrapolate effects on individuals or populations to higher levels of organization.

1. Thus, to even begin to assess risk of adverse effects in ecosystems, it is necessary to understand how we measure adverse effects or toxicity at lower levels of organization.

a. An example of this "downsizing" of our focus area relative to our goal of protecting ecosystems is illustrated in the following table (which comes from Suter and Barnthouse 1993, p. 25 in Ecological Risk Assessment, G. Suter (ed.), Lewis Publishers).

Table 1. Scenario I--The policy goal (i.e., risk management objective) is no unacceptable loss of fisheries in a southern lake when a herbicide is used for weed control. The hazard in this scenario is adverse effects on fish populations. The table provides examples of assessment endpoints, possible indicators of effects on those assessment endpoints, and possible endpoints for measurements of those indicators.

Assessment Endpoints	Indicators of Effects	Measurement Endpoints
Probability of >10% reduction in game fish production	Laboratory toxicity to fish	Fathead minnow LC50; Larval bass concentration/mortality function
	Laboratory toxicity to food-chain organisms	Daphnia magna LC50; Selenastrum capricornum (algal species) EC10
	Field toxicity to fish	% mortality of caged bass
	Population abundance in treated lakes	Catch per unit effort; Size/age ratios by age classes

Table 2. Scenario II—The policy goal is no unacceptable reductions in avian populations. The hazard is bird kills following application of an agricultural insecticide.

Assessment Endpoints	Indicators of Effects	Measurement Endpoints
Proportion of raptors killed within the region of use	Laboratory toxicity to prey	Rat LD50; Japanese quail dietary LC50
	Laboratory toxicity to raptors	Sparrow hawk dietary concentration/response function; Japanese quail dietary LC50
	Avian field toxicity	Number of prey carcasses per hectare; Number of dead or moribund raptors per hectare
Increase in the rates of decline of declining bird populations within the region of use	Avian laboratory toxicity	Japanese quail dietary LC50; Starling dietary LC50
	Avian field toxicity	Number of bird carcasses per hectare by species
	Trends in populations of declining birds	Rates of decline in areas of use as proportions of reference areas

2. For the most part, we will be talking about effects on individuals, although we need some population of these individuals to estimate toxicity.
    a. Indeed, when measuring toxicity, we must use as many individuals as possible to understand the distribution of response within the population.
        1. In other words, we want to know about the heterogeneity of the response within the population.

B. Endpoints

1. To measure toxicity, we must observe some specific endpoint. Think of an endpoint as the direct or indirect biochemical, cellular, physiological, or behavioral response following exposure to a toxicant.
2. In the above tables, the most used endpoint would be lethality or mortality, as represented by the measure called the LC50 (lethal concentration to 50% of the test population) or the LD50 (lethal dose to 50% of the test population)
    a. Note the difference between concentration and dose;
        1. The use of concentration refers to an environmental residues in some volume or mass of matrix that an organism is exposed to;
        2. In contrast, expression of exposure as a dose refers to the known mass (or total amount) of xenobiotic to which an organism is exposed;
            a. However, in mammalian toxicology, dose is often normalized to a reference point like body weight; dose relative to body weight is called dosage, which is a convenient expression for comparing exposure across different organisms or different age/sex classes of a single species.

   b. The absorbed dose is the amount of toxicant that is actually absorbed into the body, whether it is through the skin or the lungs or via absorption from the intestine.
   c. In toxicity studies wherein the amount of toxicant is expressed as a concentration, for example, as so many ppb in water, the dose can be estimated by examining the toxicant residues in the whole body at different times after exposure.
      1. If the toxicant was in the diet or in drinking water, dose could be estimated by monitoring the consumption of food or water (i.e., kg or L consumed during the observation period).
3. Other lower level or individual endpoints could be biochemical, genetic, cellular, physiological, morphological, functional, or behavioral. Indeed, any mechanism of toxic action can be the basis for using an endpoint as a qualitative or quantitative measure of toxicity.
4. Elucidating endpoints is part of the Hazard Identification process. However, not all endpoints are necessarily injurious, and some may be indicative of an interaction with a toxicant but without physiological (or biological) relevance.
5. Short descriptions of examples of endpoints applicable to individuals follow (the following list is based on a general reading of published environmental toxicology studies and is only an overview, not an exhaustive treatment):
   a. <u>Biochemical and Genetic Endpoints</u>
      1. Enzyme-toxicant interactions
         a. Induction of enzyme activity
         b. Inhibition of enzyme activity
      2. Receptor-toxicant interactions
         a. Inhibition of ability of receptor to bind with its normal biochemical substrate
         b. Increase in receptor activity by mimicking the normal biochemical substrate
      3. Unusually high or low blood titers of hormones
         a. Males exhibiting unusually high levels of female hormones like vitellogenin (e.g., in fish)
      4. DNA interactions
         a. Binding with DNA, causing mutations
      5. Chromosomal effects
         a. Clastogenicity: chromosome breakage
   b. <u>Cellular and Physiological</u>
      1. Binding to membranes, interrupting nerve signals, nutrient or ionic transport
      2. Disruption of membrane structure
      3. Increases in cell death; either necrosis (unprogrammed cell death) or apoptosis (programmed cell death)
      4. Increased levels of immunoglobulins (antibodies)
      5. Reduction of chlorophyll content (applicable to plants) leading to reduced productivity
      6. Altered respiratory metabolism energetics leading to stress

7. Reduced ability to tolerate cold temperatures
8. Reduced ability to tolerate salt water (anadromous species)

c. Morphological
1. Notable signs of irritation on the body surface or in the eyes
2. Excretory discharges
3. Developmental abnormalities (teratogenicity)
a. Skeletal abnormalities
b. Abnormalities in genitalia
c. Transgender characteristics

d. Functional and Behavioral
1. Inability to avoid predation
2. Inability to secure adequate food
3. Lack of appropriate sexual behavior leading to reduced mating success
4. Impairment of cognitive ability
5. Reduction in fertility

C. Testing Organisms
1. In mammalian toxicology studies, especially those used in regulatory toxicology, wherein data are being produced to pass review of a regulatory agency (such as approval of a drug by the FDA [Food & Drug Administration] or pesticide by the EPA), rodents (rats and mice) are the subjects of choice.
a. The EPA also accepts studies on dogs.
b. A key aspect of testing is to control for heterogeneity between individuals, so all breeding has to be carefully monitored and standardized.
2. For ecotoxicological testing, the common test species are representatives of aquatic and terrestrial organisms, encompassing invertebrates, vertebrates, and plants. (A brief description of the common ecological toxicity test organisms and their natural history is given in Landis and Yu, 1999. *Introduction to Environmental Toxicology*, Lewis Publishers, pp. 82-89.)
a. The most common aquatic invertebrate tested are microcrustaceans (Phylum Arthropoda).
1. The organism most frequently used is *Daphnia magna* or *Daphnia pulex*. *Daphnia* spp. are commonly called waterfleas.
a. *Daphnia* are used to test toxicity in the water column.
2. Periodically, in ecorisk assessments, EPA will rely on data from tests with crayfish, or aquatic insects (stoneflies, mayflies, midges).
3. Other common aquatic invertebrates include amphipods (a.k.a. scuds) (*Gammarus lacustris*, *Hyalella azteca*, and others).
a. These species are used to test toxicity in sediments.
4. One rationale for using the aquatic invertebrates commonly tested is their "role" as prey for vertebrates like fish.
a. Also, the life cycle of the aquatic invertebrates is short, making lab studies feasible.
b. The most common terrestrial invertebrates used in ecotox assessments would be insects, especially the honeybee (a beneficial pollinator).

1. Several years ago, the Monarch butterfly came into prominence as a key species with regard to the use of transgenic corn plants containing a gene encoding the synthesis of the Bt (*Bacillus thuringiensis*) toxin.
    a. For an interesting risk assessment study using Monarch butterfly, see Sears et al. 2001. Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. Proc. National Academy of Sciences 98:11937-11942.

c. The most common vertebrates used in ecological toxicity testing for aquatic risk assessments are fish, and any one of several species is commonly used. These include rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), and various species of sunfish [bluegill, *Lepomis macrochirus*; green sunfish, *Lepomis cyanellus*].
    1. Other species noted in ecorisk assessments for pesticides include bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*), or any of several species of salmon (Coho, *Oncorhynchus kisutch*, is most commonly used as well as Atlantic salmon, *Salmo salar*).

d. Birds are most commonly used for terrestrial toxicity testing. The rodent tests used in mammalian toxicology studies serve as a surrogate for mammalian wildlife in ecological risk assessments.
    1. The most common bird species include mallard duck (*Anas platyrhynchos*), northern bobwhite quail (*Colinus virginianus*), and ring-necked pheasant (*Phasianus colchicus*).

e. Both aquatic and terrestrial plants are used for ecotoxicity testing. Among aquatic plants, algae and submergent vascular plants are used. Among terrestrial plants, EPA requires root elongation and early growth studies with nontarget crop species. Tests with plants are especially important for herbicide registrations.

f. Pesticides may be tested for effects on soil microbial function (for example, denitrification).

D. The U.S. EPA has published guidelines documents for conducting several different types of water toxicity tests that would satisfy the requirements for whole effluent toxicity testing (WET) required under the Clean Water Acts National Pollution Discharge Elimination System permitting process.
    1. U.S. EPA 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition, EPA-821-R-02-012 URL http://www.epa.gov/waterscience/WET/disk2/
        a. "This manual describes test for effluents and receiving waters and includes guidelines on laboratory safety, quality assurance, facilities and equipment, dilution water, effluent sampling methods and holding times, test specied selection, data analysis, report preparation, organism culturing and handling, and mobile toxicity test laboratory design. The acute toxicity tests generally involve exposure of any of 20 test organisms to each of five effluent concentrations and a control water. The test duration ranges from 24-96 hours. This manual contains specified test conditions for 10 commonly used freshwater and marine organisms: *Ceriodaphnia dubia*, *Daphia magna*, *Daphnia pulex*, brine shrimp (*Artemia salina*), fathead minnows (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinas fontinalis*), mysids (*Mysidopsis bahia*, and

Holmesimysis costata), Bannerfish shiners (*Notropis leedsi*), sheepshead minnows (*Cyprinodon variegatus*), and sliversides (*Menida menidia, M. Beryllina, and M. Peninsulae*)."

2. EPA, U. S. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Fourth edition, EPA-821-R-02-013, URL http://www.epa.gov/waterscience/WET/disk3/
   a. "This manual describes four- to seven-day methods for estimating the chronic toxicity of effluents and receiving waters to three species: the fathead minnow, *Pimephales promelas*, the cladoceran, *Ceriodaphnia dubia*; and the alga, *Selenastrum capriconutum*. Guidelines are included on laboratory safety, quality assurance, facilities and equipment, dilution water, effluent sampling methods and holding times and temperatures, data analysis, report preparation, and organism culturing and handling."
3. EPA, U. S. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Second edition 600/R-99/064, URL http://www.epa.gov/ost/cs/freshfact.html
   a. "Procedures are described for testing freshwater organisms in the laboratory to evaluate the potential toxicity or bioaccumulation of chemicals in whole sediments. Sediments may be collected from the field or spiked with compounds in the laboratory. Toxicity methods are outlined for two organisms, the amphipod *Hyalella azteca* and the midge *Chironomus tentans*. Toxicity tests with amphipods or midges are conducted for 10 d in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and test organisms are fed during the toxicity tests. The endpoints in the 10-d toxicity test with *H. azteca* and *C. tentans* are survival and growth. The second edition includes new methods for evaluating sublethal effects of sediment-associated contaminants utilizing long-term sediment exposures with the amphipod *Hyalella azteca*, and the midge *Chironomus tentans*. The long-term sediment exposures with *H. azteca* are started with 7 to 8 day old organisms. Effect endpoints measured for *H. azteca* include survival (measured on days 28, 35, and 42), growth (measured on days 28 and 42), and reproduction (measured as number of young/female from day 28 to 42). The long-term sediment exposures with *C. tentans* start with newly hatched larvae (< 24 hours old) with effect endpoints including emergence, reproduction, and hatching of the next (F_1) generation (which requires about 60 days)."

III. Quantitative Expression of Toxicity

A. Toxicity is measured by determining the relationship between dose or concentration of a substance and the response of the test organism under specified test conditions.
   1. The response can range from death to subtle changes in enzyme activity or everything in between.
   2. The most common parameter to express toxicity is the dose or concentration causing 50% of tested organisms to respond.

a. The median population response is expressed as the LD50 (if lethality is the endpoint) or the ED50 (if other types of responses are the endpoints), or the LC50 and/or EC50 if the concentration, but not the dose is known.

3. In addition to knowing the LD50 or ED50, and more importantly for purposes of determining "safety", we also want to know the dose or concentration causing no response, known as the NOEL or NOAEL (No Observable Adverse Effect Level).
   a. In aquatic toxicity testing, the concentration of test substance would be the independent variable, so the no-effect concentration would be the NOAEC.

B. How an LD50 or LC50 (or ED50/EC50) are determined
   1. Hypothetical response of a population to a stimulus (adverse or favorable) can be described as a normal distribution ("bell-shaped" curve) if we graph the numbers responding at each dose or concentration tested (Figure 1).
      a. To generate the required data, organisms are exposed via the diet, skin, or environment (i.e., air, water [aquatic organisms], soil [worms, bacteria] to a series of increasingly higher doses, starting with zero concentration as a control. The organisms are randomly assigned to experimental groups, and each group receives one dose.

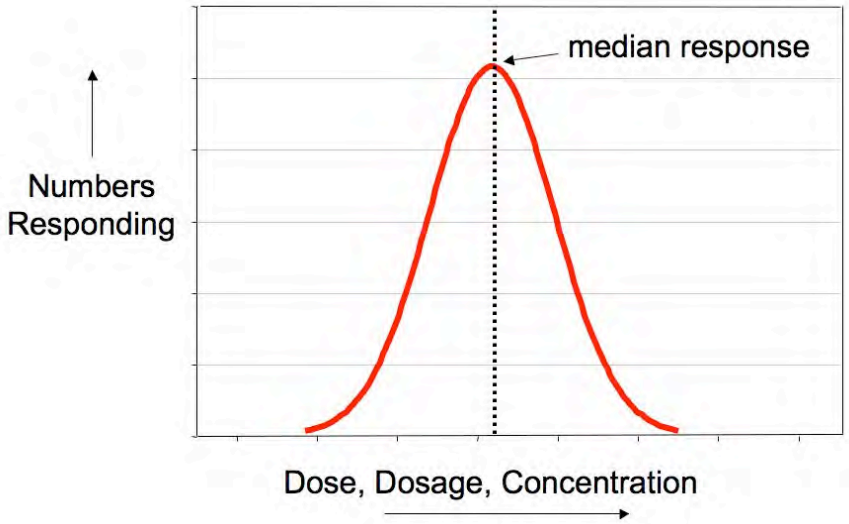


Figure 1. Normal distribution of responses to increasing dose of toxicant.

      b. The response, i.e., the endpoint, must be strictly specified; the magnitude of this endpoint is then recorded at each dose. Many times we are interested in outright death, but other effects, such as decreased weight or enzyme activity, are equally valid just as long as they are specified and measurements can be validated.
         1. Concerning endpoints, we distinguish between acute toxicity, which is usually an immediate response to the short term or single dosing of an organism, and chronic toxicity, which is a systemic effect developing over a period of time beyond the actual dosing.

a. In mammalian acute toxicity studies, a rodent is exposed usually by intubation (direct application to the stomach through a tube) to high doses; mortality is measured after 24 hours and further physiological effects monitored for the next 14 days. After 14 days the animal is sacrificed for histological observations. (Ecobichon, D. J., 2001, p. 287 in Handbook of Pesticide Toxicology, R. Krieger (ed.), Academic Press).
b. In ecological toxicity studies (i.e., testing for environmental effects), acute toxicity observations depend on the organism
    1. For fish, exposure occurs via water for 96 hours
    2. For invertebrates, exposure occurs via water for 48 hours;
    3. For birds, acute exposure can occur similarly to rats, via force-feeding directly into the stomach, and subsequent monitoring of effects 24 hours later and beyond.
c. We normally think of chronic toxicity as resulting from repeated non-acutely lethal (i.e., from sublethal) dosing.
    1. For example, in mammalian toxicity studies, chronic toxicity would be measured as developmental/reproductive effects or as carcinogenicity.
    2. For ecological toxicity studies, chronic toxicity would be measured as an adverse effect during the reproductive cycle of an invertebrate or vertebrate.
        a. The exposure would last throughout the reproductive phase.

2. The cumulative proportion responding (which can be expressed as a percent) to increasing doses can be depicted as a sigmoidal function; note that the tangent to the function would be the slope (Figure 3).
    a. The slope of the response would indicate the variability in response within the test population.
    b. The LD50, ED50, LC50, EC50 represent the dose or concentration corresponding to the median (or 50%) population response.

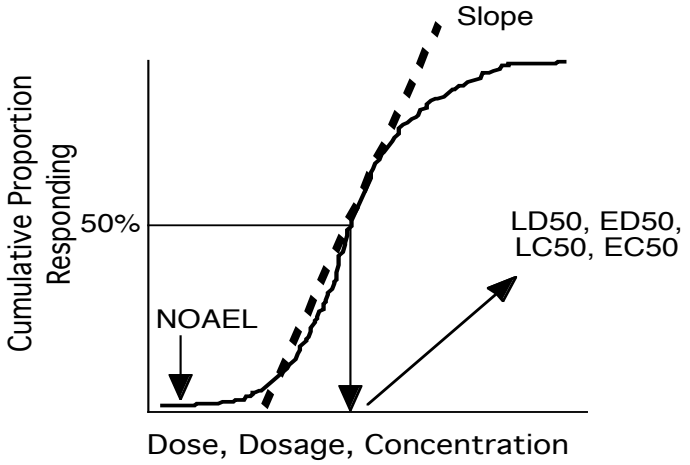
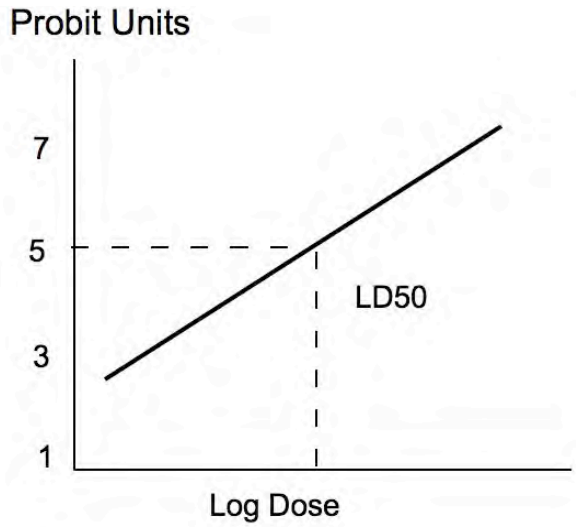


Figure 2. Sigmoidal distribution of proportion of population responding relative to dose.

3. Bliss (1935) linearized the sigmoidal function using probability units (i.e., probits, which turn out to be unit standard deviations above and below 50% mortality, or probit 5.0) plotted against the logarithmic dose [Bliss, C. I. 1935. The calculation of the dosage-mortality curve. Annals of Applied Biology 22:134-167]. (Fig. 3).
   a. The LD50/LC50 is the region having the narrowest confidence intervals, and thus the most reliable indication of response at a particular dose.
   b. See addendum starting on page 16 for an example of the computer program input and output used to estimate the dose-response function and the LC50 (or any other level of mortality).



% Mortality	Probits
10%	3.72
20	4.16
30	4.48
40	4.75
50	**5.00**
60	5.25
70	5.52
80	5.84
90	6.28

Figure 3. Transformation of the sigmoidal function to a probit function

C. Determination of the NOAEL/NOAEC
   1. Examination of Figure 2 showing the sigmoidal dose-response curve shows a concentration at which the effect being measured is essentially zero; in other words, the endpoint chosen was not found to occur among the test population.
   2. This coordinate corresponding to the dose or concentration with no measurable effect is called the no observable adverse effect level (NOAEL) if the dose is known, or the no observable adverse effect concentration (NOAEC) if the concentration but not the dose is known.
      a. Although one can model this coordinate from the empirical portion of the dose-response function, more often than not (at least in the data that US EPA uses to conduct pesticide risk assessments), the NOAEL or NOAEC is an empirical observation derived from the actual toxicity test.
   3. Note that the NOAEL or NOAEC is not usually used as an estimation of toxicity magnitude when dealing with acute exposure and lethality.
      a. For mammalian toxicology studies, the NOAEL is usually derived from either chronic toxicity testing or shorter term, multiple exposure testing known as subchronic tests.

1. For rodent toxicity tests, subchronic tests last from about one month to three months (90 days).
2. For ecological toxicity tests, the NOAEL and NOAEC is reserved as a parameter associated with life cycle (chronic) studies, which usually focus on reproductive effects.

4. The NOAEL/NOAEC are usually thought of as a threshold for toxicity, but bear in mind the threshold is only applicable to the specific endpoint being measured.
   a. It is common in mammalian toxicity testing to seek the most sensitive toxicological endpoint's NOAEL.
      1. In other words, the most sensitive endpoint would be the toxicological effect occurring at the lowest dose.
         a. The NOAEL would be determined in the experiment by comparing the response of the dosed (treated) animals with the non-dosed (control or untreated) animals, and then applying a statistical test to compare the groups.
      2. Presumably, when the threshold for the most sensitive endpoint is used, then there is protection against all effects occurring at all equal or lower doses.
   b. In ecological risk assessment, the NOAEC refers to a concentration below which no adverse effect is expected in the test organisms.
      1. Because there is a tendency to find and use the most sensitive test organisms, then there is a presumption that the NOAEC can be predictive of effects on many organisms.
         a. Unfortunately, it is impossible to know if one actually has in hand the most sensitive organism.

D. Hormesis

1. Recently, a lot of attention has been given to hormesis, a phenomenon described in the modern literature nearly 50 years ago.
   a. Hormesis is a positive or favorable physiological response to low doses of a toxicant.
   b. At low doses, the toxicant produces a stimulatory response (for example, greater growth rates) but an inhibitory response at higher doses.
2. Recent statistical examination of dose-response curves from many toxicity tests shows that a beneficial (favorable physiological effect) is common for many compounds. Following are some of the articles recently published by E. Calabrese et al.
   a. Calabrese, E. J. and L. A. Baldwin. 2002. Hormesis and high-risk groups. Regulatory Toxicology and Pharmacology 35:414-428.
   b. Calabrese, E. J. and L. A. Baldwin. 2003. The hormetic dose-response model is more common than the threshold model in toxicology. Toxicological Sciences 71:246-250.
   c. Calabrese, E. J. and L. A. Baldwin. 2003. Toxicology rethinks its central belief. Nature 421(13 February):691-692.

d. Calabrese, E. J. 2005. Pardigm lost, paradigm found: The re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. Environmental Pollution 138 379-412.

3. An example of the hormetic response can be seen in the following figure (Figure 4) taken from Calabrese and Baldwin (2002) and modified. The data was from an experiment by Ukeles 1962 (Ukeles, R. 1962. Growth of pure cultures of marine phytoplankton in the presence of toxicants. *Appl. Microbiol.* **10,** 532–537).

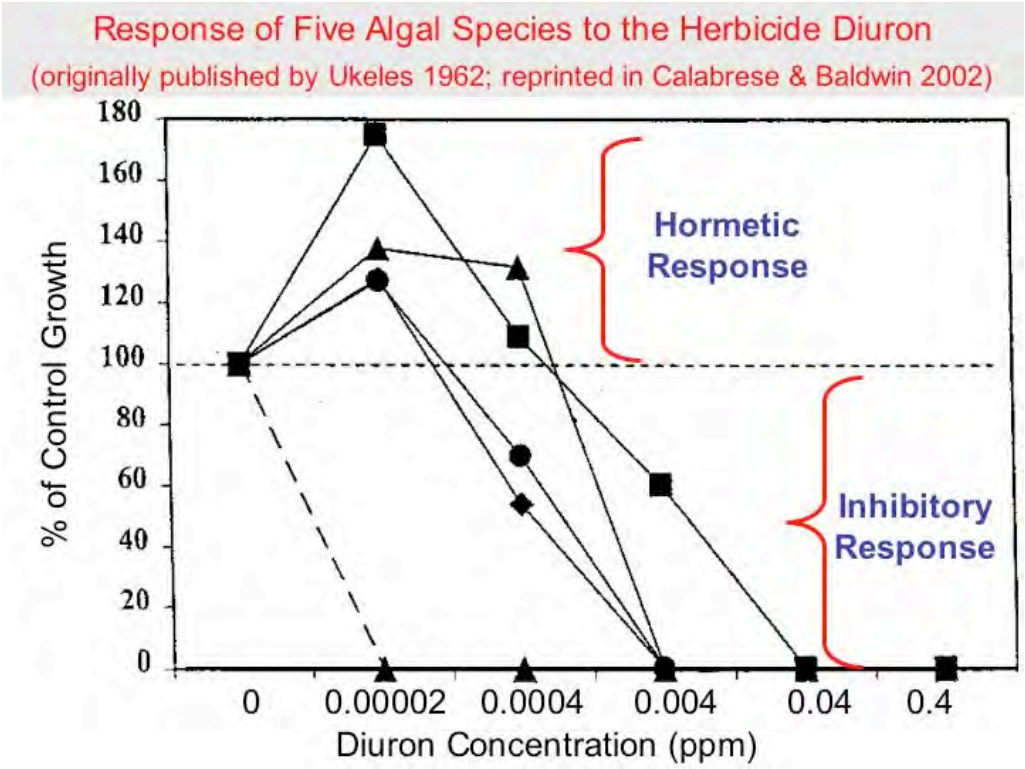


Figure 4. Response of five algal species to the herbicide diuron. Diuron, a phenylurea herbicide, is heavily used in roadside spraying to control weeds that encroach near the paved roadway. Calabrese et al. have pulled together an extensive database showing that all kinds of organisms seem to exhibit a hormetic response. However, note in the graph above, that there was one species of algal that did not exhibit this effect (represented by the dashed line). Indeed, by comparing the position of the dose-response curve to the other four algal species, you can see that its susceptibility to diuron is much greater.

IV. Factors Influencing Toxic Response

A. Two of the most important factors influencing the toxic response are dose or dosage (concentration when dealing with aquatic organisms) and time of exposure. When time is controlled or held constant in a test, then dose is the prime factor determining the appearance of injury. However, there are other

factors that can influence the expression of toxicity in addition to the dose itself. Casarett and Doull (1975, "Toxicology: the Basic Science of Poisons", Macmillan Publishing Co.; p. 134) have summarized the "toxicity-influencing factors" from the perspective of mammalian toxicology.

1. Factors related to the toxic agent
    a. Chemical composition (pH, choice of cations or anions if a salt, etc.)
    b. Physical characteristics (particle size, method of formulation, etc.)
    c. Presence of impurities or contaminants
    d. Stability and storage characteristics of the toxic agent
    e. Solubility of the toxic agent in biologic fluids
    f. Choice of the vehicle for delivering (dosing) the test organism
    g. Presence of excipients (materials used to dissolve, stabilize, and or deliver the test agent (including adjuvants, emulsifiers, surfactants, binding agents, coating agents, coloring agents, flavoring agents, preservatives, antioxidants)
2. Factors related to the exposure situation
    a. Dose, concentration, and volume of administration of the toxic agent
    b. Route, rate, and site of administration
    c. Duration and frequency of exposure
    d. Time of administration (time of day, season of the year, etc.)
3. Inherent factors related to the exposed organisms (or test subjects)
    a. Species and strain differences (i.e., taxonomic classification)
    b. Genetic status (littermate, siblings, multigenerational effects, etc.)
    c. Immunologic status
    d. Nutritional status (dietary factors, state of hydration, etc.)
    e. Hormonal status (pregnancy, etc.)
    f. Age, sex, body weight, and maturity
    g. Central nervous system status (activity, crowding, handling, presence of other species, etc.)
    h. Presence of disease or specific organ pathology
4. Environmental factors related to the subject
    a. Temperature and humidity
    b. Barometric pressure (hyper- and hypobaric effects)
    c. Ambient atmospheric composition
    d. Light and other forms of radiation
    e. Housing and caging effects
    f. Noise and other geographic influences
    g. Social factors
    h. Chemical factors
5. Note that many of the factors listed in (3) and (4) could be characterized generally as stress-producing factors.

B. With a few exceptions, most of the factors listed above have not been thoroughly studied from a quantitative perspective. For purposes of ecological toxicity testing, some of the factors have been given more attention, especially those related to age and environment. Here are some examples of quantitative data

generated regarding some of these endogenous and exogenous influences on toxicity.

1. Exposure situation: it is well known that route of administration or exposure of terrestrial organisms can influence the degree of toxicity, assuming an equal dose rate.
   a. Dermal exposures of pesticide, for example, are estimated to be four times less hazardous than oral exposures (range of ratio of toxicity of oral to dermal toxicity ranged from 0.2 to 21 with an average of 4.2; there were several compounds in which dermal exposure was more hazardous. Information was cited on p. 54 in Rozman et al. 2001). Much of what we know about differences in toxicity due to routes of exposure comes from rodent studies.
   b. Figure 5 shows a comparison of insecticide toxicity when the pesticide is administered to rodents via an acute oral dose or a patch on shaved skin. The exposure duration is 24 hours.

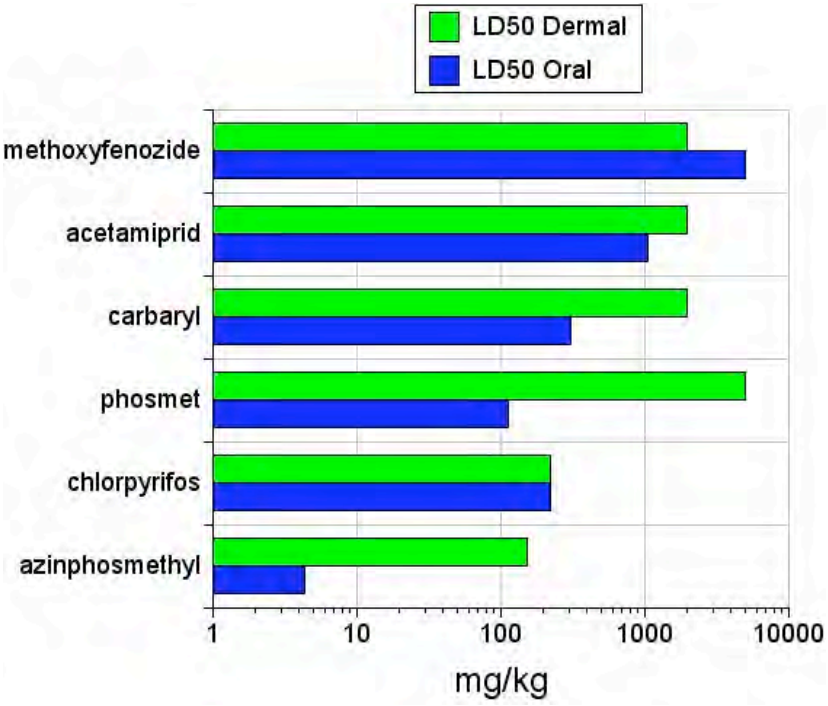


Figure 5. Comparison of toxicity of 6 insecticides by oral or dermal exposure of rodents.

   c. Dermal exposure also seems to be the most toxic route of exposure for birds. For example, Driver et al. 1991 exposed northern bobwhite quail in a wind tunnel to methyl parathion (an organophosphorus insecticide) and concluded that the routes of uptake in order of their contributionto toxicologica response from 8-48 hours after spraying were dermal > preening ≥ oral > inhalation (Driver, C. J., M. W. Ligotke, P. Van Voris, B. D. McVeety, B. J. Greenspan and D. B. Drown. 1991. Routes of uptake and their relative contribution to the toxicologic responses of northern bobwhite (colinus virginianus) to an organophosphate pesticide. Environ. Toxicol. Chem. 10 21-33.)

1. Note that the acute oral LD50 for methyl parathion to northern bobwhite quail was estimated to be 7.56 mg/kg, and the acute dermal toxicity was 9.172 mg/kg (data from the Methyl Parathion Re-registration Eligibility Decision Document released by EPA, URL http://www.epa.gov/oppsrrd1/op/methyl_parathion.htm).
   a. The bobwhites were able to tolerate 6.3 ppm methyl parathion in the diet over a longer period of time. This NOAEC was based on number of eggs laid and bodyweight.

2. Factors inherent to the organism
   a. **Species differences in response:** note in the following table that the insecticides tested putatively have the same mechanism of causing toxicity, i.e., excessive inhibition of acetylcholinesterase, the neuromodulatory enzyme present at the nerve terminal synapses in the central nervous system and at the neuromuscular junctions. Yet, azinphos-methyl is much more toxic to fish than is diazinon, but diazinon is much more toxic to birds (a notorious "bird killer"). Thus, fish and birds react differently to two different compounds with the same mode of action. (Data in the table are taken from EPA Registration Eligibility Documents; index to published documents at URL http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg#M.)

	Avian LD50 (mg/kg)	Fish LC50 (μg/L)	Ratio Avian/Fish
azinphos-methyl	75	20	3.75
diazinon	4.3	16000	0.0003

   b. **Age:** a study with starlings and redwing blackbirds exposed at different developmental stages to the organophosphorus insecticides terbufos and diazinon showed marked age susceptibility differences. Data in the table below were taken from Wolfe, M. F. and R. J. Kendall. 1998. Age-dependent toxicity of diazinon and terbufos in European starlings (*Sturnus vulgaris*) and red-winged blackbird (*Agelaius phoeniceus*). Environmental Toxicology and Chemistry 17(7):1300-1312.

Age	Terbufos Starling LD50 (mg/kg)	Diazinon Starling LD50 (mg/kg)
2	2.3	12.7
5	5	35.6
9	20.3	93.2
15	29.9	102
19	60.8	145
Adult	204	602

c. Environmental Factors

1. Some of the common environmental factors affecting toxicity that have been quantitatively studied in environmental toxicology include temperature and pH of water. Some studies have also focused on

stress related factors that could be considered related to environmental conditions. For example, starvation would be a nutritional factor imposed by environmental conditions during certain times of the year. Another possible stress factor is infection by parasites.

a. The table below shows the relationship between temperature, infection status, and toxicant exposure in clams. (Data are from Heinonen et al. 2001. Temperature- and parasite-induced changes in toxicity and lethal body burdens of pentachlorophenol in the freshwater clam *Pisidium amnicum*. Environ. Toxicol. Chem. 20(12):2778-2784.)

Temperature	Exposure (PCP, μg/L)	Infection Status	Mean Survival Time (h)
5	100	Infected	611
5		Uninfected	574
5	300	Infected	525
5		Uninfected	506
19	100	Infected	136
19		Uninfected	60
19	300	Infected	63
19		Uninfected	33

d. When the empirical database on factors affecting toxicity is adequate, than deterministically predictive model can be built using regression analysis.
   1. For example, the database for pesticide toxicity to aquatic organisms (several invertebrate species and fish species) has been analyzed for temperature effects on LC50 (Mayer and Ellersieck 1986; summarized in Suter et al.1993).

      Mayer, F. L., Jr. and M. R. Ellersieck. 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. U. S. Department of the Interior, Fish and Wildlife Service Resource Pulibcation 160, Washington, DC

      Suter, G. W., L. W. Barnthouse, S. M. Bartell, T. Mill, D. Mackay and S. Paterson. 1993. Ecological risk assessment. Lewis Publishers, Ann Arbor, MI

      a. The relationship for the temperature effect of most pesticides was modeled using linear regression; the output was the following linear function:
         1. Log LC50t±10 = log LC50t ± 0.4956, where t = temperature, ± 10°C
            (a) However, for the specific class of organophosphorus insecticides, the following regression function was more predictive:
            (b) LC50t±10 = log LC50t ± 0.7113

   b. One important objective of environmental toxicology is prediction, and the types of empirical exercises described above can help us understand specific toxicological responses under certain circumstances using "back-of-the-envelope" calculations and armchair reasoning.

Addendum: Analysis of Toxicity Data for Determination of Dose-Response Function and Estimation of LC50 using SAS (Statistical Analysis System). Five neonate codling moth larvae were placed on replicates of insecticide-treated leaf disks. After three hours, number of dead larvae were recorded. Four insecticide concentrations and an untreated control were tested.

```
Data Guthion1;   (Data input step)
      Input Dose N Dead;
      Observed=dead/N;
datalines;
0.0000   43     02
0.0099   42     13
0.0198   50     35
0.0296   36     28
0.0395   48     43
;

Proc Probit LOG10 OPTC INVERSECL;  (Programming procedure step)
   Model Dead/N=Dose;
run;

(Data Output Step)
                        Probit Procedure

Data Set         =WORK.GUTHION1
Dependent Variable=DEAD
Dependent Variable=N
Number of Observations=   5
Number of Events      =    121    Number of Trials =      219
Number of Events In Control Group =       2
Number of Trials In Control Group =      43

Log Likelihood for NORMAL -100.1627644

Probit Procedure

   Variable      DF   Estimate       Std Err     ChiSquare   Pr>Chi   Label/Value
   INTERCPT       1   5.33581127   0.870144   37.60266   0.0001     Intercept
   Log10(DOS)  1   2.92578526   0.5153      32.23771   0.0001
   _C_               1   0.04566056   0.031473                        Lower threshold

         Probit Model in Terms of Tolerance Distribution

                        MU         SIGMA
                     -1.82372      0.341789
```

Probit Procedure
Probit Analysis on Log10(DOSE)

Probability	Log10(DOSE)	95 Percent Fiducial Limits	
		Lower	Upper
0.01	-2.61884	-3.12633	-2.36712
0.02	-2.52567	-2.98501	-2.29688
0.03	-2.46655	-2.89546	-2.25221
0.04	-2.42208	-2.82817	-2.21853
0.05	-2.38591	-2.77349	-2.19108
0.06	-2.35512	-2.72699	-2.16768
0.07	-2.32813	-2.68625	-2.14712
0.08	-2.30396	-2.64981	-2.12867
0.09	-2.28197	-2.61670	-2.11187
0.10	-2.26174	-2.58625	-2.09638
0.15	-2.17796	-2.46053	-2.03188
0.20	-2.11138	-2.36113	-1.98008
0.25	-2.05425	-2.27640	-1.93512
0.30	-2.00295	-2.20089	-1.89415
0.35	-1.95542	-2.13161	-1.85549
0.40	-1.91031	-2.06671	-1.81798
0.45	-1.86667	-2.00498	-1.78061
0.50	-1.82372	-1.94563	-1.74245
0.55	-1.78077	-1.88814	-1.70242
0.60	-1.73713	-1.83221	-1.65926
0.65	-1.69202	-1.77764	-1.61140
0.70	-1.64449	-1.72414	-1.55697
0.75	-1.59319	-1.67091	-1.49372
0.80	-1.53606	-1.61626	-1.41867
0.85	-1.46948	-1.55699	-1.32675
0.90	-1.38570	-1.48669	-1.20682
0.91	-1.36546	-1.47019	-1.17738
0.92	-1.34348	-1.45242	-1.14524
0.93	-1.31931	-1.43303	-1.10974
0.94	-1.29232	-1.41156	-1.06992
0.95	-1.26153	-1.38725	-1.02432
0.96	-1.22535	-1.35891	-0.97054
0.97	-1.18089	-1.32432	-0.90415
0.98	-1.12177	-1.27868	-0.81557
0.99	-1.02860	-1.20732	-0.67538

Probit Procedure
Probit Analysis on DOSE

Probability	DOSE	95 Percent Fiducial Limits	
		Lower	Upper
0.01	0.00241	0.00075	0.00429
0.02	0.00298	0.00104	0.00505
0.03	0.00342	0.00127	0.00559
0.04	0.00378	0.00149	0.00605
0.05	0.00411	0.00168	0.00644
0.06	0.00441	0.00188	0.00680
0.07	0.00470	0.00206	0.00713
0.08	0.00497	0.00224	0.00744
0.09	0.00522	0.00242	0.00773
0.10	0.00547	0.00259	0.00801
0.15	0.00664	0.00346	0.00929
0.20	0.00774	0.00435	0.01047
0.25	0.00883	0.00529	0.01161
0.30	0.00993	0.00630	0.01276
0.35	0.01108	0.00739	0.01395
0.40	0.01229	0.00858	0.01521
0.45	0.01359	0.00989	0.01657
0.50	**0.01501**	**0.01133**	**0.01809**
0.55	0.01657	0.01294	0.01984
0.60	0.01832	0.01472	0.02192
0.65	0.02032	0.01669	0.02447
0.70	0.02267	0.01887	0.02774
0.75	0.02552	0.02133	0.03208
0.80	0.02910	0.02420	0.03814
0.85	0.03393	0.02773	0.04712
0.90	0.04114	0.03261	0.06211
0.91	0.04311	0.03387	0.06647
0.92	0.04534	0.03528	0.07157
0.93	0.04794	0.03689	0.07767
0.94	0.05101	0.03877	0.08513
0.95	0.05476	0.04100	0.09455
0.96	0.05952	0.04376	0.10702
0.97	0.06593	0.04739	0.12469
0.98	0.07555	0.05264	0.15291
0.99	0.09363	0.06204	0.21117