September 17, 2003

Lecture 6: Dose/Response I (Overview of the Dose Response Relationship)

I. Summary from Lecture 3

A. In lecture 3, we discussed how the typical dose-response relationship is derived from a normally distributed population response.
   1. The response could be any biochemical, genetic, physiological, morphological, behavioral, etc. observation that we wish.
   2. In the normal distribution, we are interested first in the median numbers responding at a specific dose; we are also concerned with the variation in individuals responding across the full regime of tested doses.

B. We also discussed that examining the normal distribution as percentage of population responding changes the bell shaped curve to a logistic or S-shaped curve.
   1. By definition the median response on the logistic curve is called the LD50 (if lethality is the toxicological endpoint or measured response and the dose is expressed on a body weight basis, usually employing the units mg/kg).
      a. If a concentration were used (such as it would be if aquatic organisms were being tested), than the median response would be the LC50.
   2. If a sublethal response is being measured, or alternatively, we are measuring a biochemical or physiological response, we could express the median response as an effective dose or concentration (ED50 or EC50).
   3. Note that we could examine any proportion or percentage of response;
      a. For example, if we were interested in 95% of the population responding, we would examine the dose-response relationship to estimate the LC95 (if a series of concentrations were being tested).
      b. Similarly, we might be interested in just the dose that gives 10% response (LD10).

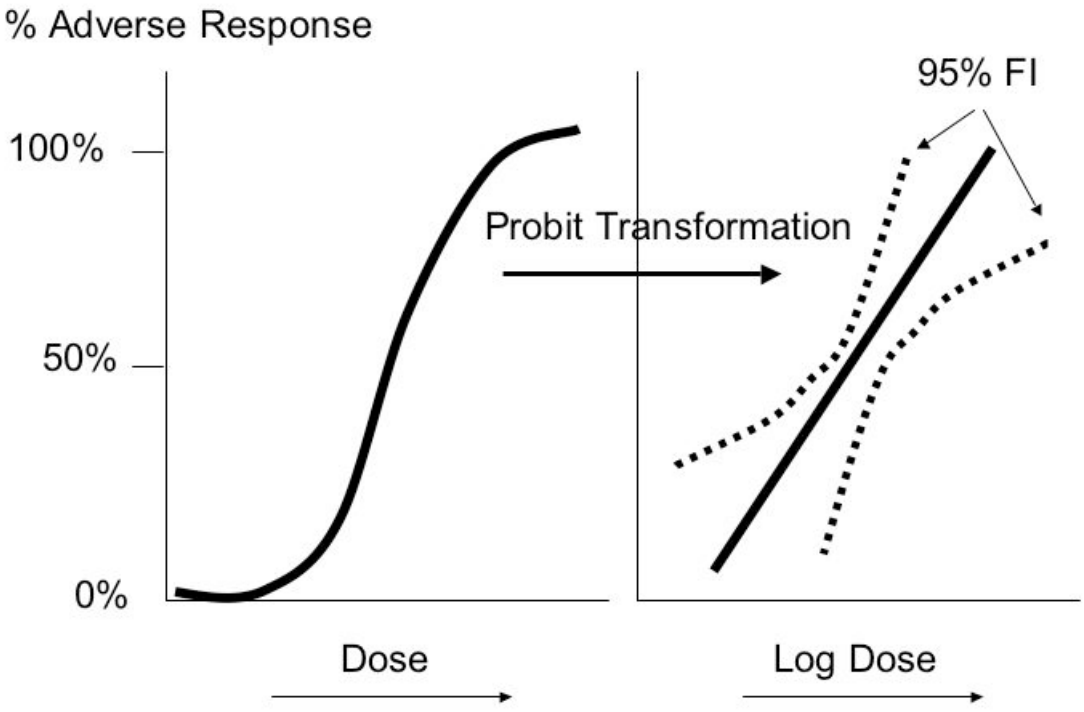
C. In addition to expressing the magnitude of population response as a relationship to dose, we could express the response in relationship to time.
   1. In this case, we might use a fixed dose or concentration and determine the time it takes to kill or adversely affect 50% of the population (LT50).

II. How the Dose-Response Relationship Is Measured and Mathematically Deduced

A. Organisms reared under standard uniform conditions (to minimize inter individual variability) are divided into separate groups and then either dosed with a series of increasing concentrations or doses of toxicant (by feeding; by topical or dermal application; by exposure to vapors, etc.). One group is not exposed to toxicant
   1. Thus the dose or concentration the different groups are exposed to is considered the independent variable in the experiment. We have control over the independent variable and know its value (magnitude) prior to the start of the experiment.
   2. At each dose level, observations of mortality or any other biological response are made. These observations are the dependent variables. Their values are unknown at the beginning of the experiment, but they are measured in response to the known independent variables.

B. The data, which are now expressed as number of organisms tested per dose, and the number responding, are fed into a computer program that can calculate one of two basic statistical techniques—probit analysis or logit analysis (logistical regression).
   1. The computer program will estimate the response at any percentage of population response.

C. Be aware that the resulting LD50 or LC50, for example, is just a statistical estimate of the median response of the population under the conditions of the experiment.
   1. The number generated is not a fixed solid characteristic of the toxicant's interaction with the population of test organisms.
      a. If the experiment was repeated again, a different estimate of LD50 or LC50 would be calculated owing to the natural variation in response from each group of individuals tested.
   2. Thus, in reality, if we kept on repeating the experiment, we would be measuring a population of potential responses of some specific level of response.
      a. Thus, to know the likelihood that we have captured in our measurements the "true" population response, the computer program also calculates confidence limits about each LD or LC estimate.
      b. In probit analysis, these confidence limits are called fiducial limits.



c. Because the fiducial limits are narrower (meaning less variation in response) about the median response (i.e., the LD50), toxicologists usually rely on this parameter for expressing comparative toxicity.
d. Thus, at the lower and higher levels of response, a lot more variability in seen and the estimates of toxicity are less reliable.
    1. One can compare the toxicity of a toxicant to two or more populations by looking for overlap between the LC50 or LD50 of the tested populations.
    2. Similarly, one can compare the influence on toxicity response of any independent variable, for example temperature effect, pH effect, second chemical in a mixture, etc. One would conduct a dose-response experiment, statistically estimate the LD50 or LC50, and then observe whether overlap has occurred about the LD/LC50 for each independent variable tested.

D. The threshold for toxicity can be estimated by mathematically extrapolating the dose-response function through the dose at which no response has occurred or been measurable. This corresponding threshold dose is the NOAEL or NOAEC.
    1. Often, however, the NOAEL or NOAEC is estimated by visual observation of which dose in the testing regime caused no significant difference in response compared to the undoes group (i.e., the control group).

III. Using the Dose-Response Relationship to Deduce Genetic Variation in a Population and Track Changes over Time—The Value of the Slope of the Curve

A. For any single compound, the slope of the dose-response line helps determine the margin of safety.
    1. Shallow slope allows greater margin of safety; in other words, comparatively larger changes in dose result in small changes in response (Figure 5A,B).
    2. The slope also tells something about the variability in the population (Figure 5A, B);
        a. This variation is actually the variation in response, largely stemming from genetic variation leading to phenotypic variation within a given population.
        b. A steep slope indicates little variation in the population response;
        c. A comparatively shallower slope indicates that the response is much more variable over a greater dose range.

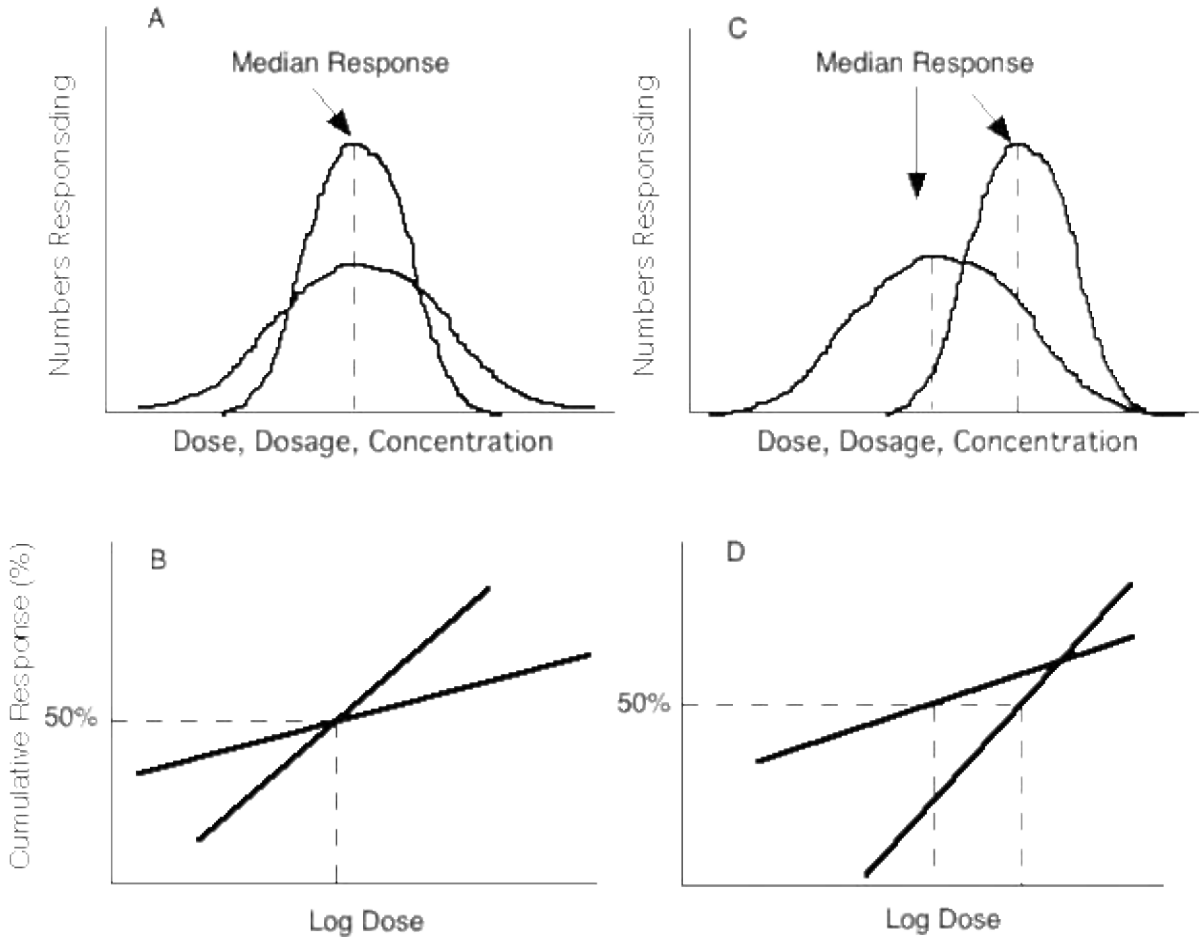


Figure 5. Relationship between slope and variability (distribution) of response of one or more populations to a single chemical, or response of a single population to two different chemicals; or response of two different species to a chemical.

B. Two different species might respond to a chemical with the same LD50/LC50, but the variation in susceptibility may differ substantially (Figure 5A,B). Alternatively, the LD50's may be substantially different, in addition to the variability being different (Figure 5C,D).
C. Note that the slope can also be used to assess the occurrence of resistance in a population. Populations naïve to a toxicant are fairly homogeneous in response. As a toxicant selects for resistant individuals, the variability in response increases (distribution flattens out), and as selection continues, most individuals will eventually become resistant, establishing a new, homogenous distribution but exhibiting a substantially higher LD50 (Figure 6).

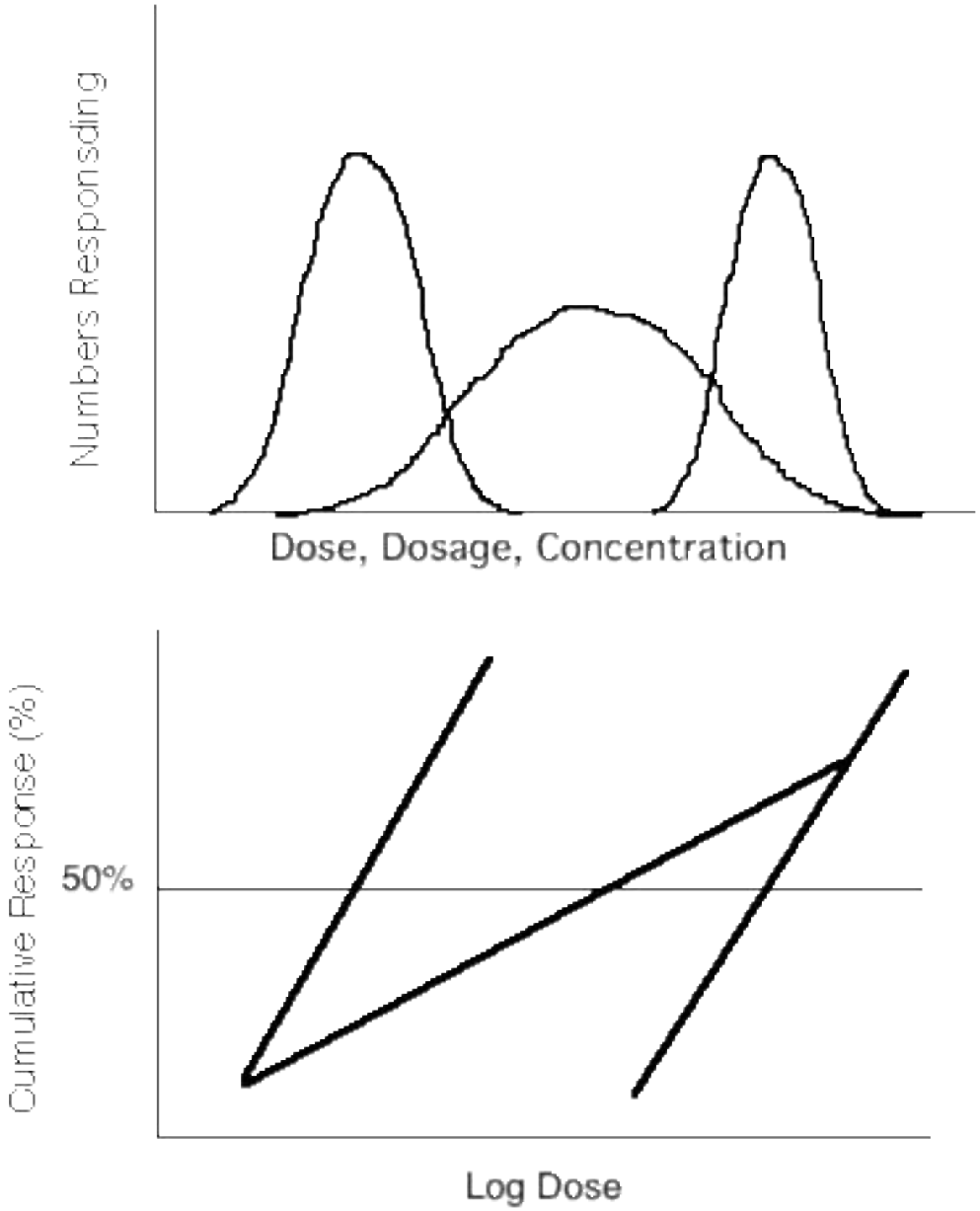


Figure 6. Change in susceptibility after repeated selection for resistant individuals.

IV. Example Computer Program and Output for Estimating LC50

A. The following data represents the input and output to determine the LC50 for an organophosphate insecticide on codling moth neonate larvae.

B. Experimental Procedure
   1. Insecticide was pipetted on leaf disks of known surface area
   2. Neonate codling moth (n=5) were placed on replicate leaf disks per dose
   3. 24 h and 48 h, dead larvae were counted
   4. The data was transferred to an Excel spreadsheet and than imported in a statistical program called SAS (Statistical Analysis System)

5. After the toxicity parameters were estimated and printed out, the data for the probability of mortality was plotted in a graphing program.

A SAS (Statistical Analysis System) Program for Estimating the LC50 of an Insecticide on Treated Leaf Surfaces Against Codling Moth Neonate Larvae

```
Data Guthion1;
      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;
   Model Dead/N=Dose;
run;
```

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	0.87014	37.60266	0.0001	Intercept
Log10(DOS)	1	2.92578526	0.5153	32.23771	0.0001	Slope
_C_	1	0.04566056	0.0314			Lower threshold

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
-1.82372	0.341789

Probit Procedure

Estimated Covariance Matrix for Tolerance Parameters

	MU	SIGMA	_C_
MU	0.002272	-0.001592	0.000500
SIGMA	-0.001592	0.003624	-0.000277
_C_	0.000500	-0.000277	0.000991

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

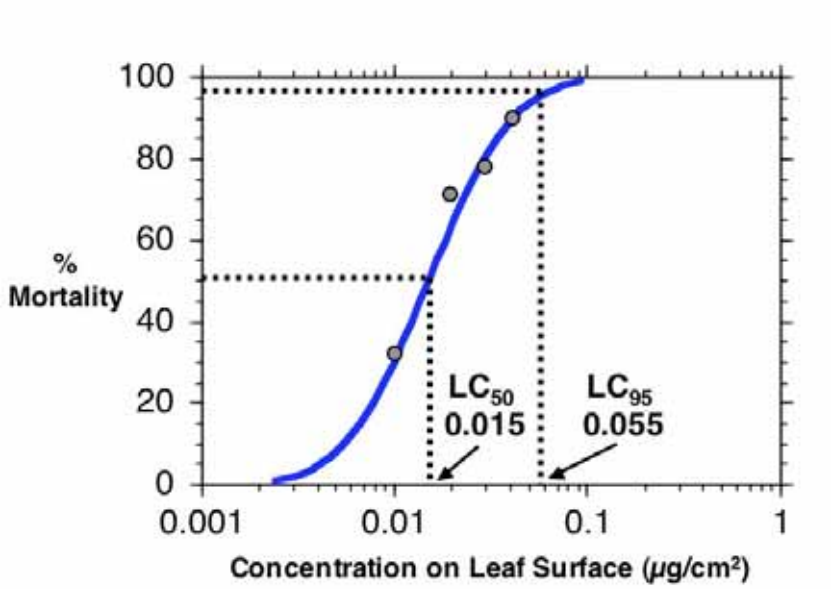
0.94	-1.29232	-1.41156	-1.06992
0.95	-1.26153	-1.38725	-1.02432
0.96	-1.22535	-1.35891	-0.97054
0.97	-1.18089	-1.32432	-0.90415
0.98	-1.12177	-1.27868	-0.81557
0.99	-1.02860	-1.20732	-0.67538

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

The first graph below represents the plotted results from the data above for Guthion (azinphos-methyl) insecticide.



The second graph below represents analysis of data for a bioassay with Intrepid (methoxyfenozide). Note that not only is methoxyfenozide less toxic to neonate codling moth larvae than azinphos-methyl, but the slope of the line is somewhat flatter. Methoxyfenozide has an entirely different pharmacodynamics action than azinphosmethyl. Also, the slope of the line suggests greater genetic variability in susceptibility to methoxyfenozide than to azinphos-methyl.