Dose - Response Relationships

Principles of Environmental Toxicology
Instructor: Gregory Möller, Ph.D.
University of Idaho

Learning Objectives

- Understand the quantitative relationship between toxicant exposure and induced effects.
- Describe frequently encountered toxic effects.
- Interpret frequency (normal distribution) and dose - response curves.
- Understand threshold effects with dosage increase.

Learning Objectives, 2

- Understand effective dose, margin-of-safety and the relationship of effective vs. toxic dose.
- Examine the use of actual data for no observed effect and lowest observed effect in risk assessments.
- Summarize effective, lethal and toxic doses.
- Understand a linearized multi-stage model for non-threshold responses.

What is a Dose?

- The amount of a substance administered at one time.
- Dosage is the amount per unit weight of the exposed individual.
- Exposure is characterized by
  - Number of doses
  - Frequency of dosing
  - The total period of time for the exposure.

Quantifying the Dose

- Gram (g) is the standard unit but mg is typical of most exposures in toxicology.
- Dosage: mg (dose) / kg (bw) / day (duration)
  - mg/kg/d
- Exposures are quantified in relation to the media.
  - mg/L in water.
  - mg/kg in food.
  - mg/m^3 in air.
- Variation in units common (ppm, ppb).

Key Concepts

- Dosage - response mathematical relationship (positive slope).
- Causal relationship.
- Observable responses.
- Statistical management of variability of individual responses.
  - Species, genetics, age, sex.

Responses (Toxic Effects)

- Inflammation.
  - Local or systemic response.
- Necrosis.
  - Cell or tissue death.
- Enzyme inhibition.
  - Biochemical pathway interruption.
  - Competitive; non-competitive.
- Biochemical uncoupling.
  - Interference with phosphate molecule synthesis (ATP)

Responses (Toxic Effects), 2

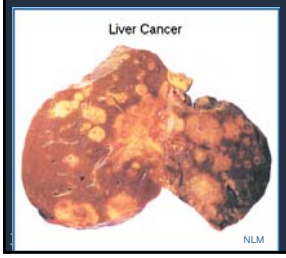
- Lethal synthesis.
  - Toxicant incorporation into a biochemical pathway.
- Lipid peroxidation.
  - Free radical oxidation of fatty acids leading to cell death.
- Covalent binding.
  - Of electrophilic reactive metabolites to nucleophillic macromolecules.

Responses (Toxic Effects), 3

- Receptor interaction.
  - Modification of normal biological effects mediated by the receptor.
- Immune-mediated hypersensitivity reactions.
  - Antigenic chemicals resulting in allergic reaction.
- Immuno-suppression.
  - Increased susceptibility to infectious agents and tumorigenesis.

Responses (Toxic Effects), 4

- Neoplasia.
  - Aberrant cell division and tissue growth.
    - Neoplasms: tumorigenesis, oncogenesis.
    - Malignant neoplasms: carcinogenesis.



Responses (Toxic Effects), 5

- Genotoxic interaction.
  - Chemical interaction with DNA possibly leading to heritable change.
    - Clastogenic (chromosomal) effects.
    - Mutagenic (base pair) effects.
- Developmental and reproductive toxicity.
  - Adverse effects on conception, and structure and function of the conceptus.

Types of Toxic Responses: Idiosyncratic

- Genetically determined sensitivity or resistance to toxicity
  - Usually lack of enzymes / factor involved in metabolism
- Primaquine (oxidative anti-malarial drug) - 10% black males / erythrocyte G-6-P dehydrogenase / hemolytic anemia
  - Glucose-6-phosphate dehydrogenase deficiency, the most common enzyme deficiency worldwide
- Nitrites - lack NADH-methemoglobin reductase / methemoglobinemia

Types of Toxic Responses: Allergic

- Immunological mediated response (memory)
- Requires sensitizing exposure
- May involve chemical/protein complex (hapten)
- Atypical dose response
  - Small doses most effective
  - Large dose tolerance
    - Ts cells (suppressor T lymphocytes)
- Contact dermatitis; anaphylaxis
- Pollens, pesticides, sulfur, penicillin

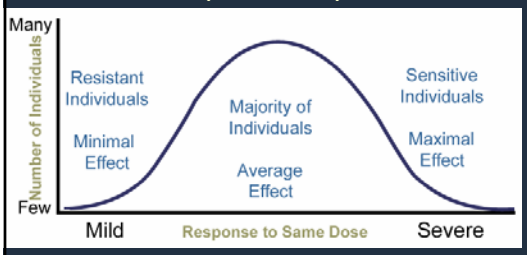
13

Dose-Response

- Quantitative analysis of incremental dose increase and occurrence of toxic end effect
- Responses follow normal frequency distribution (Gaussian)

14

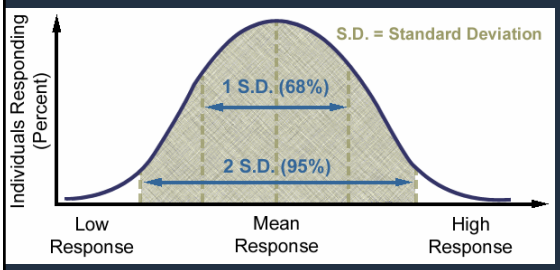
Normal (Gaussian) Distribution



- Population representation of variability.

15

Normal Distribution, 2



16

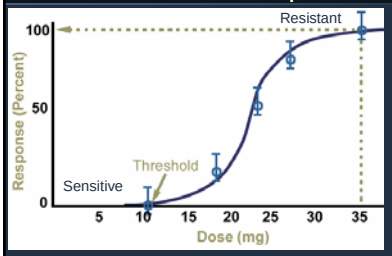
Normal Distribution Parameters

- Mean ± one SD = 68.3 % population
- Mean ± two SD = 95.5 % population
- Mean ± three SD = 99.7 % population

- Frequency converted to cumulative gives sigmoid curve

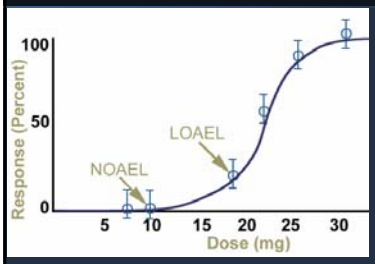
17

Dose - Response Curve

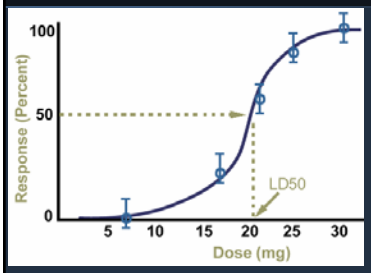


18

Observed Effects



Toxic Thresholds

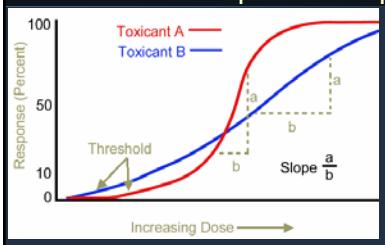


Median Lethal Dose LD_{50}

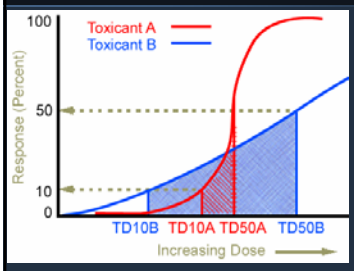
Interpretation

- Often used to compare toxicity
- Only measures lethality
- Best for quantal data
- Best for acute exposure
- Tells nothing about slope
- Specific quantifiers

Shape and Slope



Comparative Toxicity



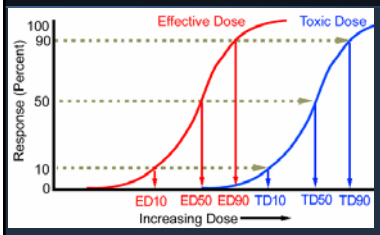
Other Thresholds: ED_{90} – EC_{50} – LC_{10} – TD_{Lo}

- ED: effective dose
  - Pharmaceuticals
- EC: effective concentration
  - Pharmaceuticals *in vivo*
    - Often blood
  - Environmental toxicology
- LC: lethal concentration
  - Environmental toxicology
- TD_{Lo}: Lowest published toxic dose
- TC_{Lo}: Lowest published toxic concentration

Therapeutic Index - TI

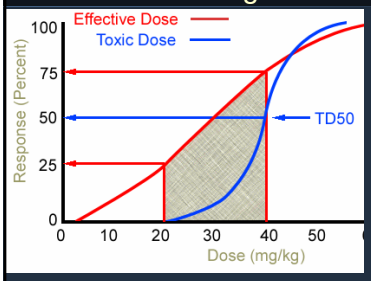
- Ratio of dose to produce toxic effect to dose to produce desired effect
- TI = LD_{50}/ED_{50}
- The larger the ratio, the greater the safety (e.g. 10)
- Slope of dose response important

Effective Dose



Therapeutic Index
(TI) = Toxic Dose/Therapeutic Dose

Margin of Safety



Margin of Safety
(MOS) = LD(01)/ED(99)

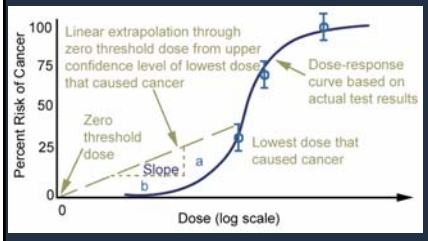
Margin of Safety - MOS

- Accounts more for slope differences
- MOS = LD_1/ED_{99}
- Neither TI or MOS works for chemicals with no beneficial effect or repeated doses

Carcinogen Risk Assessment

- Linearized Multistage Model
  - Assumes non-threshold effect.
- Linear extrapolation through zero threshold dose from upper confidence level of lowest dose that caused cancer in animal study.
- Analysis results in a cancer slope factor that can be used to predict cancer risk at a specific dose.

Linearized Multistage Model



NLM

Other Models for Risk Assessment

- One hit model (cancer)
  - Assumes a molecular event with cellular response.
- Multi hit model (cancer)
  - Assumes multiple events prior to cellular activation.
- Probit model
  - Linearization transformation that assumes log normal distribution.
- PB PK - Physiologically based pharmacokinetic model
  - Uses intensive pharmacokinetic and mechanistic data.

Transformation of Variables

- Allows better (simpler) analysis of data at points of interest such as LD_{50}.
- Transformation into an approximate normally distributed variable.
- Examples (r_j = dead animals; n_j = total animals)
- Probit transformation.
  - Based on Gaussian (Bell) curve.
  - Probit $(r_j/n_j) = \Phi^{-1} (r_j/n_j)$
  - Useful in acute lethality tests.
- Logit transformation.
  - Log odds of a quantal response.
  - Logit $(r_j/n_j) = \ln [(r_j/n_j)/1 - (r_j/n_j)]$
- Weibull transformation.
  - Exponential model used in modeling multistage processes.

Probit Transformation

- Probability units $\rightarrow$ "probits"
- Convert % response to units of deviation from the mean or "normal equivalent deviations" (NEDs).
- Hence the NED for a 50% response is 0.
- "Probit" approach adds 5 to avoid negatives.

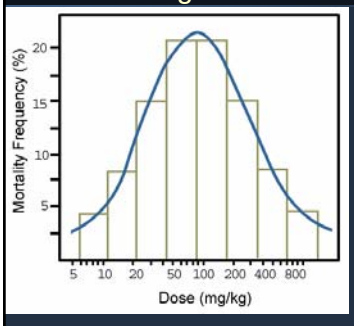
Probit Transformation, 2

% Response	SD	NED	Probit
0.1	-3	-3	2
2.3	-2	-2	3
15.9	-1	-1	4
50.0	mean	0	5
84.1	+1	+1	6
97.7	+2	+2	7
99.9	+3	+3	8

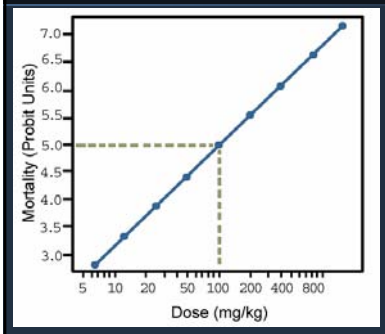
Probit Transformation, 3

- Perform $\log_{10}$ transformation of the dose.
  - Assumes log normal distribution.
- Produces an approximately linear relationship.
  - Allows linear regression analysis.

Log Normal Distribution



Probit Unit Transformation



Summary: Transformations of D-R Curve

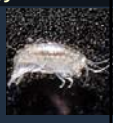
- Normal frequency distribution
- Arithmetic dose to log dose
- Frequency data to cumulative
- Probability of response to NED
  - Standard deviations of mean
- NED to probit
  - NED + 5

Dose-Response Curve Summary

Major Parameters

- Median Lethal Dose - LD_{50}
  - Other LDs, TDs or EDs
- Slope
- Thresholds
- System saturations
- Comparative toxicity
- Risk assessment

Example: Acute & Chronic Ecotoxicology Tests



- Allow for a relative indication of toxicity.
  - LC_{50} LD_{50} EC_{10}
  - Assists in QSAR development.
  - WET: whole effluent toxicity.
- Often simple, inexpensive.
  - Good reproducibility.
- Useful for defining environmental quality standards.
  - Safety factor approach 1:100
- Bacteria, algae, plants, invertebrates (i.e. insects), vertebrates (i.e. rats).