## Dose - Response Relationships

Principles of Environmental Toxicology  
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### 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/m³ 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)

- 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.

- 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.

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

- 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

Dose-Response

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

Normal (Gaussian) Distribution

- Population representation of variability.

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
Observed Effects

Toxic Thresholds

Median Lethal Dose LD₅₀

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: ED₉₀ – EC₅₀ – LC₁₀ – TD₅₀

- ED: effective dose
  - Pharmaceuticals
- EC: effective concentration
  - Pharmaceuticals in vivo
    - Often blood
  - Environmental toxicology
- LC: lethal concentration
  - Environmental toxicology
- TD₅₀: Lowest published toxic dose
- TC₅₀: Lowest published toxic concentration
**Therapeutic Index - TI**

- Ratio of dose to produce toxic effect to dose to produce desired effect
- TI = LD50/ED50
- The larger the ratio, the greater the safety (e.g. 10)
- Slope of dose response important

**Effective Dose**

![Effective Dose Graph]

Therapeutic Index
(TI) = Toxic Dose/Therapeutic Dose

**Margin of Safety**

![Margin of Safety Graph]

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

**Margin of Safety - MOS**

- Accounts more for slope differences
- MOS = LD1/ED99
- 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**

![Linearized Multistage Model Graph]
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 ($r_i$ = dead animals, $n_i$ = total animals)
  - Probit transformation:
    - Based on Gaussian (Bell) curve.
    - $\text{Probit} \left( \frac{r_i}{n_i} \right) = \Phi^{-1} \left( \frac{r_i}{n_i} \right)$
    - Useful in acute lethality tests.
  - Logit transformation:
    - Log odds of a quantal response.
    - $\text{Logit} \left( \frac{r_i}{n_i} \right) = \ln \left[ \frac{r_i/n_i}{1 - (r_i/n_i)} \right]$
  - Weibull transformation:
    - Exponential model used in modeling multistage processes.

Probit Transformation

- Probability units → “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

<table>
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<th>% Response</th>
<th>SD</th>
<th>NED</th>
<th>Probit</th>
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<tr>
<td>0.1</td>
<td>-3</td>
<td>-3</td>
<td>2</td>
</tr>
<tr>
<td>2.3</td>
<td>-2</td>
<td>-2</td>
<td>3</td>
</tr>
<tr>
<td>15.9</td>
<td>-1</td>
<td>-1</td>
<td>4</td>
</tr>
<tr>
<td>50.0</td>
<td>mean</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>84.1</td>
<td>+1</td>
<td>+1</td>
<td>6</td>
</tr>
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<td>99.9</td>
<td>+3</td>
<td>+3</td>
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</tr>
</tbody>
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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

- Exponential model used in modeling multistage processes.
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).