Dose-Response Relationship of Essential Metallic Elements

The Application of Categorical Regression

Example: Copper
Problem: Dose-response relationship for essential trace elements is complex.

- **Dose (Concentration)**
- **Response**
- **Essentiality**
  - Deficiency
  - Homeostasis
  - Excess
  - Zone of conflict

- **Very Low** → **Very High**
- **Normal** → **Highly Abnormal**
Outline

- Modeling Methods
  - NOAEL/LOAEL
  - Benchmark Modeling
- Overview of Categorical Regression
  - Background
  - Applications
  - CatReg Software
- Copper Risk Assessment
  - JTEH Review
  - Copper Database
  - Preliminary Results
  - Future Directions
Modeling Methods

- **NOAEL/LOAEL**
  - Simple and traditional method
  - Limited use of the available dose-response information
  - Single dose-time data point
  - Little uncertainty characterization

- **Benchmark Dose**
  - Entire dose-response curve is utilized instead of a single dose
  - Quantitative uncertainty characterization
  - Responses within experimental range are used
  - Empirical curve-fitting
Categorical Regression

- Can be used when mechanistic models are lacking & insufficient evidence is available to support a complex dose-response relationship
- Can be used to model multiple studies and endpoints simultaneously using a common toxicity metric
- A dose-response model may be fitted to data where only severity ratings are available
Categorical Regression & Copper

- Heterogeneity amongst available experimental studies on copper (specie, dose, endpoints, route of exposure), limits the application of traditional methods

- Traditional dose-response approaches where RfD: NOAEL/UF may bring the resulting value into the deficiency range
Advantages of Categorical Regression

- Define the relationship by increasing severity of response
- Ensures that the best available evidence is utilized and integrated into a single quantitative analysis
- Can combine data from multiple studies & utilize information on multiple species
- Curve no longer based on the most sensitive strain, specie, sex – may be more predictive of actual human risk
- Scatter demonstrated in regression provides useful information on the uncertainty in the dose-response model
CatReg Capabilities

- A computer program developed to support toxicologists & health scientists conduct exposure-response analyses
- Developed by U.S. EPA
- Executes a regression analysis of the severity scores and exposure parameters
- Three statistical models available:
  - Logistic (logit)
  - Normal (probit)
  - Gumbel (log-log)
CatReg Capabilities

- Determine whether the coefficients for concentration and time differ by severity level
- Methods for estimation and hypothesis testing of model parameters
- Range of options for sensitivity analysis
- Can produce graphical displays of data and fitted models
- Combine data sets from different experiments
  - Statistical testing for differences between combined experiments
Monograph II: Copper Risk Assessment

COPPER AND HUMAN HEALTH: BIOCHEMISTRY, GENETICS, AND STRATEGIES FOR MODELING DOSE-RESPONSE RELATIONSHIPS

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Data Collection Process

- Review scientific literature
- Identifying papers on copper toxicity due to excess and deficiency
- Review papers for quality
- Select key studies using a consensus approach
Hazard Identification Criteria

- Consensus approach reviewed over 600 papers for hazard identification and dose response assessment

- Excluded studies included:
  - Exposures in utero
  - No reliable level of exposure
  - Depletion / repletion phases
  - Only pharmacokinetic data
  - Case reports with no known exposure duration
## Criteria Used for Exclusion

<table>
<thead>
<tr>
<th>Most Useful</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Least Useful</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate Reporting</td>
<td>Multiple dose or multiple outcomes from intact animals or humans</td>
<td>Multiple or single dose from intact animals or humans</td>
<td>Single dose or clinical study / case report with indeterminate dose</td>
<td>No dose information</td>
<td>No Utility</td>
</tr>
<tr>
<td>Physiological Measures</td>
<td>Fairly Good Reporting</td>
<td>Likely to yield useful information</td>
<td>Tracer or PK Study</td>
<td>Physiological information</td>
<td>Review</td>
</tr>
<tr>
<td></td>
<td>Change in time Points</td>
<td></td>
<td>Info re. body burden or kinetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cellular effects</td>
<td></td>
<td>Mechanistic or cellular effects</td>
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</tbody>
</table>

Each criterion is rated on a scale from 1 (most useful) to 5 (least useful).
Results of the Hazard Identification

~600 Papers

Selected through criteria:
92 papers

Animal Species (6)

Systems Affected (24)

Copper species (~10)

# Doses
  • Single dose
  • Repeat dose

Routes of administration
  • Water
  • Capsule
  • Feed
Results of the Hazard Identification

- 92 papers example closely and
  - NOAELs and Benchmark Doses were determined
  - Each endpoint assigned a severity score of either 0, 1, 2, or 3 for the categorical regression, where:
    - 0 = homeostasis
    - 1 = enzyme changes
    - 2 = metabolic perturbations
    - 3 = gross toxicity or deficiency

- Both excess and deficiency studies were based on the same scoring system
Severity Scoring Summary

<table>
<thead>
<tr>
<th>Deficiency Endpoints</th>
<th>Severity Score</th>
<th>Toxicity Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper Burden; metallothionein; urine copper</td>
<td>0</td>
<td>Cu burden; metallothionein; urine Cu</td>
</tr>
<tr>
<td>Loss of Cu-dependent enzyme function (SOD); Changed blood cell # or function</td>
<td>1</td>
<td>Changes in cholesterol and triglyceride levels in blood/liver; large Cu burden; body weight; nausea; diarrhea; enzyme changes without histopathology</td>
</tr>
<tr>
<td>Organ weight changes; plasma glucose/insulin; heart rate; EKG changes; minor histopathology; white blood cell activity/counts</td>
<td>2</td>
<td>Body weight; anemia; hemolysis; vitamin levels; liver enzymes; inflammation; organ weight changes</td>
</tr>
<tr>
<td>Mortality; gross histopathology reproductive function changes</td>
<td>3</td>
<td>Death; gross histopathology</td>
</tr>
</tbody>
</table>
Copper Database

- Total of 3,844 severity scores assigned
- Maximum severity scores (312) selected from each dose group in each study

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ref.id</th>
<th>Exp</th>
<th>Group</th>
<th>Species</th>
<th>Sex</th>
<th>mg/kg bw</th>
<th>Weeks</th>
<th>GpSize</th>
<th>BestNum</th>
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<tbody>
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<tr>
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<tr>
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<td>1</td>
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<td>1.5</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
Interspecies Scaling

Interspecies scaling based on four dose metrics:

- body weight: $\text{mg/kg bw/day}$
- surface area: $\text{bw}^{2/3}$
- intermediate: $\text{bw}^{3/4}$ (Travis & White, 1988)
- total intake: $\text{mg/day}$
Dose - Duration Curves for Toxicity due to Copper Excess
ED10 Dose - Duration Curves for Severity Level 3 for Toxicity due to Copper Excess (with 95% confidence limits) for Human (n=20)
ED10 Dose - Duration Curves for Severity Level 3 for Toxicity due to Copper Excess
ED10 Dose Response Curves for Severity Level 3 for Toxicity due to Copper Excess following 100 Days Exposure for Human (n=20)
Comparison of Scaling Methods
ED10 for Severity Level 3 for Toxicity due to Copper Excess following 100 Days Exposure (with 95% confidence limits)
ED10 for Severity Level 3 for Toxicity due to Copper Excess following 100 Days Exposure (with 95% confidence limits)
ED10 for Severity Level 3 for Toxicity due to Copper Excess following 100 Days Exposure (with 95% confidence limits)
ED10 for Severity Level 3 for Toxicity due to Copper Excess following 100 Days Exposure (with 95% confidence limits)
ED10 for Severity Level 3 for Toxicity due to Copper Deficiency following 100 Days Exposure (with 95% confidence limits)
Model 1: Cumulative Odds
(parallel dose response curves)
ED10 Dose - Duration Curves for Toxicity due to Copper Excess (with 95% confidence limits) for Human (n=20); Model: Cumulative Odds
Model 2: Unrestricted Cumulative (non-parallel dose-response curves)
ED10 Dose - Duration Curves for Toxicity due to Copper Excess (with 95% confidence limits) for Human (n=20); Model: Unrestricted Cumulative Severity Level 0

Severity Level 1

Severity Level 2

Severity Level 3

Censored

Dose (mg/day)

Duration (Days)
Future Analysis

- Update the current copper database
- Include additional species
- Select Model:
  - Restricted vs unrestricted
  - Cumulative vs conditional odds
  - Exposure duration (10 – 100 days)
  - Select final dosimetry (mg/day?)
- Examine multiple levels of severity
- Conduct a qualitative analysis of all data to determine the endpoints of the homeostatic range for copper
- Consider uncertainty factors for sensitive individuals