Test Guideline 425
Up-and-Down Procedure

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Overview

- Based on staircase design
- Dose single animals in sequence
- Set initial dose at toxicologist's best estimate of the LD50
- Following each death (or moribund state), the dose is lowered
- Following each survival, the dose is increase
- After the first reversal, dose four additional animals following the up-and-down design

Example

- First animal dosed at 200 mg/kg and lives
- Second animal dosed at 260 mg/kg and dies
- Third animal dosed at 200 mg/kg and dies
- Fourth animal dosed at 154 mg/kg and lives
- Fifth animal dosed at 200 mg/kg and lives
- Sixth animal dosed at 260 mg/kg and dies

LD50 = 209 mg/kg

Protocol

- Default dose progression is 1.3
- Default is to use only females
- Observe each animal 24 hours before dosing the next animal
- Count all deaths including delayed deaths and humanely killed
- Observe for 14 days - record weekly body weights, all clinical signs and gross necropsy results

Options

- Initial dose based on all available information
- Most sensitive sex should be used
- LD50 can be confirmed in opposite sex
- Dose progression can be adapted
- Observation period between animals can be increased
- Limit study described

Study Outputs

- Test substance, vehicle, test animals, test conditions
- Individual responses including nature of signs, time of onset, severity, duration and outcome
- Time course of reversible signs
- Gross necropsy results, histopathology if warranted
- Calculated point estimate of LD50

Calculations

- Based on staircase design
- Uses maximum likelihood method to calculate LD50
- Can be run with SAS or BMDP program
- Slope is assumed and not calculated

First Test Evaluation

- First proposed by Bruce, based on Dixon's design
- Reviewed 48 standard LD50 studies
  - average value of σ was 0.121
  - 85% of animal died within 48 hours
  - Males more likely to have higher LD50 values
- Simulated 10 studies - LD50 agreed closely

First Validation

- Conducted 10 tests in parallel with 401
- Excellent agreement with 401 standard except
- potassium hydroxide a material that produced delayed deaths

Second Validation

- Conducted 5 tests in parallel with 401
- Compared results from females in both methods
- Excellent agreement with 401 standard

Third Validation

- Conducted 10 tests in parallel with 401 and FDP
- FDP sighting study was used
- Compared results from females only
- Excellent agreement with 401 standard except mercuric Cl
- 401 method - 160 mg/kg
- UDP - 12 mg/kg

- Textbook (Gosselin 1984) - 37 mg/kg

Summary of Classification Results Using EU System

- Twenty-Five Test Materials:
- Twenty-Three Identical to 401
- Two more Stringent

Strengths

- Reduced Number of Animals
- Point Estimate of LD50
- Meets all classification systems
- Death as an Endpoint
- Similar Observations as 401

Weaknesses

- Slope is given not calculated
- Females only, males may be added
- Arbitrary upper limit of 2000mg/kg
- Not suitable for delayed toxicity
- Not suitable for inhalation studies
- Increased test duration

Results of First Validation (Bruce)
Results of Second Validation
(Bonnyns, et al.)
Results of Third Validation (Yam, et al.)
Statistical Procedure
Likelihood of experimental outcome = L (given μ, σ, and n)

$L_i = 1 - F(Z_i)$ if the i^{th} animal survived or

$L_i = F(Z_i)$ if the i^{th} animal died

Where $Z = [\log(d_i) - \mu]\ \sigma/$;
μ = log LD50; and
F = cumulative, standard normal density