When and How to Demonstrate Safety and Toxicity of Potential Sterilants

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Requirements of an Ideal Sterilant

- Safe and efficacious (long-term)
- Easy to administer
- Effective in dogs and cats
- Effective in male and female
- Cost effective (affordable)

Safety Studies and GLPs

OVERVIEW OF THE HOWS AND WHYS OF REGULATIONS GOVERNING MEDICATIONS AND CHEMICALS

Drug Development

- Before marketing, must be approved by the CVM (Part of FDA) on the basis of safety and efficacy
- Generally a new veterinary drug takes 5-15 years to be developed and costs 10-50 Million (human drugs 10-15 y and 800 million on avg)

Drug Development

- Process is similar for human and animal drugs.
- Differences with animal drugs
  - Can test directly on target spp., so earlier info on safety and efficacy
  - Wider range of weights and spp. to account for
  - Feed palatability and field stability are issues

Drug Development

- Phases
  - 1.Acquisition
    - ID molecules of interest for specific molecular target
    - Preliminary in vitro
      - Potency at target
      - metabolism
    - and then in vivo studies
      - Efficacy and safety, preliminary PK
  - 2. Development
    - Create a formulation, develop analytical methods
    - Full PK studies
      - Determine dose (does it work and is it safe)
      - Also human food safety/residue studies
    - FDA review of INAD (investigational new animal drug)

Drug Development

- Registration
  - Large trials ($$$)
    - Long term safety studies
  - Submission to FDA of new animal drug application (NADA)
  - Launch after FDA approval
- 4. Support
  - Pharmacovigilance/monitoring

Structure/Activity Relationship

- Often use these tests to determine if a molecule should be investigated further
  - Structure, solubility, stability, pH, electrophilicity, volatility, reactivity
  - N-nitroso or aromatic amine groups can potentially be carcinogens

Toxicologic Tests

ACUTE TESTS
SUBCHRONIC
CHRONIC

Acute Toxicity Testing

- Acute toxicity = the adverse effects occurring within a short time of single dose of a substance or multiple doses given within 24 hrs
  - Often PO, could be injection or inhalation (depends on substance and expected use or how humans exposed)
  - Quantal response
    - All or nothing: dead or alive
  - Graded response
    - Quantitative: weight loss or feed consumption
- LD_{50} is of value for highly toxic substances primarily
- Acute toxicity is NOT = LD_{50}

Acute Toxicity Testing

- Objectives
  - Define intrinsic toxicity of a chemical
  - Hazard prediction (target and nontarget spp.)
  - Determine susceptible spp.
  - Identify target organs
  - Provide info for the design and selection of doses for prolonged studies
  - Provide info for treatment of acute overdose
  - Often, oral, dermal, and ocular acute tests are done

Acute Oral Toxicity

- In general:
  - Test substance given by gavage or by gastric intubation
  - Group of animals observed at specific intervals for clinic signs, morbidity, mortality
  - Necropsies are done, usually on all animals

Acute Oral Toxicity

- Species
  - Ideally, chosen if response is suspected to be similar to human response
    - Usually this is difficult or impossible
    - Usually chosen based on convenience, cost, and existing database of information about the species
      - Rats, mice, rabbits, guinea pigs
- Healthy young adults

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Acute Oral Toxicity

- Most regulatory guidelines suggest 10 rats
  - 5 male and 5 female
- Controlled environment
  - Temperature, light and dark cycle
  - Caged appropriately
    - Rodents may be together (usually no more than 3/cage)
    - Otherwise, housed individually
- Quarantined for at least 1 week to acclimatize
- Fasted prior to dosing
- Randomly assigned to dose groups

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Acute Oral Toxicity

- Dose levels
  - 3-5 levels usually enough to show a dose – response relationship
  - The doses should bracket the expected LD_{50}, with at least one level higher, but not causing 100% mortality and one dose lower, but not causing 0% mortality.
  - May need to do a pilot study to decide what doses to use
  - Or dose may be based on information from other spp
- In general, 5 g/kg (some say 2) is considered to be "practically nontoxic"
  - Usually no need to go higher
  - If higher doses given, could see signs that are not intrinsic to the compound itself
    - GI blockage

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Acute Oral Toxicity

- Administered in appropriate vehicle
  - If toxicity of vehicle is unknown, need to do a vehicle control group
  - Need to bear in mind that a vehicle could cause problems
    - Diarrhea from an oil, could impair absorption of the toxicant, if GI motility is increased
  - Small a volume as possible should be used
- Observation period
  - Varies depending on substance
  - Onset of signs could be very short to days
  - Usually not more than 14 days

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Other Acute Tests

- Acute dermal toxicity
- Acute hypersensitivity
- Acute ocular toxicity

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Subchronic Toxicity Testing

- 90 days is most common duration
- Usually need data from short term studies to design this type of testing
  - Doses/ dose-response
    - May need to do short-term, repeated dosing studies to determine this
    - 14-28 days
  - Target organ(s)
  - palatability

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Subchronic Toxicity Testing

- Objectives
  - ID longer term adverse effects
  - ID LOAEL and NOAEL
  - Provide information for chronic studies
    - Spp. to use, doses

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Subchronic Toxicity Testing

- Requirements/guidelines (EPA/FDA) overview
  - Animals
    - At least 3 groups and a control group +/- vehicle control group
    - At least 10 animals/sex/group
      - Weigh weekly
      - Weekly feed consumption
      - Observe daily
      - Pre and post ophthalmology exams
    - At end of study
      - Organ weights, all animals necropsied and histopathology

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Subchronic Toxicity Testing

- Test material
  - May be part of the diet or water for entire testing period and animals have free access
  - Dermal or inhalation studies
    - Intermittent exposure, usually 4-6 h per day
  - Parenteral or oral
    - Usually once a day

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Subchronic Toxicity Testing

- Dose groups (at least 3)
  - High dose should not result in more than 10% mortality, but should show evidence of toxicity
  - Mid-dose group, no more than slight toxicity
  - Low dose group, ideally should show the NOAEL
- If substance is thought to be almost nontoxic, may be only one group used

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Subchronic Toxicity Testing

- Animal models
  - Usually one rodent and one nonrodent
    - Often rats and dogs
    - Others include: rabbits, guinea pigs, mouse, hamster, swine, primates
    - Dosing is usually started at 6 w in rodents, 6 m in dogs
  - Selection based on
    - What is required by the regulatory agency
    - Similar metabolism to humans
    - Prior data
    - Sensitivity of the species, most sensitive is best
    - Cost and availability
- Daily monitoring
  - CBC, chemistries, necropsied

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Chronic Toxicity Testing

- Done much as the subchronic studies
- Defined as > 3 months
  - Duration is from 6 months to two years in rodents; 5-7 yrs in dog; duration depended on intended use of the test substance
- Used to assess the cumulative toxicity
- Usually done to fulfill a regulatory requirement

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Chronic Toxicity Testing

- Dose selection
  - Need to avoid mortality
  - Usually, goal is toxic effects at one or more dose levels, and no effect (NOEL) or NOAEL at one or more of the lower doses
  - Usually, high dose is the MTD (maximum tolerable dose)
    - MTD= the highest dose predicted not to alter the animals normal longevity
    - MTD = does in which in a 90 days study causes not more then 10% decrease in weight, no mortality, no clinical signs, no path lesions

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Chronic Toxicity Testing

- Species : rodent and nonrodent
  - Chosen much as in the subchronic studies
- Need to start with more animals to make sure that enough survive for the duration of the study
  - May need to start with twice as many as needed at the end of the study
- Similar to subchronic testing; chemistries, necropsies are done in these studies as in the subchronic studies

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Other Toxicity Tests

- Genetic tests
- Carcinogenicity
- Developmental toxicology testing
- Pharmacokinetic/analytical methods
- Environmental fate/effects
- Secondary toxicity risks
- Analytical methods
- Species differences

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Species Differences

Dogs

- Indiscriminate eater
  - More sensitive to methemoglobin formation than humans
    - 4 vs 2 sulfhydryl groups on hemoglobin
- Aceytylation limited
- Some dogs have deficient P glycoprotein on bbb
  - increased sensitivity

Cats

- More selective eating habits; grooming habits
- Concentrated urine
- Eight reactive sulfhydryl groups on hemoglobin
  - increased susceptibility of RBC to oxidative damage; Short RBC life span (66-79 days)
- Limited glucuronidation
  - highly susceptible to xenobiotics that require glucuronidation for metabolism (acetaminophen, aspirin, etc).

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Summary

- Safety and efficacy---long tedious process
- Expensive; years of efforts; millions of dollars
- Must show safety in lab animals and target animal; LD50s not enough
- Species differences important; significant metabolic differences among spp.

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Questions?

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