Toxicology for the Laboratory Animal Scientist

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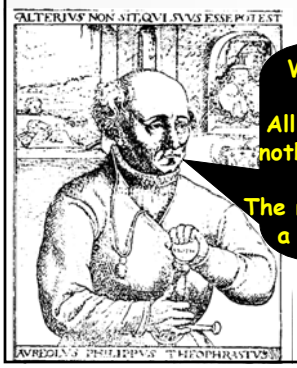
Course Objectives:

- Define terms used in the toxicology environment
- Discuss different types of toxicology studies
- Discuss the governing regulations in the toxicology environment
- Review the importance of laboratory animal science issues on toxicology data collection

Paracelsus 1493-1541

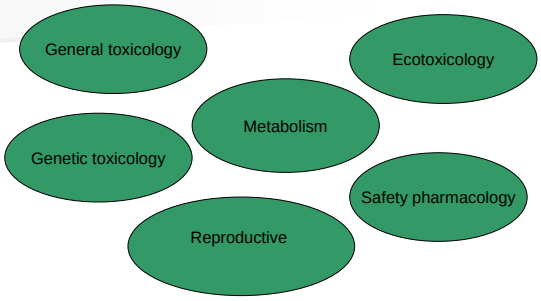


What is not a Poison?

All things are poisons and nothing is without toxicity.

The right dose differentiates a poison from a remedy.

Many Types of Toxicology Studies!!



General Toxicology Investigations

- Homogeneity and stability
- Clinical observations
- Body weights, food/water consumption
- Ophthalmoscopic examinations
- Electrocardiogram
- Hematology, clinical chemistry, urinalysis, biomarkers
- Necropsy, lesions, organ weights
- Histopathology

Reproductive Toxicology

- Fertility
- Organogenesis
- Fetal development
- Investigations
  - embryo count
  - fetal morphology
  - pup development
  - reproductive performance

Genetic Toxicology

Prediction of genetic damage to humans by:

- in vitro gene mutation studies (bacteria, mammalian cells)
- chromosome damage (in vitro and in vivo)
- Ames test, mouse lymphoma assay, SHE assay etc.

Safety Pharmacology

- Safety studies
- Acute effects
- Conscious and anaesthetized preparations
- Major functions, especially CNS and CV
- In vitro studies

Pharmacokinetics

- Dedicated to the determination of the fate of substances administered to a living organism.
- Pharmacokinetics is often divided into several areas including, but not limited to, the extent and rate of Absorption, Distribution, Metabolism and Excretion. This sometimes is referred to as the ADME scheme.

Drug Metabolism

- Absorption - how much drug gets into the body
- Distribution - where does the drug go in the body
- Metabolism - is the drug changed in the body
- Excretion - how does the drug (or metabolites) leave the body

- **Toxicokinetics** is the application of pharmacokinetics to determine the relationship between the systemic exposure of a compound in experimental animals and its toxicity.
- It is used primarily for establishing relationships between exposures in toxicology experiments in animals and the corresponding exposures in humans. Toxicokinetics measure exposure to drug (or metabolites)

Selection of Species

Designing Studies Which Route?

- Same as human
- Main routes - Oral, Intravenous
- Other routes - Inhalation, Ocular, Dermal, Intrathecal, Diet

Designing Studies What Dose?

- No dose - controls necessary
- Low dose - no toxic effect
- Mid dose - show some toxicity
- High dose - limited by toxicity or exposure

Designing Studies - For how long?

Depends upon clinical plan

1 day in humans	14 days study
7 - 14 days	14 - 28 days
1 month	1 - 6 months
1 year	6 - 12 months plus carcinogenicity studies

Toxicity Testing

- Generally conducted in healthly, experimental animals (not in animal disease models). Some *in vitro* tests.
- Required by law for international regulatory agencies.
- Highly regulated area (GLPs).

Study Design

- *Study design* is flexible and is based on regulatory agency recommendations and the International Conference on Harmonization (ICH) *Guidelines*.

Good Laboratory Practice Regulations (GLPs)

- Promulgated into law by FDA in 1978 due to documentation problems in a contract toxicology laboratory.
- ***Terms - Sponsor*** is the commercial company that conducts the preclinical toxicity study in-house or at contract. FDA does not conduct the study.
- ***Scope*** - support for marketing applications to the FDA for food and color additives, animal food additives, human and animal drugs, medical devices, and biological and electronic products.

Good Laboratory Practice Regulations (GLPs)

- ***Study Director (Sponsor)*** (21 CFR 58.33) - overall responsibility for the technical conduct of the study as well as for the interpretation, documentation and reporting of results and represents the single point of study control.
- ***Quality Assurance Unit (Sponsor)*** (21 CFR 58.35) - to assure that facilities, equipment, personnel, methods, practices, records and controls are in conformance.

Good Laboratory Practice Regulations (GLPs)

- ***Standard Operating Procedures (Sponsor)*** (21 CFR 58.81) - must have laboratory study methods in writing. Deviations authorized by Study Director.
- ***Protocol (Sponsor)*** (21 CFR 58.120) - each study shall have an approved written protocol. Requires dated signature of the Study Director. Revisions signed by Study Director and maintained with protocol.

It's critical to understand what the GLP's are and how they came about.

- A process for study conduct and documentation that leads to "reconstructability"
- One can conduct a poorly designed study with full GLP compliance and likewise some of the best studies in science would never be amenable to compliance.

There are numerous inherent conflicts in the conduct of toxicology studies that require the application of compromise and balance

- What is best for the animal may not be best for the study
- Each member of the study team has a differing role and responsibility
- Study design is often a series of "compromises" between animal welfare, personal safety, and scientific concerns

The Partnership

- Veterinarians need to understand why the study is being conducted and the desired toxic endpoint
- This is best accomplished when the veterinary and toxicology staff communicate about the project ahead of time.

ICH Guidance Documents

- Regulatory Web Sites for ICH Guidance:
  - US Food and Drug Administration
    - http://www.fda.gov/cder/guidance/index.htm
  - European Agency for the Evaluation of Medicinal Products
    - http://www.eudra.org/humandocs/humans/ICH.htm

Numerous Animal Care and Use Issues in Developing Safety Protocols

- **Species and Strains**
- **Age**
- **Source of animals for in-house studies and contract studies**
- **Group vs single housing of rodents**
- **Duration of carcinogenicity studies**
- **Selection of nonrodent species**
- **Use of non-standard species, e.g., minipigs, ferrets**
- **Use of transgenic animals**
- **Method of release of animals for use in studies**
- **Maximum dose volumes**
- **Maximum blood sample volumes**
- **Maximum intravenous injection rates**
- **Subcutaneous injection limits**
- **Diets**
- **Bedding**
- **Drinking water**
- **Environmental conditions (T/UR, lighting, caging, etc.)**

What influences data?

- Microbial status
- Diseases and lesions
- Genetics
- Environment
- Toxicology study methods
- Animal care and use program

Safety study challenges

- Regulatory requirements lead to “template studies” in protocol development
- Difficulty of putting professional judgement into the framework of working in an SOP format
- Working in the world of CRO’s
- Tendency to look at the record (data) versus the animal and the environment

The Historical Toxicology Database

- Databases are “living documents” and change all the time
- Not a reason to abandon professional judgement
- Has held back advancements in animal care and use on safety studies

Toxicologist vs Rodent

Enrichment

Table 1. Summary of recommended animal care practices for industry*

Species	Housing	Social contact	Diet	Enrichment	Human interaction
Mice	solid-bottom caging with substrate or solid inserts	variable; strain and study-dependent	diet optimization (strain-dependent)	nesting material, plastic tubing or bottles for hiding, gnawing devices	not beneficial other than for acclimation
Rats	solid-bottom caging with substrate or solid inserts	pair or group house	diet optimization	plastic box or devices for hiding, gnawing devices	beneficial
Rabbits	solid-bottom caging, oversized cages or floor pens with substrate	pair or group house juveniles and females when possible	diet optimization with occasional food treats (e.g., hay, fresh vegetables)	hiding place, shelf, toys, ball, gnawing devices	beneficial
Dogs	cages or pens with substrate	pair or group house	diet optimization with occasional food treats	platforms, chew toys, exercise	beneficial
Nonhuman primates	cages or pens	pair or group house	diet optimization supplemented with fresh fruit, vegetables, food treats, foraging opportunities	perches, mirrors, other toys rotated regularly, music/video	beneficial

*Compiled from refs. 6, 26,28.

Turner et al., JAALAS, 42 (6), p 10

Stress

- **Noise** (Turner, 2005):
  - maintenance, technical devices, animals
  - Non-auditory effects of sound in laboratory animals:
    - Cardiovascular: increased vasoconstriction and respiration in the rat
    - Hormonal: increased norepinephrine, cortisol, cholesterol and plasma corticosterone in rat
    - Other: increased microvascular permeability and disruption of the intestinal lining, decreased body weight

Stress

- Cage environment:
- Cage change: (Sharp, 2003)
  - Witnessing a routine cage change induced significant increases in HR in rates . Witnessing restraint and SQ or tail-vein injections of other rats.
- Social: mixed information

Stress

- Procedures:
  - Stress (as measured by blood glucose and corticosterone) was significantly less in animals cannulated vs. animals that were repeatedly anesthetized for blood collection (Vachon, 2001).
- Long term activation of the HPA axis has been linked to immunosuppression, insulin resistance, hypertension and arteriosclerosis, catabolism, and GI problems

Acclimation

- Facility/quarantine
- Staff
- Pre-study assessments
- Equipment
- Study procedures

Important to evaluate animals and not data alone: There is no substitution for looking at the animal in it's environment

Sentinel surveillance

- Design programs around the species, origin and duration of animal stay
  - Rodent sentinel programs: Will animals stay long enough to warrant serologic evaluation? Do you have a sense of comfort about the disease status of the animals?

What constitutes normal?

- Having a control may not suffice
- Toxicologists place too much emphasis on comparing treated with control without considering what is normal
  - Neurologic examinations in laboratory beagles

Health Surveillance

- Dogs: Vendor? Quarantine period? Stock animal management?

Dose Administration

- Avoid excessive dose
- Information search
- Pilot studies
- Modeling
- Tiered approach
- Avoid unnecessary severity
- Limit use of positive control groups
- Aggressive "moribund" sacrifice

Dosing Issues

- Database search and information background
- Vehicle and formulation considerations
- Post-procedural observations and care
- Experimental manipulations
- Define in writing – "lines of consult" and what is to be done

Establish Procedures for Post-dosing Observations

- Train personnel in species specific behaviors
- Observe immediately post-dosing and a short time interval later (30-60 minutes)
- Observe animals next morning with clinical assessment
- Schedule assessments based on toxicant, target organ of toxicity and experience

Automated Data Collection Systems

- Rapidly assess groups
- Handle large numbers of animals
- body weights, food and water consumption, clinical signs, mass tracking
- Forces standardization
- Training tool with prompts

Body Weight Determinations

- Tracking of groups
- Inherent limitations
- Trends more important than absolute
- Need to compare groups

Clinical Assessment

- Usually the cases are noted on scheduled animal observations.
- To be considered: What is the category of toxicant? When was the study started? When does it end? How many are affected? What treatment group do they represent?

Useful tools

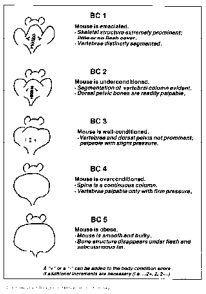
- Trends in body weights
- Food consumption information-general
- Previous data from mechanistic studies
- Previous information from DRF or MTD studies
- Data from same class of drug
- Clinical data
- pK data

Treatment modalities

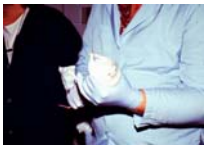
- Canned or soft food
- Nutritional supplements
- Soft bedding
- Water bowls
- Subcutaneous fluid therapy
- Others?

Humane Endpoints

- Unresponsiveness or coolness to the touch
- Progressive weight loss (Ullman-Cullere' and Foltz, 1999)
- Prolonged recumbancy
- Seizures, respiratory distress
- Chronic diarrhea.....



Decision Making



- Work with the SD
- Decide if the data that is sought has been obtained.
- Consider what is lost from the study if euthanasia occurs
- What is the justification for pain/distress?

Acknowledgements

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References:

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