TOXICITY STUDIES

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INTRODUCTION

A drug is a single substance or mixture of substances used for diagnosis, treatment, mitigation or prevention of disease ; restoring, correcting or modifying the organic functions in man or animals (W.H.O.)

Agents of these potential activities interfere with biological processes of the host or extraneous etiological agents and hence are toxic substances.

The object of toxicity testing in the laboratory is to elucidate the toxic properties of drugs

FACTORS THAT EFFECT TOXIC ACTIONS OF DRUG

- Animal factors
  - Species
  - Strain
  - Age
  - Sex
  - Nutritional state
- Route of administration
- Environmental factors
- Physical and chemical factors

ACUTE TOXICITY STUDIES

- The Globally Harmonized System (GHS),defines it as "those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours"
- The preferred species for oral and inhalation testing is the rat, and for dermal testing, the rat or rabbit
- Oral administration is the most common form of acute systemic toxicity testing.

The Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET) developed the Up-and-Down Procedure (UDP) to reduce the number of animals used in acute toxicity testing

ICCVAM endorsed two *in vitro* basal cytotoxicity assays, the Neutral Red Uptake (NRU) test with rodent cells (3T3 NRU assay) and the NRU test with normal human keratinocyte (NHK) cells (NHK NRU assay)

Five Organisation for Economic Cooperation and Development (OECD) Test Guidelines (TGs 402, 403, 420, 423, and 425) describe acute systemic testing.

Fixed Dose Procedure (OECD TG 420)

Acute Toxic Class method (OECD TG 423)

Up-and-Down Procedure (OECD TG 425)

For the OECD TG 402, Acute Dermal Toxicity, a test substance is applied to no less than 10% of the area of the skin of rats, rabbits, or guinea pigs, followed by 14 days of observation. Death of the animals is used to determine an LD_{50} value

Acute inhalation toxicity is assessed according to OECD TG 403.

NONANIMAL ALTERNATIVE METHODS

Cell Type	Endpoint(s)	Mechanism
BALB/c 3T3 - mouse fibroblast cell line	Neutral red uptake	Cell viability/cytotoxicity
Normal human keratinocytes	Neutral red uptake	Cell viability/cytotoxicity
LLC-PK1 kidney proximal tubule cell line	Transepithelial resistance (TER) and paracellular permeability	Barrier integrity/cell damage
HL-60 human acute promyelocytic leukemia cell line	Adenosine triphosphate (ATP) content	Energy production and metabolism
Change liver cell line	Morphology change followed by pH change	Cell growth/cytotoxicity
MDCK dog kidney epithelial cell line	Transepithelial resistance (TER)	Barrier integrity

SUBACUTE AND CHRONIC TOXICITY TESTING

The Globally Harmonized System (GHS)defines it as "specific target organ/systemic toxicity arising from a repeated exposure"

Repeated dose toxicity testing using oral administration of a test substance in rodents for 28 and 90 days is used to evaluate chronic toxic effects, primarily effects on various organ systems, and to establish a no observed effect level

Chronic toxicity testing consists of oral, dermal, and inhalation subacute repeated dose studies (28-day) and subchronic repeated dose studies (90-day) in rodents.

. The endpoints for repeat dose testing consist of an evaluation of clinical observations, blood analysis, whole body gross necropsy, and microscopic examination of all organs and tissues (histopathology)

Six OECD Test Guidelines describe short-term repeat-dose toxicity testing:

Repeated Dose 28-day Oral Toxicity Study in Rodents (TG407)

Repeated Dose 90-Day Oral Toxicity Study in Rodents (TG 408)

Repeated Dose Dermal Toxicity: 21/28-day Study (TG 410)

Subchronic Dermal Toxicity: 90-day Study (TG 411)

Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (TG 412)

Subchronic Inhalation Toxicity: 90-day Study (TG 413)

NON-ANIMAL ALTERNATIVE METHODS

- The need for better access to (and techniques for preservation of) high quality human tissues for the use/development of *in vitro* cultures
- The availability of reference test substances for each organ system
- The availability of *in vivo* toxicity data, including human data
- Research to identify and validate biologically relevant toxicity endpoints for each organ system

IN VITRO AND *IN SILICO* METHODS AS ALTERNATIVES TO REPEATED DOSE TESTING

- **LIVER MODELS:** isolated human hepatocytes, liver slices, and perfused liver; perfused, and bioreactor test system

- **KIDNEY MODELS:** renal epithelial primary cells and cell lines

- **CNS MODELS:** neuronal primary cells and cell lines; reaggregating brain cell cultures; astrocyte cell cultures; oligodendrocyte cell cultures; microglia cell cultures

- **PULMONARY MODELS:** isolated, perfused rat and mouse lung;human trachael/bronchial epithelial cell cultures; human alveolar cell model of Skinethic; primary rat pneumocyte type II cell culture

- **HAEMATOPOIETIC MODELS:**bone marrow cultures; culture initiating cells; myeloid-lymphoid initiating cell assay; blast colony-forming cell assay; high proliferative potential colony-forming cell assay

- **NOVEL LONG-TERM CULTURE METHODS:** hollow fiber bioreactors; perfusion culture models

REFERENCES

- www.alttox.org/trtc/chronictoxicity
- www.currentprotocols.com
- Lehman, A. J.Sewer trends in the laboratory evaluation of the safety of drugs.
- W. H. O. (1966). Principles for preclinical testing of drug safety. *Techn. Rep. Ser. 341*