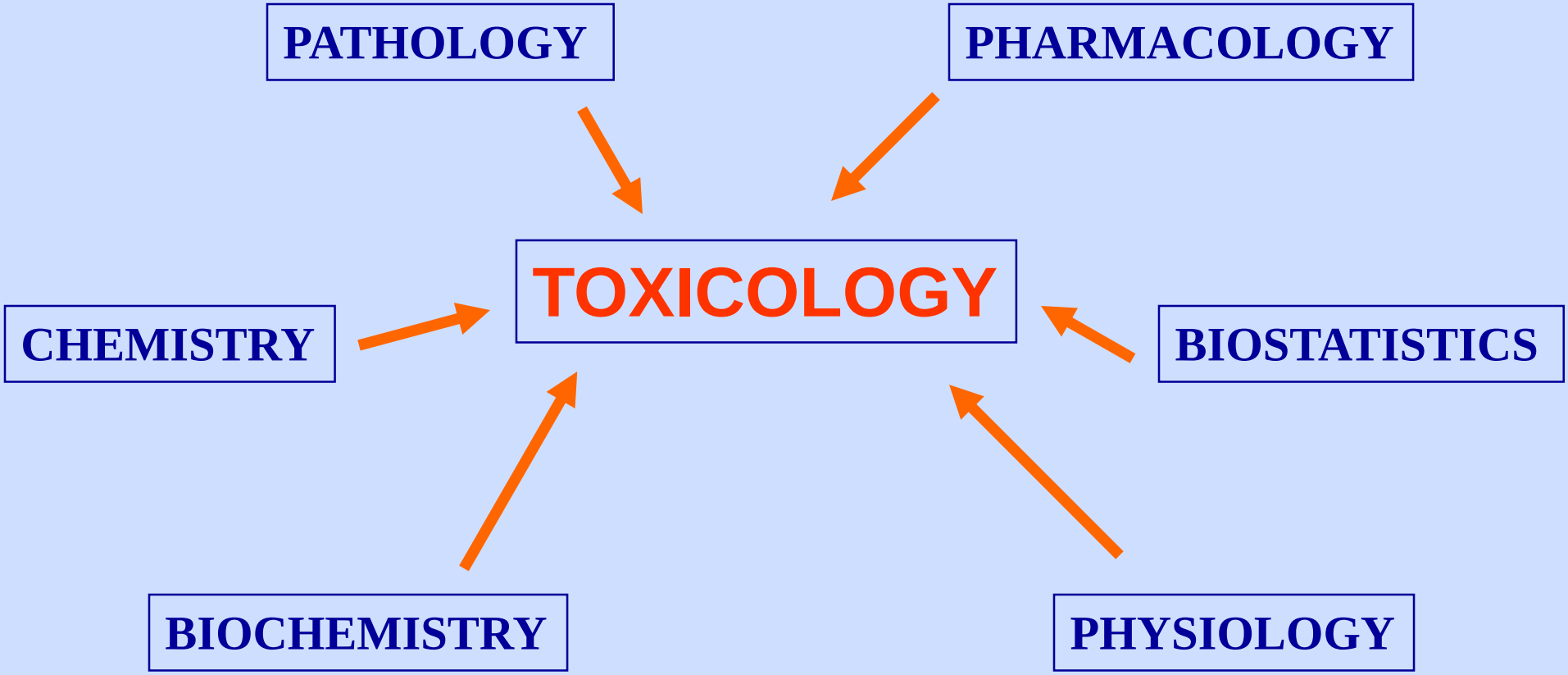
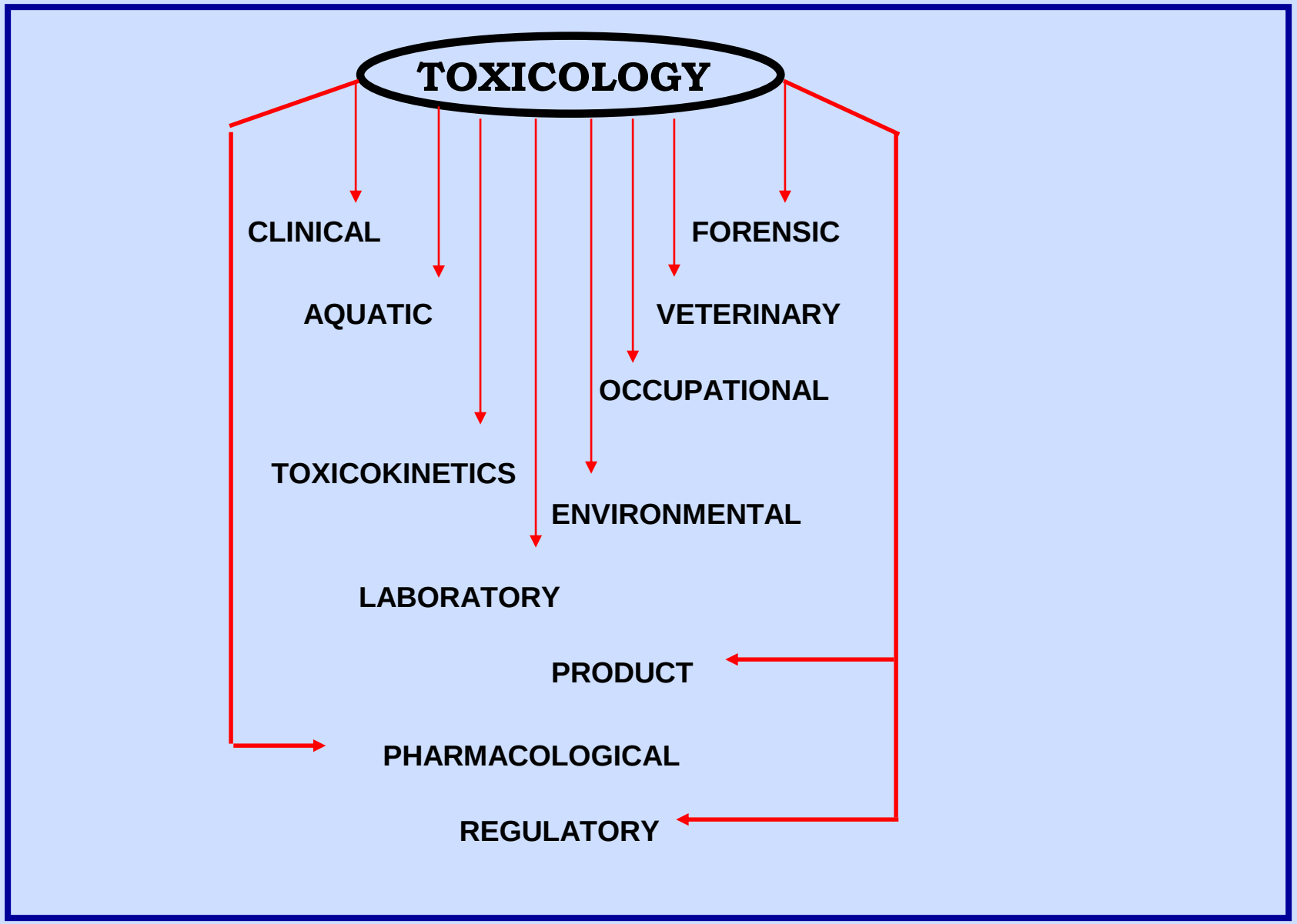
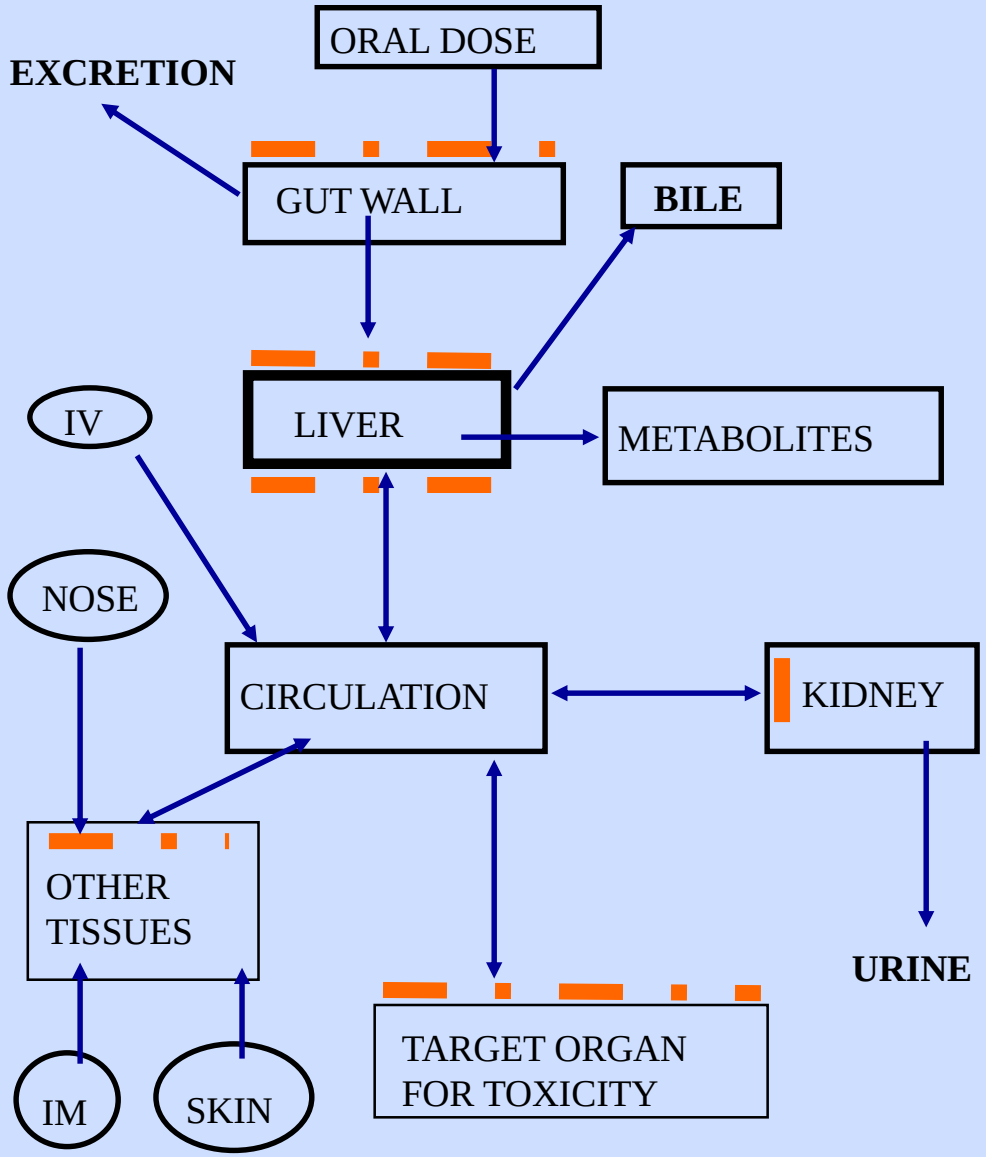
WHAT IS TOXICOLOGY ?

Toxicology is a study of the interaction between chemical and biological systems in order to quantitatively determine the potential of chemical(s) to produce injury which results in adverse effects in living organisms, and to investigate the nature, incidence, mechanism of production, factors influencing their development and reversibility of such adverse effects. (Ballantyne, 1989)

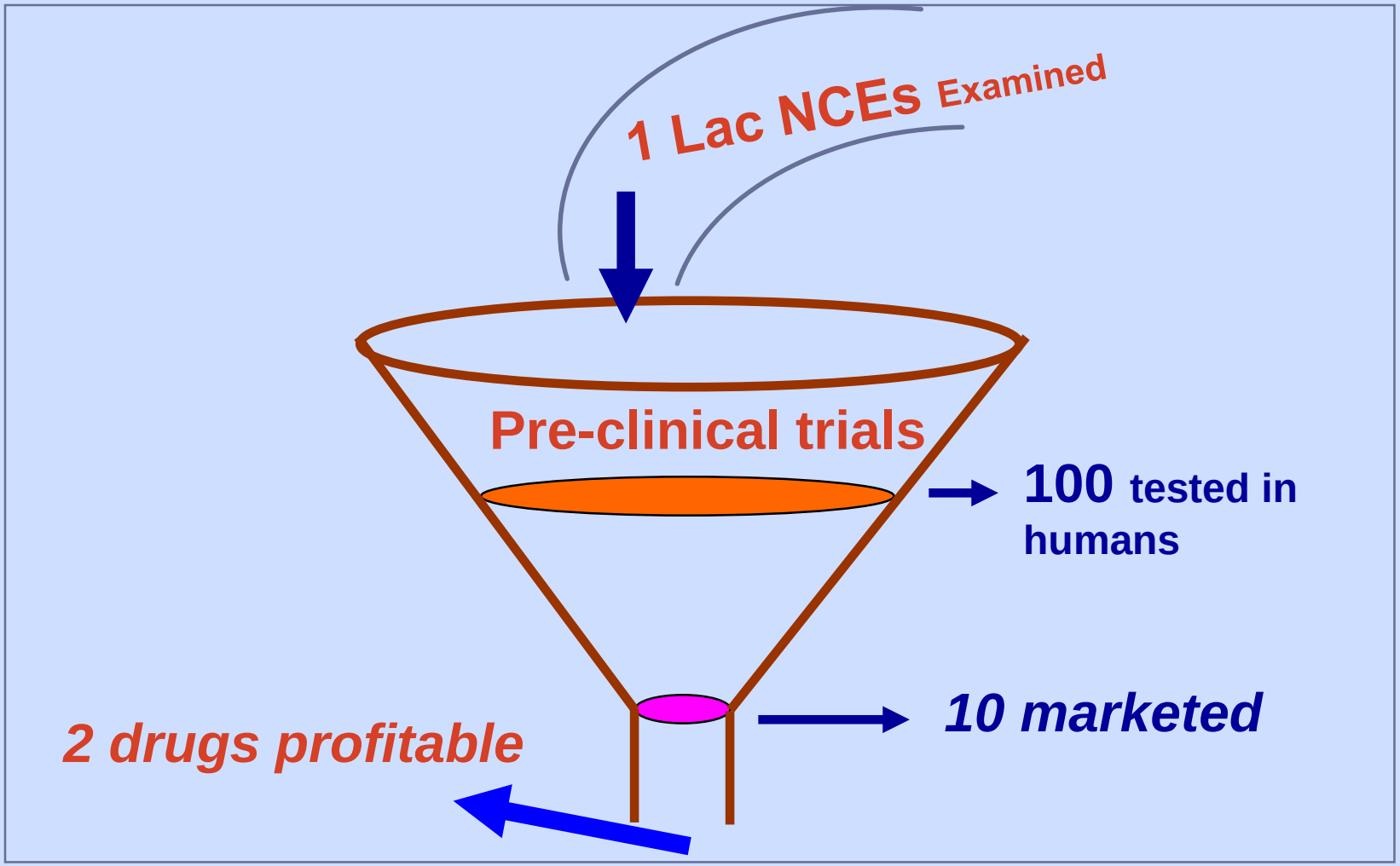

PATHOLOGY
PHARMACOLOGY
TOXICOLOGY
CHEMISTRY
BIOSTATISTICS
BIOCHEMISTRY
PHYSIOLOGY


TOXICOLOGY
CLINICAL
AQUATIC
TOXICOKINETICS
LABORATORY
PHARMACOLOGICAL
REGULATORY
PRODUCT
ENVIRONMENTAL
OCCUPATIONAL
VETERINARY
FORENSIC

TOXICITY & PHARMACOKINETICS



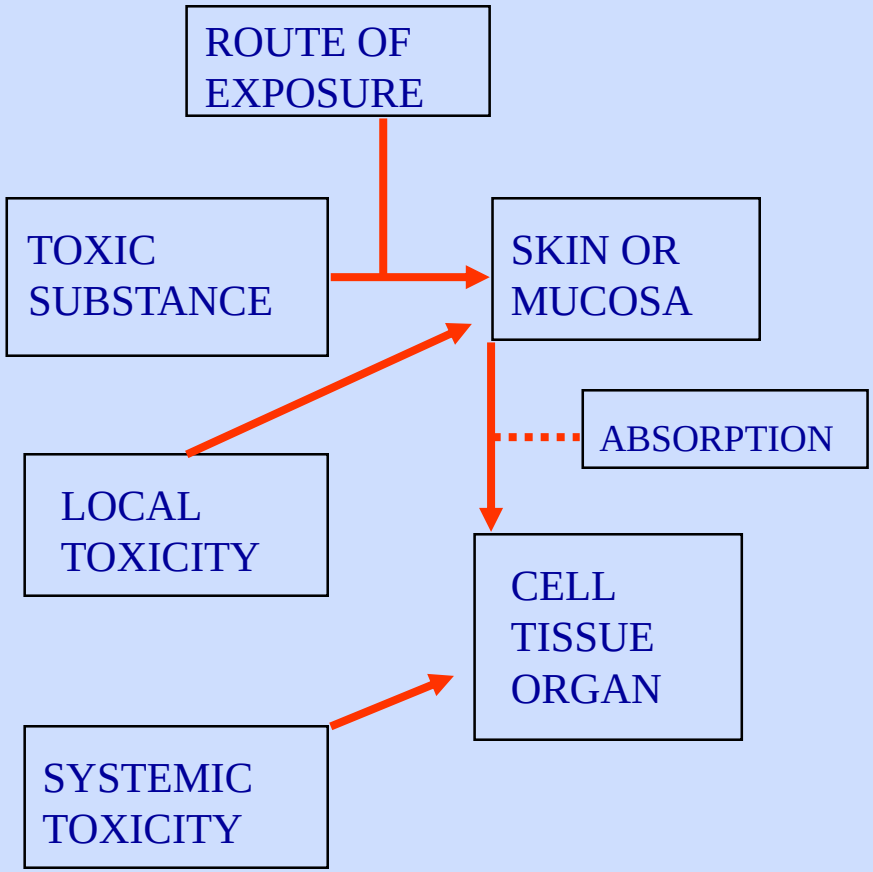
NEW DRUG DEVELOPMENT



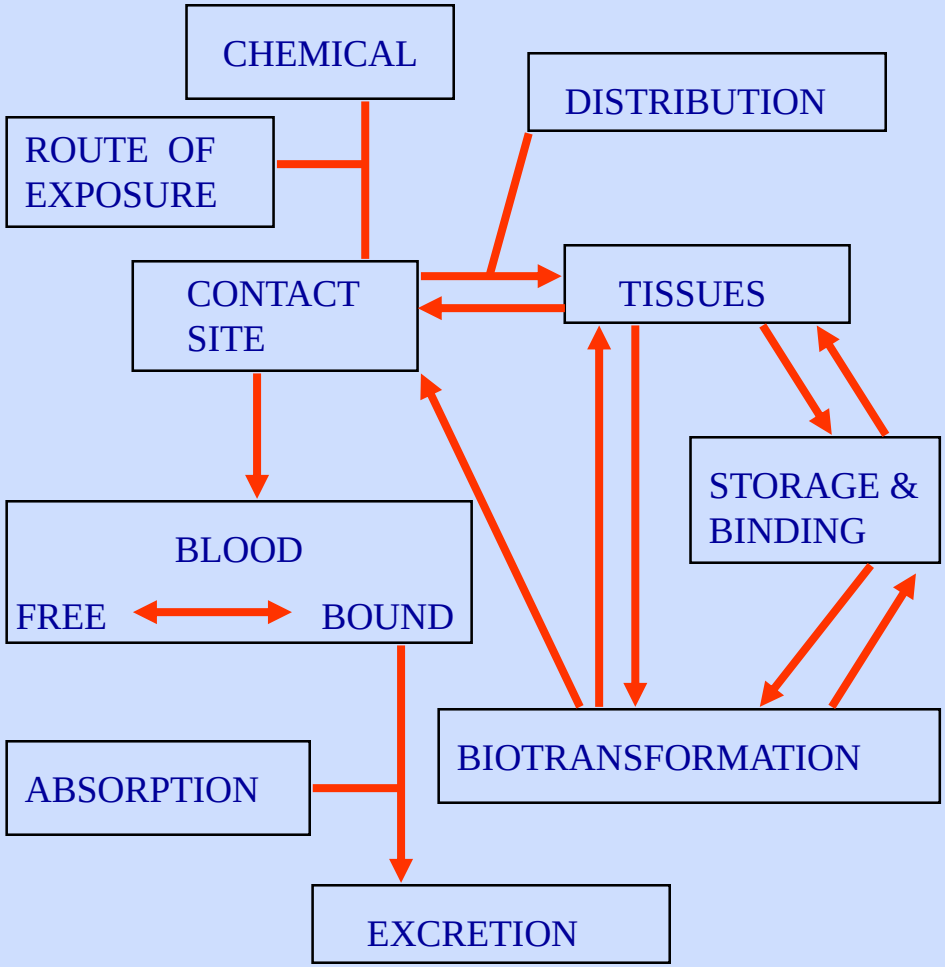
CHARACTERISTICS OF THE TOXICANT

* Basic nature of the injury produced.
* Dose - response relationship.
* The mechanism of toxicity.
* Factors that modify toxicity.
* Approach to recognise the toxicity.
* Reversibility of the toxic effect.

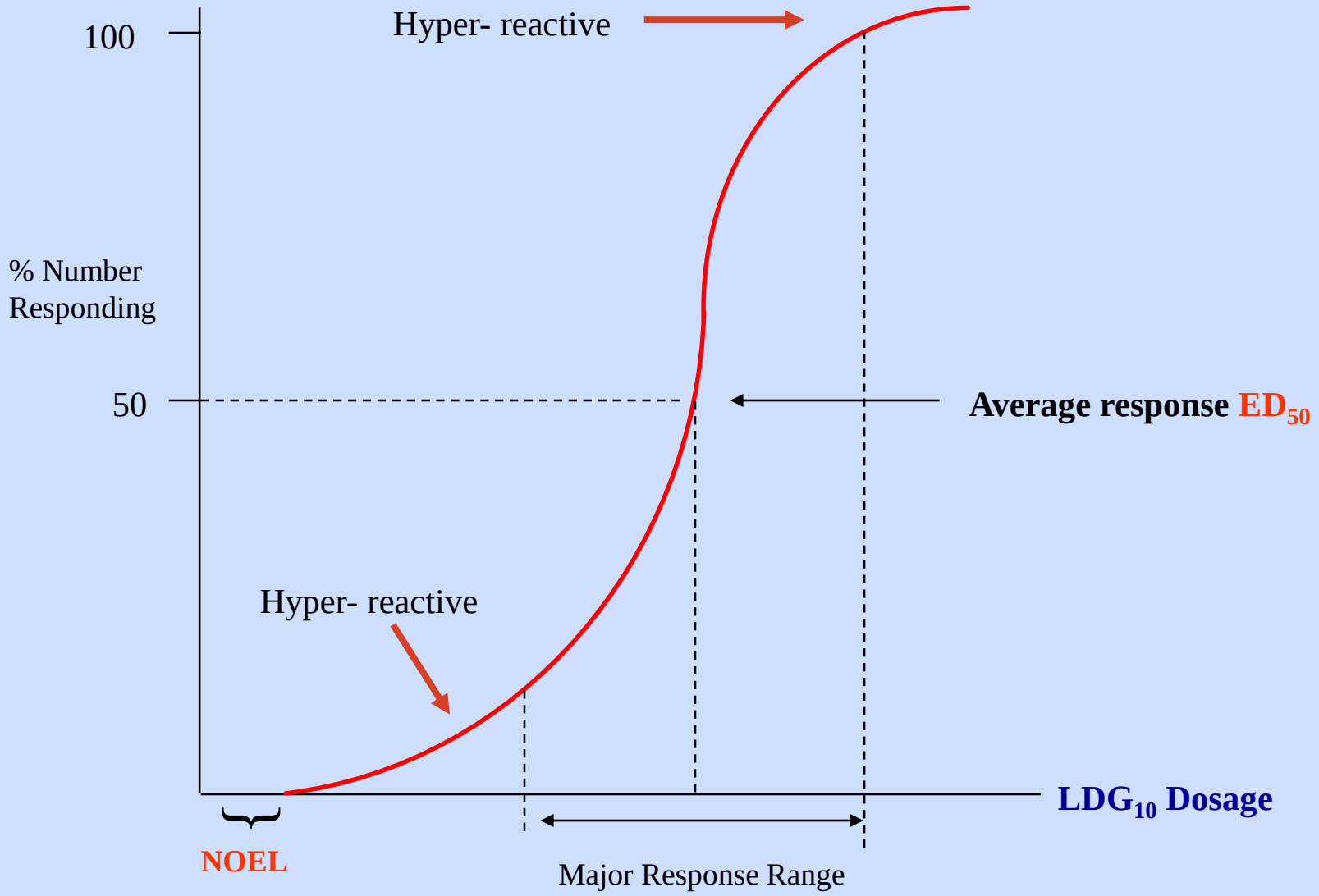
BASIS FOR CLASSIFICATION OF TOXIC EFFECTS



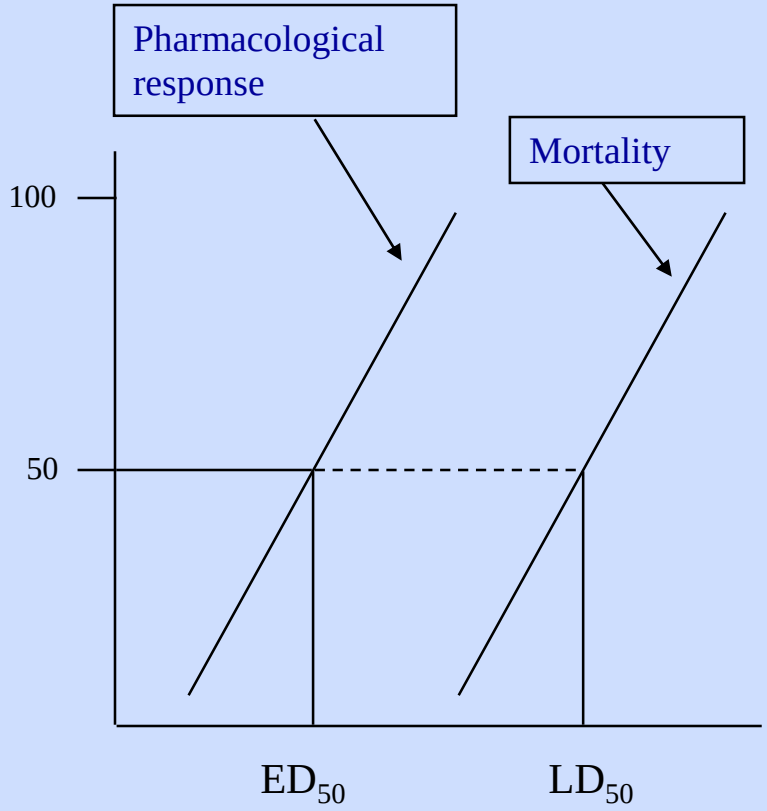
POSSIBLE FATE OF A XENOBIOTIC



DOSAGE - RESPONSE RELATIONSHIP

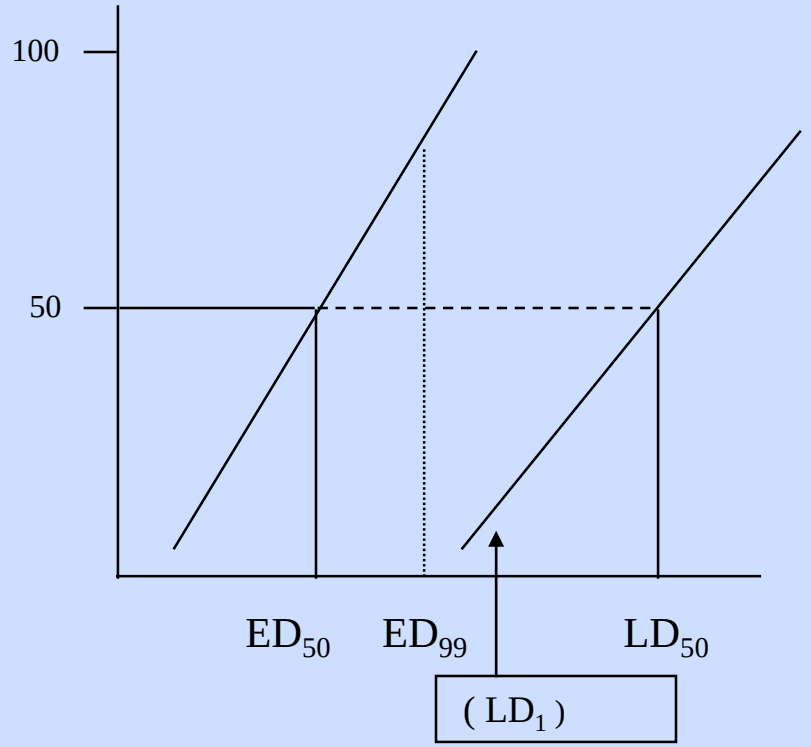


TOXICITY INDEX



$$TI_{50} = \frac{LD_{50}}{ED_{50}}$$

* TI ∝ Safety



Less TI at lower doses

Risk to hyperactive groups

$$\frac{ED_{99}}{LD_1} = \text{Margin of safety}$$

WHY UNDERTAKE TOXICITY STUDIES ?

Manipulate Exposure conditions

Relevance of animal response

Record many different responses

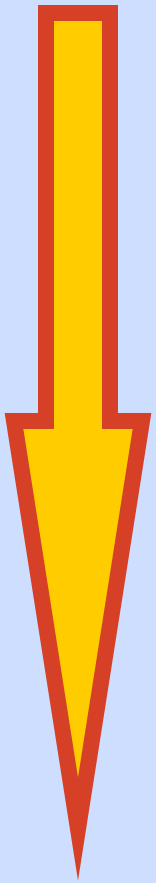
Controlled Lab. Conditions

Record various modifying factors (sex, age)

Study duration and doses different from human

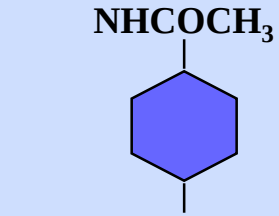
Evaluate mechanisms

Predict Potential Danger



What leads to toxicity ?

- Additive effect
- Synergistic effect
- Potentiation
- Antagonism



PARACETAMOL

Cytochrome P450

Liver glutathione

Quinoneimine

(Liver glutathione depleted)

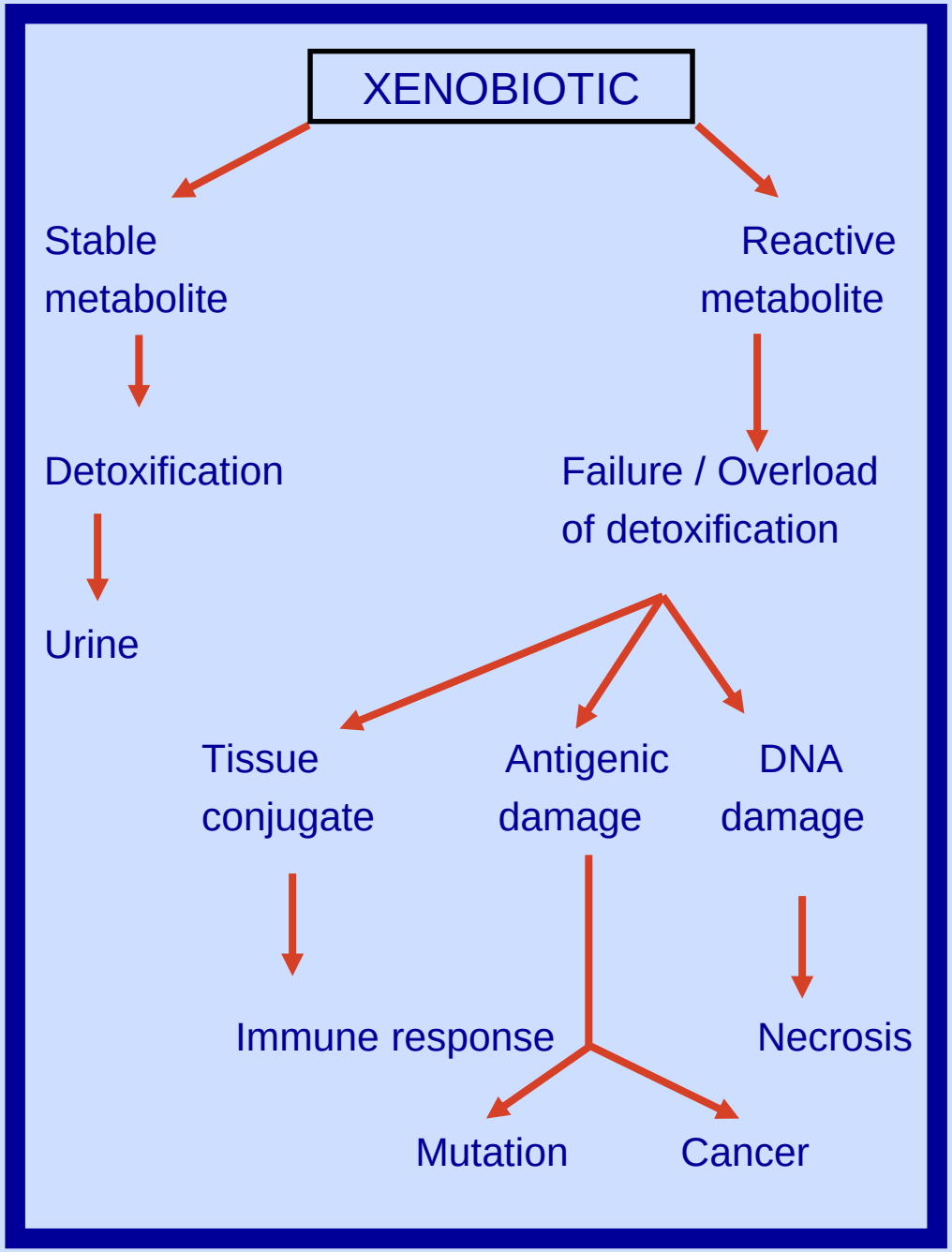
Mercapturic acid

Excreted

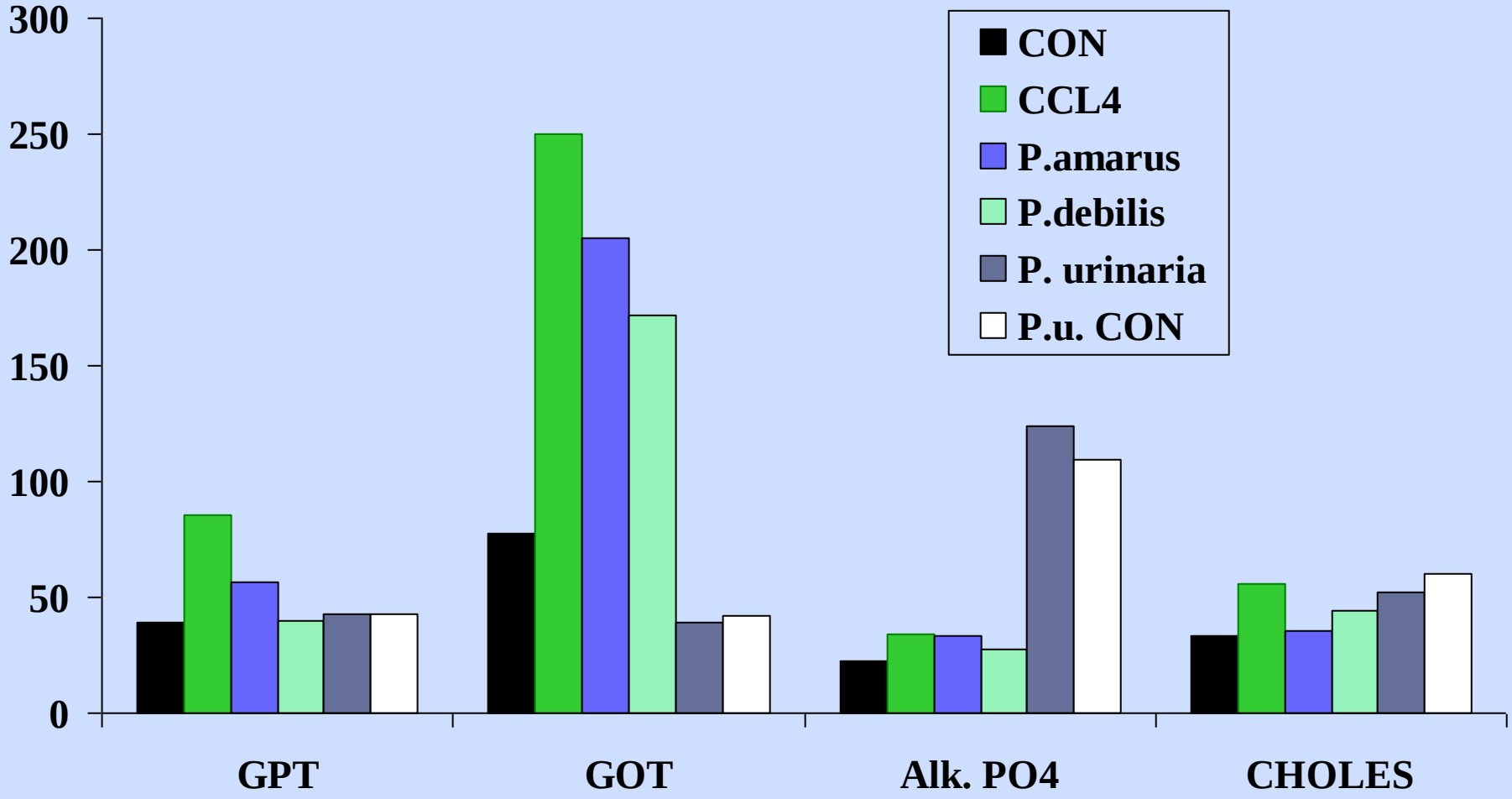
Covalent binding to liver protein

Necrosis

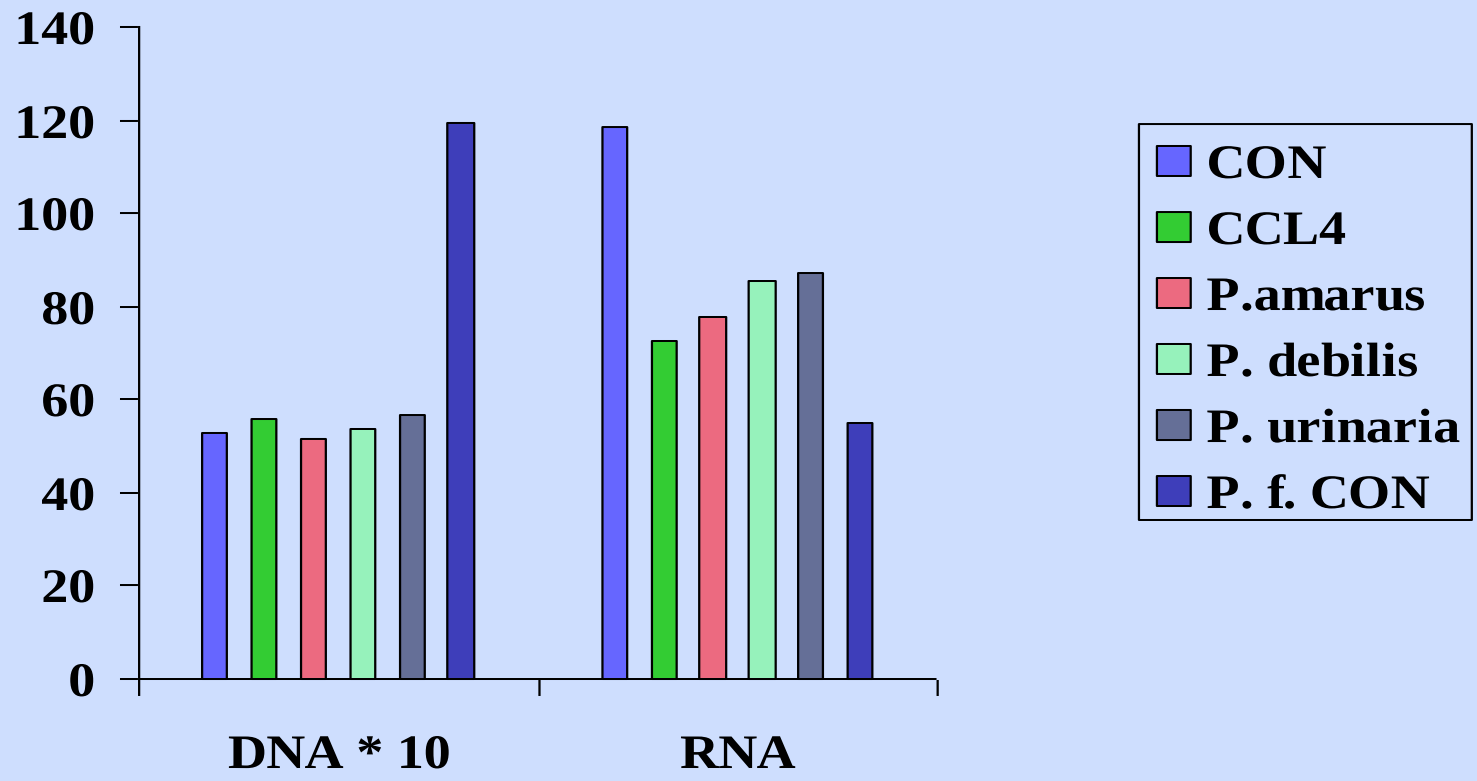
PARACETAMOL TOXICITY



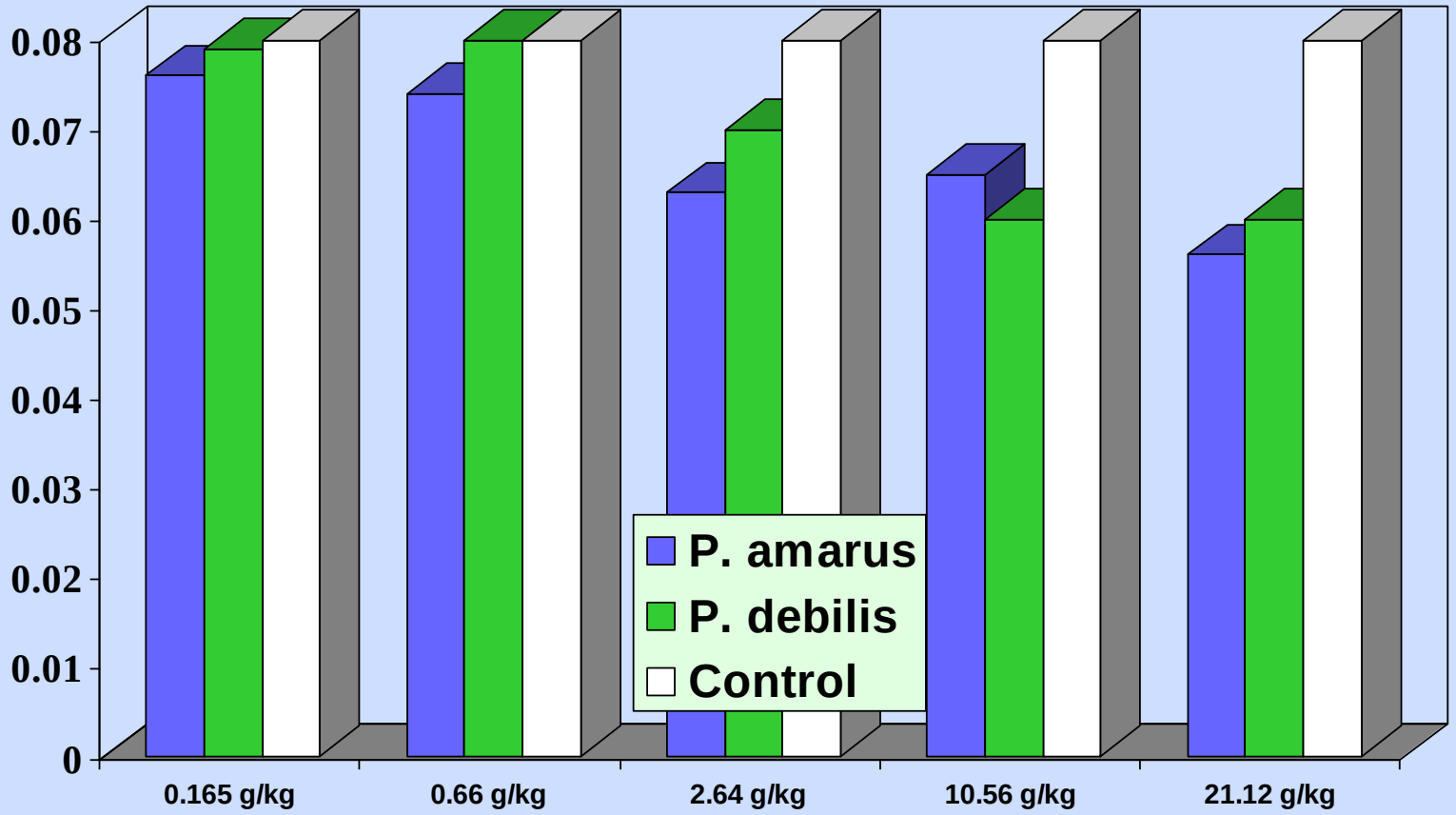
CHANGES IN PLASMA PARAMETERS



LIVER BIOCHEMISTRY



LIVER / BODY WEIGHT CHANGES



WHAT TO LOOK FOR IN TOXICITY STUDIES ?

Acute Toxicity.

Accidental Over -Exposure

Cumulative Toxicity.

Absorption from Various Routes.

Elimination.

Toxicity Studies - Purpose

- Purpose of toxicity studies are to check for any toxicity that could be manifested by the TM

If toxic

- The quantification of the same
- To understand the mode of toxicity and the organs or systems affected

Toxicity Studies
A Word of Advice

- Animals have life – Handle with love, care and respect
- Remember the 3 R's of animal experimentation – Refinement, Reduction and Replacement

TYPICAL TOXICOLOGICAL STUDY

* **OBJECTIVES**
* **PRESENTATION**
* **VEHICLE USED**
* **ANIMAL MODEL**
* **ANIMAL MAINTENANCE**
* **DOSE AND ADMINISTRATION**
* **PARAMETERS OBSERVED :** General symptoms, Mortality rate, Body weight, Water & food intake, Autopsy, Organs (liver, intestine, spleen, kidney, gonads, adrenals, etc.), Hematology (Hb, RBC, WBC) & Clinical Biochemistry (glucose, cholesterol, SGOT, SGPT, creatinine, acid & alkaline phosphatase, LDH, Triglycerides, GGT, etc.) and Urinalysis.
* **INTERPRETATION OF RESULTS**
* **EXTRAPOLATION OF RESULTS TO MAN**

Toxicity Studies - Acute

Acute Toxicity Test

- Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours.
- Death is the usual end-point and LD_{50} is the result but the signs exhibited by the animals are not to be ignored.

Toxicity Studies - Acute

Classical Acute Oral Toxicity

Species and Selection of Animals

Rat – *Rattus norvegicus*

Mice – *Mus musculus*

Healthy young adults (6 to 8 Weeks old)

Body Weight not to exceed ± 20% of mean

Acclimation – 5 days in the test room

Toxicity Studies - Acute

Test Room Conditions

- Temperature – 22 ± 3ºC
- Humidity – 30 to 70 %
- Photoperiod – 12 h light and 12 h dark

Feed and Water

- Feed - ad libitum except prior to dosing
- Water – ad libitum

Caging

- Not more than 5 animals of one sex per cage

Toxicity Studies - Acute

Range Finding Studies

2 Males and 2 Females / Group

Start with the maximum permissible dose of 2000 mg/ kg body weight.

If no signs of toxicity or death - carry out the limit test.

If mortality is observed go for lower doses to asses the dose range for the main study.

Toxicity Studies - Acute

Main Study

Observation – Minimum of once daily for 14 days
- should cover the onset and disappearance of signs and time of death were appropriate.

Body Weight – Day 0 followed by once every week

Feed Consumption - Daily

Toxicity Studies - Acute

Main Study

Randomization (On Day 0 prior to dosing)

5 Males and 5 Females per Group

Minimum of 4 Groups (except in limit test)

One Vehicle Control and three treatment groups – 10 ml/kg b.w. is the preferable dosing volume

Animals to be fasted prior to dosing

Toxicity Studies - Acute

Necropsy and Pathology

Animals to be sacrificed by humane methods (CO_2 asphyxiation) approved by the national legislative bodies eg: CPCSEA

Gross Examination

Gross pathological lesions to be observed microscopically

Toxicity Studies - Acute

Fixed Dose Method

Requires lesser number of animals

Employing fixed doses of 2000, 300, 50 and 5 mg/kg b.w.

Conditions for the test, observations and pathology – same as for classical method

Toxicity Studies – Long Term

Long Term Toxicity Tests

- Based on the expected period of clinical use

Expected period of Clinical Use	Period of Toxicity study
Single / repeated administration < a week	2 weeks to 1 month
Repeated administration 1 week <> 4 weeks	4 weeks to 3 months
Repeated administration 1 month <> 6 months	3 to 6 months
Long term administration > 6 months	9 to 12 months

Toxicity Studies
- Long Term

Species and Selection of Animals

Rat – *Rattus norvegicus*

Mice – *Mus musculus*

Healthy young adults (6 to 8 Weeks old)

Body Weight not to exceed ± 20% of mean

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Toxicity Studies
- Long Term

Test Room Conditions

Temperature – 22 ± 3ºC

Humidity – 30 to 70 %

Photoperiod – 12 h light and 12 h dark

Feed and Water

Feed - ad libitum

Water – ad libitum

Caging

Not more than 5 animals of one sex per cage

Toxicity Studies – Long Term

Administration – The expected route of clinical use

Frequency of Administration – Once daily on a seven days a week basis

Number of Animals / Group – 10 M + 10 F

Randomization – Prior to dosing

Toxicity Studies – Long Term

Selection of Doses

- Vehicle Control is required.
- One dose that causes no toxic changes (no–effect dose).
- One dose that produces overt toxic effects.
- An intermediate dose to observe a dose response relationship.

Toxicity Studies – Long Term

Observation – Minimum of once daily through out the dosing period (recovery period where appropriate)

Body Weight – Day 0 followed by once every week

Feed Consumption – Weekly

Ophthalmoscope examination – Start of experiment, Intermediate point and at termination

Other function tests like ECG, visual and auditory tests can also be carried out.

Toxicity Studies – Long Term

Hematology

Pre-test, intermediate point and termination (except in studies < 1 month duration, only terminal)

Parameters – RBC, WBC, Hb, PCV, Clotting Time, Prothrombin and 3 part differential count.

Toxicity Studies – Long Term

Necropsy and Pathology

Animals to be sacrificed by humane method

Gross Examination and Organ Weights

Histopathology of the high dose and control groups and if lesions are present the remaining groups also

Animals found dead should be autopsied

Moribund animals should be sacrificed

Toxicity Studies – Long Term

Biochemistry

Pre-test, intermediate point and termination (except in studies < 1 month duration, only terminal)

Parameters – ALT, AST, ALP, BUN, Creatinine, Glucose, Protein, Albumin and other specific parameters if required.

Toxicity Studies – Other Types

Teratology

Required for M during pregnancy

Carcinogenecity

Required for M that have to be used for years eg., Anti-Diabetics

ACUTE TOXICITY STUDIES

- HELPS DETERMINE LD_{50} VALUES
- SINGLE DOSE STUDY
- PERIOD-14 DAYS
- CAGE SIDE AND MORTALITY OBSERVATIONS

SUB ACUTE TOXICITY STUDIES

- HELPS ASSESS TOXICITY & TO EVALUATE TOXICITY AT 3 DOSE LEVELS
- DAILY DOSAGE
- PERIOD - 30 DAYS
- CAGE SIDE OBSERVATIONS, MORTALITY & VARIOUS TISSUE AND BLOOD PARAMETERS

CHRONIC STUDIES

- HELPS UNDERSTAND THE EFFECT AND CHECK SIDE EFFECTS ,IF ANY, OVER A PROLONGED PERIOD OF TIME
- DAILY DOSAGE
- PERIOD > 90 DAYS
- CAGE SIDE OBSERVATIONS , MORTALITY & VARIOUS TISSUE AND BLOOD PARAMETERS

UNDUE TOXICITY STUDIES

- HELPS DEDUCE TOXIC CONTAMINATION (IF ANY)
- SINGLE DOSE STUDY
- PERIOD - 7 DAYS
- CAGE SIDE AND MORTALITY OBSERVATIONS

DERMAL IRRITATION STUDIES/TESTS

- HELPS ASSESS IRRITANCY POTENTIAL OF TEST MATERIAL
- ONE TIME APPLICATION
- PERIOD - 3 DAYS
- TEST SITE OBSERVED FOR PRIMARY IRRITATION AND SCORED

EYE IRRITATION STUDIES / TESTS

- HELPS ASSESS THE IRRITANCY POTENTIAL TO THE EYE
- ONE TIME APPLICATION
- PERIOD - 1-21 DAYS
- EYES OBSERVED FOR IRRITANCY AND SCORED

Why are toxicity studies required ?

- **1938 - Sulfanilamide & Ethylene glycol**
- **1962 - Thalidomide**
- **1980 - Infant formulae**
- **1982 - Tylenol capsules**
- **1986 - Rye's syndrome (Aspirin)**

- **In India - J.J.Hospital deaths**

FDA has made them mandatory.

Prerequisites to Toxicity Studies

- Identification – macro and microscopic – of the plants used in TM
- The chemo-profiling of the TM is essential prior to toxicity screening – HPLC, HPTLC and GC – biomarkers of plants, contaminants
- The finished product (as to be sold in the market) and not the components should be tested for toxicity.

TOXICOLOGICAL & EFFICACY STUDIES ON DRUG PREPARATIONS

EXISTING

- Undue Toxicity
- Dermal Toxicity / Sensitivity
- Efficacy of stored Formulations
- Efficacy of stored Raw Materials
- Species variation in efficacy
- Overdose extrapolations

Batch-wise evaluations -

Undue toxicity of raw materials / formulation

NEW

- Preliminary Acute Toxicity (2 g / Kg)
- Efficacy on animal model
- Efficacy of related species
- Toxicity of related species
- Efficacy of formulation
  - known drug
  - natural recovery
- Toxicity of formulation
- Efficacy after storage
- Set limits (contaminants)

IMPEDIMENTS IN ANIMAL STUDIES ON DRUG PREPARATIONS

TOXICITY

- Long years of usage & safety record
- Dosage considerations
- Multi component composition
- Inadequate knowledge of active ingredient(s)
- Which component to follow / trace
- Toxicokinetics / toxicodynamics
- COST

EFFICACY

- Long years of usage
- Acceptability by people
- Same herb - several diseases
- Appropriate Animal model
- Appropriate artificial induction
- Definition of dysfunction
- Cost
- Holistic approach

Success Stories

- 25% of modern medicines are made from plants first used traditionally.
- Acupuncture has been proven effective in relieving postoperative pain, nausea during pregnancy, nausea and vomiting resulting from chemotherapy, and dental pain with extremely low side effects. It can also alleviate anxiety, panic disorders and insomnia.
- Yoga can reduce asthma attacks.

(WHO, Fact sheet N°134, Traditional medicine, 2003)

Success Stories

- Chinese herbal remedy *Artemisia annua*, used in China for almost 2000 years has been found to be effective against resistant malaria.
- In South Africa, the Medical Research Council is conducting studies on the efficacy of the plant Sutherlandia Microphylla in treating AIDS patients. Traditionally used as a tonic, this plant may increase energy, appetite and body mass in people living with HIV.

(WHO, Fact sheet N°134, Traditional medicine, 2003)

DIFFERENCE BETWEEN AYURVEDIC AND FOLK MEDICINES

Ayurvedic Medicines

- Plant / mineral and animal origin.
- Well laid down procedures.
- Claimed to enhances bioavalibility, reduces toxicity.

Folk Medicines

- Mostly herbal medicines used for household remedies.
- Do not have a similar systematic approach but traditionally used.
- It may not enhance bioavailibility or may not reduce toxicity.

In the United States of America

15 % growth in sale per year of herbal

remedies approximating a total of

1.5 billion dollars business.

“Much of what surrounds herbal medicine in the US is a minefield of hyperbole & hoax.”

Varro E. Tyler, Purdue University.

[Expert in plant medicine]