TOXICOLOGY

- Toxicology is a branch of science that deals with toxins, poisons, their effects and treatment.
- Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules.
- Toxicology uses the power of science to predict what, and how chemicals may cause harm and then shares that information to protect public health.

TOXICOLOGY

- A **toxic agent** is anything that can produce an adverse biological effect. It may be chemical, physical, or biological in form. For example, toxic agents may be chemical (such as cyanide), physical (such as radiation) and biological (such as snake venom).

TYPES OF TOXICANTS

- Toxic substances may be systemic toxins or organ toxins.
- A **systemic toxin** is one that affects the entire body or many organs rather than a specific site.
- A **organ toxin** is one that affects only specific tissues or organ

Main focus:

- Toxicologists mainly focus to get following important information about toxin:
- Detection of toxin
- Occurrence of toxin
- Properties of toxin
- Effects of toxin
- Treatment of toxin
- Toxin regulation

BRANCHES OF TOXICOLOGY:

There are various types of toxicology as outlined below:

- Analytical toxicology
- Applied toxicology
- Clinical toxicology
- Forensic toxicology
- Environment toxicology
- Reproductive and developmental toxicology
- Immuno-toxicology

TOXICOLOGY:

- **Analytical toxicology:** The branch of toxicology which deals with the study of detection and assay of poisonous chemicals including their metabolites that could affect the biological system.
- **Applied toxicology:** It is the application of new and modern methods or technologies for early detection of toxicants in the field setting or practice area.

TOXICOLOGY:

- **Clinical toxicology:** It is mainly involved in the study of diagnosis and treatment of poisoning that can occur in humans
- **Veterinary toxicology:** The study of diagnosis and treatment of animal poisoning including the transmission of toxin from animals to humans via milk, meat, fish, food stuff and etc

TOXICOLOGY:

- **Environmental toxicology:**
  The study of presence of different toxicants including their metabolites and degradation products in the environment and their effects on humans and animals.

- **Industrial toxicology:**
  It is the study of selective and specific are of environmental toxicology.

TOXICOLOGY:

- **IMMUNO TOXICOLOGY**
  It deals with the effect of toxicant on immune system.
- **REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY**
  It focus on the effect of chemicals or toxins on the reproductive system and the developing embryo.

TOXICOLOGY

- Mechanistic toxicology (basic biology and chemistry)

- Descriptive toxicology (testing)
- Regulatory toxicology (rule making and compliance)

MECHANISTIC TOXICOLOGY

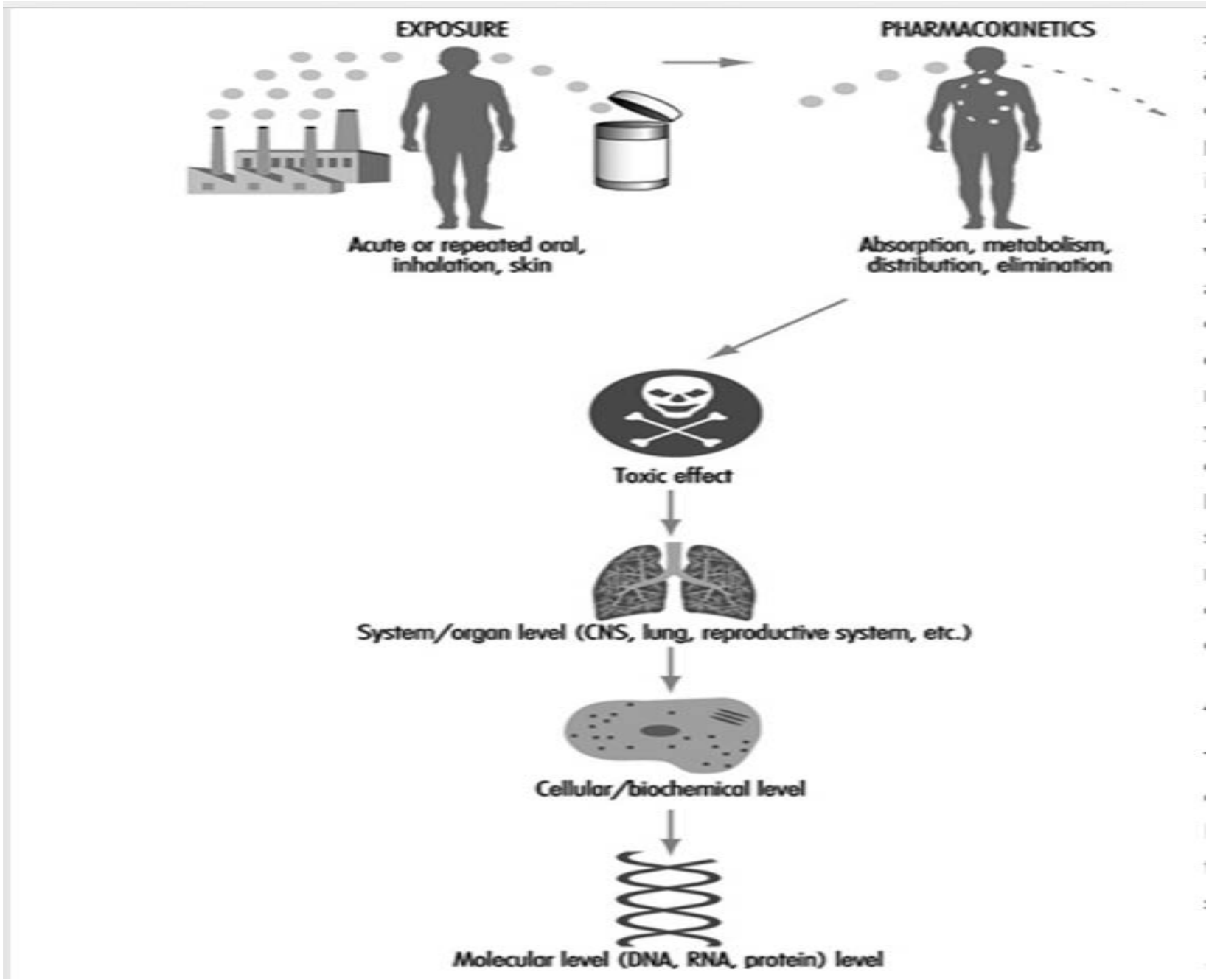
- **Mechanistic toxicology** is the study of how chemical or physical agents interact with living organisms to cause toxicity.
- Aim of mechanistic toxicology is to identify that how xenobiotics enter an organism and how these are distributed metabolize in the body.
- Knowledge of the mechanism of toxicity of a substance enhances the ability to prevent toxicity and design more desirable chemical

- Mechanistic understanding helps the governmental regulator to establish legally binding safe limits for human exposure.
- It is also useful in forming the basis for therapy and the design of new drugs for treatment of human disease.

TOXICOLOGY

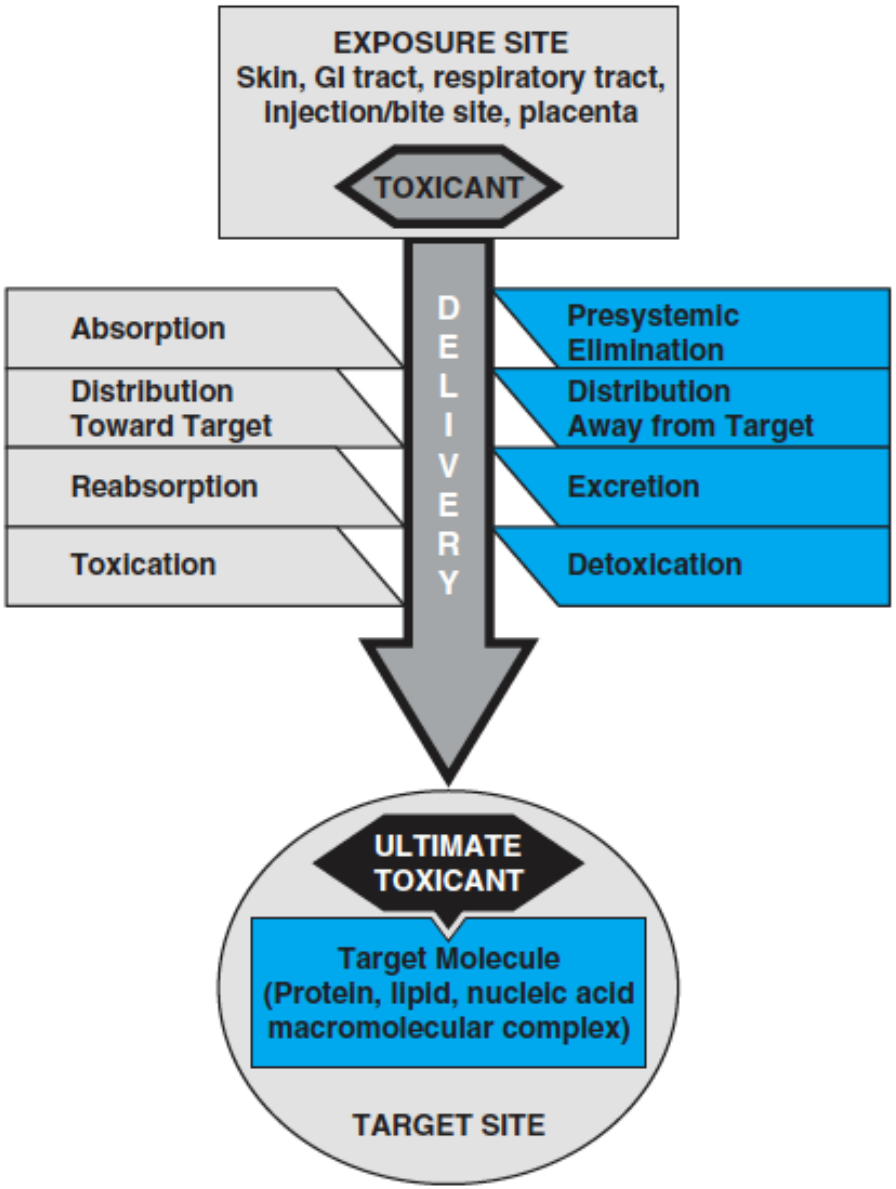
- **Toxicodynamics** refers to the molecular, biochemical, and physiological effects of toxicants or their metabolites in biological systems.

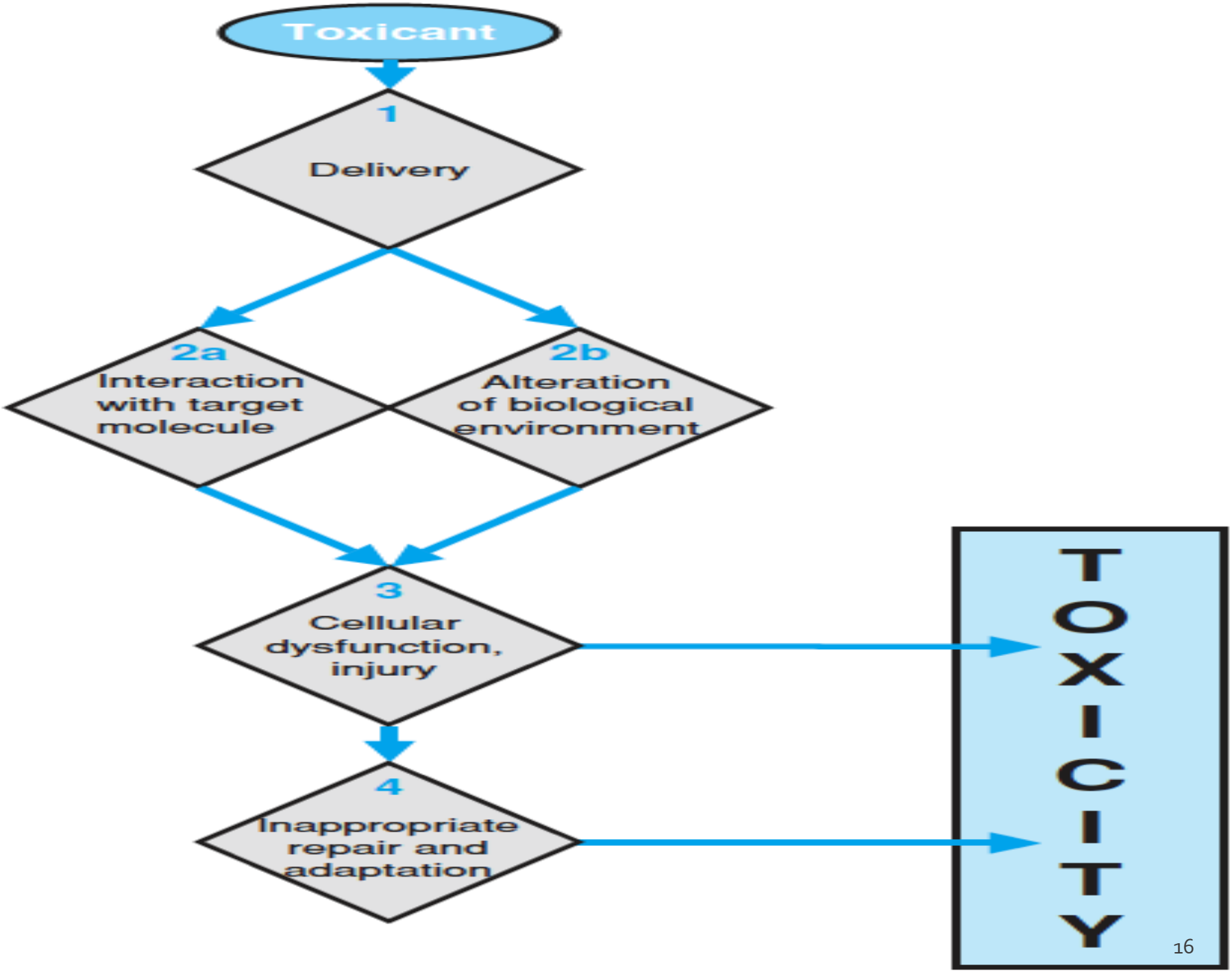
- **Toxicokinetics** is the quantitation of the time course of toxicants in the body during the processes of absorption, distribution, biotransformation, and excretion or clearance of toxicants.

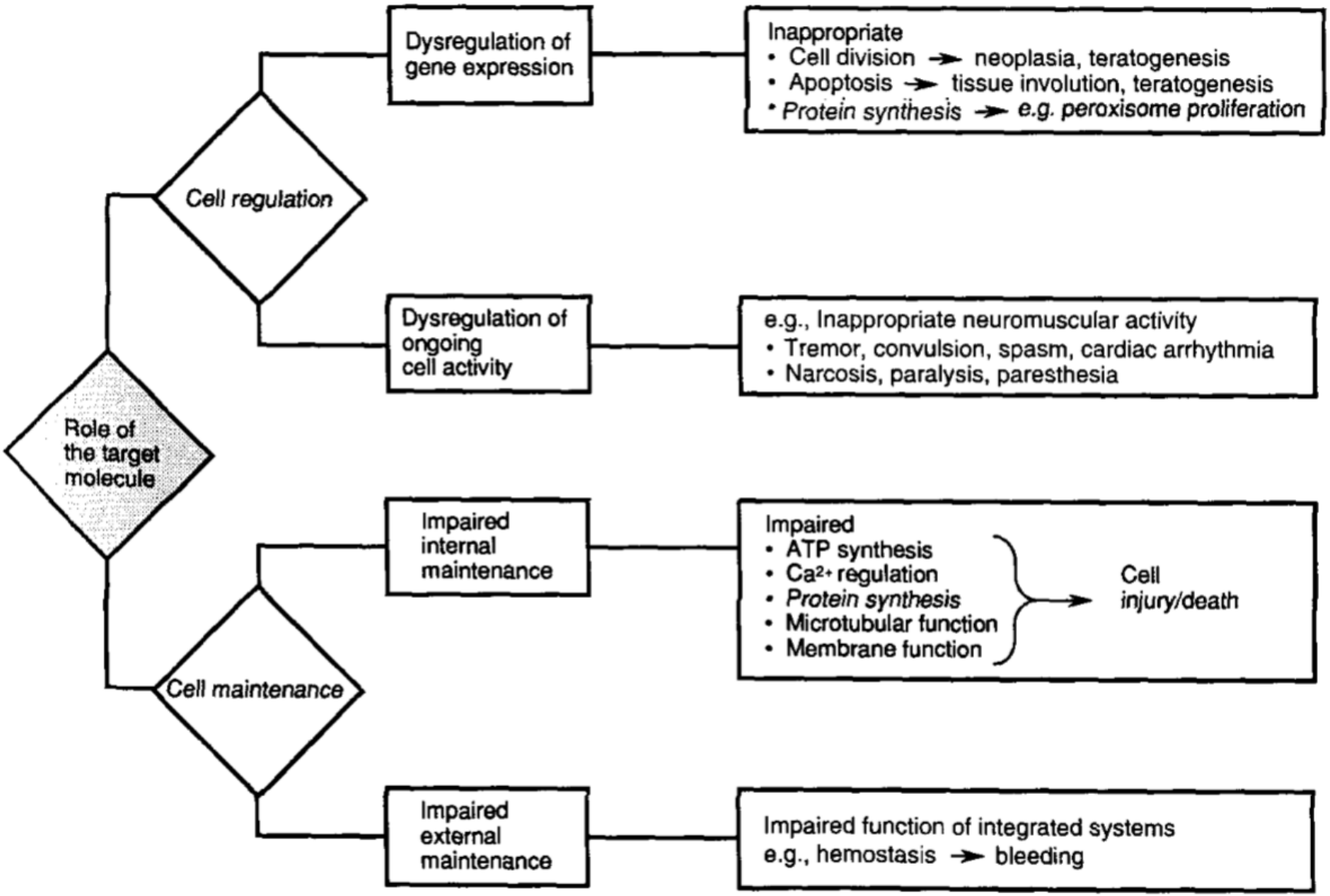

EXPOSURE
PHARMACOKINETICS
Acute or repeated oral, inhalation, skin
Absorption, metabolism, distribution, elimination
Toxic effect
System/organ level (CNS, lung, reproductive system, etc.)
Cellular/biochemical level
Molecular level (DNA, RNA, protein) level

Mechanisms of Toxicity

1. Delivery: Site of Exposure the Target
2. Reaction of the Ultimate Toxicant with the Target Molecule
3. Cellular Dysfunction and Resultant Toxicity
4. Repair or Dysrepair




Toxicant
1
Delivery
2a
Interaction with target molecule
2b
Alteration of biological environment
3
Cellular dysfunction, injury
4
Inappropriate repair and adaptation
TOXICITY


Role of the target molecule
Cell regulation
Dysregulation of gene expression
Inappropriate
• Cell division → neoplasia, teratogenesis
• Apoptosis → tissue involution, teratogenesis
• Protein synthesis → e.g. peroxisome proliferation
Dysregulation of ongoing cell activity
e.g., Inappropriate neuromuscular activity
• Tremor, convulsion, spasm, cardiac arrhythmia
• Narcosis, paralysis, paresthesia
Cell maintenance
Impaired internal maintenance
Impaired
• ATP synthesis
• Ca2+ regulation
• Protein synthesis
• Microtubular function
• Membrane function
Cell injury/death
Impaired external maintenance
Impaired function of integrated systems
e.g., hemostasis → bleeding

Chemical Factors that Cause Cellular Dysfunction

- **Chemicals that cause DNA adducts** can lead to DNA mutations which can activate cell death pathways; if mutations activate oncogenes or inactivate tumor suppressors, it can lead to uncontrolled cell proliferation and cancer (e.g. benzopyrene)
- **Chemicals that cause protein adducts** can lead to protein dysfunction which can activate cell death pathways; protein adducts can also lead to autoimmunity; if protein adducts activate oncogenes or inactivate tumor suppressors, it can lead to uncontrolled cell proliferation and cancer (e.g. diclofenac glucuronidation metabolite)
- **Chemicals that cause oxidative stress** can oxidize DNA or proteins leading to DNA mutations or protein dysfunction (e.g. benzene, CCl4)

- **Chemicals that specifically interact with protein targets**
- Chemicals that activate or inactivate ion channels can cause widespread cellular dysfunction and cause cell death and many physiological symptoms—Na+, Ca2+, K+
- levels are extremely important in neurotransmission, muscle contraction, and nearly every cellular function (e.g. tetrodotoxin closes voltage-gated Na+ channels)
- Chemicals that inhibit cellular respiration—inhibitors of proteins or enzymes involved in oxygen consumption, fuel utilization, and ATP production will cause energy depletion and cell death (e.g. cyanide inhibits cytochrome c oxidase)

- Chemicals that inhibit the production of cellular building blocks, e.g. nucleotides, lipids, amino acids (e.g. amanitin from Deathcap mushrooms)
- Chemicals that inhibit enzymatic processes of bioactive metabolites that alter ion channels and metabolism (e.g. sarin inhibits acetylcholinesterase and elevates acetylcholine levels to active signaling pathways and ion channels)

- **All of the above can also cause inflammation which can lead to cellular dysfunction**

Primary metabolic disorder which affect cell survival

I. **ATP depletion**

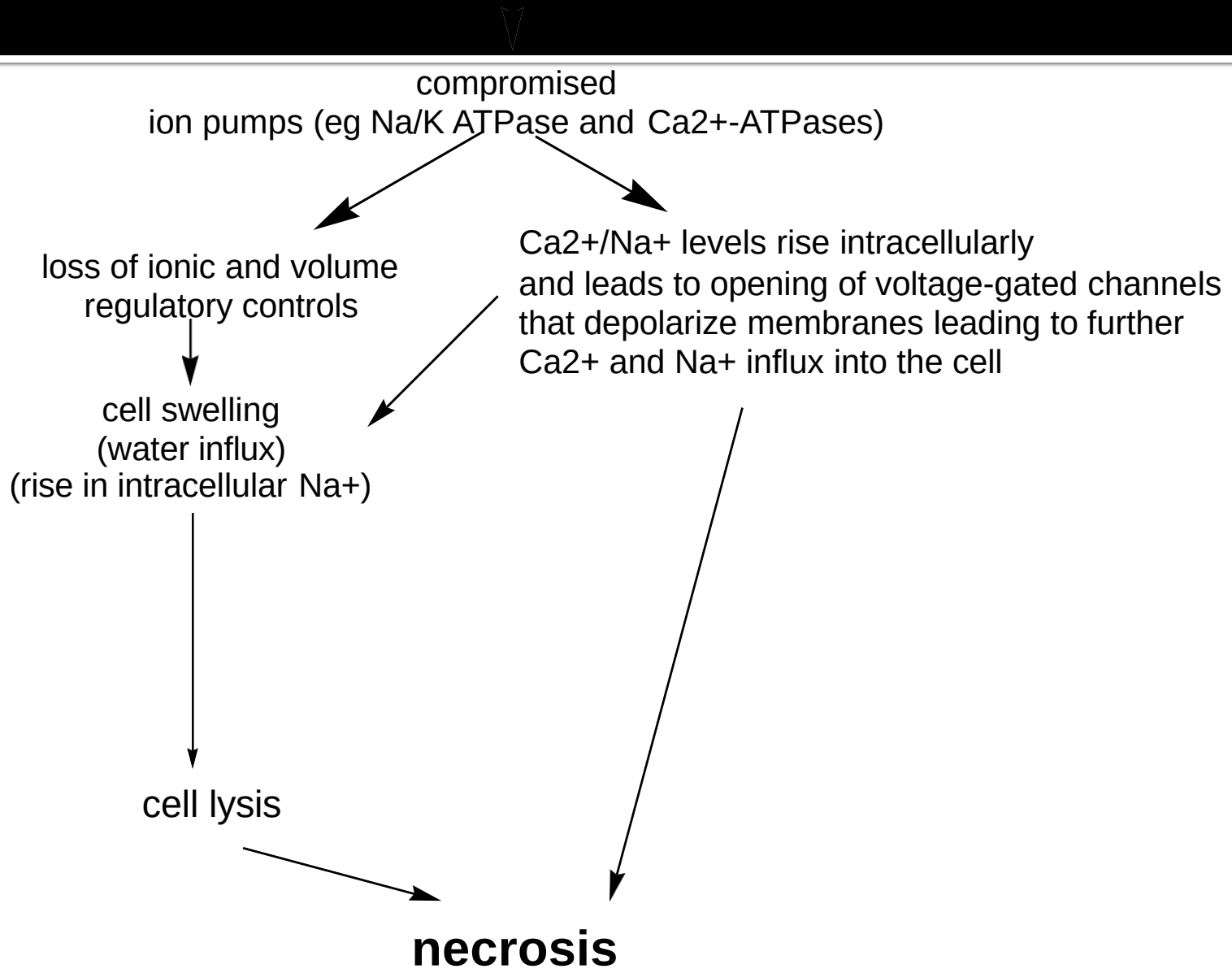
II. **Sustained rise in intracellular Ca^{2+}**

III. **Overproduction of ROS, RNS**

I. ATP Depletion

1. *ATP drives ion transporters such as Na^+/K^+-ATPase (plasma membrane), Ca^{2+} -ATPase (endoplasmic reticulum and plasma membrane) to maintain cellular ion gradients. (most important for necrosis!)*
2. *Used in biosynthetic reactions (phosphorylation and adenylation)*
3. *Used for signal transduction regulation (e.g. phosphorylation of receptor tyrosine kinase and kinase pathways)*
4. *Incorporated into DNA*
5. *Muscle contraction (actin/myosin interaction) and neurotransmission*
6. *Polymerization of cytoskeleton (actin and tubule polymerization)*
7. *Cell division*
8. *Maintenance of cell morphology*

Direct Consequences of ATP Depletion



II. Sustained Rise of Intracellular Ca^{2+}

Ca^{2+} is involved in :

1.signal transduction regulation (i.e. PKC activation by DAG and Ca^{2+}) and exocytosis

2.muscle contraction (actin/myosin interaction)

3.cytoskeletal polymerization (i.e. Ca^{2+} inhibition of actin)

4.neurotransmission (via glutamate receptor Ca^{2+} channel and voltage-gated Ca^{2+} channels) and synaptic plasticity

5.enzyme induction (i.e. citrate and α-ketoglutarate dehydrogenases from the TCA cycle)

6.Transporters (Ca^{2+}/ATPase, Na/Ca^{2+} exchanger, etc.)

Excitotoxicity: Consequence of Increased Intracellular Ca^{2+}

1. Depletion of energy reserves—decreased mitochondrial ATP production and increased loss of ATP by activation of Ca^{+2}-ATPase.
2. Dysfunction of microfilaments—impaired cell motility, disruption in cell morphology, cellular functions
3. Activation of hydrolytic enzymes—disintegration of membranes, proteins, DNA, etc.
4. Generation of ROS/RNS—disintegration of membranes, proteins, DNA, etc.

Consequences of ROS/RNS

1. ROS can directly oxidize and affect protein function and can mutate DNA leading to cellular dysfunction
2. ROS/RNS oxidatively inactivate Ca^{2+} /ATPases and elevate Ca^{2+}
3. ROS and RNS also drain ATP reserves:
    a. $NO^{\cdot}$ is a reversible inhibitor of cytochrome oxidase
    b. $ONOO^{-}$ irreversibly inactivates complexes I/II/III and aconitase
    c. ROS can disrupt mitochondrial membranes and dissipate the electrochemical gradient needed for ATP synthase.
4. $ONOO^{-}$ induces DNA single-strand breaks, which activates poly(ADP-ribose) polymerase (PARP)—PARP transfers ADP-ribose moieties from NAD^{+} to PARP; consumption of NAD^{+} compromises ATP synthesis
5. Lipid peroxidation, cell swelling, and cell rupture

Acute Toxicity Mechanisms

- Simple asphyxiants
- Chemical asphyxiants
- Central nervous system (CNS) depressants
- Skin effects
- Lung sensitization
- Eye effects.

Sub chronic and Chronic Toxicity Mechanisms

- Effect of toxicant on enzyme (anticholinestrase inhibition).
- Interaction with proteins.
- Metabolic activation.
- Effect of toxicant on receptor and ion channel
- Effect on lipids and nucleic acid.
- Cancer mechanism.
- Reproductive mechanisms.

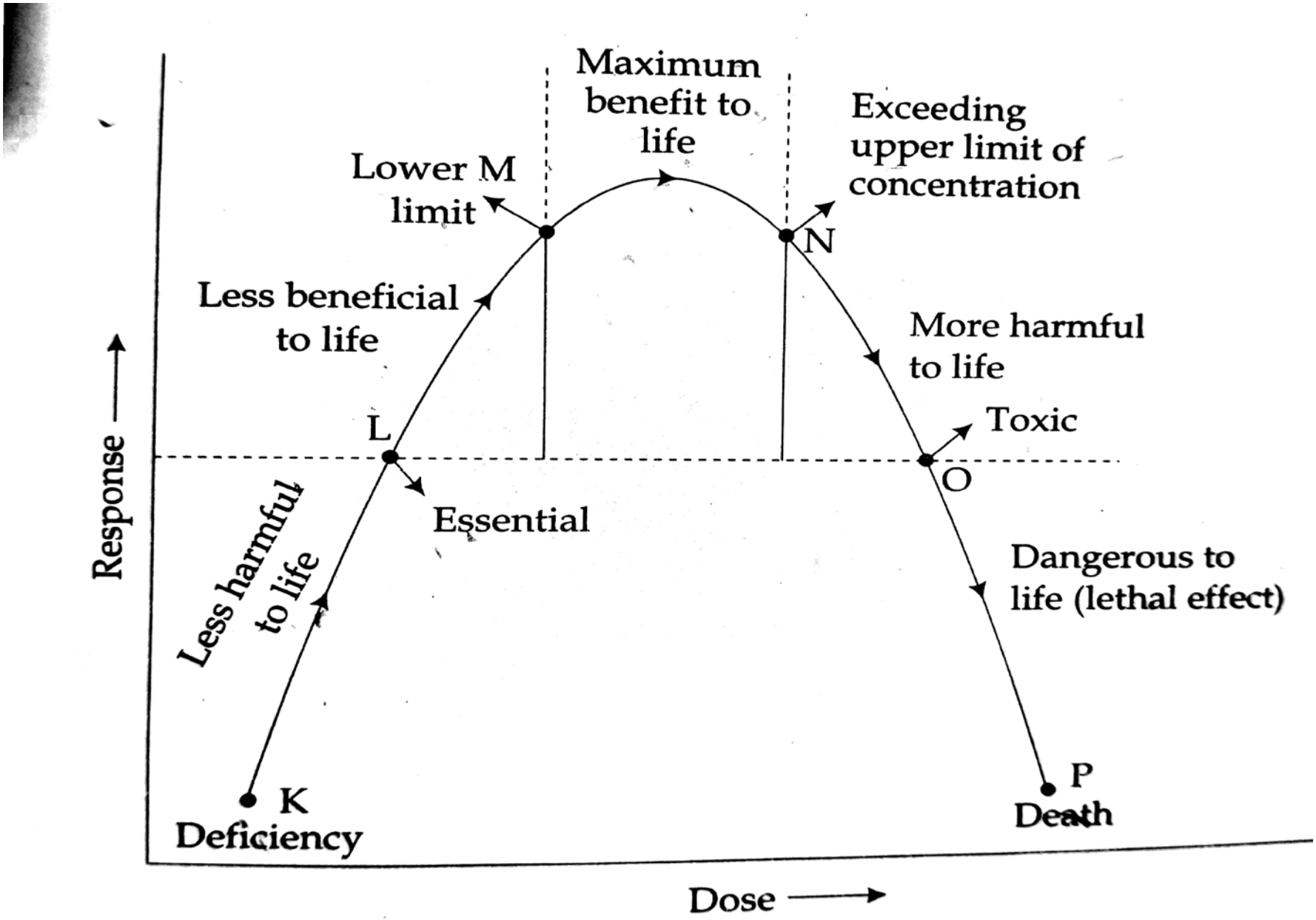
DESCRIPTIVE TOXICOLOGY

- Focuses on toxicity testing of chemicals or agents of toxicity, usually on animals and then correlated to human conditions. It provides dose-response information upon exposure to a harmful toxic agent.
- The toxicity assessment commonly involves following steps:
  1. Hazard identification
  2. Dose-response assessment
  3. Exposure assessment
  4. Risk characterization

HAZARD IDENTIFICATION

- Determines the exposure to chemical can increase the incidents of a particular adverse health effect and determines the likelihood of occurrence in human
- It is done by:
  - Hazard identification Data
  - Human epidemiology data
  - Animal bioassay (measurement of the concentration or potency of a substance by its effect on living cells or tissues.)

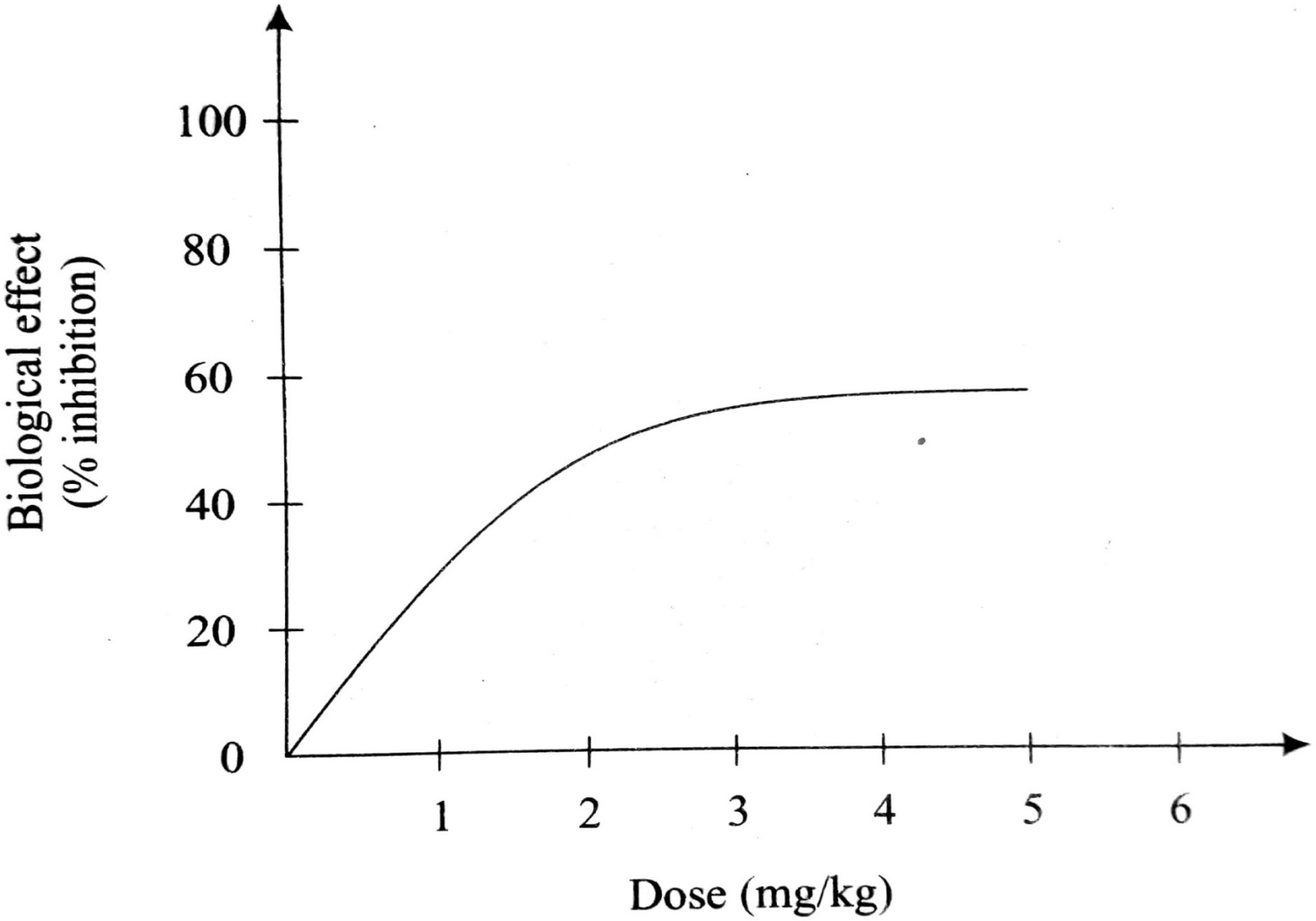
Dose response concept



Dose response relationship

- There are two types of dose response relationship.
- **Graded dose response relationship**
  It describe the response of an individual organism to varying dose of chemical
- **Quantal dose response relationship**
  characterizes of the distribution of intensity of the effect to different doses in a population of individual organisms.

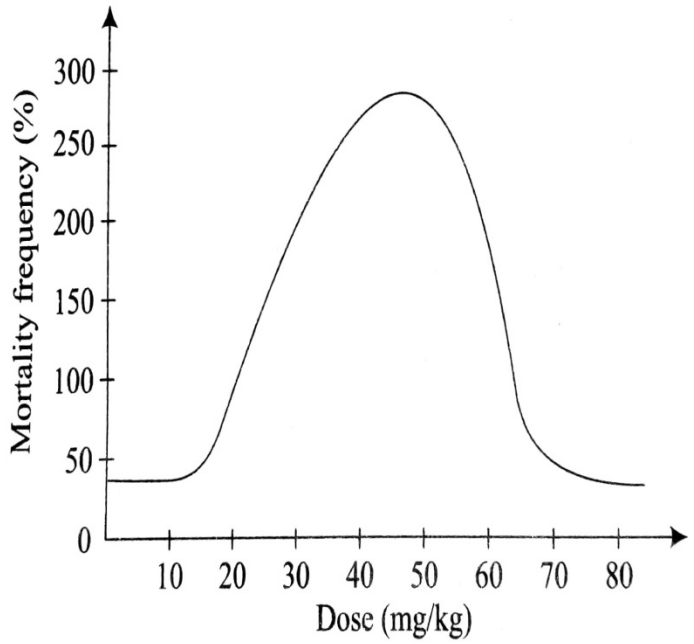
Graded dose response relationship

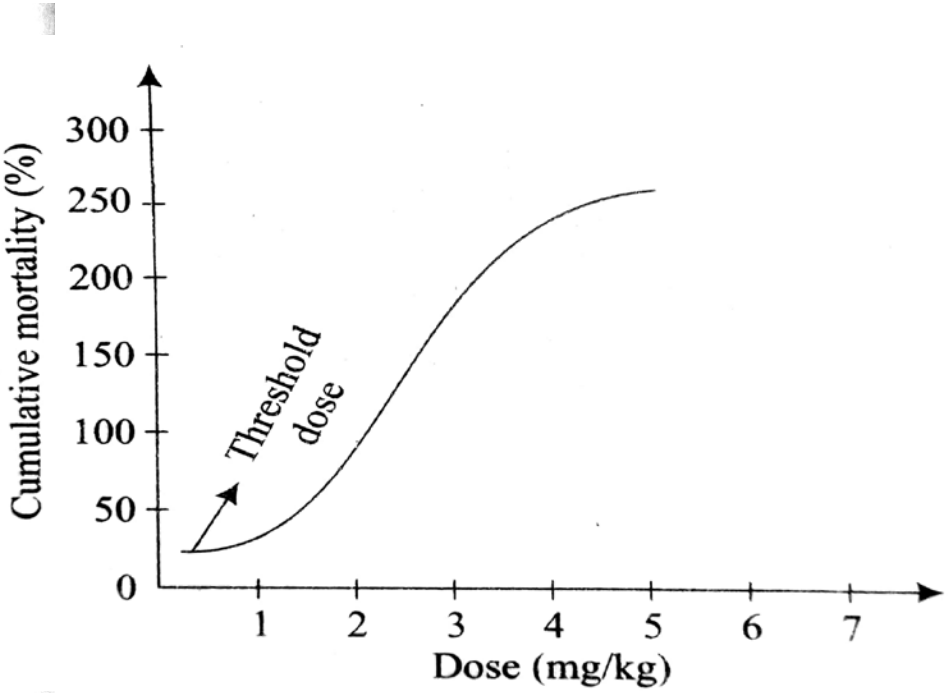


Quantal dose response relationship

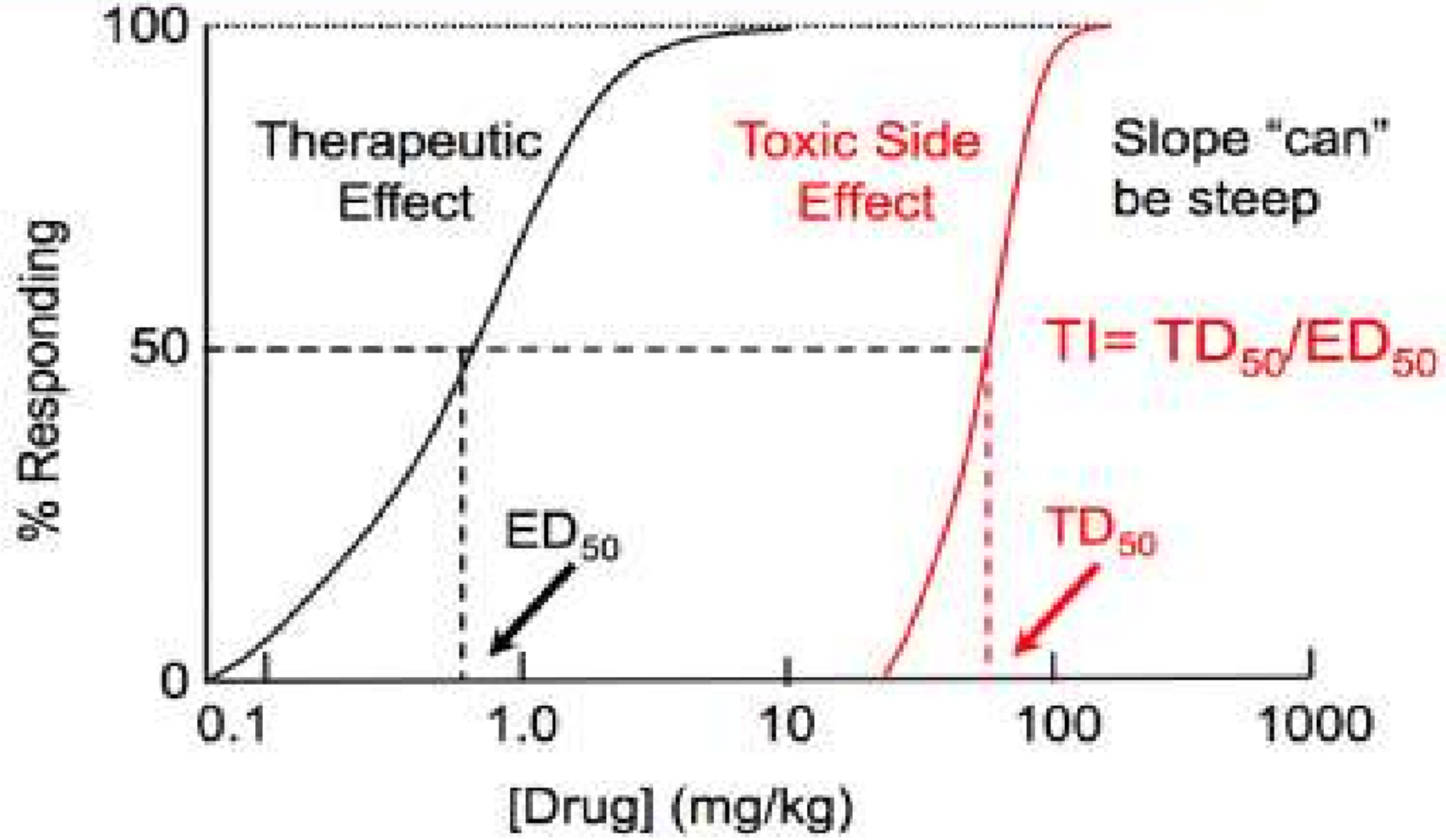
Two types-

1. Frequency quantal dose response relationship
2. Cumulative quantal dose response relationship





Drug Safety - Therapeutic Index



EXPOSURE ASSESMENT

- Exposed population (General public or selected groups)
- Types of substances(pharmaceuticals occupational chemicals or environmental pollutants)
- Single substance or mixture of substances
- Duration of exposure
- Pathways and media

Toxicology testing

- Acute
- Sub acute
- Chronic

IMPORTANCE OF TOXICITY TESTING

- The data on acute toxicity test for various chemicals against various organisms may be valuable in following ways:
- To have an idea of toxic doses of xenobiotics for certain organisms.
- Fixation of sub lethal doses for long term toxicity test.
- Evolution of safe doses of those toxicants for certain organisms.
- Recommendation of maximum permissible limits of those substances in the ambient air and drinking water.

REGULATORY TOXICOLOGY

- It deals with the relationship between the discipline of toxicology and regulatory institutions.
- The regulatory authorities have to protect the health of humans which relies on toxicological principal and toxicity evaluation data to formulate a decision.

MAIN FOCUS-

- The authority has to take a decision on Acceptable Daily Intake (ADI) of a chemical so that quantity of that chemical exposure is adjusted **safe** in terms of health.
- The authority also have the power to formulate some law or regulatory roles and to implement them rigidly.

- Principle of toxicology says **no chemical is safe** all chemicals are potentially toxic depending upon their exposure, concentration , time, frequency and nature.

- Regulators formulate the **threshold doses** to reduce exposure concentration so that risks can be minimized to highest level.

Regulatory authorities

- WHO
- ICH
- EPA
- OECD
- FDA

Introduction:

- One of the most important milestones in product development is the decision to enter into clinical trials with a candidate product. This important decision is based, in part, on data produced during non-clinical safety testing of the candidate during the preclinical phases of development.
- The quality and reproducibility of safety data are hereby key components of their utility for supporting the assumption of safety in humans.

International Guidelines:

- **ICH & WHO:** International Conference on Harmonisation & World Health Organization

ICH & WHO has produced a comprehensive set of safety Guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability(most important reason now a days to withdraw drug from market).

CARCINOGENICITY STUDIES

- **S1A: Need for Carcinogenicity Studies of Pharmaceuticals**

This document provides a consistent definition of the circumstances under which it is necessary to undertake carcinogenicity studies on new drugs. These recommendations take into account the known risk factors as well as the intended indications and duration of exposure.

Cont…

- **S1B: Testing for Carcinogenicity of Pharmaceuticals**

This document provides guidance on the need to carry out carcinogenicity studies in both mice and rats.

- **S1C(R2): Dose Selection for Carcinogenicity Studies of Pharmaceuticals**

This document addresses the criteria for the selection of the high dose to be used in carcinogenicity studies on new therapeutic agents to harmonize current practices and improve the design of studies.

Cont….

GENOTOXICITY STUDIES

- **S2(R1): Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use S2A, S2B**

- **S2A: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals**

This document provided specific guidance and recommendations for in vitro and in vivo tests and on the evaluation of test results.

- **S2B: Genotoxicity: A Standard Battery for Genotoxicity Testing for Pharmaceuticals**

This document addressed two fundamental areas of genotoxicity testing: the identification of a standard set of assays to be conducted for registration, and the extent of confirmatory experimentation in any particular genotoxicity assay in the standard battery.

Cont…

TOXICOKINETICS AND PHARMACOKINETICS

- **S3A: Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies**

This document gives guidance on developing test strategies in toxicokinetics and the need to

integrate pharmacokinetics into toxicity testing.

- **S3B: Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies**

This document gives guidance on circumstances when repeated dose tissue distribution studies

should be considered (i.e., when appropriate data cannot be derived from other sources).

Cont…

TOXICITY TESTING

- **S4: Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing)**

The text incorporates the guidance for repeat-dose toxicity tests that was agreed at the time of ICH 1, in 1991 (reduction of the duration of repeat dose toxicity studies in the rat from 12 to 6 months).

Cont…

REPRODUCTIVE TOXICOLOGY

- **S5(R2): Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility S5A,S5B(M)**

This document provides guidance on tests for reproductive toxicity. It defines the periods of treatment to be used in animals to better reflect human exposure to medical products and allow more specific identification of stages at risk.

Cont…

BIOTECHNOLOGICAL PRODUCTS

- **S6(R1): Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals**

This document covers the pre-clinical safety testing requirements for biotechnological products.

Cont…

PHARMACOLOGY STUDIES

- **S7A: Safety Pharmacology Studies for Human Pharmaceuticals**

This document addresses the definition, objectives and scope of safety pharmacology studies.

- **S7B: The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human**

This Guideline describes a non-clinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization.

Cont…

IMMUNOTOXICOLOGY STUDIES

- **S8: Immunotoxicity Studies for Human**

This Guideline addresses the recommendations on nonclinical testing for immunosuppression

induced by low molecular weight drugs (non-biologicals).

Cont…

NONCLINICAL EVALUATION FOR ANTICANCER PHARMACEUTICALS

- **S9: Nonclinical Evaluation for Anticancer**

This Guideline provides information for pharmaceuticals that are only intended to treat cancer in patients with late stage or advanced disease regardless of the route of administration, including both small molecule and biotechnology-derived pharmaceuticals.

PHOTOSAFETY EVALUATION

- **S10: Photosafety Evaluation of Pharmaceuticals (Draft Document)**

The S10 draft Guideline has been released for consultation under Step 2 of the ICH process in November 2012.

OECD

- **OECD:** Organisation for Economic Co-operation and Development

The mission of the OECD is to promote policies that will improve the economic and social well- being of people around the world.

The OECD provides a forum in which governments can work together to share experiences and seek solutions to common problems.

GUIDELINES:

- 402 Acute Dermal Toxicity
- 403 Acute Inhalation Toxicity
- 404 Acute Dermal Irritation/Corrosion
- 405 Acute Eye Irritation/Corrosion

Cont….

- 406 Skin Sensitisation
- 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
- 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents
- 409 Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents
- 410 Repeated Dose Dermal Toxicity: 21/28-day Study
- 411 Subchronic Dermal Toxicity: 90-day Study
- 412 Repeated Dose Inhalation Toxicity: 28-day or 14-day Study
- 413 Subchronic Inhalation Toxicity: 90-day Study
- 414 Prenatal Developmental Toxicity Study
- 415 One-Generation Reproduction Toxicity Study

Cont….

- 416 Two-Generation Reproduction Toxicity Study
- 417 Toxicokinetics
- 420 Acute Oral Toxicity - Fixed Dose Method
- 421 Reproduction/Developmental Toxicity Screening Test
- 422 Combined Repeated Dose Toxicity Study with the Reproduction
- 423 Acute Oral toxicity - Acute Toxic Class Method
- 424 Neurotoxicity Study in Rodents
- 425 Acute Oral Toxicity: Up-and-Down Procedure
- 451 Carcinogenicity Studies
- 452 Chronic Toxicity Studies
- 453 Combined Chronic Toxicity/Carcinogenicity Studies

Cont….

- 471 Bacterial Reverse Mutation Test
- 473 *In vitro* Mammalian Chromosomal Aberration Test
- 474 Mammalian Erythrocyte Micronucleus Test
- 475 Mammalian Bone Marrow Chromosomal Aberration Test
- 476 *In vitro* Mammalian Cell Gene Mutation Test
- 477 Genetic Toxicology: Sex-Linked Recessive Lethal Test in Drosophila melanogaster
- 478 Genetic Toxicology: Rodent Dominant Lethal Test
- 479 Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells
- 480 Genetic Toxicology: *Saccharomyces cerevisiae*, Gene Mutation Assay
- 481 Genetic Toxicology: *Saccharomyces cerevisiae*, Mitotic Recombination Assay
- 482 Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *in vitro*

Cont….

- 483 Mammalian Spermatogonial Chromosome Aberration Test
- 484 Genetic Toxicology: Mouse Spot Test
- 485 Genetic Toxicology: Mouse Heritable Translocation Assay
- 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

Cont….

DRAFT GUIDELINES

- 403 Acute Inhalation Toxicity
- 404 Acute Dermal Irritation/Corrosion
- 405 Acute Eye Irritation/Corrosion
- 426 Developmental Neurotoxicity Study
- 429 Skin Sensitisation: Local Lymph Node Assay
- 430 *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)
- 431 *In Vitro* Skin Corrosion: Human Skin Model Test
- 432 *In Vitro* 3T3 NRU Phototoxicity Test

FDA

- **FDA:** Food and Drug Administration

FDA is responsible for protecting the public health by assuring the safety, efficacy and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation.

FDA is also responsible for advancing the public health by helping to speed innovations that make medicines more effective, safer, and more affordable and by helping the public get the accurate, science-based information they need to use medicines and foods to maintain and improve their health. FDA also has responsibility for regulating the manufacturing, marketing and distribution of tobacco products to protect the public health and to reduce tobacco use by minors.

Cont….

- **Guidance Documents:**
  - Content and Format of INDs(Investigational New Drugs) for Phase 1 Studies
  - Single Dose Acute Toxicity Testing for Pharmaceuticals
  - Product Specific guidance
    - anti-virals
    - vaginal contraceptives and STD preventatives
  - Special Protocol Assessment
  - Submission in Electronic Format

Cont….

- **Draft Guidances:**
  - Carcinogenicity study protocols
  - Immunotoxicology
  - Photosafety testing
  - Statistical evaluation of carcinogenicity studies

Cont…

Types of Toxicology Studies Recommended

- General Toxicology
  - acute and repeat dose toxicology studies
- Special Toxicology Studies
  - local irritation studies, e.g., site specific, ocular
  - hypersensitivity studies for inhalation and dermal drug products
- Reproductive and Developmental Toxicology Studies
  - male and female fertility
  - embryonic and fetal development
  - post-natal reproductive and developmental effects

EPA Guidelines

- Acute toxicity test guidelines (Group A)
- Subchronic toxicity test guidelines (Group B)
- Chronic toxicity test guidelines (Group C)
- Genetic toxicity test guidelines (Group D)
- Neurotoxicity test guidelines (Group E)
- Special study test guidelines (Group F)
- Health effect chemical specific test guideline (Group G)
- Supplemental guidance.

Acute toxicity test guidelines

- 870.1000 - Acute Toxicity Testing--Background (December 2002)
- 870.1100 - Acute Oral Toxicity (December 2002)
- 870.1200 - Acute Dermal Toxicity (August 1998)
- 870.1300 - Acute Inhalation Toxicity (August 1998)
- 870.2400 - Acute Eye Irritation (August 1998)
- 870.2500 - Acute Dermal Irritation (August 1998)
- 870.2600 - Skin Sensitization (March 2003)

Subchronic toxicity test guidelines

870.3050 - Repeated Dose 28-Day Oral Toxicity Study in Rodents (July 2000)
870.3100 - 90-Day Oral Toxicity in Rodents (August 1998)
870.3150 - 90-Day Oral Toxicity in Nonrodents (August 1998)
870.3200 - 21/28-Day Dermal Toxicity (August 1998)
870.3250 - 90-Day Dermal Toxicity (August 1998
870.3465 - 90-Day Inhalation Toxicity (August 1998)
870.3550 - Reproduction/Developmental Toxicity Screening Test (July 2000)

870.3650 - Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (July 2000)

870.3700 - Prenatal Developmental Toxicity Study (August 1998)
870.3800 - Reproduction and Fertility Effects (August 1998)

Chronic toxicity test guidelies

- 870.4100 - Chronic Toxicity (August 1998)
- 870.4200 - Carcinogenicity (August 1998)
- 870.4300 - Combined Chronic Toxicity/Carcinogenicity (August 1998)

Genetic toxicity test

- 870.5100 - Bacterial Reverse Mutation Test (August 1998)
- 870.5195 - Mouse Biochemical Specific Locus Test (August 1998)
- 870.5200 - Mouse Visible Specific Locus Test (August 1998)
- 870.5250 - Gene Mutation in *Neurospora crassa* (August 1998)
- 870.5275 - Sex-linked Recessive Lethal Test in *Drosophila melanogaster* (August 1998)
- 870.5300 - *In vitro* Mammalian Cell Gene Mutation Test (August 1998)
- 870.5375 - *In vitro* Mammalian Chromosome Aberration Test (August 1998)
- 870.5380 - Mammalian Spermatogonial Chromosomal Aberration Test (August 1998))
- 870.5385 - Mammalian Bone Marrow Chromosomal Aberration Test (August 1998)
- 870.5395 - Mammalian Erythrocyte Micronucleus Test (August 1998)
- 870.5450 - Rodent Dominant Lethal Assay (August 1998)
- 870.5460 - Rodent Heritable Translocation Assays (August 1998)
- 870.5500 - Bacterial DNA Damage or Repair Tests (August 1998)
- 870.5550 - Unscheduled DNA Synthesis in Mammalian Cells in Culture (August 1998)
- 870.5575 - Mitotic Gene Conversion in *Saccharomyces cerevisiae* (August 1998)
- 870.5900 - *In vitro* Sister Chromatid Exchange Assay (August 1998)
- 870.5915 - *In vivo* Sister Chromatid Exchange Assay (August 1998)

Neurotoxicity test guidelines

- 870.6100 - Acute and 28-Day Delayed Neurotoxicity of Organophosphorus Substances (August 1998)
- 870.6200 - Neurotoxicity Screening Battery (August 1998)
- 870.6300 - Developmental Neurotoxicity Study (August 1998)
- 870.6500 - Schedule-Controlled Operant Behavior (August 1998)
- 870.6850 - Peripheral Nerve Function (August 1998)
- 870.6855 - Neurophysiology Sensory Evoked Potentials (August 1998)

Health effect chemical specific test guideline

- 870.7200 - Companion Animal Safety (August 1998)
- 870.7485 - Metabolism and Pharmacokinetics (August 1998)
- 870.7600 - Dermal Penetration (August 1998)
- 870.7800 - Immunotoxicity (August 1998)

- 870.8355 - Combined Chronic Toxicity /Carcinogenicity Testing of Respirable Fibrous Particles (July 2001)

OECD PRINCIPLES ON GLP

- Good Laboratory Practice (GLP) is a quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported

HIGHLIGHTS

- Test facility organisation and personnel
- Quality assurance programme
- Facilities
- Apparatus, material, reagent
- Test systems
- Test and reference items
- Standard operating procedures
- Performance of the study
- Reporting of the study results
- Storage and retention of records and materials

Test facility organisation and personnel

- Test facilities management responsibilities.
- Study Director responsibilities.
- **Principal Investigator's Responsibilities**
  Will ensure that the delegated phases of the study are conducted in accordance with the applicable Principles of Good Laboratory Practice
- Study Personnel's Responsibilities.

Responsibilities of test facility management

- Ensure that a sufficient number of qualified personnel, appropriate facilities, equipment, and materials are available
- Maintenance of a record of the qualifications, training, experience and job description for each professional and technical individual
- Appropriate and technically valid Standard Operating Procedures are established and followed
- For each study an individual with the appropriate qualifications, training, and experience is designated by the management as the Study Director before the study is initiated.
- Ensure, in the event of a multi-site study, that, if needed, a Principal Investigator is designated, who is appropriately trained, qualified and experienced to supervise the delegated phase(s) of the study.
- Ensure documented approval of the study plan by the Study Director
- Ensure that test facility supplies meet requirements appropriate to their use in a study
- Ensure for a multi-site study that clear lines of communication exist between the Study Director, Principal Investigator(s), the Quality Assurance Programme(s) and study personnel
- Ensure that computerised systems are suitable for their intended purpose, and are validated, operated and maintained in accordance with these Principles of Good Laboratory Practice

Study Director responsiblities

- The Study Director is the single point of study control and has the responsibility for the overall conduct of the study and for its final report.
- **The responsibility should include**
- approve the study plan and any amendments to the study plan by dated signature
- Ensure that the Quality Assurance personnel have a copy of the study plan
- Ensure that study plans and amendments and Standard Operating Procedures are available to study personnel;
- Ensure that the study plan and the final report for a multi-site study identify and define the role of any Principal Investigator(s) and any test facilities and test sites involved in the conduct of the study;
- Ensure that the procedures specified in the study plan are followed, and assess and document the impact of any deviations from the study plan on the quality and integrity of the study, and take appropriate corrective action if necessary.
- Ensure that all raw data generated are fully documented and recorded
- Ensure that computerised systems used in the study have been validated
- Sign and date the final report to indicate acceptance of responsibility for the validity of the data and to indicate the extent to which the study complies with these Principles of Good Laboratory Practice
- Ensure that after completion (including termination) of the study, the study plan, the final report, raw data and supporting material are archived.

Study personnels responsibilities

- All personnel involved in the conduct of the study must be knowledgeable in those parts of the Principles of Good Laboratory Practice which are applicable to their involvement in the study.
- Study personnel will have access to the study plan and appropriate Standard Operating Procedures applicable to their involvement in the study.
- All study personnel are responsible for recording raw data promptly and accurately and in compliance with these Principles of Good Laboratory Practice, and are responsible for the quality of their data.

Quality assurance programme

- The test facility should have a documented Quality Assurance Programme to assure that studies performed are in compliance with these Principles of Good Laboratory Practice
- The Quality Assurance Programme should be carried out by individuals designated by and directly responsible to management and who are familiar with the test procedures
- **Responsibilities of the Quality Assurance Personnel-**
- Maintain copies of all approved study plans and Standard Operating Procedures in use in the test facility
- Verify that the study plan contains the information required for compliance with these Principles of Good Laboratory Practice. This verification should be documented
- **Conduct inspections** - Study-based inspections, - Facility-based inspections, - Process-based inspections
- Inspect the final reports to confirm that the methods, procedures, and observations are accurately and completely described
- prepare and sign a statement, to be included with the final report, which specifies types of inspections and their dates, including the phase(s) of the study inspected, and the dates inspection results were reported to management and the Study Director and Principal Investigator(s)

Facilities

- The test facility should be of suitable size, construction and location to meet the requirements of the study and to minimise disturbance that would interfere with the validity of the study
- Test System Facilities
- Facilities for Handling Test and Reference Items
- Archive Facilities
- Waste Disposal

Apparatus, Material, and Reagents

- Apparatus, including validated computerised systems, used for the generation, storage and retrieval of data
- Apparatus used in a study should be periodically inspected, cleaned, maintained, and calibrated according to Standard Operating Procedures.
- Chemicals, reagents, and solutions should be labelled to indicate identity (with concentration if appropriate), expiry date and specific storage instructions. Information concerning source, preparation date and stability should be available.

Test Systems

Physical/Chemical

- Apparatus used for the generation of physical/chemical data should be suitably located and of appropriate design and adequate capacity.

Biological

- Proper conditions should be established and maintained for the storage, housing, handling and care of biological test systems, in order to ensure the quality of the data.
- Newly received animal and plant test systems should be isolated until their health status has been evaluated
- Animals should be acclimatised to the test environment for an adequate period.
- During use, housing or containers for test systems should be cleaned and sanitised at appropriate intervals. Any material that comes into contact with the test system should be free of contaminants.
- Bedding should be changed at regular interval.

Test and Reference Items

- Receipt, Handling, Sampling and Storage
- Each test and reference item should be appropriately identified (e.g., code, Chemical Abstracts Service Registry Number [CAS number], name, biological parameters).
- Purity , composition, concentration.

Standard Operating Procedures

- SOP must exist which ensure the quality and integrity of the data generated by that test facility.
- SOP must be available at performing site
- Deviations from SOP should be documented and acknowledged by the Study Director and the Principal Investigator(s), as applicable.
- **Standard Operating Procedures should be available for, but not be limited to, the following categories of test facility activities.**
- Test and Reference Items
- Apparatus, Materials and Reagents
- Record Keeping, Reporting, Storage, and Retrieval ,Test System
- Quality Assurance Procedures

Performance of the Study

- Study Plan
- **Content of the Study Plan**
  Identification of the Study, the Test Item and Reference Item
  Information Concerning the Sponsor and the Test Facility , Dates, Test Methods ,Issues, Records
- **Conduct of the Study**

1. A unique identification should be given to each study The study should be conducted in accordance with the study plan.

2. Data should be recorded directly, promptly, accurately.

3. Data generated as a direct computer input should be identified at the time of data input by the individual(s) responsible for direct data entries

Reporting of Study Results

- A final report should be prepared for each study. In the case of short term studies, a standardized final report accompanied by a study specific extension may be prepared.
- Final report should be signed by Study director, principle investigator

Content of the Final Report

- Identification of the Study, the Test Item and Reference Item
- Information Concerning the Sponsor and the Test Facility
- Dates
- Statement
- Description of Materials and Test Methods
- Results
- Storage

REFERENCES

- PANI B. Textbook of Toxicology, first edition published by international publishing house Pvt. Ltd.
- STINE.E.K Principles of toxicology, second edition CRC Press is an imprint of Taylor & Francis Group.
- http://www.iloencyclopaedia.org/part-iv-66769/toxicology-57951/mechanisms-of-toxicity/mechanisms/introduction-and-concepts?tmpl=component&print
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Thank you