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NON-CLINICAL STUDIES :

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Abstract
Nonclinical studies play a crucial role in the drug development pathway, providing essential insights into the safety and potential effectiveness of drugs, biologics, and devices. These studies, which include both in vitro and in vivo experiments, are conducted in compliance with Good Laboratory Practices (GLP) and non-GLP standards, and are designed to define the relevant efficacy and/or safety characteristics of the products under development. Nonclinical development experts, such as those at ProPharma Group, provide critical guidance to sponsors, helping to ensure that nonclinical studies are appropriately designed and conducted to support the intended indication, route of administration, and duration of treatment.
Nonclinical studies play a crucial role in the development of drugs, biologics, and devices by providing valuable insights into their safety profile and potential effectiveness. Conducting nonclinical evaluations throughout the product development process enables informed decision-making, risk assessment, and enhances confidence in the ultimate success of the clinical evaluations and regulatory approval. Nonclinical studies include pharmacology, pharmacokinetic, and toxicology programs that are appropriate to support the intended indication, route of administration and duration of treatment for the product. The nonclinical development program can help assure that time and resources are not spent on studies that fail to meet regulatory expectations.

INTRODUCTION:

Active pharmaceutical constituents(APIs) are the active factors in a pharmaceutical medicine that produce the required effect on the body to treat a condition. APIs are produced by recycling chemical composites. In natural medicine, the active component is known as a bulk process intermediate(BPI).
An active component is a component in a pharmaceutical medicine or fungicide that's biologically active. The analogous terms active pharmaceutical component and bulk active are also used in drugs, and the term active substance may be used for natural products.
Active medicinal constituents(APIs) are chemical-grounded composites that are produced substantially in the countries the USA, Europe, China, and India. APIs have pharmacological exertion substantially used with the combination of other constituents to diagnose, cure, alleviate, and treat the complaint.
These APIs can be natural or synthetic chemical-grounded active composites that are generally set up in remedial and veterinary medicines. There are chemical-grounded active composites produced using unsafe chemical routes.

- **Efficacy and Therapeutic Effectiveness:**
  Active Pharmaceutical Ingredients (APIs) are vital for the effectiveness of pharmaceutical drugs. The quality and purity of APIs have a significant impact on the efficacy of pharmaceutical drugs and the health outcomes of patients. [2]
- **Quality and Safety Assurance:**
  Ensuring quality and safety of Activated Pharmaceutical Ingredients is essential to uphold high standards and guarantee product safety and effectiveness in the pharmaceutical industry. [2]
- **Dosage Precision:**
  The precision of dosage administration is of utmost importance, as (API) Active Pharmaceutical Ingredients significantly influence the determination of the optimal therapeutic dosage. The precise measurement and control of the API quantity in each dosage form are essential to guarantee the drug's effectiveness and safety for patients.[2]
- **Customization and Tailoring:**
  Healthcare practitioners have the ability to optimize treatment outcomes by modifying the concentration and combination of Active Pharmaceutical Ingredients, thereby tailoring treatment programs to suit specific patients.[2]
- **Innovations in Drug Development:**
  Advanced Active Pharmaceutical Ingredients are being developed to selectively target specific molecular pathways. This advancement holds promise for the creation of more efficient and precise therapeutic interventions, while concurrently minimizing the occurrence of adverse consequences. [2]
- **Global Health Impact:**
  Active Pharmaceutical Ingredients (APIs) play a crucial role in the pharmaceutical sector, exerting a significant influence on global health through their facilitation of the manufacturing process for affordable and medically essential medications. The effective management of public health concerns and healthcare infrastructure on a global scale is reliant on the availability of high-quality Activated Pharmaceutical Ingredients. [2]

DRUG DEVELOPMENT PATHWAY

Before a drug can be marketed in the US, it must go through the investigational new drug (IND) application process and complete clinical testing as required by the FDA.Generally, new drug applications (NDAs) or biologics license applications (BLA) are reviewed comprehensively before approval, and then drug performance The complexity in drug development has increased manifolds over the past 40 years, requiring preclinical is resubmitted to regulatory agencies for post-marketing studies.[26]

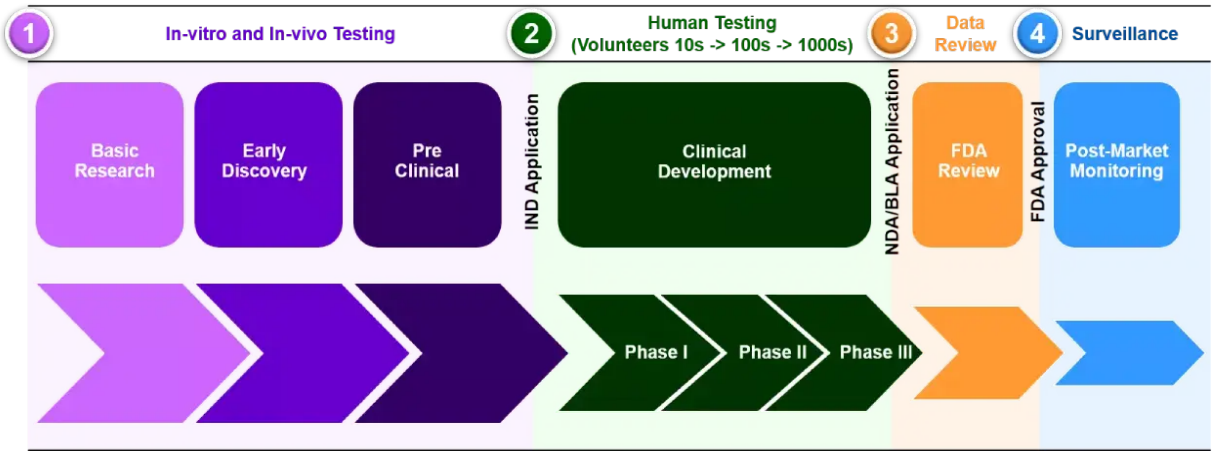


Table 1: drug development pathway

PHASES AND STAGES:

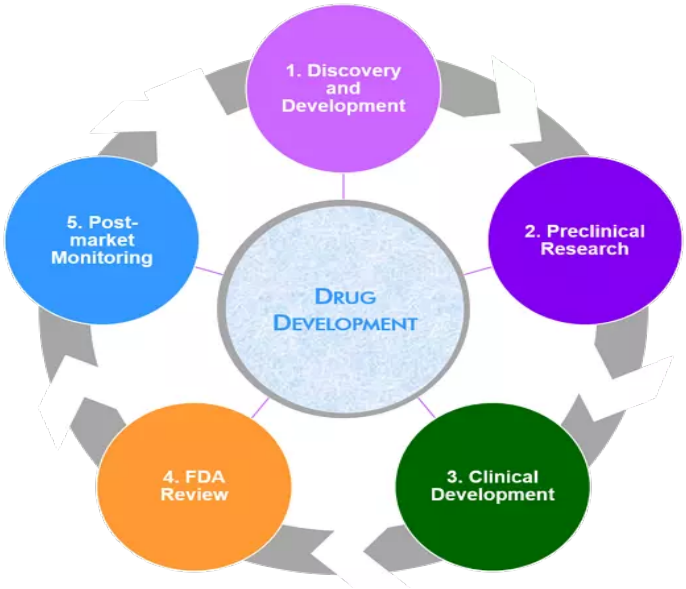


TABLE 2: drug development stages

Phase	Target Discovery	Target Validation	Lead Generation & Refinement	Preclinical Development	
Goal	Find All Targets	Eliminate Wrong Targets	Generate Molecules	Eliminate Molecules	Advance Molecules

Table 3: discovery and development

There are five critical steps in the U.S. The FDA drug development process comprises multiple phases and stages within each of them. In this discussion, we will cover all the phases and different stages of drug development to gain a comprehensive understanding of the complete process. The phases of drug development are as follows:

- **Step 1:** Discovery and Development
- **Step 2:** Preclinical Research
- **Step 3:** Clinical Development
- **Step 4:** FDA Review
- **Step 5:** FDA Post-Market Safety Monitoring.

DISCOVERY AND DEVELOPMENT:

The process of discovering and developing drugs involves several steps. These include screening hits, conducting iterative medicinal chemistry, and optimizing hits to reduce potential side effects and enhance affinity and selectivity. The drug development process also focuses on improving efficacy or potency, metabolic stability (half-life), and oral bioavailability. All of these steps are crucial in ensuring the development of safe and effective drugs.[27]

Target Identification and Validation:

Target identification finds a gene or protein (therapeutic agent) that plays a significant role in disease. Afterward, scientists and researchers record the target's therapeutic characteristics. Drug targets must be efficacious, safe, usable, and capable of meeting clinical and commercial requirements. To validate targets, researchers use various modern tools and techniques such as disease association, bioactive molecules, cell-based models, protein interactions, signaling pathway analysis, functional analysis of genes, in vitro genetic manipulation, antibodies, and chemical genomics.For example, the Sanger Whole Genome CRISPER library and Duo link PLA are excellent sources for drug discovery targets.

Numerous screening techniques can be applied to reach a "hit" compound. A "hit" is a chemical that interacts with the desired target. There are several methods to find "hits," including high-throughput screening, phenotypic screening, virtual screening, fragment-based screening, and structure-based design.

Hit Discovery Process

Following target validation, compound screening assays are developed:

- **Assay Development and Screening**: Assay development in drug discovery is a crucial component of drug discovery workflow Assays are test systems used to evaluate the effects of a new drug candidate at the cellular, molecular, and biochemical levels.
- **High Throughput Screening**: High Throughput Screening (HTS) uses robotics, data processing/control software, liquid handling devices, and sensitive detectors to rapidly conduct millions of pharmacological, chemical, and genetic tests, eliminating hours of painstaking testing by scientists. HTS identifies active compounds, genes, or antibodies that affect human molecules.

Hit To Lead

In the Hit to Lead (H2L) process, small molecule hits from an HTS are evaluated and optimized in a limited way into lead compounds. After identification, these chemical compounds undergo the process of lead optimization.The main objective is to improve a number of the most capable "hits" in order to produce candidates that are more powerful, selective, and have "optimal" pharmacokinetic features More and more pharmaceutical companies are realizing the benefits of adopting AI strategies as AI technology develops. In the process of drug discovery, the initial compounds that are tested typically have a relatively low affinity for the biological target. Researchers work to improve the affinity by several orders of magnitude.By adopting AI systems, they can enable researchers to gather large amounts of valuable biological, structural, and chemical data for faster H2L maximization.[37]

Lead Optimization

After the target validation, the next phase is lead identification. In this process, lead compounds are discovered and synthesized using the Hit-to-Lead (H2L) approach. The lead compounds are then modified and optimized to improve their potency and reduce side effects. This is done through experimental testing using animal efficacy models and ADMET tools.

The final step in the lead optimization process is designing the drug candidate.Lead compounds are often discovered and developed as collections or libraries of individual molecules that exhibit the desired properties for a new drug. Once a lead compound is identified, experimental testing is conducted on each molecule to confirm its effect on the drug target. This progresses further to lead optimization Lead optimization studies are experiments conducted on animals or in vitro, or by modifying a desired target in disease patients. These experiments are performed to compare various lead compounds, to determine how they are metabolized, and the effects they may have on the body. The information gathered from lead optimization studies is used by scientists in the pharmaceutical industry to identify the compounds that have the greatest potential to be developed into a safe and effective drug.

Active Pharmaceutical Ingredients

Active pharmaceutical ingredients (APIs) are biologically active substances within a drug that produce the desired therapeutic effects. Every medication consists of one or more APIs and excipients. Excipients are inactive substances that help to deliver the drug into the human system. High Potency Active Pharmaceutical Ingredients (HP APIs) are molecules that are effective at much lower dosage levels than standard APIs. They are classified based on toxicity, pharmacological potency, and occupational exposure limits (OELs) and are used in complex drug development that involves more than ten steps. When one lead compound is found for a drug candidate, the drug discovery process becomes narrowed, and the drug development process begins. [3]

NON-CLINICAL STUDIES

Non-clinical testing, also known as preclinical testing, is conducted during the early stages of drug development and does not involve testing in humans. Its main goal is to determine the safety of a medicine using animals and/or cells or tissues. This testing helps to identify any harmful effects of the medicine on the body, such as toxic effects, genetic changes, or potential carcinogenicity. The information from non-clinical

testing is used to plan and design subsequent clinical trials in humans, including determining the starting dose and the range of doses to be tested.

Non-clinical studies are an essential part of the drug development process, aiming to assess the safety and build solid scientific foundations before transitioning to the clinical development phase.

These studies are conducted using various protocols, including in vitro and in vivo experiments. They mostly follow the regulations of Good Laboratory Practice (GLP).

The European Medicines Agency provides scientific guidelines on non-clinical testing to help applicants prepare marketing authorization applications, reflecting a harmonized approach of the EU Member States and the Agency on how to interpret safety and efficacy set out in the Community directives.

In short, non-clinical testing is a crucial step in the development of new medicines, focusing on safety assessment before human trials, and it involves various types of studies using animals and/or cells to gather essential data for the subsequent clinical development phase.

TYPES

It is broadly classified into different types of non-clinical studies including:[12]

Pharmacodynamics (PD) Studies: These studies aim to determine what medicine does to the body, focusing on its effects and mechanisms of action. They can be conducted in vivo (in a living organism) and/or in vitro (in a controlled environment outside a living organism).

Pharmacokinetics (PK) Studies: PK studies are designed to examine how the body affects the medicine, involving the absorption, distribution, metabolism, and excretion of the compound. These studies provide crucial information about the medicine's behavior in the body

Toxicology Studies: Toxicology studies investigate the potential harmful effects of medicine on the body, such as its impact on the reproductive system, genetic changes, and carcinogenicity. They assess the toxicity in relation to different doses or duration of use of the medicine.

These types of studies are essential in the early stages of drug development to assess the safety and efficacy of a medicine before it is tested in humans. They provide valuable data for planning and designing subsequent clinical trials.[4]

NON-GLP STUDIES

Non-GLP (Good Laboratory Practice) studies refer to non-clinical health and safety studies that are not conducted in compliance with GLP standards. While GLP is required for extrapolation to humans and for assessing the safety of animal drugs, human drugs, biological products, and medical devices, non-GLP studies can be of high quality for other purposes, such as lead optimization and preclinical development. They prioritize documentation, safe procedures, and reliable data, and can be advantageous due to decreased regulatory scrutiny, shorter testing durations, and faster report delivery. However, GLP standards are absolutely necessary, especially for the evaluation of safety studies, and are a decisive factor for the acceptance of non-clinical studies in other countries where GLP is required.[5]

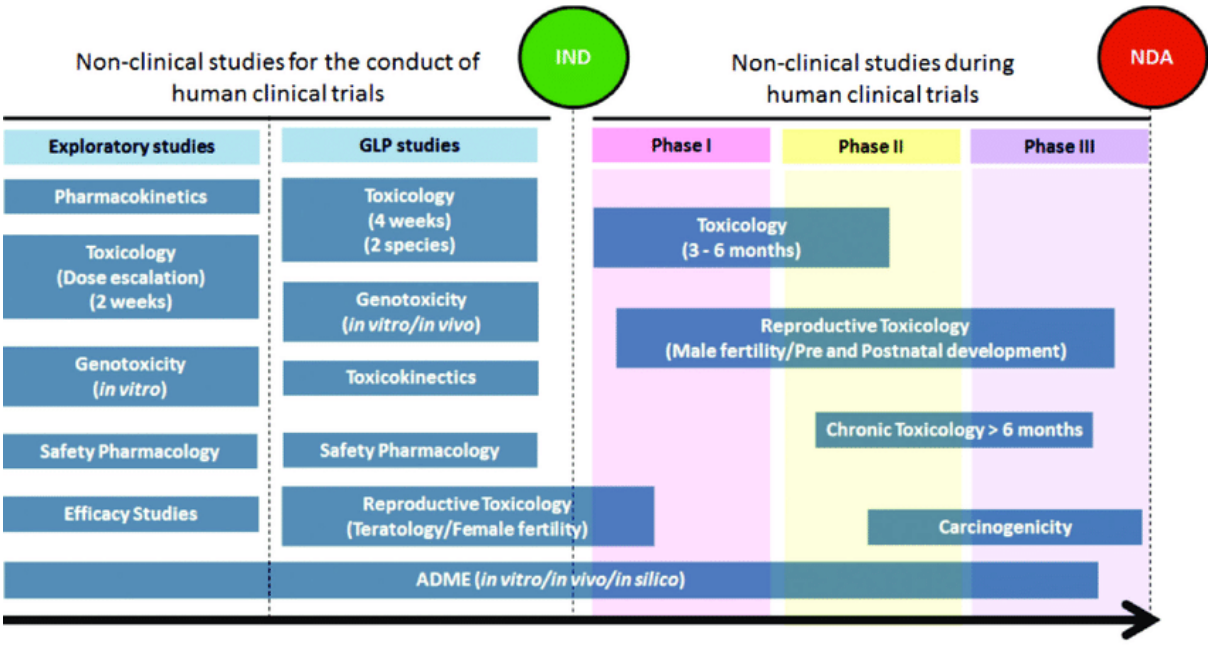


Table 4: Non-clinical and clinical studies

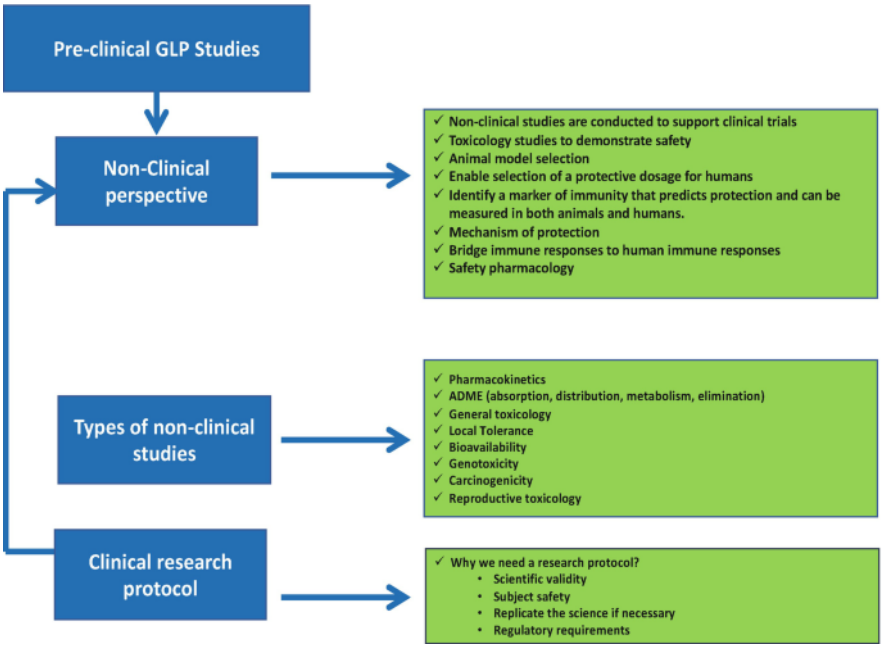


Table 5: Pre-clinical GLP studies

Non GPL		GLP
In vitro	*In vivo*	*In vivo*
1) Physical/chemical properties [lipophilicity (log P/log D), solubility, chemical stability (pKa)] 2) Metabolic stability 3) Hepatic clearance 4) Interaction between substances (inhibition/induction of CYPs) 5) Physiological characteristics (plasma protein/tissue binding) 6) Permeability 7) Plasmatic stability and total blood/plasma partition	1) Pharmacokinetic profile (concentration versus time) - Area under the curve - C_{max} - T_{max} - Distribution - Clearance - Half-life time 2) Biodisponibility bioavailability 3) Linearity 4) Metabolization 5) Routes of excretion	1) Toxicokinetic - Pharmacokinetic profile (concentration versus time) - Area under the curve - C_{max} - T_{max} - Distribution - Clearance - Half-life time 2) Biodisponibility 3) Metabolization 4) Routes of excretion 5) Quantification of biological fluids, organs, tissues, excrements and expired air (when necessary)

ADME/PK: absorption, distribution, metabolism and elimination/pharmacokinetics; GLP: Good Laboratory Practices; T_{max}: time-to-maximum; C_{max}: maximum concentration. Source: adapted from (11).

Table 6: Non clinical assays of ADME/PK

The main difference between GLP and non-GLP studies is the assessment of safety, as GLP is mandatory for studies that seek to prove the safety of certain products, while non-GLP studies do not necessarily assess safety. Non-GLP (Good Laboratory Practice) studies encompass a variety of non-clinical laboratory studies that do not need to comply with GLP regulations. These studies can include basic research, screenings, discovery studies, and other endeavors where the assessment of safety is not the primary focus. They prioritize documentation, safe procedures, and reliable data, and can provide valuable insights into dose levels, toxicity, and other factors to inform further development and studies. Examples of studies that could be exempt from GLP include exploratory genotoxicity, mutagenicity, safety pharmacology, and general in-vitro toxicology studies.

Non-GLP studies can be of high quality for purposes other than extrapolation to humans, and they can be advantageous due to decreased regulatory scrutiny, shorter testing durations, and faster report delivery.

IN VITRO STUDIES

In vitro GLP studies refer to Good Laboratory Practice (GLP) compliant studies that are conducted using in vitro test systems, which do not involve multicellular whole organisms but rather microorganisms, isolated materials, or simulations thereof as test systems. These studies are essential for assessing the safety of chemicals, pharmaceuticals, and other substances with respect to human health and the environment. In vitro studies play a significant role in drug discovery, development, and preclinical testing, providing

valuable data on the properties and safety of various substances.

Physical-chemical characteristics and physiological Properties

Several physical-chemical properties such as lipophilicity, rate of dissolution, solubility, pKa, and molecular weight, can directly interfere with the absorption, distribution, metabolism, and elimination of a substance.[6][11]

LIPOPHILICITY

Lipophilicity, commonly referred to as LogP, is a measure of the concentration of a compound in two phases, an oil and a water phase. This measure is crucial in determining how well a drug will be absorbed, transported, and distributed in the body, as well as how it should be formulated and dosed. However, it is important to note that LogD, a variant of LogP, takes into account the pH-dependent distribution of ionizable compounds at a particular pH, making it a more comprehensive measure of lipophilicity in various biological environments.{20}

RATE OF DISSOLUTION

LogD is a measure of how much a molecule is lipophilic. It considers all the different ways a molecule can be in water at a given pH. This measure is pH-dependent, which makes it suitable for practical use in various biological media that have different pH levels. LogD is more appropriate than LogP for ionizable compounds, because it considers the pH-dependent distribution of ionized substances. This is crucial for analyzing the properties of drug candidates in different biological environments.

The lipophilicity of a drug is connected with how well it dissolves in water, and a certain balance of lipophilicity and hydrophilicity is needed for a drug candidate to be successful. While water solubility is essential for a drug to dissolve in plasma and other biological fluids, lipophilicity is necessary for the drug to go through biological membranes. It is of great importance for drug absorption and distribution.{20}

METABOLIC STABILITY

Metabolic stability is a term used to describe how easily a chemical compound can be transformed by the body's enzymes, especially in the context of drug discovery and development. This is typically measured by its in vitro half-life (t(1/2)) and intrinsic clearance (CL(int In vitro metabolic stability assays evaluate the rate at which a drug candidate is eliminated by the body's enzymes. These assays help assess whether a drug candidate can be transformed into inactive or potentially toxic compounds, which can affect its efficacy and safety.

The assays measure the rate at which a test compound disappears over time to calculate intrinsic clearance. Microsomal assays assess metabolism by the cytochrome P450 system, while hepatocyte assays factor in hepatic blood flow, drug/protein binding, and other variables.. These assays play a crucial role in improving in vivo pharmacokinetic parameters, reducing systemic clearance of drugs, increasing oral bioavailability, addressing potential metabolism-related issues, and predicting the human dose. {21}

HEPATIC CLEARANCE

Hepatic clearance refers to the liver's ability to metabolize and extract drugs as they pass through.. It's determined by hepatic blood flow (Q), the free or unbound fraction (fu), and intrinsic hepatic clearance (CLint). This parameter is crucial in pharmacokinetics and drug development as it helps predict the dose, half-life, and bioavailability of drugs while also prioritizing compounds. The efficiency of drug removal from the blood and the efficiency of blood delivery to the liver are the two major determinants of hepatic clearance. Hepatic clearance is dependent on blood flow, and drugs with high hepatic extraction exhibit clearance rates directly proportional to hepatic blood flow. Understanding hepatic clearance is vital for assessing drug metabolism and elimination, as well as predicting drug behavior in the body, especially during its first pass through the liver.{8}

INTERACTION OF SUBSTANCES

Drug metabolism can be influenced by the interaction between substances. This can involve the inhibition or induction of cytochrome enzymes, also known as CYPs. Such interaction can lead to the formation of new substances or alter the metabolism of co-administered drugs. When one drug reduces the metabolic activity of CYP enzymes, this is called inhibition, which can result in increased levels of co-administered drugs. On the other hand, induction occurs when CYP enzyme activity is stimulated, leading to decreased levels of co-administered drugs. These interactions are crucial considerations in drug development and clinical practice to ensure the safety and efficacy of drug regimens.[8]

PHYSIOLOGICAL CHARACTERISTICS

Understanding the physiological characteristics of plasma proteins and tissue binding is crucial for predicting the pharmacokinetic behavior of drugs in drug development. Plasma protein binding can impact the distribution, metabolism, and excretion of drugs, as well as their potential for drug-drug interactions.

Plasma is the liquid component of blood, and it contains various essential proteins that play vital roles in the body. These proteins include albumin, globulins, and fibrinogen. Albumin, which constitutes approximately 60 percent of all plasma proteins and is synthesized by the liver, is crucial in maintaining osmotic pressure in the blood vessels. Globulins, which include antibody proteins and coagulation factors, have diverse functions such as immune response and blood clotting. Fibrinogen, found only in plasma, is involved in the clotting process. These proteins help maintain serum osmotic pressure and act as transporters for substances

such as hormones and drugs, while also playing a role in extracellular buffering.

Additionally, tissue binding is another important factor that can influence the pharmacokinetics of drugs. Tissue binding can affect the distribution of drugs to different organs and tissues, as well as their efficacy and toxicity. Therefore, it is crucial to evaluate the extent of tissue binding during the drug development process.[8]

PLASMATIC STABILITY AND TOTAL BLOOD/ PLASMA PARTITION

The total blood/plasma partition refers to how a drug spreads between the plasma and erythrocytes once it enters the bloodstream. This partitioning can impact the total drug concentration in the plasma, which is crucial in determining the drug's pharmacokinetics and pharmacodynamics.

On the other hand, plasmatic stability measures how stable a drug is in the plasma. It includes analyzing the drug's binding to plasma proteins, such as albumin and globulins, which can affect its distribution, metabolism, and excretion. Understanding plasmatic stability is crucial in predicting how the drug behaves in the body and its potential for drug-drug interactions.

The blood-to-plasma ratio assay is a common method used to determine the drug concentration in whole blood compared to plasma, providing an indication of how the drug binds to erythrocytes. This assay is useful in assessing the drug's distribution between blood and plasma, which can impact its overall pharmacokinetic profile.[8]

1. **Standard Repeated Dose Toxicity Studies:** These studies are conducted to assess the potential adverse effects of a substance that is administered repeatedly to test animals over a specified period.
2. **Genotoxicity Studies:** Genotoxicity studies are designed to evaluate the potential of a substance to damage genetic information within a cell, which can lead to mutations or cancer.
3. **Safety Pharmacology Studies:** Safety pharmacology studies are performed to assess the potential undesirable pharmacodynamic effects of a substance on physiological functions.
4. **Biocompatibility Studies:** These studies are often conducted in the context of medical devices to assess the interaction between the device and the biological system.
5. **In Vivo Toxicokinetic Studies:** These studies are designed to evaluate the absorption, distribution, metabolism, and excretion (ADME) of a substance in living organisms.
6. **In Vitro Toxicology Studies:** In vitro toxicology studies involve the assessment of the toxic effects of substances using isolated cells or tissues.

TYPES OF NON-GLP STUDIES:

IN VIVO:

PHARMACOKINETIC PROFILE:

In vivo pharmacokinetic (PK) studies allow for the quantitative evaluation of the absorption, distribution, metabolism, and elimination (ADME) of a new substance. These studies provide essential exposure (AUC) data by measuring drug concentration in the plasma of treated laboratory animals at successive time points. In vivo PK studies are critical in the early stages of drug development to determine the mechanisms of a drug's absorption, distribution, metabolism, and excretion, and to evaluate its potential effects.

To perform a PK profile analysis of a substance, it is important to determine the following parameters:

i) area under the curve (AUC).
ii) maximum drug concentration in plasma.
iii) time to reach the maximum concentration.
iv) half-life.
v) distribution volume.
vi) clearance; and
vii) bioavailability[15]

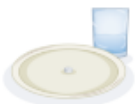
Compound	Native GLP-1	Short-acting GLP-1 RAs	Long-acting GLP-1 RAs	Oral peptide GLP-1 RAs	Oral small-molecule GLP-1 RAs
Clinical milestone	Nauck et al. 1993	Fineman et al. 2003	Nauck et al. 2006	Davies et al. 2017	Saxena et al. 2021
Approval for clinical use	NA	2005	2009	2019	?
$t_{1/2}$	~2 min	~3 h	1 week	1 week	~4–8 h
Administration	i.v. or s.c. (continuous)	s.c. BD–QD	s.c. QD–QW	p.o. QD	p.o. BD–QD
Molecular weight (Da)	~3,298	~4,187–4,860	~4,114–73,000	~4,114	~556
Clinical features	Requires continuous infusion	Predominant effect on postprandial plasma glucose	Predominant effect on fasting plasma glucose	Minimum interval of 30 min between drug intake and subsequent meal	No interval between drug intake and meal necessary
					

Table 5: plasmatic stability

GLP STUDIES:

Good Laboratory Practice (GLP) is a standardized system for conducting non-clinical health and safety studies. The system covers planning, monitoring, recording, reporting, and archiving of these studies. GLP provides regulations and standards that ensure drug safety studies are carried out in a consistent manner in non-clinical animal studies. Compliance with GLP is mandatory for studies that will be submitted with an Investigational New Drug (IND) application. GLP studies are designed to evaluate the pharmacokinetic (PK) profile of a compound, including its absorption and clearance properties. They are carried out to ensure that drug candidates have the appropriate PK properties. GLP studies follow Good Laboratory Practice (GLP) regulations and involve the determination of various PK parameters such as AUC, clearance, half-life, volume of distribution, Cmax, and Cmin.[5][15]

GLP studies are **three figures**:

Test Facility Management (TFM): TFM is crucial for managing non-clinical toxicology studies in test facilities. It ensures study integrity, compliance with GLP, resource availability, personnel understanding of functions, designating a study director, managing archives and schedules, and providing appropriate facilities and equipment. TFM's role is pivotal in upholding GLP principles and ensuring success and integrity in non-clinical drug development.[6]

Quality Assurance Unit (QAU): The Quality Assurance Unit (QAU) is an internal system that ensures that studies conducted in a test facility comply with the Good Laboratory Practice (GLP) principles. The QAU must be independent, and its activities cannot compromise its operation. No QAU member can be involved in experimental development unless they are only performing monitoring functions. The person in charge of the QAU should have direct access to different levels of management, particularly the Test Facility Management (TFM). It is the responsibility of the QAU head to report any deviation or non-compliance with GLP principles in any part of the test facility or any procedure, to establish corrective actions. For more detailed information about the functions of the QAU, please refer to section II of the NIT DICLA 035.[7]

Study Director (SD): The Study Director (SD) is responsible for managing the nonclinical health and environmental safety study. They oversee the scientific, regulatory, and administrative aspects of the study, including data collection, analysis, reporting, and conclusions.

The SD adheres to the principles of Good Laboratory Practice (GLP) and prioritizes the scientific standards of the studies related to the quality and efficacy of the experimental design, evaluation, and significance of the generated data.

The Study Director is the only point of control for the study, ensuring its integrity and validity from start to finish.[8]

Drug metabolism pharmacokinetic properties:

These are the desirable DMPK properties that a substance should possess to be considered suitable for administration through the oral route. When developing a substance to be taken orally, it is important to consider certain properties in DMPK studies. These include:

i) Water solubility
ii) High permeability and low efflux in Caco-2 cells
iii) Sufficient bioavailability to reach the desired plasma and organs to produce the intended pharmacological effect.
iv) Adequate half-life to fit the intended dosage schedule in humans.
v) Linear PK
vi) Elimination that is not dependent on a single route or a single metabolic enzyme, without forming active or reactive metabolites in large amounts, and without interacting with metabolic enzymes in significant concentrations
vii) Acceptable safety margin (therapeutic index, preferably higher than 10 times)
viii) Established PK-PD (pharmacodynamics) relation.[8]

TOXICOLOGICAL STUDIES:

Toxicology plays a crucial role in non-clinical studies by assessing the potential harmful effects of a medicine on the body. This involves various types of studies, including single-dose toxicity, repeated-dose toxicity, genotoxicity, carcinogenicity, reproductive/developmental and juvenile toxicity, and local toxicity.

The main objectives of toxicology studies are to determine.

- the toxicity of the medicinal product in relation to different doses or duration of use, as well as to assess the reversibility of any toxicity.
- The information obtained from toxicology studies is used to plan and design subsequent clinical trials in humans, including determining the starting dose and the range of doses to be tested, as well as identifying the clinical signs to be monitored for adverse effects.
- Therefore, toxicology is an essential component of non-clinical testing, providing critical data to ensure the safety of a medicine before it is tested in humans.

Types of Toxicological Studies

There are several types of toxicological studies that are conducted to determine the safety of chemicals and substances. These include acute toxicity studies, sub-acute toxicity studies, chronic toxicity studies, and reproductive toxicity studies.[16]

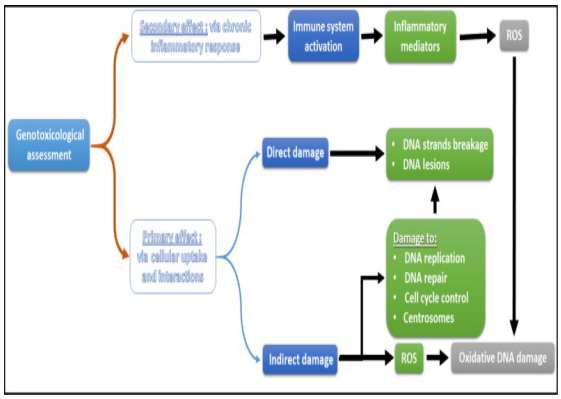
Acute toxicity studies are typically short-term studies that evaluate the effects of a substance on living organisms over a period of 24 to 48 hours. Sub-acute toxicity studies involve exposure to a substance for several weeks, while chronic toxicity studies involve exposure for several months or even years.

Reproductive toxicity studies are conducted to evaluate the potential effects of a substance on the reproductive health of living organisms. These studies may involve exposure to the substance during pregnancy or early development.

Other types of toxicological studies include genotoxicity studies, which evaluate the potential for a substance to cause genetic damage, and carcinogenicity studies, which evaluate the potential for a substance to cause cancer.

Genotoxicology:

Genotoxicology is a field of study that focuses on the effects of harmful substances on genetic material within cells, leading to mutations that can potentially result in cancer and other diseases. It involves assessing the ability of substances to damage DNA, induce mutations, and cause adverse genetic effects. Genotoxicity is the property of chemical agents that damage genetic information within a cell, causing mutations that may lead to cancer. This field encompasses various aspects, including the detection methods of genotoxicity, the relationship between genotoxic substances and human cancer, chemotherapy, germ cells, and stem cells. Researchers study genotoxic substances that induce mutations through direct or indirect mechanisms, affecting DNA sequences and leading to genetic alterations. Genotoxicity testing is crucial in evaluating the safety of chemicals, food additives, drugs, and other substances with potential human exposure. It involves assessing mutagenicity, DNA damage, and the capability of substances to damage DNA or cellular components regulating genome fidelity. Genotoxicity tests aim to identify substances that can cause genetic alterations in somatic or germ cells, providing essential information for regulatory decision-making and risk assessment.



Different types of genotoxicity tests include in vitro and in vivo assays that assess the potential of substances to induce genetic damage. Some common genotoxicity tests are:

1. Ames Test: A widely used in vitro test that detects mutations in bacteria, specifically Salmonella typhimurium, to assess the mutagenic potential of chemicals.

The Ames Test, named after its creator Dr. Bruce N. Ames, is a bacterial test that is specifically designed to detect substances that can cause mutations in DNA. This test utilizes specific strains of bacteria that lack the ability to synthesize histidine, making them histidine-dependent for growth. The procedure involves preparing the test substance in various concentrations, adding a metabolic activation system derived from mammalian liver extracts to simulate metabolic processes, and incubating the bacterial strains with the test substance. After incubation, the bacteria are spread onto agar plates lacking histidine, and colony growth is assessed. When the concentration of a substance is increased, a higher number of colonies may be observed. This may suggest a more potent mutagenic effect. When the concentration of a substance is increased, a higher number of colonies may be observed. This may suggest a more potent mutagenic effect. The Ames Test is a rapid and cost-effective method used to assess the mutagenic potential of chemicals, aiding in the identification of potential carcinogens and the determination of genotoxicity for various compounds. It is a valuable tool in toxicology for evaluating the safety of chemicals and substances with potential human exposure.[23]

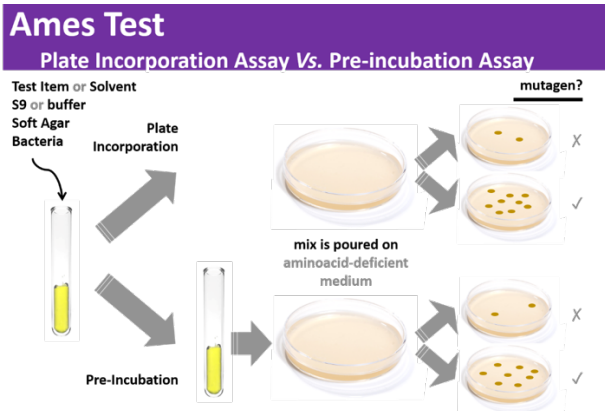


Figure : Ames test and its 2 methods: plate incorporation & pre incubation

2. Micronucleus Test: Both in vitro and in vivo, this test is crucial for evaluating genotoxicity. It is particularly reliable for assessing the induction of micronuclei, a major endpoint of mutagenicity.

The Micronucleus Test is a widely used method in toxicological screening to identify potential genotoxic compounds by assessing genetic damage. This test is recognized as one of the most successful and reliable assays for genotoxic carcinogens, particularly those that cause genetic damage. There are two major versions of the Micronucleus Test: one conducted in vivo using mouse bone marrow or peripheral blood and the other performed in vitro.

In the in vivo test, the formation of micronuclei in polychromatic erythrocytes indicates induced chromosome damage. The in vitro version involves cultured cells and has been improved over time, with advancements like the cytokinesis-block micronucleus

(CBMN) method, which uses an inhibitor to prevent cytokinesis after nuclear division. Micronuclei are small nuclei formed during cell division that contain acentric or whole chromosomes not properly segregated during anaphase. The Micronucleus Test is a valuable tool for assessing genotoxicity, offering advantages over traditional chromosomal aberration tests in terms of ease of procedures and evaluation. It is used to evaluate the potential genotoxic effects of various chemicals and is crucial in determining exposure levels and associated health risks.

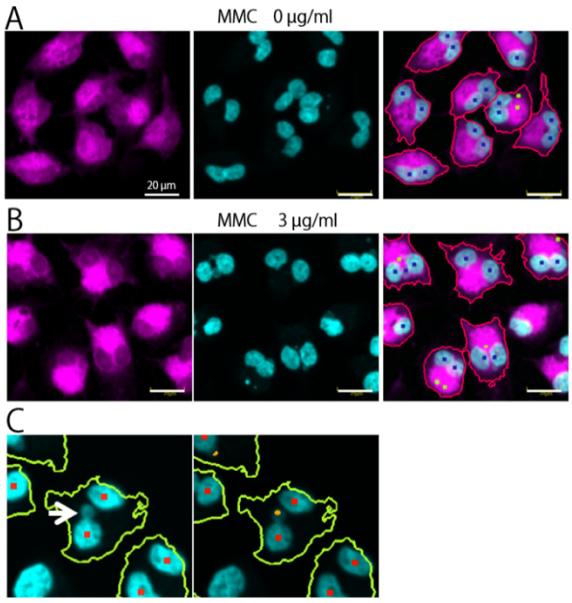


Table : Micronucleus test

- **In Vivo Mouse Bone Marrow Micronucleus Test:** An in vivo test that evaluates the genotoxic effects of substances on mouse bone marrow cells.
- **In Vitro Mammalian Cell Micronucleus Test**: This in vitro test uses mammalian cell cultures to assess the genotoxic potential of substances by detecting micronuclei formation.[24]

3. In Vivo Mouse Sperm Head Morphology Test: Another in vivo test that examines the impact of substances on the morphology of mouse sperm heads, providing insights into genotoxic effects.

The In Vivo Mouse Sperm Head Morphology Test is a method used to evaluate the morphology of sperm heads in mice, particularly focusing on abnormalities that may indicate genetic damage or mutagenicity. This test involves analyzing the structure of sperm heads from live mice to identify any deviations from normal morphology. Abnormalities in sperm head morphology can provide insights into potential genetic defects or damage that may affect fertility or lead to adverse health effects. By conducting this test, researchers can assess the impact of various factors, such as exposure to mutagenic substances, on the integrity of sperm at a cellular level. The test aims to detect and quantify abnormalities in sperm head shape, size, and structure, providing valuable information for understanding the genotoxic effects of different compounds and their potential implications for reproductive health.[21]

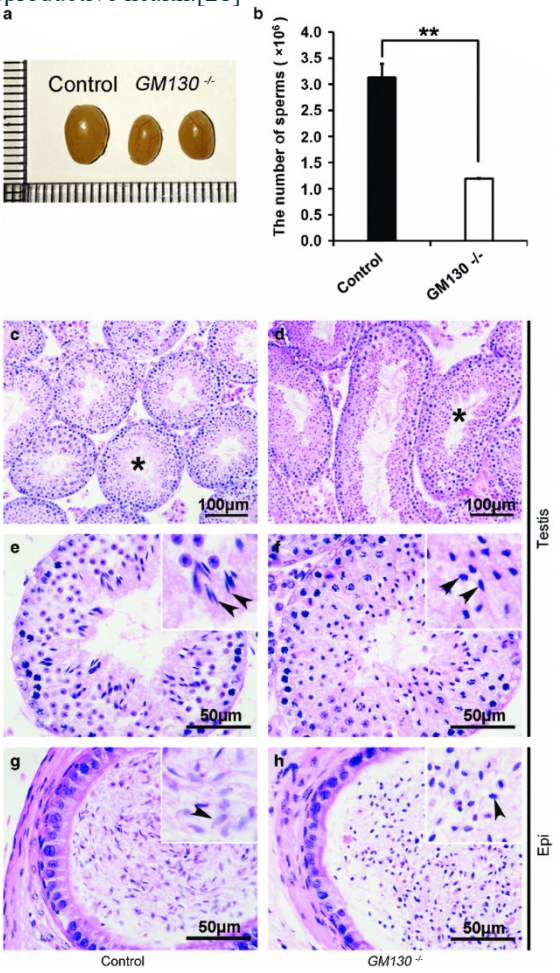


Table: in vivo mouse sperm head morphology

4. **Comet Assay**: A common test for genotoxicity that involves detecting DNA damage in cells by electrophoresis, particularly useful for assessing DNA breaks.

The Comet Assay, also known as single-cell gel electrophoresis, is a technique used to measure DNA strand breaks in eukaryotic cells. It is a sensitive and straightforward method for detecting DNA damage at the level of individual cells. The assay involves embedding cells in agarose on a microscope slide, lysing the cells, and subjecting them to electrophoresis. The term "comet" refers to the pattern of DNA migration through the gel, resembling a comet shape, with the tail indicating the extent of DNA damage. The Comet Assay is valuable for evaluating DNA damage and repair, biomonitoring, and genotoxicity testing. It has become a standard technique for assessing DNA damage and repair mechanisms, biomonitoring, and genotoxicity testing. The assay is widely used in various fields, including toxicology, pharmacology, and environmental science, to assess the impact of different factors on DNA integrity and to study genotoxic effects of various compounds.[22]

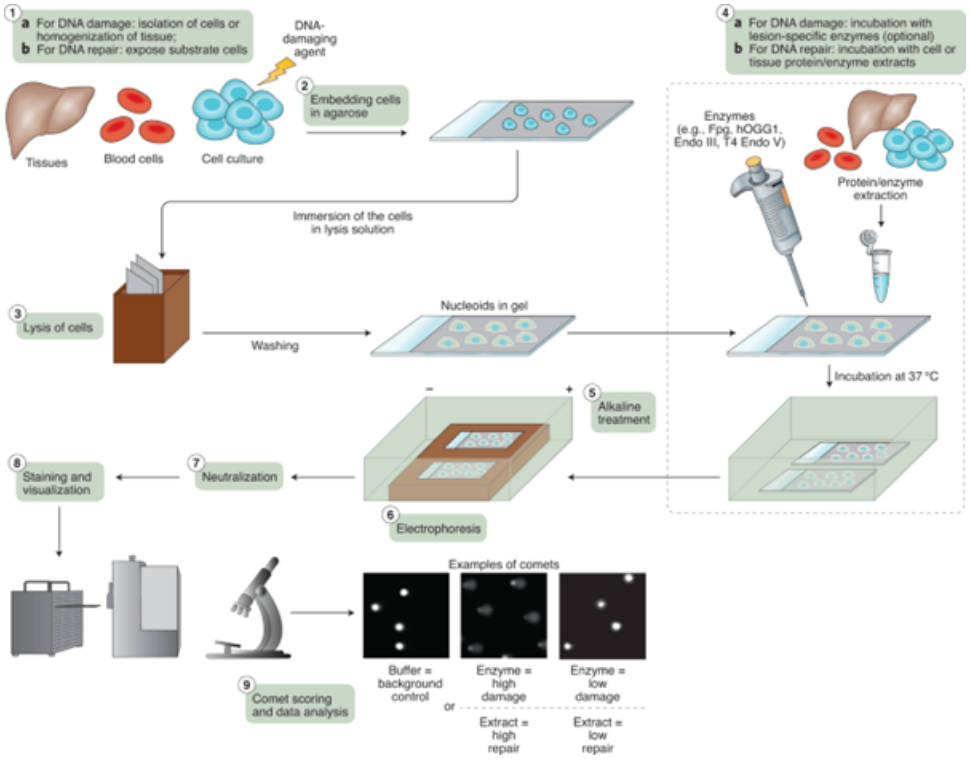


Table: comet assay

Carcinogenicity :

Carcinogenicity studies are crucial in pharmaceutical development to assess the tumorigenic potential of drugs with prolonged exposure to humans. These studies aim to identify any tumorigenic potential in animals and evaluate the relevant risk in humans.

They are typically conducted over periods ranging from six months to two years, following guidelines like the ICH S1 guideline, as part of the safety assessment for long-term use products.

Carcinogenicity studies are essential due to the limitations of clinical trials in detecting drug-related tumor emergence and assessing cancer risk, especially considering the long latency period for most human cancers.

The FDA has issued guidance to reduce animal testing in carcinogenicity assessments, introducing a "weight of evidence" approach to evaluate human cancer risk, potentially eliminating the need for certain two-year rat studies in drug assessment.

This approach considers various factors, including mechanisms of carcinogenicity, retrospective analyses, and a commitment to patient safety and animal welfare.

The tests carried out for carcinogenicity studies involve a comprehensive evaluation to determine the tumorigenic potential of substances. These tests typically include:

- **Combined Chronic Toxicity/Carcinogenicity Studies:** These studies aim to identify both chronic and carcinogenic effects following prolonged and repeated exposure to substances. They are conducted using rats and involve dose-response relationship assessments over a duration of 12 months for the chronic phase and 24 months for the carcinogenicity phase. Observations include detailed examinations, necropsy procedures, and histopathology to detect neoplastic effects and assess carcinogenic potential and general toxicity
- **Dose Selection and Group Size:** Each dose group and concurrent control group for the carcinogenicity phase should include at least 50 animals of each sex, while for the chronic toxicity phase, at least 10 animals of each sex are recommended. The study should involve at least three dose levels in addition to the control group for both phases of the study, focusing on routes of administration like oral, dermal, and inhalation
- **Observations and Measurements:** The tests involve detailed observations, measurements, and examinations to detect any preneoplastic lesions, tissue-specific proliferative effects, endocrine disturbances, and other relevant indicators of carcinogenic potential. Necropsy procedures and histopathology are crucial for identifying neoplastic effects and assessing the overall impact on the animals

These tests are essential in pharmaceutical development to ensure the safety and efficacy of drugs by thoroughly evaluating their potential carcinogenic risks through rigorous scientific methodologies and assessments.

Toxicokinetic Assays

Toxicokinetic assays are an essential part of toxicology studies and must be performed in accordance with GLP rules. These assays measure the exposure of a substance in animals and establish the relationship between the dose administered and the time course of the substance in toxicity studies. Multiple dose administrations help

to determine the PK profile of a substance, enabling better interpretation of the toxicological findings. Toxicokinetic studies evaluate the potential for a substance to accumulate in specific organs or tissues. The data generated in these studies contributes to the interpretation of toxicity test results and comparison with clinical data as part of the risk and safety evaluation in humans. Regulatory agencies recommend toxicokinetic studies as part of the non-clinical test battery.

Two protocols can be used for toxicokinetic studies: a complete protocol that aims to answer all questions or a reduced protocol, in which only the main questions are answered to corroborate the interpretation of the toxicology findings.

In 1980, the OECD and the International Conference on Harmonization (ICH) released guidelines for toxicity testing of chemical and pharmaceutical substances. These guidelines are still widely recommended by regulatory agencies. Over the years, revisions and assays were implemented to promote more predictive and ethical tests that could minimize the use of animals. The guidelines provide the basis for conducting assays, including the suggested species to be used, the duration of assays, the organs to be investigated, and the analysis to be conducted. They also specify the data that should be presented in the final report. However, some scientific and industrial communities still have doubts about the guidelines because the assays are not presented in detail.{6}

SAFETY STUDIES

The development of new drugs has posed a challenge for scientists as it requires techniques that ensure their safety in humans. Before testing on humans, safety tests are conducted on animals based on their experience and employment history. In vitro tests evaluate toxic potential of substances, besides animal studies. However, these tests are sometimes used in combination with in vivo tests. The development of new drugs has posed a challenge for scientists as it requires techniques that ensure their safety in humans. Before testing on humans, safety tests are conducted on animals based on their experience and employment history. [18][7]

Safety pharmacology is a critical discipline in drug development that plays a vital role in predicting and managing the safety of pharmaceuticals. It involves conducting assays, tests, and models to predict the clinical risk profile of new drugs before human studies. Throughout clinical development, safety pharmacology helps explore and explain both expected and unexpected side effects, enabling the refinement of the drug's risk profile. This discipline is essential for identifying potential harms, understanding risk factors, and establishing a comprehensive safety profile for drugs. Early safety monitoring through safety pharmacology is crucial for anticipating and minimizing risks, and ensuring the safety of clinical trial participants and patients using marketed products. By addressing regulatory discrepancies and enhancing safety monitoring processes, safety pharmacology contributes to the development of safe and efficient drug therapies, ultimately mitigating public health threats associated with pharmaceuticals.

Safety pharmacology assays encompass various types of studies aimed at detecting and investigating potential undesirable pharmacodynamic effects of new chemical entities. The primary organ systems, known as core battery systems, include the Central Nervous System, Cardiovascular System, and Respiratory System. Additionally, secondary organ systems of interest in safety pharmacology studies are the Gastrointestinal System and Renal System. These assays are crucial for protecting clinical trial volunteers, and patients participating in clinical trials and minimizing risks during drug development and post-marketing phases due to undesirable pharmacodynamic effects

The different types of safety pharmacology assays include:

- **Safety Pharmacology Studies:** Safety pharmacology is a discipline that focuses on the effects of drugs on physiological functions. These studies involve primary pharmacodynamic, secondary pharmacodynamic, and safety pharmacology studies, aiming to characterize the pharmacodynamic/pharmacokinetic relationship of a drug and predict the risk of rare lethal events.
- **Transportation Safety Studies:** Organizations like the NTSB conduct safety studies related to transportation, analyzing data on accidents, injuries, and emerging safety issues in transportation. These studies help in improving transportation policies, programs, and technical aspects of transportation systems.
- **General Safety and Efficacy Studies:** Safety and efficacy studies aim to estimate clinical endpoints with a specified level of precision. These studies are crucial in assessing the safety and effectiveness of various interventions, treatments, or products.

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