ALTERNATE TO ANIMAL MODELS

Dr Apurv B Patel

1st year Resident

Department of Pharmacology

10/08/2024

OVERVIEW

- Introduction
- Need for alternative
- History
- Types of alternative
- Benefits
- Organisation supporting alternate to animal models

INTRODUCTION

- Advancements in medical technology have led to a significant increase in the use of animals in experiments, with millions used worldwide each year.

- This practice raises ethical concerns due to the pain, distress, and deaths that animals endure.

- Moreover, animal experimentation requires skilled manpower, is time-consuming, costly, and often does not accurately reflect human responses.

- The Fund for the Replacement of Animals in Medical Experiments (FRAME) considers the extensive use of animals in research unacceptable.

- To address these issues, several alternatives have been proposed, including the strategy of the 3Rs (Reduction, Refinement, and Replacement), now expanded to include a 4^{th} R (Rehabilitation) and 5^{th} R (Responsibility).

- These strategies aim to minimize animal use and improve animal welfare in laboratories.

Need for Alternatives to Animal Experiments

- Cruel and Inhumane
- Time-Consuming
- Differences Between Animals and Humans
- Unreliable Safety Results & Poor Predictability for Humans
- Misleading Results
- Demand for Cruelty-Free Products

Animal Welfare

- Animal welfare includes their health and well-being, which should be considered before conducting experiments.

- The UK's Farm Animal Welfare Council (FAWC) established five freedoms in 1993 to ensure animal welfare:

- ✓ **Freedom from Thirst, Hunger, and Malnutrition**
- ✓ **Freedom from Thermal and Physical Discomfort**
- ✓ **Freedom from Pain, Injury, and Disease**
- ✓ **Freedom from Fear and Distress**
- ✓ **Freedom to Express Normal Behavior**

HISTORY OF "5R"

- The concept of the "alternatives" in animal experiments is defined by the three Rs, introduced by Russell and Burch(1992):

- **Replacement**
- **Reduction**
- **Refinement**

- In 1995, RE Banks introduced the **"4th R"** which some call "**Responsibility**" and others "**Rehabilitation**."

- In 1999, under **Mrs. Maneka Gandhi's** leadership, CPCSEA supported the 3Rs in India to reduce laboratory animal's suffering.

REPLACEMENT

Absolute Replacement	Relative Replacement
▪ **In Vitro Techniques:** Studies are conducted using cell or tissue cultures instead of whole animals. Cells can be sourced from humans or animals. ▪**Non-Animal Models:** This includes the use of dummies for educational purposes and computer simulations.	▪**Lower Animals:** Instead of using mammals like rats and mice, research may use microorganisms, plants, eggs, reptiles, amphibians, or invertebrates. ▪For example, pregnancy tests once involved killing rabbits. Now, a simple over-the-counter urine test replaces the need for animal testing.

REDUCTION

- **Objective:** Minimize the number of animals used in experiments.
- **Methods:**

1. Improve researchers' skills.
2. Conduct pilot studies.
3. Use proper experimental design.
4. Apply appropriate statistical techniques.

REFINEMENT

- Refining experimental methods involves minimizing pain, distress, and suffering for animals.
- This includes developing and applying methods to prevent or relieve discomfort and make animals more comfortable.
- All necessary information should be collected before the animal experiences any negative effects, which should be considered the endpoint of the experiment.
- Ex: Use of non invasive monitoring such as MRI

REHABILITATION

- **Objective:** Provide care for animals post-experimentation to alleviate pain and support recovery.

- **Methods:**

1. Perform procedures aseptically to prevent infection.
2. Conduct only one major procedure per animal whenever possible.
3. Ensure proper post-surgical care, including pain management and fluid balance.

RESPONSIBILITY

- **Objective:** Ensure ethical and necessary use of animals in research.

- **Guidelines:**

  - Use animals only when no alternatives are available.

  - Maintain a scientifically sound and ethical approach to animal welfare.

Alternate Methods in Animal Testing

In silico methods

In vitro methods

In Silico Methods

- "In silico" refers to experiments conducted via computer simulation.
- These methods help predict the properties and behaviors of compounds, reducing the need for animal testing.

QUANTITAVIVE STRUCTURE ACTIVITY RELATIONSHIP

- Quantitative Structure–Activity Relationship (QSAR) is a method used to predict the effects of chemical compounds based on their structure.

- It is widely used in drug discovery, environmental science, and risk assessment.

- **Key Concepts of QSAR:**

- **Predicting Effects**: QSAR models predict how a chemical's structure influences its biological and chemical properties, such as how it behaves in the body or the environment.

- **Applications**: QSAR is used in:
  - **Drug Discovery**: To find new drugs by predicting which compounds might have desired effects.
  - **Toxicology**: To assess the potential harm of chemicals without animal testing.
  - **Environmental Science**: To understand the impact of chemicals on ecosystems.
- **Benefits**:
  - Efficient Screening
  - Ethical Testing
  - Cost Savings

- **How It Works**: QSAR uses mathematical models to analyze the relationship between chemical structures and their activities. It considers factors like:

  - **Physicochemical Properties**: Such as how a compound dissolves in fat or water.
  - **Chemical Features**: Like specific groups of atoms that influence activity.

- **Mechanisms of Action**: By understanding how similar chemicals act, QSAR can predict the activity of new compounds, helping design better drugs and chemicals with desired properties.

Computer-Aided Molecular Design (CAMD)

- Computer-Aided Drug Design (CADD) is a method that uses computer software to help discover and develop new drugs.

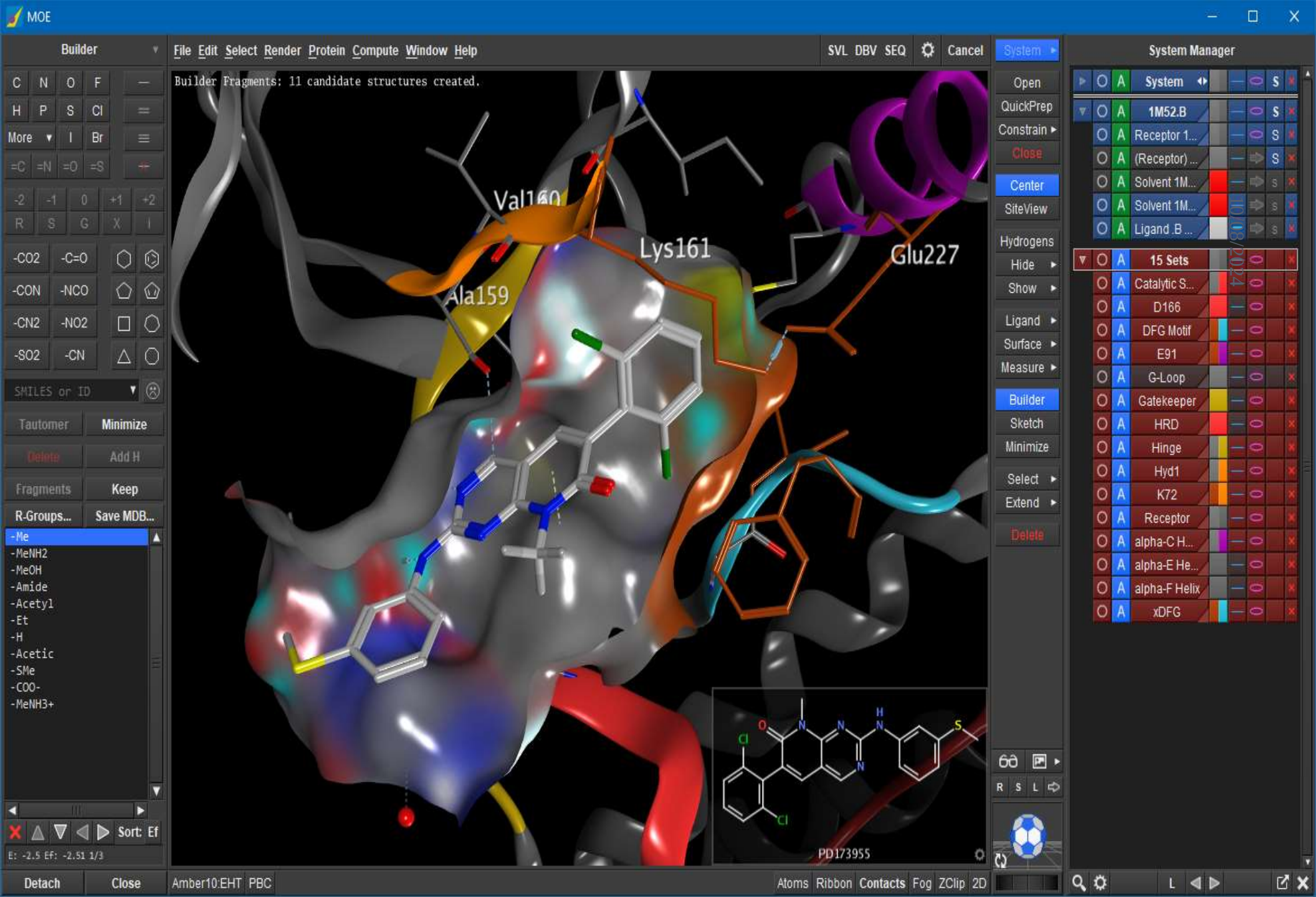
- It involves various computational techniques to predict how drugs interact with biological targets and to design new compounds with desirable properties.

- **Key Steps in CADD:**

  - **Target Identification**: Find the disease-related protein.

  - **Molecular Modeling**: Create a 3D model of the protein.

- **Virtual Screening**: Screen chemical libraries to find potential drugs.
- **Docking Studies**: Simulate how drugs bind to the protein.
- **Lead Optimization**: Improve drug candidates based on simulations.
- **Predictive Modeling**: Predict how the drug will behave in the body.


MOE
Builder
File Edit Select Render Protein Compute Window Help
SVL DBV SEQ
Cancel
System
Builder Fragments: 11 candidate structures created.
Val160
Lys161
Glu227
Ala159
PD173955
System Manager
Open
QuickPrep
Constrain
Close
Center
SiteView
Hydrogens
Hide
Show
Ligand
Surface
Measure
Builder
Sketch
Minimize
Select
Extend
Delete
Tautomer
Minimize
Add H
Fragments
Keep
R-Groups...
Save MDB...
-Me
-MeNH2
-MeOH
-Amide
-Acetyl
-Et
-H
-Acetic
-SMe
-COO-
-MeNH3+
Detach
Close
Amber10:EHT PBC
Atoms Ribbon Contacts Fog ZClip 2D

HIGH THROUGHPUT SCREENING (HTS)

- **High-Throughput Screening (HTS)** is a method that quickly tests many compounds or samples to find those that affect a specific biological target. It's mainly used in drug discovery and research.

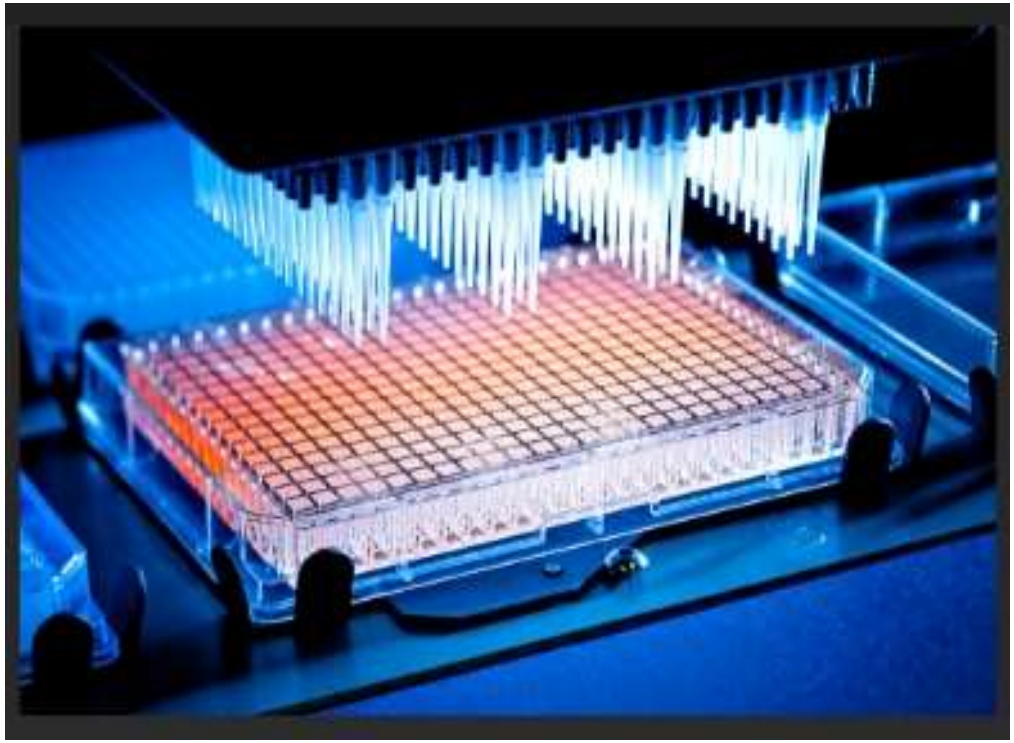
- **How It Works:**

- **Setup**: Researchers use robots to handle tests.

- **Testing Plates**: Compounds are placed in small wells on a microtiter plate, which can have thousands of wells. Each well might contain different chemicals, cells, or proteins.

- **Running the Test**: After adding the compounds, the plate is incubated to let reactions occur.

- **Observation**: Researchers use machines or sometimes manual methods to measure changes in each well. This helps identify which compounds have the desired effect.



TRAUMAMAN

- TraumaMan is a surgical simulation manikin used to teach medical professionals essential surgical skills, especially in trauma situations.

- It's widely adopted as an alternative to using animals in training. TraumaMan helps trainees practice procedures such as:

Cricothyroidotomy

Percutaneous tracheostomy

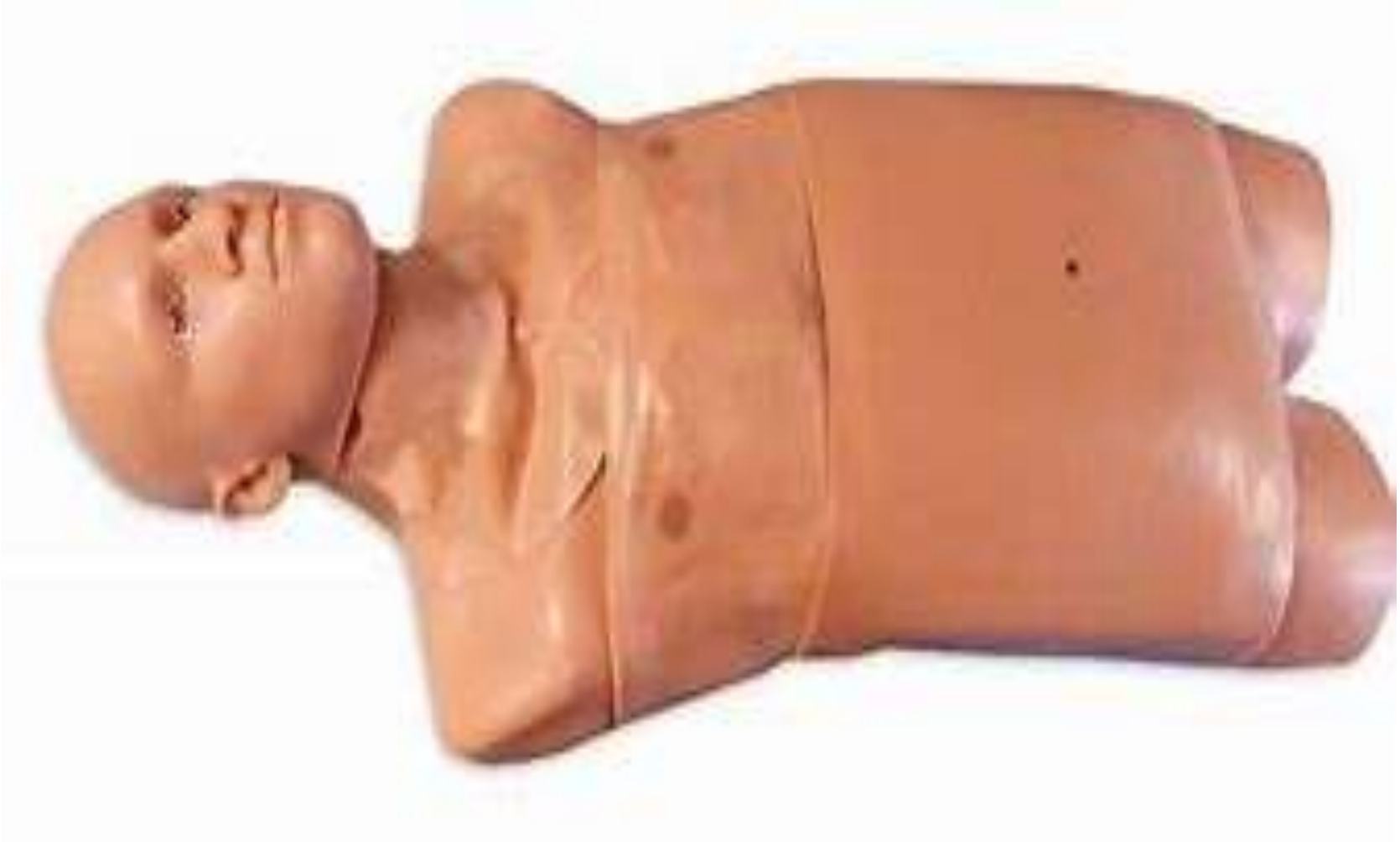
Needle decompression

Chest tube insertion

Pericardiocentesis

Diagnostic peritoneal lavage

- It's used in various programs, including the American College of Surgeons' Advanced Trauma Life Support (ATLS), and in training for combat situations.



CHEMTOX

- Chemtox is a tool used in toxicology to estimate the toxicological properties of chemicals/drugs.

- **How Chemtox Works:**

- **Input Data**: Provide the chemical structure in formats like SMILES, InChI, or molecular files.

- **Model Selection**: Chemtox uses various models, including historical data and algorithms, to estimate toxicity.

- **Prediction Process**

- **Acute Toxicity**: Harm from short-term exposure.
- **Chronic Toxicity**: Effects from long-term exposure.
- **Carcinogenicity**: Risk of cancer.
- **Mutagenicity**: Potential for genetic mutations.
- **Reproductive Toxicity**: Risks to reproductive health.

- **Output Results**: Provides risk scores or predictions for each toxicity type, aiding safety assessments.

WinSims

- WinSims is software for pharmacokinetic (PK) and pharmacodynamic (PD) modeling. It helps researchers and students predict how drugs behave in the body and understand their effects.

Key Features

Pharmacokinetic Modeling:

- **ADME Processes:** Simulates drug absorption, distribution, metabolism, and excretion.

- **Pharmacodynamic Modeling:**

- **Dose-Response:** Simulates drug effects based on concentration.

- **Mechanistic Models:** Models drug-target interactions like receptors or enzymes.

- **Advantages:**

- **Cost-Effective:** Reduces the need for expensive experiments.

- **Ethical:** Minimizes the use of animal testing.

- **Flexible:** Customizable for various research and educational needs.

- **Drug Development:**

  - **Predicts PK/PD:** Aids in designing dosage forms and planning clinical trials.

- **Academic Use:**

  - **Teaching Tool:** Facilitates hands-on learning in education.

EXPHARM

IN VITRO METHODS

- "In vitro" refers to experiments conducted outside a living organism, typically in a controlled laboratory environment.

A. Organ on chip
B. Organoids
C. In vitro pyrogen test
D. Embryonic stem cell test (EST)
E. Neutral red uptake (NRU) assay
F. Carcinogenicity test
G. Acute toxicity test
H. Repeated dose toxicity test
I. Developmental neurotoxicity test (DNT)

ORGAN ON CHIP

- Organan-on-a-chip (OOC) technology is an innovative approach to mimicking human organ functions in the lab.

What are Organ-on-a-Chip (OOC) Devices?

- **Miniature Models of Organs:**OOCs are small devices that simulate the environment and functions of human organs like the lung, heart, or kidney more accurately than traditional methods.

- **Use of Human Cells:**OOCs use human cells, providing a realistic representation of human organ function compared to using animal cells.

10/08/2024

- **Microfluidic Technology:**These devices have tiny channels that allow fluids to flow, simulating blood flow and creating a realistic environment for cells.

- **Advantages**

- **Realistic Models:**OOCs mimic complex 3D structures and dynamic environments of organs, unlike flat petri dish cultures.

- **Better Disease Models:**They help researchers study diseases more accurately, enhancing our understanding of disease effects on organs.

- **Drug Testing and Development:**OOCs allow for testing drug effects on human-like organ models, leading to safer and more effective medicines.

- **Controlled Experiments:**They offer precise control of conditions like fluid flow and mechanical forces, allowing for accurate studies of biological processes.

- **Multiple Cell Types**: To accurately mimic an organ, OOCs often use multiple types of cells. For example, a lung-on-a-chip might use both airway and blood vessel cells.

ORGANOIDS

- **Miniature Organs:**Organoids are small, lab-grown models of organs made from stem cells. They mimic the structure and function of real organs more accurately than traditional cell cultures.

- **3D Structures:** They form three-dimensional structures, resembling real organ tissues with diverse cell types.

- **How Are Organoids Developed?**

- **Stem Cells:** Created from pluripotent or adult stem cells that can develop into various cell types.

- **Growth Factors:** Specific conditions and growth factors guide stem cells to form organ-like structures.

- **Self-Organization:** Cells naturally organize into complex, 3D structures similar to real organs.

Advantages

- **Disease Modeling:** Useful for studying diseases like cancer and neurological disorders with realistic organ simulations.

- **Personalized Medicine:** Allows for testing drug responses in patient-derived models, leading to customized treatments.

- **Drug Discovery:** Provides a better platform for testing new drugs, reducing the need for animal testing.
- **Regenerative Medicine:** Potential to create replacement tissues for damaged organs.
- **Functional Complexity:** Shows real organ functions, like neuronal activity or mucus production.
- **Scalability:** Can be grown in large numbers for extensive research.

IN VITRO PYROGEN TEST

- A number of alternative cellular assays have been developed, such as Limulus amoebocyte lysate (LAL) test, monocyte activation test (MAT), etc. replacing the animal rabbit pyrogen test.

- All the test-system are based on the response of human leukocytes (primarily monocytes) that release inflammatory mediators (endogenous pyrogens) in response to pyrogenic contamination (exogenous pyrogens).

LIMULUS AMOEBOCYTE LYSATE TEST

- **Principle**

- The LAL test uses the blood of the horseshoe crab as a lal reagent. (*Limulus polyphemus*).
- It detects lipopolysaccharides (LPS), which are components of the outer membrane of Gram-negative bacteria.
- When LPS is present, it causes the blood to coagulate (clot).

- **Advantages**

- **Sensitivity**: More sensitive than the rabbit pyrogen test.

Disadvantage

- **False Negatives**: Some products can give false negative results.
- **Overestimation**: May overestimate the pyrogen content in certain products.
- **Limited Detection**: Only detects bacterial endotoxins from Gram-negative bacteria; it does not detect pyrogens from Gram-positive bacteria, viruses, or fungi.

MONOCYTE ACTIVATION TEST (MAT)

- **Principle**

- The MAT uses human mononuclear cells, such as monocytes, from human volunteers or blood banks.

- These cells release inflammatory mediators (endogenous pyrogens) in response to pyrogens (exogenous pyrogens) in the sample.

- **Advantages**

- It can detect a pyrogens which are not detected by LAL test or Rabit pyrogen test.

EMBRYONIC STEM CELL TEST (EST)

- **Principle**

- The EST uses mouse embryonic stem (ES) cells to test for embryonic toxicity.

- These stem cells can develop into different types of cells, including contracting heart cells (cardiomyocytes), which are used as a measure in the test.

- **Inhibition of Differentiation**:
  - The test checks if a substance prevents the stem cells from developing into cardiomyocytes. If differentiation is inhibited, it suggests potential embryotoxicity.

NEUTRAL RED UPTAKE (NRU) ASSAY

- The Neutral Red Uptake (NRU) assay is a lab test used to evaluate the potential of chemicals to cause eye irritation, serving as an alternative to the Draize test, which uses live animals.

- **Cell Models Used:** Conducted on cultured cells like human epidermal keratinocytes, mouse fibroblasts, and rabbit corneal cells.

- **Neutral Red Dye:** A dye that enters living cells and accumulates in lysosomes.

- **Measuring Cell Viability:** The assay measures cell uptake of the dye. Toxic chemicals inhibit dye uptake, indicating cell damage or death.

- **Other alternatives of rabit draize tests are:**

- EpiOcular Tissue Models
- Isolated Chicken Eye (ICE) Test
- Isolated Rabbit Eye (IRE) Test
- Bovine Cornea Opacity and Permeability (BCOP) Test

CARCINOGENICITY TESTS

- Carcinogenicity tests are used to determine whether a substance can cause cancer, focusing on non-genotoxic mechanisms (not directly damaging DNA).

- **Key In Vitro Tests:**

- **Cell Transformation Assays**: These tests evaluate how substances can induce cancer-like changes in cells without directly damaging DNA.

- **Syrian Hamster Embryo (SHE) Assay**: It can detect the both genotoxic and epigenetic potential of the compound.

Advantages of the SHE Assay:

- **High Sensitivity**: The SHE assay can detect 96% of known human carcinogens, both genotoxic and non-genotoxic.

- **Efficiency**: It is faster (taking about six weeks) and less costly compared to traditional rodent bioassays.

- **Reduced Animal Use**: The SHE assay uses fewer animals (up to eight embryos) compared to the 800 or more animals required in standard bioassays.

ISOLATED HUMAN OR ANIMAL LIVER MICROSOME

- **What They Are:**

- Liver microsomes are subcellular structures from liver cells that contain enzymes like cytochrome P450, essential for drug metabolism.

- **How They Work:**

- **Isolation:** Microsomes are extracted from liver tissue using centrifugation.
- **Incubation with Drugs:** The microsomes are mixed with test drugs.
- **Metabolism:** Enzymes in the microsomes metabolize the drugs, mimicking liver processing.

- **Analysis:** Scientists analyze the resulting metabolites to understand drug metabolism.

- **Advantages:**

- **In Vitro Testing:** They offer a way to study drug metabolism without using live animals.

- **Human Relevance:** They provide insights into human liver drug processing, crucial for drug safety evaluations.

ACUTE TOXICITY TEST

- Acute toxicity tests evaluate how harmful a substance can be in a short time, traditionally using animals.

- New methods focus on non-animal approaches to determine toxicity at the cellular level.

- **Key Points:**

- **Cell-Based Tests**: These tests use cultured human cells, such as normal human keratinocytes (NHK), to assess toxicity at the cellular level.They aim to replace animal testing by evaluating how chemicals affect cells.

REPEATED DOSE TOXICITY TEST

- Repeated dose toxicity tests evaluate the effects of a substance when it's repeatedly administered over time.

- Traditionally, these tests used animals to study how a chemical is absorbed, distributed, metabolized, and eliminated by the body.

- **New Approaches:**

- **Biokinetic Modeling**: Computerized models predict how a chemical distributes itself in the body, helping to identify which organs might be affected (e.g., brain, liver, kidneys).

- **Organ-Specific Cell Cultures**: Predictions from biokinetic modeling can be verified using cell cultures from specific organs, allowing researchers to study the effects on specific tissues.

- **Advantages:**

- **Reduces Animal Testing**: Combining biokinetic modeling with organ-specific in vitro assays can replace the need for repeated dose studies in animals.

- **Targeted Insights**: This approach provides detailed insights into how chemicals may affect different organs, making it more precise and humane.

DEVELOPMENTAL NEUROTOXICITY TEST

- Developmental neurotoxicity tests (DNT) assess how chemicals affect the developing nervous system.

- New methods aim to reduce costs and animal use while allowing for large-scale screening of chemicals.

- **Key Approaches:**

- **Alternative Species**: Use non-mammalian species with similar brain development processes to mammals, reducing the reliance on traditional animal models.

- **In Vitro Cell Culture Models**: Using cell cultures or organoids derived from stem cells to study the effects of substances on neuronal development and function.

- **Benefits of In Vitro Tests:**

- **Specificity and Sensitivity**: Accurately detect how chemicals affect brain development.

- **Reliability and Reproducibility**: Provide consistent results across different tests.

- **Accuracy and Predictivity**: Offer reliable predictions about a chemical's impact on brain development.

LOCAL LYMPH NODE ASSAY (LLNA)

- **Principle**

- The LLNA is based on the idea that if a substance is a sensitizer, it will cause the lymph nodes near the site of application to produce more lymphocytes.

- This increase in lymphocyte production can be measured using radioactive labeling, providing an objective and quantitative way to assess skin sensitization.

Process

- **Application**:
  - The test compound is applied to the skin of a test subject (usually mice).

- **Lymphocyte Proliferation**:
  - If the compound is a sensitizer, it will cause the lymph nodes draining the application site to produce more lymphocytes.
  - The level of lymphocyte proliferation is proportional to the dose of the test compound applied.

- **Measurement**:
  - The proliferation of lymphocytes is measured and compared to a control group that receives a vehicle.

Key Concepts

- **Stimulation Index (SI)**:
  - The ratio of lymphocyte proliferation in the treated group compared to the control group is calculated.
  - A stimulation index (SI) of at least 3 indicates that the substance is a potential skin sensitizer.

Conclusion

- The LLNA is a quantitative method for assessing skin sensitization potential of substances, helping ensure product safety for skin contact.

CLINICAL SKIN PATCH TEST

- **Principle**

- Clinical patch tests involve applying a small amount of a test substance to the skin of human volunteers, typically on the back or forearm.

- The test is designed to check for any signs of skin irritation or sensitization.

- **Other tests:**
- Human Skin Equivalent Tests
- Corrositex
- Human Epidermal Keratinocyte Culture

Steps in a Clinical Patch Test

Preliminary Safety Checks:

- Confirm the substance is non-corrosive using in vitro tests.
- Ensure it shows negative results in mutagenicity tests.

Application:

- Apply a patch with the test substance to the volunteer's skin for 24 to 48 hours.

Observation:

- Check for irritation or allergic reactions like redness, swelling, or rash after removing the patch.

- **Interpretation of Results:**

- **Positive Response:** Visible skin reaction suggests potential irritation or sensitization.
- **Negative Response:** No skin reaction indicates the substance is likely non-irritating and safe.

- **Advantages:**

- **Relevance to Humans:** Directly applicable to human skin reactions.
- **Alternative to Animal Testing:** Provides an ethical and accurate option to animal testing.

ZEBRA FISH AS AN ALTERNATIVE

- **Advantages**

- **Genetic Similarity:** Zebrafish share about 70% of their genes with humans, and 84% of human disease genes have zebrafish counterparts.

- **Rapid Development:** Zebrafish embryos develop quickly, with major organs forming within 24 hours and fully developed larvae by 5 days.

- **Transparent Embryos:** Their transparent embryos allow easy observation and manipulation of development processes without invasive techniques.

- **Genetic Manipulation:** Techniques like CRISPR-Cas9 enable easy genetic modifications, helping study gene functions and disease mechanisms.

- **Cost-Effective:** Zebrafish are cheaper and require less space than other models like mice, allowing for large-scale studies.

- **Regenerative Abilities:** Zebrafish can regenerate organs like the heart and spinal cord, useful for studying regeneration.

Research Applications

- **Developmental Biology:** Study of embryogenesis and organ development.
- **Cancer Research:** Modeling tumor development and screening drugs.
- **Cardiovascular Research:** Investigating heart development and diseases.
- **Neuroscience:** Exploring brain development and neurodegenerative diseases.

FLY AS AN EXPERIMENTAL MODEL

- Drosophila commonly known as the fruit fly, is a widely used model organism in scientific research due to its numerous advantageous characteristics.

Advantages of Drosophila as a Model Organism

- **Genetic Similarity:** Drosophila shares about 60% of its genes with humans, including many genes associated with human diseases.

- **Short Life Cycle:** They have a rapid life cycle, with development from egg to adult taking about 10 days at room temperature, allowing for quick generation turnover and studies on genetics and evolution.

- **Cost-Effective:** They are inexpensive to maintain and require minimal space and resources compared to vertebrate models.

Benefits of Alternative Methods to Animal Testing

- **Animal Welfare:**
  - They save animal lives and prevent suffering.
- **Efficiency and Reliability:**
  - These methods provide faster results.
  - They are often more reliable since they avoid species differences that can affect results.
- **Cost-Effectiveness:**
  - They are usually cheaper than animal testing.

- **Less time consuming**
  - Alternative methods take less time compared to traditional tests.

- **Environmental Friendliness:**
  - Products tested with these methods are seen as more eco-friendly and are in higher demand.

- **Improved Relevance to Humans:**
  - They are often more applicable to human biology, minimizing issues related to species differences.

ORGANIZATIONS SUPPORTING ALTERNATIVES TO ANIMAL TESTING

- Centre for Alternatives to Animal Testing
- UC Davis Center for Animal Alternatives
- Physicians Committee for Responsible Medicine
- Dr. Hadwen Trust
- National Centre for the Replacement, Refinement, and Reduction of Animals in Research
- Canadian Council on Animal Care Three Rs Microsite
- Alternatives to Animal Experimentation Laboratory, Jawaharlal Nehru Medical College
- Mahatma Gandhi-Doerenkamp Centre for Alternatives to the Use of Animals in Life Science Education, India

REFERENCES

1. Sougata sarkar,postgraduate pharmacology.Paras medical publisher.1st edition.Page:281-286
2. Ma C, Peng Y, Li H, Chen W. Organ-on-a-Chip: A New Paradigm for Drug Development. Trends in Pharmacological Sciences. 2021 Feb;42(2):119–33.
3. Bastikar VA, Alpana Bastikar, Gupta PP. Quantitative structure–activity relationship-based computational approaches. Elsevier eBooks. 2022 Jan 1;191–205.
4. Gu R, Wu F, Huang Z. Role of Computer-Aided Drug Design in Drug Development. Molecules [Internet]. 2023 Jan 1 [cited 2023 Dec 7];28(20):7160. Available from: https://www.mdpi.com/1420-3049/28/20/7160#:~:text=The%20computer-aided%20drug%20design%20method%20utilizes%20the%203D


Thank You!