ANIMAL EXPERIMENTS FOR DRUG DEVELOPMENT

Dr. Sudershan K Arora; President R&D,
Dr. Vyas M Shingatgeri, Vice-President; DSE
Ranbaxy Laboratories Limited,
Gurgaon

Through out history, scientists have been solving medical problems, developing new techniques and treatments, and curing diseases –

ALL BY USING ANIMALS OR ANIMAL TISSUES

Animal research helped relieving human suffering

- Louis Pasteur and Robert Koch : First to initiate the studies on animals (Hendriksen, Dev. Biol. Stand. 1996).
- Diphtheria Research: Quality control of immuno-biologicals arose from diphtheria research.
- Vaccination for Polio, mumps, measles, rubella, pertussis and Hepatitis
- Treatment for asthma, burns, leukemia, newborn sickness
- Antibiotics for various bacterial infections

Our children have not even heard of many of the diseases our ancestors experienced first – hand.
WHY?
They have been eradicated or can be controlled

Why are animal experiments essential

Animal use is indispensable

Fundamental reasons:

- Scientific
  - understanding of disease process
  - discover novel ways to treat
  - evaluate safety of the human, animals and environment
- Comply Regulatory requirements
- Meet social obligations

Why are animal experiments essential

Scientific Reasons

- Animals are similar to humans – similar anatomies, comparable physiologies, hormones, digestive regulations and reproductive cycles
- Shorter lifespan – can be studies for effects over entire life cycle
- Easily controllable environment reduces stress and variability to and increases predictability
- Due to complexity of the vertebrate organism, there are many in-vitro assays that are neither validated nor are relevant proposals ready for pre-validation/validation, available

[Worth et al. 2002; Rogiers 2002b, Rogiers and Pauwels 2005]

Why are animal experiments essential

Regulatory Requirements

Duration of Repeated Dose Toxicity Studies to Support Phase I and II Trials in EU and Phase I, II and III Trials in the US and Japan*

Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies	
	Rodents	Non-rodents
Single Dose	2 Weeks**	2 Weeks
Up to 2 Weeks	2 Weeks**	2 Weeks
Up to 1 Month	1 Month	1 Month
Up to 3 Months	3 Months	3 Months
Up to 6 Months	6 Months	6 Months***
> 6 Months	6 Months	Chronic***

* In Japan, if there are no Phase II clinical trials of equivalent duration to the planned Phase III trials, conduct of longer duration toxicity studies is recomennded as given in Table 2.

** In the US, as an alternative to 2 week studies, single dose toxicity studies with extended examinations can support single-dose human trials (4).

*** See (11). Data from 6 months of administration in non-rodents should be available before the initiation of clinical trials longer than 3 months. Alternatively, if applicable, data from a 9 month non-rodent study should be available before the treatment duration exceeds that which is supported by the available toxicity studies.

ICH, 2000

Why are animal experiments essential

Route of administration	Duration of proposed human administration	Human Phase(s) for which study is proposed to be conducted	Long term toxicity requirements
Oral or Parenteral or Transdermal	Single dose or several doses in one day, Upto 1wk	I, II, III	2sp,2wk
	> 1 wk but upto 2wk	I, II, III	2sp;4wk
	> 2 wk but upto 4wk	I, II, III	2sp;12wk
	Over Imo	I, II, III	2sp;24wk
Inhalation (general anaesthetics, aerosols)	Upto 2 wk	I, II, III	2sp;1 mo; (Exposure time 3h/d, 5d/wk)
	Upto 4wk	I, II, III	2sp;12wk, (Exposure time 6h/d, 5d/wk)
	> 14wk	I, II, III	2sp;24wk, (Exposure time 6h/d, 5d/wk)
Local Toxicity Studies			
Dermal	Upto 2 wk	I, II	1sp;4wk
		III	2sp,4wk
	> 2 wk	I, II, III	2sp;12wk
Ocular or Otic or Nasal	upto 2 wk	I, II	1 sp;4wk
		III	2sp,4wk
	> 2 wk	I, II, III	2sp;12wk
Vaginal or Rectal	Upto 2 wk	I, II	1 sp;4wk
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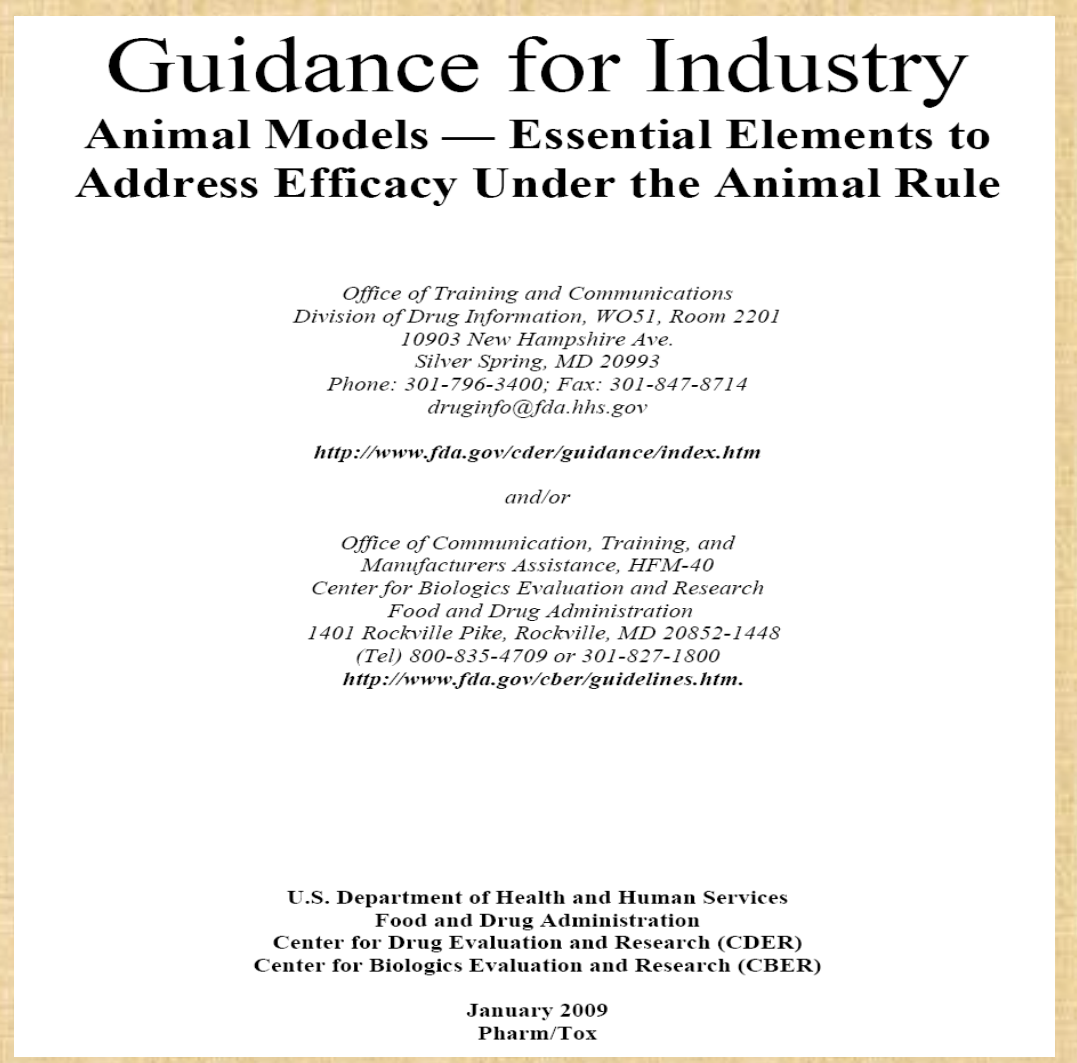
Schedule Y, 2006

Regulatory Requirements: In vivo Efficacy



Guidance for Industry

Animal Models — Essential Elements to Address Efficacy Under the Animal Rule

Office of Training and Communications
Division of Drug Information, WO51, Room 2201
10903 New Hampshire Ave.
Silver Spring, MD 20993
Phone: 301-796-3400; Fax: 301-847-8714
druginfo@fda.hhs.gov

http://www.fda.gov/cder/guidance/index.htm

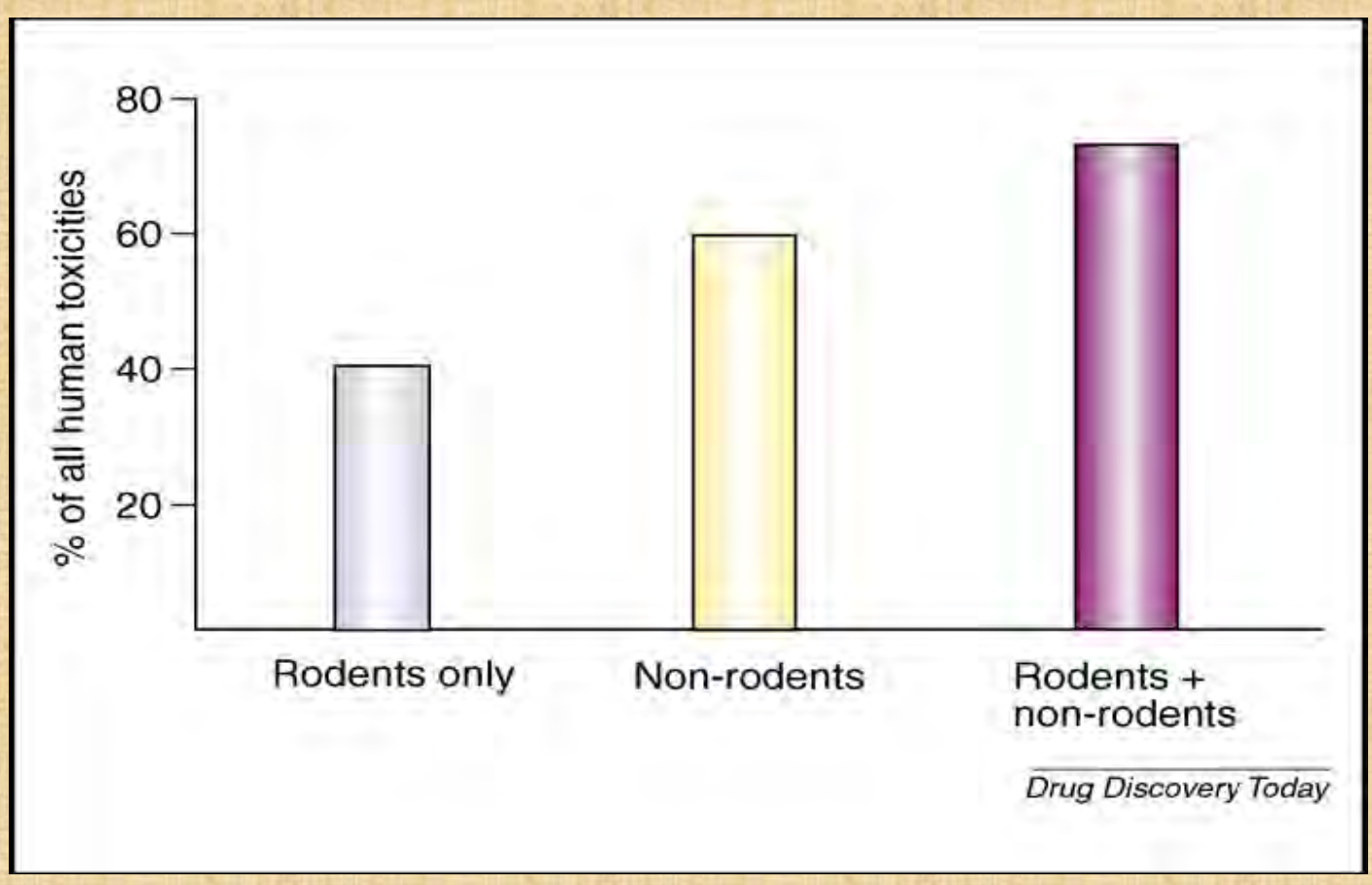
and/or

Office of Communication, Training, and
Manufacturers Assistance, HFM-40
Center for Biologics Evaluation and Research
Food and Drug Administration
1401 Rockville Pike, Rockville, MD 20852-1448
(Tel) 800-835-4709 or 301-827-1800
http://www.fda.gov/cber/guidelines.htm.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2009
Pharm/Tox

Why are animal experiments essential



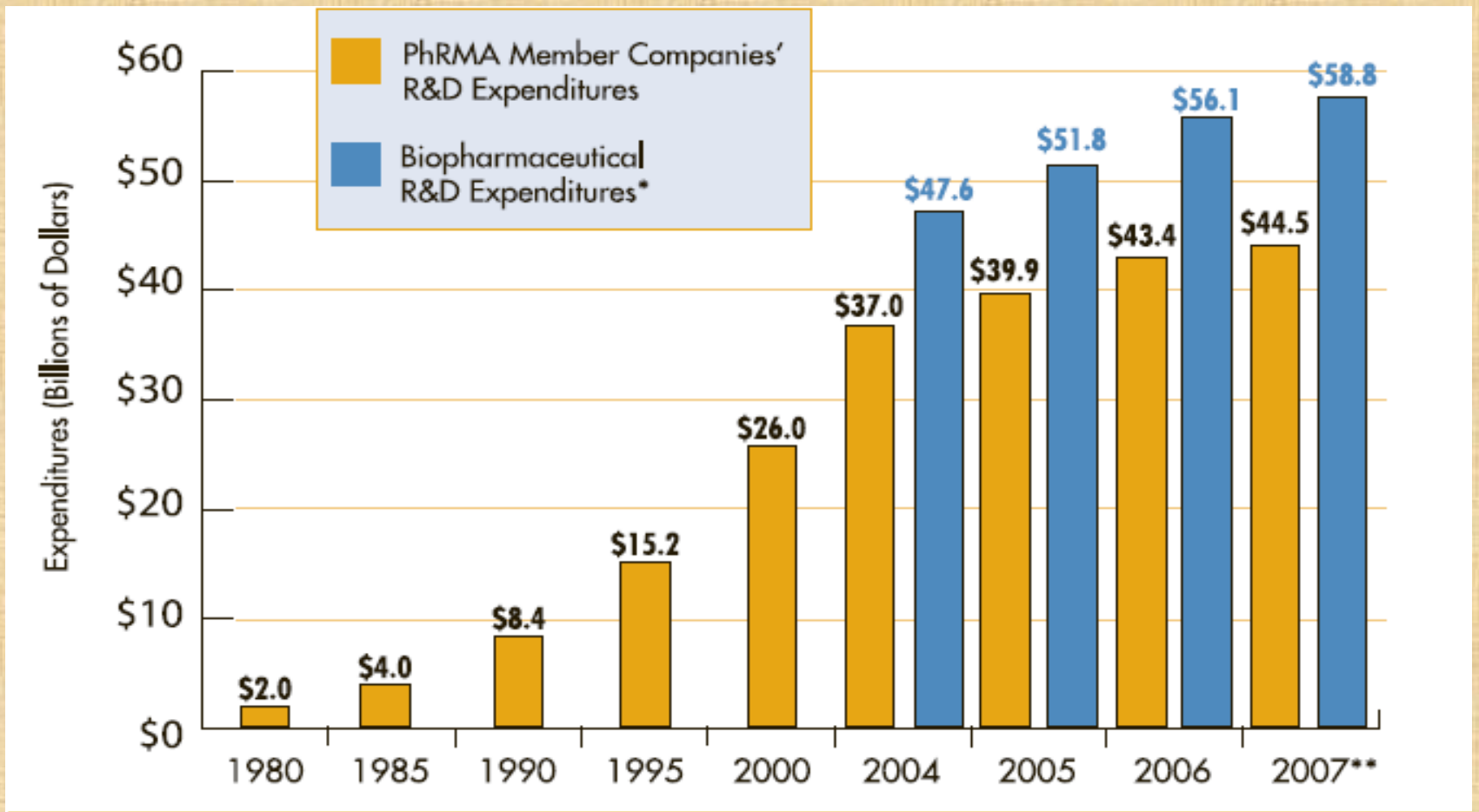
Prediction of all human toxicities from preclinical toxicology studies. Based on the review of 150 pharmaceuticals, it has been estimated that two-species (one rodent and one non-rodent) toxicology provides the best correlation

Predictivity of side effects by using various animal models

Animal model	Agent	Effect	In Man
Rabbit	Thalidomide	Phocomelia	N/Y
Rat, rabbit, dog, primate	Accutane	Developmental toxicity of CNS (Neural Tube Defects)	Y
Dog, rat, monkey	Azidothymidine (AZT)	Bone Marrow Depression	Y
Rat, Mouse, rabbit	Valproic acid	Cleft palate	Y
Rat, monkey	Cyclosporine	Nephropathy Reversible immunosuppression	Y
Monkey	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)	Parkinsonism	Y
Rat, dog	Cyclophosphamide	Hemorrhagic cystitis	Y
Rat, monkey	Methyl Mercury	Encephalopathy	Y
Rat, dog	Diethyl glycol	Nephropathy	Y
Mouse	Razoxin	Myelomonocytic leukemia	Y

Animal Models in Toxicology - S. Gad

A Costly Affair



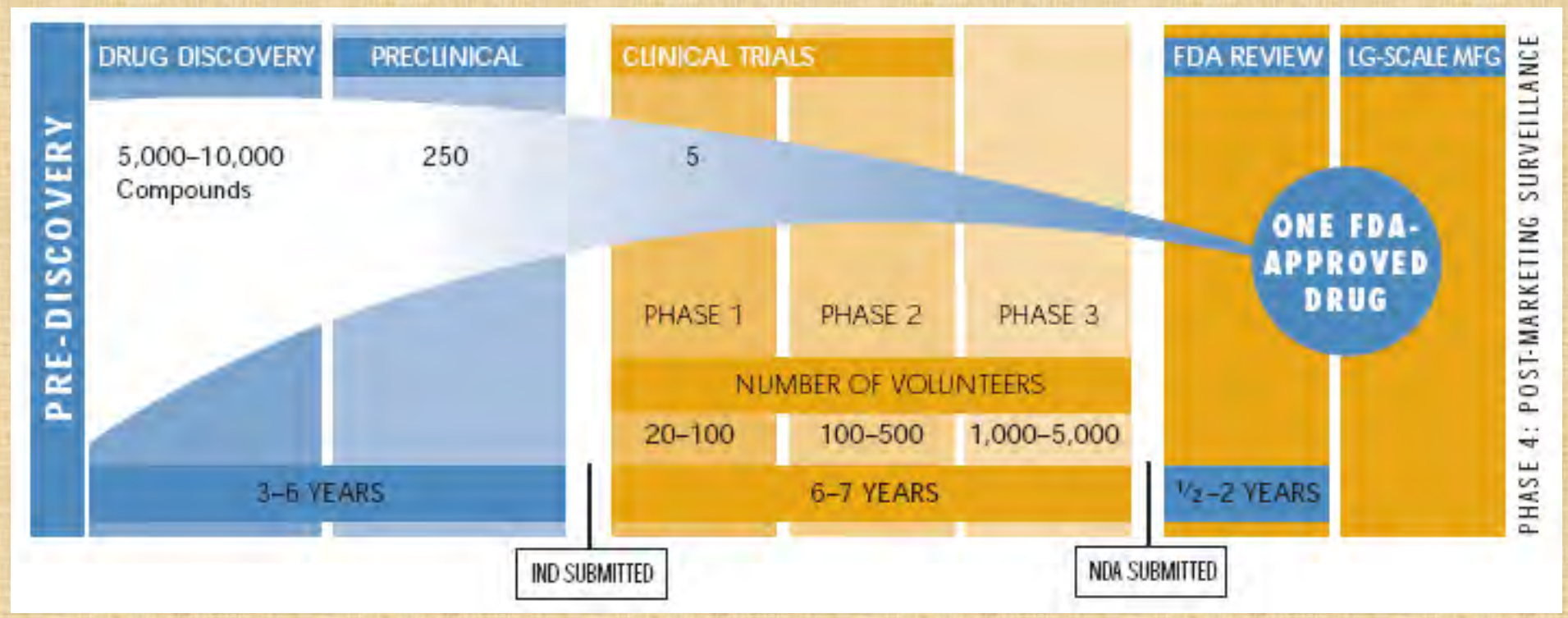
Sources: Burrill & Company, analysis for Pharmaceutical Research and Manufacturers of America, 2008; and Pharmaceutical Research and Manufacturers of America, *PhRMA Annual Member Survey* (Washington, DC: PhRMA, 2008).

*The "Biopharmaceutical R&D" figures include PhRMA research associates and nonmembers; these are not included in "PhRMA Member Companies' R&D Expenditures." PhRMA first reported this data in 2004.

** Estimated.

Drug Development Takes Longer Than it Did In The Past

Developing a new medicine takes an average of 10–15 years; the Congressional Budget Office reports that "relatively few drugs survive the drug devpt. process"



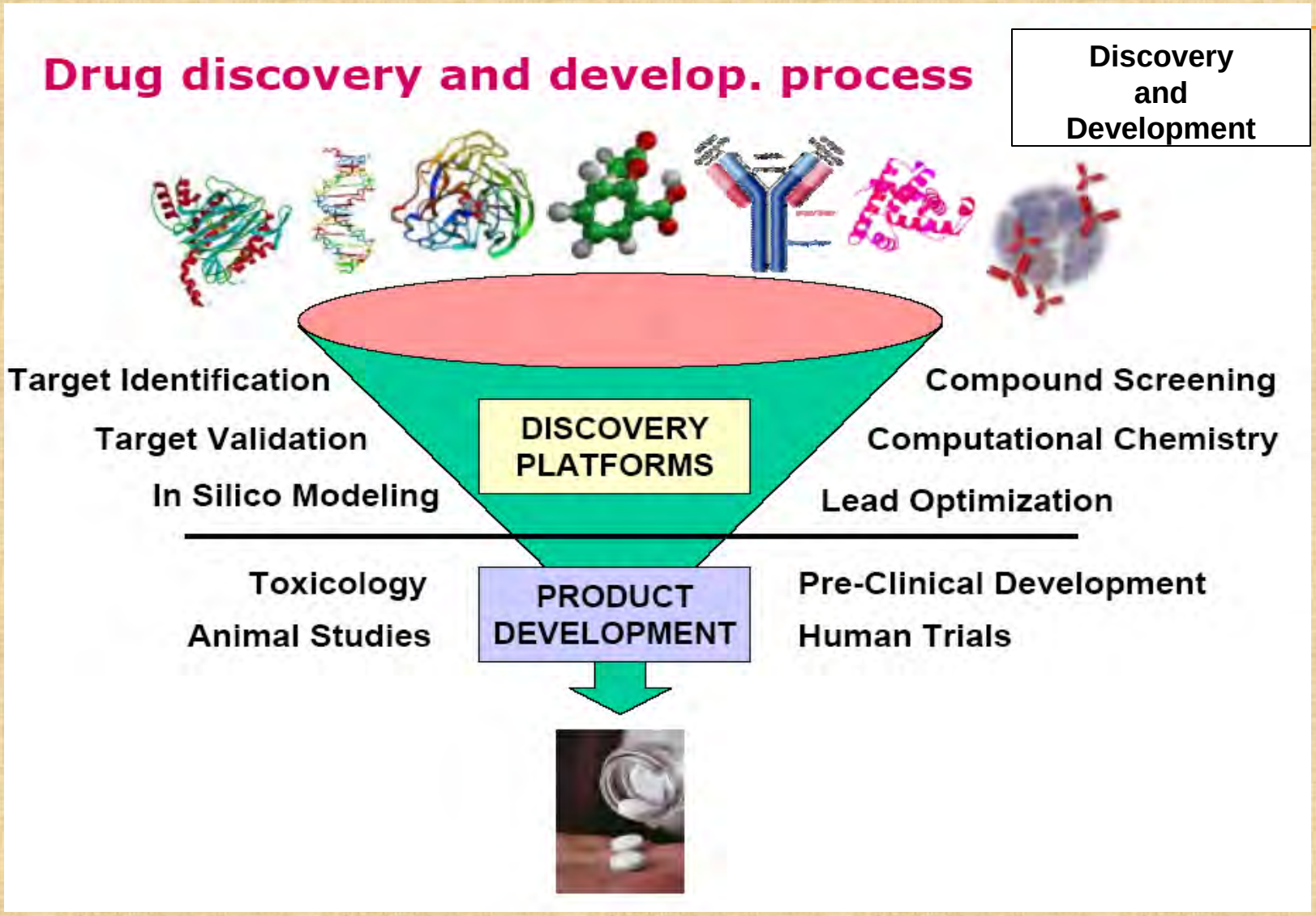
Sources: Drug Discovery and Development: Understanding the R&D Process, www.innovation.org; CBO, *Research and Development in the Pharmaceutical Industry*, 2006.

Reasons for Failure in Drug Development

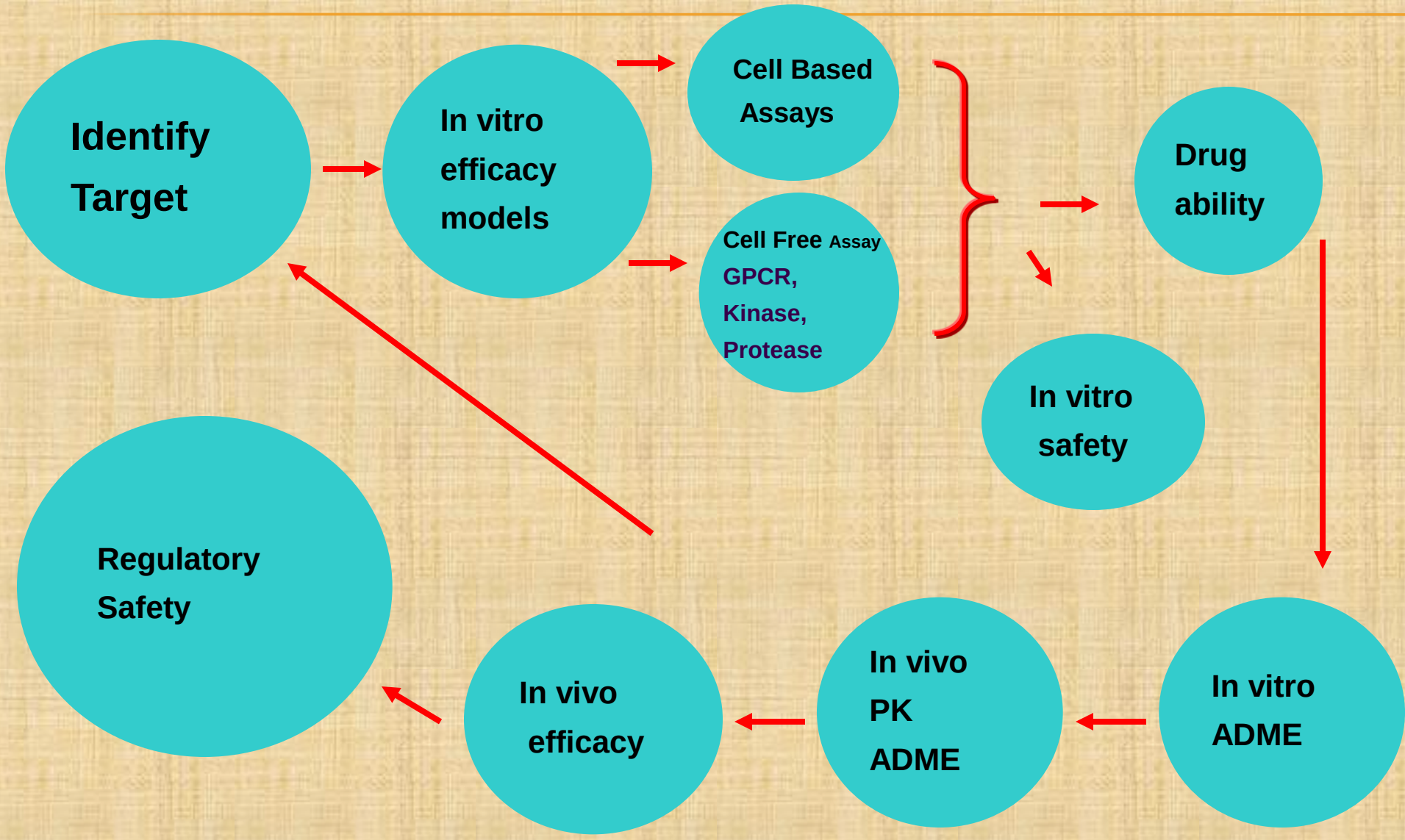
Table 1. Survey of Reasons for Failure of Compounds in Development

Industry Median	Preclinical	Phase I	Phase II	Phase III	Registration
Clinical Safety	0.5%	27.9%	13.4%	9.8%	30.0%
Efficacy	5.6%	17.5%	52.0%	72.5%	20.0%
Formulation	5.1%	5.8%	1.6%	0.0%	0.0%
Market potential	6.2%	3.9%	7.9%	3.9%	30.0%
PK/bioavailability	11.8%	14.9%	2.4%	0.0%	0.0%
Strategic	14.4%	12.3%	13.4%	5.9%	20.0%
Resources	1.5%	1.3%	0.8%	3.9%	0.0%
Toxicology	44.1%	10.4%	2.4%	3.9%	0.0%
Cost of goods	1.5%	1.3%	0.0%	0.0%	0.0%
Unknown	7.2%	1.3%	4.7%	0.0%	0.0%
Other	2.1%	3.2%	1.6%	0.0%	0.0%
Number of projects	195	154	127	51	10

Drug Discovery and Development



SCREENING NCE'S



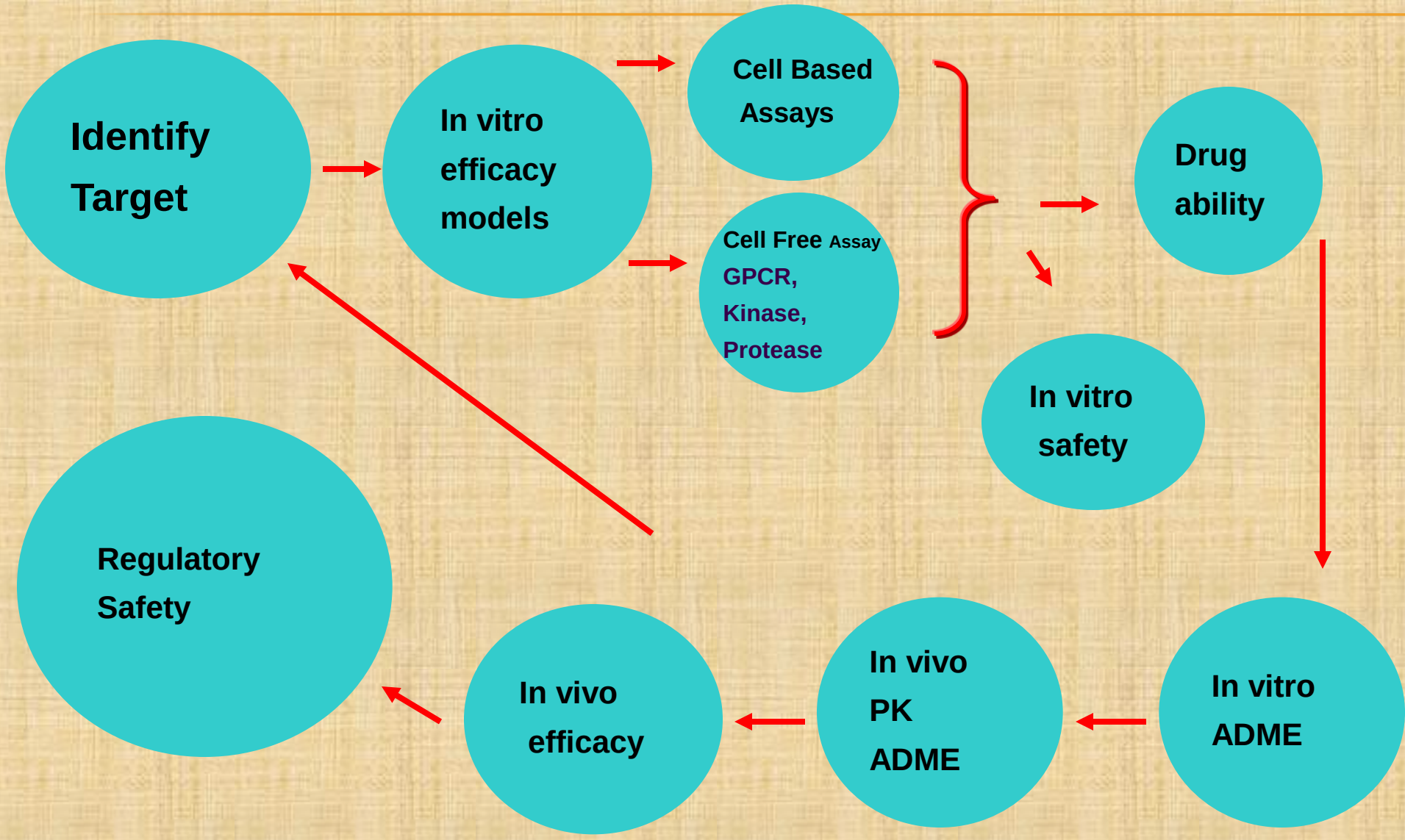
TARGETS

- Enzymes
- Receptors
- Proteins

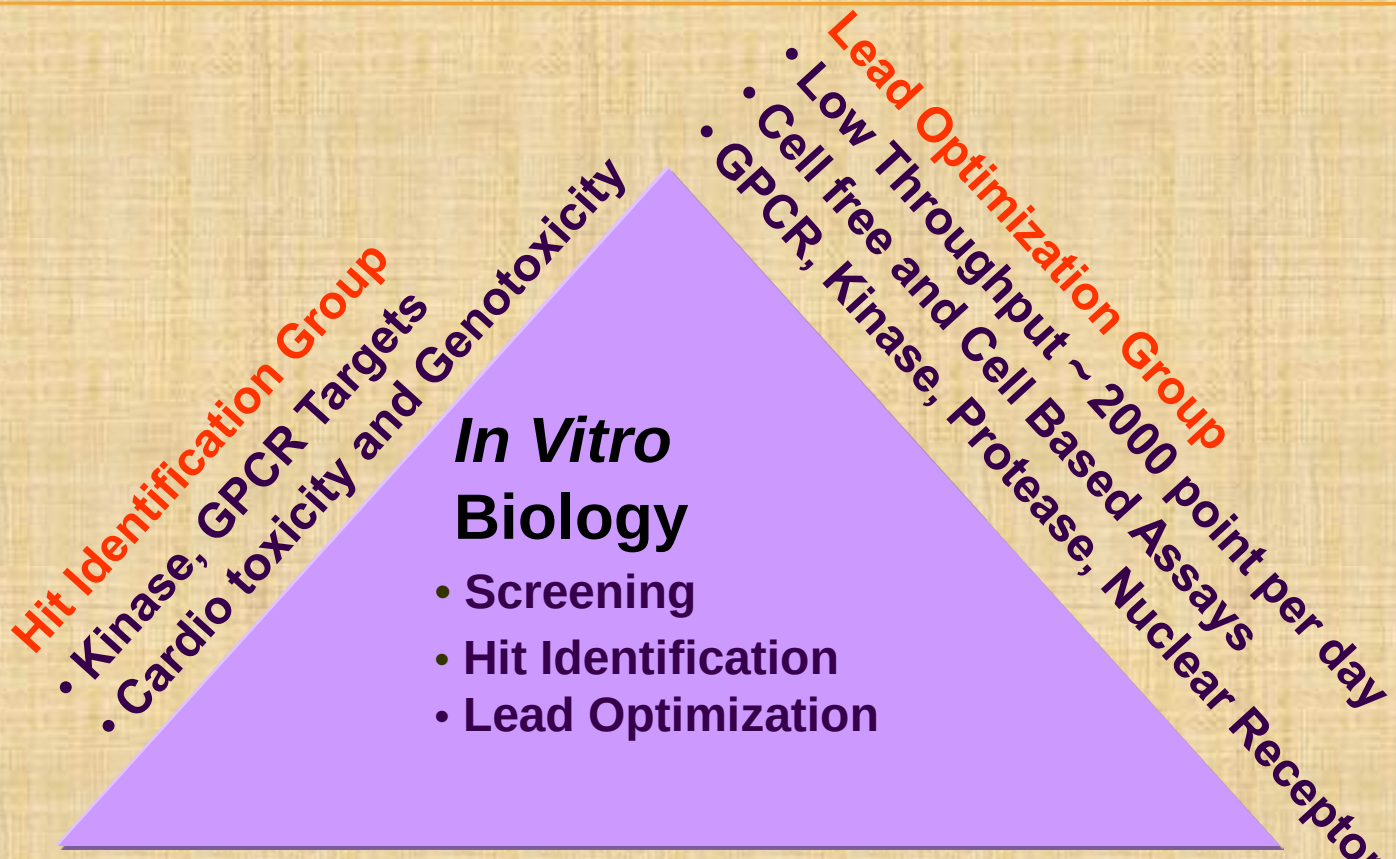
Suspected to have a role in disease

The goal is to find molecules that bind to these receptors and thus could be basis of future drugs

SCREENING NCE'S



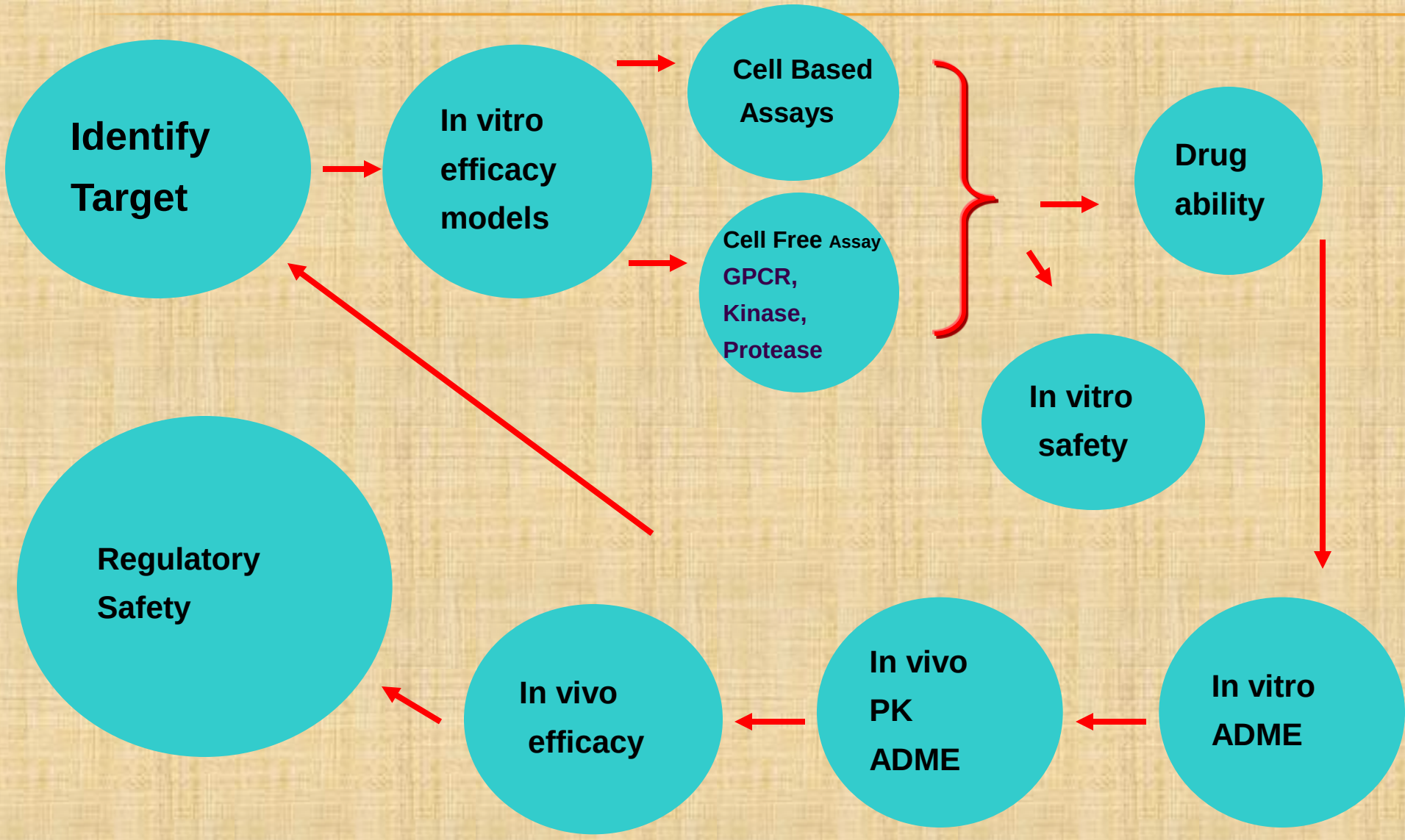
In Vitro efficacy models / Biology



Isolated Tissue Experiments

- **Biological response in a multicellular system**
- **Potency and Efficacy of NCEs**

SCREENING NCE'S



Discovery Screens for drug ability properties

Metabolism

- **Substrates**
  - Homogenates
  - S9
  - Microsomes
  - Hepatocytes
  - CYP substrates (Human 1A2, 2C9, 2C19, 2D6, 3A4)

  Rat, mouse, dog, human
- **Inhibitors**
  - CYP inhibitors (Human 1A2, 2C9, 2C19, 2D6, 3A4)

Absorption

- Caco-2 (human)
- Intestinal permeability (rat)

In silico

- clogP
- BBB penetration

Molecular Modeling (Med Chem)

In vivo

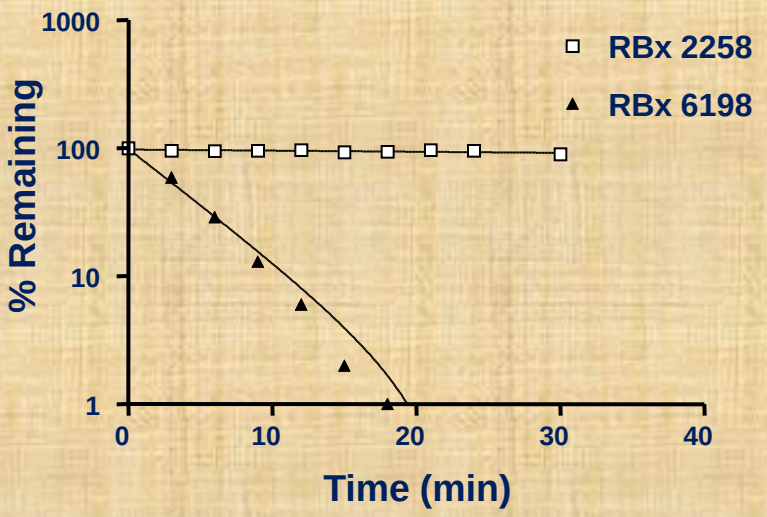
- PK in Mouse, Rat, Dog (& monkey on contract)
- Bile Duct Cannulated rat, tissue levels

Analytical

- API-4000s for quantitation
- Q-Trap for Met ID
- HPLCs

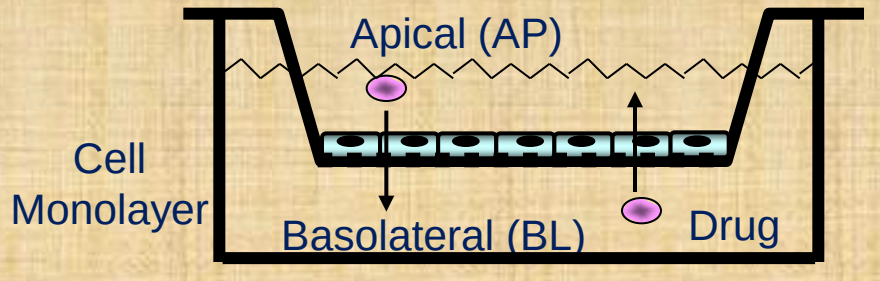
In vitro techniques for lead optimization

Intrinsic Clearance in microsomes



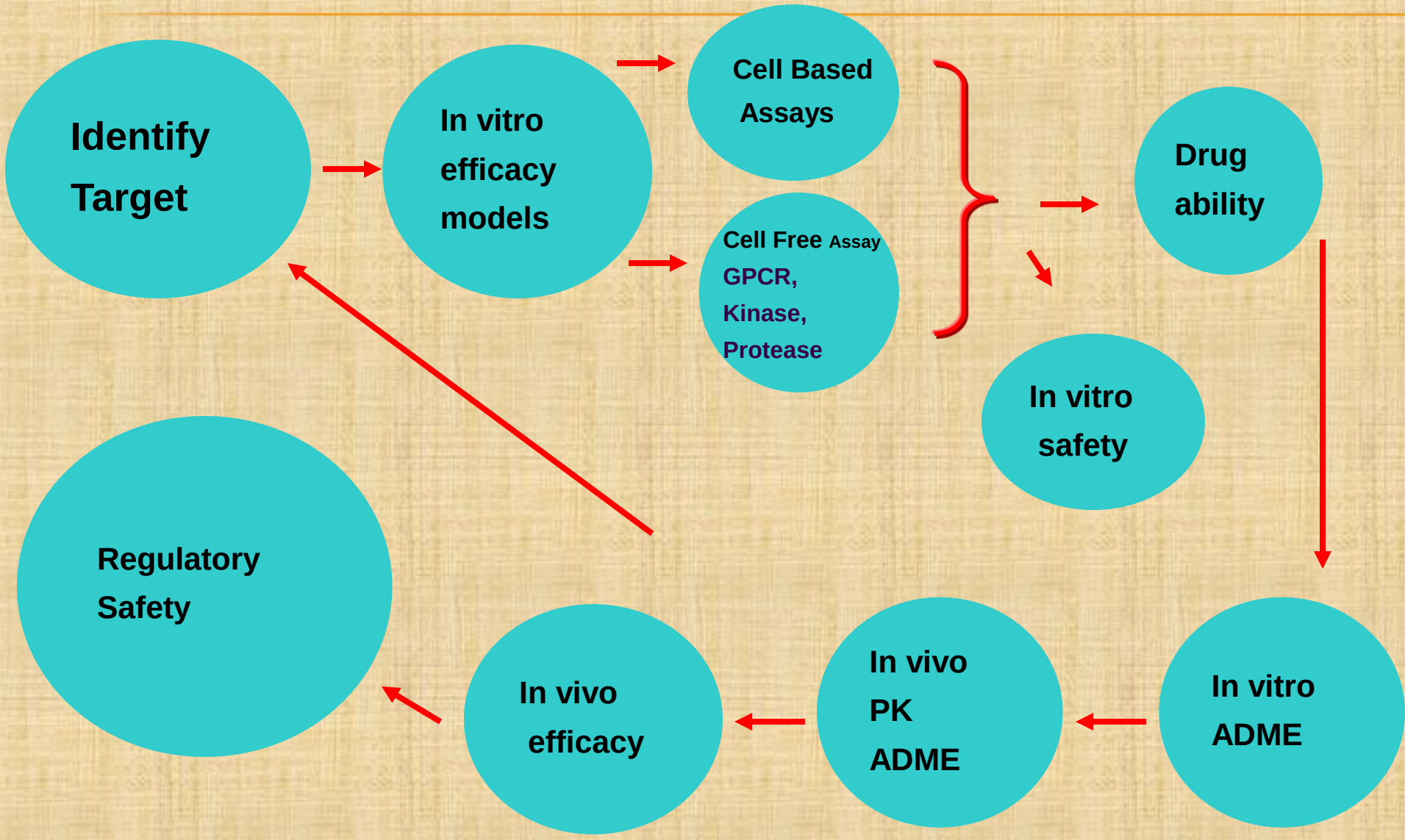
An in vitro readout for metabolic stability

Caco-2 cell Monolayer

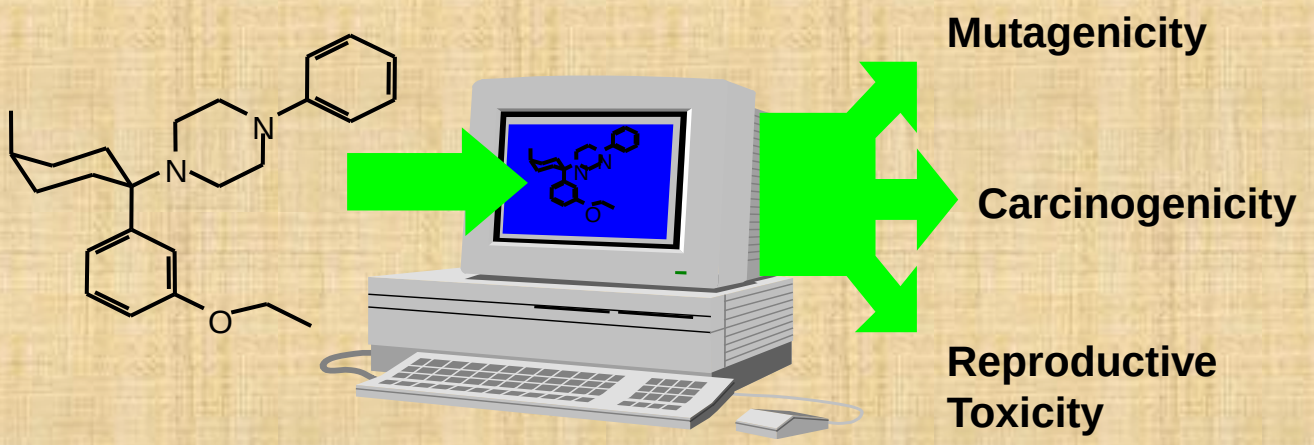


Intestinal Model for Absorption

SCREENING NCE'S



IN SILICO PREDICTIVE TOXICOLOGY



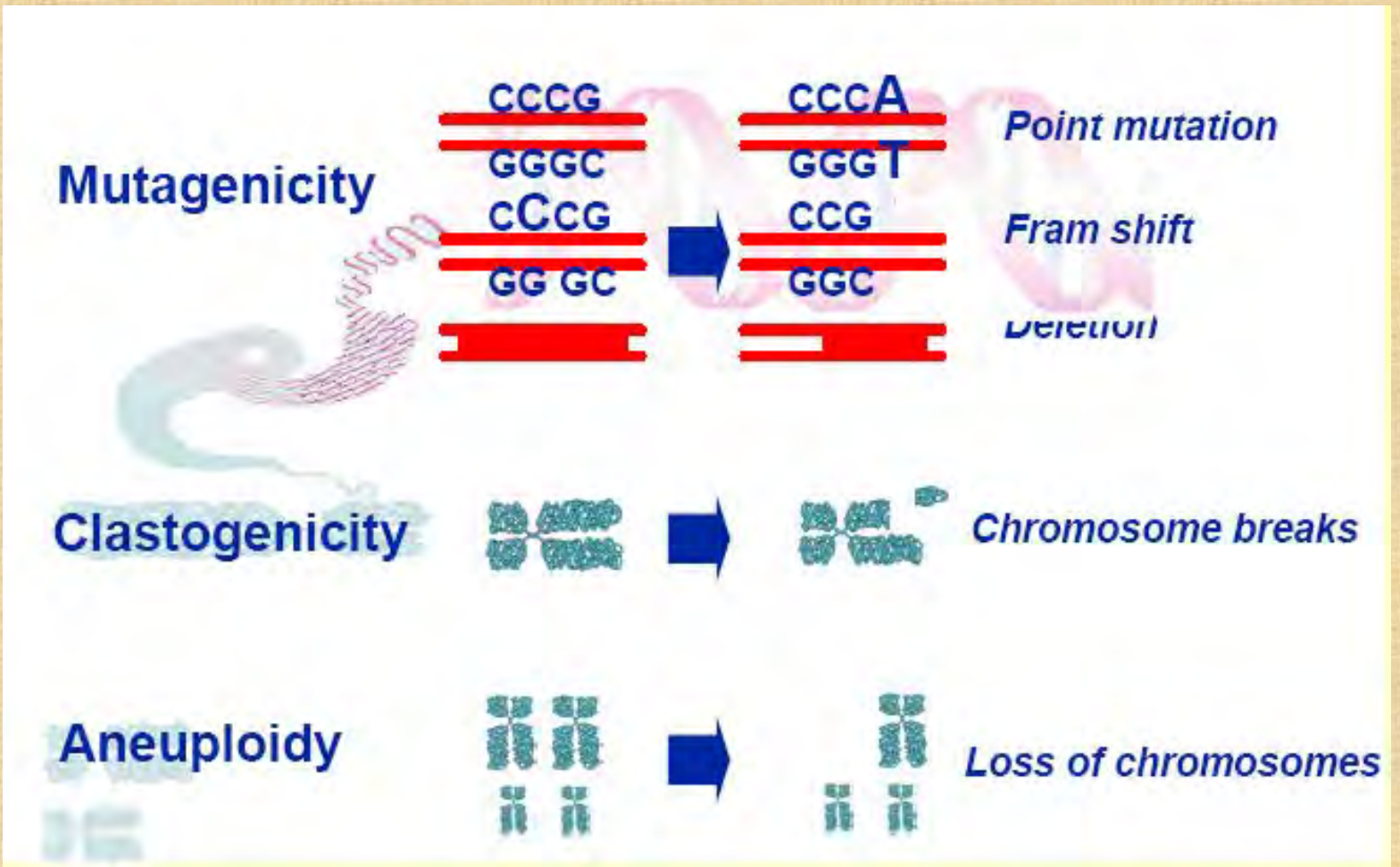
***In Silico* toxicity prediction**

Expert or Rule Based System
e.g. DEREK

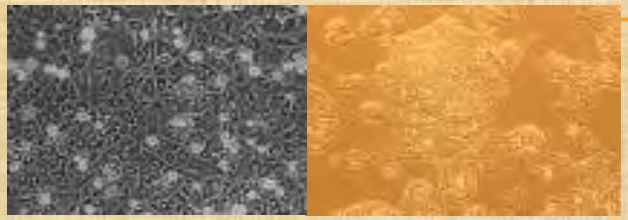
QSAR Model
e.g. TOPKAT

Such models however cannot replicate complicated interactions in the whole system

GENOTOXICITY STUDIES

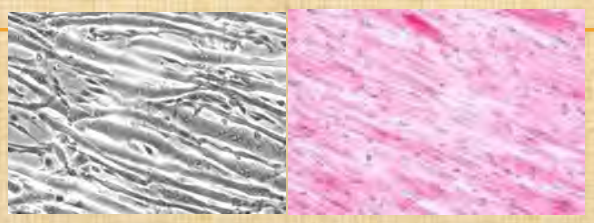


IN VITRO SAFETY SCREENS



Rat Primary Hepatocytes

Human Hep G2 cell line



Rat Myotubes

C2C12 Cell Line (Mouse)

Hepatotoxicity

End points: MTT, Neutral red uptake, LPO, enzyme leakage, etc.

Myotoxicity

End points: MTT, CK, AST, LDH leakage

Lead Optimization

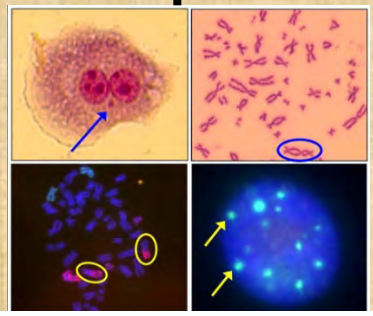
In vitro Efficacy Data (IC50, MIC)

Ranking the Molecules

Myelotoxicity

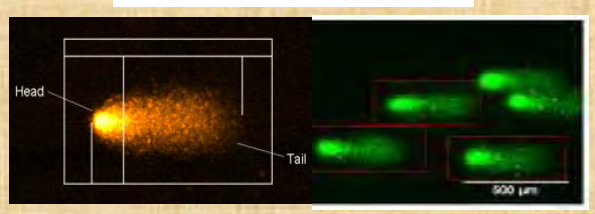
BFU-E CFU-E CFU-GM

End points: No. of colonies as a measure of cytotoxicity



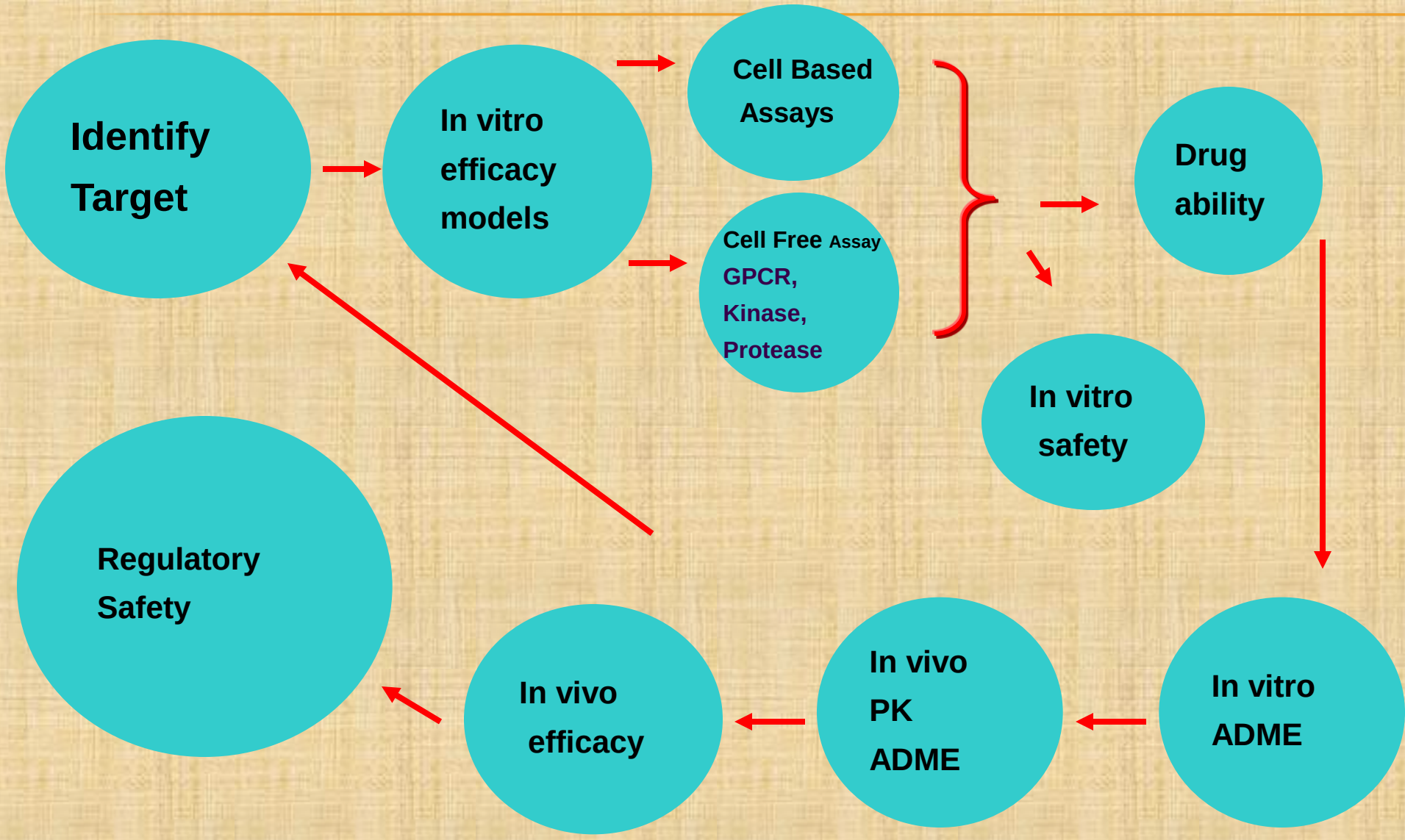
End points: Chromosomal damage

Comet assay

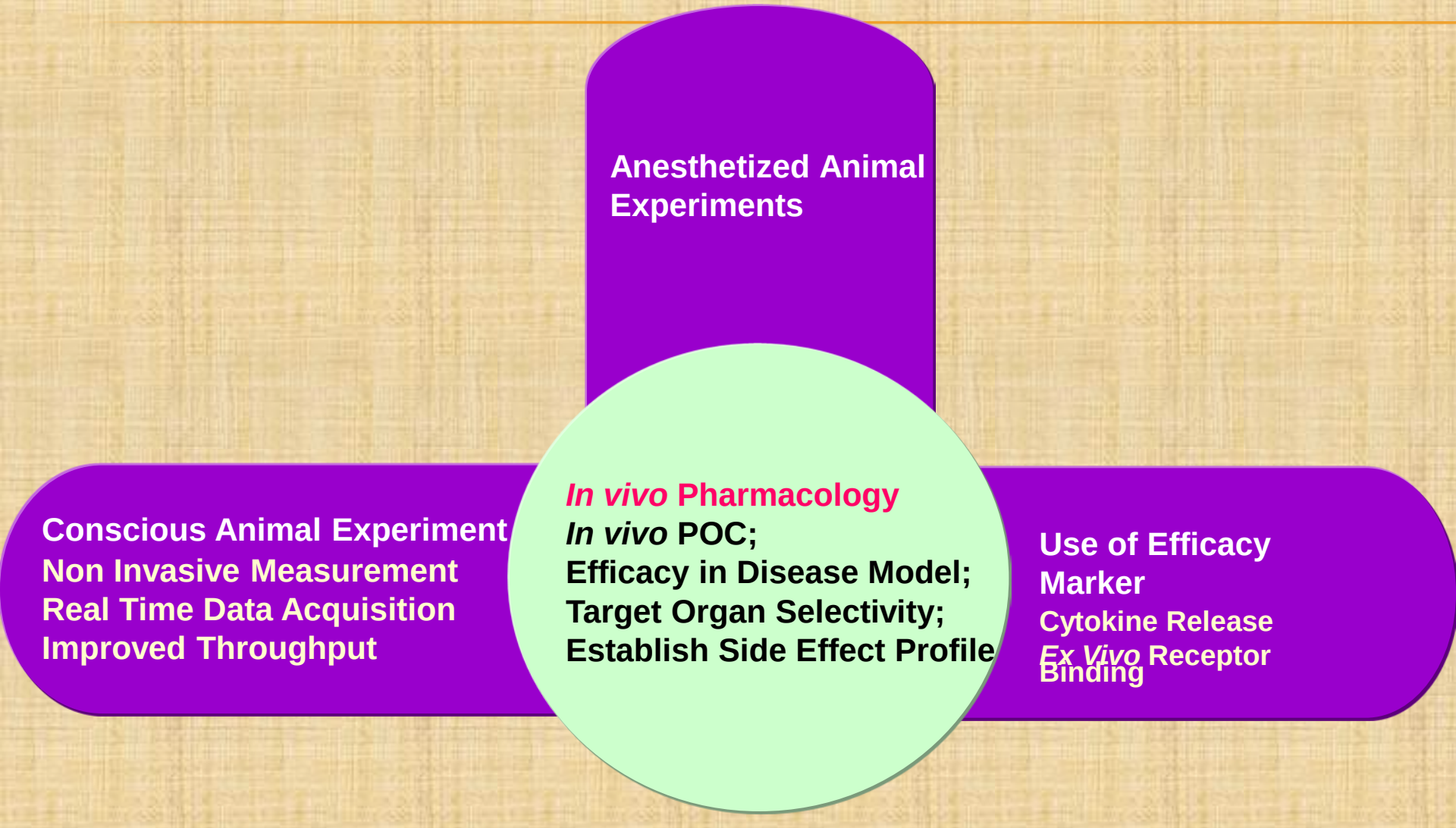


End points: DNA damage

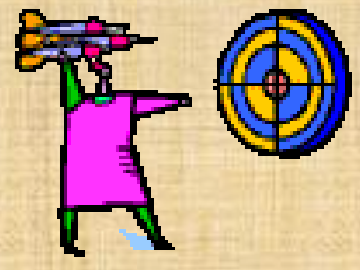
SCREENING NCE'S

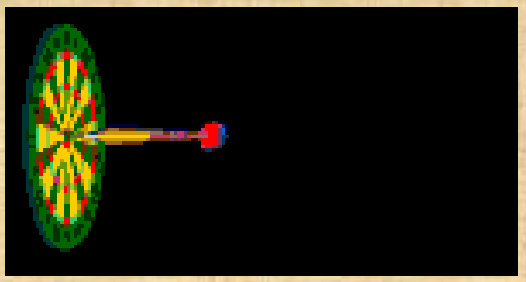


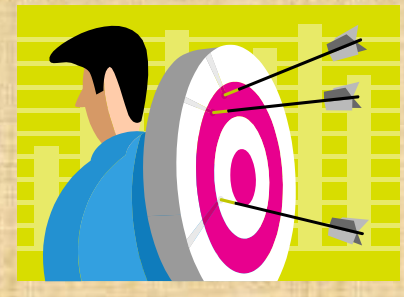
IN VIVO (ANIMAL) BIOLOGY - PHARMACOLOGY



LEAD AND ITS OPTIMIZATION







- Physicochemical
- Absorption
- Metabolism & PK
- Safety

Innovation Process Difficult

Complex Disease Targets

Not Sufficiently Selective

Too Long in Body

Most

Side Effects

Adverse Reactions

Compounds

Unsafe

Poor Absorption

Do Not Become

Unstable

Low Levels in Body

Medicines

Competition

Not Effective Enough

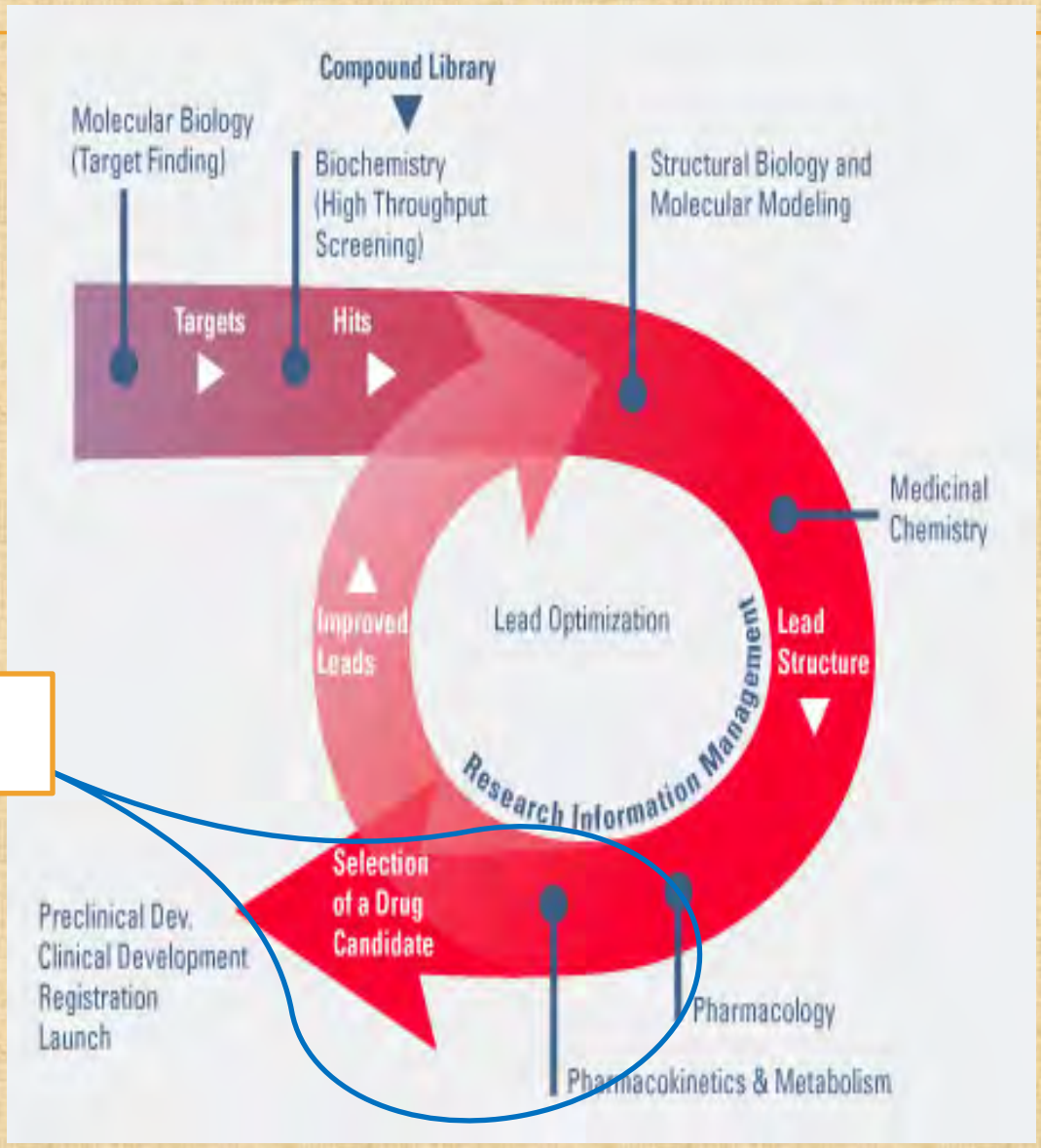
Impractical To Make

INSA: Brain Storming Meeting- Man, Animal & Science, Sept, 15th 2011

NEW DRUG DISCOVERY

Multidisciplinary

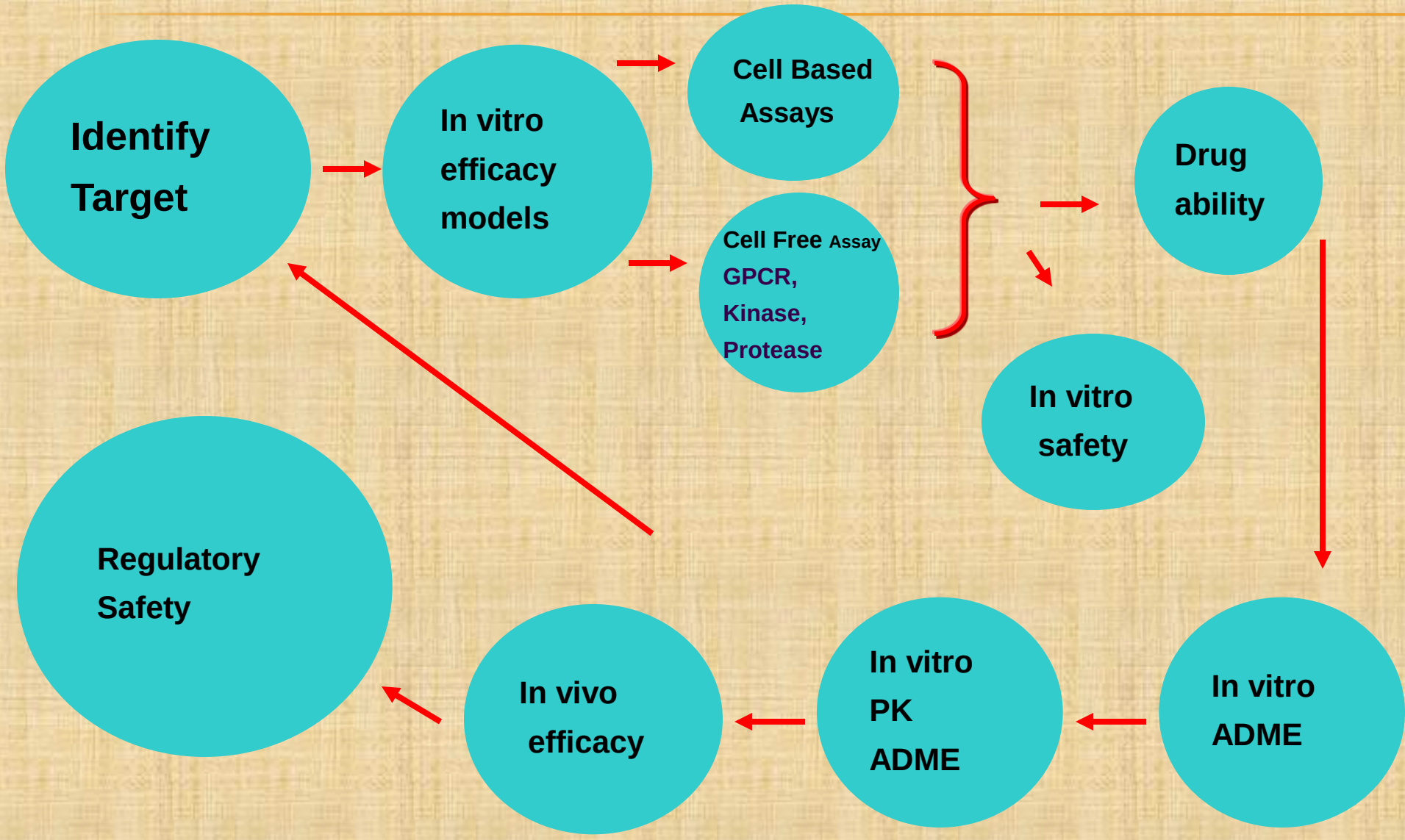
- ❖ Involves Risk
- ❖ Expensive
- ❖ Requires experience



Animals are used for experimentation

INSA: Brain Storming Meeting- Man, Animal & Science, Sept, 15th 2011

SCREENING NCE'S



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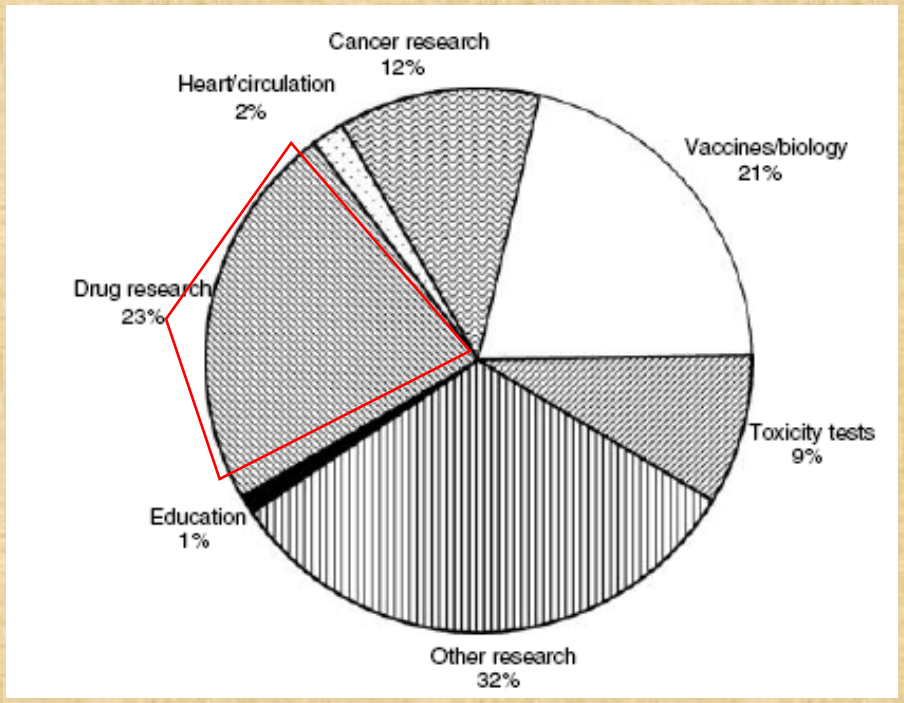
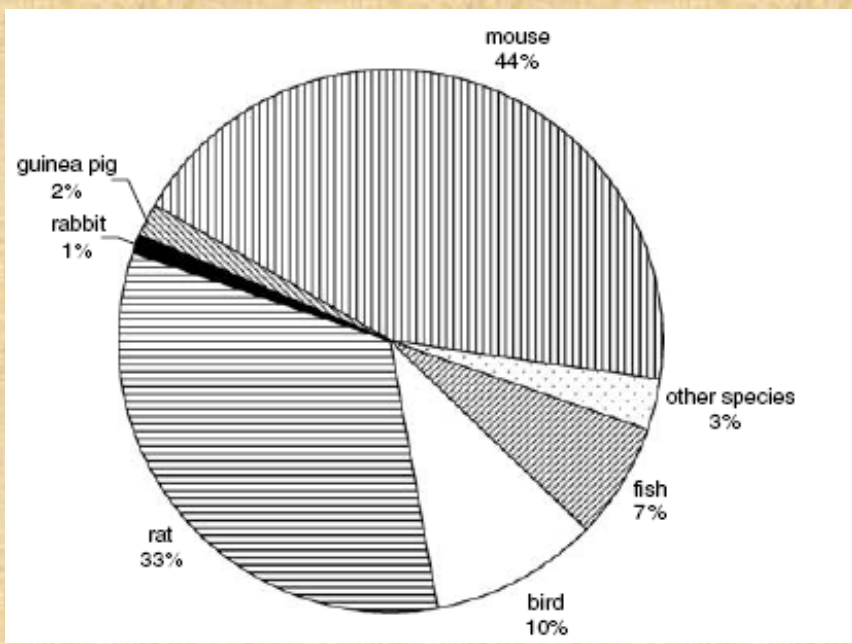
Schedule Y, 2006

ANIMAL USAGE



Distribution based on purpose of use



Distribution based on species

V Bauman's - Gene Therapy (2004) 11, S64–S66

Can Computer Models and Cell Cultures Replace Animal Research?

- Computer models and cell cultures are good for screening and are used frequently.
- Such models cannot replicate complicated interactions in the whole system.
- Final testing depends on studies in animals; sometimes it is required by law.
- Animal and non-animal models used in conjunction achieve the best answer.

Are the animals used in drug discovery & development protected ?

- Number of national and international laws, regulations ensure animals used are treated humanely
  - CPCSEA – India (under Prevention of cruelty to Animal Act- 1960)
  - Animals (Scientific Procedures) act, 1986
  - Animal Welfare Act, 1966
  - Animal Protection Act- Germany
  - European Directive 86/609/EEC
  - Experiments on Animals Act 1996 –Netherland
  - Canadian Council on Animal Care, 1968

Institutional Animal Ethics review bodies are also formed in compliance to laws to review and supervise the animal care and use

- IAEC
- IACUC

Scientific community care about animals

"Good science & good animal care go hand-in-hand."

- Use of animals in research is a privilege & animals deserve respect & best possible care
- It is in the best interest of researchers and science for animals to be well-treated and healthy
- Sick or mistreated animals don't give good research results.
- Animal research is very expensive, so lab animals are precious to scientists – they only use them when necessary, and take very good care of them
- Veterinarians get involved in care and treatment to improve health of laboratory animals and improve the human well being

Scientific community care about animals

"Good science & good animal care go hand-in-hand."

- AAALAC was originally founded by scientists and veterinarians to ensure good animal care
- Scientists have themselves come up with in-vitro assay for skin corrosion, dermal absorption, phototoxicity etc.
- They have brought alternative to test guideline on acute toxicity OECD 401 in forms of OECD 420 to refine and abandon lethality as test parameter or OECD 423 to bring significant reduction in number of animals

Ranbaxy Animal Facilities :AAALAC Accredited

Policy on Animal Experimentation

- To assure the humane care and well-being of animals used in research by providing resources and supervising and promoting quality science through responsible animal care
- Animals are required for development of drugs as per regulatory guidelines however, Ranbaxy is committed to the view that
  - The use of animals as experimental subject should be minimized
  - Scientists using these animals are morally obligated to protect them against sufferings

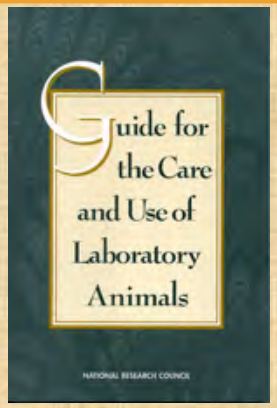
Ranbaxy is committed to

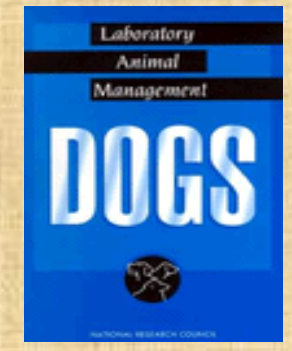
- Rigorously apply 4 R's
  - Rehabilitation –
    - Take care of animals used in non terminal experiments till their life time
  - Reduction –
    - Use 6 animals vs. 10 animals per group in regulatory studies for generics
    - Prefer rat vs. mouse in Pk studies - more time points using lesser animals
  - Replacement –
    - Use in-vitro model wherever possible
    - Use in-silico predictions
  - Refinement -
    - Club different type of studies in 1 study e.g. systemic tox in mice & MNT
    - Sequential approach and avoid un-necessary use of animals – conduct hERG assay - followed by purkinge fiber assay – and lastly canine telemetry study

Summary

- In the absence of human data, research with experimental animals is the most reliable means of developing a drug/ detecting toxic properties of a chemical and estimate risks to human, animals & environment
- Efforts should be made to develop and validate alternative models to reduce animal use and improve animal care
- Good Animal Welfare essential for Good Science
- Government , Industry and Society to work collectively to ensure animal experimentation in sensitive manner to meet medical needs

Highest Standards Followed





Breed and Supply SPF rodents

With the knowledge gained through research on animals, we can continue improving the lives of not only humans, but our pets, wildlife and other animals

INSA: Brain Storming Meeting- Man, Animal & Science, Sept, 15th 2011

THANKS