Chapter 1

Regulations for Laboratory Animals Care and Ethical Requirements

1.1 Introduction

Animal ethics includes all the specifications required to treat animal in a generous way and it ensures that minimum pain should be given to them. In 1959, William Russell and Rex Burch gave; the 3R's principles (based on reduction, refinement and replacement of animals) of Humane Experimental Technique, the scientific excellence and humane use of laboratory animals.

- **Reduction:** Use less number of animals, different group of animals can be combined together with colleagues and thus results can be obtained from less number of animals.
- **Refinement:** Use techniques to decrease pain/suffering, alteration in frequency and volume of dose, improvement of animal well-being.
- **Replacement:** Use techniques that substitute organisms with isolated organs, microorganisms, invertebrates, and sometimes mathematics and computer models.
- Previously, it was 3R's principles but now one more principle is added and it is known as 4R's principles "reuse".
- **Reuse:** Resuse of same animals in other experiments after approval from competent authority like Institutional Animal Ethics Committee (IAEC).

1.2 Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

A committee is shaped with the main aim to explore all such requirement that may be essential to guarantee that animals are not given any kind of pain before, during or after the experiment known as Committee for the

Purpose of Control and Supervision of Experiments on Animals (CPCSEA). This legal body is work according to Prevention of Cruelty to Animals Act, 1960 which is currently under the Ministry of Fisheries, Animal husbandry and dairying department of India. Various norms have been prepared by this committee to maintain quality and safety of animals. It plays role in regulation of experimental studies for proper conduction of biomedical and behavioral research along with testing of products.

1.2.1 Goal

The main aim of CPCSEA guidelines is to promote proper care of animals that is used during experiments. The major objective is to provide specifications that will improve animal well-being. It is also helpful in the search of advancement of biological knowledge that is pertinent to animals and humans.

1.2.2 Principles Adopted by CPCSEA for Animal Experimentation

- Experiments be performed on animals with the intention of progress of new drugs or discovery of physiologic knowledge that is predictable to be useful for protecting or prolonging human life or preventing suffering or against any disease, whether of human being, animals or plants.
- The selection of animals for the experiment should be based on lowest phylogenetic scale that may produce scientifically valid results. Experimental protocol must be intended in such a manner to allow minimum number of animals to provide statistically valid results at 95% level of confidence.

  [Note: Phylogenetic scale- animals are ranked according to their general complexity and ability from lowest to highest. Research protocols should be prepared in such a way that experiments on animals rank lower on the phylogenetic scale. Rodents are lower on the scale than any other animal species].
- It should be the main concern to use experimental animals properly and to prevent and reduce pain and suffering inflicted on animals.
- Welfare of animals after their use in experiments must be the moral responsibility of research personnel engaged in animal experimentation. It is the responsibility of researchers for care and rehabilitation of

animals after experiments. The animals are permitted to euthanize after study.

[Note: Euthanasia should be given to animals in following conditions when animal is paralyzed and unable to perform daily functions, locomotion, pain and suffering from long time and other life threatening conditions to human beings or other animals].

- Proper housing, feeding and caring of animals should be maintained. The conditions of living for animals should be comfortable.

  [Note: For proper caring, handling and use of the animal species in biomedical research a veterinarian or a scientist engaged in animal experimentation must be appointed].

1.2.3 Functions of CPCSEA

The following are the functions of CPCSEA:

- The organization or institute engaged in animal experimentation or breeding of animals should be registered.
- Appointment of nominees for IAEC.
- Approve any establishment or institute having animal house facilities on the basis of reports of inspections.

  Note: CPCSEA team may conduct both announced and unannounced visits to the registered establishments for the inspection of the animal house facilities in the institutes.
- To give permission for proper conduction of animals experiments on animals.
- Recommendations for import of animals for use in experiments.
- Committee can take any legal action against establishment or suspend their registration in case of violation of principles and guidelines.
- Any institution or establishment registered under CPCSEA must constitute Institutional Animals Ethics Committee (IAEC). IAEC is an organization comprised of a group of recognized persons and registered by the committee for the purpose and supervision of experiments on animals in an establishment. IAEC includes eight members Figure 1.1 depicts about composition of IAEC.

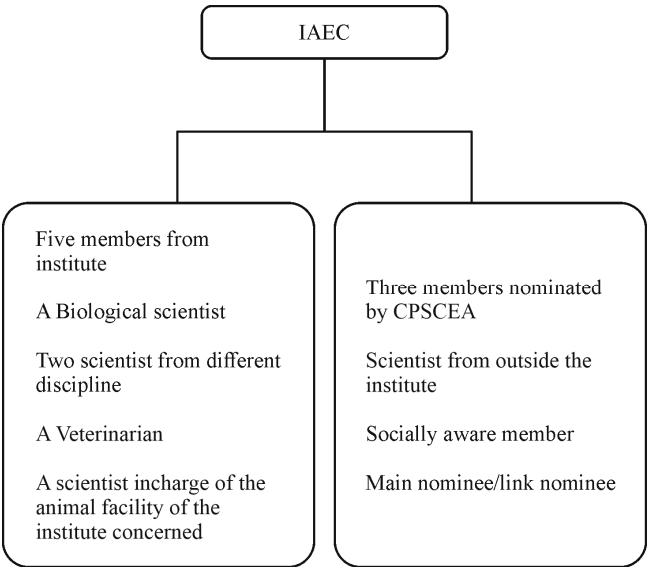


Figure 1.1 Constitution of IAEC.

1. *Five members from establishment or institute:* (two scientists from different biological disciplines, one biological scientist, one veterinarian involved in the case of animal, one scientist in charge of the animal facility of the institute concerned).
2. *Three members nominated by CPSCEA:* socially aware member (1), scientist from other institute (1), main nominee (1) and Link nominee (*)

   Link Nominee shall substitute the main nominee in case main nominee conveys his unavailability in writing to the chairperson of the IAEC in advance as per described procedure.

1.2.4 Functions of IAEC

- IAEC will come across and grant permission against feasible and appropriate proposal involving animals experimentation before start of the study. In case of proposal containing large number of animals the IAEC must forward it to CPCSEA in prescribed format with prescribed manner with its recommendation.
- IAEC gives permission for experiments on small animals only up to the phylogenetic scale of rodents. The committee does not have authority to approve any research proposal involving animals higher on the phylogenetic scale than rodents.

  Note: The committee may consider such type of proposal involving animals above sentience level of rodents, and then forward the proposal to CPCSEA for its recommendations.

- Research activities performed in any institution must be governed by IAEC periodically and after completion of study. IAEC visits the organization performing animal experiments from time to time.
- The IAEC committee is also abide to other compliances, regulatory requirements, rules and guidelines specified by CPCSEA.

  Note: IAEC is appointed for the period of 5 years (earlier it was for 3 years) and it can be reconstituted at the time of renewal of registration, if required.

1.2.5 CPCSEA Guidelines for Laboratory Animals

CPCSEA has prepared certain guidelines for welfare of animals to promote proper care of animals used in biomedical and behavioral research. Every establishment must obey these guidelines including Good Laboratory Practices (GLP) engaged in animal experimentation to assure quality maintenance and well-being of laboratory animals while conducting experiments research and testing of products. Different parameters need to fulfill or take in consideration according to these guidelines are:

(a) Veterinary care

It is the responsibility of a veterinarian or a person appointed in the institute having experience in laboratory animal handling for adequate care of animals. Animals should be observed daily by someone other than a veterinarian and communicate timely with veterinarian regarding well-being of animals or any problems in animal health and behavior. The veterinarian can participate in reviewing protocols and proposals, monitoring of occupational health hazards and control of zoonosis. Individual can establish appropriate guidelines for veterinary care, animal husbandry and animal welfare, supervising animal nutrition and sanitation.

(b) Animal procurement

All animals should be procured ethically from the registered breeder as per the guidelines of CPCSEA. The procured animals should be assessed carefully regarding its healthiness and it should be devoid of any diseases. A health surveillance program may be there to screen incoming animals. Animals should be quarantined, stabilized and separated according to procedure appropriate for the particular circumstances and species.

(c) Quarantine

Quarantine means how the new animals are kept in their housing. They must be separated from old animals .The health of the animals must be checked for any kind of infection. Time for quarantine may be a week; month or more than a month depends upon phylogenetic arrangement of the animals.

(d) Stabilization

New animals must stabilize physiologically, psychologically and nutritionally. Period of stabilization depends upon the animal's transportation duration, type of species and type of animals use.

(e) Separation

Separation should be done according to species. Separation is mainly done to inhibit disease transfer from one species to another species. It eliminates anxiety, behavioral and physiological changes due to interspecies conflict. For different groups of animals different personnel must be appointed. No other personnel should be allowed to enter the area.

(f) Monitoring, Diagnosis and Control of Disease

Animals should be kept in examination by a competent staff. Animals should be watched daily but if the animal is sick or any experiment is carried out to that particular animal then that animal must be observed carefully. Animal must be given veterinary care if needed at the proper time. If the animal is suffering from infection then it must be kept isolated from others. If bacterial infection persists in the entire room then the animal group should be kept separate and isolated during the process of observation, treatment and control.

(g) Animal care and Technical personnel

The staff trained in laboratory animal science can only be appointed by the institution for animal care. Institution may provide formal or on-job training to assure about the trained staff. Staff engaged in animal care must be aware of hygiene and must maintain cleanliness. Personnel protective equipment (PPE) like footwear, gloves, masks, coats, head covers; cloths etc. must be provided to reach the animal house to maintain cleanliness. PPE must be free of dust, toxic elements. Eating, drinking, smoking should be banned inside the animal house.

(h) Animal experimentation involving hazardous agent

Institutions engaged in testing of hazardous agents on animals must constitute a Bio-safety committee for taking care of safety issues. Bio safety committee must check the proposals in those protocols which are using hazardous substance.

(i) Duration of experiment

In most of the cases animal should be used for experiment only for 3 years but in special cases it can be used for more than 3 years.

(j) Physical restrain

Equipment used to perform experimental design should be carefully designed so that least pain is given to animals. The period of restraint

should be less. Animals are given prior training before experiment to minimize stress. Veterinarian should be there to avoid any kind of infection during experiment.

(k) Physical facilities

Animal facilities should be well planned, properly maintained and comfortable to the animals. It may vary according to the design and size to the institute, in-house animal and geographical location. The building materials used for construction of building should be moisture-resistant, durable, fire-resistant, seamless material and vermin/ pest control. Sufficient corridor space should be provided for movement of personnel and handling of equipment. Other facilities including water lines, drain pipes and electrical connections should be provided.

(l) Location of animal house

Animals must be housed in a place which is far away from the human activities for proper animal husbandry, human comfort and protection of health. Following steps should be taken into consideration:

- Animal house should be situated at a distance from human residence and it should be free from ticks, smoke, dust, noise, insects, wild rodents and birds.
- Nearby laboratories should be adequately separated using barriers such as entry locks, corridors or floors.
- Animal shelter should occupy approx. 50-60% of total constructed area; however, the remaining area should be utilized for other services like washing, stores, staff, office and quarantine, machine rooms and corridors.
- Animals should remain free from fluctuating environment, temperature, light, humidity, sound and ventilation, as animals are very sensitive to these factors.

(m) Infrastructures

Animal house doors should fit appropriately, so that the room should be free from rust, vermin and dust. Windows are optional for small animal facilities. There must be arrangement of alternate source of light and ventilation in-case of power failures and backup power is not available. Floors should remain dry. It should not absorb anything. It should be free from skid, resistant to acid, wear, solvents, adverse effects of disinfectants and detergents. It should be sufficiently strong to bear weight of racks, equipment and stored items. Walls and ceiling should be free of cracks, seepage, or damaged junctions with floors, doors and corners. Construction material should be capable to withstand on scrubbing with disinfectants, detergents and under high pressure of water.

(n) Environmental conditions

Temperature of the animal house is kept at 18-29°C and humidity of the animal house is 30% to 70% RH. Air conditioning is valuable means of regulating these environmental conditions. Ventilation system should be designed in such a way to allow 12-15 air cycles per hour. Power and lighting system should be maintained as 12 h light and 12 h dark cycle. Animal house must be made of concrete walls to avoid noise pollution. Allowed sound for rodents and non-human primates is 85 dB.

(o) Animal husbandry

Animal husbandry should be planned carefully for facilitation of animal well-being, to meet research requirements to minimize experimental variable. Cages allow meeting the biological needs of the animals like maintenance of body temperature, urination, defecation and reproduction. Cages are generally made of polypropylene, polycarbonate and stainless steel. Cage surface should be smooth and impermeable so it should not pull or keep dirt.

(p) Caging or Housing System

Caging of animals plays a vital role in social and physical behavior of animals. It should be designed appropriately for more comfort to the animals. The housing of cage animals should possess following facilities:

- Adequate space, liberty to move and adjustments of normal posture, and a resting place for appropriateness of the species.
- Provide a comfortable environment to the animals.
- Provide no escape for the animals from the cages which confines animal safety.
- Provision of food and water to the animals, must be easy.
- Provide proper ventilation.
- Confirm the biological requirement of the animals, e.g., maintenance of body temperature, urination, defecation, and reproduction.
- In cages, animals must be maintained dry and clean.
- Increase research possibility while maintaining good health of the animals.

  The caging of the animals should be made up of tough materials, strong, and designed in such a manner that cross-infection between adjoining units should be very minimum. Cages of polycarbonate, polypropylene and stainless steel should be used for laboratory animals. Cages of animals like monkeys, sheep and horses should

be made up of steel or painted mild steel. Cages should be clean and impermeable with smooth surface. The animals can be seen clearly from outside the cages without disturbing to them.

Feeding and watering devices should be clean. Handling should be easy for these devices. Cages should be comfortable to prevent injuries to animals with regular cleaning and servicing.

(q) Sheltered or Outdoor Housing

Few animals are kept outside. They are kept in runs, pens, or large enclosure. There must be proper defense mechanism to prevent from high temperature or harsh weather conditions and adequate protective escape mechanism for animals such as monkeys. Protection should be given to all animals. Proper ventilation must be there. The furnitures must be replaceable in conditions of soiled and worn out. The ground level of the housing facilities should be covered with sand, gravel, grass, absorbent bedding and other material that can be replaced or removed when needed to insure sanitation.

(r) Social Environment

The social environment is the surrounding of the animals among group of individuals to communicate. It may vary according to species and experiences of the animals. While keeping the animal in a particular environment or social interaction, the natural habitat and behavior of the animals should be kept in mind either to place single or in a group. During grouping of animals their sex, age, and social rank should be studied. Population density can affect the metabolism, behavior, reproduction and immune responses. The group composition should be constant for non-human primates, canine and other large mammals to avoid behavioral and physiological changes.

1.3 Euthanasia

Euthanasia is also popularly known as “mercy killing”. Euthanasia is the least painful death given to animal after the end of the experiment. If the animals are experiencing severe pain during the experiment then those animals are euthanized to relieve the pain. There are various types of euthanasia methods. These methods are species specific and must be appropriate for particular research. American Veterinary Medical Association (AVMA) Panel had given various justifications on techniques of Euthanasia. The justification must be given for any deviation from AVMA recommendations. Euthanasia should be performed rapidly and it is performed in a different room in which animals are housed. CPCSEA has also given guidelines for euthanasia.

1.3.1 Inhalant Euthanasia

Inhalant anesthetics are slow in action because after reaching to the alveoli when it attains a certain concentration then it starts its action. Various inhalant anesthetics are ether, sevoflurane, halothane, isoflurane. Inhalation anesthetics produce respiratory and cardiac arrest.

- Halothane is best alternative of sevoflurane in term of cost. However, action of sevoflurane is quick. These agents are safe but it should be used cautiously to avoid exposure to vapors.
- Ether irritates the respiratory tract, causing stress and is explosive hazard therefore not recommended. It should be used under fume hood.

1.3.2 Carbon Dioxide (CO_2)

Carbon dioxide (CO_2) is a good euthanasia used for all animals including rodents. Its concentration should be between 60-70% and exposure time should not be more than 5 min. CO_2 is a reversible anesthetic, its long term exposure causes respiratory arrest.

1.3.3 Non Inhalant Pharmacological Agents

(a) Barbiturates: Pentobarbital is barbiturate drug used for birds and mammalian species. It is administered intravenously in animals except rodents; in rodents intraperitoneal route is used for drug administration.

Advantages

- Quick onset of action.
- Barbiturates produce good euthanasia with least distress.
- Barbiturates are cost effective.

Disadvantage

- Barbiturates are used intravenously so it requires skilled personnel.

(b) Chloral hydrate: Chloral hydrate suppresses the cerebrum activity. It causes hypoxemia and then death. It is used for large animals.

(c) Magnesium sulfate or potassium chloride: Both magnesium sulfate or potassium chloride is used in combination for euthanasia. However, the dose can be enhanced along with potassium chloride in anesthetized animals. Potassium chloride is given in concentrated form to cause cardiac arrest.

(d) MS 222: Tricaine methane sulfonate (MS222) is used an euthanasia agent for fish and amphibians. It can be administered through injection (200-300 mg/kg of a 1% buffered solution) or as an immersion bath (2 mg/ml in H_2O, exposed for 20 min to 3 h). However, immersion in benzocaine (100-200 mg/L H_2O) is also acceptable.

[Note: Precaution should be taken while handling MS222 to wean gloves all times. It may cause retinal toxicity due to exposure].

1.3.4 Physical Methods

The use of these techniques require experience and skilled personnel.

(a) Exsanguinations is performed by giving anesthesia to all animals. It is acceptable to all species.

(b) Cervical dislocation is permissible of most of the animals including rats, birds, mice and rabbits, however, it require skill and proper technique to perform. Thus, animals should be anesthetized to do it easily.

(c) Decapitation technique is used for birds or small mammals. Carbon dioxide or phenobarbital is used to anesthetize animals. The scientific justification is required for using decapitation technique as a sole means of euthanasia. It is only performed when study required to use this technique because it is hazardous to personnel. The animals are decapitated alone to avoid anxiety to other animals with smell of blood and gloves should be changed between animals. It causes rapid loss of consciousness.

(d) Pithing is used as a sole technique of euthanasia for frogs and other amphibians. Pithing is generally followed by decapitation.

(e) Stunning, rapid freezing or air embolism is permissible for small species but scientific justification is required and used when no alternatives are available.

1.4 International Conference on Harmonization (ICH)

International Conference on Harmonization (ICH) was created in April 1990 at Brussels. It involves regulators and industry for discussing scientific and technical matters to ensure and assess quality, safety and efficacy of drugs. It was an agreement between the United States of America, Europe and Japan to harmonize different regional requirements for registration of pharmaceutical drug products.

1.4.1 Mission

The guidelines were passed with the aim to achieve greater harmony in the interpretation and application of technical guidelines. It is also subjected to reduce duplicity of testing during research and development of new drugs.

1.4.2 Objectives

The main objective is to maintain harmony in the use of animals, humans and material resources. Avoid delay in research and development of new

drugs. Maintain the safeguards on safety, quality and efficacy along with regulatory obligations to protect public health.

1.4.3 Structure of ICH

ICH structurally made up of regulatory and industry as equal partners. It is a part of discussion both scientifically and technically for testing procedures to make sure regarding quality, safety and efficacy of medicines. ICH also focuses on the technical check of medicinal products containing potent drugs. Initially, ICH was confined to applicable only for three regions i.e. United States of America, Japan and Western Europe.

ICH is comprised of six parties that act as regulatory bodies; these parties include European Union (EU), European Federation of Pharmaceutical Industries Associations (EFPIA), Ministry of Health Labour and Welfare (MHLW), Japan Pharmaceutical Manufactures Association (JPMA), US Food and Drug Administrations (USFDA) and **Pharmaceutical Research and Manufacturers of America (Phrma)**

(a) European Union (EU)

EU is represented by the 27 members of European Commission that works for the harmonization of legislation along with technical necessities with the aim to achieve a single market in pharmaceuticals. It may help in free movement of products throughout the EU.

(b) European Federation of Pharmaceutical Industries Associations (EFPIA)

EFPIA deals in manufacturing, growth and research of medicinal products for human use in Europe. It is situated in Brussels and composed of 45 leading pharmaceutical companies and 29 national pharmaceutical industries.

(c) Ministry of Health, Labour and Welfare, Japan (MHLW)

MHLW plays the role of approval and administration of drugs, cosmetics and medical devices in Japan.

(d) Japan Pharmaceutical Manufacturers Association (JPMA)

JPMA is a major research based pharmaceutical manufacturers association in Japan. It is represented by 14 committees and 75 members that includes 20 foreign affiliates.

(e) US Food and Drug Administration (USFDA)

USFDA deals in wide range of responsibilities including drugs and cosmetics, medical devices, radiological and biological products. It gives approval for drugs and drug products in USA. It consists of regulatory, scientific and administrative committees that works under the office of Commissioner and other regulating centers. Center for

Biologics Evaluation and Research (CBER) and Center for Drug Evaluation and Research (CDER) are responsible for drawing technical advice to ICH.

(f) Pharmaceutical Research and Manufacturers of America (PhRMA)

PhRMA is a research-based industry in the USA that is associated with 67 companies involved in research, development and manufacturing of medicine along with 24 research affiliates that engaged in development of vaccines and drugs. Structure of ICH is shown in Figure 1.2 (a).

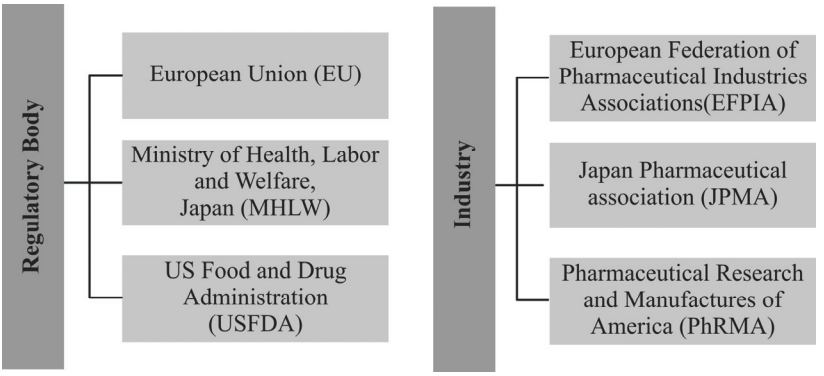


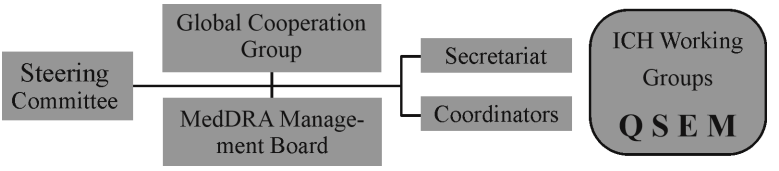
Figure 1.2 (a) Structure of ICH.

1.4.4 Purpose of ICH

- Harmonization of technical requirements
- Ensure safety, efficacy, and quality of medicines
- Avoidance of clinical trials duplication in humans
- Reduce number of animals in pre-clinical studies without compromising safety and effectiveness of the testing

1.4.5 Organization of ICH

The organization of ICH is shown in Figure 1.2 (b).



Where Med DRA = medical dictionary of regulatory activities, Q = quality, S = safety, E = efficacy, M = management

Figure 1.2 (b) Organization of ICH.

(a) Steering Committee (SC)

ICH is administered by the ICH Steering Committee which is supported by the ICH Secretariat. The Steering Committee, works with the ICH terms of reference, determines the policies and procedures for ICH, selects topics for harmonization and monitors the progress of harmonization initiatives. The steering committee meets at least twice a year with the location changing between the three regions. It has six co-sponsors that are European Union (EU), Ministry of Health, Labor and Welfare, Japan (MHLW), US Food and Drug Administration (US-FDA) and European Federation of Pharmaceutical Industries Associations (EFPIA). SC includes three observers; WHO, Health Canada and European Free Trade Association (EFTA). International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) is a non-profit, non-governmental Organization (NGO) representing national industry associations and companies from both developed and developing countries which hosts the ICH secretariat and participates as a non-voting member.

(b) Global Cooperation Group

It is a subcommittee of SC formed in 1999.

(c) Med DRA management board

This board is responsible to observe the activities of the "Maintenance and Support Services Organization" (MSSO).

(d) Secretariat and Coordinators

Secretariat of SC is located in Geneva, Switzerland operating from International Federation of Pharmaceutical Manufacturers Associations (IFPMA) offices. Coordinators are the fundamental unit responsible for smooth running of ICH.

(e) ICH working groups

ICH working groups includes Implementation Working Group (IWG), Expert Working Group (EWG), Discussion Group and Informal Working Group. It can be divided into four categories QSEM followed by assigning ICH topic codes to these categories.

(f) Quality Guidelines (Q)

Quality can be evaluated by conduction of stability studies, testing of impurities and risk management based on Good Manufacturing Practice (GMP).

Table 1.1 List of quality guidelines

Q1 A	Stability Testing of New Drug Substances and Products
Q1 B	Stability Testing Photostability Testing of New Drug Substances and Products
Q1C	Stability Testing for New Dosage Forms
Q1D	Bracketing and Matrixing Designs for Stability Testing of New Drugs Substances and Products
Q1E	Evaluation for Stability Data
Q2	Validation of Analytical Procedures: Text and Methodology
Q3A	Impurities in New Drug Substances
Q3B	Impurities in New Drug Products
Q3C	Impurities: Guidelines for Residual Solvents
Q3D	Impurities: Guidelines for Elemental Impurities
Q4	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions
Q5A	Viral safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
Q5B	Quality of Biotechnology Products: Production of rDNA Derived Protein Products
Q5C	Quality of Biotechnology Products: Stability Testing of Biotechnological/Biological Products
Q5D	Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products
Q5E	Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process
Q6A	Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances
Q6B	Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products
Q7	Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients
Q8	Pharmaceutical Development
Q9	Quality Risk Management
Q10	Pharmaceutical Quality System
Q11	Development and Manufacture of Drug Substances
Q12	Lifecycle management
Q13	Continuous manufacture of drug substances and drug products
Q14	Analytical procedure development

1.4.6 Safety Guidelines (S)

ICH has made complete set of safety guidelines, so as to minimize certain risks likes carcinogenicity, genotoxicity and reprotoxicity. A recent advancement has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years. List of safety guidelines is shown in Table 1.2.

Table 1.2 List of safety guidelines

S1A	Guidelines on the Need for Carcinogenicity Studies of Pharmaceuticals
S1B	Testing for Carcinogenicity of Pharmaceuticals
S1C	Dose Selection for Carcinogenicity Studies of Pharmaceuticals
S2	Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
S3A	Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies
S3B	Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies
S4	Duration of Chronic Toxicity Testing in Animals
S5	Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility
S6	Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
S7A	Safety Pharmacology Studies for Human Pharmaceuticals
S7B	Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarization by Human Pharmaceuticals
S8	Immunotoxicity Studies for Human Pharmaceuticals
S9	Nonclinical Evaluation for Anticancer Pharmaceuticals
S10	Photosafety Evaluation

1.4.7 Efficacy Guidelines

The work carried out by ICH under the Efficacy heading is concerned with the design, conduct, and safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/genomics techniques to produce better targeted medicines. List of efficacy guidelines is shown in Table 1.3.

Table 1.3 List of efficacy guidelines

E1	The extent of populations Exposure to Assess Clinical Safety for Drugs Intended for Long-Term Treatment of Non -Life-Threatening Conditions
E2A	Clinical Safety Data Management: Definitions and Standards for Expedited Reporting
E2B	Clinical Safety Data Management: Data Elements for Transmission of Individual Case Safety Reports
E2C	Periodic Benefit-Risk Evaluation Report

Table 1.3 *Contd…*

E2D	Post-Approval Safety Data Management
E2E	Pharmacovigilance Planning
E2F	Development Safety Update Report
E3	Structure and Content of Clinical Study Reports
E4	Dose-Response Information to Support Drug Registration
E5	Ethnic Factors in the Acceptability of Foreign Clinical Data
E6	Good Clinical Practice: Consolidated Guideline
E7	Studies in Support of Special Populations: Geriatrics
E8	General Considerations for Clinical Trials
E9	Statistical Principles for Clinical Trials
E10	Choice of Control Group and Related Issues in Clinical Trials
E11	Clinical Investigation of Medicinal Products in the Pediatric Population
E12	Principles for Clinical Evaluation of New Anti hypretensive Drugs
E14	Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Ant arrhythmic Drugs
E15	Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories
E16	Biomarkers Related to Drug or Biotechnology Product Development: Context, Structure and Format of Qualification Submissions

1.4.8 Multidisciplinary Guidelines

Those are the cross-cutting topics which do not fit uniquely into one of the Quality, Safety and Efficacy categories. It includes the ICH medical terminology, the Common Technical Document (CTD) and the development of Electronic Standards for the Transfer of Regulatory Information (ESTRI). List of multidisciplinary guidelines is shown in Table 1.4.

Table 1.4 D List of multidisciplinary guidelines

M1	MedDRA Terminology
M2	Electronic Transmission of Individual Case Safety Reports
M3	Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals
M4	Organization of the Common Technical Document for the Registration of Pharmaceuticals for Human Use
M5	Data elements and standards for drug dictionaries
M6	Gene therapy
M7	Assessment and Control of DNA Reactive Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

1.5 Organization for Economic Co-operation and Development (OECD)

Various chemicals such as pesticides, food additives, biotechnology products, industrial chemicals and pharmaceuticals arrive at the world marketplace every year. These chemicals need safety testing in most parts of the world. OECD in 1981in collaboration with its countries and its partners has developed various guidelines and these guidelines must be followed by everyone. It includes a number of advantages such as:

- It increases the validity, legality and international recognition of test data.
- Create the excellent use of existing resources in both governments and industry.
- Avoid the unnecessary use of laboratory animals.
- Decreases non-tariff trade barriers.

1.5.1 The OECD Test Guidelines

- It includes safety testing of chemicals. These testing should be in accordance with its physicochemical properties, effects on biotic systems (ecotoxicity), environmental fate properties, health effects (toxicity), and other areas such as pesticide residue chemistry and efficacy testing of biocides.
- These guidelines are internationally accepted as standard methods for safety testing and provide the common basis for the mutual acceptance of test data.
- These are essential for professionals working in industry, academia and government on the testing and assessment of chemical substances.
- Main aim is to reflect the current state-of-the-art in hazard identification and characterization testing.
- The guidelines are updated in order to keep pace with progress in science, and to address animal welfare concerns.
- The OECD accepted the need to protect animals in general and in particular those used in experimental work 25 years ago. These guidelines are followed throughout the world.

1.5.2 General Guidelines for Designing and Conducting Toxicity Studies

(a) Good Laboratory Practice

Nonclinical laboratory studies must be performed according to U.S. FDA good laboratory practice (GLP) regulations.

- **Care, maintenance and housing:** Recommendations about the care, maintenance, and housing of animals
- **Selection of species, strains and sex:** These guidelines are generally used for rodents (usually rats) and non-rodents (usually dogs). In case other species are used, modifications of these guidelines are necessary. Male and female test animals both can be used. Healthy animals (with no previous experiment) are chosen for experiments. It is necessary to think about the animal's sensitivity and the responsiveness of particular organs and tissues before performing any toxicity studies. One should be careful about rodent species, strains, and sub strains for toxicity studies.
- **Age:** Testing is usually done on young animals. These animals are acclimatized for at least 5 days, then only any experiment can be performed on them. Drug administration to rodents should begin no later than 6 to 8 weeks of age. When dogs are used, drug administration should begin no later than 4 to 6 months of age.
- **Number and sex:** Equal numbers of males and females of each species and strain should be used for the study. For sub chronic toxicity studies, experimental and control groups should have at least 20 rodents/sex/group or at least 4 dogs/sex/group. 10 rodents/sex/group may be suitable for sub chronic rodent studies when the study is considered to be range-finding in nature or when longer term studies are anticipated. These recommendations will assure that the number of animals that survive until the end of the study will be sufficient to permit significant evaluation of toxicological effects.
- **Infected animals:** Generally, it is not possible to treat animals for infection during the course of a study without risking interaction between the compound used for treatment and the test substance. This interaction may confound or complicate the interpretation of study results.
- **Animal identification:** Test animals should be characterized by reference to their species, strain (and sub strain), sex, age, and weight. Each animal must be assigned a unique identification number (e.g., ear tag, implanted identification chip, tattoo).
  Caging: Animals should be housed one per cage or run (single-caged) except during mating and lactation and for acute toxicity studies. This recommendation reflects three points of consideration:
  (i) The amount of feed consumed by each animal in the study cannot be determined when more than one animal is housed in each cage. This information is necessary in the determination

of feed efficiency (relationship of feed consumed to body weight gained).

(ii) Minimizing the possibility of confounding analyses and determining whether decreases in body weight gain are due to decreased palatability or substance mediated toxicity.

(iii) Organs and tissues from moribund and dead animals which are single-caged would not be lost due to cannibalism.

- **Diet:** In general, feed and water should be provided *ad libitum* to animals in toxicity studies, and the diets for these studies should meet the nutritional requirements of the species for normal growth and reproduction. Unless special circumstances apply which justify otherwise, care should be taken to ensure that the diets of the compound treated groups of animals are is caloric (equivalent in caloric density) with and contain the same levels of nutrients (e.g., fiber, micronutrients) as the diets of the control group. Unrecognized or inadequately controlled dietary variables may result in nutritional imbalances or caloric deprivation that could confound interpretation of the toxicity study results (e.g., lifespan, background rates of tumor incidences) and alter the outcome and reproducibility of the studies.

1.5.3 Assignment of Control and Compound Treated Animals

Animals should be assigned to control and compound treated groups in a stratified random manner. Animals in all groups should be placed on study on the same day; if this is not possible because of the large number of animals in a study, animals may be placed on study over several days. If the latter recommendation is followed, a preselected portion of the control and experimental animals should be placed on the study each day in order to maintain concurrence.

- **Mortality:** Excessive mortality due to poor animal management is unacceptable and may cause to repeat the study. For example, under normal circumstances, mortality in the control group should not exceed 10% in short and intermediate length (not lifetime) toxicity studies.
- **Autolysis:** Adequate animal husbandry practices should result in considerably less than 10% of animals and tissues or organs lost to a study because of autolysis. Autolysis in excess of this standard may result in repetition of the study.
- **Necropsy:** If the animal is found dead or sacrificed after the experiment then necropsy should be performed immediately to avoid the autolysis. When necropsy cannot be performed immediately, the animal should be refrigerated at a low temperature but temperature should not be so low

so it can cause tissue damage. If histopathological examination is necessary then tissue specimens should be taken from the animals and placed in appropriate fixatives.

1.5.4 Test Substance

The test substance should be the same that the petitioner should market. The toxicity study of the substance should be carried out. A single batch of test substance should be used throughout the study, when possible. Alternatively, batch that is as similar as possible in purity and composition should be used.

- **Identity:** The identity of the test substance or mixture of substances to be tested should be known. Petitioners should consult with the Agency in determination of test compound and to provide a Chemical Abstract Service (CAS) Registry Number or Numbers.
- **Composition/purity:** The composition of the test substance should be known including the name and quantities of all major components, known contaminants and impurities, and the percentage of unidentifiable materials.
- **Conditions of storage:** The test sample should be kept under conditions that preserve its firmness, quality, and cleanliness till the studies are going on.
- **Expiration date:** The expiration date of the test material should be well known. Test materials should not be used after the expiry date.

1.5.5 Experimental Design

- **Duration of testing:** Animals should be exposed to the test substance 7 days per week for the designated time of the study.
- **Route of administration:** The route of administration of the test substance should match with that of same route used for human if it is possible. For food ingredients (e.g., food and color additives) the oral route of administration is preferred. Proper justification should be provided when other routes are used. The same method of administration should be used for all test animals throughout the study. The test substance should be administered in one of the following ways:
  (i) In the diet: If human should take the test substance through solid food or a mixture of solid and liquid food, test substance is mixed with the diet. Animals should take the diet completely so that complete dose of the test substance is taken by the animals. If the test substance is added with ground feed, the test substance should not lose its basic properties during or after pelleting. When the test substance is administered in the diet, dietary levels should be expressed as mg of the test substance per kg of feed.

(ii) Dissolved in the drinking water: Test substance is given through liquid form when it cannot be given through diet. It is given along with water or with some suitable solvents. The amount of test substance administered in drinking water should be expressed as mg of test substance per ml of water.

(iii) By encapsulation or oral intubation: If the two previous methods are not suitable or the animals require large dose then the animal should be given dose by oral gavage. If the test substance is administered by gavage, it should be given at exact the same dose each time a day. The maximum volume of solution that can be given by gavage in one dose depends on the size of animal. For rodents, the volume ordinarily should not exceed 1 ml/100 g body weight. If the gavage vehicle is oil, then the volume should be not more than 0.4 ml/100 g of body weight. If the test substance must be given in divided doses, all doses should be administered within a 6 hour period. Doses of test substance administered by gavage should be expressed as mg of test substance per ml of gavage vehicle.

- **Dose groups:** Three to five dose levels of the test substance and concurrent control groups should be used with both males and females. Information obtained from acute and short-term toxicity studies can help determine appropriate doses for sub chronic studies.
- **Selection of treatment doses:** Dose is appropriately selected for toxicity studies. It should depend on information related to the toxicity of the test substance. A minimum of three dose levels of the test substance and a concurrent control group should be used in toxicity studies. While performing toxicity studies various factors should be considered:
  (i) The high dose should be adequately high to induce toxic responses in test animals.
  (ii) The low dose should not induce toxic responses in test animals.
  (iii) The intermediate dose should be sufficiently high to elicit minimal toxic effects in test animals (such as alterations in enzyme levels or slight decreases in body weight gains).
  (iv) No dose should cause an incidence of fatalities that prevents meaningful evaluation of the data. Administration of the test substance to all dose groups should be done concurrently.
- **Controls:** A concurrent control group of test animals is required. The control group in dietary studies should be fed the basal diet. A carrier or vehicle for the test substance should be given to control animals at a volume equal to the maximum volume of carrier or vehicle given to any dosed group of animals.

1.5.6 Computerized Systems

Computerized systems that are used in the generation, measurement, or assessment of data should be developed, validated, operated, and maintained in ways that are compliant with Good Laboratory Practice principles.

1.5.7 Observations and Clinical Tests

(a) Observations of Test Animals: Routine cage-side observations should be made on all animals at least once or twice a day throughout the study for general signs of pharmacologic and toxicologic effects, morbidity and mortality. The usual interval between observations should be at least 6 hours. Individual records should be maintained for each animal and the time of onset and the uniqueness and progression of any effects should be recorded, preferably using a scoring system. An expanded set of clinical evaluations, performed inside and outside of the cage, should be carried out in short-term and subchronic toxicity studies in rodents and non-rodents, in one-year non-rodent toxicity studies, and reproductive toxicity studies in rodents to enable detection not only of general pharmacologic and toxicologic effects but also of neurologic disorders, behavioral changes, autonomic dysfunctions, and other signs of nervous system toxicity. Signs noted should include, but not be limited to, changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, pilorection, pupil size, and unusual respiratory pattern). Additionally, changes in gait, posture and response to handling, as well as the presence of clonic or tonic movements, stereotypes (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilating, walking backwards) should be recorded. Tumor development, particularly in long-term studies, should be followed: the time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumor should be recorded. During the course of a study, toxic and pharmacologic signs may suggest the need for supplementary clinical tests or expanded post-mortem examinations.

(b) Body Weight and Feed Intake Data: Feed spillage should be noted and adjustments made in related calculations.

(c) Clinical Testing: Ophthalmological examination, hematology profiles, clinical chemistry tests, and urinalyses are few clinical tests that should be performed as described in the following sections

(d) Ophthalmological Examination: This examination should be performed by a qualified individual on all animals before the study begins and on control and high-dose animals at the end of the study. If the results of examinations at termination indicate that changes in the

eyes may be associated with administration of the test substance, ophthalmological examinations should be performed on all animals in the study.

(e) Hematology: Blood samples should be analyzed individually, and not pooled. If animals are sampled on more than one day during a study, blood should be drawn at approximately the same time each sampling day. The following determinations are recommended:

- Hematocrit
- Hemoglobin concentration
- Erythrocyte count
- Total and differential leukocyte counts
- Mean corpuscular hemoglobin
- Mean corpuscular volume
- Mean corpuscular hemoglobin concentration
- Measurement of clotting potential (such as clotting time, prothrombin time, thromboplastin time, or platelet count)

  Test compounds may produce effect on the hematopoietic system. Therefore suitable actions should be performed for evaluations of reticulocyte counts and bone marrow cytology. Reticulocyte counts should be attained for each animal using automated reticulocyte counting capabilities, or from air-dried blood smears. Bone marrow slides should be prepared from each animal for evaluating bone marrow cytology. These slides would only need to be examined microscopically if effects on the hematopoietic system were noted.

(f) Clinical Chemistry: Blood samples should be drawn at the end of the fasting time and before feeding. Fasting duration should be appropriate for the species and the analytical tests to be performed. Clinical chemistry tests that are appropriate for all test substances include measurements of electrolyte balance, carbohydrate metabolism, and liver and kidney function. Specific determinations should include:

(i) Hepatocellular evaluation: select at least 3 of the following 5 parameters

- Alanine aminotransferase (SGPT, ALT)
- Aspartate aminotransferase (SGOT, AST)
- Sorbitol dehydrogenase (SDH)
- Glutamate dehydrogenase
- Total bile acids

(ii) Hepatobiliary evaluation: select at least 3 of the following 5

- Alkaline phosphatase (ALP)

- Bilirubin (total)
- Gamma-glutamyltranspeptidase (GG transferase)
- 5' nucleotidase
- Total bile acids

(iii) Other markers of cell changes or cellular function

- Albumin
- Calcium chloride
- Total cholesterol
- Cholinesterase
- Creatinine
- Globulin
- Glucose (in fasted animals)
- Phosphorous
- Potassium
- Total Protein
- Sodium triglycerides
- Urea

(g) Urinalyses: Timed urine volume collection should be conducted during the last week of the study. The volume of urine collected, specific gravity, pH, glucose, and protein should be determined as well as conducting a microscopic evaluation of urine for sediment and presence of blood/blood cells.

(h) Neurotoxicity Screening/Testing: Screening for neurotoxic effects should be routinely carried out in short-term and subchronic toxicity studies with rodents (preferably rats) and non-rodents (preferably dogs), one-year studies in non-rodents, and reproductive toxicity studies in rodents.

(i) Immunotoxicity: For short-term, subchronic and developmental toxicity studies, results of clinical tests that are included in the list of primary indicators for immune toxicity should also be evaluated as part of an immunotoxicity screen. Additional immunotoxicity tests.

(j) Necropsy and microscopic Examination Gross necropsy: All test animals should be subjected to complete gross necropsy, including examination of external surfaces, orifices, cranial, thoracic and abdominal cavities, carcass, and all other organs. The gross necropsy should be performed by, or under the direct supervision of, a qualified pathologist, preferably the person who will later perform the microscopic examination.

(k) Organ weight: Organs like adrenals, brain, epididymis, heart, kidneys, liver, spleen, testes, thyroid/parathyroid, thymus, ovaries and uterus should be weighed. Organs should be carefully dissected and trimmed to remove fat and other contagious tissue and then be weighed immediately to minimize the effects of drying on weight of organ.

(l) Preparation of Tissues for Microscopic Examination Generally, the following tissues should be fixed in 10% buffered formalin (or another generally recognized fixative) and sections prepared and stained with hematoxylin and eosin (or another appropriate stain) in preparation for microscopic examination. Lungs should be inflated with fixative prior to immersion in fixative.

(m) Microscopic Evaluation: All gross lesions should be examined microscopically. All tissues from the animals in the control and high dose groups should be examined. If treatment related effects are noted in certain tissues, then the next lower dose level tested of those specific tissues should be examined. Successive examination of the next lower dose level continues until no effects are noted. In addition, all tissues from animals which died prematurely or were sacrificed during the study should be examined microscopically to assess any potential toxic effects.

(n) Histopathology of Lymphoid Organs: Histopathological evaluation of the lymphoid organs should be performed for all animals in short-term and sub chronic toxicity studies and developmental toxicity studies. Different OECD guidelines for testing of chemicals is shown in Table 1.5.

Table 1.5 Different OECD guidelines for testing of chemicals

Test No. 401	Acute Oral Toxicity
Test No. 402	Acute Dermal Toxicity
Test No. 403	Acute Inhalation Toxicity
Test No. 404	Acute Dermal Irritation/Corrosion
Test No. 405	Acute Eye Irritation/Corrosion
Test No. 406	Skin Sensitisation
Test No. 407	Repeated Dose 28-day Oral Toxicity Study in Rodents
Test No. 408	Repeated Dose 90-Day Oral Toxicity Study in Rodents
Test No. 409	Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents
Test No. 410	Repeated Dose Dermal Toxicity: 21/28-day Study
Test No. 411	Subchronic Dermal Toxicity: 90-day Study
Test No. 412	Repeated Dose Inhalation Toxicity: 28-day or 14-day Study
Test No. 413	Subchronic Inhalation Toxicity: 90-day Study

Table 1.5 *Contd…*

Test No. 414	Prenatal Development Toxicity Study
Test No. 415	One-Generation Reproduction Toxicity Study
Test No. 416	Two-Generation Reproduction Toxicity
Test No. 417	Toxicokinetics
Test No. 418	Delayed Neurotoxicity of Organophosphorus Substances
Test No. 419	Delayed Neurotoxicity of Organophosphorus Substances: 28-day
Test No. 420	Acute Oral Toxicity - Fixed Dose Procedure
Test No. 421	Reproduction/Developmental Toxicity Screening Test
Test No. 422	Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test
Test No. 423	Acute Oral toxicity - Acute Toxic Class Method
Test No. 424	Neurotoxicity Study in Rodents
Test No. 425	Acute Oral Toxicity: Up-and-Down Procedure
Test No. 426	Developmental Neurotoxicity Study
Test No. 427	Skin Absorption: In Vivo Method
Test No. 428	Skin Absorption: *In vitro* Method
Test No. 429	Skin Sensitisation: Local Lymph Node Assay
Test No. 430	*In vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)
Test No. 431	*In vitro* Skin Corrosion: Human Skin Model Test
Test No. 432	*In vitro* 3T3 NRU Phototoxicity Test
Test No. 435	*In vitro* Membrane Barrier Test Method for Skin Corrosion
Test No. 440	Uterotrophic Bioassay in Rodents: A short-term screening test for oestrogenic properties
Test No. 451	Carcinogenicity Studies
Test No. 452	Chronic Toxicity Studies
Test No. 453	Combined Chronic Toxicity/Carcinogenicity Studies
Test No. 471	Bacterial Reverse Mutation Test
Test No. 473	*In vitro* Mammalian Chromosome Aberration Test
Test No. 474	Mammalian Erythrocyte Micronucleus Test
Test No. 475	Mammalian Bone Marrow Chromosome Aberration Test
Test No. 476	*In vitro* Mammalian Cell Gene Mutation Test
Test No. 482	Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *in vitro*
Test No. 483	Mammalian Spermatogonial Chromosome Aberration Test
Test No. 484	Genetic Toxicology: Mouse Spot Test
Test No. 485	Genetic toxicology, Mouse Heritable Translocation Assay
Test No. 486	Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

1.6 United States Food and Drug Administration (USFDA)

The USFDA is an organization of Department of Health and Human Services (HHS) in United States. This body is responsible for due care of the public health by assuring the protection, effectiveness, and security of human and veterinary drugs, vaccines and other biological products, medical devices, national food supply, cosmetics, dietary supplements, and products that give off radiation. In order for drugs, medical devices, and other products to be approved, the FDA does not require that animals be used in product testing and drug development. Instead, it requires that certain safety and efficacy tests be met and as such has the authority to vastly reduce the number of animal tests by making the use of existing, validated alternatives mandatory.

1.6.1 FDA Fundamentals

The Food and Drug Administration (FDA) is an agency within the U.S. Department of Health and Human Services. It comprises of the Office of the Commissioner and four directorates overseeing the core functions of the agency: Medical Products and Tobacco, Foods and Veterinary Medicine, Global Regulatory Operations and Policy, and Operations.

1.6.2 Office of the Commissioner

The office collectively performs leadership of the agency's scientific activities, communication, legislative liaison, policy and planning, women's and minority health initiatives, agency operations, and toxicological research.

1.6.3 Office of Foods and Veterinary Medicine

This office addresses food and feed safety, nutrition, and other critical areas to achieve public health goals.

1.6.4 Office of Global Regulatory Operations and Policy

It provides leadership for FDA's domestic and international product quality and safety efforts.

1.6.5 Office of Medical Products and Tobacco

Advice and counsel to the Commissioner on all medical product and tobacco-related programs and issues.

1.6.6 Office of Operations

It provides agency-wide services including information technology, financial management, procurement, library services, and freedom of information, FDA history, and facilities.

1.6.7 Responsibilities of FDA

FDA is responsible for protecting the public health by assuring the safety, effectiveness, quality, and security of human and veterinary drugs, vaccines and other biological products, and medical devices. The FDA is also responsible for the safety and security of most of nation's food supply, all cosmetics, dietary supplements and products that produce any kind of radiation.

- It protects the public from electronic product radiation.
- It ensures that cosmetics and dietary supplements are safe and properly labeled
- It regulates tobacco products.
- It progress the public health by increasing product innovations.

1.6.8 FDA Structure/ Organization: Structure of FDA is Depicted in Figure 1.3 (a)

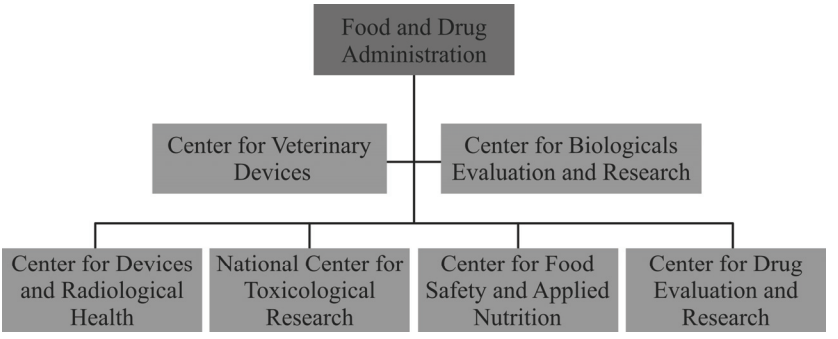


Figure 1.3 (a) Structure of FDA.

1.6.9 Scope of FDA

The scope of FDA's regulatory authority is very broad. The following is a list of traditionally-recognized product categories that fall under FDA's regulatory jurisdiction; In general, FDA regulates:

- Foods, including: dietary supplements, bottled water, food additives, infant formulas
- Drugs, including: prescription drugs (both brand-name and generic), non-prescription (over-the-counter) drugs

- Biologics, including: vaccines, blood and blood products, cellular and gene therapy products, tissue and tissue products, allergenic
- Medical Devices, including: tongue depressors and bedpans, complex technologies such as heart pacemakers, dental devices, surgical implants and prosthetics
- Electronic Products that give off radiation, including: microwave ovens, x-ray equipment, laser products, ultrasonic therapy equipment, mercury vapor lamps, sunlamps
- Cosmetics, including: color additives found in makeup and other personal care products, skin moisturizers and cleansers, nail polish and perfume
- Veterinary Products, including: livestock feeds, pet foods, veterinary drugs and devices
- Tobacco Products, including: cigarettes, cigarette tobacco, roll-your-own tobacco, smokeless tobacco

1.6.10 FDA Advisory Committee

FDA has an advisory committee. It provides special advices on problems related to human and veterinary drugs, vaccines and other biological products, medical devices, and food. In general, advisory committees include a chairperson, several members, a consumer, industry, and sometimes a patient representative. Additional experts with special knowledge may be added for individual committee meetings as needed. Although the committees provide advice to the agency, FDA has the right to take final decisions.

1.6.11 Qualifications of a Scientific Member of an Advisory Committee

Persons suggested as scientific members must be technically skilled experts in their field, such as clinical medicine, engineering, biological and physical sciences, biostatistics and food sciences. They also must have knowledge about interpretation and analysis of all scientific data, and understanding its public health significance.

1.7 Necessity of Animals for Testing of Medical Products

For testing of drugs, vaccines and other biologics, and medical devices animals are used to determine the safety of the medical product.

For drugs and biologics, the focus of animal testing is on the drug's nature, chemistry, and effects (pharmacology) and on its potential damage to the body (toxicology). Animal testing is used to measure:

- What is the bioavailability of the drug or biologics?
- What is the pharmacokinetics of the drug or biologics?
- What is toxicity of the drug and its metabolites?
- What is the excretion rate of the product?

Medical devices need biocompatibility test before using on human body because it must be biocompatible with the human tissues. Most of the devices are made of materials stainless steel or ceramic that is biocompatible with human tissues, in these cases, no animal testing is required. However, some devices with novel materials require biocompatibility testing in animals.

There are still many areas where animal testing is essential. But FDA decreases the need of animal testing. FDA has been performing various research and development efforts which reduce the need for animal testing and to work toward replacement of animal testing.

1.8 Public Health Service Policy on Humane Care and Use of Laboratory Animals

The Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) was given in 1973 and revised in 1979 and 1986. The PHS Policy (NIH, 1986) applies to all institutions that use live vertebrates in research supported by any component of PHS: the Agency for Health Care Research and Quality, the Centers for Disease Control and Prevention, the Food and Drug Administration, the Health Resources and Services Administration, the Indian Health Service, the National Institutes of Health (NIH), and the Substance Abuse and Mental Health Services Administration. The PHS Policy requires institutions to establish and maintain proper measures to ensure the appropriate care and use of animals involved in research, research training, and biologic testing activities.

1.9 US Government Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research and Training

The US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (US Government Principles) were drafted in 1985 by the Interagency Research Animal Committee (IRAC, 1985), made up of individuals drawn from federal agencies that use or require the use of animals in research or testing. Its

nine statements address compliance with the animal welfare act (AWA) and other applicable federal laws, guidelines, and policies (such as AWRs, HREA, and the Guide) and generally provide a set of overarching principles for ensuring that the use of research animals is justified and humane.

1.10 Animal Welfare Act (AWA)

The Animal Welfare Act works in U.S. This Act is mainly for wellbeing of animals in research. The Act came into force in 1966. It regulates the care and use of animals in research, testing, teaching, exhibition, transport. AWA offer least protection for certain species while excluding others such as rats, mice, and birds. It does not apply to cold-blooded animals (fish, reptiles, and amphibians). This law applies to dogs, cats, nonhuman primates, guinea pigs, hamsters, rabbits. The law makes least standard for veterinary care, handling, feeding, and housing. It also maintains the psychological well being. Various government statistics gives approximate details that U.S. labs utilize 25 millions animals in a year. U.S. labs make use of 100 million genetically engineered animals.

1.11 The U.S. Department of Agriculture (USDA)

AWA is enforced by the U.S. Department of Agriculture (USDA). A animal Care program, Animal and Plant Health Inspection Service (APHIS) administers AWA regulations and standards.

Under the AWA, businesses and individuals using regulated animals must be licensed or registered with the USDA and facilities with regulated animals must be inspected yearly by APHIS. There is no legal requirement for the inspection of federally-owned and operated research facilities. The USDA has no jurisdiction over facilities using animals not covered under the AWA.

1.12 Institutional Animal Care and Use Committee (IACUC)

Institutions or research organizations can establish an Institutional Animal Care and Use Committee (IACUC) under the AWA for monitoring and evaluation of the institution's animal care and use program. The responsibilities of IACUC's include:

- To check the facility providing for animal care and use program,
- To conduct inspection of the animal labs at least twice a year,
- To review and approve, disapprove, or modifications required in the research protocols.

- To respond, investigate and act on public complaints on animal care and use.
- To report about any shortcoming of animal care and use.
- To submit reports about the animal care and use to institution.

The IACUC is comprised of three members and including a veterinarian from the facility and one person not affiliated with the facility who will "provide representation for general community interests in the proper care and treatment of animals."

1.13 Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)

Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) is accreditation body to increase availability for government funding to the institutes or organization. It is a privately funded organization based on nonprofit membership that is financial supported from institutions it credits and inspects. It promotes humane care of animals. The aim of AAALAC is monitor and ensure the improvement and protection of the animals used in biomedical research. It follows the guidelines recommended for the Care and Use of Laboratory Animals. It is a self-policing association for accreditation.

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