Review of bioassay

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❖ ABSTRACT:

Bioassays, including immunoassays, enzyme assays, and assays using enzyme electrodes, and nucleic acid hybridization probes have been the subject of considerable industrial and academic research. New bioassay methods have applications in the medical, chemical, pharmaceutical, and food products industries. Recent US patents and scientific literature on a variety of new bioassay methods are surveyed. A description of these patents and a list of references are given.

The present study paper on the biological assays of pharmaceutical product is intended to review on the available literature that are widely applicable in the bioassay of pharmaceutical substances.

There is an urgent need for more strategies and procedures for bioassay techniques, including methods that employ multicellular and unicellular organism. The presence of small amounts of pyrogen or endotoxin in recombinant protein preparations and other pharmaceutical product can cause side effects in host organism such as endotoxin shock, tissue injury, and even death. Due to these reactions, it is essential to remove endotoxins from drugs, injectables, and other biotechnological products, an overview of this subject is provided by this paper.

An extensive review of literature with regard to methods for pyrogen, endotoxin and toxicity bioassay test of pharmaceutical and biotechnological preparation was carried out. Rabbit pyrogen test is an older, more routine, standard and official in-vivo multicellular test methods in most pharmaceutical compendia. Limulus amebocyte lysine (LAL) is an in-vitro test currently employ in the detection and quantification of endotoxins that are of bacteria and non bacterial origin in a variety of solution, and can be use for screening of starting materials.

The possible advantages of LAL test compared to Rabbit method include rapidity, reliability, ease and adaptability. Biological assays are very essential in clinical medicine and other field of science for the role it plays in evaluation and assessment of pharmaceutical and biotechnological preparation.

Keywords :- chemical quality, purity, principle, analysis, identification, pharmacological activity,

❖ INTRODUCTION :

A bioassay is an analytical method to determine the concentration or potency of a substance by its effect on living animals or plants (*in vivo*), or on living cells or tissues (*in vitro*).

A bioassay can be either quintal or quantitative, direct or indirect. If the measured response is binary, the assay is quintal, if not, it is quantitative. A bioassay may be used to detect biological hazards or to give an assessment of the quality of a mixture.

A bioassay is often used to monitor water quality as well as waste water discharges and its impact on the surroundings. It is also used to assess the environmental impact and safety of new technologies and facilities.

The development of more and more powerful analytical methods is an ongoing process and lays the foundation of many scientific, technological and medical advances. One example is the fierce advancement of the field of mass spectrometry, which had been considered as largely mature for some time. However, the analytical information explosion caused by these technological improvements may finally lead to an information overload . To pick out the relevant results out of the vast amount of data is by no means a trivial task. The emergence of the new scientific field of bioinformatics is one way to try to tame this mass of information. Unfortunately, the purely mathematical treatment of experimental data cannot replace lacking knowledge or understanding in general.

One of the ways out of this problematic situation is to abandon the purely chemical and mathematical perspective and to turn to the concept of bimolecular interaction, which is one of the most influential ideas in pharmacology and toxicology.

Paul Ehrlich (1854–1915) put it shortly in his fundamental principle *Corpora non gaunt nisi fixate* (Drugs do not act unless they are bound). Or to put it the other way round:

Only compounds binding to biological targets are of interest. Based on this rationale, it becomes evident that a “bimolecular interaction step” should be introduced into analytical methods to achieve bio-selectivity and to focus on relevant compounds. How to achieve this is the main topic of this review.

Estimation of potency of an active principle in a unit quantity of preparation. OR

Detection and measurement of the conc. Of the substance in a preparation using biological method

❖ AIM:

The primary purposes of bioassays are **to measure the pharmacological activity of new or chemically undefined substances**, as well as to determine side-effect profiles, including toxicity.

❖ OBJECTIVE:

To carry out Research and Development of medicinal plant products for anti malaria, anti cancer and anti bacteria properties.

Specific Objectives:

- To strengthen and developed many cell based assays for biological properties in vitro and efficacy studies in vivo to determine bioactivity and medicinal potential of herbal plants either as extracts or in pure chemical constituents.
- To carry out research on medicinal plant products/herbal preparations for anti malaria, anti cancer, and anti bacteria properties through in vitro biological screening, in vivo efficacy test, and mechanism of action determination.

It involves :

1. Evaluation of Internal Deposition:

The primary use of bioassay methods is to determine whether an individual has been exposed to a radioactive material in a manner that resulted in an internal deposition and, if so, to quantify the magnitude of that deposition and its dissymmetric consequences.

It plays an important role in the medical management of potentially over-exposed individuals.

Routine scheduled measurements, performed periodically after an individual is on the job, provide important input for evaluations of the extent to which the individual is adequately protected, is observing safe working practices, and is avoiding the accumulation of internally deposited radionuclide's

Bioassay results may be obtained when an individual takes up new job assignment and when an individual terminates a particular job assignment to document the estimated body or organ burden at that time .

2. Evaluation of Central Procedure

- Bioassay provides a useful tool for evaluating the general conditions of exposure throughout an operating facility.
- It gives important base line data and provides useful background information on exposures that might have occurred in past occupational assignments.
- It can indicate trends towards greater or lesser accumulations of radioactivity within the working population.
- Bioassay results can provide information on possible exposures associated with unusual procedures for which experience is not available, and on exposures that have been occurring but were not suspected.
- These results can also indicate the extent to which engineered confinement-measures and the air sampling programmed have been effective in the control of the exposures .

3. Improved Metabolic Data :

Many guidelines and standards are based, in large part, on results obtained from laboratory animals exposed to radionuclides by different routes of administration.

The models used in these documents are based on appropriate extrapolations to human exposures.

Thus, the bioassay data from human exposures are invaluable in the development of standards and the validation of extrapolations of animal data.

❖ Literature review:

L. W. Little

Research Triangle Institute, Research Triangle Park, .N. C.

(This reeled tDOS prepared for, and should be considered part of, the Annual Stature Review issue of the Journal; the manuscript was received too late for inclusion i11 tile June iSS11e.) Emphasis on bioassay proceeds rues continued. To increase in 1977 as did efforts to monitor and regulate the discharge of pollutants into the environment.

As Mount 1 put it, "Aquatic toxicologists of today are faced with an enor- mous pressure to provide decisions regarding tl1e potential effects of hundreds of chemicals should they be released into the environment." As many of the 1977 publications reveal, aqua- tic scientist's recogni1...e the inadequacy of in- formutio11 available for decision making, but recognize as well the need for expediency.

Clearly, when a decision must be made, one can only rely on the best available information. Yet, according to Doudoroff,' sometimes avail- able information is not considered in setting water quality l'riterio. Citing the criteria set by EPA for cyanide in water as an example, he strong (lee criticized EPA's "Quality Criteria for Water" document,and made an '"appeal for logic" in setting criteria and standards.

For those aquatic toxicologists and biologists who have become pessimistic in regard to their role in water pollution control, Mount's light- hearted approach to the state-of-the-art is recommended.

To make the task of testing large numbers of chemical more manageable, a number of publication s have identified batteries or tiers of screening tests. Duties outlined a pattern of laboratory testing to provide a basis for est.- mating, in a cost-effective way, the hazard of new materials to aquatic life.

He described a program of sequin tail environmental testing, including decision points and points where the most appropriate type of test can be identified and selected. Included in the program, in add it ion to bioassays, nerd assessments of the amour n t of material, its pattern of use and dies·

1852 Journal WPCF postal, its chemical and physical properties, and its fate in the environment. Kimberley el al. described a similar approach for evaluating en- vironmental effects of new industrial products.

The Industrial Environmental Research Lab- oratory of EPA published procedures '·' for Level (screening) assesment of materials, often complex mixtures, discharged lo the environment .

Included are outlines for chemical fractionation, testing, and identification; health- effects tests, including the Ames Salmonella typl1imurium reverse mutation test; and eco- logical effects tests such as bioassays with fresh- water and marine phytoplankton, invertebrates, and fish.

BIOASSAY

DEFINATION :

"Determination of the power of the drug or of a biological product by testing its effect on an animal of standard size".

OR

"The determination of the relative strength of a substance (e.g., a drug or hormone or toxicant) by comparing its effect on a test organism with that of a standard preparation." is called bioassay.

Bioassay or biological standardization is a kind of scientific experiment. A bioassay involves the usage of live animal or even plant or muscle or cell to determine the biological activity of an substance, such being a hormone or drug. Bioassays are generally conducted to measure the end results of a substance on a living organism and they are essential in the particular development of new drugs and within monitoring environmental contaminants.

Bioassay (commonly used shorthand for biological assay or assessment), or biological standardization is a type of scientific experiment.

A bioassay involves the use of live animal or plant (in vivo) or tissue or cell (in vitro) to determine the biological activity of a substance, such as a hormone or drug.

Bioassays are typically conducted to measure the effects of a substance on a living organism and are essential in the development of new drugs and in monitoring environmental pollutants. Both are procedures by which the potency or the nature of a substance is estimated by studying its effects on living matter. A bioassay can also be used to determine the concentration of a particular constitution of a mixture that may cause harmful effects on organisms or the environment.

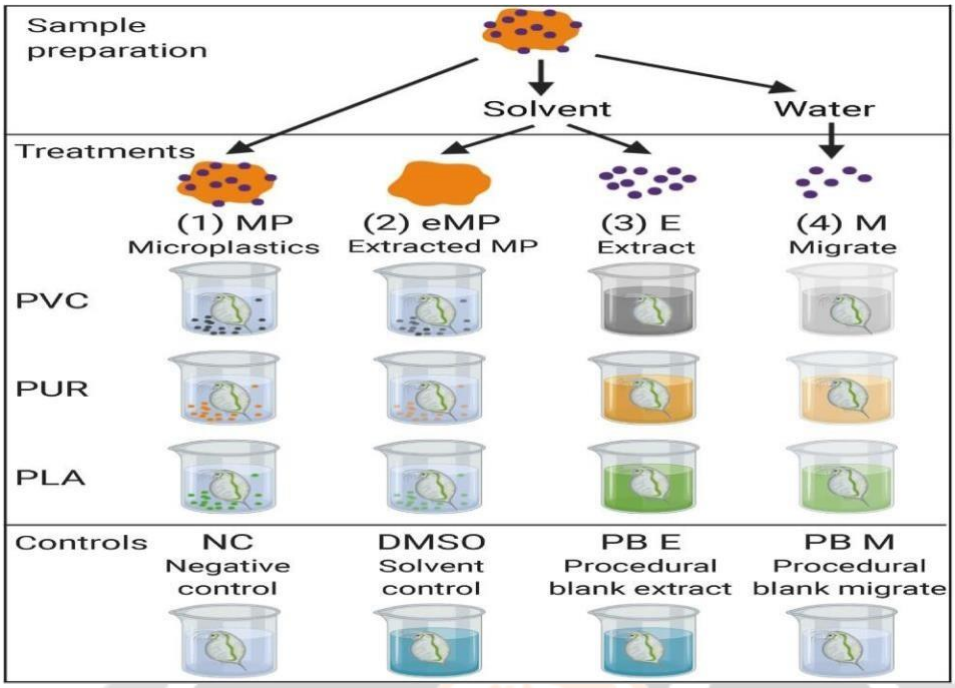
A bioassay is an analytical method to determine the concentration or potency of a substance bits effect on living animals or plants (in vivo), or on living cells or tissues (in vitro).

A bioassay can be either quintal or quantitative, direct or indirect. If the measured response is binary, the assay is quintal, if not, it is quantitative.

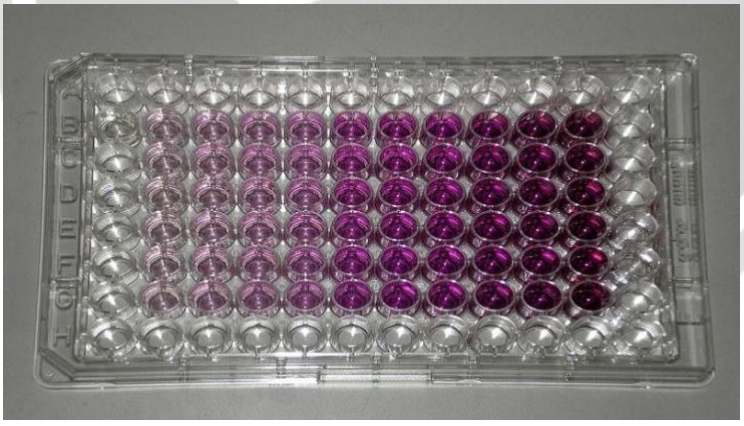
A bioassay may be used to detect biological hazards or to give an assessment of the quality of a mixture. A bioassay is often used to monitor water quality as well as wastewater discharges and its impact on the surroundings.

It is also used to assess the environmental impact and safety of new technologies and facilities.

BIOASSAY SETUP



A biological test system (here: *Daphnia magna*) is exposed to various experimental conditions (here: several microplastics preparations), to which it reacts



Some indicator of these reactions (e.g. a color change) is assessed, typically in a highly automated fashion through microplates like this.

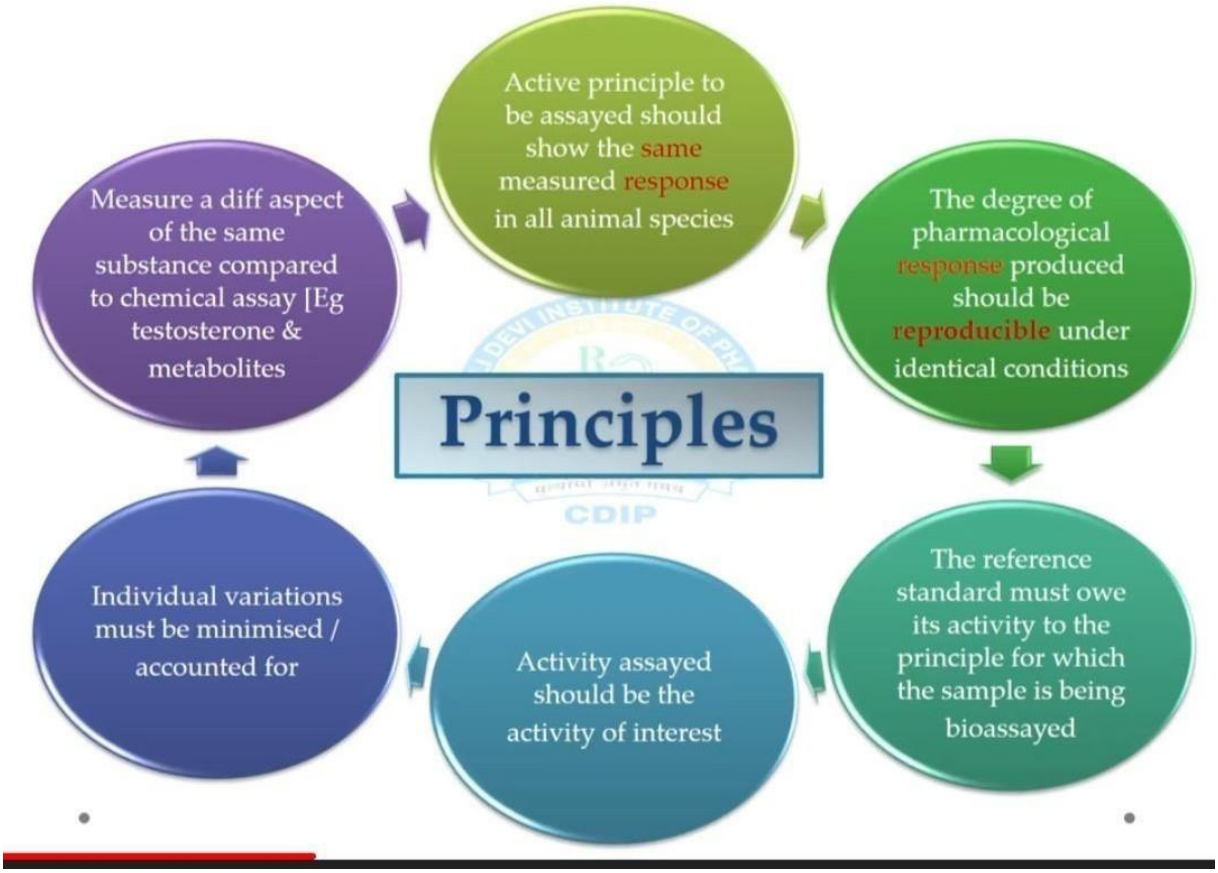
❖ Principle

A bioassay is a biochemical test to estimate the potency of a sample compound.
Usually this potency can only be measured relative to a standard compound.
A typical bioassay involves a stimulus (ex. drugs) applied to a subject (ex. animals, tissues, plants).

The corresponding response (ex. death) of the subject is thereby triggered and measured active principle to be assayed should show the same measured response in all animal species. The degree of pharmacological response produced should be reproducible under identical conditions [e.g Adrenaline shows same rise in BP in the same species under identical conditions: wt, age, sex, strain breed … etc].
The reference standard must owe its activity to the principle for which the sample is being bioassay .

Activity assayed should be the activity of interest • Individual variations must be minimized
Bioassay involves the comparison of the main pharmacological response of the unknown preparation with that of the standard.

The reference standard and test sample should have same pharmacological effect and mode of action, so that their DRC curve run parallel and their potency ratio can be calculated.



❖ History

The first use of a bioassay dates back to as early as the late 19th century, when the foundation of bioassays was laid down by German physician Paul Ehrlich.He introduced the concept of standardization by the reactions of living matter.

His bioassay on diphtheria antitoxin was the first bioassay to receive recognition. His use of bioassay was able to discover that administration of gradually increasing dose of diphtheria in animals stimulated production of antiserum.

One well known example of a bioassay is the "canary in the coal mine" experiment.

To provide advance warning of dangerous levels of methane in the air, miners would take methane-sensitive Some indicator of these reactions (e.g. a color change) is assessed, typically in a highly automated fashion through microplates like this.

Principle History canaries into coal mines. If the canary died due to a build-up of methane, the miners would leave the area as quickly as possible.

Many early examples of bioassays used animals to test the carcinogenicity of chemicals.

In 1915, Yamaigiwa Katsusaburo and Koichi Ichikawa tested the carcinogenicity of coal tar using the inner surface of rabbit's ears.

From the 1940s to the 1960s, animal bioassays were primarily used to test the toxicity and safety of drugs, food additives, and pesticides.

Uses of bioassay :

- Bioassays are procedures that can determine the concentration or purity or biological activity of a substance such as vitamin, hormone or plant growth factor by measuring the effect on an organism, tissue, cells, enzyme or receptor.
- Bioassays may be qualitative or quantitative.
- Qualitative bioassays are used for assessing the physical effects of a substance that may not be quantified, such as seeds fail to germinate or develop abnormally deformity.
- An example of a qualitative bioassay includes Arnold Adolph Berthold's famous experiment on castrated chickens.

- This analysis found that by removing the testicles of a chicken, it would not develop into a rooster because the endocrine signals necessary for this process were not available.

- Quantitative bioassays involve estimation of the concentration or potency of a substance by measurement of the biological response that it produces.

- Quantitative bioassays are typically analyzed using the methods of biostatistics.
- For more information Look up Basic and Clinical Pharmacology by Bertram G. Katzung.
- To measure the pharmacological activity of new agents isolated from plant or animal sources or prepared in the chemical laboratories or chemically undefined substances. .
- To investigate the function of endogenous mediators .
- To measure drug toxicity and unwanted side effects .
- Measurement of the concentration of known substances.
- Determination of the potency of drugs that cannot be assayed chemically because of the lack of reliable chemical assay as insulin, heparin and oxytocin.
- Determination of the therapeutic advantage of drug over another in the treatment of certain disease.

❖Purpose:

The purpose of bioassay as follows:

- Measurement of the pharmacological activity of new or chemically undefined substances
- Investigation of the function of endogenous mediators
- Determination of the side-effect profile, including the degree of drug toxicity
- Measurement of the concentration of known substances (alternatives to the use of whole animals have made this use obsolete)
- Assessing the amount of pollutants being released by a particular source, such as wastewater or urban runoff.
- Determining the specificity of certain enzymes to certain substrates. To ascertain the potency of a drug and hence it serves as the quantitative part of any screening procedure (Research).

- To standardize drugs, vaccines, toxins or poisons, disinfectants, antiseptics etc., so that each contains the uniform specified pharmacological activity.

- Helps to determine the specificity of a compound to be used e.g. Penicillin's are effective against Gram +ve but not on Gram –ve.
- Certain complex compounds like Vitamin B-12 which can't be analysed by simple assay techniques can be effectively estimated by Bioassays.
- For samples where no other methods of assays are available. Compare test sample with standard substance to determine quantity of test sample required to produce an equivalent biological response to that of the standard substance.
- Measuring pharmacological activity of new or chemically undefined substance.
- Test method employed in measuring the response of living animals to toxicity of chemical contaminant. Certain no. of individuals of sensitive specific are exposed to specific conc. of contaminant for specific period to examine toxic effects.

❖Applications of Bioassay :

- ✓ Establishing regulatory requirements for water quality.
- ✓ Ecological monitoring of sewages discharge
- ✓ State environmental monitoring of water bodies, particularly in the areas of the human exposure.
- ✓ Environmental impact assessment of new technologies, treatment facilities, reconstruction and modernization of national economic projects.
- ✓ For designing local treatment facilities assessment of aquatic ecosystems. Health care (medical),
- ✓ Crop production and agriculture,
- ✓ Non food (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels)
- ✓ Environmental uses. A bioassay is an analytical method to determine the concentration or potency of a substance by its effect on living animals or plants (in vivo), or on living cells or tissues(in vitro). A bioassay may be used to detect biological hazards or to give an assessment of the quality of a mixture.

❖ ADVANTAGES & DISADVANTAGES

ADVANTAGES OF BIOASSAY OVER CHEMICAL ASSAY :

- The active principle does not have to be known.
- The active principle may be known but its chemical composition does not have to be established.
- The active principle does not have to be in pure state.
- The sensitivity of bioassay method is far greater than that of the chemical method.
- Faster, more consistency, less acid waste; reduced labor costs; facilitates sequential analysis; better alpha peak resolution.
- Better detection limit for uranium-500 mL+ sample (vs. current 50 mL sample size)
- Cartridge technology more efficient; eliminates large sequential load solutions (evaporation steps).
- Simple & Faster
- Amount of test drug available is small ➢ Does not involve complicated calculations ➢ Does not depend on DRC.

❖ DISADVANTAGES :

- The bioassay procedures are less accurate, more time consuming and more expensive
- It is time consuming process
- It requires much skills
- Biological variation exists. The effect measured in the test animals often is not that which the drug is intended to produce in treating patients.
- The importance of this discrepancy was minimized formerly, but recent studies have shown that when several active principles are present in a crude drug, those producing the maximal therapeutic effect are not necessarily the ones chiefly responsible for the action measured in the assay.
- As a result, samples found to be of equal strength by assay may show different potencies when employed clinically.
- Biological assays leave much to be desired in several respects.
- Although some are extremely sensitive in detecting small differences in concentration, their quantitative accuracy accuracy usually falls considerably below that obtainable with chemical Techniques.

❖ Errors in bioassays :

Biological variation :

- ✓ Loss of tissue sensitivity.
- ✓ Different species/sex/age/weight/health status.
- ✓ Laboratory condition may be variable.
- ✓ Housing and handling of animals.

Methodological error:

- ✓ Lack of standardization of procedure.
- ✓ Set-up of apparatus.
- ✓ Tissue isolation/preparation for experiment.
- ✓ Drug preparation or dilution

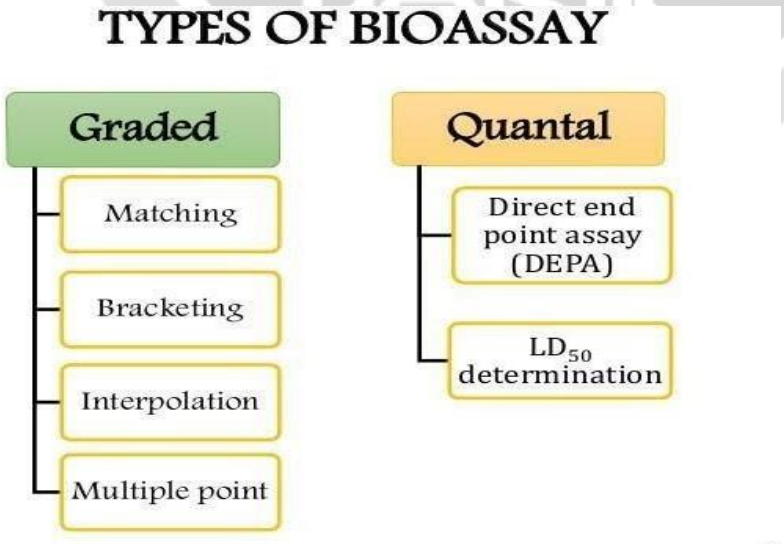
Non-Gaussian Berkson errors in bioassay :

The experimental design plays an important role in every experimental study. However, if errors in the settings of the studied factors cannot be avoided, i.e. Berkson errors occur, the estimates of the model parameters may be biased and the variability in the study increased.

Correction methods for the effect of Berkson errors are compared. The emphasis is on the study of correlated Berkson errors which follow non-Gaussian distribution as this appears to have been a neglected, yet important, area.

It is shown that the regression calibration approach bias correction methods are useful when the Berkson errors are independent. However, when these errors are dependent, the newly proposed method B-SIMEX clearly outperforms the other methods.

❖ TYPES OF BIOASSAY :



Bioassays are of two types :

1. Graded assay:

- Graded responses are those that are measured by continuous variables such as weight, body temperature, blood glucose level, blood pressure, the number and strength of contractions of heart, respiratory rate, the extent to which an isolated tissue contracts or relaxes etc., The graded responses may be assessed by using either the whole animal or a part of the animal.
- The graded bioassays are based on the observations that there is a proportionate increase in the observed response with a subsequent increase in the concentration or dose. Then the test responses are compared with that of the standard. The parameters employed in such bioassays are based on the nature of the effect of the substance that is expected to produce. For e.g., contraction of a smooth muscle preparation (guinea pig ileum) for assaying histamine; the study of blood pressure response in case of adrenaline.
- This type of assay gives almost identical results. The choice of the assay depends upon, o Precision of assay demands. o Quantity of the sample available. o Availability of experimental animals.
- Graded assays are based on the observation that there is a proportionate increase in the observed response following an increase in the concentration or dose. The parameters employed in such bioassays are based on the nature of the effect the substance is expected to produce. For example: contraction of smooth muscle preparation for assaying histamine or the study of blood pressure response in case of adrenaline.

A graded bioassay can be performed by employing any of the below-mentioned techniques. The choice of procedure depends on:

1. the precision of the assay required
2. the quantity of the sample substance available
3. the availability of the experimental animals.

There are four types of graded assay :

1. Matching Dose Bioassay:

• It is the simplest form of all graded bioassays and involves no calculations. In this type of bioassay, the response of the standard substance is recorded first and then the response of the test substance is tried to match with that of the standard by a trial and error process, until

they produce equal effects. It is also called as the analytical dilution assay as the assay involves the determination of the factor by which the test substance is either diluted or concentrated in order to produce a response that is equal to that of a known amount of the standard drug. A corresponding concentration of the test substance is then calculated. This assay is generally employed when the ample amount of sample is available. Since the assay does not involve the recording of CRC, the sensitivity of the preparation is not taken into consideration. Advantages: The assay does not depend on the assumption of a dose response relationship. Disadvantages: o Purely subjective method.

Inefficient as preliminary effects are not utilized in final assessment. Lot of experimental errors, which cannot be determined.

A crude method and not the exact method of determining the potency of a drug. o Precision and reliability are poor.

Bio assay of ach by matching tension 1 gm, Magnification 1 : 10, Batch Volume 20ml

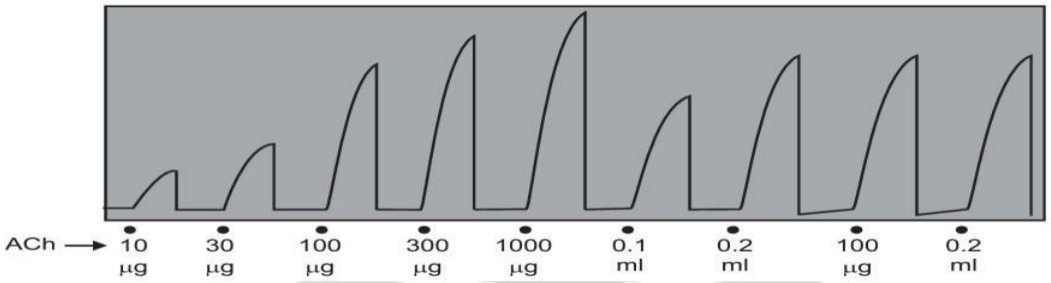


Fig : Bioassay of Ach by matching method

2. Bracketting Dose Bioassay :

• It is also a simple assay procedure, which is employed when the test sample is very small. In this method, the response due to a constant dose of the test substance is bracketed between the greater and the smaller responses due to varying doses of standard substance that provides the closest bracket. Initially, two responses of the standard substance are taken. The doses are adjusted such that one is giving response of approximately 20% and the other 70% of the maximum. The response of unknown, which lies in between the two responses of standard doses, is taken. The panel is repeated by increasing or decreasing the doses of the standard till

all three equal responses are obtained/ a closest bracket is provided for the test response by the two standard responses.

- In the end, the responses due to the double doses of the standard and the test are taken which should be equal. Concentration of the test sample can be determined as follows:

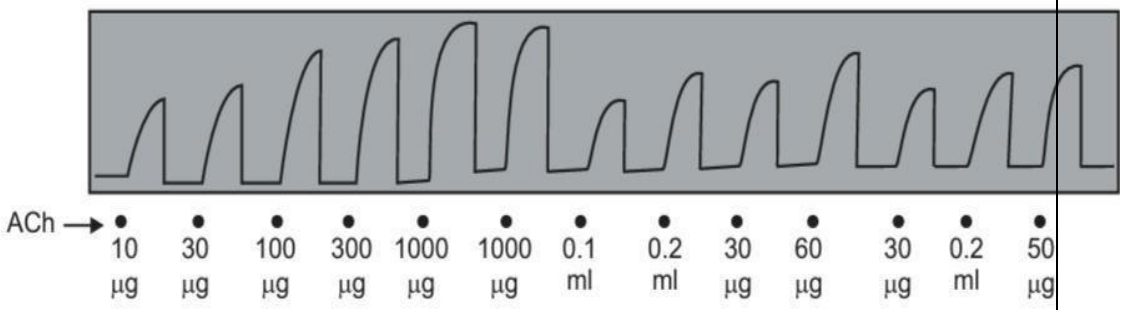
$$\text{Concentration of unknown} = \text{Dose of Standard} / \text{Dose of Test} \times \text{Concentration of Standard}$$

- This method has following limitations:

  - It occupies a larger area of the drum as far as tracings are concerned.
  - There are chances of errors that one cannot determine.
  - It does not give any idea of dose-response relationship.
  - The precision and reliability of this assay procedure is poor.

However, this method is particularly useful when the sensitivity of the preparation is not stable.

Bracketing assay of Ach
Tension 1 gm, Magnification 1 : 10 Bath volume 20 ml

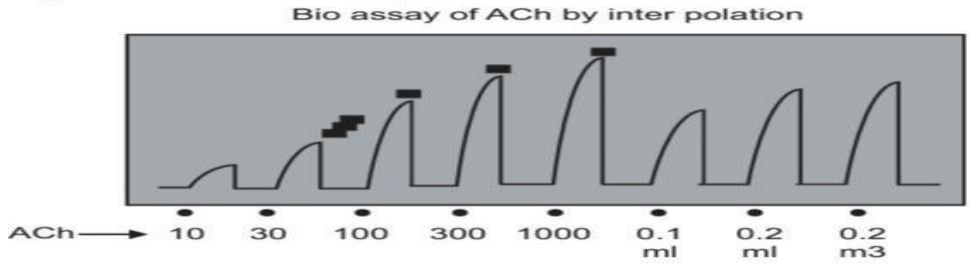


3. Interpolation Method:

- This method is based on the assumption of dose-response relationship. At first, the concentration response curve due to graded doses of a standard substance followed by the dose response curve of the test substance is recorded. Then two standard doses and one test dose are selected from the respective DRCs such that they lie on the linear portion of the DRC. The test dose is selected in such a way that its response is greater than that of smaller dose of standard and is lesser than that of larger dose of the standard. Bioassay is then carried out with the selected standard and test doses in 2-3 cycles. The height of contraction of all the standard and test doses in the bioassay is measured. Then, a log DRC is plotted with the mean values of the standard responses in the bioassay and the dose of the standard producing the same response as produced by the test sample is directly read from the graph and the concentration of the test sample is determined.

- It is a simple method and chances of errors are less if the sensitivity of the preparation is not changed. The precision and reliability of the assay is much better as compared to the

earlier methods as the sensitivity of the preparation is assessed prior to testing the unknown sample.



4. Multiple Point Bioassays:

The various types of multiple point bioassays, which are also based on doseresponse relationship, are:

- 2-point bioassay
- 3-point bioassay
- 4-point bioassay
- 6-point bioassay
- 8-point bioassay

• In these bioassays, the responses are repeated several times and the mean of each is taken. Thus, chances of error are minimized in these methods. The sequence of responses is recorded as per the Latin square method of randomization in order to avoid any bias.

(I) 2-Point Bioassay:

• Any one dose that produces from minimal to maximal response of the standard and the test substances are taken from the DRC and then their % of response is calculated.

Concentration of Test Substance = % Response of Standard / % Response of Test × Concentration of Standard .

However, this is not used practically.

(II) 3-Point Bioassay:

• This method is also based on the assumption of dose-response relationship. At first, the concentration response curve due to graded doses of a standard substance followed by the dose response curve of the test substance is recorded. Then, two standard doses and one test dose are selected from the respective DRCs such that they lie on the straightest and steepest part of the DRC. The test dose is selected in such a way that its response is greater than that of smaller dose of standard and is lesser than that of larger dose of the standard.

• Bioassay is then carried out with the chosen standard and the test doses in 3 successive cycles as per the Latin square design. The responses are recorded in the order of

- S1, S2, T;
- S2, T, S1 and
- T, S1, S2.

• The height of contraction of all the standard and test doses in the bioassay is measured. Then, a log DRC is plotted with the mean values of the standard responses in the bioassay and the dose of the standard producing the same Pharmacology - II Bio-Assays General Aspects response as produced by the test sample is directly read from the graph and the concentration of the test sample is determined. The concentration of the unknown can also be determined mathematically as follows:

$$\text{Concentration of unknown} = n2\ T \times \text{antilog}[\ T\text{-}S1\ /S2 - S1\ \times\ \log n2/\ n1\ ]Cs$$

Where, n1 = Lower Standard dose n2 = Higher Standard dose
t = Test dose S1 = Response of n1
S2 = Response of n2
T = Response of t
Cs = Concentration of Standard

Three point assay of Ach
Bath volume 20ml, Tension 1 g, Magnification 1:10

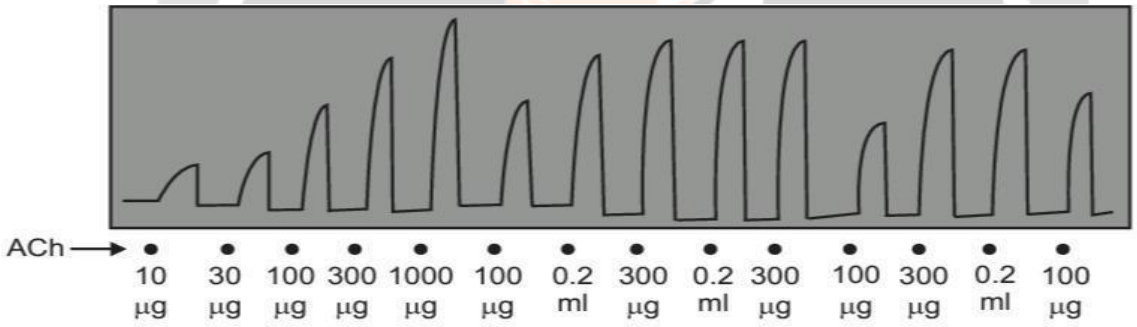


Fig . 3 Point bioassay of Ach C) 4-Point

Bioassay:

This method is almost similar to the 3-point bioassay, but in this method 2 doses of the standard and the 2 doses of the test are used. The selection of the standard doses should be such that they lie on the linear portion of the CRC and also the ratio between the smaller to greater dose should be preferably 1:2. The selection of the test doses is done by hit and trial method so that the responses fall on the linear part of the curve. By employing the Latin square design, the responses of the chosen standard and the test doses is recorded in 4 successive cycles, in the order of,

S1, S2, T1, T2;

S2, T1, T2, S1;

T1, T2, S1, S2 and T2,

S1, S2, T1.

• The height of contraction of the entire standard and the test doses in the bioassay is measured and their mean values are calculated. The concentration of the test sample can be determined by graphical method as well as mathematically by employing the following formula:

$$\text{Concentration of unknown} = n1/t1 \times \text{antilog}\,[(S1 + S2) - (T1 + T2)] / [(S2 + T2) - (S1 + T1)] \times \log n2/n1$$

Where,

n1 = Lower Standard dose

n2 = Higher Standard dose t1 = Lower Test dose

t2 = Higher Test dose

S1 = Response of n1

S2 = Response of n2

T1 = Response of t1

T2 = Response of t2

• The precision, reliability and reproducibility of this assay method are very high. Hence, it is the most commonly used bioassay for the estimation of the concentration of active substances present in the biological fluids.

➢ Advantages:

- A chemical assay finds out only the amount of active substance present in a given sample where as the bioassay measures the actual biological activity of the active substance.
- Bioassay measures small traces of compound too.
- Bioassay can establish the biological activity of a substance even when its chemical identity is not known.
- Sensitivity of a bioassay is greater than a chemical assay.
- Chemically unstable drugs can be conveniently assayed by a bioassay
- The active and the inactive isomers present in a raceme mixture can be easily distinguished by a bioassay.

➢ Disadvantages

- Complicated set up Expensive. Time consuming Too laborious.
- Requires skilled labor.
- The effect observed in animals may not be observed in humans.

The quantitative accuracy of a bioassay usually falls considerably below that attainable with most chemical assays

Four point assay of ACh on frog Rectus Abdominal muscle.

T = 0.3 ml, T = 0.6 ml S = 100 g, S = 200 1 m 2 1 2 mg

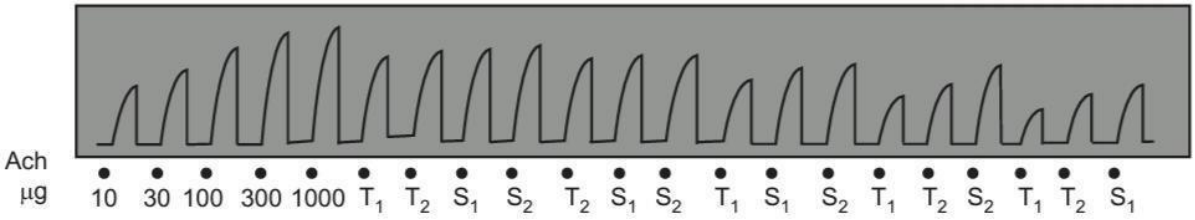


Fig . 4- Point Bioassay of Ach

Applications :

- Drugs, which are primarily of natural origin, are usually assayed by biological methods.
- When there is no suitable chemical assay is available for certain drugs such as insulin, oxytocin etc., and then bioassay is the only choice to estimate them.
- Bioassay is the only choice of analysis for some substances when the chemical assay is not a valid indication of their biological activity.
- Bioassays are useful to standardize those drugs that are composed of a complex mixture of substances of varying structure and activity. E.g., Digitalis.
- Bioassays are helpful when the purification of the crude drug sufficient for the performance of a chemical assay is not possible or practical. E.g., Vitamin D from irradiated oils.
- Bioassays are employed to find out the LD50 and ED50 of a drug under investigation.

2 . QUANTAL BIOASSAY

Definition :

A quintal response bioassay is defined as **an experiment for estimating the potency of a drug, material or process by means of the reaction** (quintal response) that follows its application to living matter The quantitative estimates of their potency are summarized by comparing these dose-response curves.

This response is in the form of "all or none" means no response or maximum response. These can be bios saved by end point method. Predetermined response is measured which is produced by threshold effect. Quantal Responses are population response based on an all-or-nothing (0 or 1-presence or absence) response such as death.

In these, response in the Indi- "visual test subject is absolute (frequently, live or die) but the critical dose of test material necessary to evoke the response is not directly determinable. Quantitation is achieved through the use of groups of test subjects and determination of the proportion responding to various dosage levels of "Unknown" and "Standard" test products. Following suitable transformation of the data (probits, angles, etc. ,) response typically is a linear function of log dose and statistical analysis is essentially similar to that employed with the graded response-parallel line bioassays.

Examples: mouse-protective potency assays of typhoid, pertussis and rabies vaccines.

(I) Direct end point assay

Threshold dose producing a required response is measured on each animal.

Eg. Bioassay of Digitalis in Cats, Hypoglycemic convulsions in mice.

Threshold dose = Period of infusion X Rate. Direct End-Point Assay

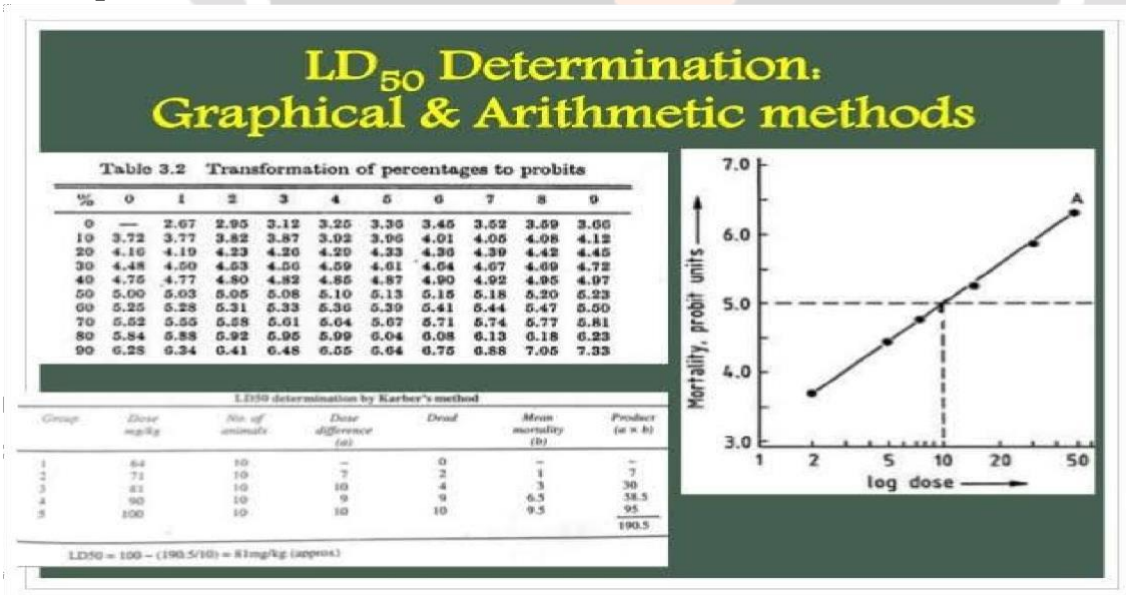
$$\text{Concentration of test} = \text{TDS}/\text{TDT} \times \text{CSD}$$

(II) LD50 Determination:

The LD50 is one way to measure the short-term poisoning potential (acute toxicity) of a material. Usually, it uses 96 hours on the test or according to requirement. We use mice (Mus musculus) as test animals.

LD50 tests Under the Animal Research Act 1985 an LD50 test (Lethal Dose 50 test) is defined as "the animal research procedure in which any material or substance is administered to animals for the purpose of determining the concentration or dose of the material or substance which will achieve any predetermined death .

➢ Graphical & Arithmetic methods :



❖ Environmental bioassays :

Environmental bioassays are generally a broad-range survey of toxicity. A toxicity identification evaluation is conducted to determine what the relevant toxicants are.

Although bioassays are beneficial in determining the biological activity within an organism, they can often be time-consuming and laborious.

Organism-specific factors may result in data that is not applicable to others in that species. For these reasons, other biological techniques are often employed, including radio immunoassays. See bio indicator.

Water pollution control requirements in the United States require some industrial dischargers and municipal sewage treatment plants to conduct bioassays. These procedures, called whole effluent toxicity tests, include acute toxicity tests as well as chronic test methods. The methods involve exposing living aquatic organisms to samples of wastewater. For example the bioassay ECOTOX uses the microalgae Euglena gracilis to test the toxicity of water samples. (See Bio indicator Microalgae as bio indicators and water quality).

❖ RESULTS & DISCUSSION :

- A bioassay is an analytical method to determine the concentration or potency of a substance by its effect on living animals or plants (in vivo), or on living cells or tissues(in vitro). ...
- A bioassay may be used to detect biological hazards or to give an assessment of the quality of a mixture.

- The study of primary purposes of bioassays are to measure the pharmacological activity of new or chemically undefined substances, as well as to determine side-effect profiles, including toxicity has been studied under the review .

- The study of bioassay has been completed.

❖ Conclusion :

- In this study of bioassay we studied different types of bioassay then its classification ,methodology as well as different kind of related concepts clearly studied under the review.
- Its applications ,procedures are also involved in the detail manner.
- It is an very important part in the pharmacy related field.
- Although bioassays are beneficial in determining the biological activity within an organism, they can often be time-consuming and laborious .

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