Abstract:
Toxicity testing is paramount in the screening of newly developed drugs before it can be used on humans. The essence of toxicity testing is not just to check how safe a test substance is; but to characterize the possible toxic effects it can produce. The guiding principles of toxicity testing is to check the effect of the test substances on laboratory animals and its direct toxic effect on human and secondly, the exposure of laboratory animals to high doses in order to evaluate its possible hazard on human that are exposed to much lower dose. The present article seeks to highlight the toxicity testing methods with respect to evaluation of acute toxicity, subchronic toxicity, chronic toxicity, teratogenicity, mutagenicity, ocular toxicity and neurotoxicity. The toxicity of substances can be observed by (a) studying the accidental exposures to a substance (b) in vitro studies using cells/ cell lines (c) in vivo exposure on experimental animals. This review mainly focuses on the various experimental animal models and methods used for toxicity testing of substances. The pre-clinical toxicity testing helps to calculate “No Observed Adverse Effect Level” which is needed to initiate the clinical evaluation of investigational products.

Key words: Toxicity testing, Laboratory animals, test substance, toxic effect, Rodents, No Observed Adverse Effect Level.

1. Introduction
Toxicology is a branch of science that deals with toxins and poisons and their effects and treatment. Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. The US-FDA states that it is essential to screen new molecules for pharmacological activity and toxicity potential in animals (21CFRPart 314). Toxicity tests are mostly used to examine specific adverse events or specific end points such as cancer, cardiotoxicity, and skin/ eye irritation. Toxicity testing also helps calculate the No
Observed Adverse Effect Level (NOAEL) dose and is helpful for clinical trials. All toxicity study is supported by; clinical analysis, autophic analysis, haematological and haematochemical analysis, histopathological analysis and statistical presentation and data interpretation.

1.1 The two basic principles guiding toxicity test in animals

- To check the effect of the test substances on laboratory animals and its direct toxic effect on human.
- Exposure of laboratory animals to high doses in order to evaluate its possible hazard on human that are exposed to much lower doses.

2. Toxicity testing methods

2.1 Acute Toxicity Studies

Acute toxicity occurs almost immediately (hours/days) after an exposure. An acute exposure is usually a single dose or a series of doses received within a 24 hour period. Death is a major concern in cases of acute exposures. Examples are:

- In 1989, 5,000 people died and 30,000 were permanently disabled due to exposure to methyl isocyanate from an industrial accident in Bhopal, India.
- Many people die each year from inhaling carbonmonoxide from faulty heaters.

Non-lethal acute effects may also occur, e.g., convulsions and respiratory irritation. This is a short term assessment and evaluation of potential hazard test substance or consequences of single dose of a test substance. Acute toxicity testing may be used in risk assessments of chemicals for humans and non-target environmental organisms. Acute toxicity study is better described as LD50, which is defined as the dose which kills 50% of animals. LD50 is used for the estimation of the toxicity of the chemical agents. Acute toxicity provides guidelines on the dose to be use in more prolonged studies and it also provides the basis for which other testing program can be design. In acute toxicity studies rodent are mostly used because they are economical and readily available and easy to handle. This test is carried out in each species of animal as the same route as intended to be use in treatment.

Acute toxicity tests are generally the first tests conducted. They provide data on the relative toxicity likely to arise from a single or brief exposure. Standardized tests are available for oral, dermal, and inhalation exposures. Basic parameters of these tests are in table.

Methods employed in acute toxicity evaluation

Graphical method of Miller and Tainter.
2.1.1 Graphical method of Miller and Tainter This method is used in the calculation of any ED50 values. This involves the administration of same volume of different doses of the test substance to various groups. The animals are divided into five groups of ten animals per group. Group one animals receive the vehicle to which the test substance is dissolved while the other groups are given different doses of the test substance. In this method log doses are plotted on a graph against probits of the percentage.

2.1.2 Arithmetical method of Reed and Muench This method is a cumulative analysis of values obtained from the result of the study. It is generally assumed that the dead of animals would have been caused by the administration of higher dose of the test substance. The cumulative dead and survivors are recorded. The % of survival is calculated and the LD50 is computed.

2.1.3 Karber’s Method This method involves the administration of different doses of test substance to various groups which has five animals each. The first group of animals receives the vehicle in which the test substance is dissolved. However, other groups receive different doses of the test substance. The animals in each group receives specific doses, while increment in dose progresses from group to group (starting from group 2 which receives the lowest dose). The interval mean of number of mortality recorded in each group and dose difference across the groups are key parameters in this method. The lethal dose is calculated using the arithmetical method of Karber which is as follows. \[ \text{LD}_{50} = \text{LD}_{100} - \frac{\sum (a \times b)}{n} \]

Where, \( \text{LD}_{50} \) = Median lethal dose
\( \text{LD}_{100} \) = Least dose required to kill 100%

a = Dose difference

b = Mean mortality
2.1.4 Lorke’s method  This test was carried out in two phases.

Phase I  This phase, nine mice divided into three groups of three mice each, are given 10, 100, 1000 mg / kg of the test substance. After administration of the test substance, observation is made at regular interval to check for the onset of adverse effect, time to death or time to recover. The period of observation in this phase I is 24 hours.

Phase II  This phase involves the use of three animals divided into three groups. In this phase, the dose level is either step up or down depending on the outcome of the result obtained from phase I. The animals are administered higher dose of 1600, 2900 and 5000 mg/kg. Toxic symptoms are observed for 24 hours as well as delayed toxic symptoms for 7-14 days. The lethal dose is calculated by the formula.

\[ LD_{50} = \sqrt{D_0 \times D_{100}} \]

Where, \( D_0 = \) Highest dose that gave no mortality, \( D_{100} = \) Lowest dose that produced mortality).

2.1.5 Fix dose procedure  The FDP is used to assess the nonlethal toxicity rather than the lethal dose. The investigational product is administered at fixed dose levels of 5, 50, 500, and 2000 mg/kg and the experimental animal is observed for a specified period. The ATC method is a sequential procedure in which three animals of the same sex are used in each step. In the ATC screening method, four pre identified starting doses may be used, and the test dose should be selected based on the Globally Harmonized Classification system.

The Fixed Dose Procedure (FDP), proposed in 1984 by the British Toxicology Society, is a method to assess a substance’s acute oral toxicity. In this procedure the test substance is given at one of the four fixed-dose levels (5, 50, 500, and 2000 mg/kg) to five male and five female rats. The objective is to identify a dose that produces clear signs of toxicity but no mortality. In comparison to the older \( LD_{50} \) test developed in 1927, this procedure produces similar results while using fewer animals and causing less pain and suffering.

2.1.6 Up and down procedure  This method involves the sequential dosing of single animals with the test substance within a time interval of 48 hours. After the administration of the first dose, the next is determined by the outcome of the subsequent dose administered. If the animal survives the subsequent dose the dose is adjusted upward, but when mortality is recorded at subsequent dose, it is adjusted downward. The adjustment of dose either upward or downward is by a constant factor. Testing is terminated when the upper limit (2000-5000 mg/kg) have been reached without mortality or when the \( LD_{50} \) have been established from the test.
The UDP testing approach is also known as the staircase design. This is the toxicological testing approach most recommended by various regulatory agencies because this method reduces the number of vertebrate animals in research. Female rodents are preferable for UDP testing.

An UDP for acute toxicity (LD50) testing has been developed and statistically evaluated. Compared with the “classical” procedure, this method permits a major reduction in the number of animals used. In the up-and-down procedure, animals are dosed one at a time. If an animal survives, the dose for the next animal is increased; if it dies, the dose is decreased. A survey of 48 acute toxicity tests in rats showed that the great majority of the animals that ultimately died did so within 1 or 2 days. Because of this, it suffices to observe each animal for 1 or 2 days before dosing the next animal. It is recommended, however, that surviving animals be monitored for delayed death for a total of 7 days.

The procedure for estimating the LD50 takes into account all deaths, and may be performed using widely available computer program packages. Testing in females alone is recommended, based on the observation that they were generally more sensitive in the survey of 48 studies; selective follow-up in males may sometimes be indicated. The procedure has been tested, by simulation, on 10 of the survey studies. It produced excellent agreement with the original studies. The 95% confidence interval for the LD50 averaged ±32% by the up-and-down method, compared with ±15% for conventional studies using 40 to 50 animals. The up-and-down procedure will require only 6 to 10 animals, provided that the initial estimate of the LD50 is within a factor of 2 of the true LD50.

2.1.7 Proposed (New) Method This method is divided into stages, with the outcome from each stage determine the next step to take (i.e, whether to terminate or proceed to the next stage).

Stage 1 This is the initial stage and it requires four animals. These animals are divided into four groups of one animal each. Then different doses of the test substance are administered to the different animals. The animals should be observed for 1 hours post-administration and then 10 minutes every 2 hours interval for 24 hours. The behavioral signs of toxicity and also mortality should be recorded. Where no mortality is recorded at this stage, the testing should proceed to stage 2.

Stage 2 This stage involves three animals, which are divided into three groups of one animal each. Different doses of the test substance (higher than those used in stage 1) are administer to the different animals and then observed for 1 hour after administration and periodically for 24 hours. Behavioral signs of toxicity should be noted and mortality as well. If no mortality occurred, testing should proceed to stage 3.
Stage 3

This stage also requires three animals which are distributed into three groups of one animal each. Various high doses of test substance (with 5000 mg/kg as the highest) are administered to the different animals. Observation is done for 1 hour after administration and then 10 minutes every 2 hours for 24 hours. Behavioral toxicity signs and also mortality should be recorded.

This is the final stage of testing and where no mortality is recorded at this stage, the LD$_{50}$ of the test substance is said to be greater than 5000 mg/kg and hence has a high degree of safety.

2.1.8 Confirmatory (or confidence) test

Where mortality was recorded at a given dose in any of the stages, a confirmatory test should be carried-out to actually validate that the test substance was the cause of such mortality.

This test simply involves the administration of the dose of test substance that caused mortality (or lowest dose that caused mortality in a situation that recorded more than one mortality) to two animals. Then observation should be done for 1 hour after administration and 10 minutes every 2 hours interval for 24 hours. Where at least a single animal from the two animals die, it should serve as a confirmation and validation of the test result.

If the main test did not show any mortality at 5000 mg/kg, a confirmatory test should also be carried-out. This can be done by administering 5000 mg/kg to two animals. Observation should be done for 1 hour after administration and 10 minutes every 2 hours interval for 24 hours. The recording of no mortality should be a confirmation of test result. The confirmatory test can also be carried-out on a substance in which literature have clearly stated its LD$_{50}$.

Calculation

After the test procedure, the formula that should be employed in the calculation of the LD$_{50}$ is shown below:

$$LD_{50} = \frac{M_0 + M_1}{2}$$

Where ($M_0$ = Highest dose of test substance that gave no mortality, $M_1$ = Lowest dose of test substance that gave mortality).

In carrying-out acute toxicity by this method, any of the two dose range in stage 1 (which is 10, 100, 300 and 600 or 50, 200, 400 and 800) may be employed. The doses recommended for stage 2 and 3 are documented. A wide range of doses have been recommended for this method so as to ensure accuracy of result. This method has some advantageous difference over other methods and have been documented. This includes that it is accurate, involves few animals, expenditure is moderate, process is simple and involves less time duration.
2.1.9 Acute toxicity testing for inhalation

Acute inhalation toxicity testing is performed for aerosol-like preparations. Rats are the most preferred animal species. The animals are acclimatized to laboratory conditions (temperature preferably 22°C ± 2°C). They are maintained in an air flow of 12–15 air changes per hour with adequate oxygen (19%/h).

The animal is exposed to the test substance for a minimum of 4 h, and then it is monitored for 14 days. Food is withheld during the exposure period, and water may be withheld under certain conditions. During the observation period, the animal is observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Mortality during the exposure and observation period is noted. Dead animals are examined for histological and pathological changes. At the end of the study, the animals are sacrificed, and pathological changes are evaluated.

2.1.10 Acute toxicity testing for topical preparations

The eye irritation test and skin irritation test are very important for topical preparations. Dermal and ophthalmic preparations can be tested using Draize tests. The Draize eye irritancy test and the Draize skin irritancy test are used to measure the harmfulness of chemicals and pharmaceutical substances in rabbits and guinea pigs. In the eye irritation test, 0.5 ml of a test substance is administered to an animal's eyes, and the animal is restrained for 4 h. Redness, swelling, discharge, ulceration, hemorrhage, and blindness are assessed and monitored for 14 days.

2.2 Skin sensitization tests

In the skin irritation test, 0.5 g of a test substance is applied to the surface of an animal's skin. During the observation period (14 days), signs such as erythema and edema are assessed. Some alternative in vitro testing methods are available that can be used in place of the Draize eye irritancy test. At the end of the study, the animals are sacrificed and pathological changes are evaluated. Skin sensitization tests are carried out using the guinea pig as a model. Skin sensitization is assessed using the Draize test, Open epicutaneous test, optimization test, split adjuvant test, guinea pig maximization test (GPMT), Buehler test, and murine local lymph node assay (LLNA). The LLNA method is used as an alternative to the guinea pig Draize test, and it is widely accepted that this method meets regulatory requirements. In the LLNA test, the test substance is applied on the surface of the ears of a mouse for three consecutive days, and the proliferation of lymphocytes in the draining lymph node is measured at the end.

2.3 Subchronic Toxicity Studies

Subchronic toxicity results from repeated exposure for several weeks or months. This is a common human exposure pattern for some pharmaceuticals and environmental agents. Examples are:

Ingestion of coumadin tablets (blood thinners) for several weeks as a treatment for venous thrombosis can cause
internal bleeding. Detailed clinical observations and pathology examinations are conducted. Basic parameters of these tests are in table.

2.4 Chronic Toxicity Studies
This study is basically to determine the organs affected and to check whether the drug is Toxic chronically or not.

This test extends over a long period of time and it involves large groups of laboratory animals. Chronic toxicity represents cumulative damage to specific organ systems and takes many months or years to become a recognizable clinical disease.

Damage due to subclinical individual exposures may go unnoticed. With repeated exposures or long-term continual exposure, the damage from these subclinical exposures slowly builds-up (cumulative damage) until the damage exceeds the threshold for chronic toxicity.

Ultimately, the damage becomes so severe that the organ can no longer function normally and a variety of chronic toxic effects may result. Examples of chronic toxic effects are: cirrhosis in alcoholics who have ingested ethanol for several years, chronic kidney disease in workmen with several years exposure to lead, chronic bronchitis in long-term cigarette smokers & pulmonary fibrosis in coal miners (black lung disease). Basic parameters of these tests are in table.

2.5 Teratogenicity:
It refers to capacity of a drug to cause fetal abnormalities when administered to pregnant mother. The placenta does not strictly constitute a barrier & any drug can cross it to a greater or lesser extent. The thalidomide disaster (1958-61) resulting in thousands of babies born with phocomelia (seal like limbs i.e. absence of development of the long bones of the arms & head) & other defects focused attention to this type of adverse effects.

2.5.1 Mechanism of Teratogenesis:
Mammalian fetal development passes through three phase.

- Blastocyst (i.e. Destruction of germ substance) formation.
- Organogenesis
- Histogenesis and maturation of function.

2.5.2 Screening for Teratogenic Activity

2.5.2.1 Evaluation of teratogenicity in animals. There are different levels of animal testing. The initial level might involve relatively large number of animals like rats.

If the test substance has no significant embryotoxicity, then it may be tested in a carnivore or an ungulate having a metabolic profile similar to man.
The higher cost and lower fecundity of this second test animal, i.e., dog or pig would dictate the use of smaller number of animals from these species than that of initial screen, at a general range of effective dosage that have been defined. The results of the second level test would then determine the necessity of further animal investigations. If overt embryotoxicity occurs at levels with moderately higher than the anticipated therapeutic level in man, the compound would probably be disqualified for consideration for use in women of reproductive age.

On the other hand, if the compound causes no teratogenic effects even at doses much higher than expected human therapeutic dose level, it might be considered ready for clinical use in the pregnant women without further animal test.

2.5.2.2 Teratologic studies in experimental animals

2.5.2.2.1. Route of administration of compound
The test substances should be administered by the same route as it is to be used clinically.

2.5.2.2.2. Duration of treatment
According to most of the protocols in use today, treatment is given throughout the span of organogenesis. This has the advantage that treatment covers the early formative stages of all organs, i.e. the time of maximal sensitivity for most organs whether they develop early or late in the overall span of organogenesis.

2.5.2.2.3. Dosage
The teratogenicity test should include a dose that produces some amount of embryotoxicity, such as intrauterine death, teratogenicity or growth retardation. The determination of an embryotoxic level of dosage is important because this is the logical starting point for extrapolating a safe level of the drugs for pregnant women. It has been demonstrated that maternal sensitivity to drugs is usually less than embryonic sensitivity and it has been recommended the use of three doses 1/3, 1/10 and 1/30 of maternal LD50. The highest doses would have to be close to the maternal toxic dose. The smaller dose should be close to the therapeutic doses.

2.5.2.2.4. Choice of animals
(a) General criteria:
Rats/mice and rabbits are the commonest species as they are readily available, inexpensive, and easy to breed and maintain in the laboratory. Ideal animals should have the following characteristics.(1) Pharmacokinetics of the test substance should be similar to that seen in man,(2) Transmit the substance and their metabolites across the placenta at the same rate as that in man and(3) Possess embryos and fetuses that have developmental schedule and metabolic pathways similar to those of human concepts.
(b) Type of species:
There is widespread tendency to select all test species from the closely related rodents (rat, mouse) and occasionally hamster or guinea pig and lagomorphs (rabbits and hares). Unfortunately all these animals possess a highly
specialised placental structure namely the inverted yolk sac placenta which is not present in higher mammals. The embryos of these species may be dependent on this atypical structure for all interchange of essential materials with maternal blood during the critical first few days of organogenesis, the time when embryos are most susceptible to embryotoxic influences. Moreover the yolk sac placenta is not only structurally but also functionally different from the typical chorioallantoic placenta that serves other mammals.

(c) **Number of animals** Usually for each dosage it seems prudent to utilise 25-30 pregnant rats or mice. If rabbits are employed, the number per dosage should be 10-12. be close to the therapeutic doses. It has been concluded by various biologists that teratogenic screen.

2.5.2.3 **Recommended Methodology In Teratology research**: Rat is the most often used species for such purposes. Regulations were formed by FDA and WHO to study reproductive toxicology for safety evaluation of new drugs for human use. Such studies are done in 3 phases.

**Phase – I**: study is designed to observe gonadal effects of the drugs both in males (n = 10) and females (n = 30). Effects on the ovarian cycle, mating, conception rates, late gestation, parturition and lactation are studied as well as it provides an overall crude screening for teratogenicity (see the flow chart).

**Phase – II**: study is specifically designed to test teratogenic potential of a drug and should be done in two species. Generally rats (n = 20) and rabbits (n = 10) are used for it (see the flow chart).

**Phase III**: study is specifically designed to test perinatal and postnatal effect of drug and rat is the species of choice (n = 20) (see the flow chart).

2.5.3 **Recent Techniques in Teratology Testing**

1. Mammalian embryo or embryo organ culture
2. Use of other vertebrate embryos
3. Chick embryo
4. Cell culture system

2.6 **Mutagenicity**:

It is the ability of chemicals to cause changes in the genetic material in the nucleus of the cells in ways that allow the changes to be transmitted during cell division

- Germinal mutation damage DNA in sperm and can undergo meiotic division and therefore have the potential for transmission of the mutation to future generations. If mutations are present at the time of fertilization in
either the egg or the sperm, the resulting combination of genetic material may not be viable and death may occur in the early stages of embryonic cell division.

- Alternatively the mutation in genetic material may not affect the early embryogenesis but may result in death of the fetus at a later developmental period, resulting in abortion.

### 2.6.1 Mechanism of Mutagenicity (1)

By the change in the structure of DNA which is responsible in inaccurate application of genome,(2) Prevent cell proliferation that fixes the DNA damage, (3) DNA repair - removal of large segments of DNA.

### 2.6.2 Evaluation Test For Mutagenicity:

**The Ames Test for mammalian environmental mutagenicity** A rat liver homogenate is prepared to produce a metabolically active extract (S9). [Above] The extract is combined with Strain 1 of his Salmonella bacteria: in the absence of histidine, the bacteria are unable to grow on minimal medium (control result). [Below] The homogenate and bacterial strain are combined with a suspected mutagenic substance (X). The induction of revertant colonies indicates that some his bacteria have mutated (reverted) to his, and therefore that substance X is a mutagen. Different bacterial strains are sensitive to different types of mutation. Use of a liver homogenate simulates the metabolic breakdown of the suspected mutagen in a mammalian system, and more accurately predicts mutagenicity of substances ingested by humans. For example, sodium nitrate (NaNO₃), which occurs naturally in smoked meat such as bacon, hot dogs, ham, etc., is not itself mutagenic. However, when acted upon by HCl in the stomach, its metabolic breakdown product nitrous acid (HNO₂) has been demonstrated to be a powerful mutagen by the Ames Test.

Bruce Ames (1928) and his undergraduate students tested large numbers of commercial products (hairspray, food colouring, etc.) in student labs at UC Berkeley when the test was first introduced in the early 1970s. Many were discovered to be mutagenic and were withdrawn from the market.

### 2.7 Current techniques for ocular toxicity testing

**2.7.1 Draize testing** Live animals have been used to assess and evaluate potentially harmful products to the eyes since the 18th century. The international standard assay for acute ocular toxicity is the rabbit in vivo Draize eye test which was developed in the 1940s by the Food and Drugs Administration (FDA). The original Draize protocol used at least six rabbits per test. The latest Draize test include the application and delivery of analgesics and anesthetics to reduce animal pain and suffering.
Rabbits are observed at selected intervals for up to 21 days for signs of irritation including redness, swelling, cloudiness, edema, hemorrhage, discharge and blindness.

2.7.2 Alternative in vivo tests

2.7.2.1 Low-volume eye-irritation test (LVET)

It developed in response to a recommendation from the National Research Council. LVET is a refinement of Draize testing developed as primary difference to the Draize test is that lower volumes of test substances (0.01 ml/0.01 g) are applied to the right-eye of the animal, with no forced eyelid closure employed and Test substances are also only applied to the corneal surface and not the conjunctival sac.

The test is believed to be less stressful to the tested animal. Pathological changes are characterized in the cornea, conjunctiva and iris/ciliary body.

Since Draize testing is often criticized for its over-prediction of human responses, it is arguable that LVET testing is more accurate.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the validity of LVET for the replacement of Draize testing.

2.7.2.2 Chicken Enucleated Eye Test (CEET) It is also known as the isolated chicken eye (ICE) protocol, which is based upon the isolated rabbit eye (IRE) protocol

2.7.3 Ex vivo test: Isolated/enucleated organ/organotypic methods

Ocular organotypic models are isolated systems that aim to maintain short-term normal physiological and biochemical function of the enucleated eye or cornea.

The protocols usually utilize opacitometric and spectroscopic methods for quantitative assessment of changes to the isolated cornea in response to a test material followed by histological analysis.

2.8 Neurotoxicity studies in rodents Neurotoxic studies may be employed to evaluate the specific histopathological and behavioral neurotoxicity of a chemical and are used to characterize neurotoxic responses such as neuropathological lesions and neurological dysfunctions (loss of memory, sensory defects, and learning and memory dysfunctions).

Usually neurotoxicological studies are carried out in adult rodents. The test substance may be administered for 28 days or even more than 90 days, and neurological changes are evaluated.
### Tables & Figures

Table 1. Acute Toxicity Studies parameters

<table>
<thead>
<tr>
<th><strong>Species</strong></th>
<th>rats preferred for oral and inhalation tests; rabbits preferred for dermal tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>young adults</td>
</tr>
<tr>
<td><strong>Number of animals</strong></td>
<td>5 of each sex per dose level</td>
</tr>
<tr>
<td><strong>Dosage</strong></td>
<td>three dose levels recommended; exposures are single doses or fractionated doses up to 24 hours for oral and dermal studies, and 4-hour exposure for inhalation studies</td>
</tr>
<tr>
<td><strong>Observation period</strong></td>
<td>14 days</td>
</tr>
</tbody>
</table>

Table 2. Subchronic Toxicity parameters

<table>
<thead>
<tr>
<th><strong>Species</strong></th>
<th>rodents (usually rats) preferred for oral and inhalation studies; rabbits for dermal studies; non-rodents (usually dogs) recommended as a second species for oral tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>young adults</td>
</tr>
<tr>
<td><strong>Number of animals</strong></td>
<td>10 of each sex for rodents, 4 of each sex for non-rodents per dose level</td>
</tr>
<tr>
<td><strong>Dosage</strong></td>
<td>three dose levels plus a control group; include a toxic dose level plus NOAEL; exposures are 90 days</td>
</tr>
<tr>
<td><strong>Observation period</strong></td>
<td>90 days (same as treatment period)</td>
</tr>
</tbody>
</table>

Table 3. Chronic Toxicity Studies parameters

<table>
<thead>
<tr>
<th><strong>Species</strong></th>
<th>two species recommended; rodent and non-rodent (<em>rat and dog</em>)</th>
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</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>young adults</td>
</tr>
<tr>
<td><strong>Number of animals</strong></td>
<td>20 of each sex for rodents, 4 of each sex for non-rodents per dose level</td>
</tr>
<tr>
<td><strong>Dosage</strong></td>
<td>three dose levels recommended; includes a toxic dose level and NOAEL; exposures generally for 12 months; FDA requests 24 months for food chemicals</td>
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<tr>
<td><strong>Observation period</strong></td>
<td>12-24 months</td>
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Table 4. Recommended Methodology In Teratology research (Flow chart)

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<table>
<thead>
<tr>
<th>Low dose (multiple of therapeutic dose)</th>
<th>High dose (maximum tolerated but asymptomatic dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female rats</td>
<td>Drug treatment for 14 days before mating</td>
</tr>
<tr>
<td>Pregnant rats</td>
<td>Treated with drugs throughout gestation</td>
</tr>
<tr>
<td>Half are killed on 13 day (mid pregnancy)</td>
<td>Other half sacrificed on 21 day (completion 21 day)</td>
</tr>
<tr>
<td>Observations - number of implants, resorption sites and corpora lutea are noted</td>
<td>Observations - conception rate, stillborn pups and litter size are noted. Study weight of pups, %, died on or survival are noted up to day 21 post partum.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low dose (multiple of therapeutic dose)</th>
<th>High dose (maximum tolerated but asymptomatic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats</td>
<td>Drug treatment for 63 days before mating</td>
</tr>
<tr>
<td>Observations - testes and epididymis are examined for any histopathological changes</td>
<td></td>
</tr>
</tbody>
</table>
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*Table 4. Recommended Methodology In Teratology research (Flow chart)*
**Figure 1.** (graph) Arithmetical method of Reed and Muench.

LD50: A figure which describes the Dose that kills 50% of the Animals

LC50: concentration (Inhalation)
Figure 2. Vision for Toxicity testing is a process that can include chemical characterization, toxicity testing and dose-response and extrapolation modeling.

![Ames Test for mutagenicity](image)

Figure 3. Evaluation Test for Mutagenicity.

Chicken Enucleated Eye Test (CEET)

![Chicken Enucleated Eye Test](image)

Figure 4. Schematic representation of the chicken enucleated eye test (CEET), also known as the isolated chicken eye (ICE) protocol, which is based upon the isolated rabbit eye (IRE) protocol.

3. Conclusion

Toxicity testing plays a crucial role in ascertaining the toxic effect and characterization of test substance. Toxicity obtained in animal studies occurs with similar incidence and severity in human. The use of animal in toxicity testing is most likely to continue for the foreseeable future because of the benefits they offer in examining Furthermore, accurate and reproducible result can be gotten through this method. We therefore recommend that the method should be considered for endorsement for the testing of toxicity by the regulatory bodies.
4. References


Corresponding Author:
Shaikh Nusrat K,
Email: nusratshaikh.pharmacist@gmail.com