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TOXICITY: a review on toxicological assessments

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ABSTRACT

Toxicology is "the science of poisons"; notably, the study of the chemical and physical features of poisons and their physiological or behavioral consequences on organisms; and both quantitative and qualitative approaches for their examination., and the creation of poisoning treatment protocols. The twentieth century saw a significant advancement in toxicological knowledge. The evaluation of the safety and toxicity profile of new chemical or biological entities is an essential component of pharmaceutical development. Despite continued attempts to better understand the mechanisms underpinning safety and toxicity, safety concerns continue to account for approximately 30% of drug discovery and development attrition rates. In this review, we will focus on the implications of current practice for drug development and consider the scientific and ethical requirements for the evaluation of safety and toxicity. Toxicological screening is critical for the development of new medications and expanding the therapeutic potential of current compounds. According to the US Food and Drug Administration (FDA), screening novel compounds for pharmacological action and toxicity in animals is critical (21CFR Part 314). In the twenty-first century, the harmful effects of chemicals, dietary items, medications, and so on have become increasingly important. This brief research emphasizes on the historical significance of toxicological screening as well as alternative and particular methodologies that employ a variety of experimental animal models.

KEYWORDS

Toxicity, Acute toxicity, Toxicology, Ethics, Screening, Mutagenic, Genetic toxicity

1. INTRODUCTION

Toxicology is "the science of poisons"; notably, the study of the chemical and physical features of poisons and their physiological or behavioral consequences on organisms; and both quantitative and qualitative approaches for their examination., and the creation of poisoning treatment protocols. Although poisons have been there since ancient times, Paracelsus (1493-1541) and Orfila (1757-1853) are Toxicology is "the science of poisons"; notably, the study of the chemical and physical properties of poisons and their physiological or behavioral consequences on organisms., qualitative and quantitative methods for their analysis credited with pioneering the study and science of toxicology. Modern In this study, we will look at the consequences of existing practices for drug development, as well as the scientific and ethical standards for assessing safety and toxicity. Toxicological screening is essential for developing novel drugs and increasing the therapeutic potential of existing substances. According to the US Food and Drug Administration (FDA), screening new chemicals for pharmacological action and toxicity in animals is crucial (21 CFR Part 314). In the twenty-first century, the adverse impacts of chemicals, dietary items, pharmaceuticals, and other substances have grown in importance. This brief research examines the historical significance of toxicological screening, as well as alternative and specific approaches that use a variety of experimental animal models. Toxicology is distinguished by advanced scientific investigations and assessments of harmful exposures. The twentieth century

saw a significant advancement in toxicological knowledge. The evaluation of the safety and toxicity profile of new chemical or biological entities is an essential component of pharmaceutical development. Despite continued efforts to better understand the mechanisms underpinning safety and toxicity, safety concerns continue to account for approximately 30% of drug discovery and development attrition. [1,2].

In addition to their impact on attrition rates, safety and toxicity results have business, legal, and societal implications, which frequently lead to speculation and increased empiricism in the evaluation and interpretation of experimental data. While a positive benefit-risk ratio should be anticipated and proved when providing novel medications to humans, the basis for drawing conclusions still lacks scientific clarity and rigor. The scientific community has not questioned the efficiency or value of the present paradigm for evaluating safety and toxicity, which is based mostly on routine battery tests at supratherapeutic exposure levels of the experimental drug. In this review, we will look at the implications of current practice for drug development, as well as the scientific and ethical requirements for assessing safety and toxicity, such as opportunities to characterize in vivo responses to mutations and DNA damage using population modeling approaches. We are particularly interested in demonstrating that, despite the assumption that preclinical safety and toxicity findings are often predictive of human toxicity [3], inefficiencies in the experimental design violate the three Rs concept (reduction, refinement, and replacement) [4]. Descriptive data summaries from empirical experimental methods must be replaced with a model-based approach that integrates pharmacokinetic, pharmacodynamic, and pathophysiological concepts in a systematic way.

1.1 History of toxicity studies

Toxicology research dates back to Paracelsus (1493-1541), who identified the particular compounds responsible for plant and animal toxicity. He revealed that poisons have both innocuous and useful effects, as well as dose-response connections for therapeutic effects. Paracelsus, a physician, alchemist, and astrologer, is largely considered as the founder of toxicology. His famous saying is: "All substances are poisons; there is none that is not a poison." "The right dose distinguishes between a poison and a remedy." [5] Mathieu Orfila (1787-1853), a Spanish physician, discovered the link between poisons and their biological features and established the specific organ damage induced by toxins. Orfila is known as the father of modern toxicology. Toxicological screening procedures and study on individual compounds emerged in the mid-1900s, whereas environmental toxicological studies emerged in the mid-twentieth century.

The use of animals in toxicity research began in 1920, when J. W. Trevan advocated using the 50% fatal dose (LD50) test to establish the lethal dose of specific substances. Following the establishment of LD50, FDA scientist John Draize created a method for assessing eye and skin irritation with rabbits, which became widely accepted for testing the effects of chemicals and medications on the eyes and skin. Later, the US National Cancer Institute (NCI) devised a test to discover carcinogenic substances by injecting rats and mice on a regular basis for two years. In the early 1960s, thalidomide caused devastating birth abnormalities in hundreds of babies. Following this, all regulatory authorities focused on identifying the toxicity profiles of all pharmacological substances available for routine patient use and mandated the submission of toxicity profiles for investigational novel medicines (IND). In the late 1980s, the Organization for Economic Cooperation and Development (OECD) and the International Conference on Harmonization (ICH) issued recommendations for pharmaceutical chemical toxicity testing.

1.2 Sources of toxic substances

Toxicants are typically classed according to their chemical composition, method of action, or class (exposure and usage class). Toxicants are classified according to whether they appear in food, air, water, or soil. Drugs are classified according to their use: drugs of abuse, therapeutic medications, agricultural chemicals, food additives, pesticides, plant poisons (phytotoxins), and cosmetics.[6]

2. TOXICITY TESTING

2.1 Acute toxicity testing

Acute toxicity testing determines the effect of a single dose on a specific animal species. In general, acute toxicity testing should be conducted with two different animal species (one rodent and one nonrodent). Acute toxicological testing involves administering the investigational product at various dose levels and observing the effect for 14 days. All deaths caused by the investigational substance during the trial time are recorded, and the morphological, biochemical, pathological, and histological changes in the dead animals are examined. Acute toxicity assessment allows

the 50% lethal dose (LD50) of the experimental product to be calculated. The LD50 was originally used to assess acute toxicity. The LD50 is determined using a large number of animals, with a high death ratio. Because of these constraints, modified approaches have been created. The fixed-dose protocol (FDP The acute toxic category (ATC) technique. The UDP (up-and-down) technique. The FDP is used to evaluate nonlethal toxicity rather than lethal doses. The investigational product is supplied at preset doses of 5, 50, 500, and 2000 mg/kg, and the experimental animal is monitored for a set amount of time. The ATC technique is a sequential approach that involves three animals of the same sex in each step. The ATC screening approach allows for the use of four predetermined starting doses, with the test dose determined by the Globally Harmonized Classification system. [7] The UDP testing approach is sometimes referred to as the staircase design. This is the most commonly suggested toxicological testing strategy by regulatory authorities since it decreases the number of vertebrate animals used in research. The UDP screening approach involves treating individual animals at 48-hour intervals. Female rodents are ideal for UDP testing. A dose less than the best-estimated LD50 dose is chosen and administered to an animal, which is then watched for 48 hours. If the animal survives, the study is resumed with a greater dose (double the initial dose); if the animal dies, testing is repeated with a lower dose on another animal of the same sex as the original. UDP testing is limited to dosages of up to 2000 mg/kg. The testing protocols for dosages ranging from 2000 to 5000 mg/kg are different. [8-10] In 1996, the Center for Drug Evaluation and Research (CDER) proposed a single dose acute toxicity testing approach for pharmaceutical drugs that employs a set safe dose that should not cause adverse effects or endanger the life of an animal. The experiment must involve at least two mammalian species, including a nonrodent species, and the animals must be monitored for 14 days.[11]

2.2 Acute toxicity testing for inhalation

Acute inhalation toxicity testing is done on aerosol-like preparations. Rats are the most popular animal species. Animals are acclimatized to laboratory conditions (ideally 22°C ± 2°C). They are kept in an air flow of 12-15 air changes per hour, with enough oxygen (19%/h). The animal is exposed to the test chemical for at least 4 hours and then monitored for 14 days. Food is withheld during the exposure period, and water may be withheld in certain circumstances. During the observation time, the animal is monitored for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Mortality during the exposure and observation periods is recorded. Dead animals are studied for histological and pathological abnormalities. At the end of the study, the animals are sacrificed, and pathological changes are evaluated.[12]

2.3 Acute toxicity testing for topical preparations

The eye irritation test and skin irritation test are critical for topical applications. Draize tests are suitable for testing dermal and ophthalmic treatments. The Draize eye irritancy test and the Draize skin irritancy test are used to determine the toxicity of chemicals and pharmaceuticals in rabbits and guinea pigs. The eye irritation test involves administering 0.5 cc of a test chemical to an animal's eyes while it is restrained for 4 hours. Redness, swelling, discharge, ulceration, bleeding, and blindness are evaluated and monitored for 14 days. The skin irritation test involves applying 0.5 g of a test material to the surface of an animal's skin. During the 14-day observation period, indications of erythema and edema are examined. There are some other in vitro testing procedures that can be employed instead of the Draize eye irritation test. [13,14] At the conclusion of the study, the animals are slaughtered and pathological alterations are assessed.

2.4 Skin sensitization tests

Skin sensitization tests are performed using guinea pigs as models. Skin sensitization is determined using the Draize test, open cutaneous 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 commonly acknowledged to meet regulatory standards. The LLNA test involves applying the test substance to the surface of a mouse's ears for three consecutive days, and then measuring the proliferation of lymphocytes in the draining lymph node.[15]

2.5 Repeated dose toxicity testing

Repeated dosage toxicity testing is carried out for at least 28 days. The test material is supplied orally once every day for a set amount of time. If this method is not practical, the test material can be supplied parenterally. The test chemical is administered on a regular basis and at a certain time. For repeated dosage toxicity testing, a male or female mouse aged 5-6 weeks is often employed. Weight variance between animals should be within ±20%. A satellite group could be included in the study protocol. This group includes both a control and a high-dose group. Animal

baseline data, such as behavior and biochemistry, should be recorded. These will be useful for determining percentage changes. The interpretation of human safety information is critical in repeated dosage toxicity investigations.[14] At the conclusion of the investigation, tissues from the majority of the organs are taken and histological changes are documented. If possible, immunotoxicity (adverse effects on the immune system) tests are carried out on the same animals. Immunotoxicological analysis is not viable beyond 14 days. Immunotoxicological investigations evaluate parameters such as delayed-type hypersensitivity (DTH), mitogen- or antigen-stimulated lymphocyte proliferative responses, macrophage function, and primary antibody response to T-cell dependent antigens. The period of repeated dose and subchronic toxicity studies differs significantly: repeated dose toxicity studies last 28 days, but subchronic toxicity studies last 90 days. [16-18]

2.6 Mutagenicity testing

Mutagenicity testing is used to detect submicroscopic alterations in DNA's base sequence, as well as chromosomal and structural abnormalities such as duplications, insertions, inversions, and translocations. Certain types of mutations cause carcinogenesis (changes in protooncogenes or tumor suppressor gene alterations), hence determining mutagenicity is critical in the medication development process. In vitro testing is performed in two or three distinct bacteria and mammalian cells to assess gene mutations, clastogenicity, and aneuploidy. The test often includes a bacterial reverse mutation experiment. The chemical structure/class of the drug determines which subsequent tests are performed. The case-by-case risk evaluation of the test compounds is based on in vivo mutagenicity, which is dose dependent. Mutagenicity studies with transgenic animals are more appropriate assay techniques to determine the toxicity of a test substance. [19,20]

2.7 Subchronic oral toxicity testing (repeated dose 90-day oral toxicity testing)

The subchronic toxicity of a chemical is studied using both rodents and nonrodents. The test drug is taken orally for 90 days, and weekly body weight fluctuations, monthly changes in biochemical and cardiovascular parameters, and behavioral alterations are examined. At the conclusion of the investigation, the experimental animals are sacrificed. Gross pathological alterations are noticed, and all tissues undergo histological examination. The permissible weight variation range is ±20%, ensuring minimal individual variance among the animals. The study procedure may contain a satellite group, which consists of a control group and a high-dose group. [21,22]

2.8 Chronic oral toxicity testing

Chronic toxicity studies involve a minimum of one rodent and one nonrodent species. The test substance is administered over a period of more than 90 days, and the animals are inspected at regular intervals. Chronic toxicological research draws conclusions regarding the long-term effects of a test chemical in animals, which can be extrapolated to the test material's safety in humans. The data on chronic oral toxicity is critical for novel pharmacological entities. Individual variance between animals should be minimal, with a weight variation range of ±20%. A satellite group could be included in the study protocol. This group includes both a control and a high-dose group. Throughout the investigation, the animals are monitored for normal physiological functioning, behavioral variances, and changes in biochemical indicators. At the conclusion of the investigation, tissues from all parts of the animal are gathered and histologically analyzed. [23]

2.9 Carcinogenicity testing

Carcinogenicity testing may involve both rodents and nonrodent animal species. The tests are carried out over the majority of an animal's lifespan. During and after exposure to test drugs, the experimental animals are monitored for indicators of toxicity and tumor formation. If these are not identified, the test may be stopped after 18 months in mice and hamsters, and 24 months in rats. If the animals are healthy, hematological analysis is performed at 12 and 18 months, respectively, and the study is concluded. The animals are slaughtered, and gross pathological alterations are seen, as well as histological investigations of all tissues.[24]

2.10 One-generation reproduction toxicity testing

The test substance is given to both male and female animals. Administration lasts for one complete spermatogenic cycle in male animals and two complete estrous cycles in female animals. Rodents are preferred for one-generation reproduction toxicity testing. The animals are allowed to mate when the drug delivery period has been completed. The test chemical is given to female animals throughout pregnancy and breastfeeding. Male animals' sperms are collected and evaluated for morphology and motility.

During the trial, the animals are monitored for signs of toxicity. Parturition, the number of offspring, and their sexes are documented. The number of dead and living puppies is recorded, and live pups are weighed in the morning and evening every day for the first four days. After the study, the animals and puppies are slaughtered and histopathologically examined. [25]

2.11 Two-generation reproduction toxicity studies

Both male and female rodents are given the test chemical. The period of administration is one complete spermatogenic cycle for males and two complete estrous cycles for females. Following the administration phase, the animals are entwined (parental mating), and the females are separated. Sperms are collected from male animals and examined for morphology and motility. The test chemical is continually administered to pregnant female animals, who are regularly evaluated for death and toxicity. Following parturition, nursing rats are given the test medication, and pup mortality (F1 generation) is observed. From the F1 generation, one male and one female animal are chosen. The operation is repeated to produce children from the second generation. F1 offspring are not permitted to mate until they have reached full sexual maturity, and pairs without a pregnancy are assessed for sterility. Necropsies and histological exams are conducted. At the conclusion of the study, the animals are sacrificed, and grosspathological and histological investigations are performed on each of them. [26-28]

2.12 Toxicokinetics

Toxicokinetics, an extension of pharmacokinetics, studies the kinetic patterns of larger dosages of chemicals, poisons, and xenobiotics. Toxicokinetics studies the metabolism and excretion patterns of xenobiotics. Animal toxicokinetic data aid in predicting physiologically based pharmacokinetics in humans. Pharmacokinetic studies are typically conducted in rats, rabbits, dogs, nonhuman primates, and swine utilizing a variety of delivery techniques. Blood samples are obtained at various time points to examine pharmacokinetic data, including the area under the curve, drug distribution ratio, Cmax, tmax, and other pharmacokinetic parameters. Toxicokinetic studies can also be performed on in vitro cell lines.[29]

2.13 Neurotoxicity studies in rodents

Neurotoxicity studies can be used to evaluate a chemical's specific histopathological and behavioral neurotoxicity, as well as to characterize neurotoxin responses like neuropathological lesions and neurological dysfunctions (e.g., memory loss, sensory defects, etc.). The peripheral nervous system is further divided into the somatic and autonomic nervous systems.

2.14 Developmental toxicity/embryotoxicity studies

Embryo toxicity can be investigated in both vivo and in vitro settings. Rodents are preferred for in vivo toxicity testing. The substance is supplied between the eighth and fourteenth days of pregnancy, and the embryo fatal effects are investigated. A caesarean section is performed at the end of the study or on the 21st day, and parameters such as fetuses with hemorrhagic bullae, limb malformations, anencephaly, cleft palates, open eyelids, and tail deformities, as well as mortality and the number of dead and live pups, are recorded. In vitro approaches for embryo toxicity testing include the embryonic stem cell test (EST), micro mass embryo toxicity assay, and whole rat embryo toxicity assay. [30-31,33]

2.15 Genetic toxicity testing

Genetic toxicity tests are used to detect gene mutations, chromosome abnormalities, and changes in DNA sequencing. These studies are typically undertaken on a variety of species, including complete animals, plants, microorganisms, and mammalian cells. In the entire animal model, rodents are preferable. Genetic toxicity is determined by the rodent chromosomal assay, dominant lethal assay, mouse-specific locus test, micronucleus test, heritable translocation assay, and sister chromatic exchange assay. [34]

3. INSTITUTE ETHICS COMMITTEE

Before doing any toxicological tests on animals or collecting tissue/cell lines from them, the study should be approved by the Institute Animal Ethics Committee (IAEC) or the methodology must follow the standards of the

local governing authority. The standards for performing experiments and regulatory requirements differ by region. In India, the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules should be followed for the care of experimental animals. To meet regulatory criteria, employ an approved fluid withdrawal method and follow schedule Y (India).

4. REGULATORY REQUIREMENTS

Before beginning any clinical investigation, the safety of the test chemical should be evaluated using animals. Preclinical investigations should determine the target organ toxicity, the dose-response relationship, important human consequences, and any issues that arise during therapy (adverse drug reactions). The toxicity research should include at least three dosages (low, medium, and high) in the experimental animals, with the toxic effect compared to data from a control group of animals. The Committee for Proprietary Medicinal Products (CPMP) has established standards for toxicological experiments on several animal species. The guideline instructs that the maximum selected dose should be sufficient to identify the target organ toxicity. The toxicological evaluation can establish the no observed effect level (NOEL) or NOAEL, which may be useful for human studies. The low dose, intermediate dose, and high dose used in the toxicity test provide the NOEL, dose-response relationship, and target organ toxicity in animals, respectively. [35]

5. CASE STUDY

History: An 18-year-old boy comes to your emergency room after consuming an unknown amount of lysergic acid diethylamide (LSD). His buddies took him in because he was "acting goofy." He is not currently suicidal and does not intend to harm himself. He claims to have taken no additional substances.

PMH: None.

SH: No previous suicide attempts and no history of depression.

Physical Examination:

T: 100.4°F HR: 124 bpm RR: 18 breaths per minute BP: 150/90 mm Hg

General: Agitated and actively hallucinating. The skin is moist and pale.

HEENT: Pupils are 4 mm bilaterally with sluggish light reaction. No nystagmus.

Pulmonary: Clear to auscultation.

CV: Regular rate and rhythm without murmur.

Abdomen: Soft and nontender with hyperactive bowel sounds.

Neurologic: GCS 14. Cranial nerves II-XII intact. Fine tremor [32]

CONCLUSION

Toxicity testing on novel compounds is critical for the drug development process. Preclinical toxicity testing on numerous biological systems shows an investigational product's hazardous effects on certain species, organs, and doses. The toxicity of compounds can be determined by (a) examining accidental exposures to the substance, (b) in vitro research utilizing cells/cell lines, and (c) in vivo exposure on experimental animals. This study focuses on the numerous experimental animal models and methodologies used to investigate the toxicity of chemicals. Pre-clinical toxicity testing aids in calculating the "No Observed Adverse Effect Level" required to begin clinical study of investigational drugs. In conclusion, our analysis has highlighted the need of empirical data creation for assessing safety and toxicity throughout medication development.

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