A review of toxicological assessment approaches for new chemical entities

Swapnali Zore

Department of Pharmacology, College of Pharmacy, King Khalid Universiy, Abha, Asir Province, Saudi Arabia

Corresponding author: Swapnali Zore

Received 19 Jul 2025; Accepted 3 Sep 2025; Published 17 Sep 2025

Abstract

The primary aim of this review is to examine the toxicological evaluation of new chemical entities. Toxicity testing of novel drug candidates is a critical component of the drug discovery and development process. This article discusses the various methods used for general toxicity assessment and provides an overview of toxicological profiling relevant to different disease conditions. Both in vitro and in vivo approaches used in toxicity studies are comprehensively reviewed.

Keywords: Toxicological studies, Drugdiscovery, New drugs, *invitro, invivo* studies

1. Introduction

A New Chemical Entity (NCE) is defined as a medication that does not contain any previously approved active moiety recognized by the United States Food and Drug Administration (USFDA) under any application. Manufacturers generally develop NCEs during the early stages of drug development, after which the NCE undergoes extensive preclinical and clinical evaluation before it is approved as a drug product.

An active moiety refers to the molecule or ion responsible for the pharmacological or physiological action of a drug substance, excluding appended portions that make the drug an ester, salt (including hydrogen or coordination bonds), or other noncovalent derivatives such as complexes, chelates, or clathrates.

Given the significant risks and adverse effects associated with therapeutic products, drug use is strictly regulated. Historically, toxicological evaluation was not mandatory prior to marketing; drugs were assessed primarily through organoleptic characteristics such as appearance, odor, and taste. However, modern drug development standards mandate comprehensive toxicological assessment before a product is released for human use.

The process of developing a new drug can be broadly divided into four major stages:

- Drug discovery
- Preclinical development
- Clinical studies
- Marketing authorization of the new chemical entity, followed by post-marketing surveillance of drug-like molecules [1-3].

2. Methods of toxicity studies

General toxicity studies

a) Acute toxicity studies

Separate single-dose or acute toxicity studies are no longer always considered essential. Acute toxicity can often be evaluated through short-term dose-ranging or dose-escalation studies, which may be conducted as non–Good Laboratory Practice (non-GLP) studies.

The primary objective of acute toxicity studies is to identify potential target organs adversely affected by the administered substance. Estimation of median lethal dose (LD50) is no longer recommended. Extended single-dose toxicity studies may support exploratory clinical trials, particularly first-dose human studies. Hematology, clinical chemistry, and histopathological evaluations can also be carried out in these studies [5].

b) Repeated dose toxicity studies

Repeated-dose toxicity studies characterize the toxicological profile of a drug after prolonged administration of high doses. These studies identify target organs affected by repeated exposure and determine no-effect levels such as the No-Observed-Adverse-Effect Level (NOAEL), which is crucial for establishing safety margins and selecting doses for subsequent studies.

These studies must be completed prior to first-in-human (FIH) trials and conducted under Good Laboratory Practice (GLP) conditions. Typically, studies are performed in two animal species—one rodent (rat or mouse) and one non-rodent (dog, minipig, or primate). Selected species should exhibit pharmacokinetic, pharmacodynamic, and metabolic patterns most similar to humans [6].

c) Sub-chronic toxicity studies

Sub-chronic toxicity testing was performed on 24 healthy rabbits of either sex weighing 1200–1800 g. Animals were divided into three groups: one control and two test groups receiving 20 mg/kg and 60 mg/kg of the herbal formulation for

60 consecutive days via oral intubation. Test doses were prepared in DMSO; the control group received DMSO alone. Animals were observed during the conditioning period for signs such as hair loss, diarrhea, edema, ulceration, and reduced activity [7].

d) Sample collection

At the end of the 60-day dosing period, approximately 6 mL of blood was collected by cardiac puncture for biochemical and hematological assessment [8].

e) Physical examination

Gross toxicity symptoms were monitored weekly, including skin ulceration, weight changes, hair loss, loss of appetite or activity, hematuria, vomiting, diarrhea, edema, lacrimation, salivation, muscle tone, tremors, and aggressive behavior. Necropsy was performed at study completion for further pathological evaluation [9].

f) Biochemical evaluation

Fasted animals were subjected to blood collection prior to necropsy. Serum was separated by centrifugation and analyzed within 3 hours using standard reagents and automated analyzers to determine biochemical parameters [10, 11].

g) Hematological evaluation

Blood samples were collected into EDTA tubes (10%, pH 7.2) and analyzed for RBC, WBC, platelet count, hemoglobin, and hematocrit using an automated veterinary hematology analyzer [12].

h) Microscopic examination

Tissue samples from the heart, liver, and kidneys were processed using standard histopathological procedures. Paraffin sections (3–4 µm) were prepared, mounted, dried, and stained before microscopic examination [13].

i) Statistical analysis

All biochemical data were expressed as mean ± SEM and evaluated using one-way ANOVA. A p-value <0.05 was considered statistically significant, while p <0.005 was considered highly significant [14].

3. Toxicological profiling of various diseases on new chemical entities

a) Genotoxicity and carcinogenicity studies

Genotoxicity testing, typically performed in rodents and mice, assesses the ability of a substance to induce DNA or chromosomal damage. Such damage may occur in germ or somatic cells and, if unrepaired, may lead to heritable mutations or carcinogenesis.

A standard test battery is described in ICH Guidance S2(R1), and validated test protocols are outlined in OECD guidelines. Genotoxicity evaluation involves both *in vitro* and *in vivo* methods.

The bacterial reverse mutation test (Ames test) using *Salmonella typhimurium* is the primary assay for detecting gene mutations. Additional in vitro tests include the mammalian cell micronucleus test and the mouse lymphoma assay. In vivo tests are used to confirm or dispute in vitro findings and detect mutagenic effects not observable in cell-based systems [15].

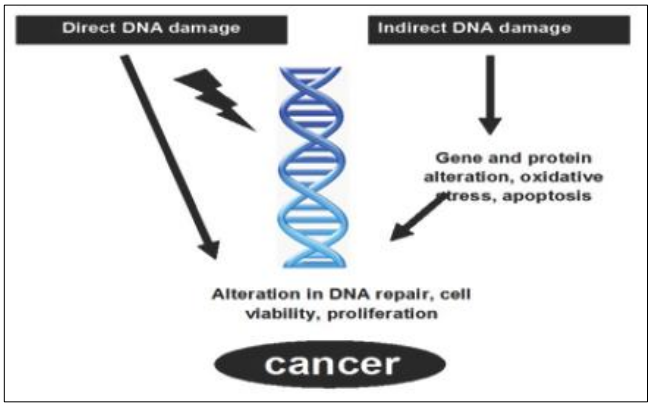

Fig 1: Relationship between carcinogenicity and Genotoxicity [16]

Dose descriptor and risk assessment

- Some chemicals are considered non-threshold carcinogens, capable of inducing cancer even at minimal exposure levels; thus, NOAEL-based approaches are not suitable.
- Carcinogenic risk assessment often uses descriptors such as T25 and BMD10, derived from long-term rodent bioassays.
- T25 refers to the chronic dose causing tumors in 25% of animals at a specific tissue after adjusting for background incidence.
- BMD10 refers to the benchmark dose that results in tumors in 10% of animals [17].

b) Acute Toxicity Testing for Inhalation

Inhalation toxicity studies are performed primarily in rodents for aerosolized formulations. Animals are exposed to the test substance for at least 4 hours and monitored for 14 days. Key observations include tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma, and mortality. Necropsy is performed to assess pathological changes [18].

Dose assessment

- LD50 (mg/kg bw/day): Dose lethal to 50% of exposed animals.
- LC50 (mg/L): Airborne concentration causing 50% mortality.

  These values are used for classification and dose selection but not for deriving NOAEL [19].

c) Acute toxicity testing for topical preparations

Topical formulations undergo eye and skin irritation tests, including Draize tests in rabbits or guinea pigs.

- **Eye Test:** 0.5 mL of test substance is applied; redness, swelling, discharge, ulceration, and opacity are monitored for 14 days.
- **Skin Test:** 0.5 g of substance is applied to the shaved skin; erythema and edema are evaluated. Alternative in vitro methods may also be used [20].

Conclusion

This review summarizes the toxicological evaluation of new chemical entities, which forms a critical component of the drug development process. With the rapid expansion of novel therapeutic agents, ensuring drug safety has become increasingly important. Insufficient toxicological assessment can lead to harmful effects, underscoring the growing need for comprehensive toxicity profiling. Although animal studies provide essential safety data, human assessment ultimately remains the most reliable method for determining the safety and efficacy of a drug before clinical use.

Reference

1. Hathaway C, Manthei J, Scherer C. Exclusivity strategies in the United States and European Union. Food Drug Law Inst Update. 2009 May;3:34–9.
2. Prajapati V, Tripathy S, Dureja H. Product lifecycle management through patents and regulatory strategies. J Med Mark. 2013 Aug;13(3):171–80.
3. Huang R, Southall N, Wang Y, Yasgar A, Shinn P, Jadhav A, et al. The NCGC pharmaceutical collection: a comprehensive resource of clinically approved drugs enabling repurposing and chemical genomics. Sci Transl Med. 2011 Apr 27;3(80):80-16.
4. Branch SK, Agranat I. “New drug” designations for new therapeutic entities: new active substance, new chemical entity, new biological entity, new molecular entity. J Med Chem. 2014 Nov 13;57(21):8729–65.
5. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov. 2004 Aug;3(8):711–6.
6. Preskorn SH. The stages of drug development and the human genome project: drug discovery. J Psychiatr Pract. 2000 Nov 1;6(6):341–4.
7. Light DW, Warburton R. Demythologizing the high costs of pharmaceutical research. BioSocieties. 2011 Mar;6(1):34–50.
8. Rahman Z, Charoo NA, Akhter S, Beg S, Reddy IK, Khan MA. Nanotechnology-based drug products: science and regulatory considerations. In: Nanoscale Fabrication, Optimization, Scale-Up and Biological Aspects of Pharmaceutical Nanotechnology. William Andrew Publishing, 2018, 619–655.
9. Coles E, Cheyne H, Rankin J, Daniel B. Getting it right for every child: a national policy framework to promote children's well-being in Scotland, United Kingdom. Milbank Q. 2016 Jun;94(2):334–65.
10. Junod V. Drug marketing exclusivity under United States and European Union law. Food Drug Law J. 2004;59(4):479–518.
11. Anthöfer J. Drug development and critical analysis of the reliability of preclinical studies [master’s thesis]. Bonn: Universität Bonn.
12. Swink M, Song M. Effects of marketing–manufacturing integration on new product development time and competitive advantage. J Oper Manag. 2007 Jan 1;25(1):203–17.
13. Andrade EL, Bento AF, Cavalli J, Oliveira SK, Freitas CS, Marcon R, et al. Non-clinical studies required for new drug development—Part I: early in silico and in vitro studies, new target discovery and validation, proof of principles and robustness of animal studies. Braz J Med Biol Res, 2016 Oct 24, 49.
14. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: challenges and opportunities to increase the knowledge base for safety assessment. Toxicol Appl Pharmacol. 2010 Mar 1;243(2):198–216.
15. Ayad MH. Rational formulation strategy from drug discovery profiling to human proof of concept. Drug Deliv. 2015 Aug 18;22(6):877–84.
16. Hann MM, Keserü GM. Finding the sweet spot: the role of nature and nurture in medicinal chemistry. Nat Rev Drug Discov. 2012 May;11(5):355–65.
17. Trenfield SJ, Madla CM, Basit AW, Gaisford S. The shape of things to come: emerging applications of 3D printing in healthcare. In: 3D Printing of Pharmaceuticals. Cham: Springer, 2018, 1–19.
18. Chaurasia G. A review on pharmaceutical preformulation studies in formulation and development of new drug molecules. Int J Pharm Sci Res. 2016 Jun 1;7(6):2313.
19. Benz KW, Neumann W. Introduction to crystal growth and characterization. Hoboken: John Wiley & Sons, 2014 Jul 28.
20. Griesser UJ. The importance of solvates. In: Polymorphism in the Pharmaceutical Industry, 2006 Feb 6, 211–33.