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Assessing Compliance with ARRIVE Guidelines in Zebrafish Herbal Toxicity Studies: Are We Meeting the Standards?

Basnayake P. I.[1], Gunatilake M.[2]

[1]Department of Physiology, Faculty of Medicine, Sabaragamuwa University of Sri Lanka.
[2]Department of Physiology, Faculty of Medicine, University of Colombo.

ABSTRACT

Transparent reporting of animal studies is essential for scientific reproducibility, whereas inadequate reporting hinders the replication of experimental methods. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were developed to standardize the reporting of animal research. This study evaluated the reporting quality of zebrafish embryotoxicity studies investigating herbal medicines, using the ARRIVE-2020 guidelines as a reference.

Original research articles published between 2020 and 2023 were assessed for compliance with the 10 Essential ARRIVE items. Eight studies from eight different journals were included, and both journal and article-related characteristics were examined. Each ARRIVE item was categorized as "fully reported", "partially reported", or "not reported", and overall compliance was classified as excellent (≥80%), moderate (50-79%), or poor (≤49%) based on the proportion of fully reported items. Among the analysed journals, 87.5% were indexed in SCI-Expanded, and 75% endorsed ARRIVE guidelines in their author instructions. The proportion of fully reported items ranged from 0% to 100%, while partially reported items ranged from 0% to 37.5%. Compliance was highest for study design, sample size, outcome measures, experimental animals, and reported outcomes. In contrast, inclusion and exclusion criteria, as well as randomization, were consistently poorly reported. These findings reveal substantial deficiencies in the reporting of key ARRIVE items in zebrafish embryotoxicity research. Although ARRIVE guidelines have the potential to improve reporting quality, their limited implementation may reflect insufficient author compliance and inadequate journal enforcement. We encourage the full and consistent application of ARRIVE guidelines to enhance research quality, reproducibility, and zebrafish welfare.

Key-words: ARRIVE guidelines; Zebrafish; *Danio rerio*; Embryotoxicity; Herbal toxicity

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INTRODUCTION

Animal experiments are a fundamental component of preclinical research. However, inadequate reporting of key methodological details remains one of the most frequently cited barriers to reproducibility in animal studies. Poorly reported studies can lead to substantial financial and ethical costs, including unnecessary animal use. Furthermore, inadequate or incorrect reporting of study design, sampling size, data collection, data analysis, and result inter-

*Corresponding author.
Basnayake P.I
Department of Physiology, Faculty of Medicine, Sabaragamuwa University of Sri Lanka.
Email- pradeepa@med.sab.ac.lk

pretation introduces unwanted heterogeneity, making comparisons across studies difficult and reducing the reliability of research findings (Liu et al., 2023).

The ARRIVE guidelines are widely recognized preclinical guidance for animal researchers, designed to standardize reporting practices across published animal studies (Kilkenny et al., 2010). They have also been described as the "gold-standard checklist", reflecting their strong alignment with animal ethics principles and adherence to the 3Rs (Replacement, Reduction, and Refinement) (Hooijmans et al., 2010). To ensure broad applicability across different animal models, the guidelines specify essential reporting elements, including study design, sample size, outcome measures, and detailed descriptions of experimental animals.

The ARRIVE reporting guidelines were first published in 2010, updated in July 2019, and formally released as ARRIVE 2.0 in July 2020 (Song et al., 2023). These guidelines apply to all areas of bioscience research involving living animals and are not specific to any particular species (Sert et al., 2020; Rodwell et al., 2023). The ARRIVE-2010 guidelines comprise 21 items intended for inclusion in animal research manuscripts, providing comprehensive reporting from study design and sample characteristics to animal welfare and translational relevance. The updated ARRIVE 2020 guidelines introduced a prioritized structure consisting of 10 Essential items and 11 Recommended items that complement the Essential 10. These revisions aim to improve animal welfare, optimize experimental conditions, and reduce the number of animals used in research through enhanced reporting quality (Sert et al., 2020). The principles of the 3Rs form the ethical foundation underpinning the ARRIVE guidelines (Liu et al., 2023). These principles promote humane experimental practices and have continued to evolve alongside advances in scientific methodology, regulatory guidance, and ethical frameworks, thereby contributing to greater scientific rigor and enhanced animal welfare.

Zebrafish have emerged as an attractive alternative animal model alongside other emerging approach. They are widely used in drug discovery and toxicology research and share approximately 87% gene homology with humans for disease-related genes (Cassar et al., 2020; Rodwell et al., 2023). In zebrafish research, adherence to ARRIVE guidelines is particularly important, as both experimental procedures and routine husbandry practices can substantially influence research outcomes (Schroeder, 2022). Accordingly, this evaluation aims to identify variations in the methods used to conduct, measure, and analyse herbal toxicity studies in zebrafish embryos. In addition, it focuses on assessing key reporting elements in the materials and methods, data analysis, and outcomes sections, which are essential for ensuring reproducibility and accurate interpretation of zebrafish embryotoxicity findings. This study aims to evaluate the reporting quality of zebrafish studies assessing the embryotoxicity of herbal medicine following the updated ARRIVE-2020 guidelines.

MATERIALS AND METHODS

Search strategy

Data were collected through electronic database searches of PubMed, Google Scholar, and Science Direct. The search strategy used combinations of keywords with Boolean operators (AND/OR) to identify relevant studies. The following search string was used: ("zebrafish" OR "Danio rerio") AND ("embryotoxicity" OR "embryo toxicity" OR "developmental toxicity") AND ("herbal toxicity" OR "herbal drugs" OR "herbal medicine" OR "plant extract"). Minor adjustments to the search syntax were made depending on the database search interface. In addition, the reference lists of the selected articles were manually screened to identify additional studies. Publication year filters were applied to identify articles published between 2020 and 2023. This timeframe was selected to evaluate studies published after the 2020 publication of the updated ARRIVE 2.0 guidelines, allowing assessment of reporting practices following their introduction.

Study selection

Studies reporting original research were screened for eligibility.

Inclusion criteria: Articles published in English that used zebrafish (*Danio rerio*) as the in vivo model and evaluated embryotoxicity for toxicity assessment. Studies were initially selected based on titles and abstracts containing relevant keywords and were restricted to publications from 2020 to 2023 to reflect research conducted after the release of the updated ARRIVE guidelines.

Exclusion criteria: Review articles, commentaries, and communications, studies not employing an in vivo model, and articles lacking sufficient data or relevant information were excluded. All citations from the selected studies were imported into a bibliographical database (EndNote 20), and duplicate records were removed.

Data extraction

Two independent reviewers conducted title, abstract, and full-text screening of the articles. Data extraction was also performed independently using a predefined data col-

lection sheet. Extracted information included author(s), article title, citation details, year of publication, journal, country of origin, name of test herb or plant, study design, sample size, inclusion, and exclusion criteria, randomization, blinding, outcome measures, statistical methods, experimental animals, experimental procedures, and reported results. Any discrepancies or disagreements between the reviewers during study selection or data extraction were resolved through discussion and agreement. If agreement could not be reached, a third reviewer was consulted to make the final decision.

Evaluation of publication quality

Articles were evaluated in accordance with the ARRIVE 2020 guidelines, with primary emphasis on the "Materials and Methods" section, as it contains the key methodological details required to assess reporting quality. However, when relevant information was not clearly presented in this section, other sections of the manuscript, including the Results, Discussion, and supplementary materials, were also examined to determine compliance with the ARRIVE Essential 10 items.

Adherence to each ARRIVE Essential 10 item was evaluated using the ARRIVE Essential 10 Compliance Questionnaire (NC3R, 2020; Sert et al., 2020). Items were categorized as "fully reported," "partially reported," or "not reported". An item was considered "fully reported" when all required information related to the ARRIVE item was clearly described in the article. An item was categorized as "partially reported" when only some components of the required information were provided, and important details were missing. An item was classified as "not reported" when no relevant information related to that ARRIVE item was presented in the article. These criteria were applied consistently by the reviewers to ensure objective assessment of reporting compliance. Overall compliance was categorized as excellent (≥80%), moderate (50-79%), or poor (≤49%), based on the proportion of items rated as "fully reported".

In addition, the influence of journal and article-related characteristics on adherence to ARRIVE standards was evaluated. Journal-related factors included indexing status (SCI-Expanded), publisher type (commercial or institutional), publication language, publication frequency (quarterly or less), and endorsement of ARRIVE guidelines in the author instructions. Article-related characteristics included publication language, number of authors, and year of publication.

Data analysis

The extracted information was summarized and presented in tabular form, with each ARRIVE Essential 10 item calculated and expressed as a percentage. No statistical analyses were performed.

RESULTS

Selection of articles

The initial database search yielded 99 records, including 36 from Google Scholar, 25 from PubMed, and 38 from ScienceDirect. After screening and eligibility assessment, a total of eight articles met the inclusion criteria and were included in the final analysis. The article selection process is summarized in Figure 1.

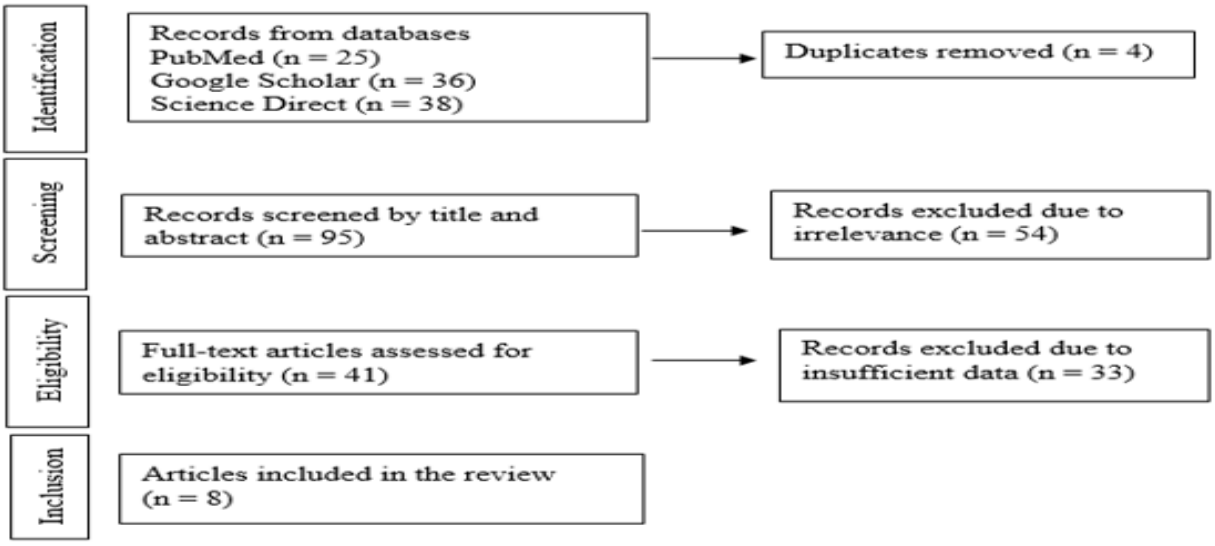

Figure 1: Flow diagram illustrating the selection of articles from the bibliographic database search

A summary of the characteristics of the selected articles is presented in **Table 1.**

Table 1: Characteristics of the included studies.

Article number	Author/s name	Country of origin	Year of publication	Journal
1	Mohamad SNAS et al.	Malaysia	2023	Pharmaceuticals
2	Nguyen TH et al.	Vietnam	2020	Journal of Ethnopharmacology
3	Veeren B et al.	France	2020	Molecules
4	Gu et al.	China	2022	Saudi Pharmaceutical Journal
5	Ahda M et al.	Indonesia	2023	Scientific Reports
6	Farooq M et al	Saudi Arabia	2020	Saudi Journal of Biological Sciences
7	Liu Y et al	China	2023	Toxins
8	Xia Z et al	China	2020	Pharmaceutical Biology

Article-related characteristics

The article-related characteristics of the included studies are summarized in **Table 2**.

Table 2: Article-related characteristics of the included studies (n = 8)

Article-related characteristic		Number of articles	Percentage (%)
Language	English	8	100
Number of authors	> 6	7	87.5
	≤ 6	1	12.5
Publication year	2020	4	50
	2022	1	12.5
	2023	3	37.5
Instruct to adhere to ARRIVE guidelines	Yes	6	75
	No	2	25
Publication frequency	> 4 per year	7	87.5
	≤ 4 per year	1	12.5
Number of authors	> 6	7	87.5
	≤ 6	1	12.5
Publication year	2020	4	50
	2022	1	12.5
	2023	3	37.5

Journal-related characteristics

The characteristics of the journals that published the selected articles are summarized in **Table 3**.

Table 3: Journal-related characteristics of the included journals (n = 8)

Journal characteristic		Number of journals	Percentage (%)
Language	English	8	100
Publisher	Commercial	5	62.5
	Institute	3	37.5
Indexing in SCI-Expanded	Yes	7	87.5
	No	1	12.5

Compliance with the ARRIVE 2020 Essential 10 Items

Compliance of the selected articles with the ARRIVE 2020 Essential 10 items is summarized in **Figure 2.** The proportion of articles that fully complied with individual ARRIVE Essential 10 items ranged from 0% to 100%. Partial compliance across items ranged from 0% to 37.5%.

Compliance level of "fully reported" Essential 10 items

The percentage of articles that fully reported each ARRIVE Essential 10 item, along with the corresponding compliance levels, is presented in **Figure 3.**

Items related to study design, sample size, outcome measures, experimental animals, and reported outcomesdemonstrated the highest compliance, ranging from 87.5% to 100%.

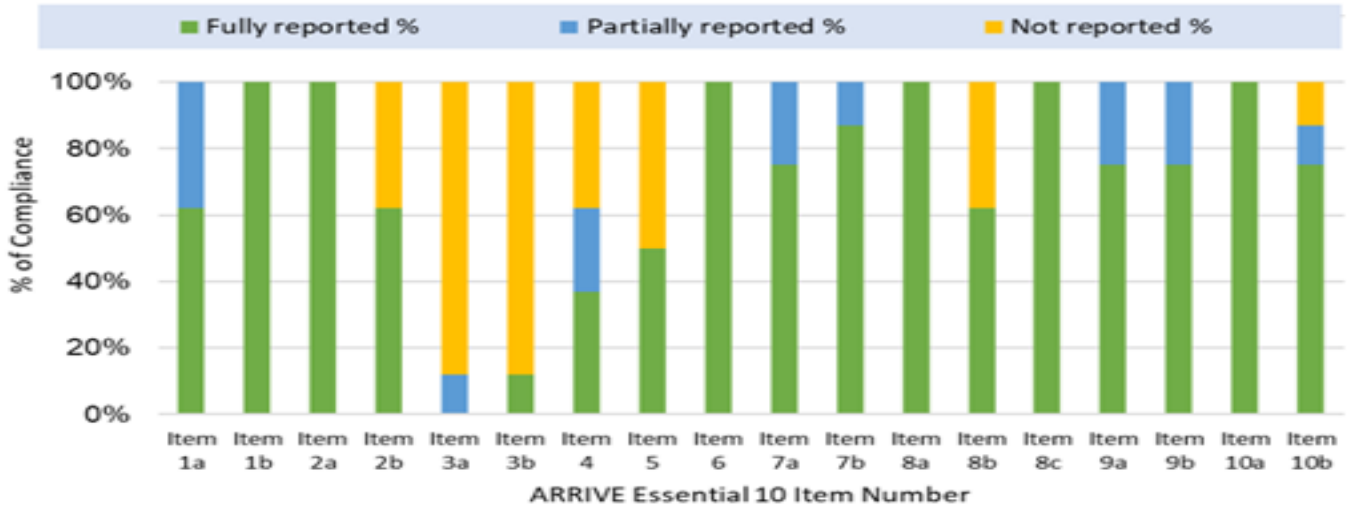

Figure 2: Compliance of articles with the ARRIVE 2020 Essential 10 items

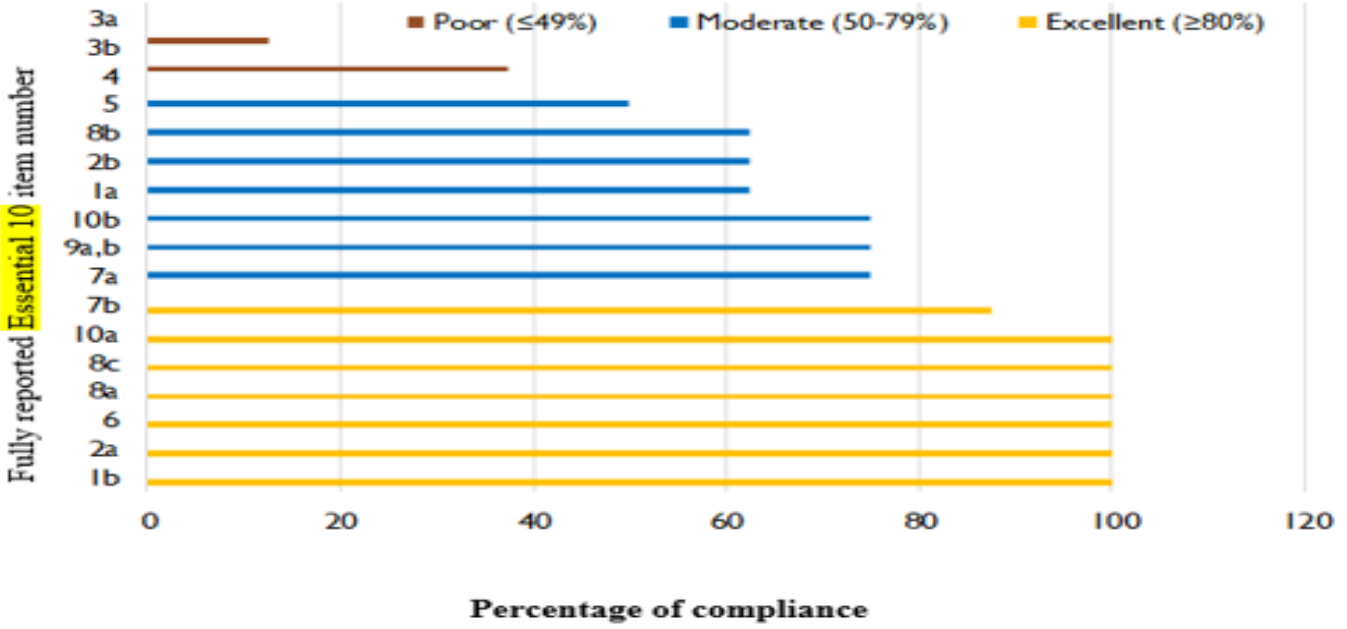

Figure 3: Compliance level of Fully reported ARRIVE 2020 Essential 10 items

Moderate compliance (50-75%) was observed for several items, whereas compliance with inclusion and exclusion criteria and randomization was notably poor, with full reporting ranging from 0% to 37.5%.

DISCUSSION

This study evaluated reporting quality in zebrafish embryotoxicity studies using the ARRIVE 2020 Essential 10 items and found that while certain aspects, such as study design, sample size, outcome measures, and descriptions of experimental animals, were generally well-reported. Critical methodological details, including inclusion and exclusion criteria, randomization, and blinding, were frequently omitted. Compliance with individual ARRIVE items varied widely, highlighting persistent gaps in transparent reporting that may compromise reproducibility, study validity, and ethical use of animals.

Interest in zebrafish (*Danio rerio*) as a model organism dates back to the early 1970s, when George Streisinger pioneered its use in vertebrate genetic research by developing the first forward genetic screening model using zebrafish larvae. This work laid the foundation for the widespread adoption of zebrafish in biomedical research (Barros et al., 2008). Over time, zebrafish have become an established model for studying embryonic and larval development,

particularly in toxicology and early-stage drug discovery, due to their suitability for rapid and efficient toxicity screening.

Several features make the zebrafish embryotoxicity model especially advantageous for toxicological research, including low maintenance costs, high reproductive capacity, optically transparent embryos, and substantial genetic homology with humans. These characteristics enable direct observation of developmental processes and facilitate the assessment of the toxic effects of pharmaceuticals, environmental contaminants, and herbal compounds (Kimmel et al., 1995; Barros et al., 2008).

As zebrafish research expands across scientific disciplines, ethical and methodological challenges related to study conduct and transparent reporting have become increasingly important. Clear, standardized reporting is essential for evaluating study validity, accurately interpreting findings, and ensuring reproducibility. Reproducibility remains a cornerstone of scientific research, yet it is frequently compromised by inadequate study design, insufficient data analysis, and poor reporting practices in animal experiments. Such deficiencies contribute to irreproducible results, unnecessary animal use, and wasted resources (Jalgaonkar et al., 2019). Moreover, poorly reported studies are difficult to include in systematic reviews and meta-analyses, thereby limiting their scientific value.

The Materials and Methods section is central to scientific reporting, as it provides the information necessary to understand experimental design, assess methodological rigor, and replicate findings. However, several systematic reviews have identified substantial deficiencies in methodological reporting across animal studies (Kilkennyet al., 2010; Gulin et al., 2015). Our findings are consistent with this evidence, demonstrating carriable compliance with the ARRIVE 2020 Essential 10 items in zebrafish embryotoxicity research.

Despite the availability of the ARRIVE guidelines, journal-level enforcement remains inconsistent. In this study, only 75% of the journals endorsed ARRIVE guidelines in their author guidelines. Similar improvements in reporting quality have been observed in journals such as PLOS and Nature following the instruction of ARRIVE guidelines, as reported by Barker et al. (2014). Journals that actively promote the use of reporting guidelines may improve transparency and methodological reporting. However, the presence of ARRIVE recommendations in journal instructions does not necessarily guarantee full compliance, suggesting that stronger editorial enforcement and reviewer awareness may be required to ensure consistent adherence to reporting standards.

In the present analysis, study design elements, such as the experimental and control groups, were fully reported in 62.5% of studies, and all studies specified the number of experimental units. However, only 62.5% adequately reported sample size determination. Justifying the sample size, including the use of power calculations, is essential to ensure statistical validity and minimize unnecessary animal use. Failure to report these details raises concerns regarding the robustness and interpretability of study findings (Kilkenny et al., 2010).

Furthermore, reporting of inclusion and exclusion criteria was particularly poor. None of the studies fully defined criteria for the inclusion or exclusion of animals, experimental units, or data points, and only 12.5% reported any exclusions. Transparent reporting of these criteria is essential to reduce bias, support reproducibility, and prevent redundant animal experimentation.

Similarly, randomization and blinding were inadequately reported. Only 37.5% of studies described randomization methods, and 50% reported blinding procedures. The absence of these methodological details increases the risk of bias and may substantially influence experimental outcomes (Alemán-Laporte et al., 2019). In zebrafish embryotoxicity studies, randomization can be implemented by randomly allocating embryos to treatment and control groups using methods such as random number generators, computer-based randomization tools, or systematic allocation procedures. These approaches help minimize selection bias and ensure that experimental groups are comparable at the start of the study.

The consistently poor reporting of items may reflect several practical and methodological challenges in zebrafish embryotoxicity research. In many embryo-based assays, researchers often follow standardized laboratory protocols and may assume that certain methodological steps are implicitly understood, leading to incomplete reporting. Additionally, high-throughput screening approaches using zebrafish embryos may lead some authors to perceive procedures such as formal randomization or explicit sample size calculations as less critical. Limited awareness of reporting guidelines and inconsistent enforcement of ARRIVE recommendations by journals may also contribute to these reporting deficiencies. Improving author awareness and strengthening editorial policies could therefore enhance compliance with reporting standards in zebrafish-based studies.

Overall, while certain aspects of zebrafish embryotoxicity studies are well-reported, critical methodological gaps persist. Inadequate reporting of essential ARRIVE items undermines scientific rigor, transparency, and reproducibility. Strengthening adherence to the ARRIVE 2020 guidelines is therefore essential for improving the quality and reliability of zebrafish-based research.

This study has several limitations. The number of included articles was small, which may limit the generalizability of the findings across the broader zebrafish embryotoxicity literature. Furthermore, the literature search was restricted to publications from 2020 to 2023 to evaluate studies conducted after the release of the ARRIVE 2.0 guidelines. In addition, the search was performed during the study period when more recent publications were not yet fully indexed across the selected databases. Although the evaluation primarily focused on the Materials and Methods section, relevant information in other sections of the manuscript was also considered when necessary. Nevertheless, some methodological details reported outside the main sections may have been overlooked, potentially leading to a slight underestimation of overall compliance. Despite these limitations, the study provides valuable insights into current reporting practices and highlights critical areas requiring improvement.

CONCLUSION

This study demonstrates that reporting compliance with the ARRIVE 2020 Essential 10 items in zebrafish embryotoxicity studies remains inconsistent, with notable deficiencies in key methodological areas such as inclusion and exclusion criteria, randomization, and blinding. While certain elements, including study design, sample size reporting, outcome measures, and descriptions of experimental animals, showed high levels of compliance, inadequate reporting of critical items undermines transparency, reproducibility, and scientific rigor. The findings underscore the continued need for strengthened implementation of ARRIVE guidelines by both authors and journals. Consistent adherence to these standards is essential to improve the quality and reliability of zebrafish-based toxicological research, facilitate reproducibility, and support ethical animal use in accordance with the principles of the 3Rs.

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