Comparative Analysis of Male Reproductive Toxicants: A Multi-Institutional Study

Ananthu Vijayakrishnan, Ranjith D*, Deepanjaly S, Bindu Shree R, Aruna B, Arpita D Patil, Diviyaanandhan VS, Mukesh Rohit V, Sanis Juliet

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala.

Corresponding Author: ranjith946@gmail.com

Male reproductive toxicity has emerged as an increasingly pressing concern, given its profound implications for fertility, population health, and the transgenerational effects of environmental exposures. A diverse array of chemicals and environmental agents—including pesticides, heavy metals, industrial compounds, and pharmaceuticals—has been identified as potential disruptors of the male reproductive system. These agents may compromise spermatogenesis, disrupt hormonal equilibrium, or inflict structural damage to reproductive organs, ultimately jeopardizing male fertility.

Acknowledging the imperative for systematic evaluation, numerous national and international organizations—including regulatory bodies, academic institutions, and public health agencies—have initiated comprehensive studies to assess the prevalence and mechanisms of male reproductive toxicity. These collaborative endeavors seek to establish standardized testing protocols, identify substances of high risk, and inform regulatory decisions aimed at safeguarding reproductive health. Various guidelines have been articulated by organizations such as the OECD, ICH, and FDA.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT GUIDELINES (OECD)

- **OECD Test Guideline 421 (Reproduction/Developmental Toxicity Screening Test)** is a standardized protocol known as TG 421 has been meticulously developed to evaluate the reproductive toxicity of chemicals, with a particular emphasis on their impact on male fertility. Male rats are administered the test substance for a minimum of two weeks prior to mating, throughout the mating process, and until sacrifice, culminating in an approximate duration of 28 days. The parameters subjected to evaluation encompass clinical manifestations, body weight, food consumption, mating behavior, fertility index, and histopathological examination of reproductive organs. Furthermore, sperm analysis may encompass assessments of motility, count, and morphology. The TG 421 test yields critical data regarding a substance's potential to disrupt spermatogenesis and compromise male reproductive health. Although it does not elucidate the mechanisms of action, it functions as an initial instrument for the identification of reproductive hazards. Should adverse effects be observed, more comprehensive assessments such as OECD TG 416 or TG 443 are advised. Consequently, TG 421 assumes a pivotal role in the preliminary safety evaluation of chemicals concerning male reproductive toxicity.
- **OECD Test Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test)** assesses potential toxic effects of a substance on systemic toxicity and male reproductive performance. It involves administering the test compound to male rats for 28 days, including mating with females, to detect adverse effects on spermatogenesis and male reproductive organs. Detailed histopathological examinations are performed on organs like the testes, epididymis, prostate, and seminal vesicles. TG 422 is useful for early safety assessments of chemicals and pharmaceuticals but may not detect subtle or long-term effects on male fertility, requiring further specialized studies like TG 416.
- **OECD Test Guideline 443 (Extended One-Generation Reproductive Toxicity Study)** assesses reproductive and developmental toxicity of chemicals, focusing on male reproductive health. It integrates key endpoints into a single study design, replacing older testing methods. Parameters include sperm count, motility, morphology, and histopathology of male reproductive organs. The study involves dosing rodents from pre-mating through postnatal period. F0 males are observed for testicular toxicity, while F1 male offspring may be evaluated for developmental impacts. Optional cohorts allow for extended assessments including

hormone analysis, neurodevelopmental, and immunotoxicity endpoints. TG 443 considers effects on sexual behavior and fertility, supporting a tiered approach to reduce animal use. In summary, OECD TG 443 offers an ethically optimized framework for evaluating male reproductive toxicity.

- **OECD Test Guideline 416 (Two Generation Reproductive Toxicity)** assesses effects of a test substance on male and female animal reproductive capability across two generations. It evaluates male fertility, organ development, and function. Male rats are dosed daily for at least 10 weeks before mating to observe effects on spermatogenesis, hormonal balance, and sexual behavior. Parameters monitored include sperm count, motility, morphology, organ weights, and histopathological changes. Hormonal evaluations may also be conducted. TG 416 evaluates the health and reproductive performance of F1 and F2 generations to assess long-term risks of chemical exposure on male fertility. It is widely used in regulatory toxicology for ensuring reproductive health safety of chemicals and drugs.

INTERNATIONAL COUNCIL FOR HARMONIZATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE GUIDELINES (ICH)

- **ICH Guideline S5(R3)** provides a framework for assessing reproductive and developmental toxicity of pharmaceutical substances. It emphasizes evaluating effects on male fertility, including impacts on spermatogenesis and hormonal regulation. Study designs like FEED studies are crucial for identifying adverse effects on male reproductive organs. S5(R3) supports alternative testing strategies and integration with toxicokinetic data. Histopathological examinations and sperm analysis are required endpoints. The guideline applies to drugs for men and women of reproductive potential, ensuring reproductive safety in drug development. S5(R3) helps identify potential male-mediated reproductive risks before human exposure.
- **ICH Guideline M3(R2)** provides a harmonized approach to non-clinical safety evaluations, including male reproductive toxicity. Fertility and early embryonic development studies are necessary before Phase III trials or long-term exposure. Specific assessments on male fertility are recommended for compounds with potential reproductive toxicity. Male fertility studies should align with clinical milestones and may be conducted earlier if needed. Integration of reproductive endpoints into general toxicity studies is encouraged to reduce animal use.ICH M3(R2) ensures a risk-based approach to evaluating male reproductive toxicity in pharmaceutical development.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA)

- The U.S. EPA's OPPTS 870.3800 guideline delineates protocols for evaluating the reproductive toxicity of chemicals, with a particular focus on male reproductive health. Employing a multi-generational study design utilizing rats, it scrutinizes sperm production, motility, morphology, mating behavior, fertility, and histopathological alterations in organs such as the testes and epididymis. Chemicals are administered orally prior to and during mating, as well as throughout gestation and lactation. Organ weights, sperm parameters, and tissue histology are meticulously documented at necropsy. This comprehensive approach is adept at detecting both reversible and irreversible effects, functioning as a regulatory instrument to identify reproductive hazards associated with environmental and occupational exposures, thereby bolstering public health protection..
- EDSP (Endocrine Disruptor Screening Program) evaluates chemicals for endocrine system interference impacting male reproductive health. It assesses substances for endocrine-disrupting properties altering hormone functions critical for male reproductive development. Male reproductive toxicity evaluates adverse effects on testes, sperm production, hormone levels, and sexual characteristics. Chemicals disrupting androgen and estrogen pathways can decrease sperm count, alter sperm motility, cause testicular atrophy, and impair fertility. The program uses tiered screening starting with in vitro assays and progressing to in vivo tests. Assays like the Hershberger assay and pubertal male assay focus on androgen-dependent tissues and male pubertal

development. These tests identify chemicals causing developmental, reproductive, and hormonal disturbances in males. EPA's EDSP systematically identifies endocrine disruptors for regulatory decisions protecting male reproductive health and preventing impacts from chemical exposures.

FDA (U.S. Food and Drug Administration (USFDA)

- Adopts ICH guidelines (S5(R3), M3(R2)) for pharmaceuticals.
- For chemicals and food additives, follows EPA/Red Book and other specific toxicology testing guidelines.

ECHA (European Chemicals Agency) – under REACH Regulation

- Follows OECD TGs for reproductive toxicity testing.
- REACH requires data based on tonnage levels; TG 421, 422, or 443 commonly applied.

WHO/IPCS (World Health Organization / International Programme on Chemical Safety)

- The WHO/IPCS aims to establish international consensus on chemical safety, including the evaluation of reproductive and developmental toxicants.
- It develops harmonized testing strategies, risk assessment principles, and health-based exposure limits for chemicals affecting reproductive health.

NTP (National Toxicology Program, USA)

- It is a part of NIH (National Institutes of Health) that conducts detailed male reproductive toxicity studies in rats, often involving Sperm count ,morphology,Hormone levels & Testicular pathology

Conclusion

Male reproductive toxicity has gained increasing attention from global regulatory bodies due to its significant implications for fertility, population health, and environmental safety. Various organizations, including the OECD, US EPA, ICH, and EFSA, have developed detailed guidelines and testing protocols to assess the impact of chemicals, pharmaceuticals, and environmental contaminants on male reproductive function. These guidelines commonly focus on evaluating parameters such as sperm count and motility, histopathological changes in reproductive organs, hormonal profiles, and mating behavior in animal models, particularly rodents. Despite differences in methodology and scope, the unified objective remains to identify potential hazards, establish safe exposure limits, and ensure public health protection. Continued harmonization and refinement of these testing standards are essential to improve sensitivity, reliability, and translational relevance of the findings, ultimately supporting informed regulatory decisions and safeguarding reproductive health.

References

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