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Impact of metal and metal oxide nanoparticles on the male reproductive system: A comprehensive review

Ajith MP[#], Rohit Gautam[#] & Paulraj Rajamani[*]
School of Environmental Sciences, Jawaharlal Nehru University, New Delhi-110 067, Delhi, India

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Technological advancements and increased use of chemically hazardous materials have become a concern for human health. Environmental pollutants such as engineered metal and metal oxide nanoparticles (M&MONPs) are reported to contribute to significant health-related problems, particularly reproductive health. Reduction in male individuals' reproductive potential is one of the important causes of concern. Literature suggests that exposure to M&MONPs considerably impacts alteration in male reproductive parameters. Therefore, it is required to analyze and understand their effects on male reproductive toxicity. Oxidative stress and changes in redox equilibrium are the significant factors *via* M&MONPs induce changes in sperm parameters or the reproductive system. M&MONPs act as endocrine disruptors and interfere with the secretion and function of reproductive hormones such as testosterone and Luteiniz ing Hormone (LH), affecting spermatogenesis. Likewise, M&MONPs accumulate in organs as they can pass through the blood-testis barrier, affect Sertoli and Leydig cells, and cause reproductive dysfunction. In this review, we have analyzed the effects of M&MONPs on the male reproductive system and the underlying mechanism of action.

Keywords: Male reproductive system, Metal and metal oxide nanoparticles, Oxidative stress, Reactive oxygen species, Sperm

Introduction

"Infertility affects an estimated 15% of couples (48.5 million) worldwide. Males are reported to be solely responsible for 20-30% of infertility cases, and also contribute half of the total infertility cases"[1]. An increase in infertility is reported to be associated with exposure to toxic environmental contaminants. Rapid industrialization, technological advancement and anthropogenic activities such as mining are the reason for environmental pollution. Nanoparticles (NPs) (1-100 nm) are nowadays widely popular due to their outstanding size-depend physical and chemical properties, as well as their potential applicability in different fields[2,3]. They are widely used in pharmacology biology, chemistry, physics, material science, industry, engineering and technology[4] Moreover, NPs are also used as a constituent of cosmetics and personal care products[5]. NPs accumulate in the air, water, and soil and enter the human body *via* inhalation, ingestion or dermal exposure. This increases the incidence of human exposure.

Metal and metal oxide NPs (M&MONPs) is distinguished as the type of flexible materials among the many NPs types[6]. Due to their tiny size and controlled properties, M&MONPs may readily pass through cells and tissues to reach the desired place inside the body[7,8]. As a result, M&MONPs are an essential tool for health care, such as cancer treatment, diabetes treatment, antibacterial treatment, imaging applications, drug administration, and even reproductive medicine[8,9]. Most metallic precursors that make up M&MONPs are often categorized as biocompatible as they are already found in human tissues, which have been shown to play a crucial part in body functioning[10]. The organism will more readily take them because they are necessary to the body[9]. Some of the elements believed to be essential for humans are manganese, molybdenum, magnesium, manganese, iron, cesium, cobalt, chromium, nickel, copper, zinc, and selenium[8-10]. However, these physiologically acceptable metals have harmful effects on mammalian cells and can even result in cell death at high doses[11]. They have two opposing effects depending on the concentration, making their application difficult[9,12]. This could contribute to the numerous contentious findings on the reproductive toxicity of M&MNONPs.

In vivo studies on the model organisms have signified the toxic effects of administered M&MONPs on several

[#]Equal first and second author
*Correspondence:
E-mail: paulrajr@yahoo.com

organs, reproductive toxicity being one of them. However, there are only a few studies on the impact of M&MONPs exposure on reproductive physiology, and they require further investigation. To determine the precise impact of these M&MONPs on the male reproductive system, this review analyzes *in vitro* and *in vivo* investigations that explore the possible reproductive toxicity of M&MONPs. Emphasis is placed on knowledge gaps and recommendations for further research.

Diversity of M&MONPs

The metallic NPs are essential in many medical applications and generally exist in several fundamental forms[13], as illustrated in (Fig. 1). The first kind of metallic NPs is metal nanoparticles, which are metal-based NPs in their purest form (*e.g.* Ag, Au, Pt, Mn, Fe NPs *etc.*). Metal oxide NPs are the other forms of metallic NPs (*e.g.* ZnO, AgO2, TiO2 *etc.*). Another kind of metallic NPs is doped metal/metal oxide NPs (*e.g.* Pt-ZnO, Au-CuO *etc.*). In addition, metal-organic frameworks (MOFs), such as those based on Zn, Cu, and Mn, as well as metal sulfide nanoparticles (NPs) such as AgS, CuS, FeS NPs have both been explored[14]. Most of the reproductive toxicity studies are focused on M&MONPs.

Route of M&MONPs exposure

Routes of exposure of M&MONPs into the reproductive system can be either directly *via* the use of M&MONPs-based drugs or imaging probes. In contrast, indirect exposure includes dermal penetration and the pulmonary and gastrointestinal route[5]. The use

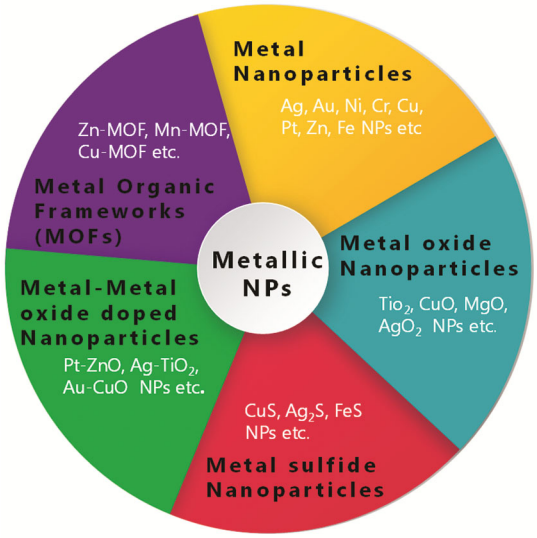

Fig. 1 — Types of metallic nanoparticles

of M&MONPs, especially silver, titanium, zinc, and gold NPs in cosmetics, food and drugs, makes oral and gastrointestinal exposure prime routes, from where they are absorbed by the blood, enter tissues and accumulate in different organs. Market commodities, including sunscreens, food additives, and paints, use ZnO NPs and TiO_2 NPs[15,16]. Eventually, ingestion and skin penetration are the main routes of their entrance into the human body, albeit they are not the only ones. Ag NPs are employed in conductive inks, textile industries, diagnostic biosensors, antimicrobial goods, water disinfectants, and food[17]. For these reasons, they can enter the human body through the skin, ingestion, and inhalation. The medical area is where Au NPs are most widely used; photothermal treatment, bioimaging, and drug administration are the main applications[16,18]. Thereby, they can quickly enter the human body. Figure 2 highlights and summarizes the primary exposure pathways to NPs in humans and other animals in the environment.

Individual effect of M&MONPs on the male reproductive system

This review section discusses the potential concerns of M&MONPs exposure and underlying reproductive issues, emphasizing the mechanism of action. According to Yan *et al*. 2016, M &MONPs toxicity is often caused by an increase in ROS production, suppression of antioxidant defence mechanisms, induction of DNA damage, and cell cycle arrest, which results in complicated pathways involving male germ cell death (Fig. 3). M&MONPs penetrate the gonads' outer membranes and produce ROS in a manner similar to Sertoli and Leydig cells, which have the function of regulating and supporting male germ cells[21]. The complex toxicity mechanisms of M&MONPs can induce the production of free radicals by damaging the antioxidant system and interfering with cellular metabolism[22].

The degree of toxicity of M&MONPs relay on several aspects such as type of metal, absorptivity and residence time in the body. While TiO_2 NPs were observed to alter the body to reproductive organ (testis and accessory male sex organs) weight ratio[23]. In another study, the administration of 70 nm Au-Si core-shell NPs did not show any changes or accumulation in testis[24]. This signifies that the metal counterpart used for M&MONPs preparation is significant in inducing toxicological effects. The toxicity of M&MONPs in the male reproductive system and the subsequent effect on sperm cells is induced when the particles trespass the

blood testes barrier[25]. M&MONPs accumulated in male reproductive organs such as the prostate gland, epididymis and testicular tissue, including spermatid[26]. A study on a mouse model showed the testis and epididymis to be the primary organs of M&MONPs accumulation[27]. The effect of TiO_2 NPs has been well analyzed. Intragastric administration of TiO_2 NPs for six months induced alterations in spermatogenesis, with marked histopathological variations in the testes of treated male offspring[28]. Abnormality in Leydig and sperm cells was also observed during the administration of TiO_2 NPs[29]. Oral administration of TiO_2 NPs in adult albino rats at 100 mg/kg/day for 8 weeks showed considerable toxic effects. Apart from a change in body-to-organ (testes) weight ratio, an increase in oxidative stress levels, micronucleated RBCs and up-regulation of the "testin gene" were reported[23].

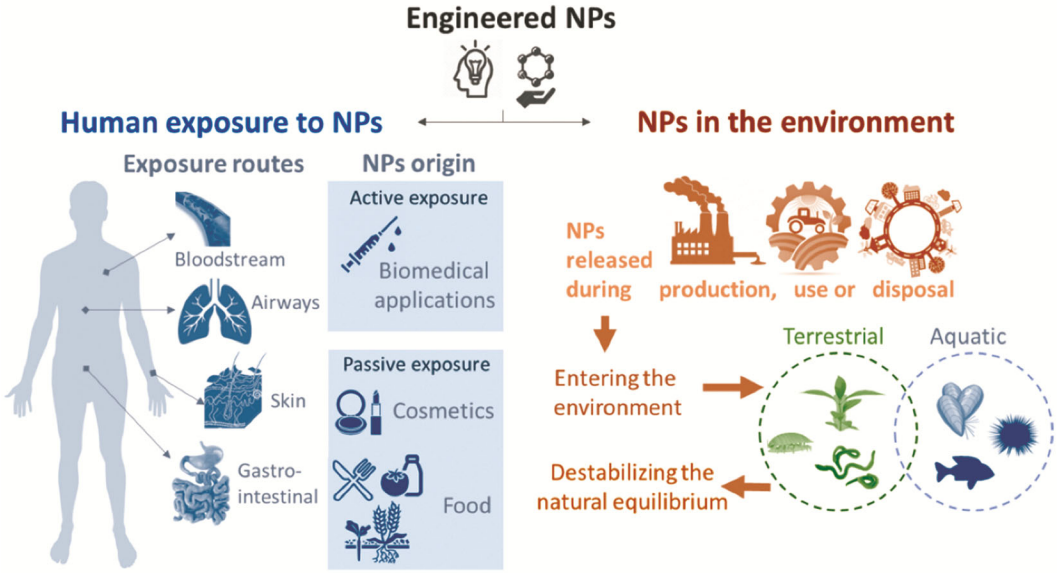

Fig. 2 — The exposure paths of NPs and interaction with humans or other environmental organisms[19]

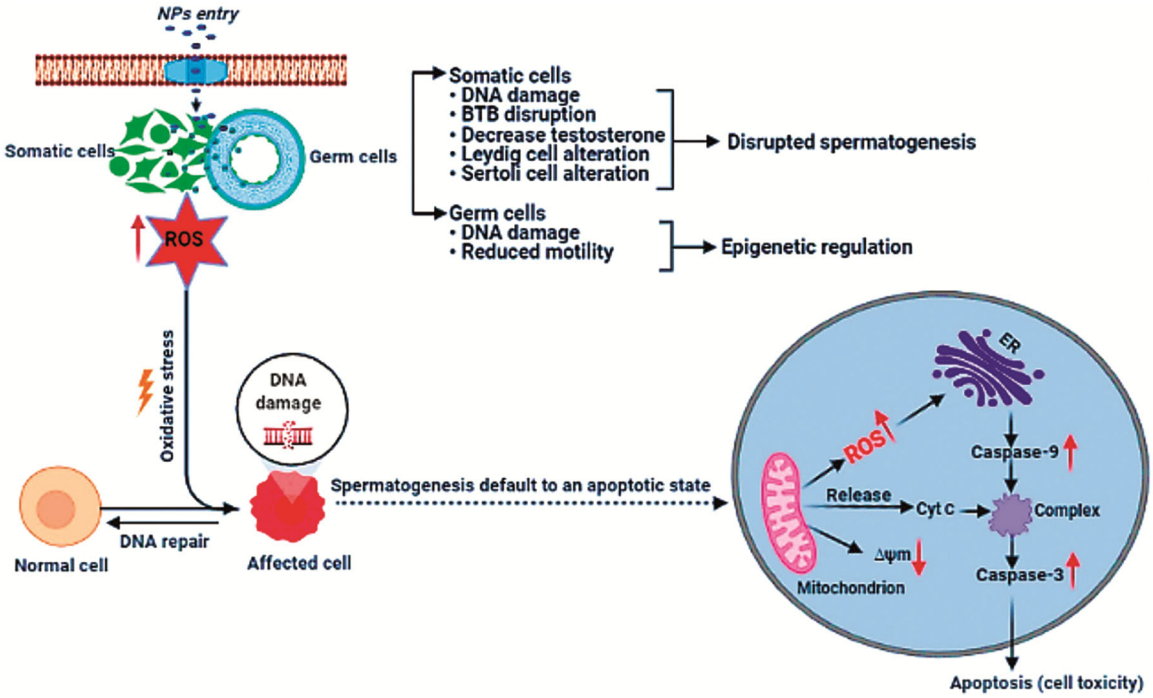

Fig. 3 — Impact of nanoparticles on male germ cells and supportive cells[22]

The toxicity of Au and Ag-based NPs have also been reported. Administration of Ag NPs in Wistar rats showed toxicological effects in decreased sperm count and viability and induced morphological changes. The alterations were prominent at a concentration of 300 mg/kg AgNPs, and noticeable variations in the number of spermatogonia and Sertoli cells were observed compared to the control set[30]. Subdermal administration of rats with AgNPs at 50 mg/kg body weight brought about haematological and hormonal variation. Considerable enhancement in superoxide dismutases (SOD), catalase, and reduced glutathione were observed because of the generation of oxidative stress by AgNPs[31] AgNPs are considered to be highly toxic to humans because of the instability and rapid oxidation followed by accumulation in the form of elemental silver. This was evident from the study conducted by Garcia *et al*., 2014[32]. They stated that AgNPs, even at a low dose (1mg/kg), considerably increased intratesticular testosterone levels. In the study, AgNPs were intravenously injected into male CD1 mice. Changes in hormone concentration were significant after 15 days of treatment, along with evident alteration in epithelium morphology, size of 3Ariat cells and germ cell apoptosis.

Further, up-regulation of Cyp11a1 and Hsd3b1 mRNA was identified. They suggested that AgNPs did not exert toxicity towards the testis but affected the functioning of Leydig cells. The size and dose-dependent toxicity of AgNPs were examined in rats after intravenous administration. Exposure to NPs resulted in decreased sperm count along with germ cell DNA damage. The extent of damage in testes seminiferous tubule morphometry was more prominent in larger-size NPs (200 nm) compared to small-sized (20 nm)[33]. In contrast, AgNPs at a very low dose of 30 µg/kg did not alter the concentration of hormones such as FSH, LH and testosterone[34].

On the contrary, administration of CeO_2 NPs and ZnO NPs induced a reduction in testosterone concentration in serum[2,35]. CeO_2NPs treated male balb/c mice showed considerable reduction in hormone (testosterone, FSH, LH and prolactin) concentration and increased testicular malondialdehyde. The congestion and disintegration of seminiferous tubules and decreased sperm count and motility were also observed. These results signify the lack of an integrated mechanism behind variation in hormone concentration; instead, the type of NPs, size, the dose of treatment and exposure time affect the hormone levels.

Significant variation in marker protein concentration is also observed on exposure to M&MONPs. Intragastric administration of TiO_2 NPs at a concentration of 50 mg/kg/day for 3 weeks showed variation in prostrate and testicular markers. At the same time, the increase in testicular gamma-glutamyl transferase and reduction in testicular steroidogenic acute regulatory protein was determined. Apart from this, up-regulation of Fas, Bax and caspase-3 gene expression and down-regulation of the Bcl-2 gene were reported. An increase in testicular and prostatic acid phosphatase, gonadotrophin and malondialdehyde levels was noticed[36]. In another study, oral administration of ZnO NPs of average size 20-30 nm altered tumour markers concentrations such as a prostate-specific antigen, 1-α-fetoprotein and carcinoembryonic antigen. The study concluded that induction of stress and free radical generation on exposure *via* a dose-dependent manner on exposure to ZnO NPs[37].

It thus suggests that M&MONPs induce a plethora of toxicological effects, including variation in hormone concentration and generation of reactive oxygen species, which subsequently affect germ cells and reproductive organs. This occurs due to the accumulation of M&MONPs in reproductive organs such as the testis and epididymis, thereby inducing stress conditions and leading to oxidative stress, which affects the normal functioning of sperm[38]. Sperm cells are more prone to oxidative stress as sperm cells as the sperm cell membrane has an abundance of polyunsaturated fatty acid. Moreover, M &MONPs can pass blood-testis and placental and epithelial barriers. Accumulating M&MONPs in organs such as the testis, epididymis, ovary, and uterus can lead to the destruction of Sertoli cells, Leydig cells, and germ cells. “They affect sperm quality, quantity, morphology, and motility and can also reduce mature oocytes number and disrupt primary and secondary follicular development”. Additionally, changes in gene expression at molecular levels can also occur. However, there is a huge disparity in the extent and incidence of toxicity by M&MONPs, which is the subject of investigation. Table 1 summarizes the impact of nanoparticles on the reproductive system.

Combined effect of two M&MONPs in the male reproductive system

The studies also reported the combinational effects of two M&MONPs. Yousef and colleagues treated

animals orally with the combination of Al_2O_3NPs and ZnONPs (70 and 100 mg/kg BW, respectively) for 75 days[50]. Semen analysis showed a significant decrease in testosterone, thyroid stimulating hormone, antioxidant and glutathione levels. Free radicals, nitric oxide, UCP2 gene, p53, tumour necrosis factor-α, interleukin-6, FSH, LH, T3 and T4 were considerably increased. Further, results of testes histopathological

Table 1 — Effect of M&MONPs exposure on the male reproductive system

Sl. No.	Nanoparticles	Organism, Age, Weight No of animals per group	Dose Duration Method of delivery	Effects	Reference
In vivo					
1.	Cerium oxide nanoparticles (CeO_2NPs)	Adult male mice, C57BL/6J, 6 weeks old, ”22 ± 2 g	10, 20 ,40 mg/kg body weight for 32 days, Orally.	• Decrease in testis weight, sperm motility, and daily sperm production. Testosterone level • Increase in sperm DNA damage • Down-regulated expression levels of steroid genesis genes.	39
2.	Manganese oxide nanoparticles (Mn_2O_3 NPs)	Wistar adult male rats 230 ± 20 g	100, 200, and 400 ppm for 14 days, Orally(gavage).	• Significant reduction in testosterone, LH and FSH levels. • Decrease in Leydig cells, spermatogonial cells, primary spermatocyte and spermatids.	40
3.	Lead selenide nanoparticles (PbSeNPs)	Male Sprague Dawley rat 6 to 7 Weeks old 170−200 g	10mg/kg/week for 60 days, Intra-peritoneally.	• Accumulation of PbSeNPs in testes. • ST degeneration with atrophy, germ-cell degeneration. Increase in the level of cytochrome c and caspase 3 activity	41
4.	Cadmium telluride Quantum Dots (CdTe QDs)	Male BALB/c mice 18-22 g	0.2 nmol and 2.0 nmol CdTe QDs in 200 mL physiologic saline per mouse, Intravenous.	• Decrease in body weight, and sperm quality. • Significant increase in sperm DNA fragmentation index	42
5.	Silver nanoparticles (Ag NPs) (60 nm in dimension)	75 male prepubertal Wistar rats	25, 50, 100, and 200 mg/kg/day, For 45 days, Orally.	• A Significant decrease in sperm motility, normal sperm morphology and Leydig cells. • Reduction in serum testosterone and increase in LH level.	43
6.	Nickel nanoparticles (Ni NPs)	Six-week-old male BALB/c mice (n = 60)	5, 15, and 45 mg/kg/week, Intratracheal instillation once a week 28 days	• A significant increase in apoptosis and damaged in testicular spermatogenic cells. • Increase in sperm deformity and expression protein related to mitochondrial fission/autophagy.	44
7.	Zinc Oxide nanoparticles (ZnO NPs)	Mice (n = 9)	100, 200, and 400mg/kg/day for 28 days	• A significant decrease in sperm density of the epididymis. • Serum testosterone levels were markedly reduced. • Disruption of seminiferous epithelium	45
In vitro					
8.	Titanium dioxide Nanoparticles (TiO_2NPs)	Human Sperm	1 and 10 µg/L for 15, 30, 45, and 90 min	• Significant loss of sperm DNA integrity. • Increase in intracellular ROS level and DNA fragmentation.	46
9.	Zinc Oxide nanoparticles (ZnO NPs)	Rabbit sperm	0, 1, 2 and 3 h	• Results showed a dose-dependent decrease in sperm viability and motility.	47
10.	Silver nanoparticles (Ag NPs)	Sperm from male BDF1 mice (8–12 weeks old	3h	• The result showed sperm physiology leading to poor fertilization and embryonic development.	48
11.	Silver nanoparticles (Ag NPs)	Sertoli cells	5, 10, or 15 µg/mL	• Increased oxidative stress. • The activation of p53, repression of bcl-2 and decrease in endogenous antioxidant enzymes level	49

investigation and level steroid genesis enzymes were also altered. In an *in vitro* study on Boar sperm semen, Fe_3O_4 NPs of diameter 40 nm (0.192 mg/mL semen) and Ag/Fe NPs of diameter 30 nm, consisted of Ag and a 5% of zero-valent Fe (0.128 mg/mL semen) were treated[52]. Samples were incubated at 17°C for 30 min. "Ag/Fe NPs demonstrated a detrimental effect on boar spermatozoa. In contrast, the used concentration of theFe_3O_4 NPs did not affect boar sperm Computer Assisted Semen Analysis (CASA) motility parameters"[51]. This indicates that the combinational effects of M&MONPs are completely different from that of the individual ones.

Protective role of M&MONPs in the male reproductive system

In contradiction to the above studies, some recent studies showed the protective role of M&MONPs. A study on male Wister rats who received SeNPs (0.5 mg/kg orally by gavage 3 times/week for 60 days; showed a protective role against *Deltamethrin* (DLM). *DLM* is an insecticide belonging to the pyrethroid family. "Result showed treatment with SeNPs improved DLM-induced negative effects on sperm parameters, testosterone and antioxidant levels and histopathological alterations. The SeNPs treated group showed improved semen parameters, antioxidant status, and sexual performance"[52].

Similarly, our laboratory reported the protective role of bovine serum albumin (BSA) coated manganese dioxide nanoparticles (Mn NPs) after exposure to 2002 MHz microwave radiation. The study showed combined exposure to Mn NPs and microwave radiation significantly reduces the reproductive alterations caused by microwave radiation. "Thus, Mn NPs mimicked as an antioxidant and showed a protective role against oxidative stress induced by microwave radiation and therefore, protects the vulnerable cells and tissues from oxidative stress-induced damages in reproductive cells"[53].

Limitation & Prospects

The inconsistency in study design, which makes it challenging to compare different techniques, is one of the primary gaps in our knowledge of how M&MONPs relate to male reproductive health. To overcome this gap, reproductive scientists and nanotoxicologists should collaborate more on the planning and analysis of investigations. Researchers may start properly addressing critical problems, such as whether there is an NP dose effect, by creating optimum strategies for exploring particular hypotheses and comprehending specific components of the study[54,55]. The ability to compare research regarding exposure plans and result evaluations is another area that must be filled. For this, it would be necessary to organize various M&MONPs according to risk assessment and search for patterns among various M&MONPs[56,57].

Few studies have examined the interactions of M&MONPs with biomaterials at the molecular level; most studies have been conducted at the macro level. Future research should thus focus on the molecular impacts of M&MONPs. The relationship between each change is rarely explored in the existing investigations of M&MONPs toxicity toward the reproductive system, which mainly concentrate on individual modifications. As a result, a deeper investigation into the molecular causes of nanotoxicity and how each modification interacts with one another has to be done. Finally, a closer examination of the dose is necessary. The significance of dosage rate and possible underlying variations for the activation of inflammation and the translocation of M&MONPs in the male reproductive system is inconsistent in many aspects.

Conclusion

The studies (*in vitro* and *in vivo)* demonstrate that engineered M&MONPs act as environmental pollutants, affecting human health. The male reproductive system induces various sperm and reproductive parameters alterations through ROS generation and oxidative stress. These studies signify that the discussed M&MONP salter sperm count viability and motility can significantly affect sperm morphology, testicular structure and spermatogenesis. Thus, exposure to such agents harms the human reproductive system, and the underlying mechanism of action has been discussed in detail in this review. However, further studies with detailed analysis of the impact of M&MONPs on different age groups, various dosages, and offspring are necessary to elucidate the mechanism and design appropriate therapeutic measures.

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Conflict of interest

All authors declare no conflict of interest.

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