Environmental Research 192 (2021) 110254

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Review article

The latest advances in the reproductive toxicity of microcystin-LR

Shiyu Zhang [a], Xingde Du [a], Haohao Liu [a], Michael D. Losiewic [c], Xinghai Chen [c], Ya Ma [a], Rui Wang [a], Zhihui Tian [a], Linjia Shi [a], Hongxiang Guo [b,**], Huizhen Zhang [a,*]

[a] College of Public Health, Zhengzhou University, Zhengzhou, Henan, China
[b] College of Life Sciences, Henan Agricultural University, Zhengzhou, Henan, China
[c] Department of Chemistry and Biochemistry, St Mary's University, San Antonio, TX, USA

ARTICLE INFO

Keywords:
Microcystin-LR
Reproductive toxicity
Environmental estrogen
Mechanisms

ABSTRACT

Microcystin-LR (MC-LR) is an emerging environmental pollutant produced by cyanobacteria that poses a threat to wild life and human health. In recent years, the reproductive toxicity of MC-LR has gained widespread attention, a large number of toxicological studies have filled the gaps in past research and more molecular mechanisms have been elucidated. Hence, this paper reviews the latest research advances on MC-LR-induced reproductive toxicity. MC-LR can damage the structure and function of the testis, ovary, prostate, placenta and other organs of animals and then reduce their fertility. Meanwhile, MC-LR can also be transmitted through the placenta to the offspring causing trans-generational and developmental toxicity including death, malformation, growth retardation, and organ dysfunction in embryos and juveniles. The mechanisms of MC-LR-induced reproductive toxicity mainly include the inhibition of protein phosphatase 1/2 A (PP1/2 A) activity and the induction of oxidative stress. On the one hand, MC-LR triggers the hyperphosphorylation of certain proteins by inhibiting intracellular PP1/2 A activity, thereby activating multiple signaling pathways that cause inflammation and blood-testis barrier destruction, etc. On the other hand, MC-LR-induced oxidative stress can result in cell programmed death via the mitochondrial and endoplasmic reticulum pathways. It is worth noting that epigenetic modifications are also involved in reproductive cell apoptosis, which may be an important direction for future research. Furthermore, this paper proposes for the first time that MC-LR can produce estrogenic effects in animals as an environmental estrogen. New findings and suggestions in this review could be areas of interest for future research.

1. Introduction

Cyanobacterial blooms produced by eutrophic water bodies have become a serious environmental issue in the world (Yang et al., 2020). When cyanobacterial blooms occur, the overgrown cyanobacteria emit an unpleasant smell that affects water quality (Fig. 1A). *Microcystis*, *Oscillatoria*, *Anabaena* and *Aphanizomenon* are the most common cyanobacteria that can produce microcystins (MCs) (Du et al., 2019"; Svirčev et al., 2019) (Fig. 1B–E). These cyanobacteria can release MCs into the water column after death, and MCs can harm aquatic animals, and indirectly affect human health through the food chain (Papadimitriou et al., 2012). Since George Francis first reported the poisoning of livestock by cyanobacterial blooms from Australia in 1878, the threat of MCs on humans and animals has attracted wide attention all over the world (Francis, 1878). Health hazards of MCs on humans have been reported in different countries including Australia (Cirés et al., 2014), China (Lin et al., 2016; Svirčev et al., 2017), Brazil (Carmichael et al., 2001; Hilborn et al., 2013), America (McCarty et al., 2016), and Canada (Kelly et al., 2019).

MCs are cyclic heptapeptide toxins and more than 200 isomers have been reported (Spoof and Catherine, 2017). Among them, microcystin-LR (MC-LR) is the most toxic and widely distributed isomer (Gupta et al., 2003). The molecular weight of MC-LR is 995.2 Da. The chemical structure of MC-LR (-D-Ala-l-Leu-D-isoMeAsp-l-Arg-l-Adda-D-Glu-Mdha) is cyclic, in which Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid) is an essential group for expressing MC-LR toxicity. The two amino acids unique to MC-LR are leucine at the second position and arginine at the fourth position (Fig. 2). The cyclic structure and presence of novel amino acids render MC-LR resistant to heat, hydrolysis, and oxidation. These chemical

* Corresponding author.
** Corresponding author.
E-mail addresses: guohongxiang06@126.com (H. Guo), huizhen18@126.com (H. Zhang).

https://doi.org/10.1016/j.envres.2020.110254
Received 30 June 2020; Received in revised form 2 September 2020; Accepted 20 September 2020
Available online 28 September 2020

properties of MC-LR enhance its stability and persistence in the environment (de la Cruz et al., 2011). MC-LR is also highly resistant to conventional drinking water treatment processes, which increases the risk of animal and human exposure (de la Cruz et al., 2011; Song et al., 2020).

MC-LR is mainly present in the water environment, and aquatic animals are most easily to be exposed to the poison. However, humans have more exposure routes than animals, such as ingestion (contaminated water, vegetables, fruits, algae supplements and other foods), inhalation (during water recreation), dermal contact and venous inflow (Lee et al., 2017) (Fig. 3). MC-LR can easily enter the body through the blood circulation and then accumulate and damage in certain target organs such as the liver (AlKahtane et al., 2020; Gorham et al., 2020; Jia et al., 2019; Lei et al., 2019; Yang et al., 2018; Zhao et al., 2019b),

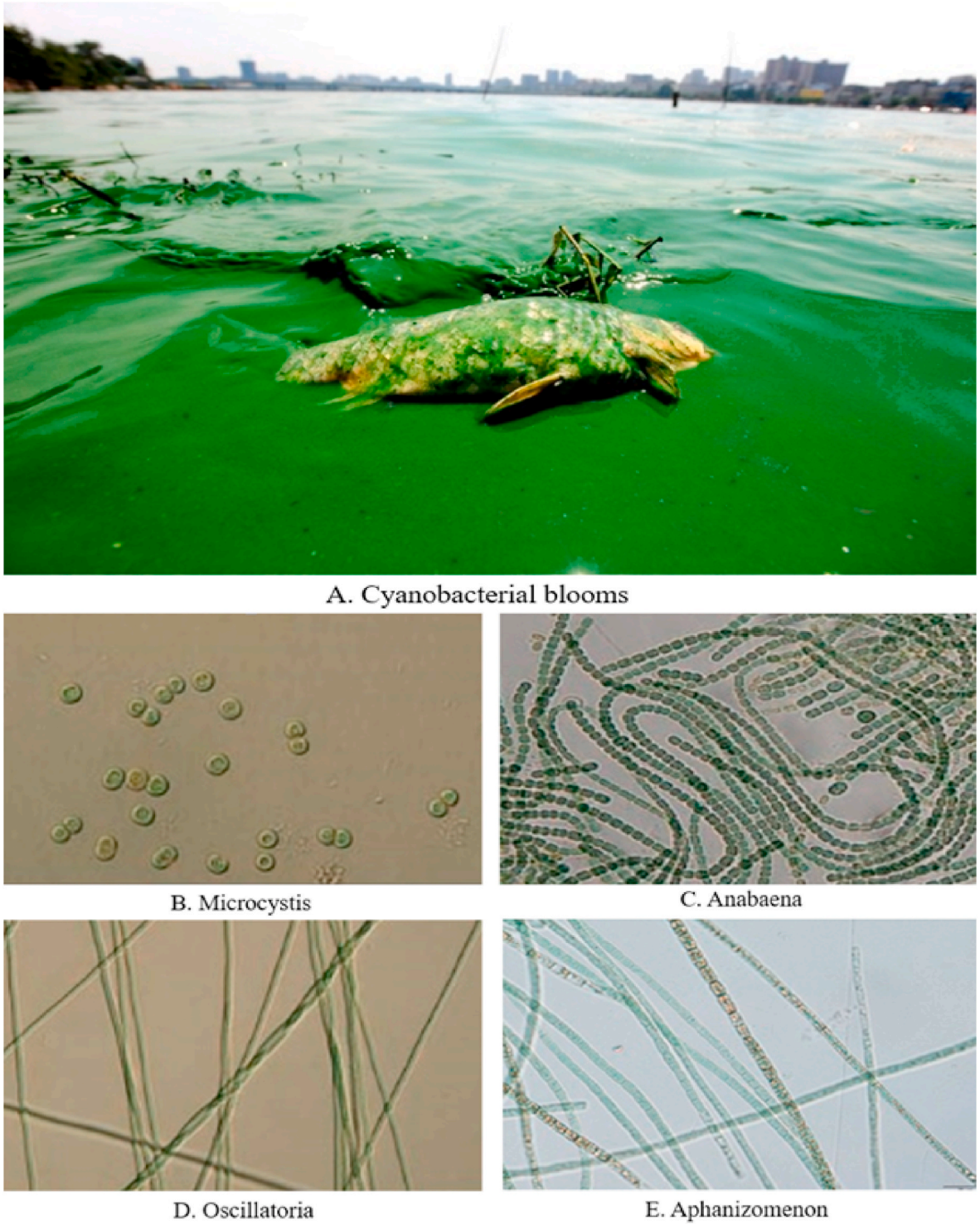

Fig. 1. Cyanobacterial blooms and common cyanobacteria that produce microcystins. (A) Cyanobacterial blooms in East Lake in Wuhan, China (From Yan zhao city network). (B) *Microcystis* is featured by the clustering of cells into a round shape. (C) *Anabaena* is featured by filaments composed of spherical cells. (D) *Oscillatoria* is featured by filaments composed of cylindrical cells that can quiver. (E) *Aphanizomenon* is featured by filaments composed of short columnar cells (From FACHB-Collection).

gonads (Zhao et al., 2018), kidneys (Lin et al., 2016; Yi et al., 2019), heart (Cao et al., 2019b), brain (Abdel-Daim et al., 2019), and intestines (Cao et al., 2019a; Díez-Quijada Jiménez et al., 2020a), etc. Acute exposure to MC-LR can cause liver enlargement, renal dysfunction, acute gastroenteritis, and even death (Bell and Codd, 1994). Furthermore, MC-LR also has potential carcinogenicity and developmental toxicity. Epidemiological and experimental studies have shown that MC-LR exposure may be related to a higher incidence of liver and colorectal cancer (Martínez Hernández et al., 2009; Ueno et al., 1996). Thus, MC-LR has been classified as a possible human carcinogen (Group 2 B) by the International Agency for Research on Cancer (IARC, 2010). The World Health Organization also has set up a temporary guidance limit of 1 μg/L MCs for human drinking water (World Health Organization, 2011).

The gonads are considered to be the second most important target organ of MC-LR after the liver (Chen and Xie, 2005). A large number of studies have reported that MC-LR can accumulate in the gonads, causing reproductive toxicity in vertebrates and invertebrates (Chen et al., 2017b; Li et al., 2008b; Zhou et al., 2020a). Recently, the study of reproductive toxicity of MC-LR has become a hot topic. Chen et al. reviewed the toxicity and mechanism of MCs on reproductive system in 2016. They pointed out that MCs can cause reproductive toxicity in mammals, fish, amphibians, and birds. This toxicity was found to result in the initiation of apoptosis, cytoskeletal destruction, DNA damage, and proliferation and tumorigenesis. They also found that the mechanism of this toxicity involved in oxidative stress and the inhibition of protein phosphatases 1 and 2 A (PP1 and PP2A) (Chen et al., 2016a). Undoubtedly, their review provides a thorough understanding of the reproductive toxicity induced by MCs. Meanwhile, they also found several areas within this research to be worth an in-depth study, such as female reproductive toxicity, trans-generational toxicity, and sex hormone disorders. The toxicity mechanism of MC-LR has also been supplemented and improved in recent years. For example, MC-LR was found to induce apoptosis in germ cells through epigenetic modification (Wang et al., 2019). The latest researches showed that MC-LR may pass on the offspring through the maternal placenta, leading to trans-generational toxicity (Chen et al., 2009; Singo et al., 2017; Zhang et al., 2007), and developmental toxicity (Wang et al., 2005; Zhao et al., 2020). A summation of the latest research data is needed for the deeper understanding of the reproductive toxicity effects and mechanisms of MC-LR.

For this purpose, data on MC-LR-induced reproductive toxicity was collected for this paper to review the latest findings. We also put forward some suggestions for future research.

2. Toxicities of microcystin-LR on the reproductive system

Current researches have shown that MC-LR can not only induce reproductive toxicity but also produce trans-generational and developmental toxicity (Fig. 4). MC-LR can damage gonads (testes, ovaries and prostate), reduce sperm quality and interfere with sex hormone homeostasis. MC-LR can also be transmitted to the next generation through the placenta, inducing placental toxicity while causing trans-generational toxicity and developmental toxicity.

2.1. Toxic effects of microcystin-LR on the male reproductive system

This section summarizes the male reproductive toxicity caused by MC-LR from *in vivo* and *in vitro* studies. *In vivo* studies include the toxic effects of MC-LR on the testis, prostate and sperm. *In vitro* studies revealed the toxic effects of MC-LR on spermatogonia, Sertoli cells and other cells. The detailed information is shown in Table 1.

2.1.1. In vivo studies

2.1.1.1. Microcystin-LR-induced testicular damage and sex hormone disorders. Testes play a vital role in the male reproductive system, which produce sperm and androgen. MC-LR has toxic effects on the testes in vertebrates and invertebrates, which can damage testicular tissue and organelles such as mitochondria and endoplasmic reticulum (Yuan et al., 2019; Zhang et al., 2019a). Meanwhile, MC-LR can interfere with the process of reproductive cell apoptosis, proliferation and differentiation, as well as cause inflammation and sex hormone disorders in testes (Chen et al., 2017b; Yuan et al., 2019).

MC-LR can induce testicular toxicities. Acute toxicity studies found that MC-LR altered epigenetic modifications, resulting in testicular tissue damage. After MC-LR was intraperitoneally injected into SD rats at doses of 9, 18 and 36 μg/kg for 14 days, the expression of apoptosis-related genes was increased by raising the levels of trimethylation of histone H3 at lysine 4 (H3K4me3) (Yuan et al., 2019). H3K4me3 is a post-translational histone modification and abnormal H3K4me3 causes epigenetic modification errors (Dawson and Kouzarides, 2012). Under the same exposure conditions, Wang et al. discovered that MC-LR activated mitochondrial apoptotic pathways and disrupted cell cycle pathways by increasing histone deacetylase (HDAC) and reducing histone acetylation (HAC) activity in testicular cells of SD rats, thereby inducing apoptosis (Wang et al., 2019). To sum up, histone methylation and acetylation modifications play roles in cell cycle disorders and apoptosis in the testes, and epigenetic modifications are closely linked to testicular

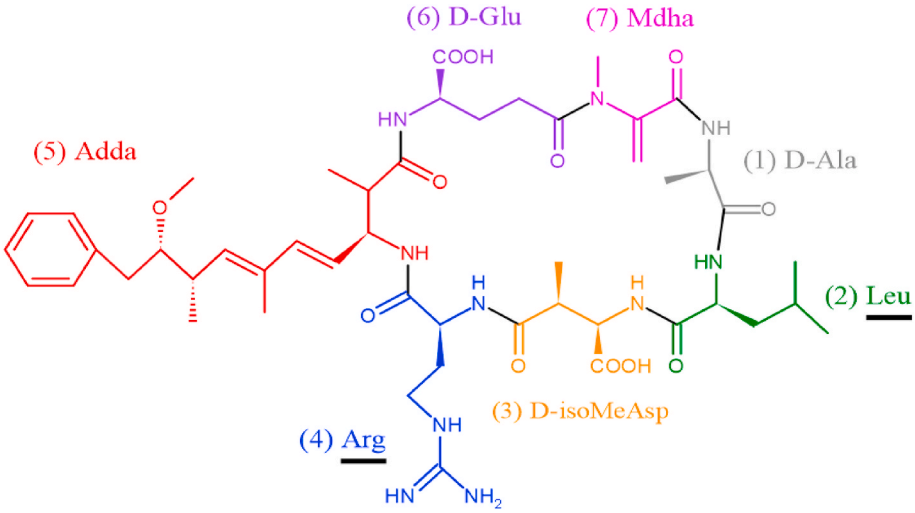

Fig. 2. Chemical structure of microcystin-LR (L and R represent leucine and arginine respectively).

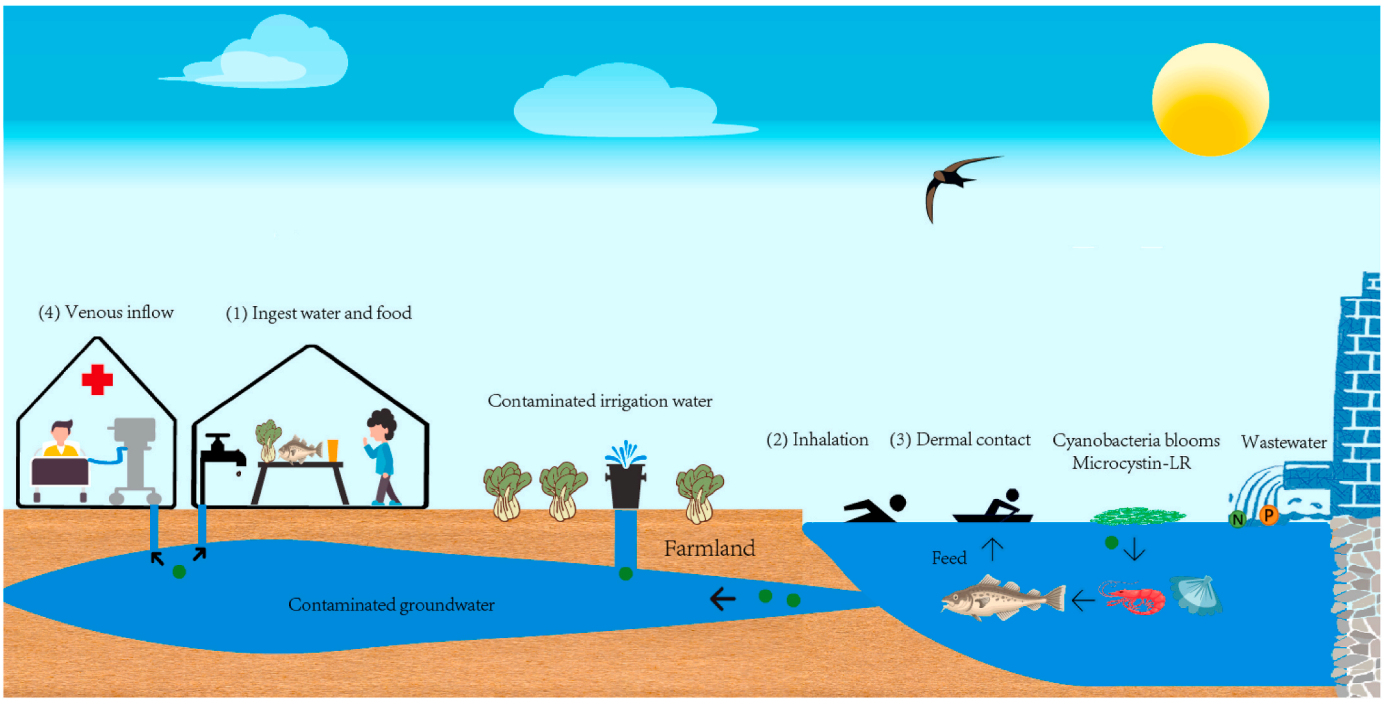

Fig. 3. Pathways of human exposure to microcystin-LR. Wastewater containing nitrogen (N) and phosphorus (P) is discharged into the lake, inducing cyanobacterial blooms. Microcystin-LR produced by cyanobacteria can be exposed to humans in many routes: ingestion of contaminated drinking water, aquatic, terrestrial animals and cyanobacterial dietary supplements, consumption of contaminated vegetables and fruits irrigated with water containing microcystin-LR, inhalation and dermal contact with microcystin-LR during water recreation, and the intravenous route of haemodialysis with contaminated water.

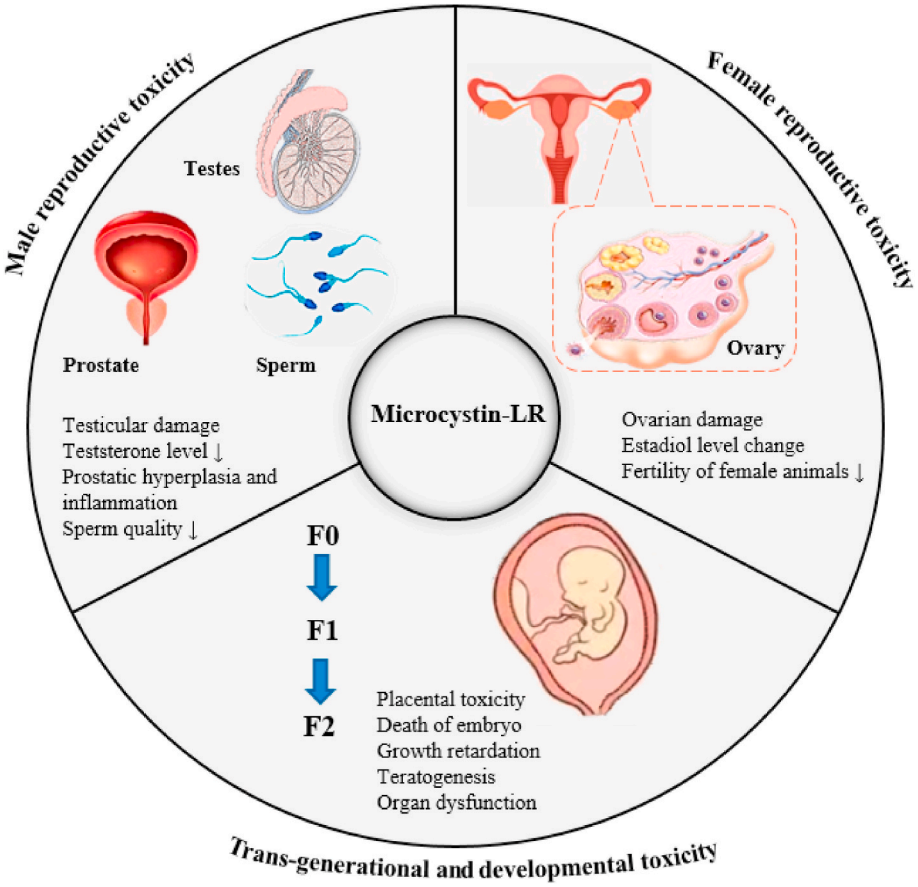

Fig. 4. Schematic diagram of microcystin-LR-induced toxic effects on various reproductive organs.

Table 1
Toxic effects of microcystin-LR on the male reproductive system.

Object	Exposure	Dose (MC-LR)	Time	Toxic effects	Reference
Rats/testis	i.p. *In vivo*	9, 18, 36 μg/kg	14 d	Testicular tissue damage, mitochondrial damage, ROS ↑, H3K4me3 ↑, apoptosis, Bcl-2 ↓, Bax and caspase-3 ↑	Yuan et al. (2019)
Rats/testis	i.p. *In vivo*	9, 18, 36 μg/kg	14 d	HDAC1 ↑, HAT ↓, apoptosis, Ac-H3 and Ac-H4 ↓, Bax, caspase-3 and caspase-8 ↑	Wang et al. (2019)
Rosenbergii/testis	Immersion *In vivo*	0.5, 5 μg/L	1, 2, 3 w	GSI ↓, disrupt testicular development, mitochondrial damage, destruction of cell junctions	Zhang et al. (2019b)
Mice/testis	Orally *In vivo*	1, 10, 100 μg/L	90, 180 d	BTB dysfunction, ZO-1 and Occludin ↓, PI3K/Akt ↑	Zhou et al. (2020b)
Rats	i.p. *In vivo*	30 μg/kg	1, 3, 5, 7, 14 d	GnRH, FSH, LH and testosterone first rise then fall, *Gnrh1* ↓	Wang et al. (2016)
Nile tilapia	Immersion *In vivo*	8.3 μg/L	28 d	Testosteron, E2 ↑, *ghrh*, *pacap*, *gh*, *chr1*, *chr2*, *igf1*, *igf2*, *cyp19a*, *cyp19b*, *20β-hsd* and *17β- hsd 8* altered	Chen et al., 2017a
Mice	Orally *In vivo*	1, 3.2, 10 μg/L	3, 6 m	Testosterone ↓, FSH, LH ↑	Chen et al. (2011)
Zebrafish	Immersion *In vivo*	0.3, 3, 30 μg/L	90 d	Testosterone ↓, E2 ↑, testosteron/E2 ↓	Su et al. (2016)
Mice/prostate	Orally *In vivo*	1, 10, 30 μg/L	90, 180 d	Prostatic hyperplasia and prostatitis, PSA and PAP ↑, TNF-α and IL-6 ↑	Pan et al. (2018)
Elegans/sperm	Immersion *In vivo*	4, 16, 64 μg/L	48 h	Abnormal sperm cell morphology and function, *spe-10*, *spe-15* and *fer-10* ↓	Li et al. (2015)
Mice/sperm	Orally *In vivo*	1, 7.5, 15, 30 μg/L	6 m	Sperm count ↓, the percent of abnormal sperm ↑, miRNA chr13_8977 and piRNA mmu_piR_027558 ↓	Meng et al. (2019)
Spermatogonia	*In vitro*	0.5, 5, 50, 500 nM	6 h	Cell viability and total antioxidant capacity ↓, ROS ↑, MMP ↑, free Ca^{2+} concentration ↑	Zhou et al. (2012)
Rats/Sertoli cells	Primary culture *In vitro*	8, 16, 32 μg/L	24 h	Apoptosis, caspase-9 and caspase-3 ↑, ROS ↑	Huang et al. (2016)
Calves/Sertoli cells	Primary culture *In vitro*	20, 40, 60, 80 μg/L	24 or 120 h	Inflammation, TLR4 and NF-kB ↑, TNF-α, IL-1β, IFN-γ, IL-4, IL-10 and IL-13 ↑, apoptosis, BTB damage	Adegoke et al. (2018)
Leydig cells	*In vitro*	500 nM	1, 3, 6, 12, 24 h	Apoptosis, TNF-α and GAS6 ↑, ROS ↑, p-p38 ↓	Chen et al., 2018
Leydig cells	*In vitro*	1 μM	1, 3, 6, 12 h	Inflammation, TNF-α, IL-6, MCP-1 and CXCL10 ↑, PI3K/Akt/NF-κB activated	Chen et al. (2017b)
Rats/co-cultured Sertoli-germ cells	Primary culture *In vitro*	9, 18, 36 μM	24 h	HDAC1 ↑, HAT ↓, Ac-H3 ↓, Ac-H4 ↓, p21 ↑, cyclinD1, cyclinE1, CDK2 and E2F1 ↓, cycle arrest in S phase, Bax, caspase-3, and caspase-8 ↑, apoptosis	Wang et al. (2019)
Rats/co-cultured Sertol-germ cells	Primary culture *In vitro*	9, 18, 36 μM	24 h	Cytochrome c from mitochondria to cytoplasm, p53 ↑, MMP ↓, apoptosis	Wu, 2019a
RWPE-1 cells	*In vitro*	5, 20, 100, 500 nM	48 h	FOXM1, β-catenin and cyclinD1 ↓, MCP-1, CXCL10, TNF-α and IL-6 ↑	Pan et al. (2018)

Abbreviation: i.p: intraperitoneal, GSI: gonadosomatic index, BTB: blood-testis barrier, MMP: mitochondrial membrane potential, GnRH: gonadotropin-releasing hormone, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: 17β-estradiol.

toxicity caused by MC-LR. Moreover, MC-LR also impairs testicular development of invertebrate. After Macrobrachium rosenbergii were exposed to MC-LR (0.5 and 5 μg/L) for three weeks, a decrease in testicular weight and gonadosmatic index (GSI) was found. It was observed that MC-LR entered rosenbergii testis, and damaged mitochondria and cell junctions in germ cells (Zhang et al., 2019b). Also, MC-LR could cause zebrafish gonad retardation. Hou et al. proved that MC-LR delays gonad maturation by disrupting the growth hormone/insulin-like growth factor system of zebrafish, and the gonadal development of males are more vulnerable than that of females to MC-LR (Hou et al., 2017).

In a chronic toxicity study (Zhou et al., 2020b), male mice were orally exposed to 1, 10 or 100 μg/L MC-LR for 90 or 180 days. Under these conditions, MC-LR caused the degradation of proteins associated with tight junctions and gap junctions, thereby destroying the blood-testis barrier (BTB) (Zhou et al. 2019b, 2020b). Furthermore, chronic exposure to MC-LR could also induce immune response and inflammation in the testis, with infiltration of macrophages. The main features of this were the increase of proinflammatory cytokines in the testis such as tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) (Chen et al. 2016b, Chen et al., 2017b).

In recent years, researchers conducted in-depth studies on the effect of MC-LR on sex hormone disorders in male animals and found that MC-LR could affect the level of sex hormones in mammals (Ding et al., 2018; Wang et al., 2016), fish (Chen et al., 2017a; Liu et al., 2016), amphibians (Jia et al., 2018) and invertebrates (Zhang et al., 2019a). After rats were injected intraperitoneally with MC-LR (30 μg/kg) for different duration (1, 3, 5, 7 and 14 days), it was observed that MC-LR increased and subsequently decreased the serum levels of gonadotropin-releasing hormone (GnRH), serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. Interestingly, *Gnrh1* expression was steadily decreased (Wang et al., 2016). These results suggested that MC-LR causes a disturbance of related hormones in the hypothalamus-pituitary-gonadal (HPG) axis. After treating Nile tilapia with MC-LR (8.3 μg/L) for 28 days, the levels of 17β-estradiol (E2) and testosterone were significantly up-regulated, and transcripts of genes (*ghrh, pacap*, *gh*, *ghr1*, *cyp19a, 3β-hsd1*, etc.) in the hypothalamus-pituitary-gonadal-liver (HPGL) axis were significantly altered (Chen et al., 2017a). MC-LR also caused sex hormone disorders in amphibians and invertebrates. Exposing rana nigromaculata and rosenbergii to MC-LR significantly decreased testosterone level and increased the level of E2 (Jia et al., 2018; Zhang et al., 2019b). In summary, MC-LR changed the sex hormone levels of male animals, which may be related to HPG and HPGL axes adjustment.

After chronic low-dose exposure of MC-LR (10 μg/L), testosterone level in male mice decreased, but LH and FSH levels increased (Chen et al., 2011). This suggests that low-dose exposure of MC-LR may change the level of testosterone, FSH and LH through HPG axis feedback regulation. However, high-dose exposure of MC-LR may disrupt the adjustment of the HPG axis, resulting in decreased levels of testosterone, FSH, and LH. Another study found that MC-LR promoted the conversion of testosterone to E2 in the blood and caused the testosterone/E2 ratio to decrease in male zebrafish (Su et al., 2016). In conclusion, MC-LR can affect the level and biotransformation of sex hormones, and alter the transcription of HPG and HPGL axes related genes. Interestingly, there are some differences in MC-LR-induced changes in sex hormone levels in different animal models. One possible explanation is the differences between species, and the method and concentration of their exposure.

2.1.1.2. Toxic effects of microcystin-LR on the prostate. The prostate is an important accessory gland in male animals. It has the function of secreting prostate fluid which can nourish and protect sperm. Male animals may suffer from prostatic hyperplasia and prostatic inflammation after long-term exposure of MC-LR. After mice were given drinking water containing 1, 10, 20 or 30 μg/L MC-LR for 90 or 180 days, mouse body weight and prostate index were decreased. MC-LR can be transported into the prostate tissues causing focal hyperplasia of the prostate, which is manifested as epithelial hyperplasia and interstitial hyperplasia. Furthermore, MC-LR was shown to increase prostate specific antigen (PSA), prostate acid phosphatase (PAP) and pro-inflammatory cytokines such as TNF-α and interleukin-6 (IL-6) in serum and prostate tissue (Pan et al., 2018). In the serum of healthy individuals, low levels of PSA and PAP can be detected, but elevated levels of PSA can be detected in people with prostate-related diseases (Elzanaty et al., 2016). This suggests that MC-LR may increase the risk of prostate disease, especially prostate inflammation. The exposure of MC-LR during the perinatal period also interfered with the prostate development of male offspring (Han et al., 2019; Zhang et al., 2017), indicating that MC-LR is not only toxic to the parental prostate, but also to the prostate of the offspring.

2.1.1.3. Toxic effects of microcystin-LR on sperm. MC-LR damages sperm in mammals (Chen et al., 2017b; Li et al., 2008b), fish (Su et al., 2016), amphibians (Jia et al., 2014; Zhang et al., 2013) and nematodes (Li et al., 2015). It has been observed in acute and chronic toxicity studies that MC-LR decreases sperm quantity, motility and vitality (Chen et al., 2017b; Li et al., 2008b). The fertility of zebrafish is affected by MC-LR, as the number of mature sperm is significantly reduced after long-term exposure to the poison (Su et al., 2016). In addition, studies also found that exposure to cyanobacteria reduced sperm motility of mussels, attesting that cyanobacteria also have an impact on the reproductive system in shellfish (Boegehold et al., 2019; Díez-Quijada Jiménez et al., 2020b). However, it cannot be confirmed whether MC-LR can cause a decrease in shellfish sperm quality.

MC-LR caused abnormal sperm morphology, abnormal sperm behavior, and suppression of sperm activation in *Caenorhabditis elegans* (Li et al., 2015). Defective sperm functions may be related to the reduced expression of *spe-10*, *spe-15* and *fer-1* genes which are involved in spermatogenesis (Gleason et al., 2006; Krajacic et al., 2009; Li et al., 2015). Also, high-throughput sequencing results revealed that MC-LR-induced abnormal spermatogenesis is related to the abnormal expression of some non-coding RNAs (ncRNAs) such as microRNAs (miRNAs), piwi-associated RNAs (piRNAs), covalently closed circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) (Meng et al., 2019). Specifically, the expression of chr13_8977 and mmu_piR_027558 in mice testis tissue decreased after exposure to MC-LR. Genetic ontology analysis found that these two types of ncRNA regulated germ cell apoptosis and sperm quality (Meng et al., 2019). Therefore, chr13_8977 and mmu_piR_027558 are involved in the MC-LR-induced sperm quality decline. Furthermore, some researchers also found that mmu_piR_003399 and miR-96 may be the target genes regulating sperm (Zhang et al., 2018; Zhou et al., 2014). In a word, MC-LR reduces sperm quality by inducing abnormal spermatogenesis, and ncRNAs regulation, especially piRNAs and miRNAs, which play a significant role in regulating spermatogenesis.

2.1.2. In vitro studies

2.1.2.1. Toxic effects of microcystin-LR on spermatogonia. Spermatogonia are primordial germ cells and eventually develop into sperm cells. MC-LR can be transported into spermatogonia through organic anion-transporting polypeptides (OATP), resulting in the decrease of cell activity, oxidative stress damage, and apoptosis (Zhou et al., 2012). Previous studies have reported that miR-96 is involved in MC-LR-induced spermatogonia toxicity (Zhou et al., 2014). In recent years, it has been discovered that MC-LR led to a high expression of miR-541 in GC-1 cells (derived from mouse spermatogonia). Overexpression of miR-541 can promote apoptosis by targeting p15 and downstream apoptosis-related proteins such as p53 and Bax (Meng et al., 2016). p15 is a member of the INK4 family of cyclin-dependent kinase inhibitors and mediates signals essential for maintaining cell cycle and apoptosis (Leeper et al., 2013; Zhang et al., 2016a). Zhang et al. have made similar findings that up-regulation of mmu_piR_003399 triggered G1 phase cell cycle arrest by reducing the expression of cyclin-dependent kinase 6 after GC-1 cells were exposed to MC-LR (Zhang et al., 2018). This suggests that MC-LR induces abnormal expression of ncRNAs, which can target cell cycle-related proteins and lead to spermatogonia toxicity. And, MC-LR-induced cell cycle disorder may cause apoptosis.

2.1.2.2. Toxic effects of microcystin-LR on Sertoli cells. Sertoli cells provide protection and nutrition for the germ cells. They protect the germ cells by creating a BTB and secreting immunomodulatory factors. It has been reported that MC-LR can enter Sertoli cells and induce apoptosis and autophagy (Chen et al. 2013, 2016b; Huang et al., 2016). After Sertoli cells were exposed to MC-LR (8, 16 and 32 μg/mL), it was found that reactive oxygen species (ROS) levels increased, which in turn triggered cell apoptosis through the mitochondrial caspase-dependent pathway (Huang et al., 2016). Chen et al. had a similar finding that MC-LR can promote caspase-3 protein expression and incur TNF-α to interact with tumor necrosis factor receptor 1 in Sertoli cells, thereby inducing apoptosis. To explore whether MC-LR can pass the BTB, they injected biotin tracer into the dissected testicular tissue. It was discovered that after the testicular tissue was exposed to MC-LR, the biotin tracer diffused through the BTB into the seminiferous tubules, confirming that MC-LR can destroy the BTB and enter the germ cells to exert toxicity (Chen et al., 2016b).

Exposure to MC-LR can bring about inflammation in Sertoli cells. After exposure of 40, 60 and 80 μg/L MC-LR for 24 or 120 h, the inflammatory response was shown to be activated through the toll-like receptor 4/nuclear factor kappa B (TLR4/NF-kB) signaling pathway in Sertoli cells (Adegoke et al., 2018). Exposure to MC-LR has been shown to up-regulate the expression of pro-inflammatory cytokines such as TNF-α, interleukin-1β (IL-1β) and interferon-γ (IFN-γ) and anti-inflammatory cytokines such as interleukin-4 (IL-4), interleukin-10 (IL-10) and interleukin-13 (IL-13) (Adegoke et al., 2018). NF-κB is a key transcription factor in the TLR4 signaling pathway, and also participates in the immune response (Wu et al., 2018). Therefore, TLR4 and NF-κB are both involved in the MC-LR-induced inflammation in Sertoli cells. In addition, some investigators believe that the inflammatory response may lead to the apoptosis of Sertoli cells, and the inflammation also seems to be related to BTB damage (Adegoke et al., 2018). In summation, MC-LR did cause an inflammatory response, the destruction of BTB and apoptosis in Sertoli cells. However, the link between MC-LR-induced inflammation and apoptosis remains controversial and needs further investigation.

2.1.2.3. Toxic effects of microcystin-LR on Leydig cells. Leydig cells are distributed in the loose interstitial tissue of seminiferous tubules, which are the main sites of androgen production. Although MC-LR cannot enter Leydig cells (Wang et al., 2013), it can still exert toxic effects in other ways (Chen et al., 2018b). After the exposure of 500 nM MC-LR to co-cultured Leydig cells and macrophages for 24 h, the number of Leydig cells was significantly decreased but the number of macrophages was increased. Studies on the mechanism of Leydig cell reduction showed that MC-LR can stimulate macrophages to secrete TNF-α and growth arrest-specific 6 (Gas6). TNF-α can result in Leydig cells apoptosis by activating the ROS-p38 mitogen-activated protein kinase (p38MAPK) signaling pathway, while Gas6 can promote macrophages to engulf Leydig cells (Chen et al., 2018b). This manifests that the reduction of Leydig cells is due to apoptosis and macrophage phagocytosis of Leydig cells.

Exposing Leydig cells to MC-LR has been shown to reduce testosterone production by inducing apoptosis and the negative feedback regulation of HPG axis (Chen et al., 2018b; Wang et al., 2016). Furthermore, MC-LR was shown to produce an innate immune response in Leydig cells which was caused by increased stimulation of toll-like receptor 2 (TLR2), NF-κB, proinflammatory cytokines and chemokines (Chen et al., 2017b). Previous research also found MC-LR could reduce cell viability, induce oxidative stress and increase the proportion of apoptotic DNA fragmentation and necrotic cells (Li et al., 2008a). However, the mechanism of this toxic effect is still not clear.

2.1.2.4. Toxic effects of microcystin-LR on co-cultured Sertoli-germ cells. Primary co-cultured Sertoli-germ cells are more adaptable to the *in vitro* environment than Sertoli or germ cells cultured alone. It has become an important model for toxicology research. Cell cycle disorder occurs in primary co-cultured Sertoli-germ cells after exposure to MC-LR. Experimental evidence has shown that MC-LR induces cell cycle arrest in the S phase and alters the expression of cell cycle-related proteins, including p21, cyclinD1, cyclinE1, cyclin-dependent kinase 2 (CDK2) and E2F transcription factor 1 (E2F1). Furthermore, apoptosis was found to occur in Sertoli-germ cells after treatment with MC-LR (Wang et al., 2019). On the one hand, MC-LR activated p53-dependent pathway-associated proteins leading to a decrease in mitochondrial membrane potential (indicating the opening of mitochondrial permeability transition pore and the release of cytochrome c from the mitochondria into the cytoplasm) and eventually the induction of apoptosis (Wu, 2019a). On the other hand, epigenetic modifications also participated in apoptosis by reducing HDAC activity and increasing the expression of histones H3, H4, and H3K4me3 (Wang et al., 2019; Yuan et al., 2019). Previous studies found that the expressions of p53 and its regulatory genes are affected by H3K4me3 (Lauberth et al., 2013; Mehta et al., 2015). Therefore, H3K4me3 may lead to apoptosis by affecting the expression of p53.

2.1.2.5. Toxic effects of microcystin-LR on human prostate epithelial cells. Human prostate epithelial (RWPE-1) cells have many characteristics of prostatic epithelial cells and have been widely used as a replacement for them. To explore the mechanism of MC-LR-induced prostate hyperplasia and inflammation, Pan et al. exposed RWPE-1 cells to concentrations of MC-LR (5, 20, 100, 500 nM) for 48 h. The results showed that MC-LR up-regulated the expression of cyclinD1 (a vital marker of cell proliferation) by FOXM1/Wnt/β-catenin signaling pathways, promoted the proliferation of prostate epithelial cells, and then resulted in prostatic hyperplasia (Pan et al., 2018). Forkhead box M1 (FOXM1) plays a crucial role in promoting the proliferation and invasion of prostate cancer (Nandi et al., 2018). When the prostate is hyper-proliferated, the prostate is likely to become cancerous, which means that MC-LR increases the risk of cancer. Furthermore, MC-LR also up-regulated MCP-1, C-X-C motif ligand (CXCL) 10 (CXCL10), TNF-α and IL-6 in RWPE-1 cells through the NF-κB pathway, thereby inducing inflammation (Pan et al., 2018).

2.2. Toxic effects of microcystin-LR on female reproductive system

MC-LR has been shown to damage ovarian tissue and interfere with estrogen secretion. Long-term exposure to MC-LR affects oocyte maturation and reduces the fertility of females. In addition, MC-LR causes oxidative stress and autophagy in the ovary as well as incurs apoptosis through the mitochondrial and endoplasmic reticulum pathways. The specific research is summarized in Table 2.

2.2.1. In vivo studies

The ovary is a vital reproductive organ of female animals, which can produce eggs and steroid sex hormones. Acute exposure to MC-LR has been shown to cause pathological damage in zebrafish ovaries and to trigger oxidative stress and endoplasmic reticulum (ER) stress (Zhan et al., 2020a; Zhang et al., 2020). MC-LR increased ROS and malondialdehyde concentration as well as the enzymatic activities and transcriptional levels of antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase (Hou et al., 2014; Liu et al., 2018b). In

Table 2
Toxic effects of microcystin-LR on the female reproductive system.

Object	Exposure	Dose (MC-LR)	Time	Toxic effects	Reference
Danio/Ovary	i.p. *In vivo*	50, 200 μg/kg	1, 3, 12, 24, 48, 168 h	Pathological damage, MDA, CAT, SOD and GPX ↑, oxidative stress	Hou et al. (2014)
Zebrafish/Ovary	Immersion *In vivo*	1, 5, 20 μg/L	30 d	Pathological damage, eggs ↓, GVBD ↑, cAMP and VTG ↓, promotes oocyte maturation	Zhan et al. (2020b)
Mice/Ovary	Orally *In vivo*	1, 10, 40 μg/L	3, 6 m	GSI ↓, follicular atresia, developmental follicles ↓, decline in female fertility	Wu et al. (2015)
Zebrafish	Immersion *In vivo*	1, 5, 20 μg/L	30 d	E2, 11-KT, testosterone and FSH ↑, transcriptional changes of 22 genes of the HPG axis	Liu et al. (2016)
Zebrafish/Oocytes	Primary culture *In vitro*	1, 10, 100 μg/L	2, 4 h	PP2A activity ↓, MPF activity ↑, ERK, p38 and JNK ↑, GVBD rates changed, cyclinB ↑	Liu et al. (2019)
KK-1 cells	*In vitro*	8.5, 17, 34 μg/mL	24 h	Pathological damage, ROS ↑, ER stress and autophagy, apoptosis	Liu et al. (2018b)
Mice/Granulosa cells	Primary culture *In vitro*	5 μM	48 h	miRNAs and mRNAs participated in apoptosis, formation of cancer, proliferation and production of hormones	Li et al. (2017)
CHO cells	*In vitro*	2.5, 5, 10 μM	24 h	ROS ↑, intracellular Ca^{2+} release, autophagic vacuoles ↑, ER stress (GRP78, ATF-6, PERK, IRE1, CHOP ↑) and autophagy (Beclin1 and LC3-II ↑, LC3-I ↓)	Zhang et al. (2016b)

Abbreviation: i.p: intraperitoneal, MDA: malondialdehyde, CAT: catalase, SOD: superoxide dismutase GPX: glutathione peroxidase, GVBD: germinal vesicle breakdown, VTG: vitellogenin, ROS: reactive oxygen species, GSI: gonadosomatic index, ER: endoplasmic reticulum, MMP: mitochondrial membrane potential, FSH: follicle stimulating hormone, E2: 17β-estradiol, 11-KT: 11-ketotestosterone, CHO: Chinese hamster ovary.

addition, glutathione (GSH) concentration was reduced after treatment with MC-LR (Hou et al., 2014). GSH is an important antioxidant, which is involved in many cellular functions, such as ROS clearance and detoxification of xenobiotics. When GSH reacts with ROS, GSH will be consumed through the binding of glutathione S-transferases (GST) or the formation of glutathione disulfide. Interestingly, after discontinuing MC-LR exposure, ovarian tissue damage and antioxidant indicators eventually recovered (Hou et al., 2014). Therefore, the decrease of GSH content after MC-LR exposure indicates that the ovarian antioxidant system may be able to overcome the damage of MC-LR in the ovary.

In a subchronic study, adult female zebrafish were exposed to MC-LR (1, 5, 20 μg/L) for 30 days. The results showed that MC-LR can cause pathological damage in fish ovaries, which is mainly characterized by enlarged intercellular spaces, detachment of follicular cells from oocytes, and vacuolation of parenchymal tissues. Moreover, MC-LR decreased the number of eggs, allowed fertilized eggs to hatch in advance, and increased the abnormal rate of offspring (Zhan et al., 2020b). Long-term exposure to environmental levels of MC-LR may stimulate follicular atresia and reduce developmental follicles, accompanied by a decrease in GSI, which ultimately leads to a decline in female fertility (Wu et al., 2015). In acute and chronic toxicity studies, MC-LR damaged the structure and function of ovaries, and triggered oxidative stress which led to apoptosis, and ultimately, to a decrease in the fertility of female animals. But this damage seems to be reversible within a period of time, which suggests that females have a strong ability to overcome MC-LR toxicity.

MC-LR has been shown to disturb hormone levels in female animals. After exposing female zebrafish to MC-LR (1, 5, and 20 μg/L) for 30 days, serum levels of E2, testosterone, 11-ketone testosterone (11-KT) and FSH were all elevated, indicating that MC-LR caused an imbalance in sex hormones. At the same time, MC-LR was shown to induce changes in the expression of HPG axis-related genes such as *gnrh2*, *gnrhr3*, *ar*, *lhr*, *hmgra*, *hmgrb* and *cyp19a*, showing that MC-LR can affect the HPG axis regulation (Liu et al., 2016). Chronic exposure of MC-LR at environmental levels decreased serum E2 and increased progesterone (Wu et al., 2015). In addition to the HPG axis, MC-LR was also shown to affect sex hormone levels through HPGL axis regulation in female animals. Qiao et al. showed that in female fish, E2 concentration was significantly reduced in the ovaries, while changes in *vtg1* transcription levels in the liver and overall vitellogenin (VTG) levels were observed (Qiao et al., 2013). VTG is a yolk precursor protein in the liver and is essential for yolk development and maturation of oocytes (Tata and Smith, 1979). All in all, hormone levels have a very important relationship with the regulation of the neuroendocrine system, especially the HPG axis and HPGL axis regulation.

2.2.2. *In vitro studies*

2.2.2.1. Toxic effects of microcystin-LR on oocytes. Oocytes participate in oogenesis and become ovum after meiosis. When MC-LR is present in oocytes, it produces reproductive toxicity to females by affecting oocyte development (Liu et al., 2019; Qiao et al., 2019). Liu et al. showed that when oocytes were isolated from zebrafish *in vitro* and treated with 10 or 100 μg/L MC-LR for 4 h, MC-LR triggered hyperphosphorylation of the MAPK pathway by inhibiting PP2A activity, and led to an early occurrence of oocyte germinal vesicle breakdown (GVBD) (Liu et al., 2019). The maturation of fish oocytes is characterized by the recovery of meiosis and GVBD is often used to morphologically determine the endpoint of oocyte maturation progression (Clelland and Peng, 2009; Maskey et al., 2019). Therefore, these results indicate that the PP2A/-MAPK pathway is involved in the process by which MC-LR interferes with oocyte maturation. In exploring the mechanism of MC-LR on oocyte maturation, some studies also showed that MC-LR could reduce the content of cyclic adenosine monophosphate (cAMP) in oocytes, increase the phosphorylation of extracellular signal-regulated kinase (ERK) and maturation promoting factor (MPF) proteins, and ultimately promote oocyte maturation (Zhan et al., 2020b). ERK is activated by c-mos (proto-oncoprotein kinase) during the maturation of oocytes in mice (Verlhac et al., 1996). In most teleost fish, a sharp drop of cyclic adenosine monophosphate (cAMP) levels in oocytes could activate protein kinase A (PKA), which further triggers the ERK pathway and ultimately promotes oocyte maturation (Lazar et al., 2002). In addition, MPF is a complex of cdc 2 and cyclinB, and its activation is essential for the maturation of most bony fish oocytes (Kondo et al., 1997; Masakane, 2000). This also explains why MC-LR promotes oocyte maturation by activating the ERK/MPF pathway in female zebrafish.

2.2.2.2. Toxic effects of microcystin-LR on ovarian granulosa cells and Chinese hamster ovary cells. Granular cells are one of the main functional cells in the follicles and play an important role in maintaining the normal growth and development of oocytes. MC-LR can enter ovarian granulosa cells, and cause ER stress, autophagy and apoptosis through oxidative stress (Liu et al., 2018b). Liu et al. observed that MC-LR damaged the mitochondria and endoplasmic reticulum, and promoted apoptosis in KK-1 cells (murine ovarian granular cells). Furthermore, it was found that antioxidant N-acetylcarnosine (NAC) pretreatment inhibited the expression of ER stress-related protein protein kinase R-like ER kinase (PERK) and autophagy-related proteins X-box binding protein 1 (XBP-1) and Beclin1 (Liu et al., 2018b), indicating that oxidative stress may mediate MC-LR-induced ER stress and autophagy in KK-1 cells.

Li et al. treated ovarian granulosa cells with MC-LR for 48 h and examined miRNA and mRNA expression in mice (Li et al., 2017). The results found that significantly altered miRNAs and mRNAs were mainly linked to proliferation, apoptosis, immunity and metabolism. Through Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, differentially expressed miRNAs and mRNAs were mainly involved in apoptosis, cancer formation, proliferation, and hormone production. In a word, MC-LR affects gene expression in mouse granulosa cells by disturbing miRNA and mRNA, thereby interfering with the normal function of mouse granulosa cells (Li et al., 2017).

MC-LR can cause toxicity in Chinese hamster ovary (CHO) cells including cytoskeletal destruction, apoptosis and necrosis, cell cycle inhibition and DNA damage (Gácsi et al., 2009; Zhang et al., 2016b). Xue et al. found that NAC could effectively alleviate oxidative damage and apoptosis caused by MC-LR (Xue et al., 2015), indicating that oxidative stress mediates MC-LR-induced apoptosis in CHO cells. MC-LR also increased the expression of ER stress-related proteins glucose related protein 78 (GRP78), activating transcription factor 6 (ATF6), PERK, inositol requiring enzyme 1 (IRE1), C/EBP homologous protein (CHOP) as well as autophagy proteins Beclin1 and microtubule-associated protein 1 light chain 3-II (LC3-II), meanwhile decreased the expression of microtubule-associated protein 1 light chain 3-I (LC3-I). And ER stress inhibitor can effectively reduce the levels of apoptosis and autophagy caused by MC-LR (Zhang et al., 2016b). This shows that ER stress plays a crucial role in MC-LR-induced apoptosis and autophagy in CHO cells. Furthermore, MC-LR was found to trigger excessive ROS production and intracellular calcium release. The ER is the main storage place for calcium ion (Ca^{2+}), ER stress may have a certain relationship with the increase of intracellular Ca^{2+}.

In brief, MC-LR can cause toxicity of ovarian granulosa cells and CHO cells. Oxidative stress is closely related to MC-LR-induced ER stress, autophagy and apoptosis.

2.3. *Trans-generational and developmental toxic effects of microcystin-LR*

In addition to affecting the female and male reproductive organs, the latest evidence shows that MC-LR can also be transmitted to the offspring through the parent, causing trans-generational toxicity. MC-LR

Table 3
Trans-generational and developmental toxicity induced by microcystin-LR.

Object	Exposure	Dose	Time	Toxic effects	Reference
Daphnia	Immersion	1 μg/L Microcystis extracts	3 generations	Delayed maturation and reproduction of the daphnia in F1 and F2	Dao et al. (2018)
Zebrafish	Immersion	1, 5, 20 μg/L MC-LR	30 d	Body weight and body length in F1 ↓, liver damage, immune function, growth-related genes (*gh*, *ghra*, *ghrb*, *igf1*, *igf1ra* and *igf1rb* ↓)	Liu et al. (2014)
Loach juveniles	Immersion	1, 3, 10, 100, 1000 μg/L MC-LR	7 d	Developmental abnormalities: tubular heart, bradycardia, poor yolk resumption, small head, curved body and tail, and abnormal hatching	Liu et al. (2002)
Pregnant mice	Orally	1, 10, 50 μg/L MC-LR	GD12-21 d after birth	AGD and prostate index ↓, interfere with the development of the prostate in the offspring	Zhang et al. (2017)
Zebrafish	Immersion	1, 5, 20 μg/L MC-LR	30 d	Deformity: spinal curvature, decreased pigmentation, and pericardial swelling in the F1	Zhan et al. (2020b)
Zebrafish embryos	Immersion	0.2, 0.5, 2, 5 mg/L MC-LR	96 h	Malformation, growth delay heart rates ↓, ROS ↑, apoptosis, Bcl-2 ↓, p53 and Bax ↑	Zeng et al. (2014)
Zebrafish	Immersion	1, 5, 25 μg/L MC-LR	60 d	MC-LR in the gonad of F0 and F1 embryos, disturbing the neurotransmitter systems and neuronal development in F0	Wu et al. (2017)
Zebrafish larvae	Immersion	4 μM MC-LR	96 h	Malformation, growth delay, heart rates ↓, ER stress, caspase-8, caspase-3, MAPK8 and Bax ↑, Bcl-2 ↓, apoptosis	Qi et al. (2016)
Pregnant mice	i.p.	5, 20 μg/kg MC-LR	GD13-GD17	Fetal weight and placental weight ↓, inhibition of proliferation and induce apoptosis in placenta, ROS ↑, ER stress	Zhao et al. (2020)

Abbreviation: i.p: intraperitoneal, ROS: reactive oxygen species, ER: endoplasmic reticulum, AGD: anal-genital distance, GD: gestational day.

can adversely affect the liver, heart, brain and other organs of the offspring (Table 3). MC-LR was found in the yolks and egg whites of birds, Nile crocodile eggs and progeny snails suggesting that it has trans-generation toxicity (Chen et al., 2009; Singo et al., 2017; Zhang et al., 2007). It is also worth noting that MC-LR not only interferes with the development of F1 generation but also affects the development of F2 generation. Daphnia was exposed to MC-LR (1 μg/L) for three consecutive generations. Although there were only some weak negative effects of the toxins on the first generation (F0), MC-LR had a strong direct, cumulative and carrying effect on the life history traits in F1 and F2 of daphnia, including the reduction of survival and reproduction ability (Dao et al., 2018).

Developmental toxicity refers to the harmful effects of offspring caused by parental exposure to exogenous physical and chemical factors before offspring birth. Parental MC-LR exposure could cause offspring and embryo malformation and growth retardation, damage to organ structure and function, and lead to the death of offspring in a dose and time-dependent manner (Wang et al., 2005; Zeng et al., 2014; Zhan et al., 2020b). Parental zebrafish were continuously exposed to MC-LR (1, 5 and 20 μg/L) for 30 days, and then the fertilized eggs were collected and the following F1 generation was reared in water without MC-LR until 60 days post fertilization. The results found that body weight and body length of F1 progeny decreased significantly and the expression of growth-related genes (*gh*, *ghra*, *ghrb*, *igf1*, *igf1ra* and *igf1rb*) decreased (Liu et al., 2014). In addition, parental exposure to MC-LR can cause liver structural damage in the offspring as well as prostate necrosis, hyperplasia, inflammation, and fibrosis damage (Han et al., 2019; Liu et al., 2014; Zhang et al., 2017). MC-LR has also been shown to affect loach embryo-larval and juvenile development such as pericardial edema and tubular heart, bradycardia, homeostasis, poor yolk resumption, small head, curved body and tail, and abnormal hatching. And, loach embryos were found to be more sensitive to this effect at a later stage of development (Liu et al., 2002). It is newly found that paternal exposure to MC-LR can cause pulmonary abnormalities in the offspring mice (Meng et al., 2020).

To explore the toxic mechanism of MC-LR on embryos, Li et al. examined the protein expression profile of zebrafish embryos after exposure to MC-LR. It was found that the altered proteins were mainly related to oxidative stress, energy metabolism, and cytoskeletal assembly (Li et al., 2011). Zeng et al. explored the role of apoptosis in MC-LR-induced developmental toxicity in zebrafish embryos. They found that the production of ROS was increased by MC-LR, thereby triggering apoptosis through the p53-Bax-Bcl-2 pathway and caspase-dependent apoptosis pathway in developing zebrafish embryos (Zeng et al., 2014). Moreover, Wu et al. considered that MC-LR could induce developmental neurotoxicity by interfering with the neurotransmitter system and the development of neurons of F1 zebrafish larvae (Wu et al., 2017). Some researchers also believed that the developmental toxicity caused by MC-LR may be attributed to diseases of the cholinergic system, dopaminergic signaling and neuronal development (Qian et al., 2018; Wu et al., 2016).

The placenta is an important organ connecting the fetus with the mother, and it is especially crucial to the development of the fetus. MC-LR has been shown to cause abnormal embryonic development, fetal morphological abnormalities, organ dysfunction and even death (Qi et al., 2016; Qian et al., 2018; Wu et al., 2016; Zhang et al., 2007). The placenta, as a material transmission channel, may be damaged by MC-LR. Zhao et al. explored the role of the placenta in MC-LR-induced growth and development disorders of offspring. Pregnant mice were intraperitoneally injected with MC-LR (5 or 20 μg/kg) from gestational day 13–17. It was found that maternal MC-LR exposure can cause fetal weight loss and inhibit placental development via the dysregulation of placental transport, placental dysplasia, suppression of proliferation and apoptosis induction in placenta of mice. And one possible mechanism is that ROS-mediated placental ER stress and impairment in placental structure and function are important factors in fetal weight loss (Zhao et al., 2020). In summary, MC-LR can be transmitted from maternal body to the offspring through the placenta. While placental function is adversely affected by MC-LR, the liver, brain, heart, prostate, and other organs of the offspring may also be damaged. These evidences fully demonstrate the trans-generational and developmental toxicity of MC-LR.

3. Some new potential mechanisms for reproductive toxicity of microcystin-LR

Through summarizing the discovered mechanisms in recent years, a mechanism diagram of MC-LR-induced reproductive toxicity is obtained (Fig. 5). In the reproductive system, MC-LR has been shown to enter cells through OATP to exert toxicity. On the one hand, MC-LR can induce the hyperphosphorylation of certain proteins by inhibiting PP1/2 A activity, resulting in BTB damage, inflammatory response, cell proliferation, and even tumorigenesis. On the other hand, MC-LR can trigger oxidative stress to cause autophagy and apoptosis. It was proved that mitochondrial damage, ER stress and epigenetic modifications are involved in MC-LR-induced apoptosis, but the regulatory mechanism of epigenetic modifications remains unclear. In addition, previous studies have elucidated the mechanism of MC-LR-induced cell proliferation, but the mechanism of DNA damage and cytoskeletal destruction is still unknown. Moreover, MC-LR has estrogenic effects and affects the

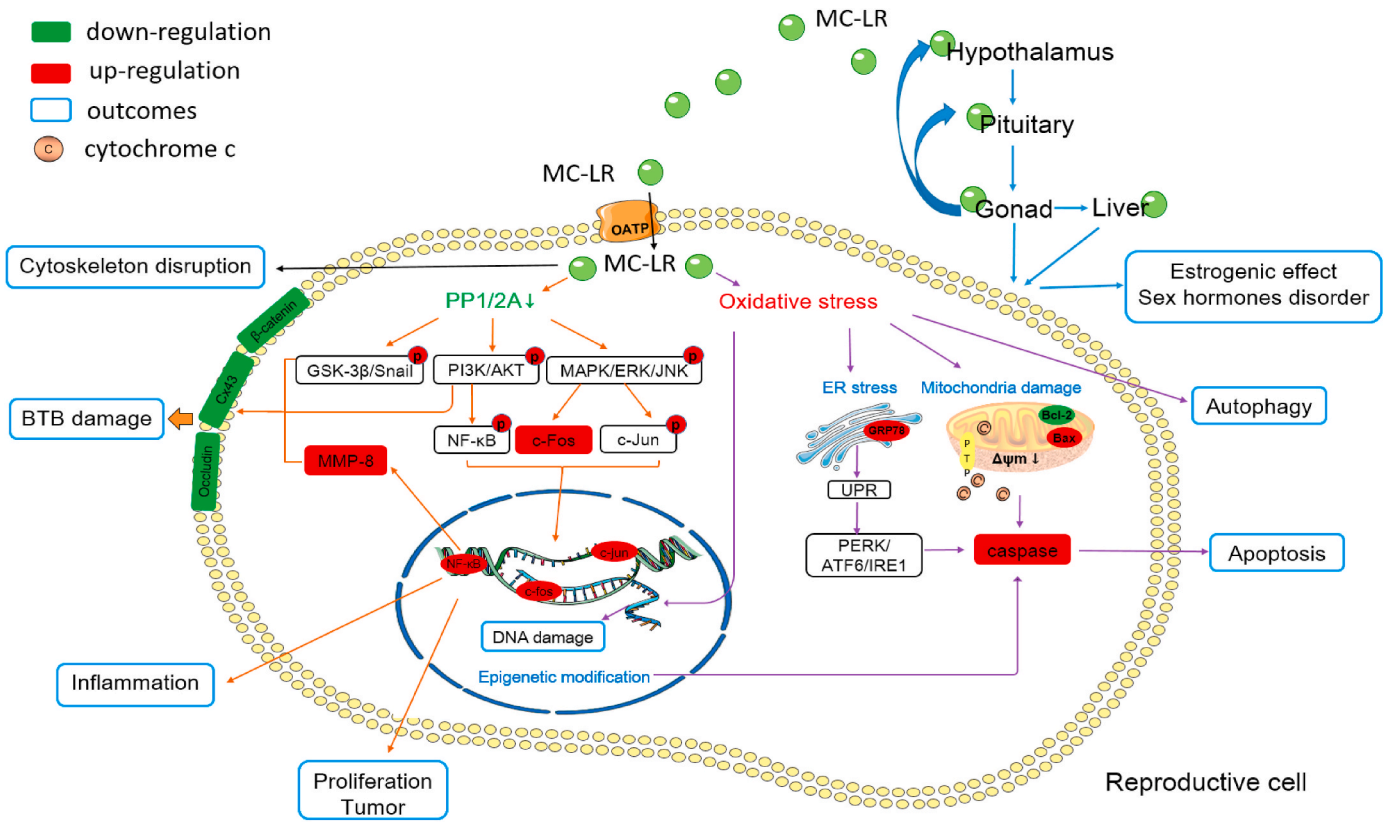

Fig. 5. Schematic diagram of the possible molecular mechanisms underlying microcystin-LR-induced reproductive toxicity. Abbreviation: OATP: organic anion transport polypeptide, PP1/2 A: protein phosphatases 1 and 2 A, BTB: blood-testis-barrier, ER: endoplasmic reticulum, UPR: unfolded protein response, Δψm: mitochondrial membrane potential.

synthesis, secretion, metabolism and transformation of sex hormones. This is closely related to the feedback regulation of the HPG/HPGL axis.

3.1. *The molecular pathways of microcystin-LR-induced apoptosis*

MC-LR can activate apoptosis-related signaling pathways to induce apoptosis. In addition to the previous discovery that MC-LR exposure leads to apoptosis by destroying the cytoskeleton, current studies on apoptosis mainly focus on the mitochondrial pathway, the endoplasmic reticulum pathway and the epigenetic modification pathway.

3.1.1. *Microcystin-LR induces apoptosis by the mitochondrial pathway*

MC-LR induces excessive intracellular ROS to act on the outer mitochondrial membrane (Huang et al., 2016; Liu et al., 2018a; Yuan et al., 2019). This causes the mitochondrial structure to destroy, resulting in the opening of mitochondrial permeability transition pore (PTP) and the decrease of the mitochondrial membrane potential (Wu, 2019a). At the same time, cytochrome c is released outside the mitochondria via PTP, which promotes the activation of the downstream caspase family proteins and initiates the apoptosis process (Wu et al., 2019b; Yuan et al., 2019). Studies found that MC-LR increased the expression of pro-apoptotic proteins caspase-3, caspase-9 and Bax in rat testis, but decreased the expression of anti-apoptotic protein Bcl-2 (Wang et al., 2019; Yuan et al., 2019). Bcl-2 can inhibit the opening of PTP by competitively binding to PTP with Bax, thereby regulating the process of apoptosis (Kuwana et al., 2020). Under normal physiological conditions, PTP is opened periodically to maintain the normal function of mitochondria, but the continuous accumulation of ROS can lead to excessive opening which will result in apoptosis (Huang et al., 2013). Some investigators believed that oxidative stress mediates MC-LR-induced apoptosis by mitochondrial pathway. This can be seen from the fact that NAC or resveratrol (antioxidant) pretreatment can significantly reduce cellular ROS production, pro-apoptotic protein expression, mitochondria damage, and the apoptosis rate, while increase anti-apoptotic protein expression (Huang et al., 2016; Liu et al., 2018a; Yuan et al., 2019).

Mitochondria, as the energy supply unit of cells, plays a crucial role in the energy supply in the process of spermatogenesis (Rajender et al., 2010). Therefore, we speculate that the apoptosis of germ cells may be caused by an insufficient supply in cells that can be attributed to mitochondrial damage. Furthermore, as the main mechanism for removing damaged mitochondria to maintain cell homeostasis, mitochondrial autophagy has gradually attracted widespread attention. Whether MC-LR can arouse mitochondrial autophagy and the relationship between mitochondrial autophagy and apoptosis still need further study.

3.1.2. *Microcystin-LR induces apoptosis by the endoplasmic reticulum pathway*

The endoplasmic reticulum is the place of protein synthesis and processing, which has crucial physiological functions. MC-LR has been shown to damage endoplasmic reticulum structure and function, which will lead to the accumulation of massive unfolded proteins or misfolded proteins, and then cause ER stress (Zhang et al. 2016b, 2020). ER stress sets off the unfolded protein response (UPR) to repair cell damage and to restore cell function. When the intensity and time of ER stress exceeds the limit, it will cause apoptosis. UPR signal transduction is regulated by three transmembrane protein transducers which are PERK, IRE1 and ATF6 (Hetz and Papa, 2018). Under normal physiological conditions, these transmembrane proteins bind to GRP78 in the endoplasmic reticulum cavity, and the ATF6, IRE1, and PERK signaling pathways are at rest. When ER stress occurs, unfolded proteins will accumulate in the endoplasmic reticulum. At the same time, GRP78 will be released from these complexes and bind to new unfolded proteins, causing the three signaling pathways of ATF6, IRE1 and PERK to be activated (Ibrahim

et al., 2019). Studies have shown that MC-LR increases the expression of ER stress-related proteins such as GRP78, ATF-6, PERK, IRE1 and CHOP. ER stress inhibitors could relieve apoptosis, indicating that MC-LR-induced apoptosis is associated with ER stress (Liu et al., 2018b; Zhang et al., 2016b). The latest study found that MC-LR triggers zebrafish ovary ER stress through the PERK and IRE1 pathways, which in turn triggers apoptosis (Zhang et al., 2020).

The endoplasmic reticulum is also the main site of Ca^{2+} storage and calcium signal transduction. Intracellular Ca^{2+} is often used as a second messenger to participate in the apoptosis process (Ureshino et al., 2019). Studies have proved that MC-LR can cause intracellular Ca^{2+} elevation, leading to imbalances in Ca^{2+} levels (Zhang et al., 2016b). In an imbalanced state, the increased Ca^{2+} levels activate calpain and Ca^{2+}/calmodulin-dependent protein kinases (Fladmark et al., 2002). This mechanism seems to play a key role in MC-LR-induced apoptosis. Furthermore, endoplasmic reticulum, as a "calcium storehouse" in the cell, is closely related to the steady state of Ca^{2+}. However, it is not clear whether the imbalance of Ca^{2+} levels caused by MC-LR can be attributed to ER stress.

3.1.3. *Microcystin-LR induces apoptosis by epigenetic modification*

Epigenetics refers to the reversible and heritable modification of gene function without changing the DNA sequence. This mainly includes DNA methylation, histone modification and the regulation of ncRNAs. H3K4me3 participates in testicular reproductive toxicity caused by MC-LR (Yuan et al., 2019). H3K4me3 refers to the histone H3 protein which is trimethylated at the 4th lysine residue. It is usually regarded as the activation signal of p53 and is closely related to the transcriptional activation of apoptosis genes (Lauberth et al., 2013). MC-LR has been shown to bring about apoptosis and increase the expression level of H3K4me3. However, after inhibiting histone methylation, the level of MC-LR-induced apoptosis was significantly reduced, suggesting that histone methylation modification mediated apoptosis induced by MC-LR (Yuan et al., 2019). In general, acetylation of histones is conducive to the dissociation of DNA and histone octamers. This action relaxes the nucleosome structure so that cooperative transcription factors can bind to specific DNA binding sites and activate genes. The deacetylation of histones has the opposite effect. It was found that MC-LR could enhance the activity of HDAC, reduce the activity of HAT, and up-regulate the expression of HDAC1 *in vitro* and *in vivo* (Wang et al., 2019). This indicates that MC-LR affects normal histone acetylation modification. It was also found that the HDAC inhibitor trichostatin A (TSA) decreased the levels of apoptosis-related proteins and apoptosis rate, demonstrating that histone acetylation modification mediates MC-LR-induced apoptosis.

A large number of studies in recent years have discovered the importance of ncRNAs in epigenetics. ncRNAs cannot translate proteins, which mainly include miRNA, piRNA, circRNA and lncRNA. piRNAs are processed by the piwi-clade argonaute protein and can degrade mRNA by imperfect base pairing with specific sites within the 3′ untranslated region (3′ UTR) of the target transcript (Gou et al., 2014). Exposure of MC-LR could lead to changes in piRNA expression profiles, increased apoptosis and decreased cell proliferation in the mice testis. It has been confirmed that MC-LR can down-regulate mmu_piR_027558 to induce apoptosis on germ cells (Meng et al., 2019). This means that MC-LR-induced apoptosis may be mediated by piRNA through the regulation of target genes. miRNAs are a novel class of small non-coding RNAs with 26–32 nucleotides in length that control a wide range of biological processes, including cell proliferation, differentiation, apoptosis, and metabolism (Correia de Sousa et al., 2019). Through KEGG pathway analysis, researchers found that the differentially expressed miRNAs in MC-LR-induced ovarian granulosa cells were mainly involved in signaling pathways such as apoptosis, cancer formation, proliferation, and hormone production (Li et al., 2017). Furthermore, after chronic exposure of MC-LR to mice, circRNAs and lncRNAs were differentially expressed in testes (Meng et al., 2019). This indicates that the apoptosis induced by MC-LR may be associated with the abnormal expression of ncRNAs, but the reason for the abnormal expression of these ncRNAs has not yet been explored.

To sum up, the above studies demonstrate that epigenetics, including histone modifications and ncRNAs changes, could be involved in MC-LR-induced reproductive toxicity. These findings have stimulated the interest of many researchers in the mechanism of epigenetic involvement in reproductive toxicity. Furthermore, studies have reported that DNA methylation modification can regulate the malignant transformation of human hepatocyte L02 cells, but the involvement of DNA methylation in the induction of germ cell toxicity has not yet been proven (Chen et al., 2018a; Zhao et al., 2019a).

3.2. *The role of autophagy in mechanism of microcystin-LR-induced reproductive toxicity*

Autophagy is a process of cell degradation and circulation that is highly conserved among all eukaryotes. Autophagy is a main cell protection mechanism, but excessive autophagy is harmful. There are three main forms of cellular autophagy: microautophagy, macroautophagy and molecular chaperone-mediated autophagy. Macroautophagy is one of the most studied in MC-LR-induced toxicity. Following autophagy activation, the UNC-51-like kinase 1/2 (Ulk1/2) complex, phosphatidylinositol-3-phosphate (PI3P), ATG12-ATG5-ATG16L1 complex and LC3-II promote the formation of autophagic vesicles encapsulating substrate proteins (Weidberg et al., 2011). The outer membrane of the autophagic vesicle will fuse with the lysosomal membrane to form an autolysosome and eventually degrade the substrate protein (Zhu et al., 2019). Studies have found that MC-LR can induce the formation of autophagic vesicles and increase expression of autophagy-related proteins such as Beclin1, ATG16, ATG5-ATG12 and LC3-II in germ cells, suggesting that MC-LR can cause autophagy (Liu et al., 2018b; Zhang et al., 2016b). However, the mechanism of MC-LR-induced autophagy is not clear. It is well known that MC-LR can cause ER stress and apoptosis. Interestingly, the autophagy inhibitor (3-methyladenine) pretreatment can increase MC-LR-induced ER stress and apoptosis (Zhang et al., 2016b). The results imply that autophagy is related to ER stress and apoptosis, and autophagy may have a protective role in germ cell apoptosis induced by MC-LR. The relationship between autophagy, ER stress and apoptosis needs further study.

3.3. *Destruction of the blood-testis barrier is a potential mechanism of microcystin-LR-induced male reproductive toxicity*

The BTB is a layer of barrier structure between spermatogenic tubules and capillary blood. It can form a microenvironment that is conducive to spermatogenesis, and also prevents sperm antigen substances from escaping outside the seminiferous tubules and causing autoimmune reactions. Researchers have demonstrated that MC-LR destroyed BTB and then exerted reproductive toxicity (Adegoke et al., 2018; Chen et al., 2016b; Zhou et al. 2018, 2019a). The BTB is mainly composed of tight junctions, desmosomes and gap junctions (Mruk and Cheng, 2015). MC-LR exposure can decrease expression of Zonula-occluden (ZO-1), Occludin, Connexin-43 (Cx43) and Catenin β1 (CTNNB1) which belong to tight, gap and adhesive junction protein respectively (Adegoke et al., 2018; Zhou et al. 2018, 2019a). This showed that MC-LR can destroy BTB through degrading intercellular junction proteins. Some researchers have made an in-depth study on the mechanism of BTB destruction by MC-LR. It was found that MC-LR inhibits PP2A activity and then breaks tight junctions through the Akt/GSK-3β/Snail pathway (Zhou et al., 2018). Akt is a family of three serine-threonine kinases, which can regulate testicular function (Wang et al., 2015). Glycogen synthase kinase (GSK-3β) is one of the downstream targets of Akt (Yoshimura et al., 2006). Snail is a zinc finger transcription factor, which directly and simultaneously inhibits the expression of two important cell adhesion molecules, E-cadherin and

Occludin (Chen et al., 2015). This reasonably explains that the Akt/GSK-3β/Snail pathway is involved in MC-LR-induced BTB damage. Also, researchers pointed out that MC-LR reduced the gap junction through the Akt pathway (Zhou et al., 2019a).

MC-LR can regulate BTB by mediating some enzymes and miRNA. Chen et al. found that miRNAs were involved in MC-LR-induced destruction of tight junctions and adherens junctions in testes (Chen et al., 2016b). MC-LR has been shown to promote the expression of matrix metalloproteinase-8 (MMP-8) by down-regulating the expression of miR-193, miR-29 b, miR-133a, and miR-184–3p (Chen et al., 2018c). MMP8 is a member of a family of zinc-dependent proteases, which is involved in the proteolytic cleavage of Occludin, causing the disassembly of cell junction components (Schubert-Unkmeir et al., 2010). This demonstrates that MMP-8 may mediate BTB destruction and degradation of tight junction proteins. In addition, the study discovered that the increased expression of *c-fos*, *c-jun* and *NF-κB* genes can positively promote the transcription of *MMP-8* (Chen et al., 2018c).

3.4. Inflammation and immune responses

The essence of the inflammatory response is a defense response to the body being stimulated, but the excessive inflammatory response can cause adverse outcomes such as chronic orchitis and prostatitis. MC-LR can induce testicular inflammation, especially in Sertoli cells and Leydig cells (Adegoke et al., 2018; Chen et al., 2017b). MC-LR has been shown to enhance testicular fibrosis and infiltration of macrophages and lymphocytes (Chen et al., 2016b). Studies have found that MC-LR could give rise to immune responses in Sertoli cells, germ cells and Leydig cells via activating the PI3K/Akt/NF-κB signaling pathway, promoting the expressions of pro-inflammatory cytokines and chemokines such as TNF-α, IL-6, MCP-1 and CXCL10 (Chen et al., 2017b). NF-κB is a family of dimeric transcription factors that plays a crucial role in mediating the immune response and inflammation (Mitchell et al., 2016). Akt is a survival kinase that has been shown to phosphorylate IκB kinase α (IKKα) and lead to the release of NF-κB. Free NF-κB can enter the nucleus and activate transcription of a large number of target genes. In addition, the activity of Akt is regulated by phosphorylation and dephosphorylation (Yang et al., 2010). It is well known that MC-LR inhibits PP2A activity by binding to the catalytic subunits of PP2A (MacKintosh et al., 1990). The decrease of PP2A phosphatase activity will increase the phosphorylation level of Akt (Yang et al., 2010). Therefore, MC-LR can activate the PI3K/Akt/NF-κB pathway by inhibiting PP2A activity. Furthermore, TLR2 and TLR4 can mediate the immune response caused by MC-LR in Leydig cells (Adegoke et al., 2018; Chen et al., 2017b). The TLR family takes part in the innate immune response and can lead to the expression of multiple inflammatory genes (Kawai and Akira, 2007). Since MC-LR cannot enter Leydig cells, the upstream regulation mechanism of TLR is not clear and should be further studied. Besides, chronic exposure of MC-LR also caused an immune response and inflammation in the prostate tissue. Studies have shown that NF-κB participates in prostate inflammation and promotes an increase of chemokines (MCP-1, CXCL10) and pro-inflammatory cytokines (TNF-α, IL-6) in RWPE-1 cells, suggesting that the release of inflammatory cytokines may be due to the activation of NF-κB (Pan et al., 2018).

In conclusion, inflammation and immune response are complex processes. Presently, there is still a large gap in the research on MC-LR-induced inflammation and immune response in the reproductive system. For example, it is unclear whether MC-LR causes inflammation of the female reproductive organs. Most proven inflammation-related pathways initiated by MC-LR play a role through phosphorylation (such as NF-κB, Akt, etc). However, there may be more pathways involved in the development of inflammatory and immune responses, and these pathways may interact and interfere with each other. A detailed mechanism of how MC-LR induces reproductive toxicity through the inflammatory response will require further study.

3.5. Estrogenic effects and sex hormone disorders

Environmental estrogens are typical environmental endocrine disruptors that can activate or inhibit the activity of endogenous estrogenic. And, they produce anti/estrogen-like effects that interfere with the normal physiological functions of endogenous estrogens. MC-LR seems to be regarded as an environmental estrogen as it exerts estrogenic effects (Mallia et al., 2020; Wang et al., 2020). After adult male zebrafish were exposed to 10 μg/L MC-LR for 30 days, E2 level was increased in the testes (Hou et al., 2018). Meanwhile, the *vtg1* gene was significantly up-regulated in the liver (Hou et al., 2018; Rogers et al., 2011). As an egg yolk precursor protein, VTG in the liver is usually expressed only in females. However, when estrogen endocrine disrupting chemicals are present, the expression of *vtg* gene in males can be used as a molecular marker of estrogen (Matozzo et al., 2008). This means that MC-LR can act as an endocrine disruptor. Environmental estrogens mainly exert estrogenic effects in two ways: by binding to the estrogen receptor or by interfering with endogenous estrogen levels (Chighizola and Meroni, 2012; Gray et al., 2002). It has been found in zebrafish and other animals that MC-LR can interfere with estrogen levels and alter the expression of estrogen receptors in a dose-independent manner (Hou et al., 2018; Liu et al., 2016; Qiao et al., 2013). Therefore, MC-LR could play an estrogenic effect as an environmental estrogen, but this estrogenic effect seems to have a dose limit. When fish are exposed to MC-LR at concentrations below 20 μg/L, the E2 level in serum or gonads was elevated. However, when fish are exposed to MC-LR at concentrations above 20 μg/L, and especially above 40 μg/L, the E2 level in fish dropped significantly (Chen et al., 2017a; Hou et al., 2018; Liu et al., 2016; Qiao et al., 2013; Zhao et al., 2015). It is not difficult to understand that E2 level can be regulated normally with low doses of MC-LR. High doses of MC-LR not only affect hormone levels but also damage the brain, as well as gonadal and liver tissues, which can synthesize, secrete and metabolize estrogen. And, MC-LR has a greater effect on these tissues than changes in estrogen levels. To sum up, MC-LR at low does can be used as an environmental estrogen to exert estrogenic effects. However, the chemical structure of MC-LR is different from estrogen-like substances such as bisphenol A and its hydrophilic character reduces the possibility of acting as a ligand for estrogenic receptors. Therefore, the mechanism of the estrogenic effect by MC-LR needs in-depth study.

MC-LR can change the levels of sex hormones by disrupting the function of the HPG and HPGL axes and affecting the synthesis, secretion and metabolism of sex hormones. In male animals, it was found that MC-LR generally reduced testosterone level in serum and testes (Wang et al., 2012). The mechanism of testosterone level decline can be explained in two aspects. On the one hand, the decline in testosterone is associated with apoptosis of Leydig cells secreting testosterone. This view was also confirmed by Li et al. After male rats were exposed to MC-LR, apoptosis of Leydig cells and a decrease in testosterone level were observed (Li et al., 2008b). Chen et al. believed that MC-LR mediated apoptosis and phagocytosis of Leydig cells by testicular macrophages, leading to a decrease in serum testosterone level. The main feature of this action is that MC-LR exposure to co-culture cells (Leydig cells and macrophages) leads to a significant increase in Leydig cell apoptosis, while macrophages were significantly increased (Chen et al., 2018b). On the other hand, MC-LR reduces serum testosterone level by affecting hormones upstream of the HPG axis. Wang et al. demonstrated that MC-LR was able to enter GnRH neurons in the hypothalamus and inhibit GnRH synthesis (Wang et al., 2016). The inhibitory effects of MC-LR on GnRH synthesis were attributed to the activation of the PKA/cyclic AMP response element-binding protein (CREB)/c-Fos signaling pathway (Wang et al., 2018). Besides, MC-LR reduced GnRH levels in mice serum and hypothalamus by PKC/PKA/MAPK signaling pathway (Ding et al., 2018). In brief, MC-LR reduced GnRH synthesis in the hypothalamus and then decreased serum LH and FSH levels (Chen et al., 2011; Wang et al., 2012), thereby reducing testosterone production through the HPG axis regulation.

The testosterone/E2 ratio indicates endocrine disruption and has been used as a sensitive biomarker for abnormal levels of sex hormones in fish (Orlando et al., 2004). MC-LR exposure can simultaneously change E2 and testosterone levels (Chen et al., 2017a; Hou et al., 2018; Su et al., 2016; Zhang et al., 2017). The regulation of testosterone and E2 synthesis is mainly achieved by transcriptional regulation of genes encoding steroid (Trant et al., 2001). The terminal enzyme of the steroid production pathway is aromatase (CYP19), which is responsible for converting testosterone to E2 (Simpson et al., 1994). *cyp19a1b* and *cyp19a1a*, two genes of *cyp 19* subfamily in teleost fish, are mainly expressed in the brain and testis (Trant et al., 2001). Experimental evidence showed that MC-LR upregulated E2 level and increased transcriptional levels of *cyp19a1b* and *cyp19a1a*, implying that the conversion of testosterone to E2 resulted in a lower testosterone/E2 ratio (Lin et al., 2018). Thus, MC-LR appears to affect the normal conversion of sex hormones by changing the expression of genes associated with steroid synthesis.

In conclusion, MC-LR could act as an endocrine disruptor which is an environmental estrogen that exerts estrogenic effects. MC-LR also affects the neuroendocrine system, damages the gonads, and interferes with the expression of sex hormone synthesis genes to cause abnormal levels of hormones in serum and gonads.

4. Future direction and summary

The toxicity of MC-LR on the reproductive system has been extensively studied, but there are still many questions to be answered. Firstly, the toxicity of MC-LR on the human reproductive system has not been confirmed. In an ecological study, 32,700 live-born neonates in southeastern Australia were studied. There may be a correlation between the intake of cyanobacterial toxins and the low birth rate in infants. However, ecological studies cannot determine causality, so there is no clear evidence that cyanobacterial contamination of drinking water sources can lead to adverse pregnancy outcomes (Pilotto et al., 1999). At present, there is no research evidence that MC-LR causes damage to the human reproductive system. However, it is urgent to confirm this possible toxicity. This is the original intention of all our research.

Secondly, the scope of future research should be expanded to include an in-depth focus on the mechanism of this toxicity. Previous studies have focused on the reproductive toxicity of testes and ovaries caused by MC-LR, but recent studies have found that it can also induce toxic effects of other reproductive organs such as the prostate and placenta. For example, the placenta has a non-negligible role in the weight loss of offspring mice caused by MC-LR. Therefore, future research should have a greater focus on other reproductive organs, in addition to the testis and ovaries. Research on the mechanism of toxicity of MC-LR can mainly be divided into three types ranging from shallow to deep. The first type of mechanism research mainly focuses on the correlation of MC-LR-induced toxicity. For example, the decrease of testosterone level caused by MC-LR is related to apoptosis of Leydig cells. The second type of mechanism research is to describe a mediating relationship. For example, MC-LR induces apoptosis by causing oxidative stress in the reproductive system. The third type of mechanism research focuses on the molecules that are targeted by MC-LR and the associated changes that occur in the process. For example, MC-LR up-regulates the expression of MMP-8 and miR-184–3p, and miR-184–3p can target MMP-8. In addition, the relationship between many mechanisms is unclear. For example, investigators have confirmed that MC-LR can cause oxidative stress and inhibit PP2A activity, but whether there is a link between oxidative stress and PP2A activity is unclear. Hence, in addition to expanding the scope of research, the continuity and depth of mechanism research should also be increased.

Thirdly, in recent years, researches have shown an increased interest in the developmental toxicity, trans-generational toxicity and estrogen interference caused by MC-LR. However, there is still much to be learned. Researchers have only confirmed that MC-LR causes the developmental and trans-generational toxicity in mice, zebrafish, fleas, etc., while other species such as amphibians, invertebrates and other animals exposed to MC-LR have not been studied. And, due to the short exposure time of MC-LR and the short observation time of progeny in these studies, some toxic manifestations may not have been observed. Thus, future research ought to expand the range of species studied and focus on the effects of long-term exposure on the reproductive structure and function of progeny animals. Furthermore, this article is the first to propose that MC-LR is an environmental estrogen. However, the mechanism of estrogen changes in animals is not fully understood and whether it affects human estrogen levels has not been confirmed. So these problems need to be solved in the future.

In summary, MC-LR has been shown to cause reproductive toxicity, trans-generational toxicity, and developmental toxicity. In male animals, MC-LR has been shown to exert testicular toxicity by damaging the BTB, triggering testicular inflammation and disrupting androgen levels. The decline of sperm quality in animals may be closely related to the impairment of spermatogenesis by MC-LR, which is regulated by ncRNAs. Furthermore, MC-LR could enter the prostate and induce hyperplasia and inflammation. In female animals, MC-LR reduces fertility by damaging ovarian tissue and interfering with estrogen levels. At the cellular level, MC-LR induces abnormalities in proliferation, differentiation, autophagy and apoptosis of reproductive-related cells. Oxidative stress-mediated ER stress and mitochondrial damage, as well as epigenetic modifications may play an important role in MC-LR-induced apoptosis. Moreover, disturbances in synthesis, secretion, and metabolism of sex hormones are associated with neuroendocrine regulation in addition to gonadal damage. We also proposed for the first time that MC-LR could act as an environmental endocrine disruptor with estrogenic effects at low doses. In addition, MC-LR can also be transmitted through the placenta to the offspring, causing growth retardation, developmental deformity and even death of embryos and juveniles. All these have fully confirmed the reproductive toxicity of MC-LR, but there are still some vacancies in the research. Whether MC-LR will cause reproductive toxicity to humans is an urgent issue to be clarified. And, the scope of the research on the reproductive toxicity caused by MC-LR needs to be expanded and the continuity of the mechanism needs to be strengthened. Also, the toxicity mechanism of MC-LR as an environmental endocrine disruptor should be considered.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to editor Chunsheng Liu and anonymous reviewers for their very constructive comments and suggestions on the manuscript. This work was supported by the National Natural Science Foundation of China (Grant Nos. 81773384).

References

Abdel-Daim, M.M., et al., 2019. Piperine enhances the antioxidant and anti-inflammatory activities of thymoquinone against microcystin-LR-induced hepatotoxicity and neurotoxicity in mice. Oxid. Med. Cellul. Long. 2019, 1309175. https://doi.org/10.1155/2019/1309175.

Adegoke, E.O., et al., 2018. Microcystin-leucine arginine (MC-LR) induced inflammatory response in bovine sertoli cell via TLR4/NF-kB signaling pathway. Environ. Toxicol. Pharmacol. 63, 115–126. https://doi.org/10.1016/j.etap.2018.09.001.

AlKahtane, A.A., et al., 2020. Fucoidan alleviates microcystin-LR-induced hepatic, renal, and cardiac oxidative stress and inflammatory injuries in mice. Environ. Sci. Pollut. Res. Int. 27, 2935–2944. https://doi.org/10.1007/s11356-019-06931-z.

Bell, S.G., Codd, G.A., 1994. Cyanobacterial Toxins and Human Health Reviews in Medical Microbiology, vol. 5.

Boegehold, A.G., Alame, K., Johnson, N.S., Kashian, D.R., 2019. Cyanobacteria reduce motility of quagga mussel (Dreissena rostriformis bugensis sperm). Environ. Toxicol. Chem. 38, 368–374. https://doi.org/10.1002/etc.4305.

Cao, L., Huang, F., Massey, I.Y., Wen, C., Zheng, S., Xu, S., Yang, F., 2019a. Effects of microcystin-LR on the microstructure and inflammation-related factors of jejunum in mice. Toxins 11. https://doi.org/10.3390/toxins11090482.

Cao, L., Massey, I.Y., Feng, H., Yang, F., 2019b. A Review of Cardiovascular Toxicity of Microcystins. Toxins. 11 https://doi.org/10.3390/toxins11090507.

Carmichael, W.W., et al., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Perspect. 109, 663–668. https://doi.org/10.1289/ehp.01109663.

Chen, H.Q., et al., 2018a. Gene expression network regulated by DNA methylation and microRNA during microcystin-leucine arginine induced malignant transformation in human hepatocyte L02 cells. Toxicol. Lett. 289, 42–53. https://doi.org/10.1016/j.toxlet.2018.03.003.

Chen, Y., Wang, J., Pan, C., Li, D., Han, X., 2018. Microcystin-leucine-arginine causes blood-testis barrier disruption and degradation of occludin mediated by matrix metalloproteinase-8. Cellul. Mol. Life CM 75, 1117–1132. https://doi.org/10.1007/s00018-017-2687-6.

Chen, Y., Wang, J., Zhang, Q., Xiang, Z., Li, D., Han, X., 2017b. Microcystin-leucine arginine exhibits immunomodulatory roles in testicular cells resulting in orchitis. Environ. Pollut. 229, 964–975. https://doi.org/10.1016/j.envpol.2017.07.081.

Chen, J., Xie, P., 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, Palaemon modestus and Macrobrachium nipponensis, from a large shallow, eutrophic lake of the subtropical China. Toxicon: official journal of the International Society on Toxinology 45, 615–625. https://doi.org/10.1016/j.toxicon.2005.01.003.

Chen, J., Zhang, D., Xie, P., Wang, Q., Ma, Z., 2009. Simultaneous determination of microcystin contaminations in various vertebrates (fish, turtle, duck and water bird) from a large eutrophic Chinese lake, Lake Taihu, with toxic Microcystis blooms. Sci. Total Environ. 407, 3317–3322. https://doi.org/10.1016/j.scitotenv.2009.02.005.

Chen, L., Chen, J., Zhang, X., Xie, P., 2016a. A review of reproductive toxicity of microcystins. J. Hazard Mater. 301, 381–399. https://doi.org/10.1016/j.jhazmat.2015.08.041.

Chen, J., Meng, S., Xu, H., Zhang, Z., Wu, X., 2017a. Effects of microcystis on hypothalamic-pituitary-gonadal-liver Axis in nile Tilapia (Oreochromis niloticus). Bull. Environ. Contam. Toxicol. 98, 562–566. https://doi.org/10.1007/s00128-017-2051-4.

Chen, Y., Wang, J., Chen, X., Li, D., Han, X., 2018b. Microcystin-leucine arginine mediates apoptosis and engulfment of Leydig cell by testicular macrophages resulting in reduced serum testosterone levels. Aquat. Toxicol. 199, 116–126. https://doi.org/10.1016/j.aquatox.2018.03.018.

Chen, Y., Xu, J., Li, Y., Han, X., 2011. Decline of sperm quality and testicular function in male mice during chronic low-dose exposure to microcystin-LR. Reprod. Toxicol. 31, 551–557. https://doi.org/10.1016/j.reprotox.2011.02.006.

Chen, Y., Zhou, Y., Wang, J., Wang, L., Xiang, Z., Li, D., Han, X., 2016b. Microcystin-Leucine Arginine Causes Cytotoxic Effects in Sertoli Cells Resulting in Reproductive Dysfunction in Male Mice Scientific Reports 6, 39238. https://doi.org/10.1038/srep39238.

Chen, Y., Zhou, Y., Wang, X., Qian, W., Han, X., 2013. Microcystin-LR induces autophagy and apoptosis in rat Sertoli cells in vitro. Toxicon : official journal of the International Society on Toxinology 76, 84–93. https://doi.org/10.1016/j.toxicon.2013.09.005.

Chen, Z.J., et al., 2015. Bisphenol A modulates colorectal cancer protein profile and promotes the metastasis via induction of epithelial to mesenchymal transitions. Arch. Toxicol. 89, 1371–1381. https://doi.org/10.1007/s00204-014-1301-z.

Chighizola, C., Meroni, P.L., 2012. The role of environmental estrogens and autoimmunity. Autoimmun. Rev. 11, A493–A501. https://doi.org/10.1016/j.autrev.2011.11.027.

Cirés, S., Alvarez-Roa, C., Wood, S.A., Puddick, J., Loza, V., Heimann, K., 2014. First report of microcystin-producing fischerella sp. (stigonematales, cyanobacteria) in tropical Australia. Toxicon : official journal of the International Society on Toxinology 88, 62–66. https://doi.org/10.1016/j.toxicon.2014.06.010.

Clelland, E., Peng, C., 2009. Endocrine/paracrine control of zebrafish ovarian development. Mol. Cell. Endocrinol. 312, 42–52. https://doi.org/10.1016/j.mce.2009.04.009.

Correia de Sousa, M., Gjorgjieva, M., Dolicka, D., Sobolewski, C., Foti, M., 2019. Deciphering miRNAs' action through miRNA editing. Int. J. Mol. Sci. 20 https://doi.org/10.3390/ijms20246249.

Dao, T.S., Vo, T.M., Wiegand, C., Bui, B.T., Dinh, K.V., 2018. Transgenerational effects of cyanobacterial toxins on a tropical micro-crustacean Daphnia lumholtzi across three generations. Environ. Pollut. 243, 791–799. https://doi.org/10.1016/j.envpol.2018.09.055.

Dawson, M.A., Kouzarides, T., 2012. Cancer epigenetics: from mechanism to therapy. Cell 150, 12–27. https://doi.org/10.1016/j.cell.2012.06.013.

de la Cruz, A.A., et al., 2011. Can we effectively degrade microcystins?–Implications on human health. Anti Canc. Agents Med. Chem. 11, 19–37. https://doi.org/10.2174/187152011794941217.

Díez-Quijada Jiménez, L., Guzmán-Guillén, R., Cascajosa Lira, A., Jos, Á., Cameán, A.M., 2020a. In vitro assessment of cyanotoxins bioaccessibility in raw and cooked mussels. Food Chem. Toxicol. 140, 111391. https://doi.org/10.1016/j.fct.2020.111391.

Díez-Quijada Jiménez, L., Guzmán-Guillén, R., Cătunescu, G.M., Campos, A., Vasconcelos, V., Jos, Á., Cameán, A.M., 2020b. A new method for the simultaneous determination of cyanotoxins (Microcystins and Cylindrospermopsin) in mussels using SPE-UPLC-MS/MS. Environ. Res. 185, 109284. https://doi.org/10.1016/j.envres.2020.109284.

Ding, J., Wang, J., Jin, H., Xia, T., Cheng, Y., Wu, J., Han, X., 2018. Microcystin-LR reduces the synthesis of gonadotropin-releasing hormone by activating multiple signaling pathways resulting in decrease of testosterone in mice. Sci. Total Environ. 643, 496–506. https://doi.org/10.1016/j.scitotenv.2018.06.123.

Du, X., et al., 2019. The diversity of cyanobacterial toxins on structural characterization, distribution and identification: A systematic review. Toxins 11. https://doi.org/10.3390/toxins11090530.

Elzanaty, S., Rezanezhad, B., Borgquist, R., 2016. Association between PSA levels and biomarkers of subclinical systemic inflammation in middle-aged healthy men from the general population. Curr. Urol. 9, 148–152. https://doi.org/10.1159/000442870.

Fladmark, K.E., et al., 2002. Ca2+/calmodulin-dependent protein kinase II is required for microcystin-induced apoptosis. J. Biol. Chem. 277, 2804–2811. https://doi.org/10.1074/jbc.M109049200.

Francis, G., 1878. Poisonous Australian Lake Nature 18, 11–12. https://doi.org/10.1038/018011d0.

Gácsi, M., et al., 2009. Comparative study of cyanotoxins affecting cytoskeletal and chromatin structures in CHO-K1 cells Toxicology in vitro. an international journal published in association with BIBRA 23, 710–718. https://doi.org/10.1016/j.tiv.2009.02.006.

Gleason, E.J., Lindsey, W.C., Kroft, T.L., Singson, A.W., L'Hernault, S.W., 2006. spe-10 encodes a DHHC-CRD zinc-finger membrane protein required for endoplasmic reticulum/Golgi membrane morphogenesis during Caenorhabditis elegans spermatogenesis. Genetics 172, 145–158. https://doi.org/10.1534/genetics.105.047340.

Gorham, T., Dowling Root, E., Jia, Y., Shum, C.K., Lee, J., 2020. Relationship between cyanobacterial bloom impacted drinking water sources and hepatocellular carcinoma incidence rates. Harmful Algae 95, 101801. https://doi.org/10.1016/j.hal.2020.101801.

Gou, L.T., et al., 2014. Pachytene piRNAs instruct massive mRNA elimination during late spermiogenesis. Cell Res. 24, 680–700. https://doi.org/10.1038/cr.2014.41.

Gray Jr., L.E., et al., 2002. Xenoendocrine disrupters-tiered screening and testing: filling key data gaps. Toxicology 181–182, 371–382. https://doi.org/10.1016/s0300-483x(02)00469-9.

Gupta, N., Pant, S.C., Vijayaraghavan, R., Rao, P.V., 2003. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology 188, 285–296. https://doi.org/10.1016/s0300-483x(03)00112-4.

Han, R., et al., 2019. piRNA-DQ722010 contributes to prostate hyperplasia of the male offspring mice after the maternal exposed to microcystin-leucine arginine. Prostate 79, 798–812. https://doi.org/10.1002/pros.23786.

Hetz, C., Papa, F.R., 2018. The unfolded protein response and cell fate control. Mol. Cell 69, 169–181. https://doi.org/10.1016/j.molcel.2017.06.017.

Hilborn, E.D., Soares, R.M., Servaites, J.C., Delgado, A.G., Magalhães, V.F., Carmichael, W.W., Azevedo, S.M., 2013. Sublethal microcystin exposure and biochemical outcomes among hemodialysis patients. PloS One 8, e69518. https://doi.org/10.1371/journal.pone.0069518.

Hou, J., et al., 2018. Estrogenic potency of MC-LR is induced via stimulating steroidogenesis: in vitro and in vivo evidence. Environ. Pollut. 240, 615–622. https://doi.org/10.1016/j.envpol.2018.04.140.

Hou, J., Li, L., Xue, T., Long, M., Su, Y., Wu, N., 2014. Damage and recovery of the ovary in female zebrafish i.p.-injected with MC-LR. Aquat. Toxicol. 155, 110–118. https://doi.org/10.1016/j.aquatox.2014.06.010.

Hou, J., et al., 2017. Microcystin-LR retards gonadal maturation through disrupting the growth hormone/insulin-like growth factors system in zebrafish Ecotoxicol. Environ Saf 139, 27–35. https://doi.org/10.1016/j.ecoenv.2017.01.025.

Huang, H., et al., 2016. Microcystin-LR induced apoptosis in rat sertoli cells via the mitochondrial caspase-dependent pathway: role of reactive oxygen species. Front. Physiol. 7, 397. https://doi.org/10.3389/fphys.2016.00397.

Huang, J., Lv, C., Hu, M., Zhong, G., 2013. The mitochondria-mediate apoptosis of Lepidopteran cells induced by azadirachtin. PloS One 8, e58499. https://doi.org/10.1371/journal.pone.0058499.

IARC, 2010. Monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. IARC Monogr. Eval. Carcinog. Risks Hum. 94, 1–412 v-vii.

Ibrahim, I.M., Abdelmalek, D.H., Elfiky, A.A., 2019. GRP78: A Cell's Response to Stress Life Sciences, vol. 226, pp. 156–163. https://doi.org/10.1016/j.lfs.2019.04.022.

Jia, X., et al., 2019. Down-regulation of GCLC is involved in microcystin-LR-induced malignant transformation of human liver cells. Toxicology 421, 49–58. https://doi.org/10.1016/j.tox.2019.03.010.

Jia, X., et al., 2018. Effects of MCLR exposure on sex hormone synthesis and reproduction-related genes expression of testis in male Rana nigromaculata. Environ. Pollut. 236, 12–20. https://doi.org/10.1016/j.envpol.2018.01.057.

Jia, X., Cai, C., Wang, J., Gao, N., Zhang, H., 2014. Endocrine-disrupting effects and reproductive toxicity of low dose MCLR on male frogs (Rana nigromaculata) in vivo. Aquatic toxicology (Amsterdam, Netherlands 155, 24–31. https://doi.org/10.1016/j.aquatox.2014.06.002.

Kawai, T., Akira, S., 2007. TLR Signaling Seminars in Immunology, vol. 19, pp. 24–32. https://doi.org/10.1016/j.smim.2006.12.004.

Kelly, N.E., Javed, A., Shimoda, Y., Zastepa, A., Watson, S., Mugalingam, S., Arhonditsis, G.B., 2019. A Bayesian risk assessment framework for microcystin violations of drinking water and recreational standards in the Bay of Quinte, Lake Ontario, Canada. Water Res. 162, 288–301. https://doi.org/10.1016/j.watres.2019.06.005.

Kondo, T., Yanagawa, T., Yoshida, N., Yamashita, M., 1997. Introduction of cyclin B induces activation of the maturation-promoting factor and breakdown of germinal vesicle in growing zebrafish oocytes unresponsive to the maturation-inducing hormone. Dev. Biol. 190, 142–152. https://doi.org/10.1006/dbio.1997.8673.

Krajacic, P., Hermanowski, J., Lozynska, O., Khurana, T.S., Lamitina, T., 2009. C. elegans dysferlin homolog fer-1 is expressed in muscle, and fer-1 mutations initiate altered gene expression of muscle enriched genes. Physiol. Genom. 40, 8–14. https://doi.org/10.1152/physiolgenomics.00106.2009.

Kuwana, T., King, L.E., Cosentino, K., Suess, J., Garcia-Saez, A.J., Gilmore, A.P., Newmeyer, D.D., 2020. Mitochondrial residence of the apoptosis inducer BAX is more important than BAX oligomerization in promoting membrane permeabilization. J. Biol. Chem. 295, 1623–1636. https://doi.org/10.1074/jbc.RA119.011635.

Lauberth, S.M., Nakayama, T., Wu, X., Ferris, A.L., Tang, Z., Hughes, S.H., Roeder, R.G., 2013. H3K4me3 interactions with TAF3 regulate preinitiation complex assembly and selective gene activation. Cell 152, 1021–1036. https://doi.org/10.1016/j.cell.2013.01.052.

Lazar, S., Galiani, D., Dekel, N., 2002. cAMP-Dependent PKA negatively regulates polyadenylation of c-mos mRNA in rat oocytes. Mol. Endocrinol. 16, 331–341. https://doi.org/10.1210/mend.16.2.0767.

Lee, J., Lee, S., Jiang, X., 2017. Cyanobacterial toxins in freshwater and food: important sources of exposure to humans. Annual review of food science and technology 8, 281–304. https://doi.org/10.1146/annurev-food-030216-030116.

Leeper, N.J., et al., 2013. Loss of CDKN2B promotes p53-dependent smooth muscle cell apoptosis and aneurysm formation. Arteriosclerosis Thrombosis. Vascul. Biol. 33, e1–e10. https://doi.org/10.1161/atvbaha.112.300399.

Lei, F., Lei, X., Li, R., Tan, H., 2019. Microcystin-LR in peripheral circulation worsens the prognosis partly through oxidative stress in patients with hepatocellular carcinoma. Clin. Exp. Med. 19, 235–243. https://doi.org/10.1007/s10238-019-00550-1.

Li, G., Chen, J., Xie, P., Jiang, Y., Wu, L., Zhang, X., 2011. Protein expression profiling in the zebrafish (Danio rerio) embryos exposed to the microcystin-LR. Proteomics 11, 2003–2018. https://doi.org/10.1002/pmic.201000442.

Li, X., et al., 2017. Expression analysis of microRNAs and mRNAs in ovarian granulosa cells after microcystin-LR exposure. Toxicon : official journal of the International Society on Toxinology 129, 11–19. https://doi.org/10.1016/j.toxicon.2017.01.022.

Li, Y., Sheng, J., Sha, J., Han, X., 2008a. The toxic effects of microcystin-LR on the reproductive system of male rats in vivo and in vitro. Reprod. Toxicol. 26, 239–245. https://doi.org/10.1016/j.reprotox.2008.09.004.

Li, Y., Sheng, J., Sha, J., Han, X., 2008b. The toxic effects of microcystin-LR on the reproductive system of male rats in vivo and in vitro. Reprod. Toxicol. 26, 239–245. https://doi.org/10.1016/j.reprotox.2008.09.004.

Li, Y., Zhang, M., Chen, P., Liu, R., Liang, G., Yin, L., Pu, Y., 2015. Effects of microcystin-LR exposure on spermiogenesis in nematode Caenorhabditis elegans. Int. J. Mol. Sci. 16, 22927–22937. https://doi.org/10.3390/ijms160922927.

Lin, H., et al., 2016. Determination of environmental exposure to microcystin and aflatoxin as a risk for renal function based on 5493 rural people in southwest China. Environ. Sci. Technol. 50, 5346–5356. https://doi.org/10.1021/acs.est.6b01062.

Lin, W., et al., 2018. Single and combined exposure of microcystin-LR and nitrite results in reproductive endocrine disruption via hypothalamic-pituitary-gonadal-liver axis. Chemosphere 211, 1137–1146. https://doi.org/10.1016/j.chemosphere.2018.08.049.

Liu, W., et al., 2016. Sex-dependent effects of microcystin-LR on hypothalamic-pituitary-gonad axis and gametogenesis of adult zebrafish. Sci. Rep. 6, 22819. https://doi.org/10.1038/srep22819.

Liu, H., et al., 2018a. Resveratrol ameliorates microcystin-LR-induced testis germ cell apoptosis in rats via SIRT1 signaling pathway activation. Toxins 10. https://doi.org/10.3390/toxins10060235.

Liu, H., et al., 2018b. Oxidative stress mediates microcystin-LR-induced endoplasmic reticulum stress and autophagy in KK-1 cells and C57bl/6 mice ovaries. Front. Physiol. 9, 1058. https://doi.org/10.3389/fphys.2018.01058.

Liu, W., Qiao, Q., Chen, Y., Wu, K., Zhang, X., 2014. Microcystin-LR exposure to adult zebrafish (Danio rerio) leads to growth inhibition and immune dysfunction in F1 offspring, a parental transmission effect of toxicity. Aquat. Toxicol. 155, 360–367. https://doi.org/10.1016/j.aquatox.2014.07.011.

Liu, Y., Song, L., Li, X., Liu, T., 2002. The toxic effects of microcystin-LR on embryo-larval and juvenile development of loach misguruns mizolepis gunthe. Toxicon : official journal of the International Society on Toxinology 40, 395–399. https://doi.org/10.1016/s0041-0101(01)00173-8.

Liu, W., Zhan, C., Zhang, T., Zhang, X., 2019. Microcystin-LR influences the in vitro oocyte maturation of zebrafish by activating the MAPK pathway. Aquat. Toxicol. 215, 105261.

MacKintosh, C., Beattie, K.A., Klumpp, S., Cohen, P., Codd, G.A., 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett. 264, 187–192. https://doi.org/10.1016/0014-5793(90)80245-e.

Mallia, V., Ivanova, L., Eriksen, G.S., Harper, E., Connolly, L., Uhlig, S., 2020. Investigation of in vitro endocrine activities of microcystis and planktothrix cyanobacterial strains. Toxins 12. https://doi.org/10.3390/toxins12040228.

Martínez Hernández, J., López-Rodas, V., Costas, E., 2009. Microcystins from tap water could be a risk factor for liver and colorectal cancer: a risk intensified by global change. Med. Hypotheses 72, 539–540. https://doi.org/10.1016/j.mehy.2008.11.041.

Masakane, Y., 2000. Toward modeling of a general mechanism of MPF formation during oocyte maturation in vertebrates. Zool. Sci. 17, 841–851. https://doi.org/10.2108/zsj.17.841.

Maskey, E., Crotty, H., Wooten, T., Khan, I.A., 2019. Disruption of oocyte maturation by selected environmental chemicals in zebrafish. Toxicol. Vitro 54, 123–129. https://doi.org/10.1016/j.tiv.2018.09.017.

Matozzo, V., Gagné, F., Marin, M.G., Ricciardi, F., Blaise, C., 2008. Vitellogenin as a biomarker of exposure to estrogenic compounds in aquatic invertebrates a review. Environ. Int. 34, 531–545. https://doi.org/10.1016/j.envint.2007.09.008.

McCarty, C.L., Nelson, L., Eitniear, S., Zgodzinski, E., Zabala, A., Billing, L., DiOrio, M., 2016. Community Needs Assessment After Microcystin Toxin Contamination of a Municipal Water Supply - Lucas County, Ohio, 2014, vol. 65. MMWR Morbidity and mortality weekly report, Lucas County, Ohio, pp. 925–929. https://doi.org/10.15585/mmwr.mm6535a1. September 2014.

Mehta, A., Dobersch, S., Romero-Olmedo, A.J., Barreto, G., 2015. Epigenetics in Lung Cancer Diagnosis and Therapy Cancer Metastasis Reviews, vol. 34, pp. 229–241. https://doi.org/10.1007/s10555-015-9563-3.

Meng, X., Peng, H., Ding, Y., Zhang, L., Yang, J., Han, X., 2019. A transcriptomic regulatory network among miRNAs, piRNAs, circRNAs, lncRNAs and mRNAs regulates microcystin-leucine arginine (MC-LR)-induced male reproductive toxicity. Sci. Total Environ. 667, 563–577. https://doi.org/10.1016/j.scitotenv.2019.02.393.

Meng, X., Zhang, L., Chen, X., Xiang, Z., Li, D., Han, X., 2016. miR-541 contributes to microcystin-LR-induced reproductive toxicity through regulating the expression of p15 in mice. Toxins 8. https://doi.org/10.3390/toxins8090260.

Meng, X., et al., 2020. The mechanisms in the altered ontogenetic development and lung-related pathology in microcystin-leucine arginine (MC-LR)-paternal-exposed offspring mice. Sci. Total Environ. 736, 139678. https://doi.org/10.1016/j.scitotenv.2020.139678.

Mitchell, S., Vargas, J., Hoffmann, A., 2016. Signaling via the NFκB System Wiley Interdisciplinary Reviews Systems Biology and Medicine, vol. 8, pp. 227–241. https://doi.org/10.1002/wsbm.1331.

Mruk, D.D., Cheng, C.Y., 2015. The Mammalian Blood-Testis Barrier: its Biology and Regulation Endocrine Reviews, vol. 36, pp. 564–591. https://doi.org/10.1210/er.2014-1101.

Nandi, D., Cheema, P.S., Jaiswal, N., Nag, A., 2018. FoxM1: repurposing an oncogene as a biomarker. Semin. Canc. Biol. 52, 74–84. https://doi.org/10.1016/j.semcancer.2017.08.009.

Orlando, E.F., et al., 2004. Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. Environ. Health Perspect. 112, 353–358. https://doi.org/10.1289/ehp.6591.

Pan, C., Chen, Y., Xu, T., Wang, J., Li, D., Han, X., 2018. Chronic exposure to microcystin-leucine-arginine promoted proliferation of prostate epithelial cells resulting in benign prostatic hyperplasia. Environ. Pollut. 242, 1535–1545. https://doi.org/10.1016/j.envpol.2018.08.024.

Papadimitriou, T., Kagalou, I., Stalikas, C., Pilidis, G., Leonardos, I.D., 2012. Assessment of microcystin distribution and biomagnification in tissues of aquatic food web compartments from a shallow lake and evaluation of potential risks to public health. Ecotoxicology 21, 1155–1166. https://doi.org/10.1007/s10646-012-0870-y.

Pilotto, L.S., Kliewer, E.V., Davies, R.D., Burch, M.D., Attewell, R.G., 1999. Cyanobacterial (blue-green algae) contamination in drinking water and perinatal outcomes Australian and New Zealand. J. Publ. Health 23, 154–158. https://doi.org/10.1111/j.1467-842x.1999.tb01226.x.

Qi, M., Dang, Y., Xu, Q., Yu, L., Liu, C., Yuan, Y., Wang, J., 2016. Microcystin-LR induced developmental toxicity and apoptosis in zebrafish (Danio rerio) larvae by activation of ER stress response. Chemosphere 157, 166–173. https://doi.org/10.1016/j.chemosphere.2016.05.038.

Qian, H., Liu, G., Lu, T., Sun, L., 2018. Developmental neurotoxicity of Microcystis aeruginosa in the early life stages of zebrafish. Ecotoxicol. Environ. Saf. 151, 35–41. https://doi.org/10.1016/j.ecoenv.2017.12.059.

Qiao, Q., et al., 2019. Subcellular localization of microcystin in the liver and the gonads of medaka fish acutely exposed to microcystin-LR. Toxicon : official journal of the International Society on Toxinology 159, 14–21. https://doi.org/10.1016/j.toxicon.2018.12.006.

Qiao, Q., et al., 2013. Female zebrafish (Danio rerio) are more vulnerable than males to microcystin-LR exposure, without exhibiting estrogenic effects. Aquat. Toxicol. 142–143, 272–282. https://doi.org/10.1016/j.aquatox.2013.07.002.

Rajender, S., Rahul, P., Mahdi, A.A., 2010. Mitochondria, spermatogenesis and male infertility. Mitochondrion 10, 419–428. https://doi.org/10.1016/j.mito.2010.05.015.

Rogers, E.D., et al., 2011. Global gene expression profiling in larval zebrafish exposed to microcystin-LR and microcystis reveals endocrine disrupting effects of Cyanobacteria. Environ. Sci. Technol. 45, 1962–1969. https://doi.org/10.1021/es103538b.

Schubert-Unkmeir, A., Konrad, C., Slanina, H., Czapek, F., Hebling, S., Frosch, M., 2010. Neisseria meningitidis induces brain microvascular endothelial cell detachment from the matrix and cleavage of occludin: a role for MMP-8. PLoS Pathog. 6, e1000874 https://doi.org/10.1371/journal.ppat.1000874.

Simpson, E.R., et al., 1994. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr. Rev. 15, 342–355. https://doi.org/10.1210/edrv-15-3-342.

Singo, A., Myburgh, J.G., Laver, P.N., Venter, E.A., Ferreira, G.C.H., Rösemann, G.M., Botha, C.J., 2017. Vertical transmission of microcystins to Nile crocodile (Crocodylus niloticus) eggs. Toxicon : official journal of the International Society on Toxinology 134, 50–56. https://doi.org/10.1016/j.toxicon.2017.05.017.

Song, Q., et al., 2020. The behaviors of Microcystis aeruginosa and microcystins during the Fe(2+)/persulfate (PS) preoxidation-coagulation and flocs storage period. Environ. Res. 186, 109549. https://doi.org/10.1016/j.envres.2020.109549.

Spoof, L., Catherine, A., 2017. Appendix 3: Tables of Microcystins and Nodularins. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119068761.app3.

Su, Y., Li, L., Hou, J., Wu, N., Lin, W., Li, G., 2016. Life-cycle exposure to microcystin-LR interferes with the reproductive endocrine system of male. zebrafish Aquat Toxicol 175, 205–212. https://doi.org/10.1016/j.aquatox.2016.03.018.

Svirčev, Z., Drobac, D., Tokodi, N., Mijović, B., Codd, G.A., Meriluoto, J., 2017. Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. Arch. Toxicol. 91, 621–650. https://doi.org/10.1007/s00204-016-1921-6.

Svirčev, Z., et al., 2019. Global geographical and historical overview of cyanotoxin distribution and cyanobacterial poisonings. Arch. Toxicol. 93, 2429–2481. https://doi.org/10.1007/s00204-019-02524-4.

Tata, J.R., Smith, D.F., 1979. Vitellogenesis: a versatile model for hormonal regulation of gene expression. Recent Prog. Horm. Res. 35, 47–95. https://doi.org/10.1016/b978-0-12-571135-7.50006-0.

Trant, J.M., Gavasso, S., Ackers, J., Chung, B.C., Place, A.R., 2001. Developmental expression of cytochrome P450 aromatase genes (CYP19a and CYP19b) in zebrafish fry (Danio rerio). J. Exp. Zool. 290, 475–483. https://doi.org/10.1002/jez.1090.

Ueno, Y., et al., 1996. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis 17, 1317–1321. https://doi.org/10.1093/carcin/17.6.1317.

Ureshino, R.P., et al., 2019. The interplay between Ca(2+) signaling pathways and neurodegeneration. Int. J. Mol. Sci. 20 https://doi.org/10.3390/ijms20236004.

Wang, C., Fu, W., Quan, C., Yan, M., Liu, C., Qi, S., Yang, K., 2015. The role of Pten/Akt signaling pathway involved in BPA-induced apoptosis of rat Sertoli cells. Environ. Toxicol. 30, 793–802. https://doi.org/10.1002/tox.21958.

Wang, J., Chen, Y., Chen, Z., Xiang, Z., Ding, J., Han, X., 2018. Microcystin-leucine arginine inhibits gonadotropin-releasing hormone synthesis in mice hypothalamus. Ecotoxicol. Environ. Saf. 163, 391–399. https://doi.org/10.1016/j.ecoenv.2018.07.094.

Wang, L., Wang, X., Geng, Z., Zhou, Y., Chen, Y., Wu, J., Han, X., 2013. Distribution of microcystin-LR to testis of male. Sprague-Dawley rats Ecotoxicology (London, England) 22, 1555–1563. https://doi.org/10.1007/s10646-013-1141-2.

Wang, P.J., Chien, M.S., Wu, F.J., Chou, H.N., Lee, S.J., 2005. Inhibition of embryonic development by microcystin-LR in zebrafish, Danio rerio. Toxicon : official journal of the International Society on Toxinology 45, 303–308. https://doi.org/10.1016/j.toxicon.2004.10.016.

Wang, X., Ding, J., Xiang, Z., Jiang, P., Du, J., Han, X., 2016. Microcystin-LR causes sexual hormone disturbance in male rat by targeting gonadotropin-releasing hormone neurons. Toxicon : official journal of the International Society on Toxinology 123, 45–55. https://doi.org/10.1016/j.toxicon.2016.10.011.

Wang, X., Ying, F., Chen, Y., Han, X., 2012. Microcystin (-LR) affects hormones level of male mice by damaging hypothalamic-pituitary system. Toxicon : official journal of the International Society on Toxinology 59, 205–214. https://doi.org/10.1016/j.toxicon.2011.12.001.

Verlhac, M.H., Kubiak, J.Z., Weber, M., Géraud, G., Colledge, W.H., Evans, M.J., Maro, B., 1996. Mos is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse. Development (Cambridge, England 122, 815–822.

Wang, Y., et al., 2019. Histone acetylation plays an important role in MC-LR-induced apoptosis and cycle disorder in SD rat testicular cells. Chemosphere 241, 125073. https://doi.org/10.1016/j.chemosphere.2019.125073.

Wang, Y., et al., 2020. A closed vitrification system enables a murine ovarian follicle bank for high-throughput ovotoxicity screening, which identifies endocrine disrupting activity of microcystins. Reprod. Toxicol. 93, 118–130. https://doi.org/10.1016/j.reprotox.2020.01.009.

Weidberg, H., Shvets, E., Elazar, Z., 2011. Biogenesis and Cargo Selectivity of Autophagosomes Annual Review of Biochemistry, vol. 80, pp. 125–156. https://doi.org/10.1146/annurev-biochem-052709-094552.

World Health Organization, 2011. Guidelines for Drinking-Water Quality, fourth ed. http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en.

Wu, J., Ding, J., Yang, J., Guo, X., Zheng, Y., 2018. MicroRNA Roles in the Nuclear Factor Kappa B Signaling Pathway in Cancer Frontiers in Immunology, vol. 9, p. 546. https://doi.org/10.3389/fimmu.2018.00546.

Wu, J., et al., 2019a. p53-Dependent pathway and the opening of mPTP mediate the apoptosis of co-cultured Sertoli-germ cells induced by microcystin-LR. Environ. Toxicol. https://doi.org/10.1002/tox.22808.

Wu, J., et al., 2019b. p53-Dependent pathway and the opening of mPTP mediate the apoptosis of co-cultured Sertoli-germ cells induced by microcystin-LR. Environ. Toxicol. 34, 1074–1084. https://doi.org/10.1002/tox.22808.

Wu, J., Yuan, M., Song, Y., Sun, F., Han, X., 2015. MC-LR exposure leads to subfertility of female mice and induces oxidative stress in granulosa cells. Toxins 7, 5212–5223. https://doi.org/10.3390/toxins7124872.

Wu, Q., Yan, W., Cheng, H., Liu, C., Hung, T.C., Guo, X., Li, G., 2017. Parental transfer of microcystin-LR induced transgenerational effects of developmental neurotoxicity in zebrafish offspring. Environ. Pollut. 231, 471–478. https://doi.org/10.1016/j.envpol.2017.08.038.

Wu, Q., Yan, W., Liu, C., Li, L., Yu, L., Zhao, S., Li, G., 2016. Microcystin-LR exposure induces developmental neurotoxicity in zebrafish embryo. Environ. Pollut. 213, 793–800. https://doi.org/10.1016/j.envpol.2016.03.048.

Xue, L., et al., 2015. N-acetylcysteine protects Chinese Hamster ovary cells from oxidative injury and apoptosis induced by microcystin-LR. Int. J. Clin. Exp. Med. 8, 4911–4921.

Yang, F., et al., 2020. A complete route for biodegradation of potentially carcinogenic cyanotoxin microcystin-LR in a novel indigenous bacterium. Water Res. 174, 115638. https://doi.org/10.1016/j.watres.2020.115638.

Yang, S., Chen, L., Wen, C., Zhang, X., Feng, X., Yang, F., 2018. MicroRNA expression profiling involved in MC-LR-induced hepatotoxicity using high-throughput sequencing analysis. J. Toxicol. Environ. Health Part A 81, 89–97. https://doi.org/10.1080/15287394.2017.1415580.

Yang, W.L., Wu, C.Y., Wu, J., Lin, H.K., 2010. Regulation of Akt signaling activation by ubiquitination. Cell Cycle 9, 487–497. https://doi.org/10.4161/cc.9.3.10508.

Yi, X., et al., 2019. Effects of chronic exposure to microcystin-LR on kidney in mice. Int. J. Environ. Res. Publ. Health 16. https://doi.org/10.3390/ijerph16245030.

Yoshimura, T., Arimura, N., Kawano, Y., Kawabata, S., Wang, S., Kaibuchi, K., 2006. Ras regulates neuronal polarity via the PI3-kinase/Akt/GSK-3beta/CRMP-2 pathway. Biochem. Biophys. Res. Commun. 340, 62–68. https://doi.org/10.1016/j.bbrc.2005.11.147.

Yuan, L., et al., 2019. Epigenetic modification of H3K4 and oxidative stress are involved in MC-LR-induced apoptosis in testicular cells of SD rats. Environ. Toxicol. https://doi.org/10.1002/tox.22865.

Zeng, C., et al., 2014. The role of apoptosis in MCLR-induced developmental toxicity in zebrafish embryos. Aquat. Toxicol. 149, 25–32. https://doi.org/10.1016/j.aquatox.2014.01.021.

Zhan, C., Liu, W., Zhang, F., Zhang, X., 2020a. Microcystin-LR triggers different endoplasmic reticulum stress pathways in the liver, ovary, and offspring of zebrafish (Danio rerio). J. Hazard Mater. 386, 121939. https://doi.org/10.1016/j.jhazmat.2019.121939.

Zhan, C., Zhang, F., Liu, W., Zhang, X., 2020b. Microcystin-LR promotes zebrafish (Danio rerio) oocyte (in vivo) maturation by activating ERK1/2-MPF signaling pathways, and cAMP is involved in this process. Environ. Pollut. 259, 113843. https://doi.org/10.1016/j.envpol.2019.113843.

Zhang, D., et al., 2020. Chronic Microcystin-LR Exposure Induces Abnormal Lipid Metabolism via Endoplasmic Reticulum Stress in Male Zebrafish Toxins, vol. 12. https://doi.org/10.3390/toxins12020107.

Zhang, D., Xie, P., Liu, Y., Chen, J., Liang, G., 2007. Bioaccumulation of the hepatotoxic microcystins in various organs of a freshwater snail from a subtropical Chinese lake, Taihu Lake, with dense toxic Microcystis blooms. Environ. Toxicol. Chem. 26, 171–176. https://doi.org/10.1897/06-222r.1.

Zhang, H., Wang, L., Shen, S., Wang, C., Xiang, Z., Han, X., Li, D., 2017. Toxic effects of microcystin-LR on the development of prostate in mice. Toxicology 380, 50–61. https://doi.org/10.1016/j.tox.2017.02.004.

Zhang, J.T., Weng, Z.H., Tsang, K.S., Tsang, L.L., Chan, H.C., Jiang, X.H., 2016a. MycN is critical for the maintenance of human embryonic stem cell-derived neural crest stem cells. PloS One 11, e0148062. https://doi.org/10.1371/journal.pone.0148062.

Zhang, L., Meng, X., Xiang, Z., Li, D., Han, X., 2018. From the cover: roles of mmu_piR_003399 in microcystin-leucine arginine-induced reproductive toxicity in the spermatogonial cells and testis toxicological sciences. an official journal of the Society of Toxicology 161, 159–170. https://doi.org/10.1093/toxsci/kfx209.

Zhang, H., et al., 2013. Toxic effects of microcystin-LR on the reproductive system of male Rana nigromaculata in vitro. Aquat. Toxicol. 126, 283–290. https://doi.org/10.1016/j.aquatox.2012.09.017.

Zhang, S., et al., 2016b. Novel Role of ER Stress and Autophagy in Microcystin-LR Induced Apoptosis in Chinese Hamster Ovary Cells Frontiers in Physiology, vol. 7, p. 527. https://doi.org/10.3389/fphys.2016.00527.

Zhang, Y., Zhuang, H., Yang, H., Xue, W., Wang, L., Wei, W., 2019a. Microcystin-LR disturbs testicular development of giant freshwater prawn Macrobrachium. Chemosphere 222, 584–592. https://doi.org/10.1016/j.chemosphere.2019.01.146.

Zhang, Y., Zhuang, H., Yang, H., Xue, W., Wang, L., Wei, W., 2019b. Microcystin-LR disturbs testicular development of giant freshwater prawn Macrobrachium rosenbergii. Chemosphere 222, 584–592. https://doi.org/10.1016/j.chemosphere.2019.01.146.

Zhao, J., et al., 2019a. Epigenetic silencing of ALX4 regulates microcystin-LR induced hepatocellular carcinoma through the P53 pathway. Sci. Total Environ. 683, 317–330. https://doi.org/10.1016/j.scitotenv.2019.05.144.

Zhao, J., et al., 2019b. Epigenetic silencing of ALX4 regulates microcystin-LR induced hepatocellular carcinoma through the P53 pathway. Sci. Total Environ. 683, 317–330. https://doi.org/10.1016/j.scitotenv.2019.05.144.

Zhao, S., Liu, Y., Wang, F., Xu, D., Xie, P., 2018. N-acetylcysteine protects against microcystin-LR-induced endoplasmic reticulum stress and germ cell apoptosis in zebrafish testes. Chemosphere 204, 463–473. https://doi.org/10.1016/j.chemosphere.2018.04.020.

Zhao, S., Zhong, S., Wang, F., Wang, H., Xu, D., Li, G., 2020. Microcystin-LR exposure decreased the fetal weight of mice by disturbance of placental development and ROS-mediated endoplasmic reticulum stress in the placenta. Environ. Pollut. 256, 113362. https://doi.org/10.1016/j.envpol.2019.113362.

Zhao, Y., Xie, L., Yan, Y., 2015. Microcystin-LR impairs zebrafish reproduction by affecting oogenesis and endocrine system. Chemosphere 120, 115–122. https://doi.org/10.1016/j.chemosphere.2014.06.028.

Zhou, Q., et al., 2020a. Daphnia enhances relative reproductive allocation in response to toxic microcystis: changes in the performance of parthenogenetic and sexual reproduction. Environ. Pollut. 259, 113890. https://doi.org/10.1016/j.envpol.2019.113890.

Zhou, Y., Chen, Y., Hu, X., Guo, J., Shi, H., Yu, G., Tang, Z., 2019a. Icariin attenuate microcystin-LR-induced gap junction injury in Sertoli cells through suppression of Akt pathways. Environ. Pollut. 251, 328–337. https://doi.org/10.1016/j.envpol.2019.04.114.

Zhou, Y., Chen, Y., Hu, X., Guo, J., Shi, H., Yu, G., Tang, Z., 2019b. Icariin attenuate microcystin-LR-induced gap junction injury in Sertoli cells through suppression of Akt pathways. Environ. Pollut. 251, 328–337. https://doi.org/10.1016/j.envpol.2019.04.114.

Zhou, Y., et al., 2018. Essential roles of Akt/Snail pathway in microcystin-LR-induced tight junction toxicity in Sertoli cell Food and chemical toxicology. an international journal published for the British Industrial Biological Research Association 112, 290–298. https://doi.org/10.1016/j.fct.2018.01.004.

Zhou, Y., et al., 2020b. Responses of the proteome in testis of mice exposed chronically to environmentally relevant concentrations of Microcystin-LR. Ecotoxicol. Environ. Saf. 187, 109824. https://doi.org/10.1016/j.ecoenv.2019.109824.

Zhou, Y., Xiang, Z., Li, D., Han, X., 2014. Regulation of microcystin-LR-induced toxicity in mouse spermatogonia by miR-96. Environ. Sci. Technol. 48, 6383–6390. https://doi.org/10.1021/es500152m.

Zhou, Y., Yuan, J., Wu, J., Han, X., 2012. The toxic effects of microcystin-LR on rat spermatogonia in vitro. Toxicol. Lett. 212, 48–56. https://doi.org/10.1016/j.toxlet.2012.05.001.

Zhu, Y., Yin, Q., Wei, D., Yang, Z., Du, Y., Ma, Y., 2019. Autophagy in Male Reproduction Systems Biology in Reproductive Medicine, vol. 65, pp. 265–272. https://doi.org/10.1080/19396368.2019.1606361.