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## A Review on the Uptake, Accumulation, Impact and Determinants of Microcystins on Intestinal Health

Isaac Yaw Massey<sup>1</sup>, Ernest Osei<sup>2</sup>, Sabina Ampon-Wireko<sup>3</sup>, Lydia Sarponmaa Asante<sup>4</sup>, Alice Ayawine<sup>5</sup>, Emmanuel Kwang<sup>6</sup>, Luke Ojo<sup>7</sup>, Samuel Yaw Opoku<sup>8</sup>.

<sup>1,2,3,4,5,6,7,8</sup>Department of Public Health, School of Public Health and Allied Science, Catholic University of Ghana  
Corresponding author: Isaac Yaw Massey. E-mail ID: isaac.massey@cug.edu.gh,

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**Abstract:** Microcystins (MCs) released during cyanobacterial blooms induce immense toxic manifestations on animals and humans. The International Agency for Research on Cancer (IARC) categorized MC as a possible carcinogen. Research indicate that aside the liver, MCs can negatively impact the intestine. Interestingly, less attention exists with respect to MCs effects on the intestine in spite of the organ being the focal site of the toxins' uptake. Compiling data from in vivo and in vitro studies, the current review summarized available literature on the impact of MCs on intestinal health. In addition, the toxins' uptake, accumulation and various methods used to determine intestinal toxicity were elucidated.

**Keywords:** Microcystins, Intestine, Transport, Toxicity and carcinogenicity, Detection

### 1. INTRODUCTION

Harmful cyanobacterial blooms contamination and the production of various cyanobacterial toxins are frequently reported during the summer months in many parts of the world (Liu et al., 2018; Feng et al., 2019; Massey et al., 2020). The culprit behind water advisory causing harm to hundreds and thousands of people and animals is a cyanotoxin called microcystin (MC). MCs are a group of monocyclic heptapeptide hepatotoxins, and are produced by several kinds of bloom-forming cyanobacterial species including *Microcystis*, *Anabaena*, *Aphanizomenon*, *Nostoc*, *Cylindrospermopsis* and *Oscillatoria* (Massey et al., 2020; Yang et al., 2020; Wei et al., 2021;). Among the over 270 documented MC variants, microcystin-LR (MC-LR), microcystin-RR (MC-RR) and microcystin-YR (MC-YR) with varying combinations of Leucine (L), Arginine (R) or Tyrosine (Y) are the most widely disseminated, exceptionally toxic and comprehensively investigated (Bouaicha et al., 2019; Massey et al., 2020; Yang et al., 2020). Regarding

the toxins health impacts, the International Agency for Research on Cancer categorized MC-LR as a possible carcinogen ([IARC, 2010](#)), and the World Health Organization (WHO) suggest a provisional guideline value of 1 µg/L as the highest acceptable concentration of the toxin in drinking water ([WHO, 1998](#)).

Microcystins which have a size of approximately 3 nm in diameter and molecular weight ranging between 800 and 1,100 Daltons, generally share a common cyclic structure cyclo-(-D-Ala-L-X-D-MeAsp-L-Z-Adda-D-Glu-Mdha). X and Z indicate highly variable amino acids, D-MeAsp indicates D-erythro-b-methylaspartic acid, Adda indicates (2S, 3S, 8S, 9S) 3- amino-9 methoxy-2,6,8-trimethyl-10-phenyldeca-4, 6-dienoic acid and Mdha indicates N-methyldehydroalanine ([Massey and Yang, 2020](#); [Guo et al., 2021](#)). It is worth noting that MC variants primarily vary in X and Z amino acids, and demethylation or methylation on MeAsp and Mdha ([Sivonen and Jones, 1999](#)).

In recent decades, MCs production and the increasing water hazards caused by these toxins primarily found in eutrophic freshwaters including lakes, streams, rivers, lagoon and reservoirs for public water supply have globally been reported ([Liu et al., 2018](#); [Feng et al., 2019](#); [Francy et al., 2020](#); [Massey et al., 2020](#)). The toxic effects of MCs have been made possible through the inhibition of protein phosphatases 1 (PP1) and PP2A, recognized as the primary mechanism for MC toxicity ([MacKintosh et al., 1990](#); [Massey and Yang, 2020](#)). Research shows that mammals are exposed to the toxins mainly through consumption of contaminated water, food and algal dietary supplements as well as body contact ([Cao et al., 2019](#); [Chia et al., 2019](#); [Massey and Yang, 2020](#)). A well-documented case of human poisoning took place in Caruaru, Brazil, when a bloom of *Microcystis* in a drinking water reservoir contaminated the water supply of a hemodialysis center in February 1996. Out of 131 patients treated, about 100 of them developed acute liver failure, 116 experienced visual disturbances, vomiting and nausea, and over 50 patients died ([Jochimsen et al., 1998](#)). Animal fatalities and severe poisonings associated with MCs bioaccumulation have also been recorded worldwide ([Tokodi et al., 2018](#); [Svircev et al., 2019](#); [Mohamed, et al., 2020](#)).

It is well established that MCs primary target organ is the liver with their main mechanism of action being induction of liver failure by inhibition of PP1 and PP2A in hepatocytes ([MacKintosh et al., 1990](#); [Jochimsen et al., 1998](#); [Fischer et al., 2005](#)). However, numerous investigators have also demonstrated that aside the liver, MCs can evoke negative significant health effects on the other organs including intestinal, renal, cardiovascular, reproductive and nervous system ([Cao et al., 2019](#); [Alosman et al., 2020](#); [Massey and Yang, 2020](#); [Xu et al., 2020](#)). Notwithstanding the intestine being the main site of MCs uptake, thus representing the first organ to come in contact with the toxins, it is strange to see the organ receiving little attention with regards to MCs toxic effects. Comparatively, the absorption, accumulation and subsequent effects of MCs are seldom characterized in the intestine. However, the scant literature available has generated convincing evidence, and has suggested the intestine as an important target organ of MCs. Thus, the present review focuses on the uptake, accumulation and impacts of MCs on intestinal health. In addition, the diverse methods utilized in assessing intestinal MCs toxicity were presented.

## 2. MICROCYSTINS UPTAKE

The esophagus, intestine and stomach form the gastrointestinal tract. The intestine is divided into various well defined anatomical regions. The duodenum, jejunum and ileum constitute the small intestine while caecum, colon, rectum and anus make up the large intestine.

The small intestine is part of the digestive system. It is a long tube-like organ that connects the stomach and colon ([Middendorp et al., 2014](#); [Volk and Lacy, 2017](#); [Kastl et al., 2020](#)). The small intestine performs a unique function in terms of absorption, digestion and immune function of the human body. The small intestine made up of duodenum, jejunum and ileum is considered as the most essential component to further absorb and digest food coming from the stomach. The duodenum is the first part of the small intestine that receives chyme (partially digested food) from the stomach and preparation of absorption begins from here.

Jejunum, which is the middle part mainly, facilitates the majority of nutrient assimilation and absorption. The ileum, identified as the third part and is attached to the colon basically absorbs residual nutrients and mediates transport of bile acids and vitamin B12 ([Middendorp et al., 2014](#); [Volk and Lacy, 2017](#); [Kastl et al., 2020](#)).

The large intestine is the terminal part of the alimentary canal. It runs from the appendix to the anus and frames the small intestine on three sides. The main function of this organ is to finish absorption of nutrients and water, synthesize certain vitamins, form feces, and eradicate feces from the body ([Hahn et al., 2019](#); [Vishy, 2019](#)). The caecum, colon, rectum and anus forms the main regions of the large intestine. The caecum reported as the first part of the large intestine, located in the right lower abdomen and connects the small intestine, colon accepts, and stores processed material from the small intestine (ileum) and passes it on to the colon. Colon, forming the major part of the large intestine is the principal place for water re-absorption and absorbs salts when required. The rectum serves as storage for leftover waste or food residue. The anal canal located in the perineum completely outside the abdominopelvic cavity forms the last section of the large intestine where leftover waste or food residue is emptied through the anus ([Hahn et al., 2019](#); [Vishy, 2019](#)).

Microcystins are one of the well-known toxins that have the ability to alter the functions of the intestine. Oral intake is the principal route of exposure to these toxins, with prolonged exposure leading to various health hazards. Once MCs are ingested, they first enter the intestine where most of these toxins are absorbed through the intestinal mucosal barrier (mucosal epithelial cells and mucosal lamina propria), and the absorbed MCs are transported through the bloodstream and distributed to the various organs ([Zhang et al., 2007a](#); [Greer et al., 2018](#)). Owing to the hydrophilic nature of these toxins, it is impossible for MCs to readily enter the cells following organism exposure. Therefore, a unique uptake mechanism is of greater importance. The hepatocytes absorb MCs mediated via the organic anion transporting peptides (OATPs) and bile acid transport system. OATPs are now being increasingly recognized to be expressed not only in the liver but also in the other organs. Evidence indicates that OATP mediated the MC-LR transport across the human blood–brain barrier (BBB) ([Fischer et al., 2005](#)). OATP demonstrated high-affinity uptake transporter for atorvastatin, exhibited in the vascular endothelium of the human heart ([Grube et al., 2006](#)). Feurstein et al. also reported that OATP was necessary for renal epithelial cells to actively uptake MCs ([Feurstein et al., 2009](#)). Of the numerous OATPs reported, OATP1A2, OATP1B1, OATP1B3 and OATP2B1 are widely studied in relation to their expression in the liver ([Drozdzik et al., 2014](#); [Thakkar et al., 2015](#); [Oswald, 2019](#)). OATP1B1 and OATP1B3 are most expressed on the sinusoidal side of hepatocytes ([Zeller et al., 2011](#); [Oswald, 2019](#)). OATP2B1 is expressed in the liver, brain and intestine. Interestingly OATP2B1 expressed in apical membrane of intestinal epithelial cells has not been classified as MCs transporter owing to scant literatures and also its poor transport. However, in a recent publication Li et al. (2019) found that the expression of OATP2B1 was positively correlated with MC-LR exposure dose. The findings suggest that OATP2B1 may be a candidate for transporting MCs to the intestine nevertheless its involvement in the toxin's uptake is still unclear. Further studies including western blot of OATP-transfected cells treated with MCs has been suggested to confirm whether OATP2B1 mediates the transfer of the toxins ([Li et al., 2019](#)). In the human intestinal tract, the localization of OATP 3A1 and 4A1 were confirmed at the cell membrane of CaCo-2 cells, and the OATPs were suggested to have played a vital role in the rapid uptake of MC-LR and MC-RR in the cell ([Zeller et al., 2011](#)). Although OATP1A2, OATP1A9, OATP2A1, OATP2B1, OATP3A1 and OATP4A1 have been discovered in the human intestinal tract, their involvement in MCs uptake needs to be determined and clarified ([Zeller et al., 2011](#); [Zhou et al., 2017](#); [Oswald, 2019](#)).

### 3. ACCUMULATION OF MICROCYSTINS IN THE INTESTINE

To evaluate the public health risk of exposure to MCs, the distribution pattern of different MC variants accumulated in the intestine was investigated. A number of studies have identified the intestine to be a potentially important target of MCs toxicity through its bioaccumulation ability under natural and

laboratory conditions (Table 1). Zhang et al. (2007a) reported different distribution of MCs concentrations in fish organs when the dominant freshwater phytoplanktivorous fish *Hypophthalmichthys molitrix* in Hangzhou, China's Tiesha River was studied. The intestines were the major organs total MCs (MC-LR and MC-RR) was found (6.49 µg/g FW). Zhang et al. (2007b) noted the presence of MC-RR, MC-YR, and MC-LR in the offspring of adult snail in Lake Taihu, China, and majority of the toxins 5.31 µg/g were identified in the intestine. In Lake Oubeira, Algeria, dominant MC variants including MC-LR, MC-YR and MC-(H-4)YR were observed, and the highest MC concentration in the intestine of omnivorous common carp (*Cyprinus carpio*) was 3,059 ng equivalent MC-LR/g DW, while that of carnivorous European eel (*Anguilla anguilla*) was from 66 to 233 ng equivalent MC-LR/g DW (Amrani et al., 2014). Ni et al. (2015) found ten MC variants (MC-LR, MC-RR, MC-YR, MC-LF, MC-LY, MC-LA and MC-LW, and other three undefined MC variants) in water samples of fish pond, and the highest MC concentration 2.28 µg/g DW was detected in the intestine of common carp (*Aristichthys nobilis*). Further using pig model to assess the effects of subchronic exposure to MC-LR through oral gavage over a period of 13 weeks, carried out at both the tolerable daily intake of 0.04 µg/kg bw per day and 2 µg/kg bw per day, free MC-LR was detected in the large intestine of two pigs from the higher dose group at 1.4 µg/kg DW (Greer et al., 2018). Exposing *Megalobrama amblycephala* to solutions with different concentrations of NH<sub>3</sub>-N (0, 0.06, 0.12 mg/L) and feeding with diets containing 15% and 30% of toxic cyanobacteria lyophilized powder for 30 days, 1.933 µg/g DW (highest concentration) of MCs was observed in the fish intestine (Xia et al., 2018). Moreover MC-RR and MC-LR at concentration 1.62 and 0.65 µg/g DW respectively, were observed in fish intestine from a man-made fishpond in Dongjituo town, Ninghe District, Tianjin, China (Bi et al., 2019). From three tropical fishponds in Egypt, free MC-LR, MC-RR and MC-YR at levels up to 11.8 ng/g was found in tilapia fish intestines (Mohamed et al., 2020).

The findings demonstrate the potential uptake and accumulation of MCs in the intestine. Presence of these toxins may cause significant damage to this organ. For dietary exposure of humans to MCs, WHO established a tolerable daily intake of 0.04 µg/kg body mass per day for the toxin (Chorus and Bartram, 1999). Majority of the studies above (summarized in Table 1) indicated higher MCs bioaccumulation exceeding the provisional tolerable daily intake set by WHO. Hence the accumulation of MCs in the intestine poses potential risks to the health of humans and other organisms who consume intoxicated animals including fish, snails, and pigs. People are therefore advised against any health risk resulting from food consumption, from MCs contaminated water bodies.

Table 1. Accumulation of microcystins in the intestine by various animals under different exposure, dose and length scenarios

Animal	MC/ concentration	Exposure route	Duration	MC detected	MC concentration detected	Organ/area identified	Reference
Mice	MC-LR (50-200 µg / kg)	I.P injection	14 days	MC-LR	-	Small intestine, caecum and colon	( <a href="#">Ito et al., 2001</a> )
Fish ( <i>Hypophthalmichthys molitrix</i> )	-	Oral (Tiesha River)	-	MC-RR and MC-LR	≥ 2 - 6.49 µg/g FW	Intestine	( <a href="#">Zhang et al., 2007a</a> )
Snail (offspring of adult snail's)	-	Oral (Lake Taihu)		MC-RR, MC-YR and MC-LR	≤ 5.31 µg/g	Intestine	( <a href="#">Zhang et al., 2007b</a> )
Fish (Mugilidae, <i>Liza</i> sp.)	-	Oral (Lake Albufera)	-	-	859 ± 128 ng/g	Intestine	( <a href="#">Romo et al., 2012</a> )
Fish ( <i>C. carpio</i> and <i>A. anguilla</i> )	-	Oral (Lake Oubeira)	-	MC-LR	≤ 3,059 ng equivalent MC-LR/g DW	Intestine	( <a href="#">Amrani et al., 2014</a> )
Fish (silver carp, bighead carp, crucian carp and common carp)	-	Oral (Lake Taihu)	-	MC-RR, MC-YR and MC-LR	1.19×10 <sup>3</sup> ± 454 ng/g DM	Intestinal walls	( <a href="#">Jia et al., 2014</a> )
Carp ( <i>Aristichthys nobilis</i> )	MC-LR, MC- RR, MC-YR, MC-LF, MC- LY, MC-LA and MC-LW, and other three undefined MC variants	Oral (Fish pond)	2 months	MC-LR, MC-RR, MC-YR, MC-LA and MC-LY	0.05 - 2.28 µg/g DW	Intestine	( <a href="#">Ni et al., 2015</a> )

Crayfish ( <i>Procambarus clarkii</i> )	MC-LR (0.1, 1, 10, 100 µg/L)	Oral	8 hr, 1, 3, 4 and 7 days	MC-LR	-	Intestine	( <a href="#">Yuan et al., 2016</a> )
Fish ( <i>Carassius gibelio</i> )	-	Oral (Lake Ludoš,)	-	MC-LR	0.08 - 0.27 ng/g DW	Intestine	( <a href="#">Tokodi et al., 2018</a> )
Pig	MC-LR ( 0.04 and 2 µg/kg bw)	Oral gavage	13 weeks	MC-LR	1.4 ng/g DW	Large intestine	( <a href="#">Greer et al., 2018</a> )
Blunt Snout Bream ( <i>Megalobrama amblycephala</i> )	-	Oral (feeding with diets)	30 days	MC-RR, MC-LR and MC-YR	≤ 1.933 µg/g DW	Intestine	( <a href="#">Xia et al., 2018</a> )
Zebrafish	MC-LR (0, 1, 5 and 20 µg/L)	Oral (fresh water containing the corresponding MC-LR)	30 days	MC-LR	0.07 ± 2.50 µg/L	Intestine	( <a href="#">Li et al., 2019</a> )
Fish	-	Oral (man-made fishpond)	-	MC-LR and MC-RR	0.65 - 1.62 µg/g DW	Intestine	( <a href="#">Bi et al., 2019</a> )
Nematodes <i>Caenorhabditis elegans</i>	MC-LR (0.1, 1 and 10 µg/L)	Oral	-	MC-LR	-	Intestine	( <a href="#">Qu et al., 2019</a> )
Tilapia fish	-	Oral (tropical fishponds)	12 months	MC-LR, MC-RR and MC-YR	≤ 11.8 ng/g FW	Intestine	( <a href="#">Mohamed et al., 2020</a> )



#### 4. IMPACTS OF MICROCYSTINS ON THE INTESTINE

Microcystins can induce different degree lesions in the intestine, which greatly depends on the toxin's variant, dosage and length of exposure (Table 2 and 3). The following are studies (both *in vivo* and *in vitro*) illustrating MCs toxicity on the intestine.

##### 4.1. Microstructure damage

Assessing MC-LR distribution in mice via oral administration, Ito et al. (2000) reported that the upper portion of the small intestine from a dead mouse (ICR, 49 weeks old), which was six times orally dosed with 500 mg/kg MC-LR, showed clearly stained surface epithelial cells of villi and lamina propria, and the surface of epithelial cells was eroded. In addition, the caecum and colon from a dead mouse (Balb/C, 24 weeks old) which was also given MC-LR orally at 500 mg/kg for one time indicated positive areas in the lamina propria, with faint staining in the epithelial cells. Goblet cells secreted positive mucus to the feces containing a large amount of positively stained material (Ito et al., 2000). Increased lipid peroxidation and decreased intraepithelial lymphocytes ( $28.7 \pm 5.0\%$  and  $44.2 \pm 8.7\%$ ) determined as a percentage of the number of enterocytes per villi was observed in small intestine of mice following a month's treatment with 50 and 100 mg MC-LR/kg (Sedan et al., 2015). Further Wu et al. (2018) reported loss of intestinal villi in mucosal layer, intestinal villi shedding in intestinal lumen, liquefaction necrosis in lamina propria, and irregular muscle fiber arrangement after mice were intraperitoneally injected with 12.5  $\mu\text{g/kg}$  MC-LR for 14 days. While disappearance of crypts, local liquefaction necrosis and shedding of villus were found in mucous layer, loose and irregular muscle fibers arrangement and much intestinal fluff tissue with necrosis or shedding were observed in the intestinal lumen following 25  $\mu\text{g/kg}$  MC-LR mice exposure. Cao et al. (2019) also found that mice exposed to 1  $\mu\text{g/L}$  MC-LR indicated increased goblet cells by 38%, and disordered arrangement of intestinal epithelial cells. Mice exposed to 30  $\mu\text{g/L}$  and 60  $\mu\text{g/L}$  MC-LR demonstrated invaginated and serrated intestinal villi, as well as disordered arrangement of intestinal epithelial cells and lymphocyte infiltration, respectively. While mice exposed to 90  $\mu\text{g/L}$  MC-LR showed obvious lymphocyte infiltration and disordered crypts, mice exposed to 120  $\mu\text{g/L}$  MC-LR indicated invaginated and serrated intestinal villi. The evidence indicates that exposure to MCs can affect mice intestinal epithelial cells, goblet cells, mucosa, villi, microvilli, crypts, lamina propria and lymphocyte.

Silver carp (*Hypophthalmichthys molitrix*) exposed to *Microcystis aeruginosa* NPLJ4 containing [D-Leu<sup>1</sup>]MC-LR for 5 days showed muscular layers cells separation due to the loss of intracellular adhesion, extravasation of blood cells in the connective tissue resulting to haemorrhage, and loss of adhesion between the columnar epithelium cells (Ferreira et al., 2010). After 10 days of fish exposure, serious deformities in blood vessels and smooth muscle layers in intestinal tract, submucosa haemorrhaging vessels and separation of epithelium cells with no clear boundaries (some in necrosis) were also found. Interestingly, the same pattern of injuries found at 10 and to a lesser extend 5 days were apparent after 15 days of toxin exposure (Ferreira et al., 2010). Chen et al. also reported that zebrafish exposed to 1  $\mu\text{g/L}$  MC-LR showed partial desquamation and epithelium with abundant microvilli. Zebrafish exposed to 5  $\mu\text{g/L}$  MC-LR revealed some enterocytes were necrotized, partial desquamation and disordered enterocytes with partial loss. Zebrafish exposed to 20  $\mu\text{g/L}$  MC-LR indicated shortened intestinal villi with necrosis in the apical area, intestinal epithelium with partial loss and total loss of microvilli (Chen et al., 2016). While Tilapia fish fed with commercial fish food mixed with 0.15 g of lyophilized *Microcystis* cells (approximately 30  $\mu\text{g}$  MC-LR/fish/day) indicated slight damages in intestinal mucosa after 14 days, Tilapia fish fed with commercial fish food mixed with 0.30 g of lyophilized *Microcystis* cells (approximately 60  $\mu\text{g}$  MC-LR/fish/day) demonstrated severe degenerative and necrotic changes in the intestinal mucosa (Preeti et al., 2016). Further fish (*common carp* and *Cyprinus carpio*) observed from blooming fishponds containing MCs demonstrated severe hyperplasia of intestinal epithelial cells which led to fusion of villi, necrosis and vacuolization in the epithelial cells, and epithelial cells in the apical part of the villi were desquamated. Edematous alterations were also found in the lamina propria which resulted in its thickening, and the cells showed nuclear lysis, pyknotic nuclei, and an unclear cell boundary (Drobac et al., 2016). In a current study, edematous alterations in lamina propria with its subsequent dilation, necrosis and subsequent desquamation of enterocytes mostly

in the apical parts of the villi and hypertrophies of goblet cells were indicated in fish from Lake Ludoš, Serbia, containing MCs (MC-LR, MC-dmLR, MC-LY, MC-LW, MC-LF, MC-RR, MC-dmRR, MC-YR, and -dmYR) (Tokodi et al., 2018). Zhang et al. also found eosinophilic granule cells, abnormal muscularis and infiltration of lamina propria by lymphocyte in the intestine of crayfish treated with 10 and 40 µg/L MC-LR (Zhang et al., 2020). The findings signify that exposure to MCs can impact fish intestinal epithelial cells, villi, microvilli, crypts, mucosa, lamina propria and lymphocyte. It should be noted that the microstructure damage induced by MCs in mice intestine is similar to that of fish, affirming the exposure manifestations of these toxins on intestinal tissue.

Tadpoles are directly exposed to and consume MCs, making them vulnerable and susceptible to the toxins. Nonetheless using frog model to determine the level of microstructure damage of intestine, various researchers suggested that some species of frog are likely to be at high risk to MCs hazards than others. *Lithobates catesbeiana* tadpoles exposed to *Microcystis aeruginosa* NPLJ4 containing [D-Leu<sup>1</sup>]MC-LR for 16 days demonstrated increased number of granulocytes through the migration of cells from the deeper layers of tissue to the base of the epithelial tissue, intense blood supply around the granulomatous areas, and increased fibrosis in the connective tissue layer beneath the epithelium. The enterocytes also indicated lesions as the presence of cytoplasmic vacuoles and clusters of melano-macrophage were found among the epithelial cells (Pires et al., 2018). In an acute short-term assessment, bullfrog (*Lithobates catesbeiana*) tadpoles exposed to 1 µg/L MCs for 7 days also revealed decreased intestinal fold heights, and increased intestinal diameter, representing pathological intestinal distension (Su et al., 2020). Conversely *Xenopus laevis* tadpoles at stage 52 exposed for 1, 3, 7, and 21 days to diets containing lyophilized cyanobacterial biomass with MC-LR at concentrations of 42.8 and 187.0 µg MC-LR/g diet, respectively exerted no toxic manifestations (Zikova et al., 2013). Interestingly at the high concentrations (2000 µg/L) of MC-LR and MC-RR up to 5 days, Fischer and Dietrich (2000) found no effect of the toxins in early life-stages of African clawed frog (*Xenopus laevis*) nor the toxins bioaccumulation. The findings necessitate further studies on long-term and short-term effects of MCs.

In the study of Yuan et al., MC-LR alone failed to alter intestinal permeability in nematodes. Conversely, combinational treatment of 0.1 µg/L MC-LR and 1 µg/L nanopolystyrene resulted to drastic enhancement in intestinal permeability of nematodes, suggesting the toxins ability to induce intestinal harm with nanopolystyrene (Yuan et al., 2016). A recent investigation also found significant decrease in the length of mice colon after orally administering 1000 µg/kg MC-LR to the animals for 14 days. In addition, the colon tissue showed continuous staining of goblet cells and mucin throughout the length of the colon. On the other hand, the mice treated with Dextran Sulfate Sodium (3% DSS) + MC-LR (1000 µg/kg) experienced significantly shorter colon lengths, sever disruption of the epithelium, segmental regions of marked acute inflammatory cell infiltration, ulceration and branching and budding of glands. An excessive decrease in goblet cells and mucin depletion were also found (Su et al., 2019). The study results also suggest that MC-LR together with other sulfate can induce serve histopathological effects on the colon.

Non-alcoholic fatty liver disease (NAFLD) many a time causes cardiovascular, renal and intestinal complications. The prevalence of NAFLD is increasing proportionately. In a sub-chronic study, changes in the microbiome were strongly found to be associated with inflammatory pathology in the intestine after NAFLD mice were administered with MC (10 µg per kg, 5 dosages per week), through intraperitoneal route for two weeks, and immortalized rat intestinal epithelial cell line (IEC-6) was exposed to MC (100 µg/mL) for 24 hr (Sarkar et al., 2019). The intestinal inflammatory pathology included extremely disturbed villi structure, distorted lumen, eroded epithelial cells, abscess crypt cell and increased crypt cells granulation. The study thus suggests that, NAFLD may influence inflammatory pathology in the intestine induced by MCs.



#### 4.2. Apoptosis

Studies have revealed the negative effects of MCs on intestinal health through inducing apoptosis. Apoptosis is characterized morphologically via the condensation of nuclear chromatin and cytoplasm, shrinkage of cell, detachment of cells from surrounding cells, membrane blebbing and development of apoptotic bodies. During apoptosis DNA is fragmented and proteases are released (Botha et al., 2004a; Li and Ma, 2017). Botha et al. investigated the apoptotic effect of intraperitoneal administered MC-LR over time on the duodenum, jejunum and ileum of mice receiving a single 75% LD<sub>50</sub> dose. The apoptotic index was found to be significantly raised in all sections at 8 hr post exposure and continued to rise for the 16, 24 and 32 hr groups. The duodenum ( $4.25 \pm 0.125\%$ ) exhibited the most significant increase in apoptotic index followed by the jejunum ( $2.5 \pm 0.15\%$ ) and ileum ( $1.75 \pm 0.125\%$ ) (Botha et al., 2004b). Resorption of enterocytes (intestinal absorptive cells) which resulted from apoptonecrosis, and associated with disorganization of the surrounding interstitial tissue (connective tissue) was reported after 5 µg MC-LR dissolved in 5 mL water per gram bodyweight was administered directly into medaka fish stomach (Djediat et al., 2010). Identifying the pro-apoptosis proteins including bax, caspase-3 and caspase-9, the content of bax, and caspase-9 were increased in intestine when the effects of MC-LR exposure on apoptosis-related proteins in small intestine of mice were investigated. Interestingly MC-LR was not successful to significantly change the expression of caspase-3 (Wu et al., 2018). Further prolonged exposure of tadpoles to [D-Leu<sup>1</sup>]MC-LR caused toxic effects within the intestines and on subsequent days of exposure, the intestine indicated signs of change as a greater number of apoptotic epithelial cells (Pires et al., 2018). In a current study, *Microcystis aeruginosa* (at a density of  $1.59 \times 10^5$  cells mL<sup>-1</sup>) and MC-LR (at concentration 100 µg/L) demonstrated distinct histological variation and apoptosis characteristics in shrimp intestine after 72 hr of exposure (Duan et al., 2020). Twisted and shed epithelial cells from the basement membrane, as well as apoptosis characteristics in the epithelial cells were found in both *Microcystis aeruginosa* and MC-LR groups. The findings suggest that *Microcystis aeruginosa* and MC-LR may exhibit similar toxic effect when exposed to them.

Taking into consideration ingestion as a foremost exposure pathway, cell lines like CaCo-2 cells that bear a resemblance to the intestine are appropriate for studying the effects of MCs. It is worthwhile knowing that the toxins have been found to evoke apoptotic cell death *in vitro*. Exposing CaCo-2 (a human colon carcinoma cell line) cells to 50 µM MC-LR, the toxin was noted to cause apoptosis in the hepatic cell line. Apoptosis was observed to occur in CaCo-2 cells after 48 hr (Botha et al., 2004a). In a comparative cellular toxicity study, CaCo-2 cells treated with MC-LW and MC-LF at concentration 1, 10 and 50 µM for 22 and 44 hr demonstrated obvious morphological changes including apoptotic features with shrinkage, blebbing and loss of cell contact which occurred in a time and dose-dependent way (Vestervik et al., 2012). Zhou et al. (2017) also examined the toxic consequences and possible mechanisms of MC-LR at a concentration of 0, 6.25, 12.5, 25 and 50 µM on barrier function of the intestinal epithelial cells and indicated significant decrease in cell viability, increase in apoptotic cells ratio (after exposure to 12.5 µM and higher MC-LR concentration), decline in occludin and zonula occludens-1 (ZO-1) expression and decrease in PP2A activity (from 12.5 µM MC-LR concentration). These results provide insight that strengthens the concept that the intestine is an important target for water contaminants like MCs to induce apoptosis.

#### 4.3. DNA damage

In a single acute MC-LR treatment, the toxin was reported to cause DNA damage which was expressed via the median analyses of Olive Tail Moment (OTM) and percentage of tail DNA in mice (Gaudin et al., 2008). Mice treated with single oral doses 2 and 4 mg MC-LR/kg bw indicated no DNA effect in the ileum after 3 and 24 hr. Although, no effect was noted on the colon after 3 hr, DNA damage was apparent after 24 hr. Mice treated with 10, 25, 40 and 50 µg MC-LR/kg bw through intraperitoneal route also showed no effect in the ileum and colon, for the lowest dose of MC-LR ( $\leq 25$  µg/kg bw) after 3 hr of treatment. Besides, no significant increase was also observed for the highest doses (40 and 50 µg/kg bw) in ileum and colon with OTM or percentage of tail DNA. However, MC-LR at doses 25 and 40 µg/kg bw induced DNA damage expressed through the median OTM and percentage of tail DNA in ileum and colon after 24 hr

(Gaudin et al., 2008). The data suggest that intestinal MC-LR DNA damage may be time and dose dependent. On the contrary, in the subchronic exposure of rats to sublethal dose of MC-YR, the toxin was indicated to induce DNA damage in brain, liver, kidney and lung but intestine (Filipic et al., 2007). The evidence suggests that intestinal DNA induction may be subjected to the MC variant exposed to.

*In vitro*, Žegura et al. demonstrated a dose and time dependent increase of DNA strand breaks when the effect of MC-LR on DNA damage in CaCo-2 cell line was studied. At 5 µg/ml the highest MC-LR concentration, a significant increase of DNA damage was evident after 2 hr of incubation (mean value of the % tail DNA was 21.6). After 4 hr at all MC-LR concentrations, the maximal DNA strand break induction was observed (mean values of the % tail DNA were 19.6, 18.1 and 22.3 at 0.2, 1.0 and 5.0 µg/ml MC-LR, respectively). Interestingly, DNA damage gradually declined with further exposure and after 12 hr, no difference between exposed and control cells were noted. A dose dependent reduction of cell viability was also observed in the cells (Žegura et al., 2008). Assessing the effect of 5 and 10 µM MC-LR on intestinal DNA damage via NCM460 intestine cell line, Wen et al. (2019) also found that the toxin invoked intestinal DNA destruction through increasing OTM, inhibiting PP2A activity, elevating reactive oxygen species levels (ROS) and enhancing gamma-H2AX and p-p53 protein expression levels after 24 hr. The findings signify that MC-LR may cause intestinal DNA damage through inhibition of PP2A activity, elevation of ROS and activation of protein. DNA damage induced in the intestinal tissues may contribute to an increased cancer risk.

#### 4.4. Inflammation

Growing evidence from *in vivo* studies has proven that MC-LR can also cause changes in the expression of inflammatory factors and induce inflammation in the intestine. It is of interest that chronic inflammation plays a vital role in early carcinogenesis. Assessing the effects of MC-LR exposure on inflammation factors in small intestine of mice, Wu et al. (2018) found that MC-LR could result in a dose-dependent down-regulation of IL-1β, IL-6 and TNF-α (pro-inflammatory factors). Higher IL-1β level was observed in mice exposed to 25 µg/kg MC-LR compared to mice exposed to 12.5 µg/kg MC-LR. Interestingly almost no IL-6 was noted in mice exposed to 25 µg/kg MC-LR. Besides, no obvious changes in TGF-β were found. Cao et al. (2019) also demonstrated that exposure to MC-LR could altered the expression levels of inflammation-related factors TNF-α, IL-1β, and IL-8 (pro-inflammatory factor) as well as IL-10 and TGF-β1 (anti-inflammatory factor) of jejunum in mice at different MC-LR concentrations (1, 30, 60, 90 and 120 µg/L). TNF-α was up-regulated in ≤ 30 µg/L MC-LR, but down-regulated in ≥ 60 µg/L MC-LR. IL-1β was up-regulated in ≤ 60 µg/L MC-LR, but down-regulated in ≥ 90 µg/L MC-LR. IL-8 was up-regulated in ≤ 30 µg/L MC-LR, down-regulated in ≤ 90 µg/L MC-LR and no significant change was noted in 120 µg/L MC-LR. Although at 1 µg/L MC-LR no significant change was found in IL-10, it was down-regulated in ≥ 30 µg/L MC-LR. TGF-β1 was up-regulated in ≤ 30 µg/L MC-LR, down-regulated in 60 µg/L MC-LR and at ≥ 90 µg/L MC-LR there were no significant change.

Su et al. (2019) reported that MC-LR together with other sulfate can alter key pro-inflammatory transcripts within colonic tissue and induce inflammation. While MC-LR exposure alone did not cause inflammation, mice treated with DSS + MC-LR resulted to a rise in the pro-inflammatory transcripts within colonic tissue (TNF-α, IL-1β, CD40 and MCP-1) and pro-fibrotic marker, PAI-1 was observed. Assessing the effects of *Microcystis aeruginosa* and MC-LR exposure on the intestinal microbiota variation and immune responses of *Litopenaeus vannamei* (shrimp), Duan et al. (2020) also noted an increase in pro-inflammatory cytokines (MyD88, Rel, TNF-α), a pattern-recognition receptor (TLR4) and a mediator of apoptosis (Casp-3).

Comparing the effects of MC-LR and MC-RR in CaCo-2 cells by assessing the cellular pro-inflammatory response IL-6 and IL-8 generation, Huguet et al. (2013) found a substantial increase in IL-6 secretion in differentiated CaCo-2 cells after 24 hr of MC-RR exposure. Compared to the solvent control, a 1.5-fold increase in IL-6 secretion was indicated at 100 µM MC-RR, while at lower concentrations no considerable

effect was noted. Interestingly, a significant rise in IL-8 secretion was also demonstrated after 24 hr of MC-LR exposure, and to a lesser extent with MC-RR. Between 50 to 100  $\mu$ M MC-LR, an increase in IL-8 secretion (33-55-fold) was shown in comparison to the solvent control. At 100  $\mu$ M MC-RR (9-fold), a considerable rise in IL-8 secretion was found (though it was significantly lower compared to that of MC-LR) ([Huguet et al., 2013](#)). Although both MC-LR and MC-RR were found to cause toxic manifestation in the human intestinal cells, the observation suggests that pro-inflammatory secretion may be dependent on the MC variants and concentration.

Table 2. Summary of intestinal toxicity induced by microcystins and determinants *in vivo* studies.

Experimental animal	Exposure route	MC variant(s)	MC concentration	Exposure time	Intestinal effect	Determinant	Reference
Mice	Oral	MC-LR	500 mg/kg	1, 6, 7, 12 and 13 weeks	Stained surface epithelial cells of villi and lamina propria, and the surface of epithelial cells was eroded. The caecum and colon from a dead mouse which was also given MC-LR orally at 500 mg/kg for one time indicated positive areas in the lamina propria, with faint staining in the epithelial cells	Immunostaining	<a href="#">(Ito et al., 2000)</a>
Mice	I.P injection	MC-LR	75% LD <sub>50</sub> dose	8, 16, 24 and 32 hr	Duodenum, jejunum and ileum exhibited apoptotic effect	Histopathological observation (HE staining)	<a href="#">(Botha et al., 2004b)</a>
Mice	Oral and I.P injection	MC-LR	2 and 4 mg/kg bw, 10, 25, 40 and 50 µg/kg bw	3 and 24 hr	DNA-damage in ileum and colon	Single-cell gel electrophoresis and comet assay	<a href="#">(Gaudin et al., 2008)</a>
Fish	Oral	MC-LR	5 µg/L bw	One time administration	Resorption of enterocytes resulting from apoptonecrosis, and associated with disorganization of the surrounding interstitial tissue	Immunohistochemical	<a href="#">(Djediat et al., 2010)</a>
Fish	Oral	[D-Leu <sup>1</sup> ]MC-LR	-	5, 10 and 15 days	Muscular layers cells separation, deformities in blood vessels, submucosa haemorrhaging vessels and separation of epithelium cells with no clear boundaries (some in necrosis)	Histopathological observation (HE staining)	<a href="#">(Ferreira et al., 2010)</a>

Medaka fish	Oral	MC-LR	5 µg/L	30 days	Several zones of lysis in intestinal epithelium and fewer goblet cells were apparent. High magnification, showed cellular disjunctions and ultrastructural observations revealed lysosomes and residual bodies within the cytoplasm of some enterocytes. Damaged microvillousities and general cellular disjunctions were found. Cells with stained granular cytoplasm were also shown	Histopathological observation	( <a href="#">Trinchet et al., 2011</a> )
Mice	Oral	MC-LR	50 and 100 µg/kg bw	1 month	Decreased intraepithelial lymphocytes	Histopathological observation (HE staining, Oil Red, Trichrome and PAS) and inhibition phosphatase assay	( <a href="#">Sedan et al., 2015</a> )
Zebrafish	Oral	MC-LR	1, 5 and 20 µg/L	3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 days	Partial desquamation and epithelium with altered microvilli, shortened intestinal villi, necrotized intestinal epithelium and enterocytes, and disordered enterocytes with partial loss	Histopathological observation (HE staining)	( <a href="#">Chen et al., 2016</a> )
Fish (Fishponds)	Oral	-	-	Observed for 4 months	Severe hyperplasia of intestinal epithelial cells. Edematous alterations were found in the lamina propria.	Histopathological observation (HE staining)	( <a href="#">Drobac et al., 2016</a> )
Fish	Oral	MC-LR	30 and 60 µg/fish/day	14 days	Severe degenerative and necrotic changes in the intestinal mucosa	Histopathological observation (HE staining)	( <a href="#">Preeti et al., 2016</a> )
Fish (Lake Ludoš)	Oral	-	-	2011 (July and August) and 2012 (March and August)	Edematous alterations in lamina propria with its subsequent dilation. Necrosis and subsequent desquamation of enterocytes. Hypertrophies of goblet cells.	Histopathological observation (HE staining) and LC-MS	( <a href="#">Tokodi et al., 2018</a> )
Mice	I.P injection	MC-LR	12.5 and 25 µg/kg	14 days	Dose-dependent pathological lesions to small intestine. MC-LR inhibited the secretion of inflammatory cytokines (TNF-α,	Histopathological observation (HE staining) and RT-	( <a href="#">Wu et al., 2018</a> )

					IL-1 $\beta$ , IL-6) with no obvious effect on anti-inflammatory factors TGF- $\beta$ . MC-LR promoted the expression of bax, caspase-3 and caspase-9 in small intestine.	PCR	
Tadpoles	Oral	[D-Leu <sup>1</sup> ]MC-LR	-	2, 4, 6, 8, 10, 12, 14 and 16 days	Increased number of granulocytes, intense blood supply around the granulomatous areas, and increased fibrosis in the connective tissue layer beneath the epithelium. The enterocytes also indicated lesions as the presence of cytoplasmic vacuoles and clusters of melano-macrophage were found among the epithelial cells	Histopathological observation (HE staining)	(Pires et al., 2018)
Mice	Oral	MC-LR	1, 30, 60, 90 and 120 $\mu$ g/L	6 months	Microstructure of the jejunum was destroyed and expression levels of inflammation-related factors IL-1 $\beta$ , IL-8, TNF- $\alpha$ , TGF $\beta$ 1 and IL-10 were altered at different MC-LR concentrations	Histopathological observation (HE staining) and qRT-PCR	(Cao et al., 2019)
NAFLD mice	I.P injection	MC	10 $\mu$ g/kg	2 weeks	Changes in the microbiome were associated with inflammatory pathology in the intestine	LAL assay, histopathological observation (HE staining), immunohistochemistry (IHC staining) and immunofluorescence microscopy	(Sarkar et al., 2019)
Mice	Oral	MC-LR	1000 $\mu$ g/kg	14 days	Significant decreases in colon length. Colon tissue showed continuous staining of goblet cells and mucin throughout the length of the colon	Histopathological observation (HE staining) and RT-qPCR	(Su et al., 2019)
Crayfish	Oral	MC-LR	10, and 40 $\mu$ g/L	96 hr	Eosinophilic granule cells, abnormal muscularis and infiltration of lamina propria by lymphocytes	Histopathological observation (HE staining)	(Zhang et al., 2020)



Shrimp	Oral	MC-LR	100 µg/L	72 hr	Distinct histological variation and apoptosis characteristics, and increased pro-inflammatory cytokines (MyD88, Rel, TNF-α)	Histopathological observation (HE staining and TUNEL analysis) and RT-qPCR	( <a href="#">Duan et al., 2020</a> )
Tadpoles	Oral	MC	1 µg/L	7 days	Increased intestinal diameter, decreased intestinal fold heights, and a constant number of intestinal folds.	Histopathological observation (HE staining)	( <a href="#">Su et al., 2020</a> )

Table 3. Summary of intestinal toxicity induced by microcystins and determinants *in vitro* studies.

Cell	MC variant(s)	MC concentration	Exposure time	Intestinal effect	Determinant	Reference
CaCo-2 (Human colon adenocarcinoma)	MC-LR	50 mM	48 hr	Induced apoptosis	MTT assay and apoptotic indices	( <a href="#">Botha et al., 2004a</a> )
CaCo-2	MC-LR	0.2, 1, 5 and 10 µg/ml	2, 4, 6, 12 and 16 hr	A dose dependent reduction of cell viability as well as dose and time dependent increase of DNA strand breaks	MTT assay and comet assay	( <a href="#">Žegura et al., 2008</a> )
CaCo-2	MC-LF and MC-LW	1, 10 and 50 µM	22 and 44 hr	Apoptotic features (including shrinkage and blebbing, and loss of cell-cell adhesion)	Cell morphology, cell proliferation, cytotoxicity assay and protein phosphatase inhibition	( <a href="#">Vestervik et al., 2012</a> )
CaCo-2	MC-LR and MC-RR	1, 5, 10, 50, 80, and 100 µM.	24 hr	Cytotoxicity was two-fold greater with MC-LR as compared to MC-RR with 100 µM MCs treatment after 24 hr. IL-6 secretion were similar following 24 hr treatment with either MCs. 100 µM MC-LR induced a five-fold greater IL-8 secretion when compared to MC-RR	Cytotoxicity assay and ELISA	( <a href="#">Huguet et al., 2013</a> )
Rat intestinal epithelial cells (IEC-6)	MC-LR	6.25, 12.5, 25 and 50 µM	6, 12 and 24 hr	Significant decrease in cell viability, increase in apoptotic cells ratio (after exposure to 12.5 µM and higher MC-LR concentration), decline in occludin and zonula occludens-1 (ZO-1) expression and decrease in PP2A activity (from 12.5 µM MC-LR concentration)	Cell viability assays, cell apoptosis assays, immunofluorescent assays, PP2A activity assay and western blot	( <a href="#">Zhou et al., 2017</a> )
Human intestinal epithelial cells (NCM460)	MC-LR	5 and 10 µM	24 hr	DNA damage	CCK-8 assay, comet assay, PP2A assay and western blot	( <a href="#">Wen et al., 2019</a> )

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Rat (IEC-6)	MC	100 µg/mL	24 hr	Changes in the microbiome were associated with inflammatory pathology in the intestine	qRTPCR, ELISA kit and immuno-fluorescence microscopy	( <a href="#">Sarkar et al., 2019</a> )
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## 5. DETERMINANTS OF MICROCYSTINS ON THE INTESTINE

Microcystins can accumulate in aquatic and terrestrial animals. Presence of these toxins in mammals and fish may result to severe damages and dysfunction in the intestine. Different researchers employ diverse methods to analyze the bioaccumulation and toxic effects of MCs on the intestine (Table 2 and 3). The following are studies demonstrating the various methods used to determine intestinal MCs bioaccumulation and/or toxic consequences.

### 5.1. In vivo studies

Ito et al. (2000) applied a slightly different immunostaining method to investigate the absorption, distribution and intestinal effect of MC-LR on mice. Gaudin et al. (2008) utilized single-cell gel electrophoresis (SCGE) or comet assay in mice to determine the DNA-damage induction by MC-LR on intestinal tissues (ileum and colon). Further, the intestinal apoptonecrosis which resulted to resorption of enterocytes following mice treatment with MC-LR was determined by immunohistochemical ([Djediati et al., 2010](#)). Several other investigators including Drobac et al. (2016), Pires et al. (2018), Wu et al. (2018), Cao et al. (2019), Sarkar et al. (2019), Su et al. (2020), Duan et al. (2020) and Zhang et al. (2020) also used histopathological observation to assesses the intestinal injuries and pathology induced by diverse MC variants (Table 2). The emergence of hematoxylin and eosin staining (HE staining) is the most widely documented recoloring system utilized in histology to determine MCs tissue or organ toxicity.

The analytical methods including biochemical (protein phosphatase inhibition assay (PPIA) and enzyme linked immunosorbent assay (ELISA)), as well as chemical (high performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS)) have also shown MCs bioaccumulation and toxic manifestations in various animals (Table 2). The inhibition phosphatase assay was one of the methods used to detect the presence of MC-LR and the subsequent decrease in intraepithelial lymphocytes in mice intestine ([Sedan et al., 2015](#)). MCs are specific inhibitors of PP1 and PP2A and thus make PPIA suitable to detect MCs and the subsequent effects. In the largest coastal Spanish Mediterranean lake (Albufera of Valencia), ELISA was used to investigate the occurrence and distribution of MCs in water, cell-bound and intestine of commercial mugilid *Liza* sp. (fish) ([Romo et al., 2012](#)). ELISA MC plate kit was employed to determine the accumulation and concentration of MC-LR in zebrafish intestine and the ex vivo intestine model developed using intestines of 6 fish species including *Aristichthys nobilis*, *Carassius auratus*, *Ctenopharyngodon idellus*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, and *Megalobrama amblycephala* ([Li et al., 2019](#)). Mohamed et al. also utilized ELISA kits to ascertain free MC-LR, MC-RR and MC-YR in tilapia fish intestines from three tropical fishponds located in Sohag province, southern Egypt ([Mohamed et al., 2020](#)). It is worthwhile knowing that the antibodies developed against  $\beta$ -amino acid Adda found in most MC variants, have made developments of ELISA and its subsequent utilization in determining biological evidence of fish and mammalian exposure to the toxins possible.

The HPLC and its linked techniques are commonly and widely used in the laboratory to analyze MCs by means of different stationary and aqueous mobile phases containing methanol or acetonitrile. Ultraviolet-visible spectroscopy (UV-Vis) absorbance and photo-diode array (PDA) detection techniques are mostly associated with HPLC system. MC-RR, MC-YR and MC-LR observed in the offspring of adult snail's intestine from Lake Taihu, China were determined by liquid chromatography electrospray ionization mass spectrum (LC-ESI-MS) ([Zhang et al., 2007b](#)). In the study of Jia et al. (2014) concentrations of MC-LR, MC-YR and MC-RR were quantified in four fish species (silver carp, bighead carp, crucian carp and common carp) from Lake Taihu using HPLC interfaced with a triple quadrupole, tandem mass spectrometer (MS/MS). Further Tokodi et al. (2018) also utilized LC-MS/MS to show the intestinal deleterious effect induced by MC-LR on fish (*Prussian carp*) from Lake Ludoš in the province of Vojvodina in northern Serbia. Xia et al. (2018) applied Finnigan LC-MS system comprising a thermo surveyor auto sampler, a surveyor MS pump, a surveyor PDA system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer equipped with an atmospheric pressure ionization fitted with an ESI to reveal the intestinal MCs concentration of *Megalobrama*

*amblycephala*. Ultra-performance liquid chromatography tandem mass spectrometry (UPLC- MS/MS) was developed and used to determine free MC-LR in the large intestine of pigs (Greer et al., 2018).

## 5.2. In vitro studies

Various other methods including the analytical have also demonstrated MCs bioaccumulation and toxic impact on diverse cell lines mainly under laboratory conditions *in vitro* (Table 3). Žegura et al. (2008) applied MTT and comet assay to illustrate the DNA effect of 0.2, 1, 5 and 10 µg/ml MC-LR on CaCo-2 cells. Treating CaCo-2 cells with MC-LR and MC-RR at concentrations ranging from 1 to 50 µM, a rapid uptake of the MC variants (with similar profile) in less than 60 min was reported, analyzed by immunolocalization using an anti-MC antibody (Zeller et al., 2011). While Vesterkvist et al. (2012) used cell morphology, cell proliferation, cytotoxicity assay and protein phosphatase inhibition to examine the harm of MC-LF, MC-LW and MC-LR on CaCo-2 cells following the toxins treatment at concentrations 1, 10 and 50 µM, Huguet et al. (2013) used cytotoxicity assay and ELISA to assess the hazard of MC-LR and MC-RR on CaCo-2 cells after the toxins exposure at concentrations 1, 5, 10, 50, 80, and 100 µM. Cell viability assays, cell apoptosis assays, immunofluorescent assays, PP2A activity assay and Western blot were also used to determine the accumulation, concentration and apoptotic effect of MC-LR in rat IEC-6 following the toxin's exposure at 6.25, 12.5, 25 and 50 µM (Zhou et al., 2017). Wen et al. (2019) also utilized CCK-8 assay, comet assay, PP2A assay and Western blot to uncover the intestinal DNA damage on NCM460 cells following 5 and 10 µM MC-LR treatment. Exposing rat IEC-6 to 100 µg/mL MC, qRT-PCR, ELISA kit and immuno-fluorescence microscopy were used to investigate the microbiome alterations (Sarkar et al., 2019).

## 6. CONCLUSION

Microcystins are generated by certain bloom-forming cyanobacteria and may harm the various mammalian and fish tissues as well as organs. In this review the impact of MCs on intestinal health were reported. Collectively, data summarized from both *in vivo* and *in vitro* studies demonstrated that MCs exert significant toxic effects on the intestine via damaging the microstructure and DNA, as well as inducing apoptosis and inflammation due to its bioaccumulation ability for the toxins. Higher MCs concentration and/or longer exposure duration to the toxins were found to extensively influence the severity of intestinal injury. Various methods including ELISA, HPLC, LC-MS, PPIA, qPCR, MTT assay, comet assay, Western blot and histopathological observation were utilized to determine the intestinal bioaccumulation and toxic manifestations induced by MCs. Figure 1 summarizes the intestinal toxicity of MCs. The observations further suggest that serious risks to animals and public health are possible to occur. Since the damage MCs inflict on intestine is undoubtedly associated to human health, this review should be valuable to analyze the potency of MCs toxicity and aid in the toxin's diagnosis. Given that the mechanism involved in MCs intestinal toxicity is not clear, it was not considered. However further studies to explore the intestinal toxic mechanism(s) is warranted for scientific study.

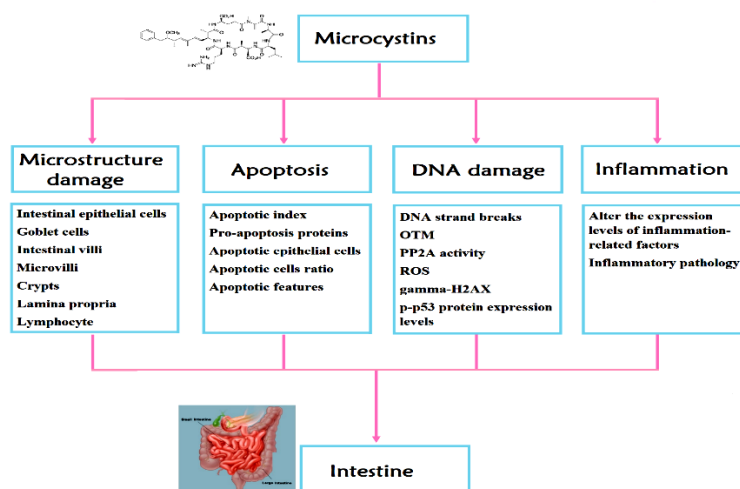


Figure 1. Intestinal toxicity induced by microcystins. The toxins are taken up into the intestines through organic anion transporting peptides (OATPs). The presence of microcystins in the intestine may damage the

microstructure and DNA, as well as induce apoptosis and inflammation.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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### Data Availability

Data used for this research is available upon request from the corresponding author.

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