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Effects of cyanobacterial toxins on the human gastrointestinal tract and the mucosal innate immune system

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Abstract: Cyanobacterial blooms occur with increasing frequency in freshwater ecosystems, posing a hazard to human and environmental health. Exposure of human to cyanobacterial metabolites occurs mostly via accidental ingestion through contaminated drinking water or during recreational activities and, most frequently, results in gastrointestinal symptoms. Despite the clinical manifestation, cyanobacterial metabolites are rather investigated for their toxicity towards specific organs or tissues, especially hepato-, nephro- and neurotoxicity, then for effects on the gastrointestinal tract and the associated lymphoid tissue.

The aim of this review was to systematically summarize available literature on the effects on the gastrointestinal tract and the mucosal innate immune system and compile the data from both, *in vitro* and *in vivo* studies, focusing on human-health relevant models. Our systematic literature review revealed significant data gaps in the understanding on metabolites breaching the gastrointestinal barrier and the role of the immune system in the establishment of clinical symptoms. Microcystins and cylindrospermopsin were linked to gastrointestinal symptoms, immune system effects or both. Furthermore, implications for cyanobacterial bloom

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lipopolysaccharides in gastrointestinal inflammation were reported in several cases, while other metabolites received only minor attention.

The collected data indicate the need for a reassessment of potential enterotoxicity of microcystins and cylindrospermopsin. Additionally, the carcinogenic potential of cyanotoxins, especially microcystins, has to be clarified, as an increasing amount of epidemiological studies show correlations between cyanobacterial blooms and gastrointestinal cancer incidence. Furthermore, other, often highly abundant bioactive metabolites like aeruginosins, have to be toxicologically evaluated at levels also accounting for (sub-)chronic exposure to low concentrations and in combination with naturally co-occurring metabolites, as can be expected in drinking water supplies.

Keywords: Cyanotoxin, cyanobacterial bloom, cylindrospermopsin, microcystin, inflammation, diarrhea, gastrointestinal illness, lipopolysaccharide, innate immune system

Background

Climate change is transforming ecosystems and their composition all over the planet. Among others, temperate climates in the northern hemisphere are experiencing longer and more intense heat periods in summer and increasing CO_2-levels are saturating surface waters with dissolved carbonate. In combination with other anthropogenic factors, such as eutrophication, this has been linked to an increase in frequency and intensity of hazardous cyanobacterial blooms in surface waters [1–4].

Cyanobacteria, also known as blue-green algae, are photosynthetic prokaryotes of ancient origin. They are able to produce a large variety of toxic secondary metabolites, cyanotoxins, heterogeneous in structure, activity and stability (reviewed in Buratti et al. [5]). These can be released to water either directly or upon cell lysis with major implications for the directly affected aquatic species, as well as for human and livestock health. For humans, Figure 1 illustrates the organs that are traditionally reported as targets of toxicity, as well as other organs/organ systems affected by cyanobacterial toxins.

As reviewed in Wood [6] and Stewart [7], fatal poisonings of livestock and pets (e.g. dogs) have been reportedly linked to cyanobacterial blooms for decades.

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Unlike animals, humans have greater capabilities to access uncontaminated drinking water (underground water or treated surface water) and tend to avoid odorous waters for drinking and recreational activities. Therefore, documented fatal human cases of acute cyanobacteria poisonings have been limited to specific exposure routes, such as hemodialysis treatment with cyanotoxin-contaminated water [8].

Nevertheless, serious adverse health outcomes have been associated also with other, more common exposure scenarios, and linked to both acute and chronic exposures to toxic cyanobacteria and cyanotoxins. During recreational water activities and sports, human beings can be exposed to contaminated water by dermal contact, inhalation, and also ingestion [5]. In the most extreme cases, recreational exposure to cyanobacteria resulted in life-threatening conditions requiring hospitalization, e.g. due to atypical pneumonia [9], pneumonia followed by dyspnea and liver damage [10], or an acute hepatic failure requiring a liver transplant [11]. Gastrointestinal symptoms, including abdominal pain, malaise, nausea, vomiting and diarrhea, were also manifested during these severe poisonings. Interestingly, such gastrointestinal symptoms have been often documented also in the other cases of acute, usually self-limited, adverse health outcomes, which followed recreational contact with cyanobacteria-contaminated water [5,12] (see also sections below). A prospective epidemiological study found an increased incidence of gastrointestinal illnesses in human populations living in the proximity of cyanobacteria-contaminated lakes, even if their recreational contact with the contaminated water was very limited [13,14].

Gastrointestinal irritation and diseases are regularly linked to the occurrence of cyanobacterial blooms, especially to the consumption of cyanobacteria-contaminated drinking water as reported by Levesque et al. [13,14] and reviewed in Svirčev et al. [12]. Acute poisonings in a larger scale have been mostly reported upon consumption of insufficiently treated surface water, often following algicidal treatment of cyanobacteria-infested freshwater reservoirs used as drinking water sources [15,16]. Lysis of cyanobacterial cells upon algicidal treatment releases the intracellular toxic metabolites to the water. While conventional treatment technologies can eliminate intact cyanobacterial cells with toxins quite effectively, these technologies have limited capability for removal of dissolved cyanobacterial toxins, which allows them to reach taps in sufficiently high

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concentrations to cause adverse effects in the gastrointestinal system (Table 1). Chronic exposures to drinking water contaminated with cyanobacterial compounds, in some instances in combination with other confounding factors, have been associated with an increased incidence of liver cancers or chronic liver damage and diseases, as reviewed and discussed by Svircev et al [12], and supported by recent findings [17–19]. Interestingly, epidemiologic studies have linked chronic exposures to drinking water contaminated with cyanobacteria and their metabolites also to the increased incidence of colorectal or small intestinal cancer in China, Portugal and Serbia, or stomach cancer mortality in China [12,20,21]. Nevertheless, other studies have not observed such associations between colorectal cancer and exposures to toxic cyanobacteria, or found negative correlation for small intestinal cancer [12,22].

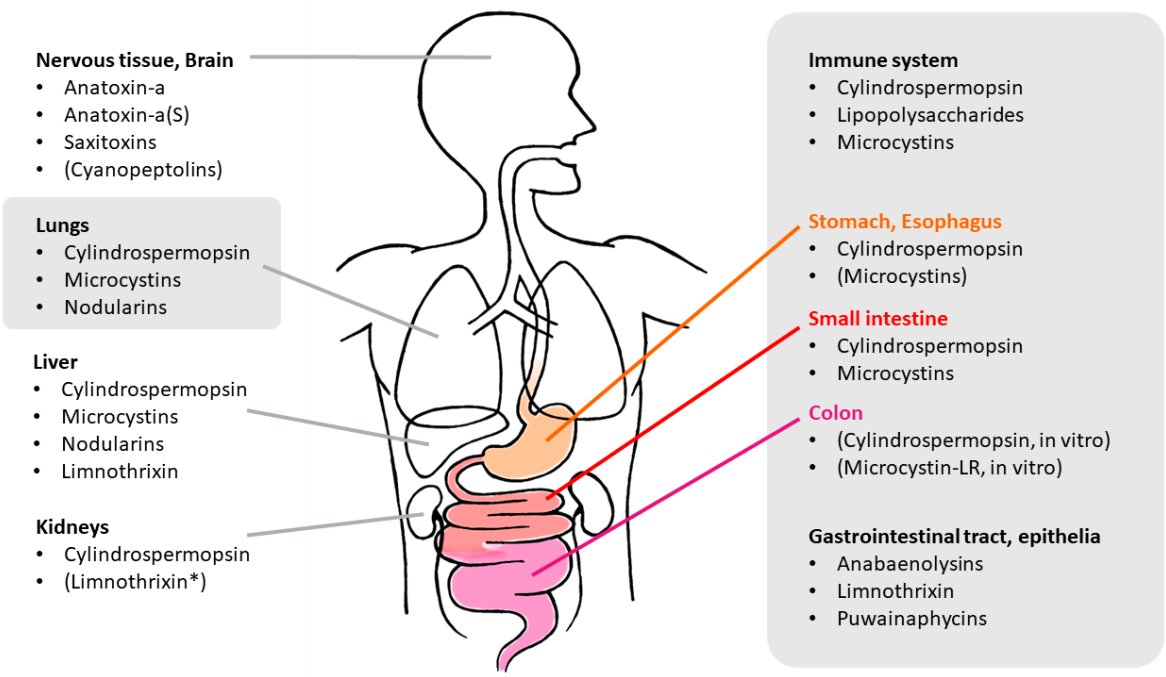

Figure 1: Organs/organ systems affected by toxic metabolites of cyanobacteria. Organs traditionally considered targets of toxicity are on the left, organs directly subjected to oral exposure are on the right. Grey background illustrates organs with mucosal surfaces that serve as primary entry portals for environmental and dietary contaminants. The figure depicts inner organs figuratively and not anatomically correct. Data based on literature review, detailed sources are provided in Tables 2-4. *: putative toxin, in parentheses: effect supported by *in vitro* data only (no *in vivo* data available).

100

101 **Table 1: Examples of incidents and case studies of acute gastrointestinal illnesses, chronic gastrointestinal illnesses connected to the consumption of cyanobacteria contaminated**
102 **water and chronic gastrointestinal illnesses connected to the recreational activities in cyanobacteria contaminated water.** Ref.: reference(s), n.a.: not assessed, MCs: microcystins,
103 NOD: nodularin, ATX: anatoxin, STX: saxitoxin, LPS: lipopolysaccharide

Country	Year	Cyanobacteria	Cyanotoxins	Description	Ref.
Acute gastrointestinal illnesses connected to the consumption of cyanobacteria contaminated water					
USA, West Virginia, Charlestone	1930	*Microcystis* bloom	n.a.	Massive water blooms of *Microcystis* sp. in the Ohio and Potomac Rivers linked to gastrointestinal illness (abdominal pain, nausea, vomiting, diarrhea) in ≈5000-8000 people out of 60000 inhabitants, despite water treatment by precipitation, filtration and chlorination.	[23]
Zimbabwe, Harare	1960-1965	*Microcystis* bloom	n.a.	Annual increases of children hospitalizations due to gastroenteritis in a city area supplied with drinking water from a water reservoir during a cyanobacterial bloom decay, no infectious agents detected, populations of children from other areas supplied with water without bloom development unaffected.	[24]
USA, Pennsylvania, Sewickley	1975	*Schizotrix calciola*	n.a.	Gastrointestinal outbreak in ≈62% of 8000 inhabitants supplied with drinking water from a water source contaminated with cyanobacterium *Schizotrix calciola*	[25,26]
Australia, Palm Island	1979	*Cylindrospermopsis raciborskii*	CYN implicated	After the use of copper sulfate to control a dense cyanobacterial bloom in water supply, 139 children and 10 adults suffered with severe, hospitalization-requiring, hepatoenteritis and gastroenteritis (malaise, nausea, vomiting, headaches, hepatomegaly, diarrhea, dehydration), with an evidence for liver and renal damage	[15,27].
Brazil, Itaparica Dam	1988	*Microcystis* sp., *Anabaena* sp.	n.a.	2000 gastroenteritis, with 88 cases resulting in death, reported over 42-day period after flooding a newly constructed dam. Cases were restricted to the areas supplied with drinking water from the dam; cyanobacteria were detected in untreated water. No other toxicants or infectious agents were identified.	[28]
Sweden, Malmo area	1994	*Planktothrix agardhii, Microcystis sp.*,	MCs	Accidental mixing of untreated river water with treated drinking water during a cyanobacterial bloom; 121 people from 304 experienced nausea, abdominal pain, vomiting, diarrhea accompanied with muscle pain, headaches and fever. No pathogens detected upon clinical examinations of patients or in the river water	[29]
Australia, South Australia. Murray River	1995	Most common: *Dolichospermum circinale, Microcystis aeruginosa, Aphanizomenon* sp., *Planktothrix* sp.	n.a.	Increased incidence of gastrointestinal symptoms in people consuming treated (chlorinated) river water during period of raised cyanobacterial cell counts in the river; gastrointestinal and dermatological symptoms in people using untreated river water for domestic purposes, when compared to people using rain water	[30]

Namibia, Windhoek	2000	*Microcystis* sp., *Oscillatoria* sp., *Anabaena* sp., *Merismopedia* sp.	MCs	Liver damage (increased serum levels of liver enzymes) and diarrhea during the occurrence of cyanotoxins and cyanobacteria in treated drinking water, positive correlation between diarrhea and chlorophyll a concentration in the water	[31]
Serbia, Uzice	2013	*Planktothrix rubescens*		Cyanobacteria and cyanotoxins found to penetrate into final treated water in December 2013; significantly higher incidence of gastrointestinal, skin and subcutaneous diseases detected during bloom periods in 2012-2015	[32]
USA, Ohio, Lucas County	2014		MCs	Microcystin levels in drinking water reached 3.19 µg/L, a do-not-drink advisory for 3 d was issued. In 16.2% out of >100000 households, at least one person reported physical health symptoms attributed to the advisory; gastrointestinal symptoms were most commonly reported (diarrhea 12.1%, nausea 9.1%, and vomiting 6.2%). Eye and skin irritation, headaches and respiratory symptomes also reported	[33]
	Chronic gastrointestinal illnesses connected to the consumption of cyanobacteria contaminated water				
China, Zhejiang, Haining,	1977-1996	n.a.	MCs	Greater incidence of colorectal cancers in patients relying on river and pond water as drinking water source in comparison to people using underground well water or tap water. Microcystins detected in river and pond water, their concentrations correlated with the cancer incidence	[21]
China, Jiangsu, Wuxi		n.a.	MCs	Microcystin in drinking water positively correlated with male overall cancer mortality and male stomach cancer mortality, but negatively correlated with male intestinal cancer mortality	[34]*
USA, Florida	1981-1998	n.a.	n.a.	No significant associations between the incidence of colorectal cancer in people living within the area supplied from surface water treatment plant and people supplied by underground wells found in geographic information system-based study	[22]*
Serbia	1999-2008	*Microcystis* sp., *Aphanizomenon* sp., *Anabaena* sp., *Planktothrix* sp.	MCs	Geographical incidence of 13 cancers (brain; bronchus and lung; heart, mediastinum, and pleura; ovary; testis; kidney; stomach; small intestine; colorectum; retroperitoneum and peritoneum; leukaemia; malignant melanoma of skin and primary liver cancer) positively correlated with the occurrence of cyanobacterial blooms and toxins	[20]
Portugal	2000-2010	*Microcystis aeruginosa,* *Aphanizomenon* sp., *Oscillatoria* sp.	n.a.	Populations exposed to cyanobacteria-contaminated drinking water had higher serum levels of liver enzymes, and higher incidence of investigated cancers (liver, colon and rectum cancer)	[35]*
USA, Ohio, Celina (Mercer County)	1996-2008	*Aphanizomenon* sp., *Microcystis* sp., *Anabaena* sp., *Planktothrix* sp.	MC, CYN, ANTX-A, STX	Pperiodically supplied with cyanobacteria-contaminated surface water from Grand Lake St. Marys; comparison of cancer incidence (hepatocellular and colorectal cancer) was inconclusive compared to two groundwater supplied cities	[36]*
	Chronic gastrointestinal illnesses connected to the recreational activities in cyanobacteria contaminated water				

Canada, Saskatchewan	1959	*Microcystis* sp., *Dolichospermum circinale*	n.a.	Ddespite animal deaths and warnings against recreational use, people swam in cyanobacteria-contaminated lake; 13 people suffered from headaches, weakness, nausea, stomach cramps, vomiting, painful diarrhea, muscle and joint pains. No pathogens detected, cyanobacteria found in the vomit and stool of one patient	[37]
USA, Pennsylvania	1979	*Anabaena* sp.	n.a.	Hay fever-like and gastrointestinal symptoms following recreational water activities in cyanobacteria-contaminated lake, no pathogenic agents detected	[38]
Great Britain, Staffordshire	1989	*Microcystis* sp.	n.a.	10 out of 20 army recruits suffered from gastrointestinal symptoms (vomiting, diarrhea, abdominal pain) and other health issues (lips blistering, sore throat) after swimming and canoe training in water with cyanobacterial bloom. Two recruits developed atypical pneumonia.	[9]
Australia, New South Wales, Victoria	1995	*Microcystis* sp., *M. aeruginosa, Anabaena* sp., *Aphanizomenon* sp., *Nodularia spumigena*	n.a. (hepato-toxicity by bioassay)	Epidemiological study (777 exposed, 75 control) reported positive correlation between exposure to cyanobacteria during recreation and diarrhea, vomiting, flu-like symptoms, skin rashes, mouth ulcers, eye and ear irritations observed 2-7 days after the exposure. Severity of the symptoms depended on bloom density and duration of the activity	[39]
Australia, New South Wales, Florida	1999-2002	cyanobacteria	n.a.; MCs, CYN, ATX	Respiratory symptoms, gastrointestinal illness, eye and ear irritation associated with recreational use of cyanobacteria and cyanotoxin contaminated water	[40]
Great Britain, Littleborough	1996	*Planktothrix agardhii*	MCs	Cyanobacterial bloom producing microcystins caused vomiting, fever, facial rashes, asthma, and dry sporadic cough in 11 cadets practicing canoe-capsizing	[41]
Finland, lakes Sompanen, Salajarvi, Iso-Kukkanen	2002-2003	*Dolichospermum lemmermannii*	STXs	Recreational activities in lakes with cyanobacterial bloom caused skin rashes, eye irritations, fever and abdominal pains in 2-10 year old children	[42]
Argentina, Salto Grande Dam	2007	*Microcystis* sp.	MCs	19-year old man practicing jet ski stayed for more than 2 h in dense cyanobacterial bloom, developed gastrointestinal malaise, nausea, vomiting, muscle weakness a few hours later, his condition worsened during next 4 days, developed into pulmonary problems and dyspnea, followed by hepatotoxicosis	[10]
Canada	2009	n.a.	MCs, LPS	Increased incidence of gastrointestinal symptoms (diarrhea, vomiting, eventually nausea, fever, abdominal cramps) in residents with full recreational contact at cyanobacteria-contaminated lake (swimming, waterskiing, windsurfing etc.). These symptoms reported also in case of limited contact with contaminated water (fishing, watercraft using but not launching). Significantly higher incidence of various other symptoms (muscle pain, skin symptoms, ear symptoms) in populations supplied with treated surface water from cyanobacteria contaminated supplies. The symptoms correlated with concentrations of lipopolysaccharides	[13,14]

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Finland	2010	n.a.	MCs, NOD, ATX, STX, LPS	Brackish and freshwater localities: health issues reported upon recreational activities in cyanobacteria-contaminated water: fever 58%, gastrointestinal symptoms 53%, nausea 34%, skin irritation 34%, headaches 32%, eye-ear-nose-throat irritation 29%, others 16%. While concentrations of cyanotoxins and LPS relatively low, *Aeromonas sp.* virulence genes were detected frequently in the bacteria isolated from the water samples	[43]
USA	2009-2010	n.a.	MCs, STX, CYN, ATX	Ohio, New York, Washington: 11 outbreaks of cyanobacterial blooms resulted in at least 61 illnesses (two hospitalizations, no known death), effects included dermatologic signs or symptoms (8); gastrointestinal signs or symptoms (8); respiratory signs or symptoms (6); fever (5); headache (4); neurologic signs or symptoms (4); ear symptoms (5); and eye irritation (3). In each of the outbreaks for which oral exposure was reported, affected persons had gastrointestinal signs or symptoms	[44]
USA	2011	*Microcystis* sp.	MCs	Kansas, Milford Lake: 7 reports of human illnesses confirmed as associated with cyanobacterial blooms, primary symptoms included: 71% eye and upper respiratory tract irritation, 29% rash, 14% gastrointestinal. The primary route of exposure included direct contact	[45]
Uruguay	2015	*Microcystis* sp.	MCs	Carrasco and Malvín beaches, Montevideo: a family with a 20-month-old child suffered gastrointestinal symptoms after recreational activities, the symptoms were self-limited except in the child, who was hospitalized with diarrhea, vomiting, fatigue, and jaundice. Serum levels of liver enzymes and bilirubin indicated liver damage resulting in acute liver failure 5 days later (liver transplant 20 days later). Microcystins detected in the liver tissue	[11]

* retrieved from [12]

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The gastrointestinal system and its link to the immune system

Upon oral exposure, which is the most relevant route of exposure to cyanobacterial metabolites [46], gastrointestinal epithelia are the first barrier to be overcome before systemic exposure, hence the gastrointestinal tract is a major entry portal for cyanobacteria and their bioactive metabolites into an organism's system. Additionally, the gastrointestinal system is highly associated with the immune system. Besides harboring commensal microbiota, ingested foodstuffs, pathogens and environmental contaminants are passed through the intestines. These give rise to many toxic or antigenic compounds that may trigger inflammatory responses. To maintain a homeostatic environment and prevent exaggerated immunological reactions when inappropriate, there are several gut-specific adaptations from all compartments involved: the intestinal epithelium, the gut-associated lymphoid tissue and the microbiome.

Interactions between the microbiome and the mucosal immune system or the gastrointestinal tract (GIT) play a critical role in the tolerance of intestinal microbiome and its specific antigens by host-defense systems to avoid permanent inflammatory states [47]. There are indications, that exposure to the cyanobacterial toxin microcystin-LR alters the taxonomic composition of the microbiome, as well as the expression of functional genes in the GIT of mammals (mice, rats) [48,49].

Anatomically, the gastrointestinal passage consists of the esophagus terminating into the stomach that connects via the pylorus to the small intestine (duodenum, jejunum, ileum in descending order). The small intestine connects to the large intestine (caecum, colon, rectum) via the ileocecal valve and terminates with the anus [50]. While the digestively highly active small intestine is characterized by structured epithelia with maximum surface area, the colon is lined by a thick mucus layer enabling effective water reabsorption while preventing tissue invasion of the essential commensal microbiota that reside predominantly in this part of the GIT [50,51]. Besides commensal bacteria residing in the gut, also the intestinal luminal content has immune-modulatory activity itself. The varying concentrations of luminal dietary constituents "along the length of the intestine are likely to have an important impact on regulating regionalized immune cell compartmentalization and functionality." [51].

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How do the GIT and the immune system accomplish the task of protective immunity and pathogen clearance while tolerating commensal microbes? In the colon, the mucus secreted by the epithelial goblet cells is antimicrobial and therefore poses a physical barrier for bacteria [50 and references therein]. The mucus production is controlled by soluble immune mediators also known as cytokines. Cytokines can be secreted by epithelial or immune cells and modulate the immune response. For example, tumor growth factor-beta (TGF-β) and retinoic acid promote regulatory T cell (Treg) differentiation in the gut, responsible for the homeostatic equilibrium in the intestines to large extent [51–53]. On the other hand, pro-inflammatory interleukin (IL) IL-1, IL-6 and tumor necrosis factor-alpha (TNF-α), are released by phagocytic cells upon activation of Toll-like receptors (TLRs) sensing pathogen-associated molecular patterns (PAMPs).

Intestinal phagocytic macrophages residing in the gut mucosa are sessile and they constitutively produce high amounts of IL-10 enabling them to limit the inflammation via effective scavenging. It results in low production of inflammatory cytokines or oxidative agents upon phagocytosis or TLR stimulation compared to macrophages in other parts of the body [54, p. 505]. Meanwhile, dendritic cells are mobile and act as major antigen presenting cells migrating to secondary lymphoid organs and priming B and T cells that then can be recruited to the gut-associated lymphoid tissue and eventually secrete the protective immunoglobulin A into the lumen [54].

These integrated mechanisms, spatially restricted to the intestinal proximity, allow the immune system to remain unresponsive to commensal microbiota and dietary constituents in the gut lumen and to launch a robust immune defense reaction when these microorganisms or compounds cross the epithelial barrier and invade tissues and the organism.

Microcystin effects

Microcystins (MCs), non-ribosomal cyclic heptapeptides, are the most common toxins in cyanobacterial blooms and most extensively studied cyanotoxins (reviewed in Testai et al [55] and Buratti et al. [5]). MCs are synthesized via non-ribosomal polyketide/peptide synthetase multienzyme complexes encoded by the *mcy* gene cluster. The general structure of MCs is a cyclo (-D-alanine[1] -L-X[2] -D-erythro-B-methylaspartic acid[3] -L-Y[4] -Adda[5] -D-glutamate[6] -N-methyldehydroalanine[7]). Adda represents (all-*S*,all-*E*)-3-Amino-9-methoxy-2,6,8-trimethyl-10-

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phenyldeca-4,6-dienoic acid, which is an unique feature characteristic for all MCs. Variable L-amino acids can be present in positions 2 and 4, for example leucine (L) and arginine (R), this structural variant is then called microcystin-LR (MC-LR). More than 240 structural variants of MCs have been reported, differing not only in the amino acids at positions 2 and 4, but also other modifications, such as demethylations of N-methyldehydroalanine, methylaspartic acid or Adda [56].

Due to their hydrophilic nature, MCs cannot readily enter the cells of exposed organisms but require an uptake mechanism. Upon swallowing, MCs overcome the gastrointestinal epithelial barrier and enter blood circulation [57–60]. Uptake into hepatocytes is facilitated by organic anion transport polypeptides (OATPs) of the bile acid transport system, particularly OATP1B1, 1B3 and, to a minor extent, OATP1A2 that are highly expressed in parenchymal hepatocytes [61,62].

Of the 12 identified OATPs only few (OATP1B1, 1B3, 2B1 and 1A2) have been extensively studied due to their expression in the tissues recognized as the main target of MC toxicity [63,64]. Of these four OATPs, only OATP2B1 that is not involved in the uptake of MCs seems to be functionally relevant in the intestines, while the expression and function of MC-transporting OATP1A2 in the intestines is still being discussed [62–65]. The expression of OATP1B1 and 1B3 is considered specific to the liver [63], hence their contribution to the intestinal uptake of MCs is probably negligible. Other OATPs functionally expressed in the human intestinal tract are OATP4A1, 3A1 and 2A1, however, these have not been examined for their involvement in MC uptake [62,63,66]. Similarly to the GIT, cells of the innate immune system only express a limited set of OATPs. Transcriptomic analyses revealed high expression of OATP2B1 mRNA and lower levels of OATP3A1, 4A1 and 4C1 while no transcriptomic indication for OATP1A1, 1A5, 1B1, 1B2, 1B3 or 1C1 expression in macrophages was found [67–69]. Furthermore, functional studies on drug transport via OATP2A1 and 2B1 in macrophages indicate their functional expression [70,71]. Moreau et al. [67] also report intermediate to low expression levels of OATP3A1 and 4C1 in monocytes, but their role in MC cellular uptake is unclear.

Once inside cells, MCs inhibit the ubiquitous protein phosphatases 1 and 2A (PP1/2A) due to the interaction of Adda-methyldehydroalalnine moiety with the catalytic site of the enzyme, including a covalent binding between methyldehydroalanine of MCs and cysteinyl residue at the catalytic site

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of the enzyme [5]. The consequence of protein PP1/2A inhibition by MCs is hyperphosphorylation of cytoskeletal proteins, disruption of intracellular signaling, inhibition of DNA repair, mitochondrial alternation and oxidative stress, DNA damage, apoptosis and necrosis induction [5,72]. Protein phosphatases play a crucial role in the intracellular signaling by dephosphorylating, and thereby deactivating, proteins like Raf, MEK, AKT (PP2A, action as tumor suppressor) [73]. Furthermore, MC-LR has been shown to stimulate pro-inflammatory cytokine production in murine macrophages (RAW 264.7) in a phosphatase-independent manner via activation of Toll-like receptors [5,68], to promote tumor growth and possibly neoplastic transformation and carcinogenesis [74–76].

Of >240 congeners, MC-LR is considered to be one of the most common, abundant and toxic structural variant, with a lethal dose killing 50% of the orally exposed mice (LD50, acute oral toxicity) of 5 mg/kg bodyweight and a no-observed adverse effect level ($NOAEL_{liver}$) of 40 µg/kg/day (chronic toxicity, 13 weeks repeated-dose exposure) [2,46,77–81]. Modifications of MC molecule, including modifications of Adda-methyldehydroalanine region as well as other parts, such as variable amino acid residues at positions 2 and 4, can lead to the reduction of PP1/2A inhibition potencies [61,62,82–86]. However, the observed differences in acute *in vivo* toxicity of MCs [86] cannot be exclusively attributed to the differences in PP1/2A inhibition among different MC variants. OATP-mediated cellular uptake of MCs seems to be also structure dependent, which contributes to the differences between cytotoxicity of individual structural variants without a clear relationship to PP1/2A inhibition potency [61]. MCs are detoxified by conjugation with glutathione (GSH) which is catalyzed by the activity of biotransformation enzyme glutathione-*S*-transferase. Reaction is followed by MC-GSH conversion with by gamma-glutamyltranspeptidase and then cysteinylglycine dipeptidase into MC-Cystein conjugate. This biotransformation reduces cellular uptake of the toxin and/or facilitates its excretion that most likely occurs via P-glycoprotein or multidrug resistance proteins, hence alters the ability to inhibit PP1/2A and decreases toxicity [84,87,88].

Depending on the MC congener, the dose and the duration of exposure, MCs cause necrotic, apoptotic or cell-proliferative changes. Due to the high abundance of OATP1B1 and 1B3 in parenchymal hepatocytes, the high metabolic activity and detoxifying function, toxic effects often manifest in the liver, even though the first site of action upon water-borne contaminants are

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probably mucosal epithelia of the GIT (Table 2). In fact, oral exposure of mice to MC-LR caused erosion of surface epithelial cells of the small intestine and accumulation of MC-LR in the villi (Table 2) [88]. DNA damage in intestine and colon of mice after intraperitoneal exposure was reported [89], as well as increased in apoptotic indices in different parts of the murine intestine [90]. In both, the small and large intestine (caecum), MC-LR was detected in the mucus of goblet cells, indicating the excretion of MCs into the GIT lumen upon blood circulation and exposure of the liver. Similarly, Falconer [91] reported lesions in the liver and the small intestine upon oral administration of microcystin-containing water to pigs. Furthermore, not only epithelial lesions but also decrease in intraepithelial lymphocytes in the intestine was shown in a murine model [92]. Moreover, changes in the activity of membrane enzymes and in the peroxidation status in rat intestine was proven [93].

Besides uptake from the GIT, lesions of the small intestine (i.e. the jejunum) have been reported in mice upon intraperitoneal exposure, indicating toxic mechanisms of the MC-LR excretion into the GIT [90].

In order to better mimic the human GIT and to account for interspecies extrapolation, a limited number of experiments on human gut-derived cell lines, especially the human colon carcinoma-derived cell line CaCo-2, that exhibits properties of the small intestine upon *in vitro* differentiation, were conducted [94]. These studies confirm a rapid uptake of MC-LR in apical-basolateral direction over a CaCo-2 monolayer [95] and indicate the susceptibility of intestinal epithelia to MC toxic effects, as observed in afore-mentioned *in vivo* studies [90]. Among others, cytotoxicity of microcystins is accompanied by oxidative stress (lipid peroxidation induction, reactive oxygen species production, reduced glutathione content etc.) and cytokine production [96]. Interestingly, MC-LR induced the same levels of IL-6 but much higher levels of IL-8 than MC-RR in CaCo-2 cells after 24 h [97]. Transcriptomic analysis showed quite similar profiles induced by MC-RR and MC-LR but overall gene expression was higher in the case of MC-LR [98]. Interestingly, the uptake of these two congeners does not differ in CaCo-2 cells and both of them are able to enter the nucleus [99]. Further, microcystin cytotoxicity could depend also on the presence of more hydrophobic amino acids in the molecule. It was published that the congeners MC-LF and MC-LW displayed higher

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246 cytotoxicity in CaCo-2 cells in comparison to MC-LR [100]. Zegura et al. even observed a comparable
247 sensitivity of CaCo-2 and hepatic HepG2 cells to MC-LR exposure [101].

248 Microcystin also plays a role in neoplastic transformation in intestinal cells. It constitutively activates
249 Akt and p38, JNK and MAPK pathways in immortalized colorectal crypt cells which leads to their
250 proliferation and anchorage-independent growth phenotype [76]. MC-LR increases migrative and
251 invasive potential of HT-29, DLD-1 and SW480 human colon cell lines increasing matrix
252 metalloproteinase-13 expression [102]. Higher migration and invasion of HT-29 cells after MC-LR
253 treatment is also connected with increase in cadherin-11 expression [103]. MC-LR also decreases E-
254 cadherin expression and increases expression of Vimentin and Snail in DLD-1 and HT-29 cells
255 promoting epithelial-mesenchymal transition [104]. Very new publication shows MC-LR ability to
256 contribute to migration of DLD-1 cells via changes in microRNA-221 expression and STAT3
257 phosphorylation [105].

258 In addition to the liver, the intestines should also be considered a target of MC toxicity [101,106].
259 Besides acute effects on animals and human colon-derived cell lines, the intestines can be dietary
260 exposed to low doses of MCs, so chronic exposure may adversely affect intestinal tissue [101]. This
261 is of special importance considering a reported tumor promoting and neoplastic transformation
262 potential of MCs [74–76,81,107].

263 **Table 2: Overview of microcystin (MC) effects on the gastrointestinal tract and/or the (gut-associated) immune system.** CYN: cylindrospermopsin; EC50: Half-maximal effective
264 concentration; ERK: extracellular signal-regulated kinase; LD50: half-maximal lethal dose; LOAEL/NOAEL: Lowest/no observed adverse effects level; LOEC/NOEC: lowest/no observed
265 effect concentration; MAPK: mitogen-activated protein kinase; MC: microcystin; OATP: organic anion transporting peptide; NF-κB: nuclear factor κB; PP2A: protein phosphatase 2A; s.p.f.:
266 special pathogen-free; TEER: trans-epithelial electric resistance.

Experimental Model	Assays performed, endpoint	Exposure conditions, Concentration ranges	Affected Tissue(s) of interest	Main Results	Reference
In vivo					
Mouse (ICR, female)	Radionuclide recovery	3H-dihydro MC-LR, 70 µg/kg bw (i.p.); detection after 3-90 min	Stomach, small intestine, large intestine, gastrointestinal tract	Detection of exposure-linked radioactivity in the gastrointestinal tract (approx. 37.6% of the total administered dose), especially in small intestine (6.4 %)	[5,108]
Mouse (aged Balb/C, ICR)	Immunohistochemistry	MC-LR, 500 µg/kg bw (p.o.); 1-13 weeks	Stomach, small intestine, caecum	Especially small intestine (villi and *lamina propria*) stained highly immunopositive; erosion of small intestine	[88]
Mouse (Balb/C, s.p.f., 7-week old female)	Histology, immunohistochemistry	75% of MC-LR LD50 (i.p.), 8-32 h	Small intestine	Apoptotic indices after 32 h exposure: 4.25 ± 0.125% (duodenum), 2.5 ± 0.125% (jejunum), 1.75 ± 0.125% (ileum)	[90]
Mouse (N:NIH-S, male)	Phosphatase inhibition assay	MC-LR, 50 µg/kg bw (p.o.) every 48 h, 30 days	Gut-associated lympohoid tissue	Decrease of intraepithelial lymphocytes by 28.7 % ± 5.0%	[92]
Mouse (Swiss albino, female)	Comet assay (single-cell gel electrophoresis; DNA-damage)	10 mL/kg bw (p.o. or i.p.), 3-24 h. oral doses: 2-4 mg MC-LR/kg bw; i.p. doses: 10-50 µg MC-LR/kg bw.	Blood, liver, kidney, small intestine (ileum), large intestine (colon)	DNA damage induced in intestinal tissues (ileum and colon) may contribute to increased cancer risk	[109]
Rat (Wistar)	Determination of MC-LR toxicokinetics by histopathology and LC-MS detection	$MC\text{-}LR_{equivalent}$, 80 µg/kg bw (i.v.); 1-24 h	stomach	Detection of MC in different tissues upon intravenous gavage; 0.010–0.058 µg/g dry weight $MC\text{-}LR_{equivalent}$ in the stomach	[110]
Fish (medaka)	Immunohistochemistry	5 µg/g MC-LR bw (p.o., direct administration to the fish stomach), 2 h	Gut-associated lymphoid-tissue, intestine (submucosa)	MC-positive staining of submucosa (penetration through the epithelium); disrupted cellular cohesion; MC-positive stained macrophages	[111]
Human (fishermen)	Epidemiology, cohort study, risk assessment	$MC\text{-}LR_{equivalent}$	Whole organism	Estimated daily intake: 2.2-3.9 µg; LOEL, tolerated daily intake: 0.28 µg/kg bw/day	[40,91]
In vitro					

Human (CaCo-2)	Apparent permeability of the pseudoepithelial cell layer to MC-LR	1-75 µM MC-LR; 0.5-24 h	Intestine (colon)	Apical-to-basolateral transport: 24-40% decrease in apical compartment/0.3-1.3% increase in basolateral compartment; low efflux from cellular to basolateral compartment. basolateral-to-apical transport: slow concentration decrease (basolateral, fast increase (apical); better efflux in basolateral-to-apical direction	[95]
Human (CaCo-2)	Immunolocalization (microcystin uptake)	1-50 µM MC-LR, -RR, 0.5-24 h	Intestine (colon)	Rapid uptake in less than 1 h of both variants (no difference in uptake profile); nuclear localization of MCs upon uptake; facilitated uptake (probably via OATPs) and active excretion	[112]
Human (CaCo-2)	Gene expression, transcriptomics	10-100 µM MC-LR, 4-24 h	Intestine (colon)	Major effects on oxidative stress, ERK/MAPK and cell cycle pathways	[98]
Human (CaCo-2)	Bradford assay (Cell number, protein content), neutral red uptake, MTS reduction (viability)	MC-LR, -RR, -YR; 50-200 µM, 24-48 h	Intestine (colon)	EC50: reduction of total protein content: 111.1 ± 3 µM MC-LR (24 h), >200 µM MC-RR (48 h); neutral red uptake: 57.3 µM MC-YR (48 h)	[106,113]
Human (CaCo-2)	MTT assay, Comet assay	0.2-10.1 µM MC-LR, 4-48 h	Intestine (colon)	40% reduced cell viability upon 48 h exposure to 10 µM MC-LR (MTT assay), 19.6% damaged DNA after 4 h exposure to 0.2 µM MC-LR	[101]
Human (CaCo-2)	Lactate dehydrogenase (LDH) leakage (cytotoxicity), cell proliferation and morphology, Protein phosphatase (PP) inhibition	1-50 µM MC-LR, -LF, LW, 22-48 h	Intestine (colon)	EC50: LDH leakage: 25 % (50 µM MC-LR, control), 36 % (MC-LW), 51 % (MC-LF); PP inhibition: 3.0 nM MC-LF, 3.8 nM MC-LW, 1.0 nM MC-LR; apoptosis and morphological changes: membrane blebbing, cell shrinkage, chromatine condensation, cytoskeletal reorganization	[114]
Human (IEC-6)	CCK-8 (cell viability), apoptosis, transepithelial electric resistance (TEER), PP2A activity, western blot	0-50 µM MC-LR, 6-24 h	Intestine (colon)	LOEC: TEER:50 µM (12 h), 12.5 µM (24 h); viability: 12.5 µM (24 h); apoptosis: 25 µM (24 h); western blot: 12.5 µM (24 h; occludin), 25 µM (24 h; ZO-1); PP2A activity: 12.5 µM (24 h)	[115]

Human (NCC)	Genechip analysis, western blot, kinase activity assays, proliferation	0.1-1005 µM MC-LR, 28 d	Intestine (colon)	Transformation (first step in carcinogenesis); constitutive upregulation of signaling pathways (PI3K, APK2, Akt, cyclin D1 and D3), of Ras GTP/GDP proteins (IQGAP-2, RabGTPase , Rap1GAP , RasGAP , R-Ras , Krev-1, TC21) and Pas/MAPK pathway (A-Raf, B-Raf, PAK); decreased proliferation of MC-LR-transformed colorectal crypt cells	[76]
Human (DLD-1, HT29)	Western blot, RT-qPCR, gene knockdown by siRNA, cell migration	0.1-50 nM MC-LR, 24 h	Intestine (colon)	Motility acquired by epithelial-mesenchymal transition through exposure to 25 nM MC-LR (LOEC) in both colorectal cancer cell lines; MC-LR is likely to aggravate (colorectal) cancer development	[74]
Mouse (Balb/C, isolated peritoneal macrophages)	mRNA expression	1-1000 nM MC-LR + 100 µg/L LPS; 6 h	Innate immune system	Reduction/alleviation of LPS-induced inflammation	[116]
Mouse (RAW 264.7, blood macrophages)	Western blot, ELISA	1-1000 nM MC-LR, 0.5-24 h	Innate immune system	LOECs upon 24 h exposure to MC-LR for: MAPK (ERK1/2) activation (100 nM), NF-kB activation (1000 nM), TNFa production (1 nM)	[68]
Human (predominantly)	Case study meta-analysis, review			References to human intoxication cases, relation between oesophagus cancer and human contact with cyanotoxins through the food chain requires further investigation	[12]

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Cylindrospermopsin effects

Cylindrospermopsin (CYN), a tricyclic guanidine alkaloid was first characterized in *Cylindrospermopsis raciborskii* [117] upon a major intoxication event in Palm Island, Australia [15]. Despite the still not entirely elucidated molecular mode of action, CYN is proven to be a potent inhibitor of protein synthesis (LOEC = 0.5 µM CYN, primary mouse hepatocytes) [5,118,119].

In addition, CYN has been found to induce tissue damage, cytotoxic effects, oxidative stress and DNA damage in a variety of organs and cell types [118].

CYN toxicity was reported to be attenuated by different inhibitors of cytochrome P450 (CYP450) *in vivo* [120], as well as in the cultured cells *in vitro* [121–124] [125,126]. Induction of CYP450 activity also increased CYN toxicity in hepatic cell lines [119,125,127]. Thus, it has been proposed that CYP450 activity is responsible for CYN bioactivation, increasing especially CYN-induced acute cytotoxicity, oxidative stress and genotoxicity, while inhibition of protein synthesis appeared to be unaffected and attributed to the parental compound [119,128]. However, no biotransformation products of CYN have been detected upon incubation with S9 liver fraction or in the presence of metabolically-competent liver cell lines [126], and also S9 liver fraction was not found to increase genotoxic potential of CYN in other studies [129,130]. Thus, the exact role of CYP450 in CYN toxicity is not completely clear and should be investigated in the future [118]. Phase II detoxification of CYN is supposedly mediated via glutathione conjugation and excretion in the liver and kidney [5,118,122,131,132]. These detoxifying and excreting organs, liver and kidney, are also the most sensitive tissues (NOAEL = 30 µg/kg/day; in mice) and the major recipients of CYN toxicity, but adverse effects on the stomach, the small intestine and on white blood cells have also been reported [133–135].

Due to the hydrophilic zwitterionic nature of CYN, similarly to MCs, it probably cannot be readily absorbed from the gastrointestinal tract upon ingestion, the main pathway of human exposure, hence, it has been hypothesized to require a facilitated or active transport [136,137]. Exposure of mice to CYN induced ulcers in the stomach and bleeding into the stomach and small intestine (2.5-8.3 mg $CYN_{equivalent}$/kg, 2-8 d; Table 3) [135]. Nevertheless, there is a lack of research addressing the gastrointestinal effects of CYN. Only two studies on colon-derived tumor cells (CaCo-2) address

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296 uptake kinetics across the intestinal epithelium, both reporting only a limited passage of the toxin
297 through the intact epithelium [94,138]. Pichardo et al. [138] furthermore conclude, that paracellular
298 diffusion is the most likely uptake mechanism of CYN from the intestinal lumen, while active
299 transport via P-glycoprotein or multidrug resistance proteins probably contributes to the secretion
300 and elimination of CYN. Despite the observed uptake across the epithelial barrier of intestinal cells
301 *in vitro*, CYN causes oxidative stress and decreases cell viability in CaCo-2 cells in a dose-dependent
302 manner (see Table 3), highlighting that enterocytes (as well as hepatocytes) also should be
303 considered a major important recipient of CYN toxicity, especially upon oral exposure [139].

304 **Table 3: Overview of cylindrospermopsin (CYN) effects on the gastrointestinal tract and/or the (gut-associated) immune system.** CYN: cylindrospermopsin; EC50: Half-maximal effective
305 concentration; GSH: glutathione; IL: interleukine; LOAEL/NOAEL: Lowest/no observed adverse effects level; LOEC/NOEC: lowest/no observed effect concentration; MC: microcystin; ROS:
306 reactive oxygen species; TDI: tolerated daily intake.

Experimental Model	Assays performed, endpoint	Exposure conditions, Concentration ranges	Affected Tissue(s)	Main Results	Reference
In vivo					
Mouse (MF1, male)	Histology	*C. raciborskii* culture extract containing 0.2% CYN; 2.5-8.3 mg/kg $CYN_{equiv.}$ (gavage), 2-8 d	Esophagus, stomach, small intestine	Stomach ulceration, fresh blood in (small) intestinal content	[135]
Mouse (Swiss albino)	Body and organ weight, urine, serum, hematology analysis, histopathology	CYN-containing cyanobacterial extract, 0-657 µg CYN/kg/day (p.o.), 10 weeks; purified CYN, 0-240 µg CYN/kg/day (p.o.), 11 weeks		NOAEL (TDI): 30 µg/kg/day; proposed guideline value for drinking water: 1 µg/L	[140]
In vitro					
Human (CaCo-2)	Cytotoxicity (Neutral Red uptake)	C. raciborskii (CYLI29, CYN/MC-free methanolic extract), C. raciborskii (AWT205; 1.1 mg CYN/g dw; methanolic extract); 0.08-1.25 mg dw/mL, 48 h	Intestine (colon)	EC50: 0.4 ± 0.1 mg dw/ml (CYLI29), 1.3 ± 0.2 mg dw/ml (AWT205)	[141]
Human (CaCo-2)	Intestinal permeability, epithelial integrity (trans-epithelial electric resistance, TEER)	1-10 µM CYN; 3-24 h	Intestine (colon)	16.7-20.5% (intestinal permeability, apical-to-basolateral, after 24 h), epithelial integrity not significantly altered	[94]
Human (CaCo-2)	Protein content (Bradford assay), cell viability (MTS reduction), oxidative stress, intracellular GSH content, ultrastructural alteration	0.72-96.3-µM CYN 24-48 h	Intestine (colon)	LOEC: 1.44 µM (cell viability, ultrastructural alteration), 3.0 µM (intracellular ROS concentration), 6.0 µM (protein content, GSH content)	[139]
Human (CaCo-2, C3A, HepG2, NCI-87, HCT-8, HuTu-80)	Cell viability (MTT assay)	0.25-5 µM CYN, 1-7 d	Intestine (colon), liver, stomach, small intestine (ileus, duodenum)	cell-line sensitivity decreased in cell lines derived from more distal regions of the gastrointestinal tract: gastric > duodenal > ileal > colonic; EC50 = 6.5 ± 3.3 µM (CaCo-2)	[142]

Human (CaCo-2)	Permeability of pseudoepithelial layer	1.9-48.1 µM CYN, 24-48 h	Intestine (colon)	Apparent permeability: 3.45 × 10−7 cm/s (absorptive direction), 6.41 × 10^{-7} cm/s (secretive direction); epithelial permeability (increase): 10-fold (absorptive), 0.7-fold (secretive); negligible transcellular passage	[138]
Human (primary lymphocytes, whole blood)	T-lymphocyte proliferation (thymidine incorporation)	1% biomass extract, 72 h (lymphocytes), 0.24 nM CYN, 72 h (whole blood)	Blood, immune system	Significant reduction of T-lymphocyte proliferation	[143]
Human (primary peripheral blood neutrophils)	Oxidative burst capacity (NADPH oxidase mediated)	0.024-2.4 µM CYN, 1 h	Blood/innate immune system (neutrophils)	Significantly decreased ROS production at all CYN concentrations tested (LOEC = 0.024 µM CYN) by decreased NADPH oxidase-mediated ROS production in neutrophils; phagocytic activity unaffected	[144]
Fish (*Cyprinus carpio*, isolated phagocytic cells)	IL-1b expression (PCR, phagocytosis, oxidative stress	0.12-2.4 µM CYN, 24 h	Immune system	32-fold increase in IL-1b expression (2.4 µM, 24 h); diminished phagocytosis (1.2 µM), ROS-production increased (0.12 µM; LOEC)	[145]

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Effects on the immune system

Besides reports of effects on gastrointestinal and hepatic cells, MC-LR is suspected to alter the immune response, especially in fish, where most of the studies addressing immunotoxic effects have been done [111,146–150]. For example, exposure to MC-LR affected the immune response of medaka fish additionally to sustained pathological changes in the GIT, liver and other organs [111]. In a mammalian (mouse) *in vivo* model, MC-LR has been shown to decrease levels of intraepithelial lymphocytes, thus affecting mucosal immunity [151]. *In vitro* exposure of murine macrophages to MC-LR significantly altered the expression of pro-inflammatory genes and the release of cytokines (Table 2) [68,116]. Adamovsky et al. [68] additionally proposed a receptor (TLR)-mediated mechanism of macrophage activation, as their model lacked specific transmembrane transporters, hence, the common mode of MC-LR action cannot be taken into regard.

Similarly to MC-LR, CYN exposure led to elevated markers of inflammation and diminished the uptake of bacteria into phagocytes in the common carp (*Cyprius carpio*) [147]. Although there are significant differences between immune systems of different groups of animals, vertebrates share some common mechanisms of innate immune system activation. These first-line defense mechanisms are, amongst others, increased expression of pro-inflammatory cytokines (e.g. IL-6 and TNFα), production of reactive oxygen species (ROS), secretion of nitric oxide and phagocytosis [68,152–154]. We recently discovered that CYN activates murine macrophages *in vitro* (unpublished data). In addition, CYN (0.1 µg/L; 0.24 µM) has been shown to significantly decrease lymphocyte proliferation *in vitro* in both isolated T cell culture and a whole-blood assay [143].

The effects on the immune system from *in vivo* studies are often evaluated as morphologically altered lymphoid follicles, changes in spleen size or weight, but the underlying mechanisms are not investigated or discussed any further [118,135].

Even though these findings have to be elaborated on and verified in a human health relevant system, disturbances in these fundamental immunological processes may lead to systemic effects that are of special concern when environmentally relevant mixture effects or effects on sensitive people with

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335 more pronounced immune reactions are evaluated and should be considered for future risk
336 assessments.

337 ## Lipopolysaccharide effects

338 Like most of the commensal and pathogenic bacteria, cyanobacteria stain Gram-negative. This
339 group of microbes shares a common structural feature: they incorporate lipopolysaccharides (LPSs)
340 in their cell wall. Eubacterial LPS is known as a model ligand of the Toll-like receptor 4 (TLR4), a
341 member of pattern recognition receptor (PRR) family expressed on epithelial, endothelial, immune
342 (particularly macrophages and dendritic cells) and other cells being an important part of the innate
343 immunity [155–157]. In order to maintain homeostasis in exposed tissues, activation of PRRs may
344 launch a robust immune reaction, eventually preventing the invasion of microbes into other tissues
345 of a healthy individual [158,159].

346 LPS binding to the TLR4-MD-2 receptor complex may initiate MyD88-dependent signaling, resulting
347 in the production of pro-inflammatory cytokines (e.g. IL-6, IL-8, IL-12, TNFα), or in MyD88-
348 independent signaling causing the release of type I interferons. Both pathways lead to activation of
349 the transcription factor NF-κB, governing the expression of pro-inflammatory cytokines
350 [155,160,161]. Even though LPS is traditionally considered one of the most potent pro-inflammatory
351 agents, there is an increasing evidence that structural variation, i.e. the degree and site of acylation
352 of the immunogenic lipid A moiety alters the immunogenicity of LPS, which may even elicit anti-
353 inflammatory properties [162–165].

354 Despite the probable oral exposure to cyanobacterial LPS (cyanoLPS) in bloom biomass, this topic
355 has received little scientific attention. Compared to LPS from heterotrophic bacteria, cyanoLPS
356 differs in structure. For example, lipid A of LPS produced by *Anabaena* spp. lacks phosphorylation
357 and glucosamine, while being acylated at up to ten sites [166–169]. Altered acylation of LPS
358 significantly influences the magnitude of LPS inflammatory potential with hexaacylation triggering
359 the strongest inflammatory responses [162,170].

360 In the gastrointestinal epithelial layer, constantly exposed to a large variety of LPSs, the expression
361 of functional TLR4 is low. Phagocytic cells of the innate immune system expressing TLR4 are rather
362 found in the submucosal *lamina propria* [170–172]. Subsequently, the immune system in healthy

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363 individuals recognizes and scavenges only bacteria invading the GIT tissue by crossing the
364 mucosal/epithelial barrier (Munford 2008 and references therein).

365 Nevertheless, the first and most widespread human acute health effect upon (accidental) ingestion
366 and intoxication of cyanobacterial bloom material is gastrointestinal illness, often with severe
367 inflammatory diarrhea (enterocolitis) [6,12,13,173]. A prime suspect for this common symptom of
368 cyanobacterial oral exposure is LPS.

369 Regardless of the strong rationale for cyanoLPS acting as a mediator of severe gut inflammation
370 upon oral exposure, experimental evidence is less conclusive. Compared to bacterial
371 (*Escherichia coli* K12) LPS standards, the pro-inflammatory potency of isolated cyanoLPS was
372 approximately 10-fold lower or protective to *E. coli* LPS-induced inflammation [164,166,174–176].
373 The reduced ability of cyanoLPS to mount a robust inflammatory response compared to LPS
374 produced by heterotrophic bacteria may be attributed to different lipid A structures and restrictions
375 in the molecular pattern recognized by the cellular receptor [170]. Interestingly, Stewart et al.
376 identified even a lack of evidence to support gastrointestinal pro-inflammatory reaction of
377 heterotrophic bacterial LPS alone, in the absence of other virulence factors [175,177]. Considering
378 the pro-inflammatory potential of bloom extracts, including bloom-associated heterotrophic
379 bacteria, a cumulative action of several (cyano-) bacterial components is very likely [166,174–
380 176,178]. For example, LPS may contribute to local GIT tissue inflammation and thereby facilitate
381 the access of other toxic compounds, like MCs, to deeper tissues and distribution to other organs
382 and targets of toxicity such as the liver [178].

383 ### Other toxins

384 Nodularins (NODs) are cyclic non-ribosomal pentapeptide toxins, produced almost exclusively by
385 *Nodularia spumigena*, that share structural and mechanistic similarities with MCs: non-
386 proteinogenic amino acids like N-methyldehydrobutyrine and D-erythro-β-methylaspartic acid are
387 incorporated into the peptide ring structure and the ADDA residue mediates toxicity [5 and
388 references therein]. *N. spumigena* favors slightly saline environments, therefore besides MCs, NOD
389 is a major concern in the Baltic Sea and other brackish habitats [179,180]. There are little data
390 available on NOD toxicokinetics, but an uptake mechanism (OATP) and mode of action (PP1 and

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391 PP2A inhibition) similar to MCs is proposed and partly supported by experimental evidence in
392 zebrafish and different hepatic cell lines [5,181,182]. Also, NOD has an experimental LD50 of 50-
393 70 µg/kg bw in rodents, which is similar to that of MC-LR (40 µg/kg bw) [2,5]. Therefore, NODs can
394 be expected to cause adverse effects on the GIT similar to MCs. Despite severely inhibiting activity
395 of protein phosphatases, NOD in contrast to MCs does not bind covalently to these ubiquitous
396 cytoplasmic enzymes [183].

397 The cyanobacterial neurotoxins anatoxin-a, anatoxin-a(S) and saxitoxin are fast actors, leading to
398 paralysis and eventually respiratory failure within minutes to few hours [184,185]. Due to the rapid
399 onset and specificity of neurological symptoms induced by inhibiting the transfer of excitatory
400 signals, gastrointestinal irritation is unlikely to be a primary effect of these toxins. Nevertheless,
401 exposure by complex cyanobacterial blooms will probably be to a mixture of many cyanobacterial
402 and bacterial components that may very well result in enterotoxic effects of other metabolites (see
403 also Table 4).

404 Except of the well-recognized cyanotoxins, there are many potentially harmful yet poorly
405 characterized bioactive secondary metabolites. Many of these, like the non-ribosomal peptides
406 aeruginosins, anabaenopeptins or cyanopeptolins, show protease inhibiting activities, the latter has
407 been also shown to cause neurotoxic effects and alter pathways linked to DNA damage and repair
408 (Table 4) [186–191].

409 Another group of poorly characterized cyanobacterial secondary metabolites are cyclic lipopeptides.
410 Representatives of this group are for example anabaenolysins and puwainaphycins, causing damage
411 to eukaryotic cell membranes also in GIT models and inducing necrotic effects (Table 4) [192–194].
412 More explicit effects on the GIT are reported by Humpage et al. (2012). They described necrotic
413 effects of the novel putative toxin “limnothrixin”, isolated from *Limnothrix* spp., upon oral gavage
414 of an aqueous extract, in liver, kidney and GIT tissue within 24 h of exposure (Table 4). Even though
415 no known toxin was detected in the aqueous extract, there may still be a variety of hydrophilic
416 compounds present and contributing to the effects observed.

417 The toxicological implications of these activities remain to be elucidated and the characterization of
418 novel putative toxins in an effect-directed screening approach along with the description of

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structural characteristics should be highly encouraged. Regardless of the unknown toxic potential, these substances may contribute to the severity of gastroenteritis upon cyanobacterial intoxication, for example by facilitating enteric hemorrhage through interference with the blood coagulation cascade [187].

Table 4: Overview of other (non-MC, non-CYN) cyanobacterial metabolites' effects on the gastrointestinal tract and/or the (gut-associated) immune system. CyP: cyanobacterial LPS-like compound; EC50: half-maximal effective concentration; ELISA: enzyme-linked immunosorbent assay; GIT: gastrointestinal tract; IFNγ: Interferone γ; IL: interleukine; LAL: *Limulus* amebocyte lysate; LOAEL/NOAEL: Lowest/no observed adverse effects level; LPS: Lipopolysaccharide; PUW: puwainaphycin; TLR: toll-like receptor.

Compound	Experimental Model	Assays performed, endpoint	Exposure conditions, Concentration ranges	Affected Tissue(s)	Main Results	Referenc
	In vivo					
Cyanobacterial LPS	Mouse (C57BL, female)	Mortality	0.025-1.5 mg bacterial LPS (i.p.), co-injection of 750-850 µg CyP	Whole organism	Protection against LPS-induced septic shock after 8 h (25 ng LPS; 58% survival, D-galactosamine-sensitized mice) and after 24-40 h (1.5 mg, 80% survival, non-sensitized mice)	[165]
"Limnothrixin" (from *Limnothrix* AC0243)	Mouse (Balb/C, male)	Histology	300 µl known-toxin-free biomass extract (i.p.; extract 1: 180 mg dw/mL, extract 2: 195 mg dw/mL), 3-24 h	Gastrointestinal tract, small intestine (duodenum)	Loss of single cells/cell sheets in the duodenum; serum-colored mucoidal material in the gastrointestinal tract 3-10 h p.i.	[195]
C. raciborskii biomass extract without detectable levels of CYN, MCs or saxitoxins	Mouse (Charles River, male)	Histology	1337-1572 mg dw/kg bw (i.p.), 8-24 h	Intestines, immune system (mucosa-associated lymphoid tissue)	Enlarged Peyer's patches	[196]
	In vitro					
Aeruginosins		In vitro inhibitory assays (biochemical)			Serine protease inhibition	[187]
Anabaenolysin A	human (primary erythrocytes)	Haemoglobin release (necrosis)	0.38-3 µM Abl A; 1 h	blood	EC50 (necrosis) ≈ 0.8 µM	[192]
Cyanobacterial LPS	Human (primary monocytes)	ELISA, RT-PCR, FACS analysis	0.1-20 µg/mL cyanobacterial LPS + 1-10 µg/mL bacterial LPS; 0-16 h	Blood (dendritic cells), innate immune system	20-fold excess of CyP compared to LPS completely inhibited cytokine production	[165]
Cyanobacterial LPS		Pyrogenicity (LAL test, PyroGene rFC assay), leukocyte activation (chemiluminescence)	Cyanobacterial LPS-extracts from *M. aeruginosa*-dominated bloom-biomass (11 naturally occurring blooms)	Immune system	10.2-78.3×104 EU/mg LPS (LAL test) ; 0.91-18.96×10^4 EU/mg LPS (PyroGene assay) ; leukocyte activation observed	[174]

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Cylindrospermopsin-containing biomass extracts		Skin sensitation	Human health risk assessment, literature review	Skin, immune system	Skin sensitation occurred in a cylindrospermopsin-independent manner, implying other irritating agents in the bloom biomass	[178]
Cyanobacterial biomass (*Spirulina platensis*) hot water extract	Human (blood, male volunteers)	Cytokine production and responsiveness, natural killer cell cytolitic activity	50 mL extract/volunteer/day, 1-8 weeks	Whole organism, blood	Internalization of yet uncharacterized *Spirulina* components via GIT; prestimulation of monocytes. Targets: monocytes (additive effect on TLR-mediated cytokine production) and natural killer (NK) cells (upregulation of cytolysis and IFN; critically affected by IL-18 levels).	[197]
Cyanopeptolin CP1020	*Danio rerio* (whole embryo)	transcriptomics	0.1-1 mg/L CP1020, 96 h	Whole embryo	Pathways altered ≥2-fold: DNA damage recognition and repair, circadian rhythm, response to light.	[190]
Puwainaphycin F	Human (CaCo-2)	ELISA	non-cytotoxic concentrations of PUW1146, PUW1118, PUW1188; 24 h	Intestine (colon)	Increased secretion of IL-8, altered expression of tight junction protein	[194]
Puwainaphycins F/G	Mouse (YAC-1 cells), human (HeLa)	MTT assay, intracellular Ca^{2+} concentration	1-10 µM PUW, 10 min - 10 h	lymphoma, cervical cancer	EC50 (necrosis) 2.2 µM	[193]

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Co-action of different factors on the GIT

While single-agent toxic effects are often reported for detoxifying organs like the liver or the kidneys in toxicological studies, the most prominent symptom of harmful algal bloom-intoxication is enterocolitis, probably mediated by coaction of a multitude of virulence factors. Besides the occasionally high abundance of already identified cyanobacterial toxins like MCs, many of the secondary compounds produced by cyanobacteria and eventually released to water are poorly characterized concerning their toxicity or not identified yet (Table 4) [178,196,197]. Furthermore, bloom-associated bacteria may contribute to the adverse effects observed upon exposure to cyanobacterial blooms, especially gastrointestinal illness [43,198]. The complex composition of cyanobacterial blooms also leaves space for additive or even synergistic effects of the multitude of compounds, which may exacerbate the impact of exposure to otherwise moderately active/toxic compounds.

Also the role of LPS of both, cyanobacterial and eubacterial origin has to be critically reflected. The controversies about pro- or anti-inflammatory activities of cyanoLPS may be explained by effects of bloom associated bacterial LPS, potentially sensitizing GIT lining epithelia for the effects of other toxins. A similar effect was observed with the pore-forming lipopeptides anabaenolysins A and B, where a transient increase in cell membrane permeability facilitated nodularin uptake, lowering the effective concentration for nodularin toxicity [192]. CyanoLPS activity on the (humoral) immune system does not sufficiently explain the GIT symptoms observed upon acute oral exposure to cyanobacterial blooms. But total bloom-LPS, including pro-inflammatory LPS from bloom-associated bacteria, may facilitate the penetration of gastrointestinal epithelia and thereby promote the uptake of other cyanobacterial toxins by macrophages or into the blood via the paracellular route as suggested for CYN [138,175].

Considering the described activity of MC-LR as a tumor promoter and CYN being suspected of carcinogenicity, the mixture of substances diverse in biological activity could finally also contribute to colorectal cancer incidents upon long-term exposure to low concentrations e.g. in drinking water [21,74,76,199].

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Conclusion

Cyanobacterial blooms are occurring more frequently and in increasing severity due to global climate change and eutrophication of water bodies, endangering the recreational value of water bodies as well as the safety of drinking water supplies. Hazards linked to cyanobacterial contamination have been recognized and addressed by regulatory authorities (WHO, EFSA, EPA). For cyanobacterial bloom management, the precautionary principle is proposed, that means the bloom is considered hazardous until proven safe [186].

Despite gastrointestinal symptoms being the most reported and wide-spread malaise upon oral exposure to cyanobacterial bloom biomass, research mostly focuses on specific organ toxicity of isolated toxins, i.e. hepatotoxic MCs and CYN. The oral route remains the most relevant exposure route for humans and gastrointestinal distress the predominant symptom. Consequently, the gut epithelia are exposed to the highest toxin concentrations and are also the first barrier that needs to be overcome for the toxins to reach the blood stream and be subsequently distributed to other organs/recipients of toxicity like the liver. In the naturally occurring complex mixture of cyanobacterial bloom material, a multitude of factors can contribute to the adverse effect observed on the GIT, probably not attributable to a single toxin or agent. Nevertheless, even isolated toxins are reported to adversely affect the (small) intestine (MC-LR, CYN) or the stomach (CYN), highlighting the importance of further investigation of this neglected yet plausible and relevant system. MC-LR, for which the scarce evidence on GIT irritation is strongest, should even be reconsidered as an enterotoxin.

Taking into consideration sensitive subpopulations, children and people with chronic gastrointestinal inflammations (e.g. Crohn's disease) are at higher risk. They suffer more often and from more severe enteritis, as their humoral immune system is still under development or in a permanently inflamed state. Also, with regard to the carcinogenic and genotoxic potential of MCs and NODs, low-level chronic exposure may contribute to colon carcinoma in later years [134,173,200,201].

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List of abbreviations

Adda: (all-*S*,all-*E*)-3-Amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid; ATX: anatoxin, EC50: Half-maximal effective concentration, EFSA: European Food Safety Agency, EPA: Environmental Protection Agency, GIT: gastrointestinal tract, IL: interleukine, LD50: half-maximal lethal dose, LOAEL/NOAEL: Lowest/no observed adverse effects level, LOEC/NOEC: lowest/no observed effect concentratio, LPS: lipopolysacharide, MAPK: mitogen-activated protein kinase, MCs: microcystins, NF-κB: nuclear factor κB, NOD: nodularin, OATP: organic anion transporting peptide, PAMP: pathogen-associated molecular pattern, PP1/2A: protein phosphatase 1/2A, PRR: pattern recognition receptor, ROS: reactive oxygen species, STX: saxitoxin, TEER: trans-epithelial electric resistence, TLR: toll-like receptor, TNFα: tumor necrosis-factor α, WHO: World Health Organization.

Declarations

a. **Availability of data and material:** Not applicable.

b. **Competing interests:** The authors declare no conflict of interest.

c. **Authors' contributions:** Conceptualization, K.H., P.B., L.Š. and B.K.; methodology, B.K.; writing—original draft preparation, B.K.; writing—review and editing, K.H., P.B., L.Š. and B.K.; supervision, K.H. and P.B.; project administration, K.H., L.Š.; funding acquisition, K.H., L.Š."

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Materials and Methods, Description of workflow

In order to obtain relevant scientific literature, a systematic review of publications was performed according to the PRISMA scheme (Preferred Reporting Items for Systematic reviews and Meta-Analyses; online supplementary Figure S1). The Scopus database, including PubMed, Web of Knowledge and Science Direct, was searched for the key words: (TITLE-ABS-KEY (cyanobacteria OR cyanotoxins OR microcystins OR bloom*) AND TITLE-ABS-KEY (epidemiology OR incidence OR health OR health AND effects OR health AND risk OR health AND impact OR health AND hazard OR adverse AND effects OR risk AND assessment OR exposure OR drinking AND water OR intoxication OR disease OR illness) AND TITLE-ABS-KEY (gastrointestinal OR gastro* OR enteric

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516 OR allerg* OR gastric OR inflamm* OR lps OR lipopolysacch*) . No further limits (Access type,
517 publication date, document type etc.) were defined; all results were in English language (last
518 searched on December 13, 2018). 87 articles were identified through the database search; 55
519 additional articles were identified by other sources. After removing duplicates (n=16), 126 articles
520 were screened for eligibility based on the abstracts. 44 publications were not eligible on the basis
521 of their abstracts, 82 underwent further full-text assessment. Of these, 66 articles were included for
522 review purposes.

523 Titles classified as highly relevant (i.e. review articles, epidemiological studies) indicated further
524 sources, not found by searching the Scopus database.

525 This review elaborates and expands on five review articles identified as highly relevant [4,6–9].

526 ## References

527 1. Codd, B.G.; Azevedo, S.; Bagchi, S.; Carmichael, W.; Harding, W.; Kaya, K.; Utkilen, H.
528 INTERNATIONAL HYDROLOGICAL PROGRAMME CYANONET A Global Network for
529 Cyanobacterial Bloom and Toxin Risk Management Initial Situation Assessment and
530 Recommendations IHP-VI Technical Document in Hydrology N°76 UNESCO Working Series
531 SC-2005/WS/55. **2005**.

532 2. Chorus, I.; Bartram, J. *Toxic cyanobacteria in water : a guide to their public health*
533 *consequences, monitoring, and management*; E & FN Spon, 1999; ISBN 0419239308.

534 3. Huisman, J.; Codd, G.A.; Paerl, H.W.; Ibelings, B.W.; Verspagen, J.M.H.; Visser, P.M.
535 Cyanobacterial blooms. *Nat. Rev. Microbiol.* **2018**, *16*, 471–483.

536 4. Verspagen, J.M.H.; Van de Waal, D.B.; Finke, J.F.; Visser, P.M.; Van Donk, E.; Huisman, J.; Orr,
537 J.; Fabry, V.; Aumont, O.; Bopp, L.; et al. Rising CO2 Levels Will Intensify Phytoplankton
538 Blooms in Eutrophic and Hypertrophic Lakes. *PLoS One* **2014**, *9*, e104325.

539 5. Buratti, F.M.; Manganelli, M.; Vichi, S.; Stefanelli, M.; Scardala, S.; Testai, E.; Funari, E.
540 Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human
541 health toxicological risk evaluation. *Arch. Toxicol.* **2017**, *91*, 1049–1130.

542 6. Wood, R. Acute animal and human poisonings from cyanotoxin exposure — A review of the
543 literature. *Environ. Int.* **2016**, *91*, 276–282.

544 7. Stewart, I.; Seawright, A.A.; Shaw, G.R. Cyanobacterial poisoning in livestock, wild mammals
545 and birds - an overview. In *Cyanobacterial Harmful Algal Blooms: State of the Science and*
546 *Research Needs*; Springer New York: New York, NY, 2008; pp. 613–637.

547 8. Azevedo, S.M.F..; Carmichael, W.W.; Jochimsen, E.M.; Rinehart, K.L.; Lau, S.; Shaw, G.R.;
548 Eaglesham, G.K. Human intoxication by microcystins during renal dialysis treatment in
549 Caruaru—Brazil. *Toxicology* **2002**, *181*, 441–446.

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

9. Turner, P.C.; Gammie, A.J.; Hollinrake, K.; Codd, G.A. Pneumonia associated with cyanobacteria. *BMJ* **1990**, *300*, 1440–1441.

10. Giannuzzi, L.; Sedan, D.; Echenique, R.; Andrinolo, D. An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande Dam, Argentina. *Mar Drugs* **2011**, *9*, 2164–2175.

11. Vidal, F.; Sedan, D.; D'Agostino, D.; Cavalieri, M.L.; Mullen, E.; Parot Varela, M.M.; Flores, C.; Caixach, J.; Andrinolo, D. Recreational Exposure during Algal Bloom in Carrasco Beach, Uruguay: A Liver Failure Case Report. *Toxins (Basel).* **2017**, *9*.

12. Svirčev, Z.; Drobac, D.; Tokodi, N.; Mijović, B.; Codd, G.A.; Meriluoto, J. Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. *Arch. Toxicol.* **2017**, *91*, 621–650.

13. Levesque, B.; Gervais, M.-C.; Chevalier, P.; Gauvin, D.; Anassour-Laouan-Sidi, E.; Gingras, S.; Fortin, N.; Brisson, G.G.; Greer, C.; Bird, D.; et al. Prospective study of acute health effects in relation to exposure to cyanobacteria. *Sci. Total Environ.* **2014**, *466–467*, 397–403.

14. Lévesque, B.; Gervais, M.-C.; Chevalier, P.; Gauvin, D.; Anassour-Laouan-Sidi, E.; Gingras, S.; Fortin, N.; Brisson, G.; Greer, C.; Bird, D. Exposure to cyanobacteria: acute health effects associated with endotoxins. *Public Health* **2016**, *134*, 98–101.

15. Bourke, A.T.C.; Hawes, R.B.; Neilson, A.; Stallman, N.D. An outbreak of hepato-enteritis (the Palm Island mystery disease) possibly caused by algal intoxication. *Toxicon* **1983**, *21*, 45–48.

16. Falconer, I.R.; Beresford, A.M.; Runnegar, M.T. Evidence of liver damage by toxin from a bloom of the blue-green alga, Microcystis aeruginosa. *Med. J. Aust.* **1983**, *1*, 511–4.

17. Zheng, C.; Zeng, H.; Lin, H.; Wang, J.; Feng, X.; Qiu, Z.; Chen, J.; Luo, J.; Luo, Y.; Huang, Y.; et al. Serum microcystin levels positively linked with risk of hepatocellular carcinoma: A case-control study in southwest China. *Hepatology* **2017**, *66*, 1519–1528.

18. Liu, W.; Wang, L.; Zheng, C.; Liu, L.; Wang, J.; Li, D.; Tan, Y.; Zhao, X.; He, L.; Shu, W. Microcystin-LR increases genotoxicity induced by aflatoxin B1 through oxidative stress and DNA base excision repair genes in human hepatic cell lines. *Environ. Pollut.* **2018**, *233*, 455–463.

19. He, L.; Huang, Y.; Guo, Q.; Zeng, H.; Zheng, C.; Wang, J.; Chen, J.; Wang, L.; Shu, W. Chronic Microcystin-LR Exposure Induces Hepatocarcinogenesis via Increased Gankyrin <i>in Vitro</i> and <i>in Vivo</i> *Cell. Physiol. Biochem.* **2018**, *49*, 1420–1430.

20. Svirčev, Z.; Drobac, D.; Tokodi, N.; Lužanin, Z.; Munjas, A.M.; Nikolin, B.; Vuleta, D.; Meriluoto, J. Epidemiology of Cancers in Serbia and Possible Connection with Cyanobacterial Blooms. *J. Environ. Sci. Heal. Part C* **2014**, *32*, 319–337.

21. Zhou, L.; Yu, H.; Chen, K. Relationship between microcystin in drinking water and colorectal cancer. *Biomed. Environ. Sci.* **2002**, *15*, 166–71.

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

22. Fleming, L.E.; Rivero, C.; Burns, J.; Williams, C.; Bean, J.A.; Shea, K.A.; Stinn, J. Blue green algal (cyanobacterial) toxins, surface drinking water, and liver cancer in Florida. *Harmful Algae* **2002**, *1*, 157–168.

23. Tisdale, E.S. Epidemic of intestinal disorders in Charleston, W. Va., occurring simultaneously with unprecedented water supply conditions. *Am. J. Public Heal.* **1931**, *21*, 198–200.

24. Zilberg, B. Gastroenteritis in Salisbury. European children--a five-year study. *Cent. Afr. J. Med.* **1966**, *12*, 164–8.

25. Keleti, G.; Sykora, J.L.; Lippy, E.C.; Shapiro, M.A. Composition and biological properties of lipopolysaccharides isolated from Schizothrix calcicola (Ag.) Gomont (Cyanobacteria). *Appl. Environ. Microbiol.* **1979**, *38*, 471–7.

26. Lippy, E.C.; Erb, J. Gastrointestinal Illness at Sewickley, Pa. *J. Am. Water Works Assoc.* **1976**, *68*, 606–610.

27. Byth, S. Palm Island mystery disease. *Med. J. Aust.* **1980**, *2*, 40, 42.

28. Teixeira, M. da G.; Costa, M. da C.; de Carvalho, V.L.; Pereira, M. dos S.; Hage, E. Gastroenteritis epidemic in the area of the Itaparica Dam, Bahia, Brazil. *Bull. Pan Am. Health Organ.* **1993**, *27*, 244–53.

29. Anadotter, H.; Cronberg, G.; Lawton, L.; Hansson, H.-B.; Gothe, U.; Skulberg, O.M. An extensive outbreak of gastroenteritis associated with the toxic cyanobacterium Planktothrix agardhii (Oscillatoriales, Cyanophyceae) in Scania, South Sweden. In *Cyanotoxins, Occurrence, Causes, Consequences*; Chorus, I., Ed.; Springer: Berlin, 2001; pp. 200–208.

30. El Saadi, O.; Esterman, A.J.; Cameron, S.; Roder, D.M. Murray River water, raised cyanobacterial cell counts, and gastrointestinal and dermatological symptoms. **1995**, *162*, 122–125.

31. Gunnarsson, H.; Sanseovic, A.-M.; 98, N. V *Possible Linkages Between Algae Toxins in Drinking Water and Related Illnesses in Windhoek, Namibia*; 2001;

32. Svirčev, Z.; Drobac, D.; Tokodi, N.; Đenić, D.; Simeunović, J.; Hiskia, A.; Kaloudis, T.; Mijović, B.; Šušak, S.; Protić, M.; et al. Lessons from the Užice Case. In *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*; John Wiley & Sons, Ltd: Chichester, UK, 2017; pp. 298–308.

33. McCarty, C.L.; Nelson, L.; Eitniear, S.; Zgodzinski, E.; Zabala, A.; Billing, L.; DiOrio, M. Community Needs Assessment After Microcystin Toxin Contamination of a Municipal Water Supply — Lucas County, Ohio, September 2014. *MMWR. Morb. Mortal. Wkly. Rep.* **2016**, *65*, 925–929.

34. Ming, X.; Jianbo, Y.; Yuzuo, L.; Lei, H.; Yan, C.; Zuo, S.; Shunzhang, Y. Microcystins in Drinking Water and Mortality of Major Cancer in a City Along Taihu Lake. *CHINESE J. Prev. Control CHRONIC NON-COMMUNICABLE Dis.* **2003**, *11*, 112–113.

35. Campos Da Cunha-Bellém, F.N. As cianobactérias na água e a morbilidade e mortalidade na

Peer-reviewed version available at *Environmental Sciences Europe* **2019**; doi:10.1186/s12302-019-0212-2

região do Alentejo, Universidade de Évora, 2014.

36. Soward, T.E. Evaluation of Cancer from Exposure to Cyanotoxins in Drinking Water at Grand Lake Saint Marys, Wright State University, Dayton, Ohio, 2011.

37. DILLENBERG, H.O.; DEHNEL, M.K. Toxic waterbloom in Saskatchewan, 1959. *Can. Med. Assoc. J.* **1960**, *83*, 1151–4.

38. Billings, W.H. Water-Associated Human Illness in Northeast Pennsylvania and its Suspected Association with Blue-Green Algae Blooms. In *The Water Environment*; Carmichael, W.W., Ed.; Springer US, 1981; pp. 243–255.

39. Pilotto, L.S.; Douglas, R.M.; Burch, M.D.; Cameron, S.; Beers, M.; Rouch, G.J.; Robinson, P.; Kirk, M.; Cowie, C.T.; Hardiman, S.; et al. Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities. *Aust. N. Z. J. Public Health* **1997**, *21*, 562–6.

40. Stewart, I.; Webb, P.M.; Schluter, P.J.; Fleming, L.E.; Burns, J.W.; Gantar, M.; Backer, L.C.; Shaw, G.R. Epidemiology of recreational exposure to freshwater cyanobacteria - an international prospective cohort study. *BMC Public Health* **2006**, *6*.

41. Codd, G.; Bell, S.; Kaya, K.; Ward, C.; Beattie, K.; Metcalf, J. Cyanobacterial toxins, exposure routes and human health. *Eur. J. Phycol.* **1999**, *34*, 405–415.

42. Rapala, J.; Robertson, A.; Negri, A.P.; Berg, K.A.; Tuomi, P.; Lyra, C.; Erkomaa, K.; Lahti, K.; Hoppu, K.; Lepistö, L. First report of saxitoxin in Finnish lakes and possible associated effects on human health. *Environ. Toxicol.* **2005**, *20*, 331–340.

43. Berg, K.A.; Lyra, C.; Niemi, R.M.; Heens, B.; Hoppu, K.; Erkomaa, K.; Sivonen, K.; Rapala, J. Virulence genes of *Aeromonas* isolates, bacterial endotoxins and cyanobacterial toxins from recreational water samples associated with human health symptoms. *J. Water Health* **2011**, *9*, 670.

44. Hilborn, E.D.; Roberts, V.A.; Backer, L.; Deconno, E.; Egan, J.S.; Hyde, J.B.; Nicholas, D.C.; Wiegert, E.J.; Billing, L.M.; Diorio, M.; et al. Algal bloom-associated disease outbreaks among users of freshwater lakes–United States, 2009-2010. *MMWR. Morb. Mortal. Wkly. Rep.* **2014**, *63*, 11–15.

45. Trevino-Garrison, I.; DeMent, J.; Ahmed, F.; Haines-Lieber, P.; Langer, T.; Ménager, H.; Neff, J.; van der Merwe, D.; Carney, E. Human Illnesses and Animal Deaths Associated with Freshwater Harmful Algal Blooms—Kansas. *Toxins (Basel).* **2015**, *7*, 353–366.

46. WHO Cyanobacterial toxins: Microcystin-LR in drinking-water background document for development of WHO guidelines for drinking-water quality. **2003**.

47. Adamovsky, O.; Buerger, A.N.; Wormington, A.M.; Ector, N.; Griffitt, R.J.; Bisesi, J.H.; Martyniuk, C.J. The gut microbiome and aquatic toxicology: An emerging concept for environmental health. *Environ. Toxicol. Chem.* **2018**, *37*, 2758–2775.

48. Lin, J.; Chen, J.; He, J.; Chen, J.; Yan, Q.; Zhou, J.; Xie, P. Effects of microcystin-LR on bacterial

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

662 and fungal functional genes profile in rat gut. *Toxicon* **2015**, *96*, 50–56.

663 49. Chen, J.; Xie, P.; Lin, J.; He, J.; Zeng, C.; Chen, J. Effects of microcystin-LR on gut microflora in
664 different gut regions of mice. *J. Toxicol. Sci.* **2015**, *40*, 485–494.

665 50. Mowat, A.M.; Agace, W.W. Regional specialization within the intestinal immune system. *Nat.*
666 *Rev. Immunol.* **2014**, *14*, 667–685.

667 51. Agace, W.W.; McCoy, K.D. Regionalized Development and Maintenance of the Intestinal
668 Adaptive Immune Landscape. *Immunity* **2017**, *46*, 532–548.

669 52. Ziegler, S.F.; Buckner, J.H. FOXP3 and the regulation of Treg/Th17 differentiation. *Microbes*
670 *Infect.* **2009**, *11*, 594–598.

671 53. Rakoff-Nahoum, S.; Paglino, J.; Eslami-Varzaneh, F.; Edberg, S.; Medzhitov, R. Recognition of
672 Commensal Microflora by Toll-Like Receptors Is Required for Intestinal Homeostasis. *Cell*
673 **2004**, *118*, 229–241.

674 54. Murphy, K.; Weaver, C. *Janeway's Immunobiology*; 9th ed.; Garland Science, 2016; ISBN
675 1315533243.

676 55. Testai, E.; Buratti, F.M.; Funari, E.; Manganelli, M.; Vichi, S.; Arnich, N.; Biré, R.; Fessard, V.;
677 Sialehaamoa, A. Review and analysis of occurrence, exposure and toxicity of cyanobacteria
678 toxins in food. *EFSA Support. Publ.* **2016**, *13*.

679 56. Spoof, L.; Catherine, A. Appendix 3: Tables of Microcystins and Nodularins. In *Handbook of*
680 *Cyanobacterial Monitoring and Cyanotoxin Analysis*; John Wiley & Sons, Ltd: Chichester, UK,
681 2017; pp. 526–537.

682 57. Hilborn, E.D.; Carmichael, W.W.; Soares, R.M.; Yuan, M.; Servaites, J.C.; Barton, H.A.;
683 Azevedo, S.M.F.O. Serologic evaluation of human microcystin exposure. *Environ. Toxicol.*
684 **2007**, *22*, 459–463.

685 58. Greer, B.; Meneely, J.P.; Elliott, C.T. Uptake and accumulation of Microcystin-LR based on
686 exposure through drinking water: An animal model assessing the human health risk. *Sci. Rep.*
687 **2018**, *8*, 4913.

688 59. Chen, J.; Xie, P.; Li, L.; Xu, J. First Identification of the Hepatotoxic Microcystins in the Serum
689 of a Chronically Exposed Human Population Together with Indication of Hepatocellular
690 Damage. *Toxicol. Sci.* **2009**, *108*, 81–89.

691 60. Mohamed, Z.A.; Bakr, A.; Soliman, H.A. Bioavailability of bound microcystins in mice orally
692 fed with contaminated tilapia edible tissues: Implications to human health. *Toxicon* **2018**,
693 *151*, 34–36.

694 61. Fischer, A.; Hoeger, S.J.; Stemmer, K.; Feurstein, D.J.; Knobeloch, D.; Nussler, A.; Dietrich, D.R.
695 The role of organic anion transporting polypeptides (OATPs/SLCOs) in the toxicity of different
696 microcystin congeners in vitro: A comparison of primary human hepatocytes and OATP-
697 transfected HEK293 cells. *Toxicol. Appl. Pharmacol.* **2010**, *245*, 9–20.

698 62. Fischer, W.J.; Altheimer, S.; Cattori, V.; Meier, P.J.; Dietrich, D.R.; Hagenbuch, B. Organic

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

699 anion transporting polypeptides expressed in liver and brain mediate uptake of microcystin.
700 *Toxicol. Appl. Pharmacol.* **2005**, *203*, 257–263.

701 63. Oswald, S. Organic Anion Transporting Polypeptide (OATP) transporter expression,
702 localization and function in the human intestine. *Pharmacol. Ther.* **2018**.

703 64. Drozdzik, M.; Gröer, C.; Penski, J.; Lapczuk, J.; Ostrowski, M.; Lai, Y.; Prasad, B.; Unadkat, J.D.;
704 Siegmund, W.; Oswald, S. Protein Abundance of Clinically Relevant Multidrug Transporters
705 along the Entire Length of the Human Intestine. *Mol. Pharm.* **2014**, *11*, 3547–3555.

706 65. Miyauchi, E.; Tachikawa, M.; Declèves, X.; Uchida, Y.; Bouillot, J.-L.; Poitou, C.; Oppert, J.-M.;
707 Mouly, S.; Bergmann, J.-F.; Terasaki, T.; et al. Quantitative Atlas of Cytochrome P450, UDP-
708 Glucuronosyltransferase, and Transporter Proteins in Jejunum of Morbidly Obese Subjects.
709 *Mol. Pharm.* **2016**, *13*, 2631–2640.

710 66. Vaessen, S.F.C.; van Lipzig, M.M.H.; Pieters, R.H.H.; Krul, C.A.M.; Wortelboer, H.M.; van de
711 Steeg, E. Regional Expression Levels of Drug Transporters and Metabolizing Enzymes along
712 the Pig and Human Intestinal Tract and Comparison with Caco-2 Cells. *Drug Metab. Dispos.*
713 **2017**, *45*, 353–360.

714 67. Moreau, A.; Le Vee, M.; Jouan, E.; Parmentier, Y.; Fardel, O. Drug transporter expression in
715 human macrophages. *Fundam. Clin. Pharmacol.* **2011**, *25*, 743–752.

716 68. Adamovsky, O.; Moosova, Z.; Pekarova, M.; Basu, A.; Babica, P.; Svihalkova Sindlerova, L.;
717 Kubala, L.; Blaha, L. Immunomodulatory potency of microcystin, an important water-
718 polluting cyanobacterial toxin. *Environ. Sci. Technol.* **2015**, *49*, 12457–12464.

719 69. Moosova, Z.; Hrouzek, P.; Kapuscik, A.; Blaha, L.; Adamovsky, O. Immunomodulatory effects
720 of selected cyanobacterial peptides in vitro. *Toxicon* **2018**, *149*, 20–25.

721 70. Shimada, H.; Nakamura, Y.; Nakanishi, T.; Tamai, I. OATP2A1/SLCO2A1-mediated
722 prostaglandin E2 loading into intracellular acidic compartments of macrophages contributes
723 to exocytotic secretion. *Biochem. Pharmacol.* **2015**, *98*, 629–638.

724 71. Nakano, T.; Katsuki, S.; Chen, M.; Decano, J.L.; Halu, A.; Lee, L.H.; Pestana, D.V.S.; Kum, A.S.T.;
725 Kuromoto, R.K.; Golden, W.S.; et al. Uremic Toxin Indoxyl Sulfate Promotes Proinflammatory
726 Macrophage Activation Via the Interplay of OATP2B1 and Dll4-Notch Signaling. *Circulation*
727 **2019**, *139*, 78–96.

728 72. Campos, A.; Vasconcelos, V. Molecular mechanisms of microcystin toxicity in animal cells.
729 *Int. J. Mol. Sci.* **2010**, *11*, 268–87.

730 73. Mumby, M. PP2A: unveiling a reluctant tumor suppressor. *Cell* **2007**, *130*, 21–4.

731 74. Ren, Y.; Yang, M.; Chen, M.; Zhu, Q.; Zhou, L.; Qin, W.; Wang, T. Microcystin-LR promotes
732 epithelial-mesenchymal transition in colorectal cancer cells through PI3-K/AKT and SMAD2.
733 *Toxicol. Lett.* **2017**, *265*, 53–60.

734 75. Ito, E.; Kondo, F.; Terao, K.; Harada, K. Neoplastic nodular formation in mouse liver induced
735 by repeated intraperitoneal injections of microcystin-LR. *Toxicon* **1997**, *35*, 1453–7.

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

76. Zhu, Y.; Zhong, X.; Zheng, S.; Ge, Z.; Du, Q.; Zhang, S. Transformation of immortalized colorectal crypt cells by microcystin involving constitutive activation of Akt and MAPK cascade. *Carcinogenesis* **2005**, *26*, 1207–1214.

77. Fawell, J.K.; Mitchell, R.E.; Everett, D.J.; Hill, R.E. The toxicity of cyanobacterial toxins in the mouse: I Microcystin-LR. *Hum. Exp. Toxicol.* **1999**, *18*, 162–167.

78. Niedermeyer, T. Microcystin congeners described in the literature 2014.

79. Meriluoto, J.; Spoof, L.; Codd, G.A. *Handbook of cyanobacterial monitoring and cyanotoxin analysis*; Wiley, 2016; ISBN 9781119068723.

80. Bain, P.; Shaw, G.; Patel, B. Induction of p53-regulated gene expression in human cell lines exposed to the cyanobacterial toxin cylindrospermopsin. *J. Toxicol. Environ. Heal. Part A* **2007**, *70*, 1687–1693.

81. Shaw, G.; Lam, P.K.S. Health aspects of freshwater cyanobacterial toxins. *Water Sci. Technol. Water Supply* **2007**, *7*, 193.

82. Garibo, D.; Flores, C.; Cetó, X.; Prieto-Simón, B.; del Valle, M.; Caixach, J.; Diogène, J.; Campàs, M. Inhibition equivalency factors for microcystin variants in recombinant and wild-type protein phosphatase 1 and 2A assays. *Environ. Sci. Pollut. Res.* **2014**, *21*, 10652–10660.

83. Chen, Y.-M.; Lee, T.-H.; Lee, S.-J.; Huang, H.-B.; Huang, R.; Chou, H.-N. Comparison of protein phosphatase inhibition activities and mouse toxicities of microcystins. *Toxicon* **2006**, *47*, 742–746.

84. Zong, W.; Wang, X.; Du, Y.; Zhang, S.; Zhang, Y.; Teng, Y. Molecular Mechanism for the Regulation of Microcystin Toxicity to Protein Phosphatase 1 by Glutathione Conjugation Pathway. *Biomed Res. Int.* **2017**, *2017*, 1–10.

85. Dietrich, D.; Hoeger, S. Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): a reasonable or misguided approach? *Toxicol. Appl. Pharmacol.* **2005**, *203*, 273–289.

86. Zurawell, R.W.; Chen, H.; Burke, J.M.; Prepas, E.E. Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *J. Toxicol. Environ. Heal. Part B* **2005**, *8*, 1–37.

87. Feurstein, D.; Holst, K.; Fischer, A.; Dietrich, D.R. OATP-associated uptake and toxicity of microcystins in primary murine whole brain cells. *Toxicol. Appl. Pharmacol.* **2009**, *234*, 247–255.

88. Ito, E.; Kondo, F.; Harada, K.-I. First report on the distribution of orally administered microcystin-LR in mouse tissue using an immunostaining method. *Toxicon* **2000**, *38*, 37–48.

89. Gaudin, J.; Huet, S.; Jarry, G.; Fessard, V. In vivo DNA damage induced by the cyanotoxin microcystin-LR: Comparison of intra-peritoneal and oral administrations by use of the comet assay. *Mutat. Res. Toxicol. Environ. Mutagen.* **2008**, *652*, 65–71.

90. Botha, N.; Venter, M. van de; Downing, T.G.; Shephard, E.G.; Gehringer, M.M. The effect of

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

773 intraperitoneally administered microcystin-LR on the gastrointestinal tract of Balb/c mice.
774 *Toxicon* **2004**, *43*, 251–254.

775 91. Falconer, I.R. Potential impact on human health of toxic cyanobacteria. **1996**, *35*, 6–11.

776 92. Sedan, D.; Laguens, M.; Copparoni, G.; Aranda, J.O.; Giannuzzi, L.; Marra, C.A.; Andrinolo, D.
777 Hepatic and intestine alterations in mice after prolonged exposure to low oral doses of
778 Microcystin-LR. *Toxicon* **2015**, *104*, 26–33.

779 93. Moreno, I.M.; Mate, A.; Repetto, G.; Vázquez, C.M.; Cameán, A.M. Influence of microcystin-
780 LR on the activity of membrane enzymes in rat intestinal mucosa. *J. Physiol. Biochem.* **2003**,
781 *59*, 293–9.

782 94. Fernández, D.A.; Louzao, M.C.; Vilariño, N.; Fraga, M.; Espiña, B.; Vieytes, M.R.; Botana, L.M.
783 Evaluation of the intestinal permeability and cytotoxic effects of cylindrospermopsin. *Toxicon*
784 **2014**, *91*, 23–34.

785 95. Henri, J.; Huguet, A.; Delmas, J.-M.; Besson, A.; Sanders, P.; Fessard, V. Low in vitro
786 permeability of the cyanotoxin microcystin-LR across a Caco-2 monolayer: with identification
787 of the limiting factors using modelling. *Toxicon* **2014**, *91*, 5–14.

788 96. Puerto, M.; Pichardo, S.; Jos, Á.; Cameán, A.M. Microcystin-LR induces toxic effects in
789 differentiated and undifferentiated Caco-2 cells. *Arch. Toxicol.* **2010**, *84*, 405–410.

790 97. Huguet, A.; Henri, J.; Petitpas, M.; Hogeveen, K.; Fessard, V. Comparative Cytotoxicity,
791 Oxidative Stress, and Cytokine Secretion Induced by Two Cyanotoxin Variants, Microcystin
792 LR and RR, in Human Intestinal Caco-2 Cells. *J. Biochem. Mol. Toxicol.* **2013**, *27*, 253–258.

793 98. Zeller, P.; Quenault, H.; Huguet, A.; Blanchard, Y.; Fessard, V. Transcriptomic comparison of
794 cyanotoxin variants in a human intestinal model revealed major differences in oxidative
795 stress response: Effects of MC-RR and MC-LR on Caco-2 cells. *Ecotoxicol. Environ. Saf.* **2012**,
796 *82*, 13–21.

797 99. Zeller, P.; Clément, M.; Fessard, V. Similar uptake profiles of microcystin-LR and -RR in an in
798 vitro human intestinal model. *Toxicology* **2011**, *290*, 7–13.

799 100. Vesterkvist, P.; Misiorek, J.; Spoof, L.; Toivola, D.; Meriluoto, J. Comparative Cellular Toxicity
800 of Hydrophilic and Hydrophobic Microcystins on Caco-2 Cells. *Toxins (Basel).* **2012**, *4*, 1008–
801 1023.

802 101. Žegura, B.; Volčič, M.; Lah, T.T.; Filipič, M. Different sensitivities of human colon
803 adenocarcinoma (CaCo-2), astrocytoma (IPDDC-A2) and lymphoblastoid (NCNC) cell lines to
804 microcystin-LR induced reactive oxygen species and DNA damage. *Toxicon* **2008**, *52*, 518–
805 525.

806 102. Miao, C.; Ren, Y.; Chen, M.; Wang, Z.; Wang, T. Microcystin-LR promotes migration and
807 invasion of colorectal cancer through matrix metalloproteinase-13 up-regulation. *Mol.*
808 *Carcinog.* **2016**, *55*, 514–524.

809 103. Zhu, Q.; Wang, Z.; Zhou, L.; Ren, Y.; Gong, Y.; Qin, W.; Bai, L.; Hu, J.; Wang, T. The role of

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

cadherin-11 in microcystin-LR-induced migration and invasion in colorectal carcinoma cells. *Oncol. Lett.* **2017**, *15*, 1417–1422.

104. Ren, Y.; Yang, M.; Chen, M.; Zhu, Q.; Zhou, L.; Qin, W.; Wang, T. Microcystin-LR promotes epithelial-mesenchymal transition in colorectal cancer cells through PI3-K/AKT and SMAD2. *Toxicol. Lett.* **2017**, *265*, 53–60.

105. Ren, Y.; Yang, M.; Ma, R.; Gong, Y.; Zou, Y.; Wang, T.; Wu, J. Microcystin-LR promotes migration via the cooperation between microRNA-221/PTEN and STAT3 signal pathway in colon cancer cell line DLD-1. *Ecotoxicol. Environ. Saf.* **2019**, *167*, 107–113.

106. Puerto, M.; Pichardo, S.; Jos, Á.; Cameán, A.M. Microcystin-LR induces toxic effects in differentiated and undifferentiated Caco-2 cells. *Arch. Toxicol.* **2010**, *84*, 405–410.

107. Falconer, I.R. Is there a human health hazard from microcystins in the drinking water supply? *Acta Hydrochim. Hydrobiol.* **2005**, *33*, 64–71.

108. Nishiwaki, R.; Ohta, T.; Sueoka, E.; Suganuma, M.; Harada, K.; Watanabe, M.F.; Fujiki, H. Two significant aspects of microcystin-LR: specific binding and liver specificity. *Cancer Lett.* **1994**, *83*, 283–289.

109. Gaudin, J.; Huet, S.; Jarry, G.; Fessard, V. In vivo DNA damage induced by the cyanotoxin microcystin-LR: Comparison of intra-peritoneal and oral administrations by use of the comet assay. *Mutat. Res. Toxicol. Environ. Mutagen.* **2008**, *652*, 65–71.

110. Wang, Q.; Xie, P.; Chen, J.; Liang, G. Distribution of microcystins in various organs (heart, liver, intestine, gonad, brain, kidney and lung) of Wistar rat via intravenous injection. *Toxicon* **2008**, *52*, 721–727.

111. Djediat, C.; Malécot, M.; de Luze, A.; Bernard, C.; Puiseux-Dao, S.; Edery, M. Localization of microcystin-LR in medaka fish tissues after cyanotoxin gavage. *Toxicon* **2010**, *55*, 531–535.

112. Zeller, P.; Clément, M.; Fessard, V. Similar uptake profiles of microcystin-LR and -RR in an in vitro human intestinal model. *Toxicology* **2011**, *290*, 7–13.

113. Puerto, M.; Pichardo, S.; Jos, Á.; Cameán, A.M. Comparison of the toxicity induced by microcystin-RR and microcystin-YR in differentiated and undifferentiated Caco-2 cells. *Toxicon* **2009**, *54*, 161–169.

114. Vesterkvist, P.; Misiorek, J.; Spoof, L.; Toivola, D.; Meriluoto, J. Comparative cellular toxicity of hydrophilic and hydrophobic microcystins on Caco-2 Cells. *Toxins (Basel).* **2012**, *4*, 1008–1023.

115. Zhou, Y.; Xu, X.; Yu, B.; Yu, G. Characterization of *in vitro* effects of microcystin-LR on intestinal epithelial cells. *Environ. Toxicol.* **2017**, *32*, 1539–1547.

116. Chen, T.; Shen, P.; Zhang, J.; Hua, Z. Effects of microcystin-LR on patterns of iNOS and cytokine mRNA expression in macrophagesin vitro. *Environ. Toxicol.* **2005**, *20*, 85–91.

117. Ohtani, I.; Moore, R.E.; Runnegar, M.T.C. Cylindrospermopsin: a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *J. Am. Chem. Soc.* **1992**, *114*, 7941–7942.

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

118. Pichardo, S.; Cameán, A.M.; Jos, A. In Vitro Toxicological Assessment of Cylindrospermopsin: A Review. *Toxins (Basel).* **2017**, *9*, 402.

119. Froscio, S.M.S.M.; Humpage, A.R.A.R.; Burcham, P.C.P.C.; Falconer, I.R.I.R. Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. *Environ. Toxicol.* **2003**, *18*, 243–251.

120. Norris, R.L.G.; Seawright, A.A.; Shaw, G.R.; Senogles, P.; Eaglesham, G.K.; Smith, M.J.; Chiswell, R.K.; Moore, M.R. Hepatic xenobiotic metabolism of cylindrospermopsin in vivo in the mouse. *Toxicon* **2002**, *40*, 471–476.

121. Runnegar, M.T.; Kong, S.; Zhong, Y.-Z.; Lu, S.C. Inhibition of reduced glutathione synthesis by cyanobacterial alkaloid cylindrospermopsin in cultured rat hepatocytes. *Biochem. Pharmacol.* **1995**, *49*, 219–225.

122. Runnegar, M.T.; Kong, S.M.; Zhong, Y.Z.; Ge, J.L.; Lu, S.C. The Role of Glutathione in the Toxicity of a Novel Cyanobacterial Alkaloid Cylindrospermopsin in Cultured Rat Hepatocytes. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 235–241.

123. Froscio, S.M.; Humpage, A.R.; Burcham, P.C.; Falconer, I.R. Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. *Environ. Toxicol.* **2003**, *18*, 243–251.

124. Humpage, A.R.; Fontaine, F.; Froscio, S.; Burcham, P.; Falconer, I.R. Cylindrospermopsin genotoxicity and cytotoxicity: Role of cytochrome P-450 and oxidative stress. *J. Toxicol. Environ. Heal. a-Current Issues* **2005**, *68*, 739–753.

125. Bazin, E.; Mourot, A.; Humpage, A.R.; Fessard, V. Genotoxicity of a Freshwater Cyanotoxin, Cylindrospermopsin, in Two Human Cell Lines: Caco-2 and HepaRG. *Environ. Mol. Mutagen.* **2010**, *51*, 251–259.

126. Kittler, K.; Hurtaud-Pessel, D.; Maul, R.; Kolrep, F.; Fessard, V. In vitro metabolism of the cyanotoxin cylindrospermopsin in HepaRG cells and liver tissue fractions. *Toxicon* **2016**, *110*, 47–50.

127. Liebel, S.; de Oliveira Ribeiro, C.A.; de Magalhaes, V.F.; da Silva Rde, C.; Rossi, S.C.; Randi, M.A.; Filipak Neto, F. Low concentrations of cylindrospermopsin induce increases of reactive oxygen species levels, metabolism and proliferation in human hepatoma cells (HepG2). *Toxicol Vitr.* **2015**, *29*, 479–488.

128. Humpage, A.R.; Fontaine, F.; Froscio, S.; Burcham, P.; Falconer, I.R. Cylindrospermopsin genotoxicity and cytotoxicity: Role of cytochrome P-450 and oxidative stress. *J. Toxicol. Environ. Heal. Part A* **2005**, *68*, 739–753.

129. Fessard, V.; Bernard, C. Cell alterations but no DNA strand breaks inducedin vitro by cylindrospermopsin in CHO K1 cells. *Environ. Toxicol.* **2003**, *18*, 353–359.

130. Lankoff, A.; Wojcik, A.; Lisowska, H.; Bialczyk, J.; Dziga, D.; Carmichael, W.W. No induction of structural chromosomal aberrations in cylindrospermopsin-treated CHO-K1 cells without and with metabolic activation. *Toxicon* **2007**, *50*, 1105–1115.

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

131. Liebel, S.; de Oliveira Ribeiro, C.A.; de Magalhães, V.F.; da Silva, R. de C.; Rossi, S.C.; Randi, M.A.F.; Filipak Neto, F. Low concentrations of cylindrospermopsin induce increases of reactive oxygen species levels, metabolism and proliferation in human hepatoma cells (HepG2). *Toxicol. Vitr.* **2015**, *29*, 479–488.

132. Kittler, K.; Hurtaud-Pessel, D.; Maul, R.; Kolrep, F.; Fessard, V. In vitro *metabolism of the cyanotoxin cylindrospermopsin in HepaRG cells and liver tissue fractions*; 2016; Vol. 110;.

133. Humpage, A.R.; Falconer, I.R. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: Determination of no observed adverse effect level for deriving a drinking water guideline value. *Environ. Toxicol.* **2003**, *18*, 94–103.

134. Falconer, I.R.; Humpage, A.R. Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsins. *Environ. Toxicol.* **2006**, *21*, 299–304.

135. Seawright, A.A.; Nolan, C.C.; Shaw, G.R.; Chiswell, R.K.; Norris, R.L.; Moore, M.R.; Smith, M.J. The oral toxicity for mice of the tropical cyanobacteriumCylindrospermopsis raciborskii (Woloszynska). *Environ. Toxicol.* **1999**, *14*, 135–142.

136. Froscio, S.M.; Cannon, E.; Lau, H.M.; Humpage, A.R. Limited uptake of the cyanobacterial toxin cylindrospermopsin by Vero cells. *Toxicon* **2009**, *54*, 862–868.

137. Chong, M.W.K.; Wong, B.S.F.; Lam, P.K.S.; Shaw, G.R.; Seawright, A.A. Toxicity and uptake mechanism of cylindrospermopsin and lophyrotomin in primary rat hepatocytes. *Toxicon* **2002**, *40*, 205–11.

138. Pichardo, S.; Devesa, V.; Puerto, M.; Vélez, D.; Cameán, A.M. Intestinal transport of Cylindrospermopsin using the Caco-2 cell line. *Toxicol. Vitr.* **2017**, *38*, 142–149.

139. Gutiérrez-Praena, D.; Pichardo, S.; Jos, Á.; Moreno, F.J.; Cameán, A.M. Biochemical and pathological toxic effects induced by the cyanotoxin Cylindrospermopsin on the human cell line Caco-2. *Water Res.* **2012**, *46*, 1566–1575.

140. Humpage, A.R.; Falconer, I.R. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: Determination of no observed adverse effect level for deriving a drinking water guideline value. *Environ. Toxicol.* **2003**, *18*, 94–103.

141. Fastner, J.; Heinze, R.; Humpage, A..; Mischke, U.; Eaglesham, G..; Chorus, I. Cylindrospermopsin occurrence in two German lakes and preliminary assessment of toxicity and toxin production of Cylindrospermopsis raciborskii (Cyanobacteria) isolates. *Toxicon* **2003**, *42*, 313–321.

142. Froscio, S.M.; Fanok, S.; Humpage, A.R. Cytotoxicity screening for the cyanobacterial toxin cylindrospermopsin. *J. Toxicol. Environ. Heal. Part A* **2009**, *72*, 345–349.

143. Poniedziałek, B.; Rzymski, P.; Kokociński, M.; Karczewski, J. Toxic potencies of metabolite(s) of non-cylindrospermopsin producing Cylindrospermopsis raciborskii isolated from temperate zone in human white cells. *Chemosphere* **2015**, *120*, 608–614.

144. Poniedziałek, B.; Rzymski, P.; Karczewski, J. Cylindrospermopsin decreases the oxidative

Peer-reviewed version available at *Environmental Sciences Europe* **2019**; doi:10.1186/s12302-019-0212-2

922 burst capacity of human neutrophils. *Toxicon* **2014**, *87*, 113–119.

923 145. Sieroslawska, A.; Rymuszka, A. Cylindrospermopsin induces oxidative stress and genotoxic
924 effects in the fish CLC cell line. *J Appl Toxicol* **2015**, *35*, 426–433.

925 146. Lone, Y.; Bhide, M.; Koiri, R.K. Microcystin-LR Induced Immunotoxicity in Mammals. *J. Toxicol.*
926 **2016**, *2016*, 1–5.

927 147. Sieroslawska, A.; Rymuszka, A.; Adaszek, Ł. Effects of cylindrospermopsin on the phagocytic
928 cells of the common carp (*Cyprinus carpio* L.). *J. Appl. Toxicol.* **2015**, *35*, 1406–1414.

929 148. Best, J..; Pflugmacher, S.; Wiegand, C.; Eddy, F..; Metcalf, J..; Codd, G.. Effects of enteric
930 bacterial and cyanobacterial lipopolysaccharides, and of microcystin-LR, on glutathione S-
931 transferase activities in zebra fish (Danio rerio). *Aquat. Toxicol.* **2002**, *60*, 223–231.

932 149. Sierosławska, A.; Rymuszka, A.; Bownik, A.; Skowroński, T. The influence of microcystin-LR on
933 fish phagocytic cells. *Hum. Exp. Toxicol.* **2007**, *26*, 603–607.

934 150. Lone, Y.; Bhide, M.; Koiri, R.K. Microcystin-LR Induced Immunotoxicity in Mammals. *J. Toxicol.*
935 **2016**, *2016*, 1–5.

936 151. Sedan, D.; Laguens, M.; Copparoni, G.; Aranda, J.O.; Giannuzzi, L.; Marra, C.A.; Andrinolo, D.
937 Hepatic and intestine alterations in mice after prolonged exposure to low oral doses of
938 Microcystin-LR. *Toxicon* **2015**, *104*, 26–33.

939 152. Marchev, A.S.; Dimitrova, P.A.; Burns, A.J.; Kostov, R. V.; Dinkova-Kostova, A.T.; Georgiev,
940 M.I. Oxidative stress and chronic inflammation in osteoarthritis: can NRF2 counteract these
941 partners in crime? *Ann. N. Y. Acad. Sci.* **2017**, *1401*, 114–135.

942 153. Vasicek, O.; Lojek, A.; Jancinova, V.; Nosal, R.; Ciz, M. Role of histamine receptors in the
943 effects of histamine on the production of reactive oxygen species by whole blood
944 phagocytes. *Life Sci.* **2014**, *100*, 67–72.

945 154. Aderem, A. Phagocytosis and the Inflammatory Response. *J. Infect. Dis.* **2003**, *187*, S340–
946 S345.

947 155. Park, B.S.; Lee, J.-O. Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp. Mol.*
948 *Med.* **2013**, *45*, e66–e66.

949 156. Barton, G.M.; Medzhitov, R. Toll-like receptor signaling pathways. *Science* **2003**, *300*, 1524–
950 5.

951 157. Vaure, C.; Liu, Y. A Comparative Review of Toll-Like Receptor 4 Expression and Functionality
952 in Different Animal Species. *Front. Immunol.* **2014**, *5*, 316.

953 158. Sansonetti, P.J.; Di Santo, J.P. Debugging how bacteria manipulate the immune response.
954 *Immunity* **2007**, *26*, 149–61.

955 159. Medzhitov, R.; Janeway, C.A. Decoding the patterns of self and nonself by the innate immune
956 system. *Science* **2002**, *296*, 298–300.

957 160. Cho, J.-S.; Kang, J.-H.; Um, J.-Y.; Han, I.-H.; Park, I.-H.; Lee, H.-M. Lipopolysaccharide Induces

Peer-reviewed version available at Environmental Sciences Europe 2019; doi:10.1186/s12302-019-0212-2

958 Pro-Inflammatory Cytokines and MMP Production via TLR4 in Nasal Polyp-Derived Fibroblast
959 and Organ Culture. *PLoS One* **2014**, *9*, e90683.

960 161. Lu, Y.-C.; Yeh, W.-C.; Ohashi, P.S. LPS/TLR4 signal transduction pathway. *Cytokine* **2008**, *42*,
961 145–151.

962 162. d'Hennezel, E.; Abubucker, S.; Murphy, L.O.; Cullen, T.W. Total Lipopolysaccharide from the
963 Human Gut Microbiome Silences Toll-Like Receptor Signaling. *mSystems* **2017**, *2*.

964 163. Arpaia, N.; Rudensky, A.Y.; Offermanns, S.; Medzhitov, R. Microbial metabolites control gut
965 inflammatory responses. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 2058–9.

966 164. Molteni, M. Glycolipid Fraction from Cyanobacteria for Treatment of Diseases of the Oral
967 Cavity. *Bluegreen Biotech S.R.I., Milan (IT), Pub. No. WO/2010/010172* **2010**, 9.

968 165. Macagno, A.; Molteni, M.; Rinaldi, A.; Bertoni, F.; Lanzavecchia, A.; Rossetti, C.; Sallusto, F. A
969 cyanobacterial LPS antagonist prevents endotoxin shock and blocks sustained TLR4
970 stimulation required for cytokine expression. *J. Exp. Med.* **2006**, *203*, 1481–1492.

971 166. Durai, P.; Batool, M.; Choi, S. Structure and Effects of Cyanobacterial Lipopolysaccharides.
972 *Mar. Drugs* **2015**, *13*, 4217–30.

973 167. Keleti, G.; Sykora, J.L. Production and properties of cyanobacterial endotoxins. *Appl. Environ.*
974 *Microbiol.* **1982**, *43*, 104–9.

975 168. Carillo, S.; Pieretti, G.; Bedini, E.; Parrilli, M.; Lanzetta, R.; Corsaro, M.M. Structural
976 investigation of the antagonist LPS from the cyanobacterium Oscillatoria planktothrix FP1.
977 *Carbohydr. Res.* **2014**, *388*, 73–80.

978 169. Gemma, S.; Molteni, M.; Rossetti, C. Lipopolysaccharides in Cyanobacteria: A Brief Overview.
979 *Adv. Microbiol.* **2016**, *06*, 391–397.

980 170. Munford, R.S. Sensing gram-negative bacterial lipopolysaccharides: a human disease
981 determinant? *Infect. Immun.* **2008**, *76*, 454–65.

982 171. Bäckhed, F.; Hornef, M. Toll-like receptor 4-mediated signaling by epithelial surfaces:
983 necessity or threat? *Microbes Infect.* **2003**, *5*, 951–959.

984 172. Abreu, M.T.; Fukata, M.; Arditi, M. TLR signaling in the gut in health and disease. *J. Immunol.*
985 **2005**, *174*, 4453–60.

986 173. Miller, T.R.; Beversdorf, L.J.; Weirich, C.A.; Bartlett, S.L. Cyanobacterial Toxins of the
987 Laurentian Great Lakes, Their Toxicological Effects, and Numerical Limits in Drinking Water.
988 *Mar. Drugs* **2017**, *15*, 160.

989 174. Bláhová, L.; Adamovský, O.; Kubala, L.; Švihálková Šindlerová, L.; Zounková, R.; Bláha, L. The
990 isolation and characterization of lipopolysaccharides from Microcystis aeruginosa, a
991 prominent toxic water bloom forming cyanobacteria. *Toxicon* **2013**, *76*, 187–196.

992 175. Stewart, I.; Schluter, P.J.; Shaw, G.R. Cyanobacterial lipopolysaccharides and human health -
993 a review. *Environ. Health* **2006**, *5*, 7.

Peer-reviewed version available at *Environmental Sciences Europe* **2019**; doi:10.1186/s12302-019-0212-2

176. Bell, S.G.; Codd, G.A. Cyanobacterial toxins and human health. *Rev. Med. Microbiol.* **1994**, *5*, 256–264.

177. Torokne, A.; Palovics, A.; Bankine, M. Allergenic (sensitization, skin and eye irritation) effects of freshwater cyanobacteria - experimental evidence. *Environ. Toxicol.* **2001**, *16*, 512–6.

178. Funari, E.; Testai, E. Human Health Risk Assessment Related to Cyanotoxins Exposure. *Crit. Rev. Toxicol.* **2008**, *38*, 97–125.

179. Stal, L.J.; Albertano, P.; Bergman, B.; Bröckel, K. von; Gallon, J.R.; Hayes, P.K.; Sivonen, K.; Walsby, A.E. BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea—responses to a changing environment. *Cont. Shelf Res.* **2003**, *23*, 1695–1714.

180. Ploug, H. Cyanobacterial surface blooms formed by Aphanizomenon sp. and Nodularia spumigena in the Baltic Sea: Small-scale fluxes, pH, and oxygen microenvironments. *Limnol. Ocean.* **2008**, *53*, 914–921.

181. Faltermann, S.; Prétôt, R.; Pernthaler, J.; Fent, K. Comparative effects of nodularin and microcystin-LR in zebrafish: 1. Uptake by organic anion transporting polypeptide Oatp1d1 (Slco1d1). *Aquat. Toxicol.* **2016**, *171*, 69–76.

182. Meili, N.; Christen, V.; Fent, K. Nodularin induces tumor necrosis factor-alpha and mitogen-activated protein kinases (MAPK) and leads to induction of endoplasmic reticulum stress. *Toxicol. Appl. Pharmacol.* **2016**, *300*, 25–33.

183. Bagu, J.R.; Sykes, B.D.; Craig, M.M.; Holmes, C.F. A molecular basis for different interactions of marine toxins with protein phosphatase-1. Molecular models for bound motuporin, microcystins, okadaic acid, and calyculin A. *J. Biol. Chem.* **1997**, *272*, 5087–97.

184. Koreivienė, J.; Anne, O.; Kasperovičienė, J.; Burškytė, V. Cyanotoxin management and human health risk mitigation in recreational waters. *Environ. Monit. Assess.* **2014**, *186*, 4443–4459.

185. Weirich, C.A.; Miller, T.R. Freshwater Harmful Algal Blooms: Toxins and Children's Health. *Curr. Probl. Pediatr. Adolesc. Health Care* **2014**, *44*, 2–24.

186. Otten, T.G.; Paerl, H.W. Health Effects of Toxic Cyanobacteria in U.S. Drinking and Recreational Waters: Our Current Understanding and Proposed Direction. *Curr. Environ. Heal. Reports* **2015**, *2*, 75–84.

187. Ersmark, K.; Del Valle, J.R.; Hanessian, S. Chemistry and Biology of the Aeruginosin Family of Serine Protease Inhibitors. *Angew. Chemie Int. Ed.* **2008**, *47*, 1202–1223.

188. Welker, M.; Von Döhren, H. Cyanobacterial peptides — Nature's own combinatorial biosynthesis. *FEMS Microbiol. Rev.* **2006**, *30*, 530–563.

189. Fewer, D.P.; Jokela, J.; Paukku, E.; Österholm, J.; Wahlsten, M.; Permi, P.; Aitio, O.; Rouhiainen, L.; Gomez-Saez, G. V.; Sivonen, K. New Structural Variants of Aeruginosin Produced by the Toxic Bloom Forming Cyanobacterium Nodularia spumigena. *PLoS One* **2013**, *8*, e73618.

190. Faltermann, S.; Zucchi, S.; Kohler, E.; Blom, J.F.; Pernthaler, J.; Fent, K. Molecular effects of the cyanobacterial toxin cyanopeptolin (CP1020) occurring in algal blooms: Global transcriptome analysis in zebrafish embryos. *Aquat. Toxicol.* **2014**, *149*, 33–39.

191. Janssen, E.M.-L. Cyanobacterial peptides beyond microcystins – A review on co-occurrence, toxicity, and challenges for risk assessment. *Water Res.* **2019**, *151*, 488–499.

192. Oftedal, L.; Myhren, L.; Jokela, J.; Gausdal, G.; Sivonen, K.; Døskeland, S.O.; Herfindal, L. The lipopeptide toxins anabaenolysin A and B target biological membranes in a cholesterol-dependent manner. *Biochim. Biophys. Acta - Biomembr.* **2012**, *1818*, 3000–3009.

193. Hrouzek, P.; Kuzma, M.; Černý, J.; Novák, P.; Fišer, R.; Šimek, P.; Lukešová, A.; Kopecký, J. The Cyanobacterial Cyclic Lipopeptides Puwainaphycins F/G Are Inducing Necrosis via Cell Membrane Permeabilization and Subsequent Unusual Actin Relocalization. *Chem. Res. Toxicol.* **2012**, *25*, 1203–1211.

194. Vašíček, O.; Hrouzek, P.; Hájek, J.; Kubala, L.; Babica, P.; Šindlerová, L.Š. The effects of puwainaphycins F on Caco-2 cell line as a model of the intestinal barrier. *Toxicol. Lett.* **2017**, *280*, S86.

195. Humpage, A.; Falconer, I.; Bernard, C.; Froscio, S.; Fabbro, L. Toxicity of the cyanobacterium Limnothrix AC0243 to male Balb/c mice. *Water Res.* **2012**, *46*, 1576–1583.

196. Saker, M.L.; Nogueira, I.C.G.; Vasconcelos, V.M.; Neilan, B.A.; Eaglesham, G.K.; Pereira, P. First report and toxicological assessment of the cyanobacterium Cylindrospermopsis raciborskii from Portuguese freshwaters. *Ecotoxicol. Environ. Saf.* **2003**, *55*, 243–250.

197. Hirahashi, T.; Matsumoto, M.; Hazeki, K.; Saeki, Y.; Ui, M.; Seya, T. Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of Spirulina platensis. *Int. Immunopharmacol.* **2002**, *2*, 423–34.

198. Bernardová, K.; Babica, P.; Maršálek, B.; Bláha, L. Isolation and endotoxin activities of lipopolysaccharides from cyanobacterial cultures and complex water blooms and comparison with the effects of heterotrophic bacteria and green alga. *J. Appl. Toxicol.* **2008**, *28*, 72–77.

199. Falconer, I.R. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. *Phycologia* **2001**, *40*, 228–233.

200. Falconer, I.R. An Overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. *Environ. Toxicol.* **1999**, *14*, 5–12.

201. Ullman, T.A.; Itzkowitz, S.H. Intestinal Inflammation and Cancer. *Gastroenterology* **2011**, *140*, 1807–1816.e1.