REVIEW ARTICLE

A review and assessment of cyanobacterial toxins as cardiovascular health hazards

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Abstract

Eutrophicated waters frequently support bloom-forming cyanobacteria, many of which produce potent cyanobacterial toxins (cyanotoxins). Cyanotoxins can cause adverse health effects in a wide range of organisms where the toxins may target the liver, other internal organs, mucous surfaces and the skin and nervous system. This review surveyed more than 100 studies concerning the cardiovascular toxicity of cyanotoxins and related topics. Over 60 studies have described various negative effects on the cardiovascular system by seven major types of cyanotoxins, i.e. the microcystin (MC), nodularin (NOD), cylindrospermopsin (CYN), anatoxin (ATX), guanitoxin (GNTX), saxitoxin (STX) and lyngbyatoxin (LTX) groups. Much of the research was done on rodents and fish using high, acutely toxin concentrations and unnatural exposure routes (such as intraperitoneal injection), and it is thus concluded that the emphasis in future studies should be on oral, chronic exposure of mammalian species at environmentally relevant concentrations. It is also suggested that future in vivo studies are conducted in parallel with studies on cells and tissues. In the light of the presented evidence, it is likely that cyanotoxins do not constitute a major risk to cardiovascular health under ordinary conditions met in everyday life. The risk of illnesses in other organs, in particular the liver, is higher under the same exposure conditions. However, adverse cardiovascular effects can be expected due to indirect effects arising from damage in other organs. In addition to risks related to extraordinary concentrations of the cyanotoxins and atypical exposure routes, chronic exposure together with co-existing diseases could make some of the cyanotoxins more dangerous to cardiovascular health.

Keywords Cyanobacteria · Cyanotoxins · Microcystins · Health · Cardiovascular system

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Introduction

Cyanobacteria (blue-green algae) are ubiquitous prokaryotes which developed the aerobic atmosphere of the Earth through oxygenic photosynthesis (Yadav et al. 2011; Cardona et al. 2018). They are commonly found throughout the world in eutrophicated freshwater lakes, rivers and reservoirs, and in brackish and marine environments. They also colonize surfaces of rocks and buildings and the top layers of soils. Cyanobacterial populations can form mass occurrences known as cyanobacterial blooms in waterbodies under favorable environmental conditions. Visible scums on water surfaces, and mats in shallow waters and along waterbody margins, may be formed by certain genera of cyanobacteria (Chorus and Bartram 1999; Whitton and Potts 2012; Huisman et al. 2018). Anthropogenic eutrophication is one of the major factors contributing to cyanobacterial dominance in many aquatic ecosystems (Bláha et al. 2009). Global climate change is expected to favor cyanobacterial populations, i.e. to increase their magnitude and promote their geographical spread, and to extend their growth periods (Codd et al. 2005; Bláhová et al. 2008; Huisman et al. 2018).

Some cyanobacterial secondary metabolites have been identified as potent toxins (cyanotoxins), which have significant adverse bioactivities at environmentally encountered concentrations. Cyanotoxins can cause illness and mortality of humans and terrestrial animals, with further toxicities to aquatic vertebrates and invertebrates, and consequent negative impacts on ecosystems (Codd et al. 1999, 2005; Sivonen and Jones 1999; Metcalf and Codd 2012; Janssen 2019; Chorus and Welker 2021).

Acutely lethal cyanotoxins can be divided into groups depending on their main targets in (mammalian) organisms (Meriluoto et al. 2017). These include hepatotoxins (microcystins–MCs and nodularins–NODs), cytotoxins (cylindrospermopsin and analogues–CYNs), and neurotoxins (anatoxin-a and analogues–ATXs, anatoxin-a(S)–ATX-a(S), and saxitoxin and analogues–STXs). Nota bene, the new name guanitoxin–GNTX has been introduced for ATX-a(S) by Fiore et al. (2020). There are also irritants of various potency (lyngbyatoxin and analogues–LTXs and lipopolysaccharides–LPSs). In addition, cyanobacteria contain neurotoxic di-amino acids (e.g. β-N-methylamino-L-alanine–BMAA and 2,4-diaminobutyric acid–DAB). The long-term effects of BMAA and DAB are under investigation (Dunlop et al. 2021). The general characteristics of common cyanotoxins are summarised in Table 1.

The toxicity of MCs is mainly mediated via the inhibition of serine/threonine protein phosphatases PP1 and PP2A activities, with PP4 and PP5 also being susceptible to inhibition (Mackintosh et al. 1990; Hastie et al. 2005; Metcalf and Codd 2012) and modulation of PP2A expression (Chen

Table 1 General information on cyanotoxins

Cyanotoxins	Chemical structure	Number of known variants	LD$_{50}$ to mice (intraperitoneal, μg/kg)	Cellular/biochemical toxic mechanism	Most commonly reported toxicity
Microcystins (MCs)	Cyclic heptapeptides	279 (Bouaïcha et al. 2019)	50 − > 1200 (Harada et al. 1990)	Inhibition of eukaryotic protein phosphatases, oxidative stress, apoptosis	Hepatotoxicity, neurotoxicity, reproductive and developmental toxicity, cardiovascular toxicity
Nodularins (NODs)	Cyclic pentapeptides	10 (Du et al. 2019)	30 − > 150 (Chen et al. 2021a)	Inhibition of eukaryotic protein phosphatases	Hepatotoxicity
Cylindrospermopsins (CYNs)	Alkaloids	5 (Kokociński et al. 2017a)	200 − 2100 (Ohtani et al. 1992)	Inhibition of protein synthesis, DNA damage, cell death	Cytotoxicity, cardiovascular toxicity
Anatoxins (ATXs)	Alkaloids	14 (Bruno et al. 2017)	260 (Stevens and Krieger 1991)	Agonist of nicotinic acetylcholine receptors at neuromuscular junctions	Neurotoxicity
Guanitoxin (GNTX), previously anatoxin-a(S) (ATX-a(S))	Organophosphate	1 (Metcalf and Bruno 2017)	40 (Mahmood and Carmichael 1987)	Inhibition of acetylcholinesterase	Neurotoxicity
Saxitoxins (STXs)	Alkaloids	57 (Ballot et al. 2017)	8 – 10 (Wiberg and Stephenson 1960)	Blockage of voltage-gated sodium channels of neurons	Neurotoxicity
Lyngbyatoxins (LTXs)	Alkaloids	3 (van Apeldoorn et al. 2007)	250 − > 300 (Ito et al. 2002)	Binding to protein kinase C	Tumour-promoting

and Xie 2016). Protein phosphatases, together with protein kinases, have key roles in the regulation of cardiac function, including the central contractile apparatus in heart muscle cells (Lorenzen-Schmidt et al. 2016). Perturbations in the fine regulation of PP1 and PP2A activities may contribute to heart pathophysiology and disease (Nicolaou et al. 2009; Lubbers and Mohler 2016). These enzymological and cardiac tissue-based observations on cardiac regulation support consideration of the potential effects of MCs and NODs on cardiac function and disease.

An increased level of reactive oxygen species (ROS) generation after MC exposure causes oxidative stress which can result in apoptosis or cell damage and genotoxicity (Svirčev et al. 2010; Žegura et al. 2011b; Chen and Xie 2016). Exposure to MCs also leads to the disturbance of cytoskeleton elements (microfilaments, intermediate filaments and microtubules). Through regulation of transcription factors and proto-oncogenes, MCs also act as tumour promoters (Svirčev et al. 2010; Žegura et al. 2011b; Valério et al. 2016; Žegura 2016).

The cyclic pentapeptide, NOD, is very similar to MCs in its modes of action. There is, however, one fundamental difference between NOD (which does not bind covalently to active site cysteine residues of protein phosphatases) and the covalently binding MCs such as the common variant, microcystin-leucine-arginine, MC-LR (which binds covalently to protein phosphatases, Bagu et al. 1997). In analogy with NOD, a dehydrobutyrine-containing MC was found to inhibit PPs but it did not bind covalently to protein phosphatases (Hastie et al. 2005). The inhibition of PP1 and PP2A triggers a cascade of cellular events associated with oxidative stress and can thereby cause a disintegration of cellular structure, cell proliferation, hepatomegaly, liver damage and hepatic hemorrhage, accompanied by an increase in phosphorylated ERK1/2, p90RSK, p70/p85S6K and p38, as well as the induction of caspase activities and anti-apoptotic Bcl-xL (Batista et al. 2003; Dittmann and Wiegand 2006; Ufelmann and Schrenk 2015; Chen et al. 2021a).

The guanidine alkaloid toxin, CYN, can be cytotoxic, immunotoxic, neurotoxic, genotoxic and carcinogenic (Falconer and Humpage 2006). It may express endocrine- and developmental toxicity (Moreira et al. 2012). CYN acts mainly through the inhibition of protein synthesis, interaction with cytochrome P450 (CYP450) and the generation of oxidative stress and DNA strand breaks. It also binds to estrogen receptors and affects acetylcholinesterase (AChE) activity (Yang et al. 2020).

The alkaloid neurotoxin, ATX-a, can passively cross most biological membranes (gastrointestinal membranes, blood–brain barrier, placenta) and quickly reach its target: nicotinic acetylcholine receptors (nAChR) in the nervous system (Hyde and Carmichael 1991). ATX-a is an agonist of these receptors, and after binding, it causes constant nAChR opening. This action compromises communication between neuronal and postsynaptic cells, leading to detrimental effects on brain, muscles, the respiratory tract and cardiovascular system (Christensen and Khan 2020; Colas et al. 2021).

GNTX (formerly, ATX-a(S); Fiore et al. 2020) is an organophosphate compound that inhibits acetylcholine esterase activity resulting in acetylcholine not being hydrolyzed at the synapse. The characteristic symptom in mammals is hypersalivation and death is due to respiratory arrest. GNTX is structurally unrelated to ATX-a.

The mode of action of the alkaloid, STX, is based on the blocking of Na^+ channels in neuronal cells and of Ca^{2+} and K^+ channels in cardiac cells (Ballot et al. 2017). In this manner, the propagation of electrical transmission is inhibited within the peripheral nerves and skeletal or cardiac muscles (Kao 1993; Wang et al. 2003; Su et al. 2004; Testai et al. 2016b; Christensen and Khan 2020).

LTXs are highly inflammatory and vesicatory, dermatotoxic alkaloids, with a cytotoxic action (Osborne et al. 2001). They are also tumour promoters which induce protein kinase C (PKC) activity (Fujiki et al. 1981; Basu et al. 1992; Jiang et al. 2014; Du et al. 2019).

A recent review (Svirčev et al. 2019) identified 1118 observations of cyanotoxins in 869 freshwater ecosystems in 66 countries throughout the world. Among the listed cyanotoxin occurrences were 183 verified or strongly suspected associated, and in some cases causative, cyanotoxin poisonings involving humans and/or animals. It is likely that cyanobacteria-related ecotoxicological and health problems are present in many more ecosystems than those mentioned in the literature in this context.

Cyanobacteria are easily observed with the naked eye in environments when they occur in higher numbers. Cyanobacterial mass occurrences, blooms, scums and mats, have a striking appearance and the cyanobacterial biomass often produces tastes and odours which may reach consumers in tap water. Poisonings of mammals, birds and fish exposed to toxic cyanobacteria have been reported from many aquatic ecosystems (Metcalf and Codd 2012; Svirčev et al. 2019). Human exposure to cyanobacteria and their toxins in either recreational or drinking waters can cause multiple symptoms including irritations and general symptoms (irritation of skin and mucous membrane of the eyes, nose and throat, weakness, fever), gastrointestinal illnesses (abdominal pain, nausea, vomiting, diarrhoea, gastroenteritis, liver damage), neurological disorders (muscle tremors, nausea, tingling in fingertips and toes, blurred vision, headache, dizziness, paralysis) and cardio-pulmonary problems (asthma-like symptoms, hypoxia, cyanosis, respiratory or cardiac arrest) which may have a fatal outcome (Moore 1984, 1996; Chorus and Bartram 1999; Metcalf and Codd 2012). The toxic effects can appear

within minutes (neurotoxins) to days (cytotoxins) after exposure. The severity of the poisonings is dependent on several factors: the particular cyanotoxins and their concentrations, the exposure media and routes involved, and body weight and age of the exposed animals or persons. Chronic exposure to cyanobacteria and their toxic metabolites, in particular to MCs, is acknowledged as a potential factor in carcinogenic processes. Epidemiological data and experimental knowledge associate cyanobacterial blooms, together with other risk factors, with a higher cancer incidence. Epidemiological studies indicate causative associations between exposure to (toxic) cyanobacteria and primary liver cancer, colorectal cancer, retroperitoneal and peritoneal cancer, kidney cancer, gastric cancer, brain cancer, heart, mediastinum and pleural cancer, ovarian cancer, testicular cancer, leukemia and malignant skin melanoma (Yu 1995; Ueno et al. 1996; Fleming et al. 2002; Zhou et al. 2002; Svirčev et al. 2013, 2014).

Although at least 279 structural variants of the cyclic heptapeptide MCs are now known (Meriluoto et al. 2017; Bouaïcha et al. 2019; Chen et al. 2021b), most of the toxicological investigations using purified MCs have been performed with MC-LR. This variant is among the most commonly found and abundant of the MCs in environmental surveys and is also one of the most toxic variants according to animal bioassays. Several organs or tissues have been reported as targets of MC toxicity (Kankaanpää et al. 2005; McLellan and Manderville 2017). These include liver (Falconer et al. 1983; Hou et al. 2015; Chen et al. 2016b, 2017; Yang et al. 2018), gastrointestinal tract (Ito et al. 2000; Cao et al. 2019a), kidneys (Piyathilaka et al. 2015), reproductive organs (Chen et al. 2013, 2016a; Zhang et al. 2019), the nervous system (Caban-Holt et al. 2005; Feurstein et al. 2010), the cardiovascular system (Zhao et al. 2008) and the endocrine system (Chen et al. 2018b, 2021c). MC-LR induces germ cell apoptosis and has a connection with a mitochondrial-reliant apoptotic pathway (Chen and Xie 2016; Li et al. 2016). MC genotoxicity has also been observed (Li et al. 2008; Žegura et al. 2011a; Žegura 2016).

While the primary target of MC-LR and other MCs in vertebrates is the liver, chronic or acute exposure to MCs also shows toxic effects on the heart (LeClaire et al. 1995; Milutinović et al. 2006; Wang et al. 2008; Qiu et al. 2009). Human cardiovascular health is thought to be affected by MCs as there is solid evidence of positive associations between MC exposure and cardiotoxicity in animal studies (Cao et al. 2019b; Alosman et al. 2020). There is, however, a scarcity of human data on the cardiovascular toxicity of MCs and other cyanotoxins. The potential impairment of cardiovascular health by cyanotoxins is thus a partially uncharacterized and underestimated risk in humans. Because of the wide distribution of toxic cyanobacteria in aquatic environments and the in vivo evidence from animal studies,

the possibility of cyanotoxin-induced cardiovascular health effects in humans fully merits investigation.

In this paper, evidence of the cardiovascular toxicity of MCs and other cyanotoxins is accounted for and evaluated. Attention has been paid to understanding whether the reported research has been representative and relevant regarding: i) type of bioassay, ii) route of exposure, iii) length of exposure and dose and iv) the animal models used in studies of cardiovascular toxicity of cyanotoxins. Detailed biochemical, physiological and medical background is presented in Tables 2, 3, 4, 5, 6 and numerical facts related to i–iv are described in Tables 7, 8, 9, 10.

Cardiovascular toxicity of cyanotoxins

Studies on cardiovascular toxicity of cyanotoxins in vertebrates

In this section, we summarize current knowledge of the cardiovascular toxicity of cyanotoxins (Tables 2, 3, 4, 5, 6). A wide range of environmental, medical and other scientific literature was explored via the Scopus database which includes PubMed, Web of Science and ScienceDirect. The search strategy was the following: (cardiotoxicity OR heart) AND (microcystin OR nodularin OR cylindrospermopsin OR anatoxin OR saxitoxin OR lyngbyatoxin). The searches resulted in an internal database from which Tables 2, 3, 4, 5, 6 were manually constructed.

Many of the most comprehensive in vivo studies concerning cyanobacterial/cyanotoxin toxicology were done during the 1990s and earlier (e.g. Falconer et al. 1994; Fawell et al. 1999). In 1995, for the first time, LeClaire et al. (1995) proved that MC-LR could be cardiotoxic. MCs are well described and systematized in their action regarding cardiovascular toxicity (Cao et al. 2019b; Alosman et al. 2020). Here, we summarize the cardiovascular actions of all of the widely recognised cyanotoxins, including MC, NOD, CYN, ATX, GNTX, STX and LTX.

Tables 2, 3, 4, 5, 6 show the reviewed results of the influence of cyanotoxins on heart function and changes in blood vessels of various vertebrates (mammals, fishes, amphibians and birds). The influence of these toxins is shown through acute and chronic action at the level of cell biochemistry and morphology and tissues of the heart and blood vessels. The activity of cyanotoxins has been followed through in vivo and in vitro exposures. Generally, cyanotoxins may have an effect on myocardial cells, specific cells of the cardiac conduction system and pericardial cells. Pathological remodeling of the extracellular matrix and adverse effects on vascular cells and blood itself can also occur.

The cardiotoxicity of cyanotoxins is observed at several cardiovascular levels: at the genetic, biochemical,

Table 2 Summary of studies on cardiovascular toxicity by whole cells and crude extracts of cyanobacteria and purified cyanotoxins in mammals in vivo

Cyanobacterial cells, extracts and purified cyanotoxins	Mammals	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
Microcystis aeruginosa NRC1	Mice	i.p	40, 80, 160 mg cyanobacteria/kg	3.8 h	Acute	Swelling, loss of cross-striation and pigmentation of myocardial fibres	Konst et al. (1965)
M. aeruginosa NRC1	Mice	Oral	3.2, 6.4, 12.8 g cyanobacteria/kg	4 h	Acute	Swelling, loss of cross-striation and pigmentation of myocardial fibres	Konst et al. (1965)
M. aeruginosa NRC1	Guinea pig	i.p	80, 160, 320 mg cyanobacteria/kg	4 h	Acute	Partly coagulated residual blood in the heart and large vessels	Konst et al. (1965)
M. aeruginosa NRC1	Guinea pig	Oral	1.6, 3.2, 6.4, 12.8 g cyanobacteria/kg	12 h	Acute	Partly coagulated residual blood in the heart and large vessels	Konst et al. (1965)
M. aeruginosa NRC1	Rabbit	Oral	1.6, 3.2, 6.4, 12.8 g cyanobacteria /kg	30 h	Acute	Tachycardia, rapid heart action, partly coagulated residual blood in the heart and large vessels, swelling, loss of cross-striation and pigmentation of myocardial fibres	Konst et al. (1965)
M. aeruginosa NRC1	Lamb	Oral	16 g cyanobacteria /kg	19 h	Acute	Accelerated heart action, petechial haemorrhages in coronary fat, swelling, loss of cross-striation and pigmentation of myocardial fibres	Konst et al. (1965)
M. aeruginosa NRC1	Calf	Oral	9.6, 32 g cyanobacteria /kg	28 h	Acute	Accelerated heart action, systolic murmurs	Konst et al. (1965)
M. aeruginosa M228 extract	Jcl:ICR mice (♂)	i.p	10–20 mg dry cells/kg	1 h	Acute	No significant changes of heart weight, contracted abdominal vascular system, decreased R-wave voltage, ST-segment depression, tachycardia development and terminal bradycardia, atrial fibrillation	Oishi and Watanabe (1986)

Table 2 (continued)

Cyanobacterial cells, extracts and purified cyanotoxins	Mammals	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
Cyanobacterial crude extracts	Wistar rats (♂)	i.v	80 μg MC-LReq/kg (1 LD_{50})	1, 2, 4, 6, 12, 24 h	Acute	Heart: MC-LR and -RR accumulation	Wang et al. (2008)
Crude extracted MCs, mainly -RR and -LR	Rabbits (♂)	i.p	12.5, 50 μg/kg	1, 3, 12, 24, 48 h	Acute	Heart mitochondria: ultra-structural damage, MDA↑, SOD and SDH activities↑, Ca^{2+}-Mg^{2+}-ATPase↓, no significant changes of NADH dehydrogenase or Na^{+}-K^{+}-ATPase	Zhao et al. (2008)
Crude extracted MCs	Wistar rats (♂)	i.v	14, 87 μg MC-LReq/kg (0.16, 1 LD_{50})	24 h	Acute	Heart rate↓, mean arterial pressure↓, plasma: ALT↑, ALP↑, AST↑, LDH↑, CK↑, cTn I↑; heart: MCs accumulation, micro- and ultra-structural damage, cytosol CAT, GST, SOD and GPX activities↑, cytosol GSH and MDA↑, mitochondrial complex I and III↓, no significant changes of complex II, mitochondrial MDA↑, CAT, GST, SOD and GPX mRNA↑	Qiu et al. (2009)
Crude extracted MCs	Wistar rats (♂)	i.v	14 μg MC-LReq/kg (0.16 LD_{50})	2, 4, 6 h	Acute	Heart: apoptosis↑, p53, Bax, Bcl-2, caspase-3 and caspase-9 mRNA↑	Qiu (2014)

Table 2 (continued)

Cyanobacterial cells, extracts and purified cyanotoxins	Mammals	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
MC-LR	Fischer 344 rats (♂)	i.v	50, 100 µg/kg	30, 60, 120, 240, 480 min	Acute	Arterial blood pressure↓, heart rate↓, cardiac output↓, stroke volume↓, body temperature↓, O_2 consumption↓, CO_2 production↓, metabolic rate↓, respiratory exchange ratio↑, no significant changes of relative tidal volume, arterial acid–base balance changes, arterial lactate↑	LeClaire et al. (1995)
MC-LR	Cross-bred, pigs (♀)	i.v	25, 72 µg/kg	45, 90, 150, 210, 300 min	Acute	Mean aortic pressure↓, central venous pressure↓, portal venous pressure↑, hepatic and renal perfusion↓, pO_2↑, O_2 saturation↑, pCO_2↓	Beasley et al. (2000)
MC-LR	Aged ICR mice (♂)	Oral	500 µg/kg/time	1, 6, 7, 12, 13 weeks	Sub-chronic	Heart: MC staining in blood plasma	Ito et al. (2000)
MC-LR	Young ICR mice (♂)	Oral	500 µg/kg/4 weeks	16 weeks	Sub-chronic	Heart: no MC staining	Ito et al. (2000)
MC-LR	Wistar rats (♂)	i.p	10 µg/kg/2 days	8 months	Chronic	Heart: micro-structural damage, loss of cell cross-striations, mono-nuclear infiltration in the interstitial tissue, cardiomyocytes↑, myofibril volume fraction↓, fibrosis↑, no significant changes of apoptosis	Milutinović et al. (2006)

Table 2 (continued)

Cyanobacterial cells, extracts and purified cyanotoxins	Mammals	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
MC-YR	Wistar rats (♂)	i.p	10 µg/kg/2 days	8 months	Chronic	Heart: micro-structural damage, fibrous proliferation, lymphocyte infiltration, bizarre-shaped nuclei, volume density↓, cardiomyocytes↑, myofibril volume fraction↓, no significant changes of apoptosis	Šuput et al. (2010)
MC-LR	N:NIH-S mice (♂)	i.p	25 µg/kg/2 days	1 month	Sub-chronic	Heart: no obvious DNA fragmentation, no significant changes of BAX, Bcl-2, α-tubulin, CaMKII or MAPK proteins	Lezcano et al. (2012)
MC-LR	KM (Kunming) mice (♂)	i.p	3.125, 6.25, 12.5, 25 µg/kg/day	7 days	Sub-chronic	Heart: no MCs accumulation	Huang et al. (2013)
MC-LR	C57 BL/6 mice (♂)	i.p	20 µg/kg/day	28 days	Sub-chronic	Liver and lung: microvascular permeability, CXCR2 and p-VE-cadherin proteins↑; liver, lung and heart: vascular leakage; serum: TNF-α, IL-1β, IL-6 and IL-8↑	Chen et al. (2018c)
MC-LR	BALB/c mice (♂)	Drinking water	1, 7.5, 15, 30 µg/L	6 months	Chronic	F1 (PD180, ♀♂): no micro-structural changes of heart	Meng et al. (2020)
Cylindrospermopsis raciborskii PHAWT/M	MF1 mice (♂)	Gavage	2.5–8.3 CYN mg/kg	48, 72 h	Acute	Heart: micro-structural damage, subepicardial and myocardial hemorrhages, loss of fiber striation, homogeneous pink staining, pyknotic nuclei	Seawright et al. (1999)
Extract of *C. raciborskii* AWQC CYP-026 J	Swiss albino mice (♂)	i.p	250, 500, 1000 mg cells/kg	48 h	Acute	Acute heart failure	Bernard et al. (2003)
CYN	ICR mice (♂)	i.p	0.2 mg/kg	16, 24, 32, 40, 48, 72, 80, 100 h	Acute	Single cell necroses	Terao et al. (1994)

Table 2 (continued)

Cyanobacterial cells, extracts and purified cyanotoxins	Mammals	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
Extract of *Anabaena flos-aquae* UTEX 2383	Wistar rats (♂)	i.v	1.5, 3, 4.5, 6, 7.5 mg extract/kg	30 min	Acute	Heart rate↑↓, mean arterial blood pressure↑	Dube et al. (1996)
(±)ATX-a	Sprague Dawley rats (♂)	i.v	10, 30, 100, 300 μg/kg	30 min	Acute	Mean arterial pressure↑, heart rate↓, cardiac index↑, no significant changes of total peripheral resistance index, hindquarter blood flow↑, no significant changes of hindquarter vascular resistance, renal and mesenteric blood flow↓, renal and mesenteric vascular resistance↑, plasma epinephrine↑, no significant changes of plasma norepinephrine	Sirén and Feurstein (1990)
(±)ATX-a	Sprague Dawley rats (♂)	i.c.v	10, 30, 100, μg/kg	30 min	Acute	Mean arterial pressure↑, heart rate↑, no significant changes of hindquarter blood flow or hindquarter vascular resistance, renal and mesenteric blood flow↓, renal and mesenteric vascular resistance↑	Sirén and Feurstein (1990)
(+)ATX-a	Sprague Dawley rats (♂)	i.v	1, 3, 10, 30, 100, 300 μg/kg	30 min	Acute	Mean arterial pressure↑, heart rate↓, pO_2↓, pCO_2↑, acidosis	Adeyemo and Sirén (1992)
(±)ATX-a	Sprague Dawley rats (♂)	i.v	1, 3, 10, 30, 100, 300 μg/kg	30 min	Acute	Mean arterial pressure↑, heart rate↓, pO_2↓, pCO_2↑, no significant changes in arterial blood acid–base balance	Adeyemo and Sirén (1992)
GNTX	Sprague Dawley rats (♂)	i.v	3.5 μg/kg/min	10 min	Acute	Blood pressure↓, ↑, no significant changes of heart rate	Cook et al. (1990)

Table 2 (continued)

Cyanobacterial cells, extracts and purified cyanotoxins	Mammals	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
STX	Hanky albino guinea pigs	i.p	10 µg/kg	10, 15 min	Acute	Blood pressure↓, incrementally dysfunctional myocardial performance, myocardial failure	Chang et al. (1993)
STX	Hanky albino guinea pigs	i.p	10 µg/kg	11 min	Acute	Blood pressure↓, blood pH↓	Benton et al. (1994)

↑ increased, ↓ decreased, *i.c.v.* intracerebroventricular, *i.p.* intraperitoneal, *i.v.* intravenous, *ALP* alkaline phosphatase, *ALT* alanine amino-transferase, *AST* aspartate amino-transferase, *ATX-a* anatoxin-a, *CAT* catalase, *CYN* cylindrospermopsin, *GNTX* guanitoxin, *GSH* glutathione, *GPX* glutathione peroxidase, *GST* glutathione S-transferase, *LDH* lactate dehydrogenase, *MC* microcystin, *MDA* malondialdehyde, *SOD* superoxide dismutase, *STX* saxitoxin. The weight of the cyanobacterial material refers to dry weight

subcellular, cellular, tissue, organ and vascular system level (Fig. 1).

Based on the reviewed papers and using the organizational levels presented in Fig. 1, a wide range of cardiovascular system injuries and medical conditions caused by cyanotoxins under acute exposure is apparent.

Summary of the cardiovascular effects by cyanotoxins

There is a similarity in the action of MCs and NOD, which is consistent with their structural relatedness and common aspects of their modes of action as protein phosphatase inhibitors. They belong to the same group of cyanotoxins, hepatotoxins. The number of studies involving MCs greatly exceeds that of the other cyanotoxins (see Tables 7, 8, 9). Hence, the overall effects of MCs on the heart and cardiovascular system are better understood than those of the other cyanotoxins.

The toxicity of CYN is principally based on its inhibition of protein synthesis. CYN often causes haemorrhage. In the case of CYN, no effect on heart rate or blood pressure was observed, while the hepatotoxins caused bradycardia and a decrease in blood pressure upon acute, high-level exposure. The effect of ATXs on heart rate and blood pressure was dose-dependent, not time-dependent in contrast to the hepatotoxins. While the molecular modes of action of ATX-a and GNTX are different, their toxico-pathological outcomes are similar. STXs acted similarly to the hepatotoxins by reducing heart rate and blood pressure. LTX induced aorta contractions in rabbits.

Supporting studies

This review also includes selected haematotoxicity studies which support the understanding of cardiovascular events related to exposure to cyanotoxins. A study where rats were dosed orally for 28 days with 700−25,000 µg MCs per kg of feed material demonstrated changes in immunological and haematological parameters (Palikova et al. 2013). The total MCs consumed was 700–15,300 µg MCs/rat/28 days. Some authors emphasize the importance of erythrocytes in the functioning of the cardiovascular system and their important role in the active regulation of vascular tone, especially during hypoxic and ischemic conditions (Pernow et al. 2019; Mahdi et al. 2021). Altered erythrocyte function has important implications for several conditions of cardiovascular disease, especially participating in cardiovascular dysfunction in pathological conditions. MCs can cause: i) an increase in erythrocyte sedimentation rate (Zhang et al. 2007); ii) deformation and damage of erythrocytes (Liu et al. 2002; Zhou et al. 2012) and iii) cell membrane damage and damage to the

Table 3 Summary of studies on the cardiovascular toxicity of purified cyanotoxins in mammals in vitro

Cyanotoxins	Cells or other models	Doses	Duration	Cardiovascular effects	References
MC-LR	Primary HUVECs	10, 20, 40 μM	24 h	Cell proliferation↓, apoptosis↑, migration↓, capillary-like structure formation↓, intracellular and mitochondrial ROS ↑, p-NF-κB↑, VCAM-1↑, ICAM-1↑, TNF-α↑	Shi et al. (2015)
MC-LR	Primary HUVECs	40 μM	24, 48 h	Cell viability↓, apoptosis↑, migration index↓, tube formation↓, ROS↑, SOD↓, GSH↓, MDA↑, nitrite↑, p-NF-κB↑, VCAM-1↑, ICAM-1↑, IL-6↑, TNF-α↑	Shi et al. (2017)
MC-LR	Primary HUVECs	2 μM	6 h	Endothelial monolayer permeability↑, morphological changes of VE-cadherin containing junctions, endocytosis of VE-cadherin↑, MCP-1, IL-6, IL-8, IL-1β, TNF-α and ICAM-1 mRNA↑, p-VE-cadherin protein↑, secreted IL-8 protein↑	Chen et al. (2018c)
MC-LR	HUVECs	40 μM	24 h	Apoptosis↑, caspase-3 and -9↑, MMP↓, mitochondrial Cyt c↓, cytoplasmic Cyt c↑, mitochondrial and cytoplasmic ROS↑, NRF2 protein and activity↓, HO-1↓	Shi et al. (2018)
MC-LR	HUVECs	50, 100, 500, 1000 nmol/L	24 h	Cell proliferation↓, G0/G1%↑, S%↓, OTM↑, SOD, CAT and GPX activities↓, MDA↑	Wang et al. (2018)
MC-LR	Rat H9C2 cardiomyocytes	10 μM	4, 8, 12, 16, 20, 24 h	Rhythmic genes bmal1, per1, per2 and rev-erbα mRNA↓, cry1 and cry2 mRNA↑, antioxidant genes ho-1 and catalase mRNA↑, SOD1 and SOD2 mRNA↑	Xu et al. (2018)
MC-LR	HUVECs	0.01, 0.05, 0.1, 0.5, 1 μM	24 h	Apoptosis↑, MMP↓, mitochondrial ROS↑, caspase-3 and -9 activities↑, cleaved caspase-3 and p53 proteins↑, PCNA protein ↑↓	Wang et al. (2019)
MC-LR	Rat thoracic aortic rings	100, 1000 nM	2 weeks	Microvessels↓, cell migration↓, cell transfer distance↓	Wang et al. (2021b)
MC-LR	HUVECs	1, 10, 100, 1000 nM	12, 24 h	Enclosed lumen↓, common node of enclosed lumen↓, circumference of enclosed lumen↓, total lumen area↓, distance of cell migration↓, morphological damage of microfilaments, hollow nucleus, fluorescence intensity of actin-myosin cytoskeleton↓, condensed chromatin	Wang et al. (2021b)
CYN	HUVECs	0.3, 0.375, 0.6, 0.75, 1.25, 1.5, 2.5, 5, 10, 20, 40 μg/mL	24, 48 h	Total protein content↓, neutral red uptake↓, MTS reduction↓, ROS↑, GCS↑, GSH↑, micro-structural and ultra-structural damage, nucleolar segregation, altered nuclei, degenerated Golgi apparatus, presence of granules and apoptosis↑	Gutiérrez-Praena et al. (2012b)

Table 3 (continued)

Cyanotoxins	Cells or other models	Doses	Duration	Cardiovascular effects	References
CYN	HUVECs	2, 20, 200, 2000 nM (0.9, 9, 90, 900 µg/L)	12, 24, 48 h	Cell viability↓, distance of migration↓, apoptosis↑, altered microfilaments morphology, cell area↓, cell perimeter, intracellular ROS↑, ITGB1, Rho, ROCK, VIM-1, Bcl-2 mRNA↓, MLC-1 and Bax mRNA↑, Bax/ Bcl-2↑	Wang et al. (2020a)
STX	Neonatal rat ventricular cardiomyocytes (NRVCs)	10, 50, 100, 200, 500, 1000 ng/mL	10 min	Field potential amplitude (FPA)↓, firing rate (FR)↓, field potential duration (FPD)↑, peak amplitude↓, rise time↑	Li et al. (2018)
LTX A	Rabbit thoracic aortic rings	1 µM	2 h	Slowly developing contraction	Robinson et al. (1991)

↑ increased, ↓ decreased, *CAT* catalase, *CYN* cylindrospermopsin, *GCS* γ-glutamyl-cysteine synthetase, *GPX* glutathione peroxidase, *HUVECs* human umbilical vein endothelial cells, *LTX* lyngbyatoxin, *MC* microcystin, *MDA* malondialdehyde, *MMP* mitochondrial membrane potential, *MTS* tetrazolium salt, *OTM* olive tail moment, *ROS* reactive oxidative species, *SOD* superoxide dismutase, *STX* saxitoxin

antioxidant system in human erythrocytes (Sicinska et al. 2006; Shi et al. 2017). In addition to erythrocytes, the importance and role of platelets in the cardiovascular system is recognised (Mahdi et al. 2021). MCs may be risk factors for disease because they cause reduction in circulating platelets (Beasley et al. 2000). Several papers have presented the effect of cyanotoxins on different types of leukocytes: an immunotoxic effect of MCs on peripheral blood lymphocytes (Lankoff et al. 2004); loss of neutrophil membrane integrity and increases in intracellular Ca^{2+} level in neutrophils and reactive oxygen species formation by rat and human neutrophils exposed to MCs (Kujbida et al. 2008, 2009). NOD can cause lymphocyte apoptosis (Zhang et al. 2012, 2013).

Indirect effects on the cardiovascular system can be achieved through changes in the function of liver, gastrointestinal tract, brain and kidney. MCs are primarily hepatotoxins and thus cause pathological changes in the structure and function of the liver (Falconer et al. 1981; Huang et al. 2013; Alosman et al. 2020). These toxins trigger hepatic interstitial hemorrhage (LeClaire et al. 1995). When the overall hemorrhage in the liver (Qiu et al. 2009) is sufficiently serious, it causes a hypovolemic shock in the affected animal. However, MCs also cause injury in other organs (Wang et al. 2008; Papadimitriou et al. 2012) and even malformation of body parts (Qi et al. 2016; Li et al. 2021). Pathological changes due to MCs in the structure and/or function of the gastrointestinal tract and kidney have been reported (Alosman et al. 2020) as well as induction of cerebral hemorrhage (Wang et al. 2019). At the molecular/cellular level, severe oxidative damage has been observed (Li et al. 2021). The consumption of oxygen and production of carbon dioxide have been reported to decrease in affected animals (LeClaire et al. 1995), coupled to a progressive hypothermia (LeClaire et al. 1995). CYN can increase lipid peroxidation in the kidney and liver, and protein oxidation in the liver (Gutiérrez-Praena et al. 2011). A reduction in glutathione (GSH) concentrations in the liver can also be observed. ATX has been reported to reduce renal and mean blood flow (Siren and Feuerstein 1990). STX binds to the Na^+ and Ca^{2+} channels of the nerve axon membranes, thus blocking the propagation of nerve impulses in cardiac muscles (Wang et al. 2003; Su et al. 2004). LTX-exposed mice died from bleeding in the small intestine where severely damaged capillaries of the intestinal villi could be observed (Ito et al. 2002).

Several papers report that neither MC nor CYN caused changes in heart function at the examined concentrations and routes of exposure (Theiss et al. 1988; Råbergh et al. 1991; Carbis et al. 1996; Tencalla and Dietrich 1997; Humpage and Falconer 2003; Lei et al. 2008a, b; Huang et al. 2013; Wu et al. 2016; Li et al. 2021). Moreover, the tissue distribution of both cyanotoxin types showed clear organotropism to the liver (and in some cases in other organs such as kidney

Table 4 Summary of studies on cardiovascular toxicity of cyanobacterial cells and extracts containing cyanotoxins and purified cyanotoxins in fish, in vivo

Cyanobacterial cells, extracts, purified toxins	Fish	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
Microcystis aeruginosa PCC 7806	Yearling carp	Oral	400 µg MC-LReq/kg	1, 3, 12, 24, 48, 72 h	Acute	Heart: no obvious micro-structural damage, no MC staining	Fischer and Dietrich (2000)
Disrupted *Microcystis*	Tenca (♂)	Oral	About 100, 220, 500, 1100 µg MC-LR/kg	96 h	Acute	Heart: micro- and ultra-structural damage, loss of myofibrils	Atencio et al. (2008a)
Disrupted *Microcystis*	Nile tilapia (♂)	Oral	About 2400 µg MC-LR/kg	24 h	Acute	Heart: micro- and ultra-structural damage, myofibrolysis, oedema, microhemorrages	Atencio et al. (2009)
Disrupted *M. aeruginosa*	Nile tilapia (♂)	Oral	About 2400 µg MC-LR/kg	24, 48, 72 h	Acute	Heart: micro- and ultra-structural damage, myofibrolysis, hemorrhages, edema, lipid accumulation	Prieto et al. (2009)
Disrupted *Microcystis*	Nile tilapia (♂)	Oral	About 2400 µg MC-LR/kg	24 h	Acute	Heart: micro- and ultra-structural damage	Puerto et al. (2010)
Microcystis blooms cultured *M. aeruginosa* LE-3	Japanese medaka Japanese medaka	Immersion Immersion	120–7000 µg MC/L 690–7838 µg MC/L	1–6 dpf 1–6 dpf	Acute Acute	Heart rate↓ Heart rate↓	Saraf et al. (2018) Saraf et al. (2018)
MC-LR	Japanese medaka	Immersion	600–6300 µg/L	1–6 dpf	Acute	Heart rate↓	Saraf et al. (2018)
M. aeruginosa FACHB-905	Zebrafish embryos	Immersion	5, 10, 20×10⁵ cells/L (6.7, 11.2, 24.6 µg MC/L)	1–96 hpf	Acute	Heart rate↓, pericardial edemas	Li et al. (2021)
Crude extracts of *M. aeruginosa* HUB 5.3	Zebrafish embryos	Immersion	0.1% dry weight of suspended matter, 40 µg MC/L	Blastula stage up to end of embryonic period	Acute	Blood circulation↓, erythrocytes accumulated in heart and edema	Oberemm et al. (1997)
Crude extracts of field *M. aeruginosa*	Zebrafish embryos	Immersion	0.1% dry weight of suspended matter, 30 µg MC/L	Blastula stage up to end of embryonic period	Acute	Blood circulation↓, erythrocytes accumulated in heart and edema	Oberemm et al. (1997)
Crude extracts of *M. aeruginosa* HUB 5–2–4	Zebrafish embryos	Immersion	0.02%, 0.1 w/v suspended dry matter, 40 µg MC/L	–	Acute	Blood circulation↓, erythrocytes accumulated in heart, hematomas, edema	Oberemm et al. (1999)
Crude extracts of field *M. aeruginosa*	Zebrafish embryos	Immersion	0.02%, 0.1 w/v suspended dry matter, 30 µg MC/L	–	Acute	Blood circulation↓, erythrocytes accumulated in heart, hematomas, edema	Oberemm et al. (1999)
MC-LR	Zebrafish embryos	Immersion	10 mg/L	2 h (eleuthero-embryos)	Acute	Heart rate↓, pectoral edema	Oberemm et al. (1999)

Table 4 (continued)

Cyanobacterial cells, extracts, purified toxins	Fish	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
Extracts of *Microcystis* PCC 7813	Newly hatched brown trout alevins (2–7 dpf)	Immersion	5, 50, 500 µg MC-LReq/L	1, 2, 3 min, 24, 72 h	Acute	Heart rate↑, stroke volume↑, cardiac output↑	Best et al. (2001)
MC-LR	Newly hatched brown trout alevins (2–7 dpf)	Immersion	5, 50, 500 µg/L	1, 2, 3 min, 24, 72 h	Acute	No significant changes of heart rate, stroke volume↑, cardiac output↑	Best et al. (2001)
Extracts of *M. aeruginosa* KMF	Nile tilapia (♂)	i.p	725 µg MC-LR/kg	24 h, 7 days	Acute	Heart: LPO↑, SOD↓	Pichardo et al. (2008)
Extracts of *Microcystis* spp. bloom	Crucian carp	i.p	50, 200 MC-LReq/kg	1, 3, 12, 24, 48 h	Acute	Mean arterial blood pressure↓, heart rate↑, circulating blood volume↓	Li et al. (2009)
Crude extracts of *M. aeruginosa* bloom	Zebrafish	Immersion	30 mg biomass/L	36 h	Acute	Heart: micro-structural damage, edema, dissociation, congestion of blood vessels, cytoplasmic vacuolation, degenerative nuclei of myocardiocytes, DNA laddering, % double-stranded DNA↓	Shahi et al. (2012)
Exudates of *M. aeruginosa* FACHB-905	*Sinocyclocheilus graham* embryos	Immersion	0.4–4×10^6 cells/mL	1 hpf–10 dpf	Acute	Swollen pericardium heart dysplasia, protein S100A1↑, myosin light chain↓	Zi et al. (2018)
Extracts of *M. aeruginosa* RST9501	Trahira	i.p	100 µg MC-LR/kg	24 h	Acute	No significant changes of oxygen uptake, O$_2$ extraction↓, heart rate↑	Martins et al. (2019)
MC-LR	Loach embryos	Immersion	0–500 µg/L	Post-fertilization, 32-cells, gastrula	Acute	Pericardial edema, tubular heart, bradycardia	Liu et al. (2002)
MC-LR	Nile tilapia (♂)	i.p	500 µg/kg	7 days	Acute	Heart: micro-structural damage, myopathy, fibrolysis	Atencio et al. (2008b)
MC-RR	Nile tilapia (♂)	i.p	500 µg/kg	7 days	Acute	Heart: micro-structural damage, myopathy, fibrolysis	Atencio et al. (2008b)
MC-LR	Zebrafish embryos	Immersion	0.2, 0.5, 2, 5 mg/L	0.5–96 hpf	Acute	Heart rate↓, heart malformations	Li et al. (2011)
MC-LR	Nile tilapia	i.p	100 µg/kg	48 h	Acute	Metabolic rate↓, critical O$_2$ tension↑, heart rate↓	Martins et al. (2011)
MC-LR	Zebrafish embryos	Immersion	0.2, 0.5, 2, 5 mg/L	0.5–96 hpf	Acute	Apoptosis in heart area	Zeng et al. (2014)

Table 4 (continued)

Cyanobacterial cells, extracts, purified toxins	Fish	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
MC-RR	Zebrafish embryos	Immersion	0.3, 1, 3 mg/L	0.5–96 hpf	Acute	Heart rate↓	Xie et al. (2015)
MC-LR	Zebrafish embryos	Immersion	0.5,1, 2, 4, 6 μM	0.5–96 hpf	Acute	Heart rate↓, apoptosis in heart area	Qi et al. (2016)
MC-LR	Zebrafish (♀♂)	Immersion	1, 5, 25 μg/L	60 days	Sub-chronic	F1 (5 dpf): pericardial edema	Wu et al. (2017)
MC-LR	Zebrafish embryos	Immersion	1, 10, 100, 300 μg/L	4–72 hpf	Acute	Heart rate↓	Chen et al. (2018b)
MC-LR	Transgenic zebrafish (TG flk-1: GFP) embryos	Immersion	50, 100, 500, 1000 nmol/L	24–72 hpf	Acute	Caudal vein regions↓, deficiencies of dorsal longitudinal anastomotic vessels, inter-segmental arteries and veins	Wang et al. (2018)
MC-LR	Transgenic zebrafish Tg (Flk1:GFP)	Immersion	0.1, 1 μM	24–72 hpf	Acute	Vessel: angiodysplasia, damaged vascular structures, suppressed and malformed blood vessels↑, lumen size↓, blood flow area↓, brain hemorrhage	Wang et al. (2019)
MC-LR	Transgenic zebrafish Tg (flk-1: EGFP) embryos	Immersion	100, 1000 nM	2–24 hpf	Acute	Dorsal aorta: rougher appearance and curved shape, curved PCV and growth inhibition, suppressed MceV, ISV sprouting↓	Wang et al. (2021b)
MC-LR	Transgenic zebrafish Tg (fli1a: NGFP) embryos	Immersion	100, 1000 nM	2–72 hpf	Acute	EC migration from CHT↓	Wang et al. (2021b)
MC-LR	Zebrafish (♀♂)	Immersion	1, 5, 25 μg/L	45 days	Sub-chronic	F1 (5 dpf): heart rate↓	Cheng et al. (2017)
MC-LR	Zebrafish (♀♂)	Immersion	0.9, 4.5, 22.5 μg/L	21 days	Sub-chronic	F1 (5 dpf): heart rate↓	Zuo et al. (2021)
MC-LR	Zebrafish (♂)	Immersion	5, 20 μg/L	6 weeks	Sub-chronic	F1 (96 hpf): heart rate↓	Zhao et al. (2021)
NOD	Transgenic zebrafish Tg (flk-1: EGFP) embryos	Immersion	0.5, 1, 2, 4 μM	3–24, 48, 72, 96 hpf	Acute	Heart rate↑↓, pericardial edema, area of peri-cardium area↑, SV-BA distance↑, SV-BA distance/body length↑, DA-DLAV distance↓, ISV↓, SIV area↓, CCV area↑	Chen et al. (2020b)
NOD	Transgenic zebrafish Tg (fli1a: NGFP) embryos	Immersion	0.5, 1, 2, 4 μM	3–72 hpf	Acute	EC migration from CHT↓	Chen et al. (2020b)

Table 4 (continued)

Cyanobacterial cells, extracts, purified toxins	Fish	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
NOD	Zebrafish embryos	Immersion	0.5, 1, 2, 4 μM	3–24, 120 hpf	Acute	Apoptosis in heart↑, vascular development-related genes DLL4 and VEGFC mRNA↑, CDH5 and VEGFA mRNA↓	Chen et al. (2020b)
lyophilized Aphanizomenon ovalisporum LEGE-X001	Nile tilapia (♂)	Oral	200 μg CYN/kg	24 h	Acute	Heart: micro- and ultrastructural damage, myofibrolysis, edema, hemorrhage	Gutiérrez-Praena et al. (2014)
CYN	Nile tilapia (♂)	Oral	200 μg/kg	24 h	Acute	Heart: micro- and ultrastructural damage, myofibrolysis, edema, hemorrhage	Gutiérrez-Praena et al. (2014)
Aphanizomenon ovalisporum LEGE-X001	Nile tilapia (♂)	Immersion	10 μg CYN/L and 0.46 μg deoxy-CYN/L, 100 μg CYN/L and 4.6 μg deoxy-CYN/L	7, 14 days	Sub-chronic	Heart: micro- and ultrastructural damage, myofibrolysis, edema	Guzmán-Guillén et al. (2015)
lyophilized Aphanizomenon ovalisporum LEGE-X001	Nile tilapia (♂)	Oral	400 μg CYN/kg	24 h	Acute	Heart: micro- and ultrastructural damage, myofibrolysis, loss of myofibrils, edema and hemorrhage, no significant changes of cardiac fibers diameter or capillaries diameter	Guzmán-Guillén et al. (2017)
CYN	Nile tilapia (♂)	Oral	400 μg/kg	24 h	Acute	Heart: micro- and ultrastructural damage, myofibrolysis, loss of myofibrils, edema and hemorrhage, no significant changes of cardiac fibers diameter or capillaries diameter	Guzmán-Guillén et al. (2017)
Oscillatoria sp. FACHB-528	Zebrafish embryos	Immersion	5, 10, 20×10^5 cells/L (8.5, 17.1, 35.1 μg CYN/L)	1–96 hpf	Acute	No significant changes of heart rate	Li et al. (2021)
CYN	Nile tilapia (♂)	Gavage	200 μg/kg	24 h, 5d	Acute	Heart: micro- and ultrastructural damage, myofibrolysis, edema	Gutiérrez-Praena et al. (2012a)

Table 4 (continued)

Cyanobacterial cells, extracts, purified toxins	Fish	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
CYN	Nile tilapia (♂)	i.p	200 µg/kg	24 h, 5d	Acute	Heart: micro- and ultra-structural damage, myofibrolysis, edema	Gutiérrez-Praena et al. (2012a)
CYN	Nile tilapia (♂)	gavage	200, 400 µg/kg	24 h	Acute	Heart: micro- and ultra-structural damage, fibrolysis	Puerto et al. (2014)
CYN	Nile tilapia (♂)	oral	400 µg/kg	24 h	Acute	Heart: myofibrolysis, edema, cardiac fibers diameter↓, capillaries diameter↑	Guzmán-Guillén et al. (2016)
CYN	Transgenic zebrafish Tg (flk-1: EGFP) embryos	Immersion	2, 20, 200, 2000 nM (0.9, 9, 90, 900 µg/L)	48, 72 hpf	Acute	Damaged blood vessels in head, trunk and caudal tail region, deformed PCeV, CtA and PHS, incomplete and irregular development of ISV and DLAV, CCV area↑, ventral diameter↑, abnormal blood vessels↑, CCV remolding delay rate↑	Wang et al. (2020a)
CYN	Zebrafish embryos	Immersion	2, 20, 200, 2000 nM	2–48, 72, 96 hpf	Acute	Area of pericardial edema↑, SV-BA distance↑, heart beat↓	Wang et al. (2020b)
CYN	Transgenic zebrafish Tg (flk-1: EGFP) embryos	Immersion	2, 20, 200, 2000 nM	24–72 hpf	Acute	Blood vessels in brain↓, missing PCeV and PHS, deformity of DLV, CtA, PCeV and PHS	Wang et al. (2020b)
ATX-a	Zebrafish embryos	Immersion	400 µg/L	Pec-fin stage (55 hpf), protruding-mouth stage (80 hpf)	Acute	Heart rate↓↑	Oberemm et al. (1999)
STX	Transgenic zebrafish Tg (flk-1: EGFP) embryos	Immersion	0.05, 0.1 µM	3–24, 48, 72, 96 hpf	Acute	Cardiac rate↓, pericardial edema, DA width↑, DA-DLAV distance↓, ISV↓, ISV length↓, SIV area↓, CCV area↑	Chen et al. (2020a)

Table 4 (continued)

Cyanobacterial cells, extracts, purified toxins	Fish	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
STX	Zebrafish embryos	Immersion	0.05, 0.1 µM	3–24 hpf	Acute	DLL4 and VEGFC mRNA↑, no significant changes of CDH5 mRNA, VEGFA mRNA↓	Chen et al. (2020a)

↑ increased, ↓ decreased, *dpf* day(s) post-fertilization, *hpf* hour(s) post-fertilization, *i.p.* intraperitoneal, *ATX-a* anatoxin-a, *CCV* common cardinal vein, *CHT* caudal hematopoietic tissue, *CtA* central artery, *CYN* cylindrospermopsin, *DA* dorsal aorta, *DLAV* dorsal longitudinal anastomotic vessel, *DLV* dorsal longitudinal vein, *EC* endothelial cell, *ISV* inter-segmental vessel, *LPO* lipid peroxidation, *MceV* mid-cerebral veins, *NOD* nodularin, *PCV* posterior cardinal vein, *PCeV* posterior cerebral vein, *PHS* primary head sinus, *SIV* sub-intestinal vessels, *SOD* superoxide dismutase, *STX* saxitoxin, *SV-BA* sinus venosus–bulbus arteriosus

and gonads) but not to the heart (Norris et al. 2001; Lei et al. 2008a, b).

Discussion

Involvement of different cyanobacteria and cyanotoxins in cardiovascular toxicity studies

According to a recent global survey of published findings (Svirčev et al. 2019) the most commonly found toxic cyanobacterial genera worldwide were *Microcystis* spp. (669 reports), *Anabaena* spp. (397), *Aphanizomenon* spp. (100), *Planktothrix* spp. (98) and *Oscillatoria* spp. (75 reports).

Among the 112 studies in Tables 2, 3, 4, 5, 6 that examined effects on the cardiovascular system (Table 7), most were based on the use of purified cyanotoxins (67 studies, 60%): MCs (39 studies), NOD (3 studies), CYN (13 studies), ATX-a (5 studies), GNTX (1 study), STX (5 studies) and LTX (1 study). Natural blooms as crude sources of cyanotoxins were used in 7 (6%) of the 112 examined studies, principally containing *Microcystis* spp. (including *M. aeruginosa*), consistent with species of this genus being often encountered in natural and controlled freshwaters. When culture collections were considered as a source of toxic materials (20 studies, 18%), again *M. aeruginosa* was the most frequently listed (14 studies). Although also often present, *Aphanizomenon ovalisporum* as a source of cyanotoxins was represented in only three studies. *Cylindrospermopsis raciborskii* was the third most often recorded (2 studies), perhaps acknowledging the high interest in this species as a highly invasive organism which is increasing its distribution range from tropical and subtropical to temperate regions, and presenting a spreading environmental health risk to aquatic ecosystems and humans due to the cyanotoxins which it releases (Kokociński et al. 2017b). Finally, *Oscillatoria* spp., despite occurring in several environmental cyanotoxin surveys (Svirčev et al. 2019), and as a source of culture material was represented in only one study.

Over the last 2 decades there has been a considerable growth of interest in the analysis and ecotoxicology of cyanotoxins. A global geographical and historical assessment (of 468 articles, including 1118 cyanotoxin identifications, 869 freshwater ecosystems and 66 countries) of cyanotoxin distribution and cyanobacterial poisonings revealed that, of the cyanotoxins included, MCs were the most often recorded worldwide (63%; 669 of 1118), followed by CYN (10%; 107), ATXs (9%; 100) and STXs (8%; 93), while NODs were the least-often detected cyanotoxins (2%; 19). However, it should be noted that there were also blooms or poisoning reports where cyanotoxins were not analysed or specified (9%) (Svirčev et al. 2019). Similarly, Du et al. (2019) determined that the most widely distributed

Table 5 Summary of studies on cardiovascular toxicity of cyanobacterial cells and extracts containing cyanotoxins in amphibians in vivo and in vitro

Cyanobacterial cells, extracts purified toxins	Amphibian models	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
Cylindrospermopsis raciborskii	Cane toad tadpoles	Immersion	200 µg cylindrospermopsin (CYN)/L	48, 96, 168 h	Acute	Heart: thickened walls, blood cells clumping, presence of smaller, pink casts, possibly cellular or proteinaceous	Kinnear et al. (2007)
Extracts of *C. raciborskii*	Cane toad tadpoles	Immersion	107 µg CYN/L	48, 96, 168 h	Acute	Heart: thickened walls, blood cells clumping, presence of smaller, pink casts, possibly cellular or proteinaceous	Kinnear et al. (2007)
CYN	Excised *Pelophylax ridibundus* heart	Immersion	1 µM	60 min	Acute	No significant changes of heart contractions	Chichova et al. (2021)

Table 6 Summary of studies on the cardiovascular toxicity of cyanobacterial cells containing cyanotoxins in birds in vivo

Cyanotoxins	Birds	Route	Doses	Duration	Acute/chronic	Cardiovascular effects	References
Microcystis aeruginosa NRC1	Chicken	i.p	80 mg cyanobacteria /kg	12 h	Acute	Petechial haemorrhages in myocardium, welling, loss of cross-striation and pigmentation of myocardial fibres	Konst et al. (1965)
M. aeruginosa NRC1	Chicken	Oral	2.2, 8, 16 g cyanobacteria /kg	24.5 h	Acute	Petechial haemorrhages in myocardium, swelling, loss of cross-striation and pigmentation of myocardial fibres	Konst et al. (1965)
M. aeruginosa NRC1	Duck	i.p	80, 320 mg cyanobacteria /kg	12 h	Acute	Petechial haemorrhages in myocardium	Konst et al. (1965)
M. aeruginosa NRC1	Duck	Oral	2.2, 16 g cyanobacteria /kg	12 h	Acute	Petechial haemorrhages in myocardium	Konst et al. (1965)
M. aeruginosa	Japanese quail	Oral	0.2, 2.24, 22.46, 224.6 µg MC/kg/day	10, 30 days	Sub-chronic	Heart: EROD↑, GST↑, GSH↑, GR↑, LPO↑	Pašková et al. (2008)
M. aeruginosa 90%, *M. ichthyoblabe* 10%	Japanese quail (♂)	Oral	about 210 µg MC/kg/day	30 days	Sub-chronic	Heart: GSH↑, GR↑	Pašková et al. (2011)

↑ increased, *i.p.* intraperitoneal, *EROD* 7-ethoxyresorufin O-deethylase, *GSH* glutathione, *GR* glutathione reductase, *GST* glutathione S-transferase, *LPO* lipid peroxidation, *MC* microcystin

Table 7 Sources of surveyed cyanotoxins and their numerical representation in studies on cardiovascular effects

Sources	Toxin/species/strains	Number of papers	Total
Cyanobacterial blooms	*Microcystis* spp. (including *M. aeruginosa*)	7	7
Cyanobacterial cultures	*Microcystis aeruginosa* NRC1	11	20
	Microcystis aeruginosa PCC 7806	1	
	Microcystis aeruginosa LE-3	1	
	Microcystis aeruginosa FACHB-905	1	
	Cylindrospermopsis raciborskii PHAWT/M	1	
	Cylindrospermopsis raciborskii (strain not specified)	1	
	Aphanizomenon ovalisporum LEGE-X001	3	
	Oscillatoria sp. FACHB-528	1	
Extracts of cyanobacterial blooms	*Microcystis* spp. (including *M. aeruginosa*)	8	8
Extracts of cyanobacterial cultures	*Microcystis aeruginosa* 228	1	9
	Microcystis aeruginosa HUB 5.3	1	
	Microcystis aeruginosa HUB 5-2-4	1	
	Microcystis aeruginosa PCC 7813	1	
	Microcystis aeruginosa KMF	1	
	Microcystis aeruginosa RST9501	1	
	Cylindrospermopsis raciborskii AWQC CYP-026 J	1	
	Cylindrospermopsis raciborskii (strain not specified)	1	
	Anabaena flos-aquae UTEX 2383	1	
Exudates of cyanobacterial cultures	*Microcystis aeruginosa* FACHB-905	1	1
Pure cyanotoxin	Microcystins	39	67
	Nodularin	3	
	Cylindrospermopsin	13	
	Anatoxins	5	
	Guanitoxin	1	
	Saxitoxin	5	
	Lyngbyatoxin	1	
Total		112	112

cyanotoxins analyzed were also MCs (57 of 60 countries), then CYN (31), STXs (29), ATX (26), BMAA (16) and NOD (13 of 60). A comparable order of identified cyanotoxin prevalence is also observed in the overall literature, with MCs encompassing more than half (56%; 2971 of 5293) of the available literature, succeeded by STXs (27%; 1439), ATXs (9%; 467), NODs (9%; 452), CYN (7%; 364), BMAA (2%; 112), LTX (2%; 101) and aplysiatoxin (APTX) (1%; 70) (Merel et al. 2013).

Some of the aforementioned results on cyanotoxin occurrence may, unavoidably, reflect the development, economic capacity and environmental analysis capabilities of individual regions and countries, i.e. including available methods, analytical standards, economic factors and technical expertise. Consequently, the true occurrence of cyanotoxins is unknown. Nonetheless, based on this review (Tables 7 and 8), it can be seen that the distribution of 67 published studies examining the effects of cyanotoxins on the cardiovascular system approximately corresponds to the published data on cyanotoxin occurrence, since most of the papers examined

the effects of MCs (39 studies, 58%) followed by CYN (13 studies, 19%), ATX-a (5 studies, 7%), STX (5 studies, 7%), NOD (3 studies, 5%) and finally GNTX (1 study, 1%) and LTX (1 study, 1%). Although they have been less frequently sought or detected, additional cyanotoxins can be present and can be harmful, but they are far less studied than MCs, especially the MC-LR variant, so it is necessary to pay more attention to the wider range of the environmentally occurring cyanotoxins in future research.

Many authors have used purified cyanotoxins in their research (67 studies; 60% of the 112 surveyed studies), an essential contribution to understanding cyanotoxin toxicology. However, the use of purified toxins also presents a limitation in toxicity studies, since they do not represent a natural exposure scenario presented by cyanobacteria and their toxic metabolites. In natural conditions, cyanobacterial blooms can simultaneously produce several different cyanotoxins and other bioactive secondary metabolites. Such natural populations can also include further environmental health hazards, e.g. microbial pathogens, synthetic

Table 8 Number of studies in the review reporting cardiovascular effects in different groups of organisms depending on the cyanotoxin tested

Toxins	Groups of organisms	Number of studies	Total number of studies
MCs	Mammals in vivo	10	39
	Mammals in vitro	9	
	Fish in vivo	20	
	Amphibians in vivo	–	
	Amphibians in vitro	–	
	Birds in vivo	–	
NOD	Mammals in vivo	–	3
	Mammals in vitro	–	
	Fish in vivo	3	
	Amphibians in vivo	–	
	Amphibians in vitro	–	
	Birds in vivo	–	
CYN	Mammals in vivo	1	13
	Mammals in vitro	2	
	Fish in vivo	9	
	Amphibians in vivo	–	
	Amphibians in vitro	1	
	Birds in vivo	–	
ATX	Mammals in vivo	4	5
	Mammals in vitro	–	
	Fish in vivo	1	
	Amphibians in vivo	–	
	Amphibians in vitro	–	
	Birds in vivo	–	
GNTX	Mammals in vivo	1	1
	Mammals in vitro	–	
	Fish in vivo	–	
	Amphibians in vivo	–	
	Amphibians in vitro	–	
	Birds in vivo	–	
STX	Mammals in vivo	2	5
	Mammals in vitro	1	
	Fish in vivo	2	
	Amphibians in vivo	–	
	Amphibians in vitro	–	
	Birds in vivo	–	
LTX	Mammals in vivo	–	1
	Mammals in vitro	1	
	Fish in vivo	–	
	Amphibians in vivo	–	
	Amphibians in vitro	–	
	Birds in vivo	–	
Total		67	67

Table 9 Summary of exposure routes, duration of exposure and concentrations of pure cyanotoxins applied in papers examining effects on the cardiovascular system

Cyanotoxin	Exposure route	Number of studies		
		Acute	Chronic	Total
MCs	Intraperitoneal	3	5*	8
	Oral	–	3**	3
	Intravenous	2	–	2
	Immersion	13	4***	17
	Cellular, in vitro	9	–	9
NOD	Immersion	3	–	3
CYN	Intraperitoneal	2	–	2
	Oral	5	–	5
	Immersion	3	–	3
	Cellular, in vitro	3	–	3
ATX	Intravenous	3	–	3
	Intracerebroventricular	1	–	1
	Immersion	1	–	1
GNTX	Intravenous	1	–	1
STX	Intraperitoneal	2	–	2
	Immersion	2	–	2
	Cellular, in vitro	1	–	1
LTX	Cellular, in vitro	1	–	1
Total		55	12	67

*7 days–8 months, **7 days –6 months, ***21–60 days

and natural chemicals, metals and microplastics. These in combination with cyanotoxins can exert additive, synergistic and antagonistic toxicities (Metcalf and Codd 2020; Chen et al., 2021b). Indeed, it is probably due to these interactions that crude cyanobacterial extracts containing known concentrations of specific cyanotoxin such as MC-LR can be more (or less) toxic than the same concentrations of the pure cyanotoxin (Testai et al. 2016a; Metcalf and Codd 2020). Also, accurate risk assessment of cyanotoxins is difficult when only partially characterized samples containing cyanobacteria or their products are used in this type of research (Testai et al. 2016a).

Pathways of exposure used in studies of cardiovascular toxicity of cyanotoxins

In natural and man-made environments, humans can be exposed to cyanobacteria and their toxins present in water, food, air and dust, via several different pathways: by ingestion, intravenously, direct dermal contact and/or inhalation (Codd et al. 1999; Drobac et al. 2013; Buratti et al. 2017; Massey et al. 2018). One of the most frequently involved exposure routes is the oral, with cyanotoxins being ingested via drinking water, incidental drinking during recreation and showering and via food (aquatic animals, edible plants,

Table 10 List of test organisms and corresponding numbers of studies examining effects of cyanobacteria and cyanotoxins on cardiovascular systems

Group	Tested organisms and in vitro models (number of studies)	Number of organisms	Number of studies
Mammals	Wistar rats (6), Sprague–Dawley rats (5), Fischer 344 rats (1), Swiss Albino mice (1), ICR mice (4), BALB mice (1), KM mice (1), MF1 mice (1), C57BL/6 mice (1), NIH-S mice (1), mice (strain not specified) (2), rabbits (2), guinea pigs (4), cross-bred pigs (1), lamb (1), calf (1), HUVECs (9), rat H9C2 cardiomyocytes (1), rat thoracic rings (1), NRVCs (1), rabbit thoracic rings (1)	21	46
Birds	chicken (2), duck (2), Japanese quail (2),	3	6
Fish	*Danio rerio* (30), *Oreochromis niloticus* (Tilapia) (16), *Cyprinus carpio* (1), *Carassius carassius* (1), *Hoplias malabaricus* (1), *Misgurnus mizolepis* (1), *Oryzias latipes* (3), *Salmo trutta* (2), *Sinocyclocheilus grahami* (1), *Tinca tinca* (1)	10	57
Amphibia	*Bufo marinus* (2), frogs heart in vitro (1)	2	3
Total		36	112

Fig. 1 Organizational levels of cardiovascular toxicity of cyanotoxins
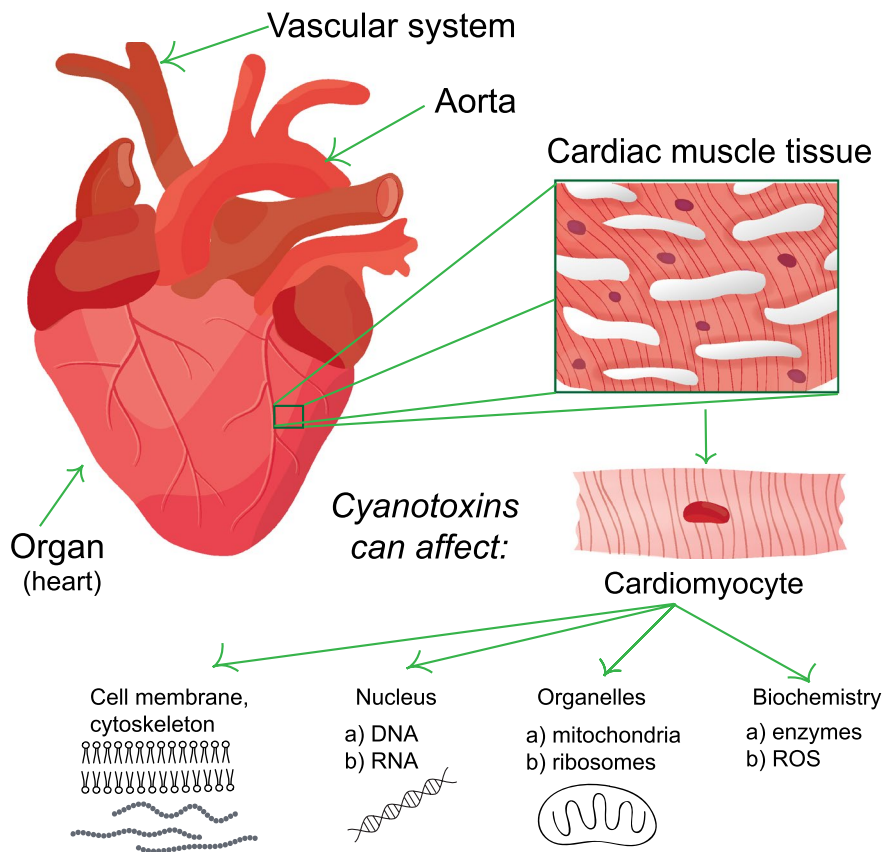
cyanobacteria-based food supplements). For this reason, further studies examining effects and consequences of cyanobacterial toxicity should pay increasing attention to the oral exposure route of the test organisms to cyanotoxins. However, based on the collected data (Table 9) only around 12% (8 from 67 studies, including 3 on MCs and 5 on CYNs) of the studies have used oral exposure in assaying the toxicity of cyanotoxins to the cardiovascular system.

The most frequently used exposure route in in vivo research has been via immersion (26 studies, 39%), and although this is a useful strategy to monitor the effects of toxins (immersion scenarios for the exposure of fish and of amphibians obviously exist in natural conditions), the effects observed may vary from those via oral exposure only. Oral (8 studies, 12%) exposure is less represented in the literature. Only a few papers have compared the results of using

different exposure routes (Carbis et al. 1996; Navratil et al. 1998; Ito et al. 2002; Gaudin et al. 2008; Gutiérrez-Praena et al. 2012a). For example, Carbis et al. (1996) found different degrees of histological change in carp (*Cyprinus carpio* L.) tissues after exposure to MCs by i.p. administration, gavage and immersion. Heart lesions were not observed in the fish in any of the treatment groups. However, an i.p. injection of 50 µg/kg of MC was lethal to all fish within 8 h, while gavaging with 250 µg/kg caused minimal damage in the carp tissues. Navratil et al. (1998) applied purified MC-LR, and MC-LR in cyanobacterial biomass, i.p. and orally to juvenile carp to examine effects on red blood cells and activities of plasma enzymes. As anticipated, the results depended on the route of administration, character of the material and the cyanotoxin concentrations given. Fish (tilapia, *Oreochromis niloticus*) were also exposed to an acute high dose of CYN (200 µg/kg) by i.p. injection, and the effects were compared to those involving oral dosing (gavage). The histological alterations of tissues (including heart) were more pronounced after i.p. administration, except for the gastrointestinal tract, where lesions were more severe in fish exposed orally (Gutiérrez-Praena et al. 2012a). Ito et al. (2002) investigated the pathological effects of lyngbyatoxin A in mice. Much higher lethal doses were observed for the cyanotoxin applied orally (no deaths at 1000 µg/kg) in comparison to i.p. administration (250 µg/kg for young mice). The higher toxicity found via i.p. exposure in contrast to oral dosing implies a difference in the bioavailability of the cyanotoxins such as MC, as i.p. or i.v. administration leads to a more rapid uptake into the liver (over 70%), while oral administration results in less than 1% uptake into this organ (Gaudin et al. 2008). Parental exposure of zebrafish (*Danio rerio*) to MCs via immersion also resulted in decreased heart rate of F1 larvae (Cheng et al. 2017; Zuo et al. 2021).

Intraperitoneal (12 studies, 18%), intravenous (6 studies, 9%) and intracerebroventricular (1 study, 1%) exposures are less represented in the literature. Human hemodialysis patients in a treatment clinic (at Caruaru, NE Brazil) were accidentally exposed intravenously to cyanotoxins in an ineffectively treated dialysate water originating from locally sourced surface waters contaminated with cyanobacteria (Azevedo et al. 2002). Also, dermal and intranasal exposure have been observed during training and recreational activities in blooming waters (e.g. Turner et al. 1990; Vidal et al. 2017).

In recent years more research has been performed on the cells and tissues of the cardiovascular system (14 studies, 21%; Table 9). Animal and human cells are helpful tools in elucidating complex interactions and signaling pathways involved in MC toxicity at cellular and molecular levels (Campos and Vasconcelos 2010; Chen and Xie 2016). Nonetheless, cells used in bioassays in vitro cannot directly characterize the toxicity of compounds towards multicellular organisms, and thus cyanotoxins still need to be tested in vivo (Orbach et al. 2018; Khoshnamvand et al. 2020).

Duration of exposure and toxicologically relevant concentrations of cyanotoxins, with special consideration of human toxicology

The division between acute and chronic exposure is not clear in all of the papers examined. Most of the so-called chronic exposure experiments (7–14 days) could be rather classified as subchronic. To obtain data on the relative toxicity arising from a single dose or a brief exposure (e.g. to determine LD_{50}), acute toxicity tests are the first ones to be used (Bhardwaj and Gupta 2012). Repeated dosing is typically done to establish the resulting effects from repeated administration of a toxin at lower concentrations than those applied in acute toxicity studies. In these chronic tests, organisms may be dosed for weeks or months, or even for 1 to 2 years, making the exposure a considerable part of a subject's life. Chronic toxicity tests are similar to the subchronic tests except that they may span over a longer time period and include larger groups of organisms (Bhardwaj and Gupta 2012). In research conducted on the effects of cyanotoxins on the cardiovascular system, these types of studies are very disproportionately represented, with single exposure tests accounting for most of the research. Only 12 studies have included chronic exposure (18%; with study lengths from 1 week to 8 months). Accordingly, it is clear that the accumulated bulk of research does not extend to the actual human situation which would typically involve repeated oral exposure, contrary to a single i.p. injection (Testai et al. 2016a).

Another important issue is the relevance of applied concentrations: the principle propounded since the fifteenth Century by Paracelsus is that "the dose makes the poison" (e.g. Grandjean 2016; Chen et al. 2018a). Too high or too low concentrations may be misleading, making health risks presented by cyanotoxins to be overestimated or underestimated. The WHO-proposed provisional guideline value for MC-LR in drinking water (Chorus and Welker 2021) includes an estimate of the tolerable daily intake (TDI) and the amount of MC-LR as a harmful substance, which can be consumed daily over the lifetime of a human adult, with a negligible risk of adverse health effects. According to a 13-week mouse oral study with pure MC-LR and consequential liver histopathology and serum enzyme changes, a no-observed adverse effect level (NOAEL) of 40 µg/kg body weight (b.w.) per day was derived (Fawell et al. 1994, 1999; WHO 1998; Chorus and Bartram 1999). By applying a total uncertainty factor of 1000 ($\times 10$ for inter-species variability, $\times 10$ for intra-species variability and $\times 10$ for limitations in the database: in particular a lack of data on chronic toxicity and carcinogenicity), and assuming an average daily water intake of 2 L for a human adult, with 0.8 of

the water requirement derived from the drinking water, a TDI of 0.04 µg MC-LR/kg b.w. per day was derived. This TDI was supported by a 44-day study, in which groups of five pigs were given extracts of *M. aeruginosa* in their drinking water at dose levels calculated from potency estimates using the mouse i.p. bioassay to be equivalent to 280, 800 or 1,310 µg/kg b.w. per day of MCs (assuming an average i.p. LD_{50} for MCs of 100 µg/kg b.w.) (Falconer et al. 1994; WHO 1998; Chorus and Bartram 1999). The dosed extracts contained at least seven MC variants, with MC-YR tentatively identified by high-performance liquid chromatography (HPLC) as the major constituent. After the exposure of the pigs, a comprehensive postmortem examination followed. Histopathological tissue samples were collected from the oesophageal and pyloric ends of the stomach, the duodenum, upper small intestine, colon, cecum, three regions of liver, kidney, testis, lung, heart and brain. There were no changes in the appearance of the organs in the exposed pigs. No changes related to cyanotoxin exposure could be seen by histopathological examination of samples of gastrointestinal tract, kidney, testis, lung, heart or brain. The liver samples showed damage at cell and tissue level in a dose-dependent manner. A lowest-observed adverse effect level (LOAEL) of 280 µg/kg b.w. per day of MCs was identified, with general liver injury (evident from histopathology and changes in serum enzymes) observed at the two higher dose levels, of 800 and 1,310 µg/kg b.w. per day. At the lowest dose level, 280 µg/kg b.w. per day, one pig was affected. The authors determined the potency of their extract by mouse i.p. LD_{50} bioassay, HPLC analysis and by in vitro protein phosphatase inhibition assay (Falconer et al. 1994). Summation of the peak areas from the HPLC identification of MC variants, standardised against MC-LR, indicated that the LOAEL equated with 100 µg MC-LR equivalents per kg b.w. per day. To this LOAEL an overall uncertainty factor of 1,500 was applied, arrived at using 3 rather than 10 for interspecies variability (because pigs physiologically resemble humans more closely than rodents), 10 for intra-species variability, 5 for extrapolating from a LOAEL to a NOAEL (10 was considered inappropriate due to the low incidence of effects in the lowest dose group and the deduced shape of the dose–response curve) and 10 for the less-than-lifetime exposure. This resulted in a provisional TDI of 0.067 µg/kg b.w. per day. The lower of these two values, 0.04 µg/kg b.w. per day, has been used in deriving a provisional WHO drinking water guideline value (Chorus and Bartram 1999; Chen et al. 2018a; Chorus and Welker 2021).

Three examples of exposure that led to human poisonings highlight the toxicologically relevant concentrations of cyanotoxins. In the first case, an estimated concentration of 19.5 µg MC/L of water used during haemodialysis treatment, i.e. the patients were i.v.-exposed to MC. A total of 116 patients experienced the 'Caruaru Syndrome', 100 of them developed acute liver failure, and more than half of them died (Azevedo et al. 2002). In the second case, long-term consumption of cyanobacteria-based food supplements with 2.62—4.06 µg MC-LR/g dry weight (DW) by a 34-year-old woman preceded her death due to liver failure. Although a causal relationship was not definitely established, MC-positive immunostaining was observed in the patient's liver (Dietrich et al. 2007). In the third case, a young man manifesting nausea, abdominal pain, fever, dyspnea, respiratory distress, atypical pneumonia and hepatic damage was hospitalized and received intensive care after recreational exposure to- and immersion in a bloom of *Microcystis* sp. containing 48.6 µg MC-LR/L of lake water. He finally recovered completely 20 days later (Giannuzzi et al. 2011).

In one study conducted by i.v. dosing, two concentrations of MC were tested (14 and 87 µg MC-LReq/kg) on rats (Qiu et al. 2009). The 14 µg MC-LReq/kg b.w. slightly lowered heart rate and significantly reduced blood pressure, enhanced GST activity, slightly increased the cytosolic MDA level (malondialdehyde as a measurement of lipid peroxidation) and caused myocardial damage in the form of enlarged cells with enlarged and abnormally shaped nuclei, occasional cytoplasmic vacuolization and partially degenerated muscle fibres in the exposed rats. The heart rate was significantly decreased by 87 MC-LReq/kg. The authors (Qiu et al. 2009) concluded that the cardiotoxic effects of MC aggravated the pathogenesis of hypovolemic shock, and could thus present a new contributory factor in the haemodialysis patient deaths in Caruaru. The human poisoning case (Dietrich et al. 2007) of long-term oral consumption of dietary supplements containing 2,620–4,060 µg MC-LR/kg DW is comparable to the 28 days' exposure of rats to feed containing 700–25,000 µg MCs/kg feed (Palikova et al. 2013). Significant changes in the red blood cell parameters were induced in the MC-exposed rats. The exposure also especially influenced the innate immune system represented by natural killer cells and by gamma–delta T cells, which were significantly increased in number in peripheral blood in the MC-exposed group (Palikova et al. 2013). Other human studies have been based on acute exposure: e.g. immersion in 48.6 µg MC-LR/L of recreational water resulting in acute intoxication (Giannuzzi et al. 2011) is within the range of one study in which brown trout (*Salmo trutta* L.) were exposed to 5–500 µg MC-LR/L. The concentration of 50 µg MC-LR/L caused increased heart rate, stroke volume and cardiac output of fish (Best et al. 2001). In general, the comparisons are rather difficult because the methods of research varied greatly, including the type of toxin, dose, manner and duration of exposure and the range of organisms used (i.e. inter-species variation should also be taken into account).

The acute i.p. toxicity of MC-LR is about 50 µg/kg in mice. Assuming the same toxic potency in humans, a 60-kg person would thus die from a 3000 µg i.p. dose of MC-LR.

As the typical concentration of MC in pelagic lake water is less than 10 µg/L (Fastner et al. 1999) the 60-kg person would thus need to be i.p. injected with a minimum of 300 L of lake water to die. The WHO-derived provisional guideline value of MC-LR in drinking water is 1 µg/L. The same person would thus need to be i.p. injected with 3000 L of such safe drinking water to die. The oral toxicity of MCs is even much lower than the i.p. toxicity (Falconer et al. 1994; WHO 1998; Chorus and Bartram 1999). These examples illustrate the need to conduct exposure studies at environmentally relevant concentrations to understand the true risks of cardiovascular diseases and other health problems caused by cyanotoxins.

Animal models used in studies of the cardiovascular toxicity of cyanotoxins

Since animal species differ in their responses to toxins, ideally at least two species (e.g. a rodent and a non-rodent) should be used in toxicity tests. If both express a similar adverse effect, it is possible that such an effect could appear in humans also. On the contrary, if the effect is exhibited in one species only, the reaction could be species-specific (Bhardwaj and Gupta 2012). Due to the behavioural practice of wild and domestic animals of drinking from natural and controlled water resources (i.e. the untreated water of lakes, rivers etc.) they may be more frequently and/or severely exposed to cyanobacteria and cyanotoxins than humans, making them good sentinels for potential human exposures. Resulting animal observations can give an approximation of what humans could experience (Backer and Miller 2016). Animal studies reported in the surveyed publications include (Table 10) mammals (46 studies: 41%), birds (6 studies: 5%), fish (57 studies: 51%) and amphibia (3 studies: 3%). The studies of invertebrates were not listed in the tables, but some studies on crustacea (*Penaeus monodon*, *Daphnia magna*, *Daphnia similis*) (Bownik and Pawlik-Skowrońska 2019; Ferrão-Filho and da Silva 2019) and on mollusca (*Elliptio complanata*, *Helix pomatia*) (Vehovszky et al. 2012) have been performed. Recently, mass mortalities of sea otters (Miller et al. 2010), dolphins (Brown et al. 2018) and African elephants (Wang et al. 2021a) have been attributed to cyanotoxin poisonings, although confirmatory investigations into the elephant deaths are required. If cyanotoxins can cause the deaths of megafauna, it is clear that humans can also be endangered, inter-species variability being taken into account. Extrapolation of a dose from animals to humans needs a consideration of the body surface area and body weight, life span, water-based behavioral characteristics and differences in toxicokinetics and toxicodynamics between species. Nair and Jacob (2016) have provided a guide for dose exchange between species during research, experiments and clinical trials.

For MC toxicity characterization the most detailed research has been conducted on rodents (Table 2), especially mice, since the latter have a comparatively higher sensitivity to MCs than other rodents such as rats (Fawell et al. 1999). Pigs have also been used as an experimental model in the determination of in vivo responses to cyanotoxins, since their gastrointestinal tract, liver and kidney functions, metabolic rates and body weights are similar to those of humans (Falconer et al. 1994; Swindle et al. 2012). Recent pig studies (Greer et al. 2018) investigated the effects of subchronic oral (gavage) exposure to MC-LR at a TDI of 0.04 µg/kg b.w. per day (98 days) and at 2 µg/kg b.w. per day (35 days): 50 times the TDI. MC-LR was not found in the serum of the gavaged animals, possibly due to the cyanotoxin being rapidly processed from the blood. However, free MC-LR was found in the large intestine and kidney, while bound MC-LR was detected in the pig livers, indicating a possible accumulation in human livers after oral, chronic and sublethal exposure. Concerning research on effects of MC and MC-producing cyanobacteria on the cardiovascular system in vivo (22 studies, Table 2), few studies have involved pigs (both acute, i.v. high doses) (Stotts et al. 1997; Beasley et al. 2000), while the majority of the studies have been with rodents, including nine investigations on mice and six studies on rats. The findings with pigs showed a rapid clearance of MC from the blood by the liver. Only a small fraction of the dose was rapidly secreted into the bile. At a potentially lethal dose, clearance was reduced (Stotts et al. 1997). Finally, the lethal effect from an acute MC-LR toxicosis can be considered a consequence of hypotensive, hypovolemic shock resulting from an obstruction of blood flow through the liver, severe haemorrhage and the destruction of liver parenchyma. The shock syndrome is further complicated by a reduction in circulating platelets, a partially compensated metabolic (lactic) acidosis, reduced renal perfusion and terminal hyperkalemia, as well as hypoglycemia (Beasley et al. 2000).

Although mammals should ideally be predominantly used in research to determine risks of cyanobacteria and cyanotoxins to humans via cardiotoxicity, the target organisms most studied are fish (57 studies, Table 4). Indeed, as aquatic vertebrates, they can serve as early sentinels for potential adverse effects from cyanobacteria and their toxins (Backer and Miller 2016), especially in natural conditions. One of the most researched fish regarding the effects of cyanotoxins on the cardiovascular system has been the zebrafish (*D. rerio*; 30 studies: 53% of all 57 fish studies), which can be considered an excellent vertebrate model and is extensively utilized in wider toxicity assessments (Shen and Zuo 2020). In the last 2 decades zebrafish have become increasingly popular in toxicology due to their small size, low maintenance cost, high fecundity, fast embryonic development, embryo transparency and some

similarities to mammalian systems (Selderslaghs et al. 2013). It seems likely that *Danio* spp. will continue to find wide-scale applications in vertebrate toxicology and with the contribution of zebrafish to characterizing cyanotoxin cardiotoxicity already having been established (Table 10). Another much-used fish species in this research is the tilapia (*Oreochromis niloticus*; 16 studies: 28% of all fish spp. investigated).

Conclusions and gaps in knowledge

Specific conclusions

Involvement of cyanotoxins in cardiovascular toxicity

The best studied group of cyanotoxins with cardiovascular effects are the MCs. Based on the reviewed papers, high enough acute doses of MCs (and some other cyanotoxins) have a toxic effect on myocardial cells, specific cells of the cardiac conduction system and pericardial cells. Pathological changes of the extracellular matrix and effects on vascular cells and blood cells also occur. Human cardiovascular health and other aspects of health can be further endangered during chronic exposure to low concentrations of cyanotoxins. The effects of MCs on the heart have been observed mainly through changes in heart rate (MCs interfering with blood flow and the rhythm of blood pumping), blood pressure (MCs increasing vascular permeability due to endothelial injuries) and effects on the heart muscles. Upon prolonged exposure, MCs can cause significant cytoskeletal alterations including enlargement of cardiomyocytes, loss of cell cross-striations, fibrosis and abnormal nuclei. Taken together, these results suggest that long-term exposure to relatively low doses of MCs can induce myocardial atrophy and fibrosis. The changes in heart rate are basically caused by mitochondrial dysfunction, whereas the changes in blood pressure are caused by increased protein content in blood capillaries (because of increased vascular permeability) and the damage to heart muscles is caused by ROS production and oxidative stress. All of these cellular and subcellular changes, together with damage to the endoplasmic reticulum caused by MCs, can lead to cardiomyopathy and heart failure.

Although less frequently detected and investigated, further cyanotoxins are present and can be harmful (Tables 2, 3, 4, 5, 6). Other cyanotoxins are far less studied than MCs (especially MC-LR) and it is necessary to study them more intensively in future research.

Purified cyanotoxins, cyanobacterial cell extracts and cyanobacterial biomass

Purified cyanotoxins are frequently used in toxicological research (Table 7) but this type of approach presents a limitation in toxicity studies, since it does not correspond to a natural exposure scenario where a mixture of toxic metabolites (and other compounds of various characteristics) are typically present. On the other hand, the use of cyanobacterial cell extracts may lead to confusing results as the attribution of toxicity among the mixture of diverse and potentially bioactive compounds cannot be unambiguous. Combinations can exert e.g. additive, synergistic and antagonistic toxicities and a certain concentration of a known toxin may have a different potency in a matrix. For these reasons it is encouraged to conduct studies with both pure toxins and cyanobacterial cell extracts.

Localization methods for cyanotoxins in cardiovascular systems

Recognition and understanding of the involvement of cyanotoxins in cardiotoxicity and -pathology could be aided by the application of more modern cyanotoxin-related analytical and localization methods to cardiac cells and tissues. For example, by analysis for cardiac protein phosphatase-MC covalent associations, and the subcellular localization of cyanotoxins by immunogold-electron microscopy (Young et al. 2005).

Exposure route

The most frequently involved natural and hitherto recognized exposure route is the oral route, with cyanotoxins occurring in environmental untreated- and ineffectively treated drinking waters, or recreational waters, or in food items. For this reason, further studies examining cyanobacterial toxicity should pay more attention to cyanotoxin exposure via the oral route. However, based on the collected data (Table 9), only 8 of 67 studies (12%) have employed oral exposure. The bulk of research is thus not directly comparable to the typical human exposure scenario which would typically involve repeated oral exposure through ingestion of drinking water and foodstuffs instead of e.g. a single i.p. injection.

Exposure to environmentally relevant cyanotoxin concentrations and chronic exposure

Tables 2, 3, 4, 5, 6 and 9 also show that research approaches vary greatly in the type of cyanotoxin, dose, manner and duration of exposure and the organisms used (i.e. interspecies variation should also be taken into account). Many of the concentrations used are much above any realistic

concentration found in a natural setting. There is thus a need to conduct the exposure studies at environmentally relevant cyanotoxin concentrations if the goal is to assess the real risks of cardiovascular and other health problems caused by cyanotoxins. There is also no real consensus about which durations of exposure should be understood as chronic and subchronic.

Human epidemiological research

The majority of the tested organisms have been rodents and fish (Table 10), while further species which are phylogenetically and physiologically closer to humans should also be included. Such an approach is needed to obtain a relevant picture of cardiovascular toxicity to humans. There are only a few case studies of human health problems known to have been associated with-, or caused by contact with cyanobacteria and their toxins. Medical professionals have not been employed in most cases to an optimal extent. Some cases have been described by non-medical professionals and postmortem and other pathological examinations are mostly missing. As there are plenty of populations which are naturally exposed to cyanotoxins in their drinking water one way forward in understanding the cardiovascular toxicity of cyanotoxins is to conduct epidemiological research.

Gaps in knowledge

Whilst this review has focused on the impacts of cyanotoxins on cardiovascular structure and function, it is recognised that these toxins can cause damage to multiple structural and physiological systems in the vertebrate body (causing hepatotoxicity, nephrotoxicity, neurotoxicity, genotoxicity, etc.). The degree to which these multiple outcomes are interlinked, with cardiovascular toxicity being a direct consequence of cyanotoxin exposure, or as part of a cascade of damage to the body's physiological systems requires investigation.

Whether the adverse effects of MCs, and potentially of NOD, on vertebrate cardiovascular structure and function arise only from an initial inhibition of protein phosphatases in vivo by these cyanotoxins also requires investigation. Indeed, understanding of whether such actions do include mechanisms without involving protein phosphatase inhibition is needed: in vitro studies have shown that purified MC-LR and NOD cause pore formation, weakening and electrical conductivity changes in synthetic lipid bilayer membranes (Petrov et al. 1991; Mellor et al. 1993), with no protein phosphatases in the assay systems.

Organic anion transporter polypeptides (OATPs) are expressed in several tissues including kidney, liver and brain (Nigam et al. 2015). They have a crucial role in the uptake and excretion of many xenobiotics and endogenous substances. It has been shown that the isoforms OATP1B1

and OATP1B3 mediate the uptake of MCs in hepatocytes (Fischer et al. 2010). As OATP1B1 and OATP1B3 are selectively expressed in the liver (Roth et al. 2012) and other OATP isoforms appear to have no or less affinity for MCs, the effects of these toxins are more pronounced in the liver tissue. As there are tissues where OATPs with high affinity for MCs are not present, but effects still can be seen, it is plausible to assume that either other transporters or other (passive) uptake mechanisms for MCs are in place in these tissues. MCs are relatively polar molecules while the more hydrophobic amino acid residues in some of them could be expected to have an influence on their toxicokinetics and possibly also on their toxicity (Ward and Codd 1999). Indeed, MC-LW and MC-LF showed a higher surface activity than MC-LR on a phosphatidylcholine-cholesterol monolayer when tested by biophysical methods (Vesterkvist and Meriluoto 2003). A follow-up study showed that MC-LW and MC-LF induced stronger cytotoxic effects on Caco-2 cells than MC-LR (Vesterkvist et al. 2012). By analogy, it could be hypothesized that the more hydrophobic MCs could have a higher cardiovascular toxicity than the more hydrophilic congeners.

It is likely that there are additional toxic substances and medical conditions which might potentiate the adverse (cardiovascular) effects of cyanotoxins but the data on this topic are scarce. One interesting aspect is whether the COVID-19 disease known to have cardiovascular effects (Salabei et al. 2022; Xie et al. 2022) may have any interactions with cyanobacterial toxicity.

Overall conclusions

In the light of the presented evidence, it is likely that cyanotoxins do not constitute a major risk to cardiovascular health under ordinary conditions met in everyday life. The risk of illnesses in other organs, in particular the liver, is higher under the same exposure conditions. However, cardiovascular effects could be expected due to indirect effects arising from damage in other organs. In addition to risks related to extraordinary concentrations of the cyanotoxins and atypical exposure routes, chronic exposure and co-existing diseases could make some of the cyanotoxins more hazardous to cardiovascular health.

It is generally concluded that the emphasis in future research should thus be on oral, chronic exposure of mammalian species, including at environmentally relevant concentrations. It is also necessary that in vivo experiments are conducted in parallel with studies on cells and tissues. It would be extremely beneficial to attract more medical professionals to cyanotoxin research ranging from molecular level studies to epidemiology. The efforts should finally lead to environmental health guidelines aiming at human health protection.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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