Review

Microcystins in South American aquatic ecosystems: Occurrence, toxicity and toxicological assays

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Abstract

The acute poisoning of chronic renal patients during hemodialysis sessions in 1996 in Caruaru City (Pernambuco State, Brazil) stimulated an intensive search for the cause of this severe complication. This search culminated in the identification of microcystins (MC), hepatotoxic cyclic heptapeptides produced by cyanobacteria, as the causative agents. More than ten years later, additional research data provides us with a better understanding of the factors related to cyanobacterial bloom occurrence and production of MC in Brazil and other South American countries. The contamination of water bodies and formation of toxic blooms remains a very serious concern, especially in countries in which surface water is used as the main source for human consumption. The purpose of this review is to highlight the discoveries of the past 15 years that have brought South American researchers to their current level of understanding of toxic cyanobacteria species and that have contributed to their knowledge of factors related to MC production, mechanisms of action and consequences for human health and the environment.

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1. Introduction

A possible consequence of anthropogenic pollution and the eutrophication of water bodies is the appearance of toxic algal red tide in oceans (e.g. dinoflagellates and diatoms) (Harvell et al., 1999; Van Dolah, 2000) and cyanobacterial blooms in freshwater (Landsberg, 2002). The presence of algae and cyanobacteria can result in decreased water quality, reflected in altered color, odor and taste, as well as in potential contamination by released toxins. As a consequence, water may become unsuitable for bathing and drinking, limiting its use for human activities (Pitois et al., 2000). Moreover, through the food chain, some toxins and pollutants can accumulate in seafood or freshwater fish to concentrations toxic to humans and animals (Ibelings and Chorus, 2007; Smith et al., 2008; Galvao et al., 2009; Martins and Vasconcelos, 2009). In this context, agencies and companies responsible for the production and distribution of potable water have constantly been challenged to improve purification processes to continue to provide safe drinking water for consumers (Hitzfeld et al., 2000; Svrcek and Smith, 2004; Schmidt et al., 2008).

Cyanotoxins represent a class of compounds that vary widely in chemical structure; they are often divided into three classes: cyclic peptides, alkaloids and lipopolysaccharides (van Apeldoorn et al., 2007). The structural diversity of the cyanotoxins is reflected in their mechanisms of toxicity;
hepatotoxic, neurotoxic and dermatotoxic cyanotoxins, as well as cyanotoxins capable of inhibiting protein synthesis, have been described (Ohtani et al., 1992; Dawson, 1998; Carmichael, 2001; Wiegand and Pfugmacher, 2005; Chen et al., 2009a).

The most notable known occurrence of a harmful effect of cyanotoxins is sometimes referred to as “the Caruaru Incident”; this event represents the first confirmed case of human death caused by cyanotoxins. In early 1996, 130 chronic renal patients were poisoned during hemodialysis sessions in a clinic in Caruaru city in the state of Pernambuco, Brazil. Later, 70 patients died as a result of direct exposure to cyanotoxins (Jochimsen et al., 1998). Biological and chemical analyses confirmed the presence of microcystins (MC) and cylindrospermopsin in the activated carbon filter used in the clinic’s water purification system. MCs were also detected in samples of blood and liver tissue from the affected patients (Jochimsen et al., 1998; Carmichael et al., 2001; Azevedo et al., 2002). In a pioneer study by Chen et al. (2009a), microcystins were found to be transferred mainly from contaminated aquatic organisms to a human population (in China) through chronic exposure. They identified the presence of microcystins in serum samples (average 0.39 ng mL⁻¹) and found positive relationships between MC serum concentration and major liver function biochemical indices, suggesting substantial hepatocellular damage.

Numerous cities in Brazil and in other South American countries use surface reservoirs, including lakes and rivers, as a main source of drinking water. In many cases, these water bodies are subjected to anthropogenic pollution and become eutrophicated ecosystems, showing recurrent cyanobacterial outbreaks. Some recent reports on toxic cyanobacterial blooms in South America partially reveal the extent of this problem and reaffirm it as an emerging concern to public health authorities (De Leon and Yunes, 2001; Azevedo et al., 2002; Frias et al., 2006; Echenique et al., 2008).

In the present review, South American toxic bloom episodes are discussed with special emphasis on the occurrence of MCs. The identification of toxic cyanobacterial species, methods for detection of MCs, determination of the mechanism of action of cyanotoxins, and consequences for human health and the environment are also commented.

2. Cyanobacterial blooms and production of toxins in South America

The cyanobacterial toxic bloom phenomenon is well-known in several countries. However, a lack of information exists with respect to South America, with very few official reports and/or published data for the majority of countries. Table 1 summarizes the occurrence of cyanobacterial blooms and the presence of toxins in South America.

In Argentina, according to Ringuellet et al. (1955), toxic cyanobacterial blooms have been observed since 1947. That pioneering paper described massive fish mortality caused by *Anabaena inaequalis*, *Anabaena circinalis* and *Polycystis flos-aquae* in a lagoon. In the past decades, several blooms have been observed in rivers, reservoirs, lakes, coastal lagoons and estuaries from North to South Argentina (25°–55° S). *Microcystis* and *Anabaena* were the most common genera found. According to the literature, concern about public health risks is increasing in parallel with the increasing occurrence of cyanobacterial blooms (Scaria et al., 1995; Ame et al., 2003; Conti et al., 2004). Contamination of fish and drinking water by MCs at levels above those considered safe (1 ng mL⁻¹) has already been described (Cazenave et al., 2005; Echenique et al., 2008). Fish and duck mortality was also reported during a *Microcystis* bloom in an urban recreational lake in Buenos Aires (Ehrenhaus and Vigna, 2006). Such events clearly show the challenges of controlling the mass development of toxic cyanobacteria.

There are reports of cyanobacterial blooms in Brazil during the 1980s. A review of data in the Brazilian literature on phytoplankton ecology that considered studies with databases maintained for at least one year showed that aquatic environments located in areas with anthropic influence presented a high percentage of bloom occurrences. Although some of these occurrences can be considered to be naturally occurring events, an increasing number of environments in which cyanobacteria represent the predominant species in the phytoplankton community are noticeable (Beyruth, 2000; Bittencourt-Oliveira et al., 2001; Magalhães et al., 2001; Bittencourt-Oliveira et al., 2005; Branco et al., 2006; Costa et al., 2006; dos Anjos et al., 2006; Bittencourt-Oliveira et al., 2009).

Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>Samples/species/method</th>
<th>MC analog</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Environmental samples from San Roque Reservoir (Cordoba)/LC-MS</td>
<td>MC-LR and MC-RR</td>
<td>(Ame et al., 2003; Ame and Wunderlin, 2005;</td>
</tr>
<tr>
<td>Brazil</td>
<td>Cyanobacterial blooms and strains isolated from lakes/FAB-MS, LC-MS, MALDI-TOF</td>
<td>MC-LR, MC-LF, MC-RR, MC-YR, [Asp₃]-MC-LR, [Leu₁]-MC-LR, MC-hRhr</td>
<td>(Cazenave et al., 2005)</td>
</tr>
<tr>
<td>Chile</td>
<td>Cyanobacterial bloom, Lake Tres Pacuallas, Concepcion/LC-MS</td>
<td>MC-RR, MC-FR, [Asp₃]-MC-LR</td>
<td>(Campos et al., 1999; Neumann et al., 2000)</td>
</tr>
<tr>
<td>Uruguay</td>
<td>Environmental samples from La Plata River/ELISA</td>
<td>MC-LR and [Asp₃]-MC-YR</td>
<td>Only positive using ELISA</td>
</tr>
</tbody>
</table>

No official data or references (WebofScience® and Scifinder®) were found to prove the presence of microcystin in Colombia, French Guyana, Guyana, Paraguay, Peru, Suriname and Venezuela.
Outbreaks of cyanobacteria have been documented in 11 of the 26 Brazilian states, from the North to the South region. Although these outbreaks were commonly observed in artificial reservoirs, especially in the northeast part of the country, several coastal lagoons, natural lakes, rivers and estuaries were also affected. According to a review by Sant’Anna and Azevedo (2000), the most common genera encountered are Microcystis and Anabaena; an increased dominance of Cylindrospermopsis has been detected over the last decade (Huszar et al., 1998; Bouvy et al., 2000).

The isolation of toxic nanoplanktonic cyanobacteria (Synechocystis aquatilis) from coastal areas and toxic picoplanktonic cyanobacteria strains from reservoirs in the northeast region of Brazil define a new challenge for public health and water treatment authorities (Domingos et al., 1999; Komarek et al., 2002). Due to the very small size of these cells, their identification requires special care; removal by traditional methods of water treatment can be difficult. Therefore, the potential toxicity of these species must be considered and the risk of picoplanktonic cyanobacteria development in water supplies must be monitored to minimize the hazards of cyanotoxins.

The few existing reports of blooms of cyanobacteria in Colombia are mainly related to aquaculture activities in coastal lagoons, floodplain lakes and estuaries (Mancera and Vidal, 1994). There are also some reports of cyanobacterial blooms in recently constructed reservoirs. The main genera described are Microcystis and Cylindrospermopsis. These data represent information from the last decade; earlier reports are not available. Nevertheless, many personal communications from Colombian researchers describe cyanobacterial blooming as a well-known event in several places.

In Chile, the first reported cyanobacterial bloom occurred in 1995. It happened in a natural lake in the Concepción region, where another event was documented in 1998. In both cases, the main genus was Microcystis (Campos et al., 1999; Neumann et al., 2000).

In Uruguay, cyanobacterial blooms have been observed in rivers, reservoirs, lakes, coastal lagoons and estuaries (De Leon and Yunes, 2001; Scasso et al., 2001; Brena et al., 2006; Piccini et al., 2006; Chalar, 2009). These events are related to increased eutrophication and to changes in river hydrodynamics resulting from the construction of reservoirs in cascade, which interferes with water retention time, favoring bloom formation. The most common genera are Microcystis, Nodularia and Anabaena.

There are few data on the occurrence of cyanobacterial blooms in Venezuela, and the events appear to be restricted to reservoirs and lakes. However, the early (1978) documented occurrence of these events in lakes and reservoirs used as the water supply for large cities, including Caracas and Valencia, show their important impact. The main genera reported are Microcystis, Anabaena, Cylindrospermopsis and Synechocystis (Gardner et al., 1998; Gonzalez and Ortiz, 1998; Gonzalez, 2000; Gonzalez et al., 2002, 2004; Thunell et al., 2004).

The data presented above suggest that toxic cyanobacterial bloom events in South America are underestimated and that they are generally not widely noticed by the international scientific community. This may be attributed, at least in part, to deficient water monitoring programs in some countries and/or missing or unreported data in countries where such programs are carried out. However, despite the lack of official data, unpublished communications from these countries confirm the occurrence of toxic cyanobacterial blooms as an emerging concern to public health authorities.

3. Variants of microcystins found in South America

MCs are cyclic heptapeptides (Fig. 1) with molecular weights of approximately 1000 Da and the following general structure: cyclic (aa1-aa2-aa3-aa4-aa5-aa6-aa7), where aa1 = 1-D-alanine, aa2 = variable L-amino acids (side chain, R1), aa3 = 3-ethynyl-β-methyl-aspartic acid (R2 = CH3) or 3-aspartic acid (R2 = H), aa4 = variable L-amino acids (side chain, R3), aa5 = (2S,3S,5S,8S)-3-amino-2,6,8-trimethyl-10-phenilenecac-4(6E,8E)-dienoic acid (Adda), aa6 = D-glutamate acid and aa7 = N-methyl-dehydroalanine (Mdha – where R4 = CH3 and R5 = H) or 2-amino-2-butenolic acid (dehydrobutyrine – Dhb – where R4 = H and R5 = CH3) or dehydroalanine (Dha, where R4 and R5 = H). With some rare exceptions, the amino acid Adda is part of the common structure of all MCs. The letters L and R of MC-LR correspond to the amino acids leucine (L) and arginine (R) in positions aa2 and aa4, respectively (Quilliam, 1999).

Variations in the chemical structure of MCs are very common and more than 70 different congeners have been characterized (Dawson, 1998; Quilliam, 1999; dos Anjos

![Fig. 1. General structures of nodularins and microcystins. In the case of MC-LR, aa2 = leucine (L) (R1 = side chain of leucine), R2 = CH3, aa4 = arginine (R) (R3 = side chain of arginine), R4 = CH3 and R5 = H.](image-url)
et al., 2006; Kruger et al., 2009). Depending on their specific chemical structures, MC vary in toxicity from highly toxic to non-toxic, but most are toxic (Dawson, 1998). The most frequent variations detected are the replacement of the α-amino acids at positions aa2 and aa4 and the demethylation of the amino acids at positions R2 and/or R4 (Kruger et al., 2009).

In samples taken from Uruguayan water bodies during cyanobacterial bloom events, ELISA detection indicated the presence of MCs in samples from La Plata River and Lake Rodo (De Leon and Yunes, 2001; Scasso et al., 2001; Brena et al., 2006). However, no evidence on their specific structural identification is available.

In Argentina, recent experiments carried out using LC-MS-TOF with environmental samples from the San Roque Reservoir (Cordoba) showed the presence of MC-LR and MC-RR variants (Ame et al., 2003; Ame and Wunderlin, 2005; Cazenave et al., 2005).

A cyanobacterial bloom that occurred in 1998 in Lake Tres Pascualas (Concepcion/Chile) was found to contain the analogs MC-RR, MC-FR, [Asp3]-MC-LR and [Asp3]-MC-YR, as well as cyanopeptolins, another class of peptides frequently found in cyanobacteria (Neumann et al., 2000).

At least six MC variants were described in Brazil. MC-LR and the analog MC-LF were isolated and confirmed by RP-HPLC and fast atom bombardment mass spectrometry (FAB-MS) from a colony isolate (NPJB-1) of Microcystis aeruginosa collected from Lagoa das Garças, São Paulo (Azevedo et al., 1994). Concerning the incident in Caruaru City, the variants MC-LR, MC-YR and MC-AR were identified from blood sera and liver tissues of poisoned patients by HPLC-PDA, MALDI-TOF and ESI/MS (Carmichael et al., 2001). The demethylated variant [Asp3]-MC-LR was also identified in a strain of cyanobacteria isolated from Barra Bonita, a eutrophicated water reservoir in São Paulo state, Brazil (Bittencourt-Oliveira et al., 2005).

Two new variants were discovered in South America, specifically in Brazil. These included the analog [Leu1]-MC-LR (Matthiensen et al., 2000; Schripsema and Dagnino, 2002), where aa1 = leucine, aa2 = leucine (L), R1 = side chain of leucine), R2 = CH3, aa4 = arginine (R) (R3 = side chain of arginine), R4 = CH3 and R5 = CH3 (Frias et al., 2006).

Nodularins (Fig. 1) are cyclic pentapeptides that are usually produced by the genus Nodularia. These compounds are closely related to MCs in structure and mechanism of action. Although they are commonly found in northern Europe (Mazur-Marzec and Plinski, 2009), the presence of nodularins in South America has not been yet reported.

4. Strategies for the investigation of potential toxic cyanobacterial samples in South America

Several analytical methods for MC determination and identification have been reported in the literature; a detailed discussion of these methods is outside the scope of this article. Instead, the most commonly used methodologies in the South American context are discussed. Comprehensive reviews can be found elsewhere (Hawkins et al., 2005; Msagati et al., 2006; Sangolkar et al., 2006).

Biological assays such as the mouse bioassay, phosphatase inhibition and particularly the enzyme-linked immunosorbent assay (ELISA) are widely used for screening and toxicity evaluation in many laboratories. In the mouse bioassay, samples are injected intraperitoneally and the toxic response, if any, is observed to identify the class of toxin involved. Although this test suffers from poor sensitivity and precision, it can provide information about general mammalian toxicity and is useful for toxicity evaluation of samples with unknown toxin composition. In addition, because it does not require expensive or complex equipment, this test is accessible to most laboratories in South America. However, restrictions on its use are increasing due to ethical concerns regarding animal bioassays.

Phosphatase inhibition assays are based on the capacity of MC to inhibit Type 1 and Type 2A protein phosphatases (Mackintosh et al., 1990). Although the amount of inhibition measured in the reaction can be related to toxin concentration (Ward et al., 1997; Heresztyn and Nicholson, 2001), this test is also sensitive to inhibitors other than MC, such as nodularin (An and Carmichael, 1994). Moreover, cyanobacterial material can show endogenous phosphatase activity, possibly leading to an underestimation of MC concentration (Sim and Mudge, 1993). Nevertheless, this method is simple, rapid and sensitive enough for use in evaluating the toxicity of water samples and bloom material (Matthiensen et al., 2000; Almeida et al., 2006).

Immunoassay procedures are extensively described in the literature as both screening and quantitative methods for determination of MCs. ELISA-type assays using polyclonal or monoclonal antibodies are well-established, sensitive and specific procedures (Nagata et al., 1999; Zeck et al., 2001; Metcalf et al., 2002; Sheng et al., 2006; Young et al., 2006) and are commercially available from different suppliers (Table 2). However, care should be taken when interpreting results because the cross-reactivity of antibodies is different for the wide variety of MC structural congeners (An and Carmichael, 1994; Mikhailov et al., 2001). Furthermore, results obtained using commercial kits can be susceptible to interference from various sources (Metcalf et al., 2000). Nonetheless, ELISA assays have been widely employed to detect MCs in raw water (Hirooka et al., 1999; Vieira et al., 2005), cyanobacterial material (Domingos et al., 1999; Sotero-Santos et al., 2008; Furtado et al., 2009), zooplankton (Ferrão et al., 2002), fish and crustaceans (Magalhães et al., 2001, 2003; Soares et al., 2004) and even human tissues (Hilborn et al., 2005, 2007; Soares et al., 2006).

Alternative assays either for biomonitoring cyanotoxins or to indicate genes involved in MC biosynthesis have also been proposed. For example, Ferrão-Filho et al. (2009) used native cladocerans to biomonitor toxins in Brazilian reservoirs. Although it has not been a proof of MC presence itself, Bittencourt-Oliveira (2003) detected potentially toxic strains of cyanobacteria using the polymerase chain reaction (PCR) to determine the presence of mcyB, a gene in the operon that encodes an MC synthetase.
### Table 2
Commercially available diagnostic test kits for microcystin, suppliers and mechanism of detection.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Supplier</th>
<th>Method/Mechanism of detection</th>
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<tbody>
<tr>
<td>Microcystin tube</td>
<td>Abraxis&lt;sup&gt;a&lt;/sup&gt;, Enzo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ELISA/Coated Tube Kit uses a polyclonal antibody that binds both microcystins and a microcystin-enzyme conjugate.</td>
</tr>
<tr>
<td>Microcystins – Adda</td>
<td>Abraxis&lt;sup&gt;a&lt;/sup&gt;, Enzo&lt;sup&gt;b&lt;/sup&gt;, Biosense&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ELISA/Based on the recognition of microcystins, nodularins and their congeners by specific antibodies against Adda&lt;sup&gt;b&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Microcystins-DM</td>
<td>Abraxis&lt;sup&gt;a&lt;/sup&gt;, Enzo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ELISA/Direct competitive ELISA that allows the detection of microcystins and nodularins by using a monoclonal antibody. ELISA/Coated plates/strips use antibody that binds microcystins.</td>
</tr>
<tr>
<td>Plate – strips</td>
<td>Beacon Analytical Systems Inc.; EnviroLogix&lt;sup&gt;e&lt;/sup&gt;; Strategic Diagnostics Inc.</td>
<td>Inhibition of the enzymes PP1 and PP2A enzyme/Colorimetric reaction monitored by a spectrophotometer or &lt;sup&gt;32&lt;/sup&gt;P decay by β-scintillation spectrometry.</td>
</tr>
<tr>
<td>PP1 and PP2A protein phosphatases&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Sigma-Aldrich&lt;sup&gt;a&lt;/sup&gt;, Gibco&lt;sup&gt;a&lt;/sup&gt;, Calbiochem&lt;sup&gt;a&lt;/sup&gt;, Boehringer-Mannheim&lt;sup&gt;a&lt;/sup&gt;, several others</td>
<td>Inhibition of the enzymes PP1 and PP2A enzyme/Colorimetric reaction monitored by a spectrophotometer or &lt;sup&gt;32&lt;/sup&gt;P decay by β-scintillation spectrometry.</td>
</tr>
</tbody>
</table>

<sup>a</sup> Although PP1 and PP2A protein phosphatases are available from several suppliers worldwide, the inhibition assay with microcystin is normally performed in-house (Carmichael and An, 1999).

The most common physicochemical methods of MC investigation are based on liquid chromatography coupled to different detection systems, such as UV absorption and mass spectrometry. Undoubtedly, HPLC-UV is the most widely used technique for MC quantification. Toxin congeners are usually separated on reversed-phase columns employing mobile phases that combine methanol or acetonitrile with acified water or buffers. Further benefits are obtained when diode array detectors are used. In this case, the characteristic absorption spectrum of MC, i.e., between 190 and 300 nm with a maximum at 238 nm, is a valuable identification tool. On the other hand, this absorption profile is similar for the most commonly occurring variants, limiting the ability of the method to distinguish between them. Tryptophan-containing variants have an absorption maximum at 222 nm (Lawton et al., 1994; Moollan et al., 1996; Meriluoto, 1997; Spoof et al., 2001; Barco et al., 2005).

Methods based on mass spectrometric (MS) detection of cyanotoxins are extensively used due to their sensitivity, specificity and the possibility of providing structural information (Dahllmann et al., 2003; Meriluoto et al., 2004; McElhinney and Lawton, 2005; Dorr et al., 2008, 2010). MC is easily ionized by electrospray (ESI), the most common ionization technique, and mass spectra have different features depending on the amino acid residues present. Single (M + H)<sup>+</sup> and doubly charged (M + 2H)<sup>2+</sup> ions are frequently observed in the positive mode, especially for variants containing basic arginine residues, such as MC-RR (Poon et al., 1993; Yuan et al., 1999a).

Important structural information can also be obtained by fragmentation of MC ions in collision-induced dissociation (CID) experiments. In this case, an invaluable diagnostic ion at m/z 135 is produced from all MCs containing the Adda residue (Yuan et al., 1999b) (Fig. 2). This ion has been used for quantitation of known variants in multiple reaction monitoring (MRM) experiments (Cong et al., 2006; Allis et al., 2007; Mekebri et al., 2009) and for screening of unknown MCs using precursor ion scan experiments (Hiller et al., 2007). Further structural characterization is possible using strategies that determine the amino acid composition of linear peptides, such as immonium ions and ions formed via b or y reactions (Bittencourt-Oliveira et al., 2005; Diehmelt et al., 2005, 2006; dos Anjos et al., 2006).

Another approach to the analysis of MCs in environmental samples is the detection of 3-methoxy-2-methyl-4-phenylbutanoic acid (MMPB), an oxidation product of the lateral chain of the amino acid Adda, via gas or liquid chromatography (Sano et al., 1992; Kaya and Sano, 1999; Tsuji et al., 2001; Ott and Carmichael, 2006). This method is especially well-suited to analysis of animal tissues and sediments where toxins are bound to matrix components.

![Fig. 2. Alpha-cleavage of aa5-Adda from microcystin-LR and formation of ion m/z 135, analyzed by ESI-MS positive mode.](image-url)
such as proteins. After sample extraction and oxidation, all toxin variants containing the Adda moiety give rise to the same product (MMPB); hence, the total amount of MC is quantified. Recently, Wu et al. (2009) modified and improved this methodology.

All the methods briefly reviewed here are already in routine use in a limited number of laboratories throughout South America. However, the absence of reference laboratories to validate results and to provide reference material, as well as the requirement for sophisticated and expensive equipment and high levels of technical expertise, are the main limitations to improving our knowledge regarding MC occurrence in these countries.

Apart from the methods used for qualitative and quantitative toxin analysis, analytical standards are a key necessity. In this regard, the limited number of commercially available MC standards hampers further research development in this field. Regional efforts have been made to produce standards for local supply. In 2006, the National Council for Scientific and Technological Development (CNPq), a Brazilian Federal Foundation for Science, issued a call for specific proposals on the investigation of cyanotoxins and the production of analytical standards (http://www.cnpq.br/editais/ct/2006/docs/047.pdf).

5. Microcystin exposure routes

Human health hazards resulting from cyanobacterial toxins mainly occur through three different exposure routes: direct contact, oral ingestion and inhalation (Chorus et al., 2000).

Oral ingestion is the most widely considered exposure route in risk assessment of cyanotoxins because the majority of documented health effects are related to water consumption (Carmichael, 1992; Dawson, 1998; Ito et al., 2000). However, potential intoxication through the food web must also be considered. It is well-known that there is a possible difference of MC accumulation among multiple vertebrate groups (i.e., fishes, turtle, domestic duck and water bird). Nasri et al. (2008) reported a case of turtle deaths during a toxic Microcystis spp. bloom in Lake Oubeira, Algeria. A recent study performed in the Lake Taihu in China reported for the first time MC contamination in domestic duck and identified high MC concentrations in the spleen of duck and water bird (Chen et al., 2009b). Previously, a study carried out in Egypt demonstrated that Ochrochromis niloticus cultivated in a fish farm containing Microcystis bloom could have accumulated MC (Mohamed et al., 2003). The consumption of freshwater aquaculture products has increased significantly during the last two decades, and the bioaccumulation of cyanotoxins by a variety of aquatic organisms has already been confirmed by many authors (Ibelings et al., 2005; Xie et al., 2005; Ibelings and Chorus, 2007; Galvao et al., 2009). Very little is known about the toxicokinetics of MC after consumption of contaminated aquatic organisms, and no estimation of human exposure can presently be obtained in South America (Magalhães et al., 2001, 2003; Soares et al., 2004).

Recreational activities are another potential route of oral exposure to cyanotoxins and can represent a significant risk if age and frequency of exposure are considered. Reports of skin irritation and allergies are common when recreational activities involve water bodies presenting cyanobacterial blooms, showing that direct contact is also an important exposure route (Falconer, 1999; Torokne et al., 2001). Indeed, some of these incidents were reported in Argentina and Brazil (Soares et al., 2007).

Inhalation is an exposure route not properly considered in some risk-assessment programs. When we consider that the toxicity of some cyanotoxins can be 10 or 100 times higher by this route when compared to oral exposure, it is clear that cyanotoxin inhalation by recreational and professional (labor) exposure represents an important risk for human health (Codd et al., 1999; Benson et al., 2005; Cheng et al., 2007).

The probability of acute poisoning by ingestion of water containing cyanotoxins is lower than that of poisoning caused by low-level prolonged exposure. However, much less is known about the health risks resulting from repeated low-level exposure as compared to those related to acute intoxication (Bouaicha et al., 2005; Grosse et al., 2006). Experimental data have shown that mice develop acute lung injury when exposed to MC-LR at sublethal doses. Both toxic Microcystis extract and pure MC-LR generate similar damage through intratracheal and intra-peritoneal routes of exposure (Hooser, 2000). It was observed that MC-induced impairment of respiratory mechanics was caused mainly by an acute inflammatory process in the lungs (Picanço et al., 2004; Soares et al., 2007). Some in vitro experiments with human and rat neutrophils demonstrated that MCs may affect polymorphonuclear lymphocytes (PMN). MC variants can cause migration of neutrophils in a chemotaxis chamber, suggesting that PMNs may migrate from the bloodstream to organs that concentrate MCs, such as the liver. In addition, MCs may cause a dose-related increase in reactive oxygen species (ROS) production, as measured by chemiluminescence of PMN degranulation products that accompany ROS production. These findings suggest the possibility that PMNs may mediate some of the toxic effects of MCs (Kujbida et al., 2006, 2008, 2009). Considering some technical reports describing coastal lagoon and beach water contamination with cyanobacteria and MCs as regular events in Rio de Janeiro, a better understanding of respiratory damage related to exposure to aerosols containing MCs is of paramount importance.

6. Concluding remarks and future prospects

MCs display a broad spectrum of action that is not restricted to their hepatotoxic mechanism of phosphatase inhibition. Known MC variants are diverse not only in their species of origin but also in their functions and targets. Although peptides have been identified and characterized from only a few of the toxic cyanobacterial species found in South America, cyanobacterial bloom samples have shown the presence of several MC analogs. 10 MC variants were described in this review: MC-LR, MC-RR, MC-FR, MC-YR, MC-AR, MC-LF, MC-hRhR, [Asp3]-MC-LR, [Asp3]-MC-YR and [Leu1]-MC-LR. Chronic and acute toxicological experiments have been carried out only for the most common variants. This highlights the research potential and public
health risks that lie dormant in South American water bodies.

The chemical synthesis of specific toxins can be important in the investigation of chronic exposure. However, because of the complexity of the chemical structure of cyanotoxins, this approach is economically not feasible. An understanding of the structure–function relationship of MCs is also important for the potential use of these toxins as drugs. There is a continuous need for new and improved pharmacological tools and a more precise understanding about the structure–function relationships of MCs. The production of recombinant MCs and the elucidation of the molecular basis of MC effects require more investigation; the development of better methods to screen and identify MCs in different biological and environmental samples is also essential. This will improve existing databases on toxic cyanobacterial blooms and enable easier comparison of toxins. In summary, toxins from cyanobacteria of South America should be further investigated because very little is known about their presence in surface water and their consequences for human health and the environment.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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