Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems

Jean-François Briand, Stéphan Jacquet, Cécile Bernard, Jean Francois Humbert

Vet. Res. 34 (2003) 361–377

DOI: 10.1051/vetres:2003019

Review article

Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems

Jean-François BRIAND[a], Stéphan JACQUET[a], Cécile BERNARD[b], Jean-François HUMBERT[a]*

[a] INRA, UMR CARRTEL, Équipe Microbiologie Aquatique, BP 511, 74203 Thonon Cedex, France
[b] MNHN, USM 505, Écosystèmes et Interactions Toxiques, 12 rue Buffon, 75005 Paris Cedex 05, France

(Received 5 November 2002; accepted 21 March 2003)

Abstract – Toxigenic cyanobacteria are photosynthetic prokaryotes that are most often recognized in marine and freshwater systems, such as lakes, ponds, rivers, and estuaries. When environmental conditions (such as light, nutrients, water column stability, etc.) are suitable for their growth, cyanobacteria may proliferate and form toxic blooms in the upper, sunlit layers. The biology and ecology of cyanobacteria have been extensively studied throughout the world during the last two decades, but we still know little about the factors and processes involved in regulating toxin production for many cyanobacterial species. In this minireview, we discuss these microorganisms, and more especially the toxins they produce, as a potential and important health risk for wild and domestic animals.

cyanobacteria / aquatic ecosystem / toxin / health risk / mammal

Table of contents

1. Introduction 362
2. Biology and ecology 362
3. Cyanobacterial toxins and health effects 365
   3.1. Hepatotoxins 365
      3.1.1. Microcystins and nodularins 366
      3.1.2. Cylindrospermopsins 366
   3.2. Neurotoxins 368
      3.2.1. Anatoxins 368
      3.2.2. Saxitoxins 369
   3.3. Dermatotoxins/irritant toxins 369
4. Cyanobacterial poisoning of terrestrial vertebrates 369
5. Conclusion 371

* Correspondence and reprints
Tel.: (33) 4 50 26 78 09; fax: (33) 4 50 26 07 60; e-mail: humbert@thonon.inra.fr

1. INTRODUCTION

The quality of surface water is currently arousing considerable interest, due to the importance of this resource for human life and activities. However, progressive deterioration of many surface water ecosystems has been reported for many years, both in Europe and beyond. One important form of pollution is nutrient pollution, especially with phosphorus but also with nitrogen, that leads to the eutrophication of these systems, i.e. an accelerated growth of algae (including cyanobacteria) and higher forms of plant life capable of upsetting the balance of organisms present in the water and impairing the quality of the water concerned. These nutrients result principally from insufficiently treated sewage, runoff from fertilized agricultural areas and lawns, manure and more complex effluent from livestock industries [12]. In Europe, Asia and America, more than 40% of lakes are now eutrophic and hence subject to algal proliferations [4].

Cyanobacterial proliferations, also known as blooms, can have major impacts on ecosystem functioning (disturbances of relationships among organisms, biodiversity, oxygen concentrations…) and on the health of animals and humans living in or using these systems for drinking water and/or recreational purposes. Interest in cyanobacteria has surged in recent years with the rising frequency and widening distribution of incidents related to toxic cyanobacteria [20, 30]. Many cyanobacterial species are able to synthesize a wide range of noxious products or toxins that make it dangerous to consume contaminated water (40 of the almost 2000 species identified have been documented to be toxigenic). The first scientific report of cyanotoxin poisoning in animals, including cattle, dogs, horses, was made by Francis [40] in 1878, but a much earlier indication of animal cyanotoxin poisoning could date from the Pleistocene age (i.e. about 150 000 years BC) [9]. For the last twenty years, many studies have investigated impacts of cyanotoxins on animal behavior and health, especially those of aquatic organisms. Palm Island human disease in Australia [8, 49] or the recent case of 50 fatal human poisonings by microcystins at a hemodialysis center in Brazil [15, 59] highlighted impacts of these toxins on terrestrial species. In 1998, risks to human lead the World Health Organization (WHO) to propose a provisional guideline value of 1.0 $\mu g \cdot L^{-1}$ for the level of one of the most common cyanotoxins, the microcystin-LR [124] in drinking water, and to support the publication of a book about water-borne toxic cyanobacteria [18]. Some countries, including France, have now accepted this value (1.0 $\mu g \cdot L^{-1}$) as a legal standard for their drinking water. However it should be noted that more than 60 different analogues of microcystin have been described so far, and none of the neurotoxins produced by cyanobacteria has yet been taken into account by regulatory agencies [31].

The health risks for wild and domestic terrestrial vertebrates resulting from the presence of cyanotoxins in water are still ignored almost everywhere, although the number of publications describing livestock morbidity and mortality exposed to cyanotoxins is increasing. The most probable explanation for this lack of concern is the ignorance of this emerging problem of many veterinary and health authorities responsible for surveying domestic and wild terrestrial vertebrates. The goal of this minireview is to present a synthesis of what is known about freshwater cyanobacteria (plus the only known species of interest in the marine field), their toxins and impacts on the health of terrestrial vertebrates.

2. BIOLOGY AND ECOLOGY

Cyanobacteria (or blue-green algae) are photosynthetic prokaryotes that were originally classified as microalgae, because they contain photosynthetic pigments (chlorophyll *a* and accessory pigments such as

phycocyanin, allo phycocyanin and phycoerythrin), making them colored, like higher plants. Fossil records suggest that these microorganisms have been present on earth since at least 3.5 billion years [105], and they are distributed worldwide, from polar to equatorial latitudes (e.g. [121, 128]). Cyanobacteria colonize both terrestrial and aquatic biotopes where they are found in both marine and freshwater (rivers, lakes, ponds and estuaries) ecosystems [80, 123].

The morphology of cyanobacteria is very diverse, including spherical, ovoid and cylindrical unicellular species, as well as multi-cellular colonial and filamentous forms (Fig. 1) (e.g. [22]). Some species are able to differentiate specialized cells: the heterocysts that are able to fix nitrogen in water under N-limited conditions, and the akinetes that allow them to survive when confronted with stressful survival conditions such as periods of high temperature or drought. Species are sometimes difficult to identify mainly because of high phenotypic plasticity. Molecular studies suggest that the taxonomic validity of numerous species and genera could change in the future (e.g. [45, 53, 94]).

In aquatic ecosystems, cyanobacteria are primary producers that use light energy to synthesize organic matter from mineral nutrients and CO_2 (photosynthesis). When environmental conditions are appropriate for growth and competitive advantage over other species of the phytoplanktonic community, blooms can occur (Fig. 1). In such a context, one (or two) species dominates the community (of microalgae and cyanobacteria) and its biomass increases significantly over a relatively short time (within a few days to one or two weeks). Environmental factors that lead to such proliferations have now been well identified, although their importance may be species-dependent. Blooms of cyanobacteria (and microalgae) usually occur in eutrophic environments (i.e. in water containing high concentrations of mineral nutrients, and more particularly of phosphorus in the case of freshwater) [4]. Stability of the water column is another pre-requisite for bloom enhancement. Hence, most blooms occur in summer after a long period of sustained sunshine, with calm and relatively warm conditions [90].

Control of these cyanobacteria depends on competition among species for food and available light (which are known as bottom-up controls), and predation and parasitism (top-down control mechanisms). Specific physiologic capabilities of cyanobacteria enable them to compete very efficiently with other photosynthetic microorganisms. Most cyanobacterial species regulate their buoyancy (by means of gas-vacuoles) and this allows them to colonize different depths in the water column depending on the localization of nutrients and the availability of light [63, 122]. Possession of accessory pigments, such as phycoerythrin, allows several species to carry out photosynthesis at depths that receive only green light and where, in addition, nutrients are more abundant than on the surface (surface waters are rapidly depleted following spring algal proliferations). Cyanobacterial pigments as well as mycosporin-like amino acids are also involved in their great capacity to resist ultraviolet radiation in surface waters, and this could give them another advantage over some phytoplankton [16]. Some species possess heterocysts, thus giving them a further competitive advantage over other microorganisms that are capable of photosynthesis but do not fix nitrogen. With regard to top-down control, cyanobacteria are usually organized in filaments and/or colonies, and may produce mucilage layers, that make it much more difficult for zooplankton to feed (graze) on cyanobacteria than on single cell structures (e.g. [38]). These authors have recently shown that the mode of defense adopted by cyanobacteria also depends on grazer pressure; i.e. cyanobacteria are able to modify their defense reaction according to the actual risk of grazing. The synthesis of toxins by many cyanobacterial blooms can also be

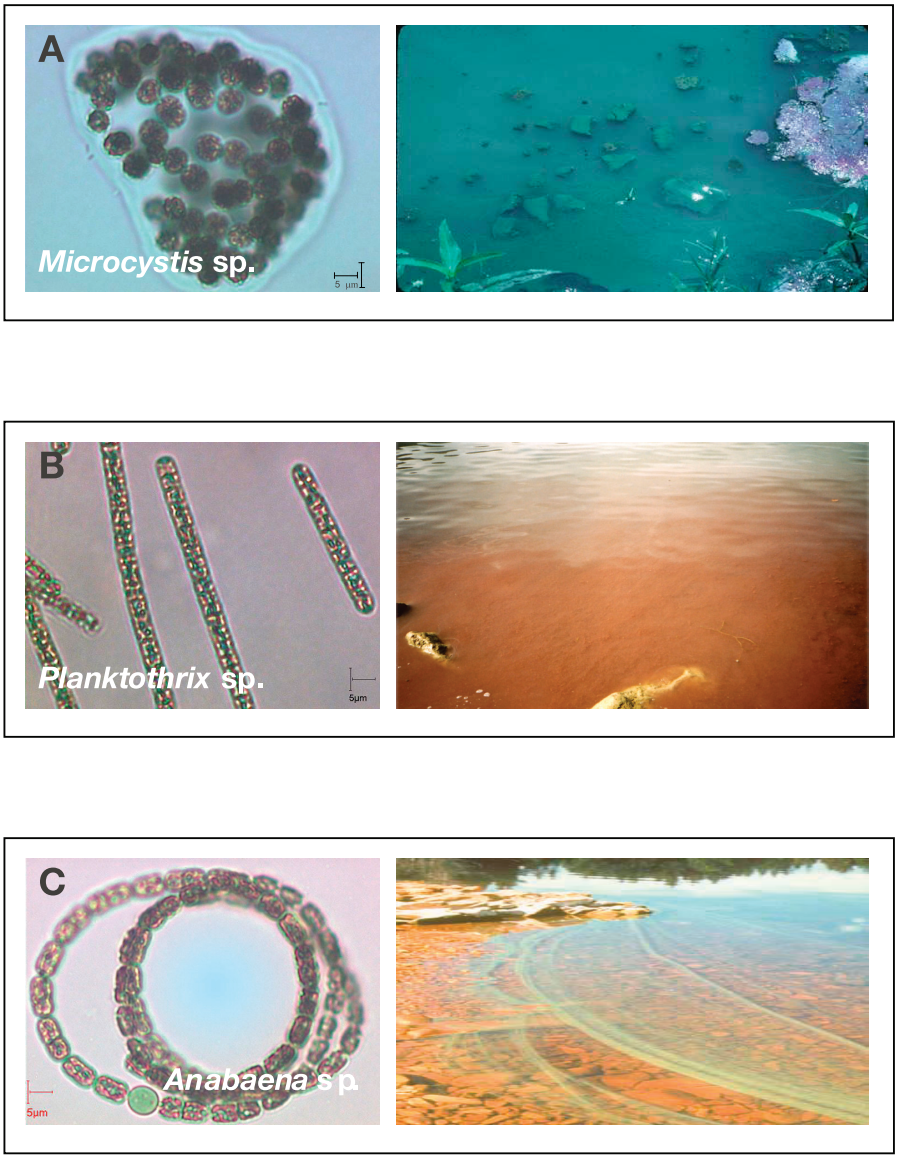

Figure 1. Photomicrographs of a colonial (**A**: *Microcystis aeruginosa*) and filamentous (**B**: *Planktothrix rubescens*; **C**: *Anabaena flos aquae*) toxigenic cyanobacteria (left panels) and their corresponding blooms in the field (right panel). Microscopic images A & C courtesy of Luc Brient (University of Rennes 1, France). Pictures of blooms courtesy of David Frogmann (Purdue University, USA), Mark Schneegurt (Wichita State University, USA) and Cyanosite (www.cyanosite.bio.purdue.edu) for photo A, Michel Roux (Conseil Supérieur de la Pêche, France) for photo B, and J.C. Druart (INRA, France) for photo C.

Figure 2. Macroscopic pathology of fixed livers from Male Swiss Albino mice (IOPS OF1 strain) treated with toxic extracts (unpublished results from Mathilde Harvey, AFSSA Paris). Top row from left to right: two mouse livers injected with 0.9% NaCl and two non-perfused mice livers as controls. Bottom row: liver of mouse injected with an extract (20 mg lyophilised sample/mL, 0.9% NaCl used as extraction solvant, i.e. 1000 mg lyo/kg of body weight) of a field sample of *Planktothrix agardhii* (producing microcystins) and with culture extracts (5, 10 and 20 mg lyophilised sample/mL, 0.9% NaCl, i.e. 250, 500 and 1000 mg lyo/kg of body weight) of *Cylindrospermopsis raciborskii* (producing cylindrospermopsin).

viewed as giving a selective advantage, since some zooplanktonic predators are susceptible to these toxins and avoid eating cyanobacteria [7, 23]. Finally, the control of cyanobacteria by parasites, typically viruses, is probably very slight ([114], Jacquet S., unpublished data).

3. CYANOBACTERIAL TOXINS AND HEALTH EFFECTS

Cyanotoxins can be characterized by their chemical or toxicological properties. We have chosen to present them in terms of their toxicological target. In this context, there are hepatotoxins (microcystins, nodularins and cylindrospermopsins), neurotoxins (anatoxins and saxitoxins) and dermatotoxins/irritant toxins (lyngbyatoxin A, aplysiatoxins and lipopolysaccharides).

3.1. Hepatotoxins

Three families of toxins mainly target the liver (Fig. 2): the microcystins, which include more than 60 molecules, the nodularins and the cylindrospermopsins.

3.1.1. Microcystins and nodularins

Microcystins are the most widely distributed cyanotoxins and the ones most often implicated in human and animal poisonings. They are produced by several genera, including the planktonic *Microcystis*, *Planktothrix*, *Anabaena* species and the benthic *Oscillatoria*. Nodularins are only produced by the species *Nodularia spumigena,* which occurs in brackish waters, essentially in the Baltic Sea, Australia and New Zealand [30]. Nevertheless, motuporin, a nodularin analogue, was first isolated from a marine sponge [24].

These two families of toxins are both cyclic peptides, with the same basic cyclic structure involving an amino-acid called ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) plus six (microcystins) or four (nodularins) other amino acids (Fig. 3A). Only six nodularins have so far been identified. In contrast, the presence of two variable amino-acids (X and Z), two groups (R1, R2) and two demethylated positions (3 and 7) results in the existence of more than 60 microcystins [20]. Their molecular weights range from 800 to 1100 Daltons (Fig. 3A).

These hepatotoxins range from extremely toxic compounds such as microcystin-LR ($L.D._{50} = 50\ \mu g \cdot kg^{-1}$ bodyweight in mice by intraperitoneal (i.p.) injection) and nodularin ($L.D._{50} = 30\ \mu g \cdot kg^{-1}$ bodyweight in mice by i.p. injection) to non-toxic forms of microcystin [99]. Then, oral toxicity to mice was reported 30 to 100 fold less than toxicity after i.p., depending on the mixture of microcystins [35].

Clinical signs of microcystin or nodularin intoxication in mammals are diarrhoea, vomiting, piloerection, weakness and pallor [28, 33]. The hepatospecificity of these toxins is due to the requirement for uptake by a bile acid transporter [29, 100, 102]. Microcystins and nodularins have been shown to be inhibitors of serine/threonine protein phosphatase 1 and 2A [52, 77]. This inhibition leads to hyperphosphorylation of proteins associated with the cytoskeleton in hepatocytes [117]. The rapid loss of the sinusoidal architecture and attachment to one another leads to the accumulation of blood in the liver, and death most often results from hemorrhagic shock. Hypoglycemia and hyperkalemia can also be terminal events [5]. Lower doses of microcystins cause progressive changes in liver tissue, including chronic inflammation, focal degeneration of hepatocytes and the accumulation of metabolites such as bilirubin in the blood, and tend to increase mortality [28, 51].

Microcystins are also tumor-promoting substances when combined with compounds that are able to initiate the cancer process (usually by causing DNA-damage) [32, 55, 85]. There is evidence that the pentapetide toxin nodularin is a more potent tumor promoter than microcystin. This is supported by the lower concentration of nodularin required to inhibit phosphatase enzymes in vitro [88].

3.1.2. Cylindrospermopsins

To date, cylindrospermopsins are known to be produced only by *Cylindrospermopsis raciborskii* [50], *Aphanizomenon ovalisporum* [2, 108], *Umezakia natans* [47] and *Raphidiopsis curvata* [67], mainly in tropical areas. In addition, cylindrospermopsin (CYN) was identified in New Zealand field extracts without identification of the toxin-producing cyanobacterium [113].

CYN is an alkaloid containing a tricyclic guanidine combined with hydroxymethyl uracyl (Fig. 3B) with a molecular weight of 415 Daltons. This molecule acts mainly as an inhibitor of proteins synthesis, but other actions have also been described [30, 47, 115]. The $L.D._{50}$ of CYN is 2100 $\mu g \cdot kg^{-1}$ bodyweight in mice at 24 h and only 200 $\mu g \cdot kg^{-1}$ at 5–6 days by i.p. injection [89]. However, in the present context it should be noted that a number of papers have reported that crude extracts of *Cylindrospermopsis raciborskii* can have greater 24 h toxicities than expected from the known CYN content [36, 50]. The oral

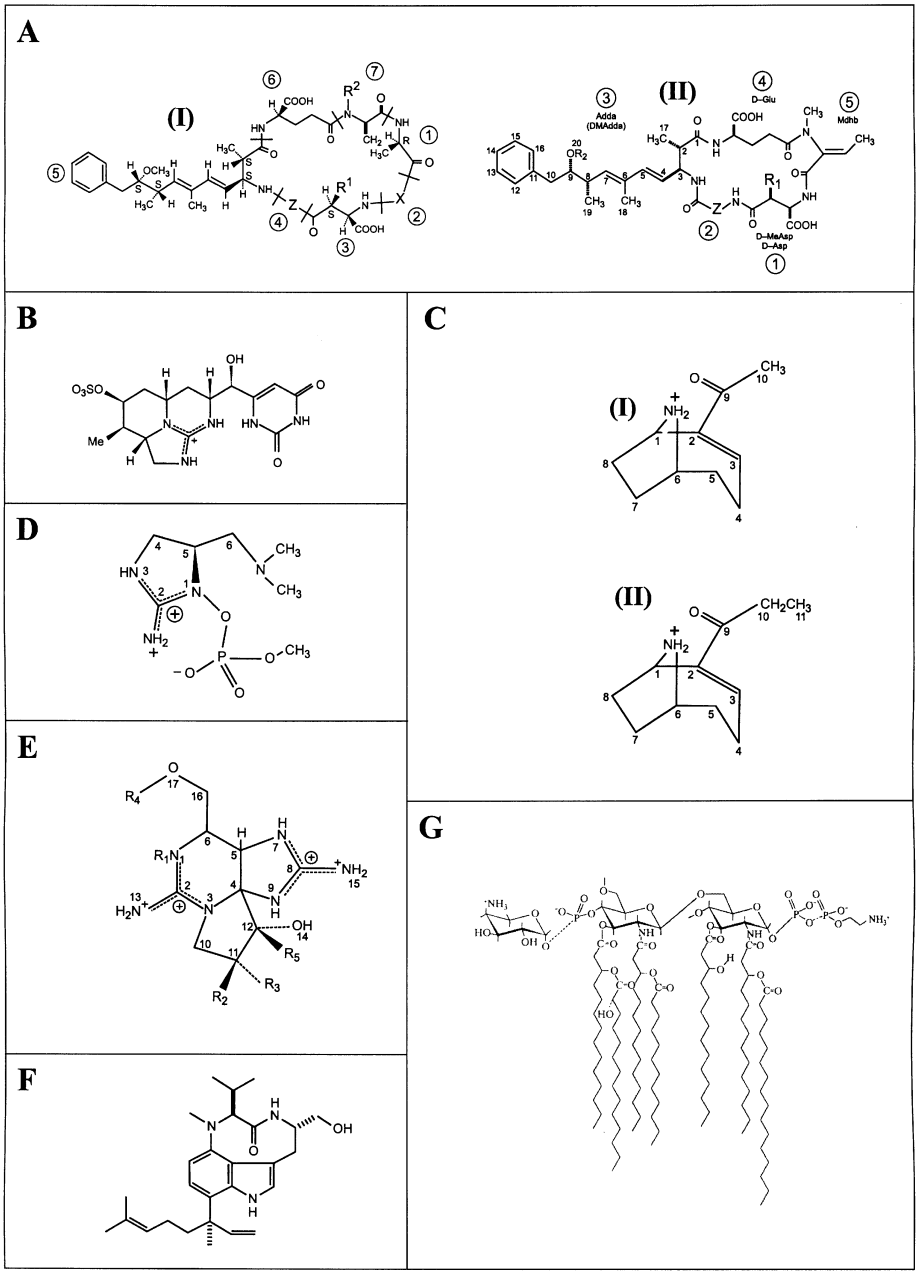

Figure 3. Chemical structures of **A**: microcystins (I) and nodularins (II) (X and Z are variable amino acids, R = H or CH_3), **B**: cylindrospermopsin, **C**: anatoxin-a (I) and homoanatoxin-a (II), **D**: anatoxin-a(s), **E**: PSPs, **F**: lyngbyatoxin A, **G**: lipopolysaccharides (LPS).

toxicity is about 30 fold less than toxicity by i.p. [106]. CYN has two known derivatives, one of them is toxic, 7-epicylindrospermopsin [1] and the other virtually non-toxic, deoxy-cylindrospermosin [86].

The main target of this toxin is the liver, but unlike the microcystins, it can affect other organs such as the kidney, thymus or heart. Acute poisoning induces death probably due to heart failure, as suggested by Seawright et al. [106]. At toxic concentrations, lesion development is quite minor compared to the speed of clinical evolution. The liver is dark in color with rib marks at the highest concentrations. The kidneys are pale, and palor increases at higher doses. At lower doses, death occurs less rapidly and specific injuries in different organs such as the liver, kidneys, thymus, spleen and immune system are observed. The kidneys exhibit considerable granular degeneration and coagulation necrosis of the tubular epithelium. Necrosis in the convoluted tubules is most severe and extensive. In the thymus, there is widespread necrosis of the cortical lymphocytes. The spleen is characterized by necrosis with phagocytosis of cellular fragments by macrophages [6, 36, 106]. Recently CYN has been found to display genotoxic activity [56, 109].

3.2. Neurotoxins

The neurotoxins known to be produced by freshwater cyanobacteria include anatoxin-a, anatoxin-a(s) and saxitoxins. Their target is the neuromuscular system, and they can paralyze peripheral, skeletal, including respiratory muscles. Death occurs as a result of respiratory arrest within a few minutes to a few hours (see review by Duy et al. [26] and Kuiper-Goodman et al. [65]).

3.2.1. Anatoxins

Anatoxins are produced mainly by *Anabaena* species, but also by *Aphanizomenon*, *Microcystis* and the benthic *Oscillatoria* [111].

Anatoxin-a is a secondary amine with a molecular weight of 165 Daltons (Fig. 3C). Anatoxin-a is a cholinergic agonist that binds to nicotinic acetylcholine receptors in nerves and neuromuscular junctions. The molecule is not easily cleared or displaced from tissue. This results in local depolarization that opens voltage-sensitive Ca^{++} and Na^{+} channels. As a result of extreme depolarization, there is a blockade of further electrical transmission, leading to muscle paralysis and death by respiratory failure in mammals [30]. The $L.D._{50}$ is 200 $\mu g \cdot kg^{-1}$ bodyweight in mice by i.p. injection. Oral toxicity for sonicated cells suspension of anatoxin-a-containing *Anabaena* cells is about one hundred to one thousand times higher [11]. Homoanatoxin-a is a methyl derivative of anatoxin-a (Fig. 3C(II)), with a molecular weight of 179 Daltons and lower potency ($L.D._{50}$ = 250 $\mu g \cdot kg^{-1}$ bodyweight in mice by i.p. injection).

Anatoxin-a(s) is unrelated to anatoxin-a. It is a unique N-hydroxyguanidine methyl phosphate ester with a molecular weight of 252 Daltons (Fig. 3D). Anatoxin-a(s) is a acethylcholinesterase inhibitor with a mechanism similar to that of the organophosphorus insecticides. However, anatoxin-a(s) acts only in the periphery and thus brain and retinal cholinesterase activities remain normal even in lethally poisoned animals [21]. The neurological effects in mice given acute doses are muscle weakness, respiratory distress (dyspnea) and convulsions (effect on seizure threshold) preceding death. In pigs and mice, anatoxin-a(s) can cause viscous mucoid hypersalivation. Death often occurs from respiratory arrest [74]. The "s" in the name of the toxin stands for "salivation", because of the additional hypersalivation observed in mice. This neurotoxin is significantly more potent than anatoxin-a, with an $L.D._{50}$ in mice of only 20 $\mu g \cdot kg^{-1}$ bodyweight by i.p. injection [30].

3.2.2. Saxitoxins

Saxitoxins are well-known marine toxins produced by dinoflagellates, especially *Alexandrium* spp. and *Gymnodinium* spp. [119, 127], and by heterotrophic bacteria [44]. Saxitoxins have been recently identified in five freshwater cyanobacterial species: *Aphanizomenon flos-aquae* [37, 57, 70, 95], *Anabaena circinalis* [54, 83], *Lyngbya wollei* [14, 91, 130] *Cylindrospermopsis raciborskii* [66] and *Planktothrix* sp. [96]. STXs were also identified in Danish freshwater field extracts without unambiguous identification of toxin-producing species (*Anabaena lemmermannii* being very common in these lakes) [60].

The saxitoxins or paralytic shellfish poisons (PSPs) consist of a family of more than 20 molecules with a tetrahydropurine structure (molecular weights from 241 to 491 Daltons). They can be divided in four groups, depending on the substitutions in the five variable positions R1 to R5 (Fig. 3E): saxitoxins (STX, dcSTX, neoSTX) [54, 57, 60, 66, 70, 83, 95, 96], gonyautoxins (GTX 1 to 6 [54, 60, 66, 83, 95] and dcGTX 2 and 3 [54, 83, 91, 130]), C-toxins (C 1 and 2) [37, 54, 82], and variants identified in American strains of *Lyngbya wollei* (LWTX 1 to 6) [91]. Depending on the variants, the toxicity in the mouse can differ considerably. Saxitoxin is the most potent PSP (LD_{50} = 10 $\mu g \cdot kg^{-1}$ mouse, i.p.) and LWTX 1,4 and 6 can be more than 165 times less toxic [93].

All these toxins act in the same way: nervous transmission is blocked when the PSP binds to site 1 of the sodium channels [17], and this induces muscle paralysis. In animals, typical neurologic effects induced by this toxin include nervousness, jumping, jerking, ataxia, convulsions and paralysis. The paralysis of respiratory muscles leads to the death of animals within a few minutes [101].

3.3. Dermatotoxins/irritant toxins

Although they are produced by marine cyanobacteria, the dermatotoxins lyngbyatoxin A (Fig. 3F) and aplysiatoxin were related to acute dermatitis, poisoning and animal death, especially in Japan and Hawaii [3, 92, 129]. These toxins are indeed produced by the marine benthic cyanobacterium *Lyngbya majuscula*. However, this species synthesizes a variety of chemicals that exert a range of biological effects through phospholipid-dependent protein kinase C activation, inducing contraction of vascular smooth muscles. Clinical signs include skin, eye and respiratory irritation [92]. In addition, lynbyatoxin A is a potent tumor promoter [42].

Lipopolysaccharides (LPS) are common compounds in the outer cell walls of gram-negative bacteria, including cyanobacteria. They consist of condensed products of a sugar, usually a hexose, and a lipid, usually a hydroxy C_{14}-C_{18} fatty acid (Fig. 3G). The sugar and fatty acid composition may vary among the cyanobacterial species; they differ from those of the Enterobacteria, largely related to phylogeny. The irritant or allergenic effects are mainly due to the fatty acid component [61, 104]. Few studies deal with cyanobacterial LPS. They seem less potent than LPS from pathogenic gram-negative bacteria, but could be implicated in human health problems encountered as a result of bathing for example [20, 24]. In addition, Glutathione S-transferase genes, which are responsible in part for detoxication of microcystins, may be inhibited by LPS [125].

4. CYANOBACTERIAL POISONING OF TERRESTRIAL VERTEBRATES

Terrestrial vertebrate poisoning usually occurs as a result of drinking contaminated water or by the accidental ingestion of contaminated water during swimming. Poisoning resulting from eating contaminated food is well known with marine shellfishes [119]. While bioaccumulation of four different cyanotoxins in the trophic chain is demonstrated (nodularin [34], microcystins

[68], PSPs [82] and cylindrospermosin [103]), intoxication has not yet been clearly demonstrated to occur. National and international programs devoted to this field of research should clarify the situation in the future.

The first report of cyanotoxin poisoning of domestic animals was based on an incident at Lake Alexandrina in Australia [40]. This country seems particularly subject to bloom-forming toxic cyanobacteria as shown by reports of Carbis et al. [10], Main et al. [72], Mc Barron and May [75], Mc Barron et al. [76], Mulhearn [79], Negri et al. [83] and Thomas et al. [116]. In these studies, prolonged morbidity and delayed mortality in sheep and cattle have been attributed to cyanotoxins produced by *Microcystis aeruginosa* (microcystins), *Nodularia spumigena* (nodularins), *Anabaena circinalis* (PSPs), and *Cylindrospermopsis raciborskii* (cylindrospermopsin). This last species is a typical tropical cyanobacterium frequently proliferating in Australia. The paper of Thomas et al. [116] links this microorganism to the death of three cows and ten calves in a herd of 300 cattle. One animal showed signs of staggering and weakness before its death. Abdominal and thoracic haemorrhagic effusion, hyperemic mesenteries, and pale and swollen liver were found at necropsy, with nothing abnormal observed in the brain, lungs, spleen or kidney. The most dramatic intoxication event documented to date occurred in 1992 along a 1 000-km stretch of the Darling river where 10 000 livestock died after a massive bloom of neurotoxic *Anabaena circinalis* [30]. In Australia, all types of cyanotoxins are of concern: hepatotoxins including the microcystins, the nodularins and cylindrospermopsin, and also neurotoxins with the PSPs [31].

In Europe, there is little documentation of animal poisoning by cyanobacteria although toxic cyanobacterial blooms have been observed in many countries [111]. Deaths of cattle and dogs have been attributed to *M. aeruginosa* (microcystins) and *N. spumigena* (nodularins) in England and Norway, respectively [19, 27, 46, 84], and to *Nodularia* sp. (probably nodularins) and *Anabaena flos-aquae* (neurotoxin) in Sweden [126], but the main report of such poisoning has been in Switzerland, where more than 100 cattle deaths have been attributed to microcystin poisoning during the last two decades [78, 81]. Clinical signs variably combined in leading to these deaths were tremor, recumbency, foam in front of the mouth, teeth chattering, convulsions, staggering gait, restlessness, loss of appetite and drowsiness. The histopathologic examination of liver revealed acute centrilobular to panlobular coagulative necrosis with dissociation of hepatocytes. The poisoning was due to the development of benthic cyanobacteria belonging to the *Oscillatoria* genus in oligotrophic high alpine lakes which are theoretically not favorable to cyanobacterial proliferations. This shows that cyanotoxin problems can occur in environments where they are not expected. A similar benthic *Oscillatoria*, but one producing neurotoxic anatoxin-a, has been responsible for the death of dogs in Scotland [27] and Ireland [58].

In North America, numerous studies have reported cattle poisoning by cyanobacteria (*Anabaena circinalis* and *Microcystis* spp. [25], microcystins produced by *M. Aeruginosa* [39], *Microcystis* toxicoses [41], hepatotoxicosis due to *M. Aeruginosa* [43, 62, 64], *Anabaena circinalis* and *Microcystis* spp. [107], *Microcystis* spp. producing microcystins [110]). In some cases, this poisoning has led to the deaths of animals, for example Puschner et al. [97]. In one field study, the authors reported the rapid death of 24 out of 175 heifers. Six cattle were found dead near a pond containing an algal bloom. The other 18 heifers died three days after the first clinical signs (nervousness, recumbency, weakness, anorexia and hypersensitivity to noise). Necropsy of one heifer revealed a liver that was markedly larger than normal, with a cut surface that was friable, dark and hemorrhagic. Dissociation and necrosis of the

hepatocytes were also found. There were small foci of hemorrhage in the hearth subepicardium and mild edema in the lungs. The kidney was unaffected. In most of these cases, *M. aeruginosa* hepatotoxins were incriminated. Furthermore, the neurotoxins anatoxin-a produced by *Anabaena flos-aquae* have been implicated in the death of cattle in Canada [13]. Anatoxin-a(s) also produced by *A. flos-aquae* was responsible for the death of dogs, pigs and ducks in USA [71] and waterfowl in Canada [98]. However, Cook et al. [21] suggested that cattle could be resistant to ingestion of anatoxin-a(s). In South America, *M. aeruginosa* (producing microcystins) has been implicated in the sudden death of 72 cows in Argentina [87].

Other continents and other animals have also been involved in vertebrate cyanotoxin poisoning problems. In Africa, poisoning of livestock by hepatotoxic *M. aeruginosa* and *Nodularia spumigena* [120], dogs by nodularin produced by *N. spumigena* [48] and white rhinoceros by *M. aeruginosa* [112] has been observed. But although the climatic conditions in this continent could be expected to support the massive development of cyanobacteria, there is little information about cyanobacteria impact probably due to lack of investigation. Similarly, very little information is available from the Asian continent. The only report concerns the mass death of spot-billed ducks in a pond located in Japan. Matsunaga et al. [73] attributed the deaths of these wild birds to high concentrations of three kinds of microcystins in a bloom of *Microcystis aeruginosa*. Histological study of one of the bird victims revealed a necrotic liver, severely jaundiced and dark green in color. Surprisingly in regards to links established between drinking water from reservoirs that contained toxigenic blue-green algae and increased risks of liver cancer in human populations [65, 118], no animal poisoning was reported from China.

One probable explanation of the numerous fatalities among livestock is that mammals do not avoid drinking concentrated cyanobacteria scum. In their experiments, Lopez Rodas and Costas [69] found that mice preferred to consume dense cultures of a toxic strain of *Microcystis aeruginosa* than low-density cultures or clear water. They have confirmed their findings in the field with observations from several reservoirs in Spain, where the consumption of concentrated *M. aeruginosa* scum by domestic and wild animals has often been reported.

As we have seen in this short review, most of the literature on the impacts of cyanotoxins on terrestrial vertebrate animals concerns domestic animals. This is because their economic importance prompts investigations into the cause of death. Thus, the morbidity and mortality of wild animals due to cyanotoxin intoxication is probably considerably underestimated.

5. CONCLUSION

By reviewing the existing information dealing with toxic cyanobacteria and cyanobacterial toxins, our goal was to introduce to the veterinary profession (practitioners, diagnosticians, researchers, etc.) an important health hazard for terrestrial vertebrates (especially reported with domestic animals like dogs and cattle) that is unlikely widely known at present and may well be an important consideration to have in mind. We highlighted the environmental conditions in which animal intoxications might occur and also the fact that these conditions are increasing in frequency nowadays. Typically, problems due to cyanobacteria can be linked to the increase in nutrient pollution of lakes, ponds and rivers as a consequence of (industrial, agricultural and domestic) human activities. Periods of cool weather related to high water column stratification and warm temperatures also enhance cyanobacterial proliferations. Differently said, the risk is higher in summer and professionals

should be more careful at this period of time.

When unexplained deaths of wild or domestic animals occur in locations with water bodies, the veterinary and health authorities in charge of monitoring animal health should consider the possibility of poisoning by cyanobacterial toxins, as well as infectious diseases, anthropogenic chemical poisoning and the other causes usually investigated. Then, water quality should be tested for cyanobacterial presence and cyanotoxins (for both intracellular and extracellular metabolites). Necropsy of dead animals should be done and search of hepatotoxin in the liver should also be performed when suspected.

Hence, by increasing awareness among the veterinary audience, we hope this contribution may help to save more domestic (with eventually economic implications) but also wild animals and perhaps human lives in the future.

ACKNOWLEDGEMENTS

The authors want to thank the two anonymous reviewers for their precise and constructive comments.

REFERENCES

[1] Banker R., Carmeli S., Hadas O., Telsch B., Porat R., Sukenik A., Identification of Cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from lake Kinneret, Israël, J. Phycol. 33 (1997) 613–616.

[2] Banker R., Telsch B., Sukenik A., Carmeli S., 7-Epicylindrospermopsin, a toxic minor metabolite of the cyanobacterium *Aphanizomenon ovalisporum* from lake Kinneret, Israel, J. Nat. Prod. 63 (2000) 387–389.

[3] Banner A.H., A dermatitis-producing alga in Hawaï, Hawaï Med. J. 19 (1959) 35–36.

[4] Bartram J., Carmichael W.W., Chorus I., Jones G., Skulberg O.M., Introduction, in: Chorus I., Bartram J. (Eds.),Toxic cyanobacteria in water, E & FN Spon, London and New-York, 1999, pp. 1–14.

[5] Beasley V.R., Lovell R.A., Holmes K.R., Walcott H.E., Schaeffer D.J., Hoffmann, W.E., Carmichael W.W., Microcystin-LR decreases hepatic and renal perfusion, and causes circulatory shock, severe hypoglycemia, and terminal hyperkalemia in intravascularly dosed swine, J. Toxicol. Environ. Health A 61 (2000) 281–303.

[6] Bernard C., Harvey M., Briand J.-F., Biré R., Fontaine J.-J., Krys S., Liver damage caused by a French *Cylindrospermopsis raciborskii* strain: comparison with diverse *C. raciborskii* toxic strains, Environ. Toxicol. 18 (2003) 176–186.

[7] Berthon J.L., Brousse S., Modification of migratory behaviour of planktonic crustacea in the presence of a bloom of *Microcystis aeruginosa* (cyanobacteria), Hydrobiologia 300/301 (1995) 185–193.

[8] Bourke A.T.C., Hawes R.B., Neilson A., Stallman N.D., An outbreak of hepatoenteritis (the Palm Island mystery disease) possibly caused by algal intoxication, Toxicon Suppl. 3 (1983) 45–48.

[9] Braun A., Pfeiffer T., Cyanobacterial blooms as the cause of a Pleistocene large mammal assemblage, Paleobiology 28 (2002) 139–154.

[10] Carbis C.R., Waldron D.L., Mitchell G.F., Anderson J.W., McCauley I., Recovery of hepatic function and latent mortalities in sheep exposed to the blue-green alga *Microcystis aeruginosa*, Vet. Rec. 137 (1995) 12–15.

[11] Carmichael W.W., Cyanobacteria secondary metabolites: the cyanotoxins, J. Appl. Bacteriol. 72 (1992) 445–459.

[12] Carmichael W.W., Falconer I.R., Diseases related to freshwater blue-green algal toxins, and control measures, in: Falconer I.R. (Ed.), Algal toxins in seafood and drinking water, Academic Press, 1993, pp. 187–209.

[13] Carmichael W.W., Gorham P.R., Anatoxins from clones of *Anabaena flos-aquae* isolated from lakes in western Canada, Mitt. Int. Ver. Theor. Angew. Limnol. 21 (1978) 285–295.

[14] Carmichael W.W., Evans W.R., Yin Q.Q., Bell P., Moczydlowsky E., Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov., Appl. Environ. Microbiol. 63 (1997) 3104–3110.

[15] Carmichael W.W., Azevedo S.M.F.O., An J.S., Molica R.J., Jochimsen E.M., Lau S., Rinehart K.L., Shaw G.R., Eaglesham G.K., Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins, Environ. Health Perspect. 109 (2001) 663–668.

[16] Castenholz R.W., Garcia-Pichel F., Cyanobacterial responses to UV-radiation, in: Whitton B.A., Potts M. (Eds.), The Ecology of cyanobacteria, their diversity in time and space, Kluwer Academic Publishers, Dordrecht, 2000, pp. 591–611.

[17] Catterall W.A., Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes, Annu. Rev. Pharmacol. Toxicol. 20 (1980) 15–43.

[18] Chorus I., Bartram J., Toxic Cyanobacteria in water: A guide to their public health consequences, monitoring and management, E & FN Spon, London & New York, 1999.

[19] Codd G.A., Edwards C., Beattie K.A., Barr W.M., Gunn G.J., Fatal attraction to cyanobacteria?, Nature 359 (1992) 110–111.

[20] Codd G.A., Bell S.G., Kaya K., Ward C.J., Beattie K.A., Metcalf J.S., Cyanobacterial toxins, exposure routes and human health, Eur. J. Phycol. 34 (1999) 405–415.

[21] Cook W.O., Beasley V.R., Lovell R.A., Dahlem A.M., Hooser S.B., Mahmood N.A., Carmichael W.W., Consistent inhibition of peripheral cholinesterases by neurotoxins from the freshwater cyanobacterium *Anabaena flos-aquae*: studies of duck, swine, mice and a steer, Environ. Toxicol. Chem. 8 (1989) 915–922.

[22] Couté A., Bernard C., Les cyanobactéries toxiques, in: Frémy J.M., Lassus P. (Coord.), Toxines d'algues dans l'alimentation, Ed. Ifremer, Paris, 2001, pp. 89–108.

[23] Demott W., Zhang Q.X., Carmichael W.W., Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*, Limnol. Oceanogr. 36 (1991) 1346–1357.

[24] de Silva E.D., Williams D.E., Andersen R.J., Klix H., Holmes C.F.B., Allen T.M., Motuporin, a potent protein phosphatase inhibitor isolated from the Papua New Guinea sponge *Theonella swinhoei* Gray, Tetrahedron Lett. 33 (1992) 1561–1564.

[25] Dillenberg H.O., Dehnel M.K., Toxic waterbloom in Saskatchewan, 1959, Can. Med. Assoc. J. 83 (1960) 151.

[26] Duy T.N., Lam P.K.S., Shaw G.R., Connell D.W., Toxicology and Risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water, Rev. Environ. Contam. Toxicol. 163 (2000) 113–186.

[27] Edwards C., Beattie K.A., Scrimgeour C.M., Codd G.A., Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland, Toxicon 30 (1992) 1165–1175.

[28] Elleman T.C., Falconer I.R., Jackson A.R.B., Runnegar M.T., Isolation, characterization and pathology of the toxin from a *Microcystis aeruginosa* = (*Anacystis cyanea*) bloom, Aust. J. Biol. Sci. 31 (1978) 209–218.

[29] Eriksson J., Gronberg L., Nygard S., Slotte J.P., Meriluoto J., Hepatocellular uptake of ^{3}H-diydromicrocystin-LR, a cyclic peptide toxin. Biochim. Biophys. Acta 1025 (1990) 60–66.

[30] Falconer I.R., Algal toxins and human health, in: The handbook of environmental chemistry, J. HRUBEC, Berlin Heidelberg, Springer-Verlag, 5 Part C Quality and treatment of drinking water II, Berlin, 1998, pp. 53–82.

[31] Falconer I.R., Toxic cyanobacteria bloom problems in Australian waters: risks and impacts on human health, Phycologia 40 (2001) 228–233.

[32] Falconer I.R., Buckley T.H., Tumour promotion by *Microcystis* sp. a blue-green alga occurring in water supplies, Med. J. Aust. 150 (1989) 351.

[33] Falconer I.R., Jackson A.R.B., Langley J., Runnegar M.T.C., Liver pathology in mice in poisoning by the blue-green alga in *Microcystis aeruginosa*, Aust. J. Biol. Sci. 34 (1981) 179–187.

[34] Falconer I.R., Choice A., Hosja W., Toxicity of edible mussels (*Mytilus edulis*) growing naturally in an estuary during a water bloom of the blue-green alga *Nodularia spumigena*, Env. Toxicol. Water Qual. 7 (1992) 119–123.

[35] Falconer I.R., Burch M.D., Steffensen D.A., Choice M., Coverdale O.R., Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment, Env. Toxicol. Water Qual. 9 (1994) 131–139.

[36] Falconer I.R., Hardy S.J., Humpage A.R., Froscio S.M., Tozer G.J., Hawkins P.R., Hepatic and renal toxicity of the blue-green alga (cyanobacterium) *Cylindrospermopsis raciborskii* in male Swiss Albino mice, Environ. Toxicol. 14 (1999) 143–150.

[37] Ferreira F.M.B., Soler J.M.F., Fidalgo M.L., Fernández-Vila P., PSP toxins from *Aphanizomenon flos-aquae* (cyanobacteria) collected in the Crestuma-Lever reservoir (Douro river, Northern Portugal), Toxicon 39 (2001) 757–761.

[38] Fialkowsak E., Pajdak-Stos A., Dependence of cyanobacteria defense mode on grazer pressure, Aquat. Microb. Ecol. 27 (2002) 149–157.

[39] Fitzgerald S.D., Poppenga R.H., Toxicosis due to microcystin hepatotoxicosis in three

Holstein heifers, J. Vet. Diagn. Invest. 5 (1993) 651–653.

[40] Francis G., Poisonous Australian lake, Nature 18 (1878) 11–12.

[41] Frazier K., Colvin B., Styer E., Hullinger G., Garcia R., *Microcystis* toxicosis in cattle due to overgrowth of blue-green algae, Vet. Hum. Toxicol. 40 (1998) 23–24.

[42] Fujiki H., Suganuma M., Hakii H., Bartolini G., Moore R.E., Takayama S., Sugimura T., A two-stage mouse skin carcinogenesis study of lyngbyatoxin A, J. Cancer Res. Clin. Oncol. 108 (1984) 74–176.

[43] Galey F.D., Beasley V.R., Carmichael W.W., Kleppe G., Hooser S.B., Haschek W.M., Blue-green algae (*Microcystis aeruginosa*) hepatotoxicosis in dairy cows, Am. J. Vet. Res. 48 (1987) 1415–1420.

[44] Gallacher S., Flynn K.J., Franco J.M., Brueggemann E.E., Hines H.B., Evidence for production of paralytic shellfish toxins by bacteria associated with *Alexandrium* spp. (Dinophyta) in culture, Appl. Environ. Microbiol. 63 (1997) 239–245.

[45] Gugger M., Lyra C., Henriksen P., Couté A., Humbert J.F., Sivonen K., Phylogenetic comparison of the genera *Anabaena* and *Aphanizomenon*, Int. J. Syst. Evol. Microbiol. 52 (2002) 1867–1880.

[46] Gunn G.J., Rafferty A.G., Rafferty G.C., Cockburn N., Edwards C., Beattie K.A., Codd G.A., Fatal canine neurotoxicosis attributed to blue-green algae (cyanobacteria), Vet. Rec. 4 (1992) 301–302.

[47] Harada K.I., Othani I., Iwamoto K., Suzuki M., Watanabe M.F., Watanabe M., Terao K., Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method, Toxicon 32 (1994) 73–84.

[48] Harding W.R., Rowe N., Wessels J.C., Beattie K.A., Codd G.A., Death of dog attributed to the cyanobacterial (blue-green algal) hepatotoxin nodularin in South Africa, J. S. Afr. Vet. Assoc. 66 (1995) 256–259.

[49] Hawkins P.R., Runnegar M.T.C., Jackson A.R.B., Falconer I., Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic supply reservoir, Appl. Environ. Microbiol. 50 (1985) 1292–1295.

[50] Hawkins P.R., Chandrasena N.R., Jones G.J., Humpage A.R., Falconer I., Isolation of *Cylindrospermopsis raciborskii* from an ornamental lake, Toxicon 35 (1997) 341–346.

[51] Hermansky S.J., Stohs S.J., Markin R.S., Murray W.J., Hepatic lipid peroxidation, sulfhydryl status, and toxicity of the blue-green algal toxin microcystin-LR in mice, J. Toxicol. Environ. Health 31 (1990) 71–91.

[52] Honkanan R.E., Codispoti B.A., Tse K.T., Boynton A., Characterization of natural toxins with inhibitory activity against serine/threonine protein phosphatases, Toxicon 32 (1994) 339–350.

[53] Humbert J.F., Le Berre B., Genetic diversity among two freshwater cyanobacteria species, *Planktothrix (Oscillatoria) rubescens* and *P. agardhii*, Arch. Hydrobiol. 2 (2001) 197–206.

[54] Humpage A.R., Rositano J., Bretag A.H., Brown R., Baker P.D., Nicholson B.C., Steffensen D.A., Paralytic shellfish poisons from Australian cyanobacterial blooms, Aust. J. Mar. Freshw. Res. 45 (1994) 761–771.

[55] Humpage A.R., Hardy S.J., Moore E.J., Froscio S.M., Falconer I.R., Microcystins (cyanobacterial toxins) in drinking water enhance the growth of aberrant crypt foci in the mouse colon, J. Toxicol. Environ. Health A 61 (2000) 155–165.

[56] Humpage A.R., Fenech M., Thomas P., Falconer I.R., Micronucleus induction and chromosome loss in transformed human white cells indicate clastogenic and aneugenic action of the cyanobacterial toxin, cylindrospermopsin, Mut. Res. 472 (2000) 155–161.

[57] Ikawa M., Wegener K., Foxall T.L., Sasner J.J., Comparison of the toxins of the blue-green alga *Aphanizomenon flos-aquae* with the *Gonyaulax* toxins, Toxicon 20 (1982) 747–752.

[58] James K.J., Sherlock I.R., Stack M.A., Anatoxin-a in Irish freshwater and cyanobacteria, determined using a new fluorimetric liquid chromatographic method, Toxicon 32 (1997) 963–971.

[59] Jochimsen E.M., Carmichael W.W., An J., Cardo D.M., Cookson S.T., Holmes C.E.M., Antunes M.B.d.C., Filho D.A.d.M., Lyra T.M., Barreto V.S.T., Azevedo S.M.F.O., Jarvis W.R., Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil, New Engl. J. Med. 338 (1998) 873–878.

[60] Kaas H., Henriksen P., Saxitoxins (PSP toxins) in Danish lakes, Water Res. 34 (2000) 2089–2097.

[61] Keleti G., Sykora J.L., Production and properties of cyanobacterial endotoxins, Appl. Environ. Microbiol. 43 (1982) 104–109.

[62] Kerr L.A., McCoy C.P., Eaves D., Blue-green algae toxicosis in five dairy cows, J. Am. Vet. Med. Assoc. 191 (1987) 829–830.

[63] Klemer A.R., Feuillade J., Feuillade M., Cyanobacterial blooms: carbon and nitrogen have opposite effects on the buoyancy of *Oscillatoria*, Science 215 (1982) 1629–1631.

[64] Konst H., McKercher P.D., Gorham P.R., Robertson A., Howell J., Symptoms and pathology produced by toxic *Microcystis aeruginosa* NRC-1 in laboratory and domestic animals, Can. J. Comp. Med. Vet. Sci. 29 (1965) 221–228.

[65] Kuiper-Goodman T., Falconer I., Fitzgerald J., Human health aspects, in: Chorus I., Bartram J. (Eds.), Toxic cyanobacteria in water, E & FN Spon, London and New-York, 1999, pp. 41–111.

[66] Lagos N., Onodera H., Zagatto P.A., Andrinolo D., Azevedo S.M.F.Q., Oshima Y., The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil, Toxicon 37 (1999) 1359–1373.

[67] Li R., Carmichael W.W., Brittain S., Eaglesham G.K., Shaw G.R., Liu Y., Watanabe M.M., First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (cyanobacteria), J. Phycol. 37 (2001) 1121–1126.

[68] Lindholm T., Eriksson J.E., Meriluoto J.A.O., Toxic cyanobacteria and water quality problems – Examples from a eutrophic lake on Aland, South West Finland, Water Res. 23 (1989) 481–486.

[69] Lopez Rodas V., Costas E., Preference of mice to consume *Microcystis aeruginosa* (Toxin-producing cyanobacteria): A possible explanation for numerous fatalities of livestock and wildlife, Res. Vet. Sci. 67 (1999) 107–110.

[70] Mahmood N.A., Carmichael W.W., Paralytic shelfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* NH-5, Toxicon 24 (1986) 175–186.

[71] Mahmood N.A., Carmichael W.W., Pfahler D., Anticholinesterase poisonings in dogs from a cyanobacterial (blue-green algae) bloom dominated by *Anabaena flos-aquae*, Am. J. Vet. Res. 49 (1988) 500–503.

[72] Main D.C., Berry P.H., Peet R.L., Robertson J.P., Sheep mortalities associated with the blue-green alga *Nodularia spumigena*, Aust. Vet. J. 53 (1977) 578–581.

[73] Matsunaga H., Harada K.I., Senma M., Ito Y., Yasuda N., Ushida S., Kimura Y., Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan: Sudden appearance of toxic cyanobacteria, Nat. Toxins 7 (1999) 81–84.

[74] Matsunaga S., Moore R.E., Niemczura W.P., Carmichael W.W., Anatoxin-a(s), a potent anticholinesterase from *Anabaena flos-aquae*, J. Am. Chem. Soc. 111 (1989) 8021–8023.

[75] McBarron E.J., May V., Poisoning of sheep in New South Wales by the blue-green alga *Anacystis cyanea* (Kuetz.) Dr. and Dail, Aust. Vet. J. 42 (1966) 449–453.

[76] McBarron E.J., Walker R.I., Gardner I., Walker K.H., Toxicity to livestock of the blue-green algae *Anabaena circinalis,* Aust. Vet. J. 51 (1975) 587–588.

[77] MacKintosh C., Beattie K.A., Klumpp S., Cohen P., Codd G.A., Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants, FEBS Lett. 264 (1990) 187–192.

[78] Mez K., Beattie K.A., Codd G.A., Hanselmann K., Hauser B., Naegeli H., Preisig H.R., Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland, Eur. J. Phycol. 32 (1997) 111–117.

[79] Mulhearn C.J., Beware algae! They can poison livestock, J. Agricult. 62 (1959) 406–408.

[80] Mur L., Skulberg O., Utkilen H., Cyanobacteria in the environment, in: Chorus I., Bartram J. (Eds.), Toxic cyanobacteria in water, E & FN Spon, London and New-York, 1999, pp. 15–40.

[81] Naegeli H., Sahin A., Braun U., Hauser B., Mez K., Hanselmann K., Preisig H.R., Bivetti A., Eitel J., Sudden death of alpine cattle in the canton Graubunden, Schweiz. Arch. Tierheilkd. 139 (1997) 201–209.

[82] Negri A.P., Jones G.J., Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*, Toxicon 33 (1995) 667–678.

[83] Negri A.P., Jones G.J., Hindmarsh M., Sheep mortality associated with paralytic shellfish poisons from the cyanobacterium *Anabaena circinalis*, Toxicon 33 (1995) 1321–1329.

[84] Nehring S., Mortality of dogs associated with a mass development of *Nodularia spumigena* (Cyanophyceae) in a brackish lake at the German North Sea coast, J. Plankton Res. 15 (1993) 867–872.

[85] Nishiwaki-Matsushima R., Ohta T., Nishiwaki S., Suganuma M., Kohyama K., Ishikawa T., Carmichael W.W., Fujiki H., Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR, J. Cancer Res. Clin. Oncol. 118 (1992) 420–424.

[86] Norris R.L., Eaglesham G.K., Pierens G., Shaw G.R., Smith M.J., Chiswell R.K., Seawright A.A., Moore M.R., Deoxycylindrospermopsin, an analog of cylindrospermosin from *Cylindrospermopsis raciborskii*, Environ. Toxicol. 14 (1999) 163–165.

[87] Odriozola E., Ballabene N., Salamanco A., Poisoning in cattle by blue-green algae, Rev. Argent. Microbiol. 16 (1984) 219–224.

[88] Ohta T., Sueoka E., Iida N., Komori A., Suganuma M., Nishiwaki R., Tatematsu M., Kim S.J., Carmichael W.W., Fujiki H., Nodularin, a potent inhibitor of protein phosphatases 1 and 2A, is a new environmental carcinogen in male F344 rat liver, Cancer Res. 54 (1994) 6402–6406.

[89] Ohtani I., Moore R.E., Runnegar M.T.C., Cylindrospermopsin: A potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*, J. Am. Chem. Soc. 114 (1992) 7941–7942.

[90] Oliver R.L., Ganf G.G., Freshwater blooms, in: Whitton B.A., Potts M. (Eds.), The ecology of Cyanobacteria, Kluwer Academic Publishers, Dordrecht, 2000, pp. 149–194.

[91] Onodera H., Satake M., Oshima Y., Yasumoto T., Carmichael W.W., New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*, Nat. Toxins 5 (1997) 146–151.

[92] Osborne N.J., Webb P.M., Shaw G.R., The toxins of *Lyngbya majuscula* and their human and ecological health effects, Environ. Int. 27 (2001) 381–392.

[93] Oshima Y., Postcolumn derivatization liquid chromatography method for paralytic shellfish toxins, J. AOAC Int. 78 (1995) 528–532.

[94] Otsuka S., Suda S., Shibata S., Oyaizu H., Matsumoto S., Watanabe M.M., A proposal for the unification of five species of the cyanobacterial genus *Microcystis* Kutzing ex Lemmermann 1907 under the rules of the Bacteriological Code, Int. J. Syst. Evol. Microbiol. 51 (2001) 873–879.

[95] Pereira P., Onodera H., Andrinolo D., Franca S., Araujo F., Lagos N., Oshima Y., Paralytic shellfish toxins in the freshwater cyanobacterium *Aphanizomenon flos-aquae*, isolated from Montargil reservoir, Portugal, Toxicon 38 (2000) 1689–1702.

[96] Pomati F., Sacchi S., Rossetti C., Giovannardi S., Onodera H., Oshima Y., Neilan B.A., The freshwater cyanobacterium *Planktothrix sp.* FP1: molecular identification and detection of paralytic shellfish poisoning toxins, J. Phycol. 36 (2000) 553–562.

[97] Puschner B., Galey F.D., Johnson B., Dickie C.W., Vondy M., Francis T., Holstege D.M., Blue-green algae toxicosis in cattle, J. Am. Vet. Med. Assoc. 213 (1998) 1605–1607.

[98] Pybus M.J., Hobson D.P., Mass mortality of bats due to probable blue-green algal toxicity, J. Wildl. Dis. 22 (1986) 449–450.

[99] Rinehart K.L., Namikoshi M., Choi B.W., Structure and biosynthesis of toxins from blue-green algae (cyanobacteria), J. Appl. Phycol. 6 (1994) 159–176.

[100] Runnegar M., Berndt N., Kaplowitz N., Microcystin uptake and inhibition of protein phosphatases: effects of chemoprotectants and self-inhibition in relation to known hepatic transporters, Toxicol. Appl. Pharmacol. 134 (1995) 264–272.

[101] Runnegar M.T., Jackson A.R.B., Falconer I.R., Toxicity to mice and sheep of a bloom of the cyanobacterium (blue-green alga) *Anabaena circinalis*, Toxicon 26 (1988) 599–602.

[102] Runnegar M.T., Gerdes R.G., Falconer I.R., The uptake of the cyanobacterial hepatotoxin microcystin by isolated rat hepatocytes, Toxicon 29 (1991) 43–51.

[103] Saker M.L., Eaglesham G.F., The accumulation of cylindrospermopsin from the cyanobacterium *Cylindrospermopsis raciborskii* in tissues of the redclaw crayfish *Cherax quadricarinatus*, Toxicon 37 (1999) 1065–1077.

[104] Schmidt W., Drews G., Weckesser J., Fromme I., Borowiak D., Characterization of the Lipopolysaccharides from Eight Strains of the Cyanobacterium *Synechococcus*, Arch. Microbiol. 127 (1980) 222.

[105] Schopf J.W., Microfossils of the early Archean apex chert: New evidence of the antiquity of life, Science 260 (1993) 640–646.

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

[107] Senior V.E., Algal poisoning in Saskatchewan, Can. J. Comp. Med. 14 (1960) 26.

[108] Shaw G.R., Sukenik A., Livne A., Chiswell R.K., Smith M.J., Seawright A.A., Norris R.L., Eaglesham G.K., Moore M.R., Blooms of the cylindrospermopsin containing cyanobacterium, *Aphanizomenon ovalisporum* (Forti), in newly constructed lakes, Queensland, Australia, Environ. Toxicol. 14 (1999) 167–177.

[109] Shaw G.R., Seawright A.A., Moore M.R., Lam P.K.S., Cylindrospermopsin, a cyanobacterial alkaloid : evaluation of its toxicologic activity, Ther. Drug Monit. 22 (2000) 89–92.

[110] Short S.B., Edwards W.C., Blue-green algae toxicosis in Oklahoma, Vet. Hum. Toxicol. 32 (1990) 558–560.

[111] Sivonen K., Jones G., Cyanobacterial toxins, in: Chorus I., Bartram J. (Eds.), Toxic cyanobacteria in water, E & FN Spon, London and New York, 1999, pp. 41–111.

[112] Soll M.D., Williams M.C., Mortality of white rhinoceros (*Ceratotherium simum*) suspected to be associated with the blue-green alga *Microcystis aeruginosa*, J.S. Afr. Vet. Assoc. 56 (1985) 49–51.

[113] Stirling D.J., Quilliam M.A., First report of the cyanobacterial toxin cylindrospermopsin in New Zealand, Toxicon 39 (2001) 1219–1222.

[114] Suttle C.A., Cyanophages and their role in the ecology of cyanobacteria, in: Whitton B.A., Potts M. (Eds.), The ecology of cyanobacteria, Kluwer Academic Publishers, Dordrecht, 2000, pp. 563–589.

[115] Terao K., Ohmori S., Igarashi K., Othani I., Watanabe M.F., Harada K.I., Ito E., Watanabe M., Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*, Toxicon 32 (1994) 833–843.

[116] Thomas A.D., Saker M.L., Norton J.H., Olsen R.D., Cyanobacterium *Cylindrospermopsis raciborskii* as a probable cause of death in cattle in northern Queensland, Aust. Vet. J. 76 (1998) 592–594.

[117] Toivola D.M., Goldman R.D., Garrod D.R., Eriksson J.E., Protein phosphatases maintain the organization and structural interactions of hepatic keratin intermediate filaments, J. Cell Sci. 110 (1997) 23–33.

[118] Ueno Y., Nagata S., Tsutsumi T., Hasegawa A., Watanabe M.F., Park H.-D., Chen G.-C., Chen G., Yu S.-Z., Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay, Carcinogenesis 17 (1996) 1317–1321.

[119] Van Dolah F.M., Marine algal toxins: origins, health effects, and their increased occurrence, Environ. Health Perspect. 108 (2000) 133–141.

[120] Van Halderen A., Harding W.R., Wessels J.C., Schneider D.J., Heine E.W., Van der Merwe J., Fourie J.M., Cyanobacterial (blue-green algae) poisoning of livestock in the Western Cape Province of South Africa, J. S. Afr. Vet. Assoc. 66 (1995) 260–264.

[121] Vincent W.F., Cyanobacterial dominance in the polar regions, in: Whitton B.A., Potts M. (Eds.), The ecology of cyanobacteria, their diversity in time and space, Kluwer Academic Publishers, Dordrecht, 2000, pp. 321–340.

[122] Walsby A.E., Dubinsky Z., Kromkamp J.C., Lehmann C., Schanz F., The effects of diel changes in photosynthetic coefficients and depth of *Planktothrix rubescens* on the daily integral of photosynthesis in Lake Zurich, Aquat. Sci. 63 (2001) 326–349.

[123] Whitton B.A., Potts M., Introduction to cyanobacteria, in: Whitton B.A., Potts M. (Eds.), The ecology of cyanobacteria, Kluwer Academic Publishers, Dordrecht, 2000, pp. 1–11.

[124] WHO, Guidelines for Drinking-water Quality, second edition, *Addendum* to volume 2, Health Criteria and Other Supporting Information, World Health Organization, Geneva, 1998.

[125] Wiegand C., Pflugmacher S., Oberemm A., Meems N., Beattie K.A., Steinberg C.E.W., Codd G.A., Uptake and effects of microcystin-LR on detoxication enzymes of early life stages of the zebrafish (*Danio rerio*), Environ. Toxicol. 14 (1999) 89–95.

[126] Willen T., Mattsson R., Water-blooming and toxin-producing cyanobacteria in Swedish fresh and brackish waters, 1981–1995, Hydrobiologia 353 (1997) 181–192.

[127] Wright J.L.C., Cembella A.D., Ecophysiology and biosynthesis of polyether marine biotoxins, in: D.M. Anderson, A.D. Cembella, G.M. Hallegraeff, Physiological ecology of harmful algal blooms, Heidelberg, NATO-Advanced Study Institute Series, Springer-Verlag, 41 (1998) 427–452.

[128] Wynn-Williams D.D., Cyanobacteria in deserts – life at the limit?, in: Whitton B.A., Potts M. (Eds.), The Ecology of cyanobacteria, their diversity in time and space, Kluwer Academic Publishers, Dordrecht, 2000, pp. 341–366.

[129] Yasumoto T., Fish poisoning due to toxins of microalgal origins in the Pacific, Toxicon 39 (1998) 1515–1518.

[130] Yin Q., Carmichael W.W., Evans W.R., Factors influencing growth and toxin production by cultures of the freshwater cyanobacterium *Lyngbya wollei* Farlow ex Gomont, J. Appl. Phycol. 9 (1997) 55–63.