Summary

This paper is based on an unreviewed draft publication prepared by Dr. Ingrid Chorus for the World Health Organization (WHO) Expert Consultation on Health Impacts of Recreational Water and Bathing Beach Quality, held in Bad Elster, Germany, 20-22 June 1996. The WHO guideline document on this subject will be forthcoming in late 1997.

This publication presents an overview of the health impacts of freshwater algae based on an extensive review of the available literature beginning with the first documented case of lethal intoxications published in the last century. Increased levels of eutrophication combined with recently developed practical methods for detection and surveillance have resulted in a greater awareness of algal toxins in the environment. Notwithstanding, investigations on algal toxins in recreational waters and drinking water supplies have been quite limited until recently, and are currently gaining impetus in a number of countries.

The cyanobacteria or “blue-green algae” are defined and their ability to form scums or mats described. Three neurotoxins, one general cytotoxin and a group of toxins termed microcystins (hepatotoxic) are described along with their mode of acute or chronic toxic action on humans. LD₅₀ acute toxicity levels are presented as well as case studies and reports on acute intoxications with cyanobacteria. Guidelines values are suggested along with their derivation.

In a highly eutrophic water body, lethal intoxications of humans are unlikely, even if transparency amounts to only half a meter, unless scum material is consumed. Whereas adults are unlikely to ingest this unappetizing material, it may pose serious hazard to children who are prone to ingest larger volumes of water, especially when romping around in shallow waters amidst the accumulated scums. Lethal doses are estimated for children.

Key words CYANOBACTERIA, EUTROPHICATION, RECREATION, TOXINS, ALGAE

1. Introduction: why are health impacts of freshwater algae a fairly recent issue of concern?

The first documented case of lethal intoxications of livestock, waterfowl and wildlife by algae was published in the last century (box), and numerous cases have been recorded since. In lesser number, cases of injury of humans after exposure to freshwater algae through drinking and bathing waters have also been documented. During the past decades, the chemical structures of a number of toxins have been identified. Among the marine algae, dinoflagellates and Chrysochromulina spp. are the most important toxin producers, whereas in the freshwater environment, toxins have almost exclusively been identified from Cyanobacteria, which formerly were termed “Cyanophyta”, or “blue-green algae” (see 2.1). Toxin production by other groups of freshwater phytoplankton has scarcely been investigated, but has been: "Francis (1878) described “a thick scum like green oil paint, some two to six inches thick ... unwholesome for cattle and other animals to drink at the surface, bringing on a rapid and sometimes terrible death” of cattle, sheep, pigs and dogs that had consumed the scum of a Nodularia spumigena-bloom."
shown for freshwater species of *Dynophyceae* and *Chrysochromulina* (see 2.2), and it is indeed reasonable to expect toxic species among these groups in freshwaters as well as in the marine environment. However, though species of these groups may also proliferate quite intensively in eutrophic ("fertilized") waters, they do not form as dense mass developments or surface scums as do Cyanobacteria, and therefore present a lesser risk in recreational waters.

Algal toxins are natural substances, but human activity has lead to excessive fertilization ("eutrophication") of many water bodies, especially during the past three decades. This in turn causes unnatural proliferation of algae, in freshwaters especially of blue-green algae, and thus considerably changes recreational water quality. Many regions (especially in lowland areas with slowly flowing or impounded rivers and low discharge) have always had some naturally eutrophic water bodies together with a number of clear ones, but presently report more or less total coverage with eutrophicated waters dominated by blue-green algae for several summer months, usually during the chief bathing season, and can no longer provide clear waters for swimming and bathing. Whereas common knowledge used to be not to swim "where the water is blooming", people have now (due to the present lack of alternatives in many regions) gotten accustomed to swimming in green waters with a transparency of 0.5 meters or less. Therefore, recreational hazards due to algae have increased substantially in many parts of the world.

Further, algal toxins are perceived as an issue of concern only recently because our knowledge of their occurrence and distribution has been strongly limited due to the lack of adequate methods for detection and surveillance: from the 1960s to the end of the 1980s, detection was primarily done with the mouse bioassay. Due to the rather high expenditures necessary (as well as ethical limitations of applicability), this is not suitable for large screening or monitoring programs. However, effective methods of chemical analysis are now available for the known toxins, and immuno-assays as well as enzyme assays have become commercially available for the most important ones (e.g. microcystins and saxitoxin, see below). This opens new possibilities for screening programs targeted at assessment of the potential risk, as well as for regular surveillance.

Ongoing screening programs in several countries are likely to show that algal toxins present a greater health hazard than a number of industrial and anthropogenic chemicals currently in the focus of public attention and addressed by legislation.

"One can only speculate as to why the United States has remained reactive rather than proactive on the issue of cyanobacterial toxins. ... it is unlikely that natural toxins would ever receive the same attention, either by the public or the media, as do man-made contaminants or pollutants. As described in the literature on risk communication there are a number of "outrage factors" that contribute to the public's perceptions about a particular risk. These include whether the risk is voluntary versus involuntary, natural versus industrial, or familiar versus exotic ... . Cyanobacterial toxins are generally less newsworthy than industrial chemicals because they are natural and not as morally relevant or dreaded." (Yoo, Carmichael, Hoehn & Hrudey 1995, p. 8-9).

2. **Risk assessment: what do we know about hazards caused by freshwater algae?**

Freshwater algal toxins have been in the focus of research in a small number of profiled working groups spread over a few countries, especially in Australia, Scandinavia, Japan, United Kingdom, South Africa and the USA. Internationally, these substances were first discussed as a potential health hazard at the symposium "Algae, Man, and the Environment" (Jackson, ed. 1968). In 1981, the First International Conference was dedicated to algal toxins (Carmichael et al, 1981) and in 1993, a meeting focused on detection methods (Codd et al. 1994). The first comprehensive international congress on toxic cyanobacteria took place only in August 1995 (published as a volume of *Phycologia* in 1997). Again, reflecting the state of awareness, this conference focused on one group of toxins, the microcystins, currently considered to be the most hazardous algal metabolites (see below). However, a number of contributions indicated that further, as of yet unidentified cyanobacterial metabolites should not be dismissed as potential hazards. While it is generally agreed that the most important algal toxins are
known in their chemical structure and mode of action, further research on cyanotoxins as well as on toxins and allergens produced by other groups of algae is needed for comprehensive risk assessment.

2. 1 Cyanobacteria and Cyanobacterial toxins (cyanotoxins)

What are cyanobacteria: Cyanobacteria are single-celled, very widespread organisms which grow planktonically (i.e. dispersed in water), on surfaces in water bodies, as well as on moist terrestrial surfaces. Whilst the single cells are very small (a few µm in diameter), many species form filaments or colonies. Some colonies become large enough to be seen with the naked eye. Benthic species (i.e. those which live on the sediment surface) may form dense mats.

Because of their pigment content and capability of photosynthesis, they were first classified as Cyanophyta or "blue-green algae", but later insight into the ultrastructure of their cells showed that they resemble bacteria much more closely than they resemble other algae. Therefore, they are now either termed Cyanobacteria, or classified as a separate group (Cyanoprokaryotes).

However, the ecological function especially of planktonic cyanobacteria is similar to that of algae. Therefore, they may be included in the term "phytoplankton" and are frequently loosely termed "algae". Depending upon their pigment content, cyanobacteria may appear green or bluish green, decaying populations may even occasionally turn bright blue or turquoise.

In contrast to other algae, many species of planktonic cyanobacteria possess gas vesicles. These enable them to regulate their buoyancy and thus to actively seek water depths with optimal growth conditions. However, regulation of buoyancy by changing the amount of gas in the vesicles is slow. Cells adapted to turbulent mixing by enlarged gas vesicles will take a few days to reduce their buoyancy in order to adapt to more quiescent conditions. Thus, especially when the weather changes from stormy to fair (i.e. mixing conditions in the water from turbulent to strongly stratified), many excessively buoyant cells or colonies may accumulate at the surface. Light winds may then drive them to leeward shores and bays, where they form so-called "blooms" (National Rivers Authority 1990). In extreme cases, such agglomerations may become very dense and even acquire a gelatinous consistency. More frequently, they are seen as streaks or slimy scums.

Whilst agglomerations of cyanobacteria are usually caused by planktonic species in eutrophic waters, benthic mats in oligotrophic waters occasionally also cause problems: these surface-covering mats can grow only in clear water, in which sunlight penetrates to the bottom. During sunny days, their photosynthesis may lead to high rates of oxygen production, causing bubbles which loosen parts of the mats and drive them to the surface. Mats of benthic cyanobacteria washed to the shore and scavenged by dogs have been lethal (Edwards et al. 1992), and cattle deaths on Swiss alpine meadows may also be caused by benthic cyanobacteria (Metz, et al 1995). Though relevant for pets and livestock, the human health impact of these cyanobacteria on beaches will be considerably lower than that of scums in the water.

What toxins do cyanobacteria contain? Progress in analytical chemistry during the past two decades has enabled the isolation and structural identification of three neurotoxins with somewhat different modes of blocking neuronal signal transmission (anatoxin-a, anatoxin-a(s), and aphantoxin or saxitoxin), one general cytotoxin which inhibits protein synthesis (cylindrospermopsin), and a group of toxins termed

<table>
<thead>
<tr>
<th>Steps in development of cyanotoxin-knowledge</th>
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<tbody>
<tr>
<td>1878: first publication on toxic effects of cyano-bacteria</td>
</tr>
<tr>
<td>up to the mid 1940s: reports of intoxications of livestock and wildlife after ingesting cyano-bacteria</td>
</tr>
<tr>
<td>1948-1950: causal connection between intoxication and cyanobacteria established by feeding experiments (Carmichael 1994)</td>
</tr>
<tr>
<td>1950-1970: numerous toxicity tests (mouse assay, i.p.) with cyanobacterial bloom material and cultures, lists of potentially toxic cyanobacteria, description of effects, differentiated by hepato- and neurotoxins</td>
</tr>
<tr>
<td>1972: first cyanotoxin identified (Anatoxin-a)</td>
</tr>
<tr>
<td>1994: Adequate analytical methods for routine microcystin-assays available (Lawton et al. for HPLC, An &amp; Carmichael for ELISA and enzyme-assay)</td>
</tr>
</tbody>
</table>
microcystins which inhibit protein phosphatases. This mechanism would also be generally cytotoxic, but microcystins are primarily hepatotoxic because they use the bile acid carrier to enter the cells. These toxins were named after the organism from which they were first isolated, but most of them have meanwhile been found in a wider array of genera. Aphantoxin has proven to be structurally analogous to saxitoxin known from marine dinoflagellates. Table 1 presents an overview of the currently known cyanotoxins and their mode of acute action.

Tab. 1: Cyanobacterial toxins and their acute toxicity

<table>
<thead>
<tr>
<th>toxin (effect and class of substances) or species of toxic Cyanobacteria</th>
<th>LD$_{50}$ (i.p.) of pure toxin</th>
<th>LD$_{50}$ (i.p.) of crude extracts (examples; toxin content varies)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>protein-phosphatase-blockers (cyclic peptides with ADDA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcystins in general (ca. 50 known structural variants)</td>
<td>45-&gt;1000 µg/kg</td>
<td>Rinehart et al. 1994</td>
<td></td>
</tr>
<tr>
<td>Microcystin-LR</td>
<td>50 µg/kg</td>
<td>Nat. Rivers Auth. 1990</td>
<td></td>
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<tr>
<td>Microcystin-YR</td>
<td>70 µg/kg</td>
<td>Watanabe et al. 1988</td>
<td></td>
</tr>
<tr>
<td>Microcystin-RR</td>
<td>300-600 µg/kg</td>
<td>Nat. Rivers Auth. 1990</td>
<td></td>
</tr>
<tr>
<td>Nodularin (from Nodularia spumigena)</td>
<td>30-50 µg/kg</td>
<td>Nat. Rivers Auth. 1990</td>
<td></td>
</tr>
<tr>
<td>Microcystis aeruginosa</td>
<td>55 mg/kg</td>
<td>Carmichael 1994</td>
<td></td>
</tr>
<tr>
<td>neurotoxins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a (blocks post-synaptic depolarization; alkaloid)</td>
<td>200 µg/kg</td>
<td>Codd 1992</td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a(s) (blocks acetylcholinesterase; organophosphate)</td>
<td>20 µg/kg</td>
<td>Codd 1992</td>
<td></td>
</tr>
<tr>
<td>aphantoxin / saxitoxin (block sodium channels)</td>
<td>10 µg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anabaena flos aquae</td>
<td>10 mg/kg</td>
<td>Carmichael et al. 1985</td>
<td></td>
</tr>
<tr>
<td>Aphanizomenon flos aquae</td>
<td>10 mg/kg</td>
<td>Sasner 1981</td>
<td></td>
</tr>
<tr>
<td>proteinsynthesis-blocker (cytotoxin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cylindrospermopsis (alkaloid)</td>
<td>64 mg/kg</td>
<td>Falconer 1993</td>
<td></td>
</tr>
<tr>
<td>damage or irritation of skin and mucous membranes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cell-wall lipopolysaccharides:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anabaena flos aquae</td>
<td>125 mg/kg 500 µg/kg</td>
<td>Nat. Rivers Auth. 1990</td>
<td></td>
</tr>
<tr>
<td>Aphanizomenon fl. aquae</td>
<td></td>
<td>Nat. Rivers Auth. 1990</td>
<td></td>
</tr>
</tbody>
</table>

Though the toxins listed in Tab. 1 are assumed to be the substances most significant for human health, it is unlikely that all of the important cyanotoxins have been discovered. Yoo et al. (1995) point out that an increasing variety of individual toxins is continually being discovered. In Germany alone, two pharmacological working groups are conducting research for pharmacologically active substances from cyanobacteria (Falch et al. 1995, Mundt & Teuscher 1988). At the first international conference on toxic cyanobacteria (see 1), twelve lectures indicated the presence of further (largely unidentified) biochemically active compounds in crude extracts of cyanobacteria. Results of Fastner et al. (1995) show that primary rat hepatocytes react to microcystins in crude extracts of cyanobacteria in close correlation to their content of microcystins, but that this reaction is enhanced by some further, unknown factor. Thus, further cyanotoxins may well be found.
How hazardous are cyanotoxins for human health?

Inspite of somewhat different modes of action, all three neurotoxins may be lethal by causing suffocation, anatoxin-a through cramps, anatoxin-a(s) through paralysis. Artificial respiration may enable survival. Anatoxin-a(s) is the only known naturally occurring organophosphate and causes strong salivation (s stands for salivation), cramps, tremor, diarrhea, vomiting and an extremely rapid death within minutes. Anatoxin-a and anatoxin-a(s) are among the most neurotoxic substances known. However, evidence is accumulating that they are not as frequent as microcystin. This applies especially to anatoxin-a(s): to date it has only been found in a small number of Anabaena-blooms in North America (Carmichael, pers. comm.). Further, concentrations even of these highly toxic substances in scums will scarcely reach levels acutely neurotoxic to a human ingesting a mouthful. (In contrast, livestock will drink many liters, and pets - especially dogs - gather scum material in their fur and ingest it through grooming with their tongue.).

After ingestion of a sublethal dose of neurotoxins, recovery is complete, and no chronic effects have been observed to date. For these reasons, the neurotoxins are a hazard to be aware of when using waters infested with cyanobacteria for recreation, but they are considered less dangerous than microcystins or cylindrospermopsin.

Microcystins are the most frequent and most widespread cyanotoxins. They are cyclic heptapeptides containing a specific amino acid (ADDA) side chain which to date has only been found in microcystins and in nodularin (a cyclic pentapeptide toxin Nodularia spumigena; a predominantly marine species). About 50 structural variations of microcystin are known so far (Rinehart et al. 1994). They vary with respect to methyl groups and two amino acids within the ring. This has consequences for the tertiary structure of the molecule and results in pronounced differences in toxicity (Rinehart et al. 1994) as well as in hydrophobic/hydrophilic properties. Microcystins block the protein phosphatases 1 and 2a, which are important "molecular switches" in all eukaryotic cells, with an irreversible covalent bond (MacKintosh et al. 1990).

The chief pathway for microcystins into cells is the bile acid carrier, which is found in liver cells, but to a lesser extent also in intestinal epithelia (Falconer 1993). Thus, for vertebrates, a lethal dose of microcystin causes death by liver necrosis within hours up to days. Permeability of other cell membranes for microcystins is still controversial. Possibly, hydrophobic structural variants can penetrate into some types of cells even without the bile acid carrier (Codd 1995). Further, Fitzgeorge et al. (1994) published strong evidence for disruption of nasal tissues even by the hydrophilic variant microcystin-LR. Whilst toxicity by oral uptake is generally an order of magnitude lower than intraperitoneal injection, intranasal application in these experiments was equally toxic as i.p. injection, and membrane damage by microcystin enhanced toxicity of anatoxin-a.

**Chronic toxicity of microcystins:** Fitzgeorge et al. (1994) further showed that microcystin-toxicity is cumulative: a single oral dose of 31.4 mg/kg mouse showed no increase in liver weight, whereas the same dose applied daily over 7 days caused an increase of liver weight by 84 % and thus had the same effect as a single oral dose of 500 mg/kg. This may be explained by the irreversible covalent bond of microcystin to the protein phosphatases and subsequent substantial damage to cell structure (Falconer 1993): healing of the liver probably requires growth of new liver cells.

Equally important is that tumor promoting activity of microcystins is well documented, though microcystins alone are not carcinogenic. This has been shown in experiments applying microcystin and a carcinogen (dimethylbenzanthracene) separately and simultaneously (Falconer and Buckley 1989). It is further supported by epidemiological data from China (Yu 1995) which show that in regions with a groundwater supply for drinking water, the incidence of liver cancer is lower by orders of magnitude than in regions which use eutrophic ponds and ditches heavily infested with microcystin for drinking water (the only treatment performed being chlorination; Carmichael and Falconer, pers. comm.). Studies on the mechanism of toxicity show that microcystin interferes with mitosis, and this may contribute to explaining the tumor promoting activity (Kaya 1995).
Cylindrospermopsin is a newly identified alkaloid isolated from *Cylindrospermopsis raciborskii*. It is a general cytotoxin which blocks protein synthesis, the first clinical symptoms being kidney and liver failure, but also injury to the lungs, adrenals and intestine. It is especially dangerous because clinical symptoms may become manifest until several days after exposure (Falconer, pers. comm.). Thus, cause and effect will often be difficult to relate. Patients intoxicated with cylindrospermopsin via drinking water in an incident in Australia escaped death only through skilled and intensive hospital care (Falconer 1995). *Cylindrospermopsis raciborskii* is considered to be a tropical and sub-tropical species, but recently it has been reported to form blooms as far north as Vienna (Roschitz 1996) and substantial populations in north-eastern Germany (Wiedner, pers. comm.). Thus, it is unclear whether the toxin is limited to warmer climates.

2. 2 Dinoflagellates, chrysophytes, chlorophytes, and other algae

As mentioned above, toxin production in other groups of freshwater algae has scarcely been investigated. Oshima et al. (1989) isolated and identified three ichtyotoxins (polonicumtoxins A, B, and C) from a dinoflagelate, *Peridinium polonicum*, suspected to be responsible for fish-kills. Toxicity in the mouse bioassay was 1.5 - 2 mg/kg and thus several orders of magnitude lower than the toxicity of microcystin. The Ames test showed no mutagenicity, but the authors emphasize the need for studies on chronic toxicity to evaluate the potential health risk of these toxins.

Hansen et al. (1994) describe a case study of a fish kill in a small Danish lake during an enormous mass development of *Chrysochromulina parva* (614 000 cells/ml) with little other phytoplankton present. The authors consider the total lack of any other detrimental conditions as a strong indication for toxicity of this species, especially as marine species of the genus *Chrysochromulina* contain potent toxins.

Allergic reactions to algae and cyanobacteria are frequently reported on the level of "anecdotal evidence" from eutrophic bathing waters. Yoo et al. (1995) point out that "allergic reactions to cyanobacteria are relatively common" (p. 77). However, these are rarely investigated in scientific studies or published. Among the small number of publications available, Heise (1949 and 1951) described ocular and nasal irritations in swimmers exposed to Oscillatoriaceae. McElhenny et al. (1962) applied extracts from four different algal species (cyanobacteria and chlorophyceae) as intracutaneous skin tests to 20 non-allergic children, none of which responded, and to 120 children with respiratory allergies, 98 of which showed clear positive reactions to at least one of the test strains. Mittal et al. (1979) tested 4000 patients in India with respiratory allergies, 25 % of which showed positive reactions either to chlorophyceae or to cyanobacteria, or to both. Further, pronounced skin reactions in response to a bloom of *Uroglena* spp. were observed in a small number of bathers, especially under bathing suits where cells were accumulated and partially disrupted during swimming (Chorus 1993).

Thus, allergic reactions are not at all confined to cyanobacteria and the substances which provoke these reactions are likely to be others than the toxins described above. However, allergic reactions require elevated cell densities in bathing water, and in freshwaters, mass developments are most frequently due to cyanobacteria. Further, other groups of algae do not accumulate to surface scums and therefore their metabolites will not occur in comparably high concentrations.

Algae have further caused irritative coughs in personnel and patients of a physiotherapeutic unit supplied with coarsely filtered surface water for underwater massage treatment. In October 1986, the water body contained 4600 to 58 000 cells/ml of the desmid *Staurastrum gracile*. This species was not effectively eliminated by the filter, and has a strong cell walls armed with spines and hooks which may well cause irritations of mucous membranes (Naglitsch 1988). Whilst this incident may be more of a curiosity than a serious health threat, it does highlight the benefit of regular microscopic examination of bathing and therapeutic waters in order to recognize algae as a potential cause of health reactions.

2. 3 How often and in which types of recreational waters are risks to be expected?

Data from surveys in a number of countries show that toxicity is to be expected in far more than half of all samples containing cyanobacteria (Tab. 2.). Further, an increasing amount of data is showing that very
many species of cyanobacteria are potentially toxic; at least 30 species have currently been shown to contain toxins (Volterra 1993; Yoo et al. 1995). Microcystins are not only produced by the genus *Microcystis*, but also by several species of *Planktothrix*, *Anabaena*, and *Nostoc*. Anatoxin-a is produced by some species of *Anabaena*, but may also be found in *Aphanizomenon*, *Cylindrospermum*, and *Oscillatoria*. Some species contain neurotoxin and microcystin simultaneously. The most common bloom-forming genus, *Microcystis*, is almost always toxic (Carmichael 1995), but non-toxic strains do occur. Generally, toxicity is not a trait specific for certain species, rather, most species comprise toxic and non-toxic strains. Evidence is compiling for genetic differences between strains containing microcystin and strains without, within taxonomic categories otherwise identified as one-and-the-same species (Dittmann et al. 1995, Rouhainen et al. 1995). Experience with cultures also shows that toxicity is a fairly constant trait of a given species, only somewhat modified by environmental conditions.

Tab. 2: Surveys of frequency of cyanobacterial toxicity

<table>
<thead>
<tr>
<th>country</th>
<th>number of sites sampled</th>
<th>% toxic</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>England</td>
<td>78</td>
<td>70 %</td>
<td>NRA report 1990</td>
</tr>
<tr>
<td>Scandinavia</td>
<td>51</td>
<td>59 %</td>
<td>Codd et al. 1989</td>
</tr>
<tr>
<td>Finland</td>
<td>188</td>
<td>44 %</td>
<td>Sivonen et al. 1990</td>
</tr>
<tr>
<td>Baltic Sea</td>
<td>25</td>
<td>72 %</td>
<td>Sivonen et al. 1989</td>
</tr>
<tr>
<td>Wisconsin, USA</td>
<td>102</td>
<td>27 %</td>
<td>Repavich et al. 1990</td>
</tr>
<tr>
<td>Netherlands</td>
<td>10</td>
<td>90 %</td>
<td>Leeuwangh et al. 1983</td>
</tr>
<tr>
<td>Netherlands</td>
<td>29</td>
<td>79 %</td>
<td>RIZA 1994</td>
</tr>
<tr>
<td>Hungary</td>
<td>35</td>
<td>82 %</td>
<td>Törökné-Kozma &amp; Gábor 1988</td>
</tr>
<tr>
<td>Germany (GDR)</td>
<td>6</td>
<td>67 %</td>
<td>Henning &amp; Kohl 1981</td>
</tr>
<tr>
<td>Germany 1993</td>
<td>12</td>
<td>83 %</td>
<td>Fastner et al. 1995</td>
</tr>
<tr>
<td>Denmark</td>
<td>96</td>
<td>72 %</td>
<td>Henriksen 1995</td>
</tr>
</tbody>
</table>

However, as of yet it is unclear how often and in which types of waters toxic strains predominate and cause high toxin concentrations. A new study of more than 90 Danish water bodies showed that 40 % of the samples contained less than 100 µg/g dry weight of cells, and only 11 % of the samples contained more than 500 µg/g (Henriksen 1995). Generally, very few studies include the variability of toxin content in the course of the development of cyanobacterial populations (Benn-dorf and Henning 1989, Jungmann 1995, Kotak et al. 1995, Fastner et al. in prep.), although this knowledge would be important for risk assessment: due to cumulative toxicity of microcystins (see above), hazards are highest for persons exposed on several consecutive days.

For practical purposes, the present state of knowledge implies that health authorities should regard any mass development of cyanobacteria as a potential health hazard, an approach currently followed by a number of water resource and supply organizations in the United Kingdom and Australia. A number of case studies are documented quite well and show that humans have become quite ill through ingestion of toxic cyanobacteria from recreational or even drinking water (Tab. 3). "In addition, anecdotal reports are numerous, though few appear in the literature" (Yoo et al. 1995). Symptoms reported include "abdominal pain, nausea, vomiting, diarrhea, sore throat, dry cough, headache, blistering of the mouth, atypical pneumonia, and elevated liver enzymes in the serum (especially gamma-glutamyl transferase)" (Carmichael 1995, p. 9) as well as hay-fever symptoms, dizziness, fatigue, skin and eye irritations; these symptoms are likely to have diverse causes with several classes of toxins and genera of cyanobacteria involved (ibid, p. 7).

Further, contact irritation has been reported from a number of freshwater cyanobacterial genera (*Anabaena*, *Aphanizomenon*, *Nodularia*, *Oscillatoria*, *Gloeotrichia*), and a case of severe pneumonia is attributed to he inhalation of a *Microcystis*-toxin while canoeing (Yoo et al. 1995).

In summary, there is ample evidence to show that toxic cyanobacteria are a serious health hazard in recreational waters, whereas other freshwater algae may cause allergic or irritative reaction of lesser severity and frequency. However, quantification of this hazard is as of yet hampered by a lack of knowledge on chronic health impacts and on the frequency as well as the duration of highly toxic mass developments.
Table 3: Case studies and reports on acute intoxications with cyanobacteria

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Event</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1931</td>
<td>USA</td>
<td>Massive Microcystis-bloom in the Ohio and Potomac Rivers</td>
<td>5000 - 8000 persons fell ill due to drinking water affected by the bloom. Drinking water treatment was not sufficient to remove the toxins. (Tisdale)</td>
</tr>
<tr>
<td>1959</td>
<td>Saskatchewan</td>
<td>Inspite of a kill of livestock and warnings against recreational use, people did swim in a lake infested with cyanobacteria.</td>
<td>13 persons became ill (headaches, nausea, muscular pains, painful diarrhea). In the excreta of a patient - a medical doctor who had accidentally ingested 300 ml of water- numerous cells of Microcystis spp. and some trichomes of Anabaena circinalis could be clearly identified. (Dillenberg 1960).</td>
</tr>
<tr>
<td>1968</td>
<td>Australia</td>
<td>Numerous cases of gastrointestinal illness after exposure to mass developments of cyanobacteria</td>
<td>Compiled by Schwimmer &amp; Schwimmer.</td>
</tr>
<tr>
<td>1975</td>
<td>USA</td>
<td>Endotoxic shock of 23 dialysis-patients in Washington DC</td>
<td>attributed to a cyanobacterial bloom in a drinking-water reservoir (Hindman et al. 1975).</td>
</tr>
<tr>
<td>1979</td>
<td>Australia</td>
<td>Combating a bloom of Cylindrospermopsis raciborskii in a drinking water reservoir</td>
<td>On Palm Island with copper sulfate lead to liberation of toxins from the cells into the water and thus caused serious illness with hospitalization of 149 persons supplied from this reservoir (Falconer 1993 and 1994).</td>
</tr>
<tr>
<td>1981</td>
<td>Australia</td>
<td>In city of Armandale liver enzyme activities were elevated in the blood of the population supplied from surface water polluted by microcystis spp</td>
<td>Falconer et al, 1983).</td>
</tr>
<tr>
<td>1985</td>
<td>USA</td>
<td>Carmichael compiled case studies on nausea, vomiting, diarrhea, fever, eye-, ear-, and throat-infections after exposure to mass developments of cyanobacteria.</td>
<td></td>
</tr>
<tr>
<td>1989</td>
<td>England</td>
<td>10 of 20 soldiers became ill after swimming and canoe-training in water with a heavy bloom of Microcystis spp.; two of them needed hospitalization and intensive care</td>
<td>(National Rivers Authority 1990). Swimming skills and the amount of water ingested appear to have related to the degree of illness.</td>
</tr>
<tr>
<td>1993</td>
<td>China</td>
<td>The incidence of liver cancer relates clearly to water sources and is significantly higher for populations using cyanobacteria-infested surface waters</td>
<td>often without any treatment except chlorination) as compared to those using groundwater (Yu 1995).</td>
</tr>
<tr>
<td>1993</td>
<td>Australia</td>
<td>Falconer estimates that due toxic cyanobacterial blooms, more than 600 000 person days are lost for drinking water abstraction annually.</td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td>Sweden</td>
<td>Illegal use of untreated river water in a sugar factory led to an accidental cross-connection with the drinking water supply for an uncertain number of hours. The river water was densely populated by Planktothrix agardhii, and samples taken a few days before and a few days after the incident showed these cyanobacteria to contain microcystins. 121 of 304 inhabitants of the village (as well as some dogs and cats) became ill with vomiting, diarrhea, muscular cramps, nausea (Cronberg et al. 1997).</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>Caruaru, Brazil</td>
<td>55 hemodialysis patients died of liver disease, seizures, or acute hemorrhaging after receiving dialysis treatment using water collected at the city’s reservoir and transported by tanker truck where it was chlorinated. The water contained high levels of hepatotoxins (microcystin), released from the cyanobacteria cells that were lysed by chlorination. These toxins were not removed by the inadequately maintained in-house treatment facility at the hemodialysis center. (Ely 1996)</td>
<td></td>
</tr>
</tbody>
</table>
3. Risk management: surveillance, immediate measures, and risk minimization

Risk management must take the current uncertainties of risk assessment into account. Nonetheless, several countries have attempted to derive guideline values. The very heterogeneous approaches introduced below may serve as preliminary orientation. A question of debate is which consequences to choose at high toxin concentrations. Short-term measures encompass warning of the public, closing of bathing sites and canceling water sports activities such as competitions. Medium- to long-term measures are identification of the sources of nutrient (usually phosphate) pollution and significant reduction of nutrient input in order to effectively reduce cyanobacterial growth.

3.1 Guideline values and their derivation

The international discussion on guidelines for cyanotoxins is focusing on microcystins because the neurotoxins are neither considered to be as widespread nor as hazardous (due to their lack of chronic toxicity). Generally, cyanobacteria containing microcystins at levels more than 1 mg/g of cells should be considered as highly toxic (see box).

As compared to other toxins, cyanotoxins are quite potent, considering LD \( \text{LD}_{50} \) (i.p.) values of 10 to 50 µg/kg. However, their occurrence in the freshwater environment is usually rather dilute. Further, oral uptake is less toxic than intraperitoneal application by about an order of magnitude. The model calculation given in Tab. 4 may serve to illustrate the ranges of microcystin concentration to be expected in a eutrophic water body with a high population density of quite toxic cyanobacteria, and the acute dose to which this may lead. Thus, in a eutrophic water body of less than half a meter of transparency, lethal intoxications of humans are unlikely, unless scum material is consumed. Scum material may readily contain enrichments of cyanobacteria by a factor of one thousand or more, as compared to density in open waters. Whilst it is generally assumed that humans would be deterred from swallowing surface scums, accidental ingestion of hazardous amounts may occur in intensive water sports (swimming with submersion of the head, jumping from diving boards, sail-boarding, water skiing). Further, wearing wet suits may lead to intensive skin exposure to algae trapped and accumulated under the suit (National Rivers Authority 1990). The most serious hazard, however, is that to children because they tend to ingest higher volumes of water, especially when romping around in shallow waters amidst the accumulated scums.

Tab. 4: Estimated amount of water required for a lethal dose of cyanotoxins

| assumptions: |  
| --- | --- |
| \( \text{LD}_{50} \) (i.p.) | 10 µg/kg |
| \( \text{LD}_{50} \) (oral) | 100 µg/kg |
| toxin content of cyanobacteria | 1 mg/g dry weight ( = 1 µg/mg dry weight) |
| cyanobacterial density | 50 mg/L of fresh weight |
| = 10 mg/L of dry weight (corresponding to about 200 µg/L of chlorophyll-a) |
| It follows that 1 Liter contains 10 µg of toxin. |

Per kg of body weight a human would need to ingest 10 L of water for a lethal dose.

With scum enriching cyanobacterial density by a factor of 1000, 10 ml per kg would be sufficient for a lethal dose, and 200 ml could kill a child of 20 kg body weight.
A first move to establish a guideline for microcystins was made by Falconer (1994) on the basis of a six-week exposure study with pigs and other toxicological data. Falconer is suggesting 1 µg/L for microcystins (and nodularins) in drinking water, a concentration which may be reached by 5000 cells of *Microcystis* per ml of water. Canada and Great Britain are also discussing establishment of this guideline value for drinking water.

Current discussion for bathing water safety address the occurrence of cyanobacteria as such rather than toxin concentrations, for several reasons:

- representative quantitative sampling is difficult due to the potentially very heterogeneous distribution of cyanobacteria in time and space,
- toxin content of cyanobacterial cells may vary quite rapidly,
- it is as of yet unclear whether all important cyanotoxins have been identified, so monitoring of toxicity with a suitable biotest would have to supplement monitoring of toxin concentration with chemical analysis.

The Australian New South Wales Blue-Green Algae Task force recommended 15 000 cells/ml as "acceptable contact exposure to cyanobacteria". Ressom et al. (1994) question the feasibility of determining "safe" levels of cyanobacteria in bathing waters, but suggest 20 000 cells per ml as a threshold. This is a density that will show as a slight discoloration of the water and thus may be recognizable to the informed public.

The bathing water directive of the European Union (EC 1976) requires a minimum of 1 m transparency (secchi disc reading) for bathing waters. Of all of the parameters of the directive, this one is the least enforced. For example in Germany and the Netherlands, almost half of the freshwater bathing sites reported less than 1 m of transparency, in most cases due to algal turbidity. The original intention of this parameter was a purely physical one: persons drowning are easier to see and rescue in clear water. However, this parameter is at present the only one in the directive which reflects algal density: algae and cyanobacteria as such are not regulated by the directive. The German recommendation on cyanotoxins in bathing waters picks up this transparency criterion, which is measured in the context of routine bathing site monitoring according to the EC directive at intervals of 14 days, and suggests a hierarchical approach:

1. **Transparency**: if transparency (secchi disc readings) complies to the guide value of the directive (> 2 m), mass developments of cyanobacteria are unlikely. At lower readings, samples for further investigations should be taken (see step 2.).

2. **Nutrients**: if total phosphate concentrations are below 0.02 - 0.04 mg/L P, mass developments of cyanobacteria are unlikely, and high turbidities may have other causes. At higher concentrations of total phosphate, phytoplankton should be checked for mass developments of cyanobacteria (see steps 3 and 4).

3. **Mass developments of algae**: if concentrations of chlorophyll-a remain below 0.04 mg/L, hazardous densities of cyanobacteria are unlikely. At higher concentrations, microscopic investigations for dominance of cyanobacteria are required (see step 4).

4. **Dominance of potentially toxic cyanobacteria**: If microscopic investigations show dominance of cyanobacteria at algal densities, immediate measures are to be taken and long-term measures for restoration of bathing water quality should be planned (see chapters 3.3 and 4).

This approach aims at maximizing safety while reducing effort of investigation to situations likely to present a hazard.

Aside from acute intoxications, chronic liver damage may be a serious problem for persons repeatedly exposed to microcystins, i.e. during a holiday with regular water sports activities. No epidemiological studies have been published on this issue, but from toxicological evidence on the chronic effects of
microcystins, it cannot be excluded that regular chronic exposure may be more of a risk than acute intoxications through accidental ingestion of scum material.

In summary, because the scientific rationale for deriving guideline values at present is weakened by the lack of quantitative epidemiological and toxicological data on chronic exposure as well as on the temporal and spatial development of toxic blooms, the initiatives existing in several countries are based on a general precautionary approach: exposure to high cyanotoxin concentrations should be avoided and the public must be informed about this hazard.

Carmichael (1995) emphasizes the point that factors which govern the spatial and temporal distribution of cyanobacteria are fairly predictable in many water bodies, and can be learnt by regular observation. The need to deal with the hazard of toxic cyanobacteria can lead to better knowledge of patterns of the occurrence of mass developments and thus to a basis for an adequately differentiated approach to handling potential risks according to the specific situation of each water body.

3.2 Methods of monitoring the occurrence of cyanobacteria and of cyanotoxin-analysis

Yoo et al. (1995) point out that the increasing variety and number of individual cyanotoxins being discovered make "the goal of very specific and sensitive analytical methods that would detect all relevant toxins increasingly complex and ultimately unachievable" (p. 93). Thus, as outlined in chapter 3.1, monitoring the development of blooms rather than toxins is a more rational approach. Further, good familiarity with the limnology of the respective bathing water body will facilitate alertness and well-targeted action.

Sampling cyanobacteria must take into account that their horizontal and vertical distribution may be extremely heterogeneous and variable within short periods of time. To assess health risks, dense blooms and surface scums should be sought and sampled. This is difficult to do in the context of routine bathing site sampling. For this reason, it is recommended to include the concentration of total phosphorus into the monitoring program, as the analytical method is simple and the result provides a basis for estimating the potential population development of cyanobacteria (see 3.1). Cyanobacterial density itself may be assessed by counting cells or filaments in an inverted microscope (Cronberg 1982, Utermöhl 1958). Algal biomass may also be estimated with sufficient accuracy by determining the concentration of chlorophyll-a in the water. On the basis of chlorophyll-data, microscopical analysis can be limited to a brief investigation of dominant species, rather than conducting labor-intensive cell counts. General experience shows that at chlorophyll-a-concentrations of more than 40 µg/L and dominance of cyanobacteria, surface scums may form. Severe scums are observed at concentrations above 100 - 150 µg/L of chlorophyll-a (measured in the open water, outside of shoreline scums). Enrichments in scums by a factor of 1000 as compared to the open water have been observed (Oehms, pers. comm.). The larger the surface area of the water body, the higher is the potential concentration of cyanobacterial scums through wind action.

Sampling for toxins must take into account that especially microcystins are usually contained within the cells and released into the surrounding water only upon lysis. This may not apply to anatoxin-a (Bumke-Vogt et al. in print). Most analytical methods address intracellular content. Therefore, rapid filtration of samples before onset of lysis is necessary to prevent underestimation of toxin content. On-site enrichment with plankton nets has proven to be very effective for "harvesting" larger amounts for toxin analysis. In situations in which seston (particle) concentrations largely consist of cyanobacteria, the dry weight of concentrated bloom samples may easily be related to the water volume sampled by determining the seston concentration in the lake water.

In countries following the directive of the European Union for bathing waters (EC 1976), sampling is usually undertaken fortnightly. This will usually be sufficient when no cyanobacterial populations are detected. However, as soon as cyanobacterial population growth begins (often in mid-summer), sampling frequency should be condensed to weekly sampling.

For the identified cyanotoxins, analytical methods suitable for routine analysis have been established, some of them quite recently (Codd et al. 1994). A good review of methods, their sensitivity and fields of
application is given by Harada (1994). Health authorities will largely focus on microcystins; analysis for neurotoxins may be indicated in specific situations (e.g. with animal kills). Microcystins may be effectively and quite accurately analyzed within 1 - 2 days according to the HPLC-method of Lawton et al. (1994). A rapid preliminary assessment is possible with the immuno-assay (commercially available); reliability is greatly augmented by parallel conduction of an enzyme assay (An and Carmichael 1994). Beyond these methods which address known substances, the mouse bioassay will show an integrated response to known as well as unknown toxins in cyanobacteria. It is rapid and easy to perform for laboratories routinely doing mouse assays, but ethically controversial in many countries, and usually more expensive than analytical methods. A suborganismic biotest has been developed using primary rat hepatocytes in comparison to a cell line to test for hepatotoxicity and general cytotoxicity (Heinze, 1997).

3.3 Immediate measures

Perception of risks due to toxic cyanobacteria has lead to different reactions: In Great Britain, water sports events have been canceled because of cyanobacterial blooms (National Rivers Authority 1990), but experience is also being gathered with alternatives, such as close observation of scum formation and protecting bathers by "fencing" scums in with buoyant barriers or launching boats and swimmers only from piers rather than from shorelines (Codd, pers. comm.). In Australia, responsibility for reacting to warnings is largely left to the public: bathing sites are not closed, but care is taken to post warnings in a clearly understandable way (with pictograms to overcome language barriers in this multi-cultural society). In Australia, this policy can be based on an especially high degree of public awareness towards cyanotoxins, because a small number of human intoxication incidents as well as ongoing problems with intoxications of livestock have launched the topic into the public media. In Germany, a two-step reaction is being proposed:

- If microscopic investigations show dominance of cyanobacteria at algal densities of > 0.04 mg/L chlorophyll-a, warning notices should be published at the bathing site and in the local media (see box for an example). Further, investigation frequency must be intensified at least to weekly intervals.

- If microscopic investigations show dominance of cyanobacteria at algal densities > 0.150 mg/L chlorophyll, very dense scums may form. Several options may be considered:
  1. Close the bathing site to the public until the situation is no longer given.
  2. Investigate the bathing site daily for surface scum formation; close as long as scums appear.
  3. Investigate for microcystin-content of cyanobacteria twice a week. Close the bathing site if microcystin content exceeds 0.1 mg/L until this situation is no longer given.

Carmichael (1995) emphasizes differentiation between the degree of water contact in different types of water sports. This should also be conveyed to the public in warning notices.

In principle, information of the public on this risk is of critical importance. Awareness of a potential hazard is not only a prerequisite for avoiding it, but also for understanding symptoms potentially caused by

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Warning to all visitors of this beach

A massive bloom of blue-green algae is presently developing in Lake _______. You can recognize it by the bluish-green discoloration of the water, and at times also by scums on the surface.

These algae may be toxic.

Toxic effects will increase if water is swallowed on several consecutive days.

Therefore:
- Avoid swallowing water when swimming!
- Children are especially at risk!
- If symptoms appear after bathing (e.g. nausea, vomiting, diarrhea, skin or eye irritations, difficulties with breathing) see your physician and please notify your health authority!

Pets, especially dogs, are also at risk!
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exposure and identifying their cause. Just as people are aware of the toxicity of toadstools or laburnum, people should know that cyanobacterial scums must in no case be ingested.

4. Risk avoidance: how can we maintain or achieve low population densities of harmful algae?

The aim of measures to minimize risks due to toxic algae is not to close bathing sites, but rather the restoration of bathing water quality with transparencies of > 2 m (secchi disc reading). Where turbidity is due to algae, this can be achieved by keeping total phosphorus concentrations below 0.03 mg/L P (Chorus & Heinzmann 1995). This threshold may be difficult to reach in water bodies with multiple sources of nutrient pollution. However, nutrient sources are locally very variable. Therefore, identifying the chief sources (see figure below) and developing restoration strategies is strongly recommended and may in many cases prove to be more feasible than originally assumed. Particularly nutrient input from agricultural runoff may in many cases easily be reduced without cost and even at economic gain by reducing the application of fertilizers to the actual demand of the crop, and by planting shrubs along a strip of about 20 m along the shoreline, rather than ploughing and fertilizing to the very edge of the water. Health authorities can initiate substantial improvement in such situations.

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