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# Cyanobacterial toxins: Microcystin-LR in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

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## Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as International Standards for Drinking-Water. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO Guidelines for drinking-water quality (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared/updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants examined in drinking-water.

For each chemical contaminant or substance considered, a lead institution prepared a health criteria document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the requested health criteria documents.

Under the responsibility of the coordinators for a group of chemicals considered in the guidelines, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the experts meetings. A "final task force" meeting reviewed the health risk assessments and public and peer review comments and, where appropriate, decided upon guideline values. During preparation of the third edition of the GDWQ, it was decided to include a public review via the world wide web in the process of development of the health criteria documents.

During the preparation of health criteria documents and at experts meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the joint FAO/WHO Meetings on Pesticide Residues, and the joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO internet site and in the current edition of the GDWQ.

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## GENERAL DESCRIPTION

### *Identity*

The cyanobacteria, also known as blue-green algae, owe their name to the presence of photosynthetic pigments. Cyanobacteria are a major group of bacteria that occur throughout the world. Freshwater cyanobacteria may accumulate in surface water supplies as "blooms" and may concentrate on the surface as blue-green "scums."

Some species of cyanobacteria produce toxins, which are classified according to their mode of action into hepatotoxins (e.g. microcystins), neurotoxins (e.g. anatoxins), skin irritants, and other toxins. Both hepatotoxins and neurotoxins are produced by cyanobacteria commonly found in surface water and therefore are of relevance to water supplies (Carmichael, 1992; Fawell et al., 1993).

The hepatotoxins are produced by various species within the genera *Microcystis*, *Anabaena*, *Oscillatoria*, *Nodularia*, *Nostoc*, *Cylindrospermopsis*, and *Umezakia*, although not all strains do so (Fawell et al., 1993; AWWA, 1995). Most hepatotoxins (all cyclic heptapeptides) are microcystins. At least 50 congeners of microcystins are known (Carmichael, 1994), and several of these may be produced during a bloom. The chemical structure of microcystins includes two variable amino acids and an unusual aromatic amino acid, ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), containing a substituted phenyldecadienoic acid (Botes et al., 1985). Different microcystins have different lipophilicities and polarities, which could affect their toxicity. Microcystin-LR was the first microcystin chemically identified; to date, most work has been conducted using this microcystin. It has been associated with most of the incidents of toxicity involving microcystins in most countries (Fawell et al., 1993). Microcystin-LR is a cyclic heptapeptide with a molecular weight of about 1000 daltons.

Neurotoxins are not considered as widespread in water supplies, and they do not appear to pose the same degree of risk from chronic exposure as microcystins (Fawell et al., 1993; AWWA, 1995). The neurotoxins, such as anatoxin-a and -a(s), are highly toxic nerve poisons but have short biological half-lives. On acute exposure, the neurotoxins cause death within minutes to a few hours, depending on the species, the amount of toxin ingested, and the amount of food in the stomach (Carmichael, 1992). Dog poisonings in Scotland were reported to be due to the consumption of *Oscillatoria* containing anatoxin-a (Codd, 1992).

Toxic cyanobacteria also produce cytotoxic alkaloids, the most recently described being from the species *Cylindrospermopsis raciborskii*, which occurs in freshwater lakes, rivers, and drinking-water storage reservoirs in tropical areas. The molecular structure is a tricyclic guanidine linked to a hydroxymethyl uracil with a molecular weight of 415 daltons (Ohtani et al., 1992). These alkaloids have been implicated in a variety of health effects, ranging from gastroenteritis to kidney disease (Falconer, 1994).

### *Occurrence and growth of cyanobacteria*

The occurrence of a particular genus and species of cyanobacteria around the world is apparently influenced by regional differences in water chemistry and climatic conditions. For example, *Cylindrospermopsis* is produced in tropical waters but has not been found in temperate climates. Similarly, *Microcystis* and *Anabaena* blooms occur widely in the temperate regions of the world (AWWA, 1995). In general, 50–75% of bloom isolates can produce toxins, often with more than one toxin being present. Toxic and non-toxic blooms of the same species can be found together (Skulberg et al., 1993; AWWA, 1995; Codd & Bell, 1996). The overall toxicity of a bloom can be uncertain, because variations can occur in toxin concentration over a short time and spatially within a water body experiencing a bloom

(Hrudey et al., 1994). There is no simple method to distinguish the toxic from the non-toxic forms. The unpredictability of toxin production within any given bloom "renders them potentially dangerous and suspect at all times" (Ressom et al., 1994), and prevention of cyanobacterial blooms is therefore the key to the control of toxic blooms.

The growth of cyanobacteria and the formation of blooms are influenced by physical, chemical, and biological factors, which were recently reviewed by Pearson et al. (1990), Ressom et al. (1994), and AWWA (1995) and are discussed below. As a result of the interplay of these factors, there may be large yearly fluctuations in the levels of cyanobacteria and their toxins. There is also a seasonal variation in predominating species.

Cyanobacterial blooms persist in water supplies that contain adequate levels of essential inorganic nutrients such as nitrogen and phosphorus, water temperatures generally between 15 and 30°C, and pH levels between 6 and 9. Blooms usually occur in late summer or early fall and are most common in eutrophic or hypereutrophic bodies of water.

The amount of daylight needed to optimize growth depends on the species. In addition, some cyanobacteria, such as *Microcystis aeruginosa*, can regulate their buoyancy in response to available light. This characteristic allows cyanobacteria to migrate through thermal gradients and use nutrients confined to cooler deeper water below. Buoyancy is controlled mainly through the production of carbohydrates from photosynthesis. This control mechanism breaks down if there is too little carbon dioxide available. Although buoyancy cannot be adjusted during the night, the organisms will float to the surface because of their reduced carbohydrate content as a result of respiration at night.

Turbulence and high water flows are unfavourable to the growth of cyanobacteria, as they interfere with the organisms' ability to maintain a position in the water column. Heavy rain storms can increase runoff and nutrient levels in the water, which encourages the formation of blooms.

The formation of surface scum is enhanced by calm weather conditions. Initially, there may be high barometric pressure and light to moderate winds, accompanied by constant circulation in a water body in which large numbers of cyanobacteria are maintaining their position in the water column to take advantage of those conditions. If the wind stops and circulation also stops, the cyanobacteria may suddenly become "overbuoyant." If they cannot adjust their buoyancy fast enough or at all (at night), then the blooms will float to the surface and form surface scum. Thus, scums are often formed overnight. The scum may drift downwind and may settle at lee shores and quiet bays, where the cyanobacteria may release their toxins and eventually die (Ressom et al., 1994).

***Toxin production and persistence***

The two main factors that have been shown to affect toxin production are light and temperature. The optimum temperature for toxin production in cyanobacteria is between 20 and 25°C (Gorham, 1964; van der Westhuizen & Eloff, 1985; Watanabe & Oishi, 1985), which suggests that cyanobacteria are most toxic during periods with warm weather and in areas with warm climates. However, the optimum temperature may change from country to country. Light intensity more than light quality is an important factor in toxin production in *M. aeruginosa*. Both toxicity and the ratio of the toxin to protein production are enhanced by both red and green light compared with white light (Utkilen & Gjølme, 1992). The toxicity of cyanobacteria increases with an increase in light intensity below 40 microeinsteins/$m^2$ per second (van der Westhuizen & Eloff, 1985; Watanabe & Oishi, 1985; Utkilen & Gjølme, 1992) and therefore decreases with water depth (Utkilen & Gjølme, 1992). However, when mixing of water from different depths occurs, especially during periods of high winds, this may not be true.

Some laboratory studies have shown that pH, nitrogen, phosphorus, and carbon dioxide could also influence the growth of microcystins. The presence of six different microcystins in floating scums of *M. aeruginosa* in Hartbeespoort Dam, South Africa, was monitored for 2.5 years. The toxins were either not detectable or in low concentrations during the winter and reached maximum concentrations during the summer. The total concentrations of four of the toxins (5–415 µg/g dry scum) were directly correlated to solar radiation, surface water temperature, pH, and per cent oxygen saturation. No significant correlations were found between total toxin concentrations in scum samples and organic and inorganic nutrient concentrations in surface water (Wicks & Theil, 1990).

Kotak et al. (1995) studied the patterns of occurrence of microcystin-LR (measured as µg/g biomass of *M. aeruginosa* by high-performance liquid chromatography, or HPLC) in three hypereutrophic hard-water lakes in central Alberta, Canada, over three seasons. *Microcystis aeruginosa* was highly variable temporally (differences up to 3 orders of magnitude) within each lake over 1 year, between years in an individual lake, and between lakes in a year. Seasonal changes in microcystin-LR concentration were positively correlated to the abundance and biomass of the *M. aeruginosa,* total and total dissolved phosphorus concentration, pH, and chlorophyll. Surprisingly, there was a negative correlation between microcystin-LR concentration and nitrate concentration and no correlation with water temperature. Over a 24-hour period, the concentration of microcystin-LR in *M. aeruginosa* decreased more than 6-fold at night compared with concentrations during the day. Codd & Bell (1996) determined the effect of temperature and nutrient supply on microcystin levels in cultures of *M. aeruginosa* in water bodies in the United Kingdom. The amount of microcystin per unit cyanobacterial dry weight was higher when nitrate levels were in excess and highest between 20 and 25°C, but was reduced above 25°C and below 20°C.

As toxin production varies greatly among different strains of the same species, genetic differences and metabolic processes may also be important in the production of these toxins. Studies have shown that the ability to produce toxins can vary temporally and spatially at a particular site or within the bloom itself (Hrudey et al., 1994; Ressom et al., 1994).

Cyanobacterial toxins either are membrane-bound or occur free within the cells. In laboratory studies, most of the toxin release occurs as cells age and die and passively leak their cellular contents, although active release of toxins can also occur from young growing cells (Pearson et al., 1990). Watanabe & Oishi (1983) investigated the toxicity of a cultured strain of *M. aeruginosa* through lag, exponential, and stationary growth phases. Maximum toxicity was observed between the exponential and stationary growth phases. The maximum cellular content of the toxin in two cultured *Microcystis* strains occurred at the late stage of exponential growth (Watanabe et al., 1989).

Microcystins and alkaloid toxins are degraded in natural waters, but there may be a lag phase before significant degradation takes place. Studies conducted using microcystin-LR at 10 µg/litre in reservoir water in the United Kingdom suggest a half-life of less than a week (Cousins et al., 1996). Codd & Bell (1996) also found that microcystin was readily biodegraded in ambient waters, with a half-life of about 1 week. Generally, if a lag phase exists, it is about 9 or 10 days long. In one study, microcystin was present up to 21 days following treatment of a bloom with an algicide (Jones & Orr, 1994). This could have been due to the shock dose with copper sulfate. Microcystin-LR is very stable in water and resistant to pH extremes and temperatures up to 300°C (Wannemacher, 1989). Biodegradation and photolysis are means by which released microcystin-LR can naturally degrade in water (Kenefick et al., 1993; Tsuji et al., 1994).

**Analytical methods**

Ressom et al. (1994), Lambert et al. (1994b), and AWWA (1995) reviewed the methods available for the analysis of microcystins in drinking-water. In comparing the various analytical methods being used for microcystin-LR and other microcystins, including their detection limits, it may be useful to distinguish screening methods, such as the mouse bioassay, enzyme-linked immunosorbent assay (ELISA), and phosphatase bioassay, which are conducted before clean-up and indicate the presence of toxins in samples, from methods that are conducted for the identification and quantification of the various individual microcystins (Harada, 1994). Often, more than one toxin may be present in a sample. The consensus among those using analytical methods is that a single method will not suffice for the identification and accurate quantification of many microcystins. The best approach for monitoring is to use a combination of screening and more sophisticated quantification methods.

It is important to measure *total* microcystins, which includes microcystins occurring free in water and microcystins bound to or inside cyanobacterial cells and which includes all microcystins, not just microcystin-LR. Thus, sample preparation may need to include sonification (to break up cells) and a variety of extraction procedures in order to isolate the different (i.e. more lipophilic or polar) microcystins. Most of the existing studies on the levels of microcystins in water supplies have not clearly indicated whether total or free microcystins were measured.

The mouse bioassay plays an important role as a screening tool, as it gives the total toxic potential of the sample within a few hours and it can distinguish hepatotoxins from neurotoxins. The assay determines the minimum amount of toxin required to kill a mouse and compares this value with lethal doses of a known amount of toxin. The disadvantage is that it does not detect toxins at low levels, especially in finished drinking-water, and it does not identify the specific toxic agent (Lambert et al., 1994a). As some cyanobacteria can produce both microcystin and anatoxin, the presence of microcystin can be overlooked with the mouse bioassay. The problem arises because anatoxin can kill the mouse within minutes, whereas microcystin can kill the animal within an hour. Bhattacharya and colleagues (1996) used a modified mouse liver slice culture technique for rapidly screening large numbers of cultures and bloom samples of cyanobacterial species for cytotoxicity and hepatotoxicity. Following microcystin-LR treatment, the hepatocytes were swollen with granulated cytoplasm. Congestion and haemorrhage were also evidenced by eosinophilic debris. The method is sensitive enough to detect toxins at the microgram level (Bhattacharya et al., 1996).

The protein phosphatase bioassay is another screening method for the quantification of microcystin-LR in water samples (Lambert et al., 1994a). This method is sensitive to subnanogram levels of microcystins in finished and raw water samples. This is a quick method, and many samples can be quantified in a few hours. However, it is not specific to microcystins and will indicate the presence of other substances inhibiting protein phosphatases. This should not be a problem when monitoring a particular area where the potentially occurring species and their possible toxins are known.

An ELISA using polyclonal antibodies and with a detection limit of 0.2 ng/ml has been published (Chu et al., 1990). It is likely that methods using monoclonal or polyclonal antibodies raised against a single toxin (e.g. microcystin-LR) will have problems of cross-reactivity with other microcystins.

There are several HPLC methods for the identification and quantification of microcystins and other cyanobacterial toxins. Many HPLC methods are variations on methods developed by Siegelman et al. (1984) and Harada et al. (1988). HPLC can distinguish between microcystin analogues, provided standards are available for reference (Lambert et al., 1994b). The HPLC–ultraviolet (UV) detection method is more sensitive than the mouse bioassay, but it does not

detect microcystin at levels lower than 1 µg/litre in waters containing high levels of natural organic matter (Lambert et al., 1994b). In the United Kingdom, an official HPLC–UV method with a detection limit of 0.5 µg/litre has been developed by the Water Research Centre. This method is designed to measure only free microcystin-LR; it does not measure "total" microcystin (free plus cell-bound) or microcystins other than microcystin-LR. It could be modified, however, to measure total microcystins (Fawell et al., 1993).

Lawton et al. (1994) developed a reverse-phase HPLC method to determine numerous variants of microcystin and nodularin (a hepatotoxin) in both raw and treated water by a single procedure within 24 hours. This method involves filtration to separate cyanobacterial cells from water, allowing intracellular and extracellular toxin levels to be assessed. The filtered water is subjected to trace enrichment using a $^{18}C$ solid-phase extraction cartridge, followed by identification and determination by photodiode array HPLC. Recoveries of microcystin-LR, -RR, -LY, -LF, and nodularin were good when raw and treated water samples were spiked with a mixture of microcystins and nodularin at concentrations as low as 0.25 µg/litre.

Liquid chromatography–mass spectrometry can analyse microcystin-LR, -YR, and -RR separately at 37, 42, and 23 ng/litre, respectively. It is a simple method that does not require a clean-up procedure because of its high selectivity (Tomoyasu & Keiji, 1996).

## ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

The major route of human exposure to cyanobacterial toxins is the consumption of drinking-water. A minor exposure route is the recreational use of lakes and rivers; for microcystin-LR, however, absorption through skin contact is unlikely, as the toxin does not readily cross cell membranes (Eriksson et al., 1990). Some people are also exposed to cyanobacterial toxins through the consumption of certain algal food tablets. An additional, minor route of exposure is through inhalation while taking showers; microcystin-LR, however, is very water soluble and non-volatile, so inhalation and absorption through the lungs are unlikely, unless the toxin is inhaled as an aqueous aerosol in air (Lambert et al., 1994b). The extent to which cyanobacterial toxins move up the food-chain (e.g. freshwater mussels and fish) has been investigated recently (Falconer et al., 1992b; Negri & Jones, 1995). The duration of toxin exposure would generally be shorter in colder countries than in those with milder climates.

The levels of microcystin-LR in the lakes and dugout ponds of Alberta, Canada, ranged from 4 to 605 µg/g dry weight of biomass (Kotak et al., 1993) or up to 1500 µg/g (Hrudey et al., 1994). More than 70% of over 380 bloom biomass samples from 19 lakes in Alberta between 1990 and 1992 showed detectable levels (>1 µg of microcystin-LR per g dry weight of biomass) of toxin (Hrudey et al., 1994). Similarly, levels of microcystin-LR from natural blooms of *Microcystis* in Japan, between 1989 and 1991, ranged from 27 to 622 µg/g dry weight of biomass (Park et al., 1993). In the same blooms, the levels of microcystin-RR and microcystin-YR ranged from 11 to 979 µg/g dry weight of biomass and from 9 to 356 µg/g dry weight of biomass, respectively, with a total maximum level of microcystins of 1732 µg/g dry weight of biomass (Park et al., 1993).

For two Alberta drinking-water supplies, the raw water intake levels of microcystin ranged from 0.15 to 4.3 µg/litre, with a large coefficient of variation of 59% for hourly fluctuations over an 11.5-hour period. In treated water, levels ranged from 0.09 to 0.64 µg/litre, with a small coefficient of variation of 10%. Over a 5-week period, similar coefficients of variation were obtained in the two types of samples (Hrudey et al., 1994).

In the summer of 1993, microcystin-LR was detected (>0.5 µg/litre) in water samples collected from Shoal Lake, Manitoba, Canada, and from within the drinking-water distribution system following the presence of *M. aeruginosa* blooms in Shoal Lake (Jones,

1996). Following this, in 1995, 160 surface water supplies, located mainly in southwestern Manitoba, were chosen for algal study. Treated water samples were analysed only for those sites in which raw water supplies were found to have detectable levels of toxins (detection limit 0.1 µg/litre). Toxin was present in 68% of the treated water samples collected from both the municipal water supply and dugouts used for domestic and livestock consumption. Thus, it appears that conventional treatment methods may be only partially successful in removing the toxins. Toxin concentrations ranged from <0.1 to 1.0 µg/litre in raw water samples and from <0.1 to 0.6 µg/litre in treated water samples.

Fastner et al. (1995) detected seven different microcystins in 9 of 12 eutrophic water bodies in Germany in 1993. The microcystin concentration was up to 800 µg/g dry weight for a single microcystin. Cytototoxins were also observed in six field samples.

With appropriate water treatment, maximum exposure to total microcystins is probably less than 1 µg/litre, based on the above data. Average exposure generally would probably be well below this level. Not all water supplies, however, are treated by filtration or adsorption; many are untreated or simply chlorinated.

*Cylindrospermopsis* cultured from a drinking-water supply reservoir (Hawkins et al., 1985) has been shown to contain the toxic alkaloid cylindrospermopsin at 0.5% dry weight of algae (Ohtani et al., 1992).

## KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

The most likely route of exposure to cyanobacterial toxins is via oral ingestion. However, there have been no pharmacokinetic studies with orally administered microcystins. After intravenous or intraperitoneal injection of sublethal doses of variously radiolabelled toxins in mice and rats, microcystin appears to be transported by bile acids transporter in both the intestine (Falconer et al., 1992a) and the liver (Runnegar et al., 1991). About 70% of the toxin is rapidly localized in the liver (Brooks & Codd, 1987; Meriluoto et al., 1990; Lin & Chu, 1994). The kidney and intestine also accumulate significant amounts of microcystin-LR (Meriluoto et al., 1990; Robinson et al., 1991a). Plasma half-lives of microcystin-LR, after intravenous administration, were 0.8 and 6.9 minutes for the alpha and beta phases of elimination, but the concentration of radioactive ($^{3}$H-microcystin-LR) label in the liver did not change throughout a 6-day study period (Robinson et al., 1991a). Microcystin-LR was excreted rapidly, with 75% of the total excretion occurring within 12 hours. The remaining 24% of the administered dose was excreted after 6 days, about 9% via the urinary route and 15% slowly (1% per day) via the faecal route.

Microcystin-LR does not readily cross cell membranes and does not enter most tissues. It crosses the ileum through the multispecific organic ion transport system (Runnegar et al., 1991) and mainly enters hepatocytes, where it is covalently bound to a 40 000-dalton protein (protein phosphatase 2A and possibly protein phosphatase 1) in the cytosol (Robinson et al., 1991b).

The liver plays a large role in the detoxification of microcystins (Brooks & Codd, 1987; Robinson et al., 1991b). Detoxification products were seen in urine, faeces, and liver cytosolic fractions (Robinson et al., 1991a), but these products have not been structurally identified. The detoxification products of microcystin-LR are more water soluble than the parent toxin.

## EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO SYSTEMS

### *Acute exposure*

Fatalities in animals have been reported following the consumption of water containing large numbers (>$10^6$/ml) of cyanobacterial cells (Beasley et al., 1989; Carmichael, 1992).

Microcystin-LR is an extremely acute toxin. The $LD_{50}$ by the intraperitoneal route is approximately 25–150 µg/kg of body weight in mice; the oral (by gavage) $LD_{50}$ is 5000 µg/kg of body weight in mice, and higher in rats (Fawell et al., 1994). The intraperitoneal $LD_{50}$s of several of the commonly occurring microcystins (microcystin-LA, -YR, and -YM) are similar to that of microcystin-LR, but the intraperitoneal $LD_{50}$ for microcystin-RR is about 10-fold higher (Kotak et al., 1993; Rinehart et al., 1994).

The microcystins are primarily hepatotoxins. After acute exposure to microcystin by intravenous or intraperitoneal injection, severe liver damage occurs, characterized by a disruption of liver cell structure, a loss of sinusoidal structure, increases in liver weight due to intrahepatic haemorrhage, haemodynamic shock, heart failure, and death. Other organs affected include the kidney and lungs (Hooser et al., 1990).

Intestinal damage is a consequence of the transport of microcystins through the lining cells, which are damaged in a similar manner to hepatocytes (Falconer et al., 1992a).

### *Short-term exposure*

Microcystin-LR was administered orally by gavage to groups of 15 male and 15 female mice at 0, 40, 200, or 1000 µg/kg of body weight per day for 13 weeks. No definite treatment-related changes were noted at the lowest dose. At 200 µg/kg of body weight per day, there was slight liver pathology in some male and female mice. At the highest dose level, all male and most female mice showed liver changes, which included chronic inflammation, focal degeneration of hepatocytes, and haemosiderin deposits. In male mice at the two highest dose levels, serum transaminases were significantly elevated, serum gamma glutamyl transferase was significantly reduced, and there were small but significant reductions in total serum protein and serum albumin. In female mice, changes in transaminases were observed, but only at the highest dose level. Also at the highest dose level, food consumption in male and female mice was increased by 14 and 20%, respectively, but body weight was 7% lower in both sexes compared with control mice. The NOAEL for microcystin-LR was considered to be 40 µg/kg of body weight per day (Fawell et al., 1994).

In another study, extract from *M. aeruginosa* was given to groups of five pigs in their drinking-water for 44 days at dose levels calculated to be equivalent to microcystin doses of 280, 800, or 1310 µg/kg of body weight per day (Falconer et al., 1994). The extract contained at least seven microcystin variants, with microcystin-YR tentatively identified as the major constituent. A no-adverse-effect level (NAEL) for microcystins of 280 µg/kg of body weight per day was reported by the authors, with liver injury (evident from histopathology and changes in serum enzymes) observed at the two highest dose levels. However, one pig was also affected at the lowest dose level, and it is appropriate to consider the 280 µg/kg of body weight per day dose level as a LOAEL.

### *Long-term exposure*

An oral repeated-dose study was conducted with *M. aeruginosa* extract supplied to mice at five concentrations (equivalent to 750–12 000 µg of microcystin-YM per kg of body weight per day) in their drinking-water for up to 1 year. At the higher concentrations, increased mortality, increased incidences of bronchopneumonia, and chronic liver injury were noted.

No liver cancer was seen, but the authors indicated that there may have been some evidence of tumour promotion. No clear NOAEL was established in this study (Falconer et al., 1988).

***Reproductive and developmental toxicity***

To investigate the effects of microcystin-LR on the embryonic and fetal development of the mouse, four groups of 26 time-mated female mice of the Cr1:CD-1 (ICR) BR strain were dosed once daily by oral gavage with aqueous solutions of microcystin-LR from days 6 to 15 of pregnancy, inclusive. The dose levels were 0, 200, 600, or 2000 µg/kg of body weight per day. Maternal clinical signs, body weights, and food consumption were recorded. On day 18 of pregnancy, the females were killed, a necropsy was performed, and the fetuses were examined for abnormalities. Only treatment at 2000 µg/kg of body weight per day was associated with maternal toxicity and mortality. Nine of the 26 females died or were sacrificed prematurely during the dosing period. At necropsy, a number of females had abnormal livers, and retardation of fetal weight and skeletal ossification were observed at the maximum dose. There was no evidence of embryolethality, teratogenicity, or embryonic growth retardation at all dose levels. There was no apparent effect of treatment at any dose level on litter size, post-implantation loss, or the sex distribution of the live fetuses. The NOAEL for any aspect of developmental toxicity was 600 µg/kg of body weight per day (Fawell et al., 1994). These data are in agreement with those of Falconer et al. (1988), which similarly provided no evidence of teratogenicity, embryonic mortality, or reduction in fertility in mice exposed to microcystin at 750 µg/kg of body weight per day in drinking-water from weaning (for 17 weeks prior to mating) through mating.

***Mutagenicity and related end-points***

No mutagenic response was observed for purified toxins derived from *Microcystis* in the Ames *Salmonella* assay (strains TA98, TA100, and TA102) with or without S9 activation. The *Bacillus subtilis* multigene sporulation test was also negative with regard to mutagenicity using both the 168 and hcr-9 strains (Repavich et al., 1990). In contrast, results of a study in which the purified toxins were tested against human lymphocytes suggested that the toxins may be clastogenic, as indicated by dose-related increases in chromosomal breakage (Repavich et al., 1990).

***Carcinogenicity***

There has been some evidence of tumour promotion in animal studies. In a modified two-stage carcinogenesis mouse skin bioassay, dimethylbenzanthracene (DMBA) (500 µg) in acetone was applied to the skin of four out of six groups of 20 3-month-old Swiss female mice. After 1 week, the DMBA-treated mice received drinking-water, *Microcystis* extract in drinking-water (actual microcystin-YM dose not provided), croton oil (as a positive control) applied to the skin (0.5% in 0.1 ml acetone twice a week) plus drinking-water, or croton oil plus *Microcystin* extract; the control mice received drinking-water or *Microcystis* extract in drinking-water. After 52 days from initiation, substantial skin tumours and ulcers were visible on the DMBA-treated mice consuming *Microcystis* extract. There was a significant increase in the mean weight of skin tumours per mouse in treated mice given the *Microcystis* extract compared with water. The actual number of tumours per mouse and the weights of the tumours in relation to the weights of the animals were not provided. It was concluded by the authors that oral consumption of *Microcystis* extract in drinking-water may act as a promoter. However, the mechanism of action is not clear, as microcystins have difficulty penetrating epidermal cells. The tumour weight per mouse in DMBA-treated mice given both croton oil and the algal extract was slightly lower than in those given croton oil and drinking-water. These latter findings could not be explained by the author (Falconer, 1991).

In a two-stage carcinogenicity bioassay, groups of 10–19 7-week-old male Fischer 344 rats were initiated by intraperitoneal injection with 200 mg of diethylnitrosamine (DEN) per kg of body weight, followed by partial hepatectomy at the end of the third week. Tumour promotion was assessed by intraperitoneal injection of microcystin-LR at 1 or 10 µg/kg of body weight from the third week of the experiment, 3 or 5 times per week. Tumour promotion, as indicated by an increase in glutathione *S*-transferase placental form (GST-P) positive liver foci, was seen after 8 weeks in animals dosed with 10 µg of microcystin-LR per kg of body weight (Nishiwaki-Matsushima et al., 1992). In a second similar experiment, the same authors used dose levels of 10 µg/kg of body weight per day before, and 10, 25, or 50 µg/kg of body weight per day after, partial hepatectomy. It was found that the increase in GST-positive foci was dose related. However, these results should be interpreted with caution, because the doses used were very high and could have caused significant damage to hepatocytes. Microcystin-LR had no effect when given to non-initiated rats or to rats that had received partial hepatectomy but no promotion dose of microcystin-LR.

Microcystin-LR was found to be a potent inhibitor of eukaryotic protein serine/threonine phosphatases 1 and 2A both *in vitro* (Honkanen et al., 1990; Mackintosh et al., 1990) and *in vivo* (Runnegar et al., 1993). Such substances are considered to be non-phorbol ester (TPA) type tumour promoters. The inhibition of protein phosphatase 2A by microcystin-LR can be effectively reversed in the presence of polyclonal antibodies against microcystin-LR (Lin & Chu, 1994). The inhibition of protein phosphatase type 1 and type 2 activities by microcystin-LR is similar to that of the known protein phosphatase inhibitor and tumour promoter okadaic acid (Nishiwaki-Matsushima et al., 1992). The implications for low-level chronic exposure to microcystins are not known.

## EFFECTS ON HUMANS

Blue-green algae have been known to cause animal and human poisoning in lakes, ponds, and dugouts in various parts of the world for over 100 years.

Through the recreational use of contaminated water, cyanobacterial blooms of *Microcystis*, *Anabaena*, and others have been linked to incidence of human illness in many countries, but no fatalities have been reported (Lambert et al., 1994b). In Canada, human illnesses have been reported in Saskatchewan, with symptoms including stomach cramps, vomiting, diarrhoea, fever, headache, pains in muscles and joints, and weakness (Dillenberg & Dehnel, 1960). Similar symptoms as well as skin, eye, and throat irritation and allergic responses to cyanobacterial toxins in water have also been reported in other countries (Ressom et al., 1994). The reported instances of illnesses are few, but, because they are difficult to diagnose, such illnesses may in fact be more common than has been reported (Carmichael & Falconer, 1993).

In Saskatchewan, Canada, 10 children became sick with diarrhoea after swimming in a lake covered with cyanobacteria. *Anabaena* cells, which produce microcystin-LR, were found in the stools of one child (Dillenberg & Dehnel, 1960). In the United Kingdom, 10 of 18 army recruits on a military exercise in a reservoir with a bloom of *M. aeruginosa* suffered abdominal pain, nausea, vomiting, diarrhoea, sore throat, dry cough, blistering at the mouth, and headache. Two were hospitalized and developed an atypical pneumonia. Serum enzymes indicating liver damage were elevated. Microcystin-LR was identified in the bloom material (Pearson et al., 1990). Nevertheless, substances other than microcystin-LR may have been present, and some of the observed effects were probably due to other materials in the water.

In the USA and Australia, several different cyanobacterial toxins have been implicated in human illness from certain municipal water supplies, often after algal blooms had been treated with copper sulfate (Bourke et al., 1983; Falconer, 1989; Ressom et al., 1994). In most

cases, the cyanobacteria and sometimes the toxins involved have been identified, but the levels of toxin associated with illness have not been established in any of the outbreaks. The Palm Island mystery disease, affecting about 140 people, largely children, in Australia, occurred after a dense cyanobacterial bloom on a water supply was treated with copper sulfate. Within a week, severe illness was seen, characterized by vomiting, hepatomegaly, and kidney dysfunction, with loss of electrolytes, glucose, and plasma protein; recovery took 1–3 weeks (Byth, 1980). The cause of illness was not identified until a subsequent cyanobacterial bloom in the same water supply reservoir was shown to be highly toxic (Hawkins et al., 1985). The causative organism was *C. raciborskii*, containing the cytotoxic alkaloid cylindrospermopsin, a powerful inhibitor of protein synthesis (Terao et al., 1994).

Possible liver damage, as evidenced by significant increases in gamma glutamyl transferase, was seen in persons drinking water from supplies containing blooms of *Microcystis* after treatment with copper sulfate (Malpus Dam, Armidale, Australia) compared with persons drinking uncontaminated water (Falconer, 1989).

In Salisbury, Rhodesia, seasonal acute childhood gastroenteritis during the years 1960–1965 was linked to annual blooms of *Microcystis* in the lake serving as the water supply. An adjacent water supply was not similarly affected and was not associated with this disease (Zilberg, 1966).

El Saadi & Cameron (1993) reported on 26 cases (aged 1–64 years) with skin diseases and multiple systemic symptoms associated with exposure (some via drinking-water) to river water or rainwater in Australia during 1991–1992. The water was stored in open tanks and contained *Anabaena* blooms. Further case–control studies in the same area are ongoing.

Recently, an epidemiological survey in Haimen city (Jian-Su province) and Fusui county (Guangxi province) in China found a close relationship between the incidence of primary liver cancer and the use of drinking-water from ponds and ditches (Ueno et al., 1996). In 1993 and 1994, microcystin concentrations ranged from 0.058 to 0.460 µg/litre; the highest concentrations occurred from June to September. Microcystin was not detected in deep well-water. A similar survey on 26 drinking-water samples in the Guangxi province showed a high frequency of microcystins in the water of ponds/ditches and rivers, but no microcystins were found in shallow and deep wells. According to Ueno et al. (1996), the combined effect of microcystin toxin from the drinking-water of ponds/ditches and rivers or both and other carcinogens such as aflatoxin B1 found in food may be the cause of the high incidence of primary liver cancer in Haimen city and other areas in China.

Cyanobacterial blooms tend to occur repeatedly in the same water supply. Therefore, some human populations are at risk of repeated ingestion of cyanobacterial toxins. However, the available data are not sufficient to allow a quantitative assessment of human exposure.

## PROVISIONAL GUIDELINE VALUE

There are insufficient data to derive a guideline value for cyanobacterial toxins other than microcystin-LR. Only a guideline value for this compound is derived.

A 13-week study in mice with microcystin-LR (Fawell et al., 1994) is considered the most suitable for the derivation of a guideline value. In this study, a NOAEL of 40 µg/kg of body weight per day was determined for liver pathology. A TDI of 0.04 µg/kg of body weight per day can be calculated by applying an uncertainty factor of 1000 (100 for intra- and interspecies variation, 10 for limitations in the database, in particular lack of data on chronic toxicity and carcinogenicity) to the NOAEL. An allocation factor of 0.80 is used for the proportion of daily exposure arising from drinking-water, because there is little exposure from

any other source and route. The resulting guideline value for total microcystin-LR (free plus cell-bound) is 1 µg/litre (rounded figure) in drinking-water.

The guideline value thus calculated is supported by a 44-day study in which pigs were exposed, in their drinking-water, to an extract from *M. aeruginosa* containing microcystin-LR.

The guideline value of 1 µg/litre for microcystin-LR is provisional, as the database is limited, new data for the toxicity of cyanobacterial toxins are being generated, and the guideline value covers only microcystin-LR.

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