

CYANOBACTERIAL PEPTIDE TOXINS

Microcystin-LR (Group 2B) ***Microcystis* extracts (Group 3)** **Nodularins (Group 3)**

For definition of Groups, see Preamble Evaluation.

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5. Summary of Data Reported

[The text of these Summaries and Evaluations may be edited for language and clarity during the checking of the main text of the Monographs.]

5.1 Exposure data

Microcystins and nodularins are cyclic peptide toxins that have a ring structure of seven and five amino acids, respectively, which comprise one unique phenyl deca-dienoic acid, four invariable D-amino acids and, in microcystins, two variable L-amino acids. There are approximately 70 variants of microcystin and several variants of nodularin. These peptides are produced naturally by cyanobacteria, an evolutionarily very ancient group of photosynthetic prokaryotic organisms. The bacteria occur in filamentous and coccoid forms in free suspension in water or form layers (scums) on surfaces; they are distributed worldwide in water and soils, and on rock and plant surfaces.

Microcystin-LR (lysine-arginine) is the most investigated cyanobacterial peptide toxin because it is frequently present in cyanobacterial blooms in rivers and lakes. Nodularin occurs in brackish waters. The concentration of toxin in water depends on the cell content of microcystins or nodularins and the concentration of cells in the water. The peptide toxins are contained primarily within the cyanobacterial cells and are rarely released before the cells die or are destroyed (for instance, following water treatment or in the stomach, after ingestion). Due to widespread eutrophication in many settings, these toxins may occur more frequently and at unnaturally high concentration. In natural water bodies and in water storage reservoirs, the concentrations of toxin vary widely from undetectable to several milligrams per litre in cyanobacterial scums.

A number of analytical techniques are used for the quantification of cyanobacterial peptide toxins, and include high-performance liquid chromatography, liquid chromatography–mass spectrometry, enzyme inhibition assays and the enzyme-linked immunosorbent assay. Genetic probes are also available for the detection of genes involved in the biosynthesis of these toxins.

Human exposure to these toxins occurs most frequently through the ingestion of water, i.e. through drinking or during recreational activities in which water is swallowed. Furthermore, cyanobacterial dietary supplements (mistakenly referred to as blue-green algae supplements) are now commercially available, and consumption of these toxins occurs from this source. Fish, shellfish and crustaceans accumulate microcystins and nodularins, which remain stable and unchanged during cooking; this leads to their ingestion by humans.

WHO has adopted a provisional guideline value for drinking-water supplies of 1 µg/L microcystin-LR, based on the subacute toxicity of microcystin-LR in mice. The toxicity of this microcystin variant is representative of that of other variants and of nodularin, and therefore provides a reasonable approximation of the toxicity of naturally occurring mixtures of these variants in water bodies. A

number of countries have adopted similar guideline values for microcystins, some of which refer to the total concentration of microcystins present in water samples.

Guidelines for recreational exposure to cyanobacterial toxins have also been proposed by WHO; they are derived from the drinking-water guideline but refer to the concentrations of cyanobacteria found in water. They have also been implemented by a number of countries, and include the emission of warnings or the closure of recreational water sites when the specified levels of cyanobacterial contamination are reached.

5.2 Human carcinogenicity data

The Working Group reviewed several reports on ecological, cohort and case–control studies of the risk for hepatocellular carcinoma and source of drinking-water, some of which contained information on concentrations of microcystins in the water. The Working Group had to rely in many instances on summary information in review articles that lacked detailed descriptions of study methods and results. The studies of hepatocellular carcinoma included 14 ecological studies (12 from a review), two cohort studies (one in the same review), two meta-analyses of case–control studies and one additional case–control study. All studies conducted in the area of Southeast China that is endemic for hepatocellular carcinoma showed a positive association between risk for hepatocellular carcinoma and drinking-water source; the use of surface waters (pond, ditch or river waters) was associated with higher risks compared with that of either shallow or deep wells. The few studies that reported concentrations of microcystin indicated that levels were much higher in surface than in well waters, but no study estimated the level of microcystin on an individual basis. In an analysis of pooled data from six case–control studies, the relative risk for hepatocellular carcinoma was 1.6; estimates of relative risk from other studies were generally in the range of 1.5 to 4. Exposure to aflatoxin was not generally considered, and other contaminants or organisms in surface waters or factors related to water source were not evaluated. Some studies controlled for hepatitis B viral antigen or a history of hepatitis, which decreased the likelihood of confounding from this strong risk factor.

An ecological study of colorectal cancer showed an association with concentration of microcystins, but confounding could not be ruled out.

In summary, although many studies of hepatocellular carcinoma and one study of colorectal cancer found intriguing, positive associations with consumption of surface waters, in light of the quality of the published material available to the Working Group, it was not possible to associate the excess risk specifically with exposure to microcystins.

5.3 Animal carcinogenicity data

In one study in male mice, repeated intraperitoneal injections of microcystin-LR induced liver foci, which were probably benign tumours. The study was found to be inadequate for evaluation.

In three experiments in male rats that were initiated with *N*-nitrosodiethylamine and one experiment in male rats that were initiated with aflatoxin B₁, multiple intraperitoneal injections of microcystin-LR increased the incidence in the liver of glutathione *S*-transferase placental form-positive foci, which are considered to be preneoplastic lesions.

In one experiment in mice that were initiated by skin application of 7,12-dimethylbenz[*a*]anthracene, *Microcystis* extracts given in the drinking-water increased the weight of skin papillomas per mouse.

One study in mice that were initiated with *N*-nitroso-*N*-methylurea and given *Microcystis* extracts in the drinking-water gave negative results.

In one experiment in mice that were initiated by intraperitoneal injection of azoxymethane, exposure to *Microcystis* extracts in the drinking-water resulted in an increase in the area of aberrant crypt foci in the colon.

In two studies in male rats that were initiated with *N*-nitrosodiethylamine, multiple intraperitoneal injections of nodularin increased the incidence of glutathione *S*-transferase placental form-positive foci in the liver.

5.4 Mechanistic and other relevant data

Studies on the distribution of microcystins and nodularins have been carried out after intravenous and intraperitoneal administration of radiolabelled microcystins or nodularins to mice, rats and pigs. Kinetic studies showed rapid distribution into the liver and low accumulation in other tissues. The cellular metabolism of microcystins has not been elucidated but some data suggest that the conjugation of microcystins and possibly nodularins by glutathione occurs. Excretion of microcystins occurs primarily in faeces. Renal excretion clears insignificant amounts of microcystins.

Microcystins do not permeate into cells. Cell uptake studies with microcystins have demonstrated the requirement for cell membrane-associated organic anion transporter proteins. In-vitro studies in hepatocytes which express these transporters have shown competitive inhibition of microcystin uptake by endogenous transporter substrates (e.g. bile acids and steroids) and xenobiotics (e.g. antibiotics).

Microcystins and nodularins have a similar mechanism of toxicity, that is the specific inhibition of protein phosphatases 1 and 2A, which occurs at picomolar concentrations of the toxin. This inhibition results in hyperphosphorylation of intracellular proteins. In human and rodent hepatocytes this leads to the rapid disaggregation of intermediate filaments (cytokeratins) that form the cellular scaffold. Microfilaments become detached from the cytoplasmic membrane, which results in cell cytoskeletal deformation and bleb formation. Cell lysis and apoptosis follow, depending on dose. Acute death results from dissolution of the liver structure and intrahepatic pooling of blood, which lead to overall haemorrhagic shock. Doses that are not immediately lethal can result in death from liver failure in animals and humans several months after the initial exposure to microcystin.

The acute toxicity of microcystin in humans was shown in the intoxication and subsequent death of more than 50 haemodialysis patients in Caruaru, Brazil, who were exposed to microcystin in the dialysis water. Significant amounts of microcystin were detected in the liver and serum of these patients. In addition, several incidents of hepatic disease and/or gastroenteritis have been reported after subacute intoxication with microcystins.

The acute toxicity of microcystins and nodularins has been described in rodents after intraperitoneal administration. The main injury was to the liver. Similar toxicity was also demonstrated in sheep and pigs; the hepatic damage was sustained for long periods after exposure.

Subchronic toxicity studies of microcystin have been undertaken in mice and pigs by oral exposure and led to liver injury. These studies resulted in the establishment of the WHO guideline value of 1 µg/L of water.

Cyanobacterial extracts containing microcystin were given in the drinking-water to mice over a period of 1 year. Liver damage was observed, and male mice were more susceptible than females.

There are indications that chronic administration of microcystin results in immunotoxicity and some reproductive effects.

There is no clear evidence that microcystins or nodularins are mutagenic or clastogenic in non-mammalian, mammalian or human cell systems. Both toxins modulate the expression of oncogenes, early-response genes and tumour necrosis factor- α , affect cell survival and/or apoptosis, and inhibit

DNA repair. An increased frequency of polyploid cells and centromere-positive micronuclei was observed, which suggests that both microcystins and nodularins are aneugenic.

6. Evaluation and Rationale

There is *inadequate evidence* in humans for the carcinogenicity of microcystin-LR.

There is *inadequate evidence* in humans for the carcinogenicity of nodularins.

There is *inadequate evidence* in experimental animals for the carcinogenicity of microcystin-LR.

There is *inadequate evidence* in experimental animals for the carcinogenicity of *Microcystis* extracts.

There is *inadequate evidence* in experimental animals for the carcinogenicity of nodularins.

Overall evaluation

Microcystin-LR is *possibly carcinogenic to humans (Group 2B)*.

Microcystis extracts are *not classifiable as to their carcinogenicity to humans (Group 3)*.

Nodularins are *not classifiable as to their carcinogenicity to humans (Group 3)*.

In reaching the overall evaluation of microcystin-LR, the Working Group noted:

- Human epidemiological studies in China showed elevated incidences of hepatocellular carcinoma and colorectal cancer associated with drinking surface water compared with drinking well water, though no clear correlation with exposure to microcystins could be ascertained.
- Studies in rodents given microcystin-LR or *Microcystis* extracts were indicative of promotion of liver and colon preneoplastic foci, as was a subchronic study with microcystin-LR that resulted in persistent neoplastic nodules in mouse liver.
- Several studies strongly supported a plausible tumour promoter mechanism for microcystin-LR. This mechanism is mediated through the inhibition of protein phosphatases 1 and 2A, an effect demonstrated in rodent liver and hepatocytes. The resulting hyperphosphorylation of intracellular proteins leads to disruption of intermediate filaments forming the cellular scaffold in human and rodent hepatocytes. Microcystin-LR modulates the expression of oncogenes, early-response genes and tumour necrosis factor- α and affects cell division, cell survival and apoptosis.

In reaching the overall evaluation of nodularins, the Working Group noted that although a tumour promoter mechanism was plausible, few studies had been conducted to demonstrate this.