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Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): a reasonable or misguided approach?

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Abstract

This article reviews current scientific knowledge on the toxicity and carcinogenicity of microcystins and compares this to the guidance values proposed for microcystins in water by the World Health Organization, and for blue-green algal food supplements by the Oregon State Department of Health. The basis of the risk assessment underlying these guidance values is viewed as being critical due to overt deficiencies in the data used for its generation: (i) use of one microcystin congener only (microcystin-LR), while the other presently known nearly 80 congeners are largely disregarded, (ii) new knowledge regarding potential neuro and renal toxicity of microcystins in humans and (iii) the inadequacies of assessing realistic microcystin exposures in humans and especially in children via blue-green algal food supplements. In reiterating the state-of-the-art toxicology database on microcystins and in the light of new data on the high degree of toxin contamination of algal food supplements, this review clearly demonstrates the need for improved kinetic data of microcystins in humans and for discussion concerning uncertainty factors, which may result in a lowering of the present guidance values and an increased routine control of water bodies and food supplements for toxin contamination. Similar to the approach taken previously by authorities for dioxin or PCB risk assessment, the use of a toxin equivalent approach to the risk assessment of microcystins is proposed.

Keywords: Microcystin; Risk assessment; Cyanotoxins

Introduction

Cyanobacteria are responsible for oxygenic life on earth through their photosynthetic activity and have had more than 3.5 billion years to develop the production of a broad variety of molecules. Peptides such as anabaenopeptins, aeruginosins, microcystins (MCs), nodularins, microginins or microviridins and alkaloids such as anatoxin-a, saxitoxins and cylindrospermopsins do not appear to be necessary for primary metabolism. Although some hypotheses exist (Kaebernick and Neilan, 2001), the physiological function(s) of these molecules either within or outside of the cell is presently unknown. Hundreds of molecules from these families have been structurally identified and many more new compounds will probably be discovered within the next years. Dozens of cyanobacterial species and families produce these secondary metabolites and in principle, every surface water can act as a habitat for cyanobacteria. Furthermore, cyanobacteria occur even in very arid climates (Hitzfeld et al., 2000a; Wynn-Williams, 2000). Parameters such as climate, trophic status and the morphology of the water body determine the composition of the cyanobacterial community. Although there are well-known advantages of these organisms as nitrogen fixers, as succession pioneers, as a first link in the food chain or as potential sources for pharmaceutically relevant compounds (Mundt et al., 2001), cyanobacteria as toxin producers can also have grave disadvantages for humans and their environment, be these through direct or indirect exposure.

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The scope of this review focuses on the possible direct and indirect risks of MCs for human health, while disregarding the additional potential risk presented by other cyanobacterial toxins potentially simultaneously present, which may act synergistically (Fitzgeorge et al., 1994). Prerequisite for any human health risk assessment of chemicals, whether these be of natural or anthropogenic origin (IPCS, 1995), are not only the chemical/biochemical characteristics, kinetics and dynamics (toxicity/carcinogenicity) of the compound(s) in question, but also an understanding of the main routes and possibilities for human contact with the chemical compound(s), in this case MCs. The following paragraphs present merely a brief and condensed overview of the current knowledge on MCs and thus, the reader is referred to the original publications for more detailed information.

Microcystins: chemical and biochemical characteristics

MCs, cyclic peptides with a molecular weight between 900 and 1100 Daltons, are the most common of the cyanobacterial toxins found in water, as well as being those most often responsible for poisoning animals and humans who come into contact with toxic blooms. Because of their chemical structure, MCs are extremely stable in water and can tolerate radical changes in water chemistry, including pH (Harada and Tsuji, 1998; Harada et al., 1996). Structurally, MCs are monocyclic heptapeptides, containing two variable L-amino acids and two novel D-amino acids: *N*-methyl-dehydroalanine (Mdha), which hydrolyses to methylamine, and a unique non-polar-linked amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, also known as Adda. One of the key components for biological activity appears to be linked with the Adda side chain, as cleavage of the Adda side chain from the cyclic peptide seems to render MCs inactive with regard to protein phosphatase inhibition (Harada et al., 1990a, 1990b). However, the Adda side chain alone has been shown not to inhibit the catalytic subunit of proteinphosphatase 1 even at the extremely high concentration of 10 μM and not to be toxic to mice at i.p. doses of up to 10 mg/kg bw (Harada et al., 2004). MCs are named according to their variable L-amino acid: for example, MC-LR contains leucine and arginine (Carmichael et al., 1988). To date, nearly 80 different congeners have been identified (Sivonen and Jones, 1999).

Microcystins: toxicity and carcinogenicity

Acute toxicity

MCs have been shown to be acutely toxic to animals and humans (Annadotter et al., 2001; Azevedo et al., 2002; Beasley et al., 1989a; Duy et al., 2000; Hooser et al., 1989; Mez et al., 1997; Pouria et al., 1998; WHO, 1998; Yoshida et al., 1997) with LD_{50}s of the individual MC congeners ranging between 50 (MC-LR) and 600 (MC-RR) μg/kg bw following i.p. injection in mice (Sivonen and Jones, 1999). The primary mechanism of toxicity is probably the inhibition of protein phosphatases 1 and 2a followed by loss of cytoskeletal integrity and subsequent cytolysis or apoptosis, primarily of hepatocytes but also of glomeruli and renal proximal tubule cells (Eriksson et al., 1990b, 1992; Fischer and Dietrich, 2000a; Fischer et al., 2000b; Hooser et al., 1989; MacKintosh et al., 1990; Wickstrom et al., 1995). The oral LD_{50} in mice (5000 μg/kg bw) appears to be approximately a factor 100 lower than the i.p. LD_{50}, while the oral LD_{50} in rats was reported as >5000 μg/kg bw, suggesting an even greater difference between p.o. and i.p. application in this species (Fawell et al., 1994, 1999). In addition, Miura et al. (1991) reported a 25-h i.p. LD_{50} of 122 μg MC-LR/kg bw in fed versus an i.p. LD_{50} of 72 μg MC-LR/kg bw in fasted rats. Taken together, the data of Fawell et al. (1994, 1999) and Miura et al. (1991) clearly point to the potential weaknesses of such investigations for use in human risk assessment, especially as the oral bioavailability and thus toxicity of MCs appears largely dependent on whether the animals were fasted or not. Indeed, the OECD 401 LD_{50} protocol requires fasting of animals 24 h prior to oral dosing, with food ad libitum for up to 14 days post-dosing (observation period). If gastrointestinal as well as hepatic uptake of compounds, for example, MCs, is rate-limited via a carrier mediated uptake process (Eriksson et al., 1990a; Fischer and Dietrich, 2000b; Hermansky et al., 1990, 1991; Hooser et al., 1991; Kuiper-Goodman et al., 1994; Kullak-Ublick et al., 1996; Meriluoto et al., 1990; Runnegar et al., 1991, 1995), the 24 h pre-dosing fasting period may not suffice to provide for a high bioavailability of the compound as the rodents will immediately resume consuming food during the observation period. Furthermore, the question arises whether the rodent food composition is comparable to the food composition generally applicable for humans, that is, whether the bioavailability of MCs is similar in rodent and in human food. In view of this uncertainty and based on the fact that acute intoxication situations in humans are less likely to occur in conjunction with food mixtures (except perhaps blue green algal supplements: (BGAS)), but more likely to occur in conjunction with contaminated water (Annadotter et al., 2001; Hitzfeld et al., 2000a,b; Kuiper-Goodman et al., 1999), thus similar to a “fasted” situation in the rodent, the rodent i.p. LD_{50} values appear more representative for calculations of an acute intoxication risk in humans than the corresponding p.o. LD_{50} values.

Subchronic and chronic toxicity

In studies conducted at the Water Research Center (WRc) in the United Kingdom, MC-LR was also administered orally by gavage to groups of 15 male and 15

female mice at 0, 40, 200 or 1000 μg/kg bw per day for 13 weeks (Fawell et al., 1994, 1999). The no-observed-effect level (NOEL) for liver toxicity was 40 μg/kg bw per day. At the next highest dose level, slight liver pathology was noted in one male and two female mice. At the highest dose level, all mice showed liver changes, which included chronic inflammation, degeneration of hepatocytes and hemosiderin deposits. In addition to the findings of Fawell, Schaeffer et al. (1999), in an effort to prove the lack of health risks stemming from Upper Lake Klamath *Aphanizomenon flos-aquae* (AFA) bloom material, re-evaluated the following study: young adult mice were treated with AFA bloom material, which corresponded to doses of up to 333 μg MC-LR/kg bw day, in the rodent feed for up to 43 days. The authors stated that no pathological changes or alterations in blood enzyme levels were observed, thus suggesting a daily intake of 333 μg MC-LR/kg bw day to have no effect at all in mice. In a similar study, mice were treated with microcystins containing bloom extract in drinking water for up to 52 weeks (Falconer et al., 1988). The treatment with a high concentration of extract in the drinking water resulted in increased mortality, liver enzyme levels, liver damage and possibly increased renal pathology. No neoplastic nodules were observed in the liver, although a slightly higher number of tumours (carcinomas of the lung and abdomen) were noted in the high-dose group animals when compared to the control. Unfortunately, the toxin concentration was never determined properly, thus a direct comparison with the studies of Fawell or Schaeffer is not possible. In general, it appears that gastrointestinal uptake of MCs in mice is very slow and limited; an observation also supported by the organ distribution studies of Nishiwaki et al. (1994) and Robinson et al. (1989), where the comparison between i.p and p.o. applications resulted in factor 80 lower amount of radio-labeled dihydro-MC-LR in the liver following p.o. application and comparable time frame of observation post-application (Table 1).

In a follow-up study with pigs (Falconer et al., 1994) using *Microcystis aeruginosa* bloom material, animals were exposed to MCs (approximately 7 different congeners including MC-YR but excluding MC-LR and MC-RR) at 1312, 796, 280 and 0 μg MC-LR equiv. per kg bw and day for 44 days in drinking water. All pigs presented with histopathological alterations of the liver even at the lowest dose applied (one animal of five). Consequently, the dose level of 280 μg MC-LR equiv./kg bw day should be considered as a lowest-observed-adverse-effect level (LOAEL), when using the toxin concentration estimated from the i.p. equivalent of 100 μg MC-LR. If however, the HPLC and the protein phosphatase inhibition test derived MC equivalent concentrations (summation of visible MC peaks) are employed, the LOAELs are 184 and 88 μg MC-LR equiv. /kg bw, respectively.

Although the above studies appear to provide conflicting results mainly caused by the different experimental setups, prudence would dictate that the NOEL of 40 μg/kg bw per day, derived from the Fawell study with mice, be used for human health risk assessment purposes. Despite this, the main issue seems, as already noted for the acute toxicity situation, to lie with the bioavailability of MCs. Indeed, MC application within the food appears to provide for the least, within drinking water commensurate with food ad libitum a moderate, and oral gavage of MC, commensurate with water and food ad libitum the highest bioavailability of MCs and consequently liver and possibly

Table 1
Organ distribution of radiolabeled microcystin (MC) congeners, in various species, application routes and time-points post-application

Species	MC-form	Application form	Time [a] (h)	Liver (%)[b]	Kidney (%)[b]	Brain (%)[b]	Lung (%)[b]	Spleen (%)[b]	Heart (%)[b]	Gall Bladder (%)[b]	GI-Tract (%)[b]	Muscle (%)[b]	Carcass[c] (%)[b]	Ref.
Mouse	^{3}H-dhMC-LR	i.p.	1.0	71.5	0.5	–	0.4	–	–	0.5	2.2[d]	–	–	1
	^{3}H-dhMC-LR	p.o.	6.0	0.65	0.05	0.02	n.d.	n.d.	n.d.	n.d.	0.69	n.d.	n.d.	1
	^{3}H-dhMC-LR	p.o.	144.0	0.4	0.01	–	n.d.	n.d.	n.d.	n.d.	–	n.d.	n.d.	1
	^{3}H-MC-LR	i.v.	1.0	67.0	0.8	n.d.	<0.1	<0.1	<0.1	n.d.	8.6	n.d.	6.0	2
	^{3}H-MC-LR	i.p.	6.0	56.0	0.9	n.d.	<1.0	<1.0	<1.0	n.d.	7.0	<1.0	10.0	3
	^{3}H-dhMC-LR	i.v.	0.45	70.0	6.0	2.0	n.d.	2.0	n.d.	n.d.	10	2.0	4.0	4
Rat	^{125}I-MC-YM	i.v.	2.0	19.2	5.3	n.d.	n.d.	n.d.	n.d.	n.d.	9.4	n.d.	n.d.	5
	^{3}H-MC-LR	i.v.	6.0	78.0	2.5	n.d.	n.d.	n.d.	n.d.	n.d.	3.5	n.d.	6.5	6
Swine	^{3}H-dhMC-LR	i.v.	5.0	64.60	1.20	n.d.	1.75	0.04	0.22	n.d.	0.13[e]	n.d.	n.d.	7[f]
	^{3}H-dhMC-LR	i.v.	5.0	46.99	2.19	n.d.	0.55	0.07	0.23	n.d.	0.20[e]	n.d.	n.d.	7[g]
	^{3}H-dhMC-LR	i.i.l.	5.0	49.50	1.04	n.d.	0.65	0.16	0.81	n.d.	33.94 [e]	n.d.	n.d.	7[g]

n.d., not determined; – not detectable; i.p., intraperitoneally; p.o., perorally; i.v., intravenously; i.i.l., intra-isolated ileal loop; bw, body weight. (1) Nishiwaki et al., 1994; (2) Robinson et al., 1991; (3) Robinson et al., 1989; (4) Meriluoto et al., 1990; (5) Falconer et al., 1986; (6) Pace et al., 1990; (7) Stotts et al., 1997.

[a] Time post initial application.

[b] [%] denotes % of dose administered.

[c] Carcass denotes the sum of all organs if not all organs were individually analysed.

[d] Small intestine, large intestine and stomach were summed.

[e] only ileum.

[f] 25 μg/kg bw.

[g] 75 μg/kg bw.

other organ pathologies in rodents and pigs. Thus, for human risk assessment purposes, a detailed understanding of the actual MC exposure scenario becomes crucial for allowing any kind of extrapolation from the animal data presently available.

Tumour promotion

There has been some evidence of tumour promotion in animal studies, mostly presented in the reviews by Duy et al. (2000) and Kuiper-Goodman et al. (1999). Briefly, when MC-LR was applied to mice i.p. 100 times over 28 weeks, a dose as low as 20 μg/kg bw produced neoplastic liver nodules. These neoplastic lesions appeared to persist 2 months after cessation of exposure (Ito et al., 1997). Conversely, when given 100 times p.o. at a dose of 80 μg/kg bw over a period of 28 days, the mice presented with neither liver damage nor neoplastic nodules. Furthermore, Sano et al. (2004) treated mice for 14 months with i.p. injections of MC-LR and Dhb-MC-LR (0, 12.5 and 25 μg/kg bw, once a week), without previous initiation with a DNA damaging compound. While a dose-related increase in hepatic adenomas and adenocarcinomas was observed with MC-LR, no such increase was observed with the Dhb-MC-LR. It is interesting to note in this context that Dhb-MC-RR was demonstrated to inhibit protein phosphatase 2a less effectively than MC-LR, MC-RR and D-Asp-MC-RR (Schmid et al., 2004). Sano et al. (2004) also determined the generation of 8-OHdG in the liver of mice and observed similar if not higher amounts of 8-OHdG levels in mice treated with Dhb-MC-LR than in mice treated with MC-LR. These findings suggest that the generation of reactive oxygen species and the resulting oxidative DNA damage and transient DNA strand breaks may not be as important in the generation of liver tumours as proposed (Bouaicha and Maatouk, 2004a, 2004b; Zegura et al., 2003) but rather that the inhibition of protein phosphatases plays the central role in MC liver tumourigenesis.

From a risk assessment stand-point, it is important to understand whether MC-LR is biologically available or not, especially when given as a part of the daily food ration. Indeed, in a modified two-stage carcinogenesis mouse skin bioassay, *Microcystis* extract consumed in drinking water appeared to act as a skin tumour promoter and to induce pronounced liver damage (Falconer, 1991). This highlights the importance of MC bioavailability for the induction of chronic toxicity and tumour promotion. In another two-stage carcinogenicity bioassay, groups of 9–15 seven-week-old male Fischer 344 rats were initiated by i.p. injection with diethylnitrosamine (DEN) (200 mg/kg bw), followed by partial hepatectomy at the end of the third week. Tumour promotion was assessed by intraperitoneal injection of MC-LR at 1 or 10 μg/kg bw twice per week from the third week of the experiment. 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 MC-LR at 10 μg/kg bw (Nishiwaki-Matsushima et al., 1992). MC-LR had no effect when given to non-initiated rats. Furthermore, treatment of initiated rats with 1 μg/kg bw did not provide for any significant increase of (GST-P) positive liver foci. To confirm the tumour-promoting activity of MC-LR, the same authors administered MC-LR at a dose of 10 μg/kg bw before partial hepatectomy and 10, 25 or 50 μg/kg bw twice a week after partial hepatectomy to groups of 14–19 male rats. It was found that the increase in GST-P-positive foci following repeated i.p. injections of MC-LR was dose-related. According to the authors, the results suggested MC to be a strong liver tumour promoter. The latter findings were corroborated by Charbonneau et al. (2004), who used aged rats in a similar exposure protocol: a single i.p injection of 200 mg DEN / kg bw 2 weeks prior to MC treatment for an additional 7 weeks with a partial hepatectomy at week 3. As only difference in the Charbonneau study, MC-LR was applied via oral gavage for 7 weeks at doses of 10, 40 and 80 μg/kg bw, or 3 times per week with 10 μg/kg bw via i.p. injection. Increased GST-P-positive foci were observed at the end of the study (67 days) at a dose of 80 μg/kg bw by gavage and 10 μg/kg bw via i.p. injection, demonstrating that chronic oral application of MC-LR can promote preneoplastic lesions in the liver of aged Sprague–Dawley rats. Furthermore, the comparison between the rat and the mouse studies (Charbonneau et al., 2004; Ito et al., 1997; Nishiwaki-Matsushima et al., 1992) strongly suggest that mice may either be generally much less susceptible to oral uptake of MC-LR and thus, to chronic liver damage and tumour promotion than rats or that bioavailability as well as the distribution and elimination pathways and kinetics of the toxin play a much greater role than previously suspected.

Genotoxicity

MCs, primarily MC-LR, have been tested in the routine type of genotoxicity assays. The results of these assays strongly suggest that moderate to high concentrations of MC-LR are not directly genotoxic (Bouaicha and Maatouk, 2004a; Fessard et al., 2004; Zegura et al., 2003), however, do promote the generation of reactive oxygen species and subsequently lead to oxidative DNA damage and transient DNA strand breaks (Zegura et al., 2003; Bouaicha and Maatouk, 2004a, 2004b). Consequently a tumour initiating capacity of MC-LR has been suggested. This view was corroborated by the findings of Sano et al. (2004), who expressed the opinion that MC-LR induces lipid peroxidative reactions and thus, oxidative DNA damage (mainly 8-OH-dG) but not DNA adducts. A study by Lankoff et al. (2004) suggested that MC-LR-induced DNA damage may be related to the early stages of apoptosis due to cytotoxicity but not genotoxicity and that MC-LR reduces the capability of DNA repair in human peripheral

lymphocytes following UV damage. However, due to the use of relatively high doses of MC-LR, the relevance of these results is somewhat questionable.

Microcystins: organ specificity/distribution and elimination

The fact that MCs are termed "hepatotoxins" by many researchers in the field has biased the understanding of organ distribution toward the notion that primarily, if not exclusively, toxicity would and could only occur in the liver. Most acute intoxications, whether in animals or humans, do indeed present with liver pathology (Azevedo et al., 2002; Beasley et al., 2000; Falconer et al., 1981; Fawell et al., 1999; Fischer and Dietrich, 2000a; Fischer et al., 2000b; Harding et al., 1995; Hooser, 2000; Hooser et al., 1991; Mez et al., 1997; Pouria et al., 1998; Puschner et al., 1998). However, despite that the organ distribution studies reported to date are somewhat controversial, due to the variant application modes and the use of different MC congeners (Table 1), most studies have clearly demonstrated that, for example, MC-LR can be also found in the kidneys and the brain. This is not only corroborated by the fact that MC protein adducts can be found in several organs, for example, kidneys, brain, muscle (Fischer et al., 2000a), but also by the pathological and symptological evidence for renal and/or neurological damage in animals and humans (Azevedo et al., 2002; Beasley et al., 2000; Fischer and Dietrich, 2000a; Krienitz et al., 2003; Milutinovic et al., 2002; Nobre et al., 1999, 2001; Pennycott et al., 2004). A most likely explanation for the assumption that primarily hepatotoxicity would occur is the fact that it was realised very early on that MCs cannot permeate across cell membranes but are transported actively into hepatocytes via organic anion transporters (Runnegar et al., 1991), for which bile acid salts (cholate and taurocholate) amongst others, are the "natural" substrates (Frimmer and Ziegler, 1988; Meier, 1996; Meier and Stieger, 2002; Meier et al., 1997; Takikawa, 2002). However, these organic anion transporters are not only expressed in the liver but also in the gastrointestinal tract, the kidney and the brain (blood–brain barrier) (Craddock et al., 1998; Hagenbuch and Meier, 2003; Kullak-Ublick et al., 1998; Kusuhara et al., 1999; Nobre et al., 1999). Indeed, the most recent evidence strongly suggests that MC-LR can be transported across the human blood–brain barrier (Fischer et al., 2004), thus potentially explaining some of the observed neurological symptoms observed in the fatal incident at the renal dialysis station in Cuaruaru, Brazil (Azevedo et al., 2002; Pouria et al., 1998). It may be assumed at present that the organ distribution of MCs is governed by the presence/absence, type and expression level (number of functional transporters per cell) of organic anion transporters. As saturation kinetics have only been carried out with ^{3}H-dihydro-MC-LR, this implies that similar experiments should be carried out with a representative subset of the nearly 80 different MC congeners known to date in order to gain a better understanding of the distribution and elimination kinetics of these toxins. Indeed, when using different epimers of ^{3}H-dihydro-MC-LR, Meriluoto et al. (1990) found one of the epimers to be taken up and distributed across the different organs three to four times faster than the other epimer. This strongly suggests that minimal structural changes in the MC molecule can have major implications for the uptake, organ distribution and excretion kinetics, which are most likely governed by the affinity of the respective MC for the organic anion transport proteins. Furthermore, the analysis of MC metabolism has demonstrated that MCs are primarily conjugated and then excreted either as parent compound or conjugate via the bile or the urine (Dahlem et al., 1989; Falconer et al., 1986; Hermansky et al., 1991; Kondo et al., 1992; Milutinovic et al., 2002; Nishiwaki et al., 1994; Robinson et al., 1989, 1991; Takenaka, 2001), probably involving the same organic anion transport proteins. Of importance is also that after moderate liver damage, for example, in MC-LR-dosed pigs, a reduced MC-LR clearance can be observed in the blood, resulting in an extended availability of MC-LR for uptake into other organs such as the kidneys (Stotts et al., 1997). Consequently, pathological changes are observed in those organs. This may be of importance especially in the chronic exposure scenario, where humans with impaired liver function or lowered expression of organic anion transporters in the liver could display a longer blood half-life of MCs and could thus be prone to a higher risk for kidney or brain pathology.

When comparing the blood half-lives of MC in different species, quite clearly a congener-dependent effect is evident. Indeed, while a plasma half-life of ^{125}I-MC-YM of 42 min (i.v. application) was reported for adult female rats (Falconer et al., 1986), an application of ^{3}H-MC-LR (i.v. in rats) resulted in a half-life of 70 min (Pace et al., 1990). In contrast, an i.v. application of ^{3}H-MC-LR in mice resulted in a plasma half-life of 6.9 min (Robinson et al., 1991), thus strongly suggesting that for MC plasma half-life species-specific effects may be as important as the MC-structure. In view of these species and congener specificities, a better understanding of congener-specific MC kinetics in general, and especially in humans, appears crucial for an improved assessment of risk in a chronic exposure setting.

The principles of assessing human health risks applied to microcystins

In order to highlight the possible problems in the risk assessment of MCs, the current knowledge on MCs is put into context with the principles and type of information employed for assessing human health risk of chemicals

(IPCS, 1995), by first describing the techniques underlying the risk assessment process followed by a discussion of possible weaknesses in the MC data set.

The aim of the IPCS risk assessment process is to derive a tolerable daily intake (TDI) of a compound for various routes of exposure for effects considered to have a threshold. "The TDI is defined as an estimate of the intake of a substance over a lifetime that is considered without appreciable health risk. Its units depend on the route of administration (e.g., mg/l water; mg/kg food, etc.)". Within this context, estimation of the TDI involves the application of uncertainty factors, generally to the no-observed-adverse-effect-level (NOAEL), for critical effects in the most relevant study or studies (Fig. 1). Of essence with regard to MCs is the fact that the IPCS document emphasises that a TDI can only be developed on the basis of a NOAEL "for substances where the critical effect is considered to have a threshold (including non-genotoxic carcinogenesis for which there is adequate mechanistic data)" (IPCS, 1995). Although MC-LR was shown not to have genotoxic

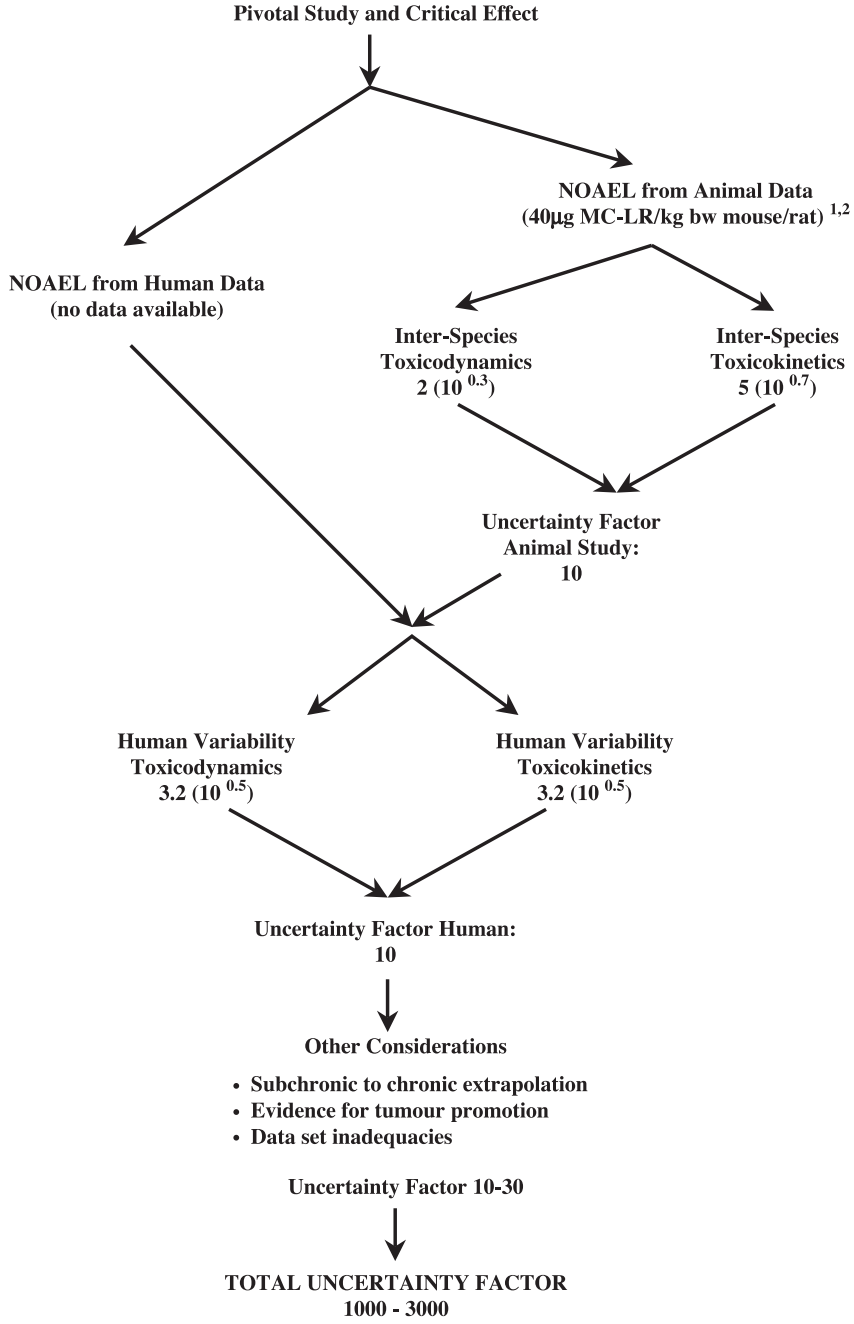

Fig. 1. Procedures for the derivation of uncertainty factors for the development of a tolerable daily intake (TDI) of microcystin-LR (adapted from IPCS, 1995. 1: Fawell et al., 1994, 1999; 2: Charbonneau et al., 2004).

activities and a threshold (NOAEL) of 40 μg MC-LR/kg bw for the generation of liver preneoplastic lesions was observed in mice and rats (Fawell et al., 1999; Charbonneau et al., 2004), the mechanistic understanding of MC-LR-induced liver tumours and the associated data appears at present far from adequate. Despite this and in view of the fact that both the mouse and rat data for MC-LR demonstrated a NOAEL of 40 μg MC-LR/kg bw for chronic liver toxicity and preneoplastic lesions in the liver, it appears reasonable to use this data to derive a TDI for MC-LR (Fig. 1). Although the mouse to rat NOAEL and acute toxicity comparison a priori would not indicate large species differences in toxicodynamics (uncertainty factor 2), differences in the kinetics due to variant expression of organic anion transporters (uncertainty factor 5) suggest that a total uncertainty factor of 10 is advisable. In case of human (intra-species) variability, equal uncertainty (total uncertainty of 10) was attributed to dynamics and kinetics, especially as in vitro assays with primary hepatocytes indicated similar if not higher susceptibility of human hepatocytes to MC-LR than rat primary hepatocytes (Batista et al., 2003) and human organic anion transporter analysis has suggested a higher transport in human than in rat liver (Fischer et al., 2004). These uncertainty factors should also account for potential enzyme polymorphisms (e.g., glutathione transferase isoenzymes (Landi, 2000; Habdous et al., 2004)) that could substantially alter the metabolism and excretion of MC-LR. Finally, an additional uncertainty factor of 10–13 can be applied for additional issues, for example, inadequacies in the durations of the pivotal studies (subchronic to chronic extrapolation), tumour promotion and lack of appropriate developmental studies. The degrees of uncertainty attributed to these other considerations have been intensively discussed (Table 1) and have led to diverse opinions, some considering an uncertainty factor of 10 (total of 1000) to be sufficient (Falconer et al., 1994; Gilroy et al., 2000), while others support a factor of 10 plus an additional factor 3 (total of 3000) for tumour-promoting activity (Duy et al., 2000). Some agreement is found in the literature suggesting that the total uncertainty factor to be applied to the data set should range between 1000 and 3000, although this difference would amount to a TDI of 0.013–0.04 μg MC-LR/kg bw/day (Eq. (1)). The provisional WHO-guideline (WHO, 1998) for drinking water as well as the Oregon Health Division (Gilroy et al., 2000) for BGAS currently use 0.04 MC-LR/kg bw/day as the TDI for calculation of guidance values (Table 2).

$$TDI = \frac{NOAEL \text{ or } LOEL}{UF} = TDI(MC-LR) = \frac{40\ \mu g/kg\ bw}{1000(3000)} = 0.04(0.013)\mu g/kg\ bw \quad (1)$$

TDI: Tolerable Daily Intake; NOAEL: Nxo Observed Adverse Effect Level; LOEL: Lowest Observed Effect Level; UF: Uncertainty Factors.

In a second step, the proportion of total intake that originates from various sources of exposure (e.g., drinking water, food, etc.) is estimated, based on exposure estimates from a consistent set of assumed volumes of intake and representative concentrations in the general environment, for a given situation. These proportions, often termed "allocation factors", are then multiplied by the body weight of an "international unit adult" (60 or 70 kg) and the TDI and divided by the daily intake of the source (water, food, etc.) thus providing a "guidance value" (GV) for the maximal acceptable concentration (MAC) in a given exposure source (Eq. (2)).

$$GV(MAC) = \frac{TDI \times bw \times AF}{C} \quad (2)$$

GV: guidance value (e.g., μg MC-LR/l water, kg food or g BGAS); MAC: maximal acceptable concentration; bw: body weight of an "international unit adult" (60 or 70 kg); AF: allocation factor, or percentage of the total exposure from a given source; C: amount of daily exposure from a given source (e.g., 2 l drinking water, 0.1 kg fish, 2 g BGAS).

Thus, using the TDIs calculated above and varying bodyweights for adults and children, diverse guidance values (GV) are derived for various sources of exposure, for example, water and food (Table 3). The guidance values largely depend on how strongly the individual experts evaluated the degree of uncertainty in the data sets at hand. However, from the presently available data, it can be stated that the toxicity and carcinogenicity data on MC-LR are the best available, while all other data sets have strong confounders (Table 2). Of utmost importance with regard to actual exposure and hence risk determination is the paucity of data with regard to MC congeners other than MC-LR. Especially congener-specific uptake and distribution, as discussed above, may strongly govern the subsequent toxicodynamics and perhaps most importantly the development of chronic toxic effects and tumour promotion. In addition, as the current TDI was based on the occurrence of chronic liver toxicity or the presence of preneoplastic lesions in the liver, other apical endpoints, for example, neuropathy or nephropathy may be severely underestimated. Furthermore, it is at present unclear how best to incorporate the simultaneous occurrence of several different MC congeners into a risk assessment framework. One possibility would be to deal with the MC congener mixtures as was established for dioxins and PCBs in that a toxicity equivalent approach was taken while including kinetic determinants of each congener for the derivation of equivalents. This would however assume additivity of toxicity of the various congeners and demand the establishment of the individual kinetic values for many if not all of the MC congeners. Irrespective of these caveats, the TDIs developed for MC-LR should be employed to derive guidance values for various exposure scenarios and sources.

Table 2
Studies on MC toxicity after oral administration with food or drinking water

	Test organism	Administration of	Duration	NOAEL	Uncertainty Factors (UF)					Sum of UF	Calc. TDI	Guideline value
					Intraspecies variability	Interspecies variability	TPC	Lack of data on chronic toxicity	LOEL to NOAEL			
Falconer et al., 1988, Carmichael and Falconer, 1993	mice	*Microcystis sp.*	1 year	500	10,000					10,000	0.05	1.5 µg/l[a]
Falconer et al., 1994	pigs	*M. aeruginosa* extract	44 days	280 (LOEL)	10	10	–	–	10	1000	0.28	8.4 µg/l[a]
							10		10	10,000	0.028	0.84 µg/l[a]
Fawell et al., 1994, 1999	mice	MC-LR	13 weeks	40	10	10	10		–	1000	0.04	0.96 µg/l[b,c]
Schaeffer et al., 1999	mice	BGAS	43 days	333	1000					1000	0.333	10 µg/g[d]
Duy et al., 2000	Based on data of (Fawell et al., 1999) study			40	10	10	3	10	–	3000	0.0133	0.32 µg/l[c] 0.11 µg/l[e] 0.07 µg/l[f]
	Based on data of (Falconer et al., 1994) study			186	10	10		10	5	5000	0.0368	0.88 µg/l[c] 0.29 µg/l[e] 0.20 µg/l[f]
Kuiper-Goodman et al., 1999	Based on data of (Falconer et al., 1994) study			100	10	3		10	5	1500	0.067	1.0 µg/l[c]
Gilroy et al., 2000	Based on data of (Fawell et al., 1999) study			40	10	10	10		–	1000	0.04	1.0 µg/g[d]

Data from Fawell et al. (1994, 1999) together with data from Falconer et al. (1994) form the basis for the WHO provisional guideline for MC-LR in drinking water of 1.0 µg/l. Australia, Brazil, Canada, France, the Czech Republic, Poland, Spain and New Zealand assumed this guideline value, partly with slight changes due to different assumptions concerning the average body weight of consumers and the average daily consumption of drinking water.
[a] 60 kg; 2 l/day; allocation factor (AF) 1 (drinking water).
[b] Calculation by WHO (WHO, 1998).
[c] 60 kg, 2 l/day, AF 0.8 (drinking water).
[d] 60 kg; 2 g/day; AF 1 (BGAS).
[e] 10 kg, 1 l/day, AF 0.8 (drinking water).

Microcystin: risk assessment and guidance values for various exposure scenarios

Microcystins: main routes of human contact (exposure scenarios)

In order to evaluate the potential risk associated with MC exposure in humans, various exposure scenarios need to be distinguished:

I. accumulation of cyanobacterial toxins in the food chain, for example, contaminated food (e.g., lettuce) after irrigation with toxin-rich water, cyanobacterial blooms in rice fields and MCs accumulated in fish, crayfish and shellfish.
II. dermal, nasal or oral (accidental ingestion) contact during recreational use of water.
III. drinking water and intoxication during hemodialysis.
IV. involuntary exposure via contaminated blue-green algal food supplements (BGAS).

The order of importance (exposure) of the individual routes varies between countries and largely depends on factors such as climatic conditions, eating habits of the local population, drinking water source and the drinking water treatment in the individual regions, and last but not least, the economical affluence of the population in question (e.g., North America, Western Europe) that allows spending vast amounts of funds for seemingly healthy and health-promoting bio-products (Willett et al., 2004), of which

Table 3
Daily tolerable total microcystin-LR exposure based on two different TDIs, concurrent calculations of guideline values (GV) for food (fish: 0.1 kg day) and water (0.75 l/day for infants, 1.0 l/day for children; 2 l/day for adults)

	TDI (MC-LR/kg bw/day)	GV MC-LR/d exposure (μg)	GV MC-LR/kg food (μg)	GV MC-LR/l drinking water (μg)
Infants	0.04	0.20	0.40	0.21
5 kg	0.013[a]	0.065	0.13	0.07
Children	0.04	0.80	1.60	0.64
20 kg	0.013[a]	0.26	0.52	0.21
Adults	0.04	2.40	4.80	0.96
60 kg	0.013[a]	0.78	1.56	0.31
Adults	0.04	2.80	5.60	1.12
70 kg	0.013[a]	0.91	1.82	0.36

The allocation factor (AF) for food was estimated as 0.2 in case of primarily fish and shellfish consuming populations in contaminated areas; the allocation factor for drinking water was estimated as 0.8.
[a] This TDI includes an additional uncertainty factor of 3 for tumour promoting capacity, i.e. a total uncertainty factor (UF) of 1300).

blue-green algae supplements (BGAS) are an economically important representative. The potential exposure routes are listed here in order of perceived increasing importance, although it must be noted that exposure routes III and IV at present appear to be of equal importance, when considering the risk of acute and chronic exposure to potentially toxic/carcinogenic concentrations of MCs.

I. MCs are known to be taken up by commercially cultivated plants such as lettuce (*Lactuca sativa*) (Codd et al., 1999) and the common bean (*Phaseolus vulgaris*) (Abe et al., 1996), provided that the toxins are present in the irrigation water or the growing media. As some cyanobacteria can fix nitrogen from the atmosphere and hence provide a valuable nitrogen source for the growing rice plants after lysis of the cyanobacterial cells, cyanobacteria are welcome in rice fields (Rahman et al., 1996). However, little is known about the uptake mechanism(s) of MCs into plants and the concentration of toxic cyanobacterial compounds in rice fields. Thus, the level of contribution to the overall exposure of humans to cyanobacterial toxins and hence the risk for human health arising from these sources is presently difficult to estimate. MCs (and other cyanobacterial toxins) can also accumulate in fish, crayfish and shellfish (Ernst et al., 2000, 2001; Kankaanpää et al., 2004; Magalhaes et al., 2001, 2003; Mohamed et al., 2003; Vasconcelos, 1999) with maximum MC-concentrations of 300 μg/kg in the edible parts of fish, 2700 μg/kg in crayfish and 16000 μg MC-LR/kg in mussels. At present, little data exist concerning the accumulation of cyanobacterial toxins in livestock (e.g., cattle, swine, sheep). It is known that, for example, in Australia, the USA and alpine regions of Switzerland (Beasley et al., 1983, 1989a, 1989b; Mez et al., 1997) livestock may be frequently exposed to MCs and other cyanobacterial toxins via consumption of water contaminated with cyanobacteria. However, current studies suggest no carry-over of MCs into milk (Orr et al., 2001) or meat (Orr et al., 2003) in cows after oral application of toxic *M. aeruginosa* via drinking water.

II. A number of the toxin producing cyanobacteria demonstrate mass development during late spring, summer and early autumn months, that is, during the time many people use water bodies for recreational purposes. In 2% of 128 samples from recreation sites near Berlin, Germany, more than 100 μg MC-LR equiv./l water could be detected, 74% contained >1.0 μg MC-LR equiv./l (Fromme et al., 2000). It has generally been found that 50–75% of bloom isolates are capable of producing toxins, with often more than one toxin (e.g., MCs and anatoxin-a) and several MC congeners being simultaneously produced. Concentrations of more than 24,000 μg MCs/l have been reported from the shores of the Havel River, Berlin, Germany (Fastner et al., 1999) and Lake Akersvatn, Norway (Berg et al., 1987). Germany could exemplify for the widespread occurrence of toxic cyanobacterial blooms as documented in the literature (Wiedner et al., 2001). In addition, the overall toxicity of a bloom cannot be defined because of variations in toxin concentration temporally and spatially within a water body experiencing the bloom. There may be also large year-to-year fluctuations in the levels of cyanobacteria and their toxins (Hoeger, 2003; Hoeger et al., 2004b; Park et al., 1993) and seasonal variations with regard to the dominant species of toxic cyanobacteria and hence, the toxins present (Henriksen and Moestrup, 1997; Hoeger et al., 2004b; Xu et al., 2000).

III. Of all exposure scenarios, drinking water is, in a worldwide view, the main source for the incorporation of cyanobacterial toxins for humans. The published cases of cyanobacterial toxins in raw water, during drinking water treatment and even in finished water are numerous and problematic worldwide. MCs have been reported in final water in Argentina, Australia, Bangladesh, Canada, Czech Republic, China, Finland, France, Germany, Latvia, Poland, Thailand, Turkey, Spain, Switzerland and USA (Westrick, 2003; Hoeger et al., 2004a, 2004b). Up to 9000 cells/ml were detectable even after treatment in Argentina, Australia, Finland, Germany, Israel and Italy. It must be assumed that this is only the tip of the iceberg, especially as water works are not primarily interested in publishing reports regarding toxins or cyanobacterial cells in their raw or final water. In many cases worldwide, the drinking water is simply not screened for cyanotoxins. In addition, more than one billion people have no access to “treated” drinking water, in many cases,

the drinking water is simply boiled, which does not destroy most of the known cyanobacterial toxins. In fact, most of the people affected by gastroenteritis at Itaparica dam/Brasilia (Teixera et al., 1993) boiled the drinking water before use. It is likely that the boiling actually exacerbated the problem by causing lysis of the cyanobacterial cells resulting in toxin release. In a sugar refinery in Scania, Sweden, the drinking water distribution system was erroneously coupled to untreated river water (Annadotter et al., 2001). Coincidentally, a high density of *P. agardhii* occurred in this river and the water contained approximately 1.0 μg MC-LR equiv./l. In the following days, 121 persons, who consumed the contaminated water, developed numerous symptoms including diarrhoea, headache, vomiting, fever and muscular and abdominal pain. Pathogenic bacteria or viruses could be excluded as the cause for the illnesses. As another interesting fact, 100% of the tea-drinkers of the sugar refinery were sick in the days following the accident, while none of the coffee-drinkers were affected. It is likely that the toxins were released from the cells by boiling the water and, in the case of the coffee-drinkers, filtered out by the use of a coffee filter and the coffee grinds. The tea-drinkers just put the teabag in the boiled water and consumed the whole cocktail of heat-resistant cyanobacterial metabolites. Thus, it can be assumed that cyanotoxins at least participated in the observed symptoms. Other cases of human illnesses are described in the literature, where cyanobacterial toxins are suspected to be at least involved in the intoxication of the affected population (de Olivera Araújo, 1995; Falconer et al., 1983; Yu, 1995; Zilberg, 1966). However, intoxication may not only occur via consumption of drinking water, but also through hemodialysis as was reported from Portugal (Pereira et al., 2000), USA (Hindman et al., 1975) and Brazil (Azevedo et al., 2002; Pouria et al., 1998), where the severe intoxication and death of patients were most likely caused by intravenous exposure to cyanobacterial toxins.

IV. Several regions worldwide such as Mexico, northern Africa and China have a history of the use of blue-green algae (*Spirulina* and *Nostoc* spp.) as a food source (Carmichael et al., 2000; Jensen et al., 2001). Nowadays, blue-green algal supplements (BGAS), mainly products of *Aphanizomenon flos-aquae* and *Spirulina* spp., represent an important economic branch (Carmichael et al., 2000), while being sold mainly in the industrialised countries. These supplements are commonly consumed for their putative beneficial health effects, for example, increased alertness, increased energy, "detoxification", elevated mood and weight loss (Jensen et al., 2001). Moreover, some of the products are specifically marketed for use by children as a replacement or alternative for the pharmacological therapy of Attention Deficit Hyperactivity Disorder (ADHD) (Lindermann, 1995). Although the providers of *Aphanizomenon flos-aquae* based BGAS state that they screen out MC-levels of more than 1.0 μg/g dw in their products (Carmichael et al., 2000), independent investigations into the MC contamination of BGAS products have demonstrated toxin concentrations of up to 35 μg/g dw (Gilroy et al., 2000; Lawrence et al., 2001). Although samples with toxin contaminations of more than 10 μg MC-LR equivalents/g dw are the exception, 8 of 13 blue-green algae products from the German and Swiss markets tested recently have shown more than 1.0 μg MC-LR equiv./g DW in concurrent analyses (Hoeger and Dietrich, 2004) carried out with an Adda-ELISA (Fischer et al., 2001) and cPPA (Heresztyn and Nicholson, 2001). Our own studies (Hoeger and Dietrich, 2004) and the study of Lawrence et al. (2001) have shown differences in detectable toxin amounts when employing ELISA, PPA and LC-MS/MS. These differences appear to stem from the lack of certified standards for 5 to 10 of the MC congeners commonly detected in BGAS, but may also be due to some differences in the MC congener cross-reactivity of some of the respective antibodies in the ELISAs employed. Thus, the values obtained can only be taken as an estimation of the MC content in BGAS. Within this context it is also important to understand that not all *Aphanizomenon flos-aquae*-based BGAS show high levels of MCs (above 1.0 μg MC-LR equiv./g dw) and that the levels of MCs in a given brand name can vary extensively from batch to batch (Gilroy et al., 2000; Hoeger and Dietrich, 2004).

Table 4
Daily tolerable total microcystin-LR exposure based on two different TDIs, concurrent calculations of guideline values (GV) for different blue green algal supplements (BGAS)—doses taken per day

	TDI (MC-LR/ kg bw/day)	GV MC-LR/d exposure (μg), daily consumption 1 g	GV MC-LR/g dw BGAS (μg), daily consumption 2 g	GV MC-LR/g dw BGAS (μg), daily consumption 10 g
Infants	0.04	0.20	0.10	0.02
5 kg	0.013[a]	0.065	0.03	0.0065
Children	0.04	0.80	0.40	0.08
20 kg	0.013[a]	0.26	0.13	0.026
Adults	0.04	2.4	1.20	0.24
60 kg	0.013[a]	0.78	0.39	0.078
Adults	0.04	2.8	1.40	0.28
70 kg	0.013[a]	0.91	0.46	0.091

The allocation factor (AF) for BGAS was estimated as 1.
[a] This TDI includes an additional uncertainty factor of 3 for tumour promoting capacity, that is, a total uncertainty factor (UF) of 3000.

Table 5
Calculated possible daily ingestion to avoid acute health problems according to the calculations of Fromme et al. (2000)

Ingestion route	MC concentrations	Infants 5 kg = 12.5µg	Children 20 kg = 50 µg	Adults 60 kg = 150 µg
Food (1)	100 µg/kg	125 g	500 g	1500 g
	10,000 µg/kg	1.25 g	5 g	15 g
Cyanobacterial bloom in lake/river (2)	100 µg/l	125 ml	500 ml	1500 ml
	1000 µg/l	12.5 ml	50 ml	150 ml
Drinking water (3)	1.0 µg/l	12,500 ml	50,000 ml	150,000 ml
	100 µg/l	125 ml	500 ml	1500 ml
BGAS (4)	1.0 µg/g	12.5 g	50 g	150 g
	10 µg/g	1.25 g	5 g	15 g

Therefore, the lowest dose with no hepatotoxic effects after i.p. injection was chosen (25 µg/kg bw, (Kotak et al., 1993), multiplied by 10 for i.p. to p.o. extrapolation, divided by 10 for inter- and 10 for intra-species differences, ergo 2.5 µg/kg bw). The MC concentrations of the individual ingestion routes are based on literature information and discussed in the text. (1) Magalhaes et al., 2003; Mohamed et al., 2003; Vasconcelos, 1999; (2) Fastner et al., 1999; Fromme et al., 2000; Ueno et al., 1996; (3) Burns, 2004; Hitzfeld et al., 2000a,b; Hoeger et al., 2004a; Westrick, 2003; (4) Gilroy et al., 2000; Hoeger and Dietrich, 2004; Lawrence et al., 2001.

Acute intoxication scenario

The harmful concentrations for an acute intoxication with the most abundant cyanobacterial toxin in freshwater can be calculated as follows (Fromme et al., 2000): On the basis of mouse studies (Hooser et al., 1989; Kotak et al., 1993; Fawell et al., 1994; Yoshida et al., 1997), it can be assumed that a single intake of 12.5, 50 or 150µg MC-LR equiv. should not have adverse effects in toddlers (5 kg), young children (20 kg) and adults (60 kg), respectively. Both groups of children appear to be the age cohorts with the highest risks, because the MC levels are—as a result of the relatively low weight—within a range, which could be reached in different scenarios (summarised in Tables 3–5):

I. It is possible and in some regions worldwide the normal case that children consume more than 0.1 kg fish or shellfish per day (Mohamed et al., 2003). Calculated with the actual contaminations of fish and shellfish reported by Vasconcelos (1999), Magalhaes et al. (2001, 2003) and Mohamed et al. (2003) with MC-levels of up to 300 µg/kg edible fish, 2700 µg/kg crayfish and 16000 µg/kg mussels, it becomes obvious that in some regions worldwide, a risk for an acute poisoning of children through MCs in fish and shellfish exists (Table 5).
II. Children would most likely be playing and bathing in the shallow areas of surface waters where the waters are contaminated with MC- concentrations >100 µg/l and consumption of 125 to 500 ml of this highly contaminated water could already potentially result in an acute intoxication (Table 5). Infants playing in shallow water with a high density of perhaps decomposing cyanobacterial bloom could also potentially be highly endangered with respect to an intoxication with MCs, as bloom material may contain > 1000 µg MC-LR equiv./l (Ueno et al., 1996).
III. It is unlikely, that concentrations of 25 µg MC-LR equiv. or higher are incorporated via drinking water by children. However, concentrations of up to 100 µg cylindrospermopsin, another highly potent cyanobacterial toxin, per litre final drinking water in Florida, USA (Burns, 2004), and the cases of death in Itaparica, Brasil ((Teixera et al., 1993), see above) show that there may be a further risk of an acute intoxication (Table 5).
IV. Infants and children are one of the target groups of BGAS, which contain up to 35 µg MC-LR equiv./g dw (Lawrence et al., 2001). But even if the MC concentration is distinctly lower (1–10 µg/g), overzealous parents may potentially intoxicate their child, if they administer several grams of the BGAS daily (Table 5). Indeed, consumption of up to 20 g/day have been reported in case of an adult (Gilroy et al., 2000).

The above calculation is fraught with difficulties as it is presently unclear whether humans absorb MCs via the GI tract in amounts comparable to mice. However, it also should be considered that mice may be less prone to oral MC intoxications due to uptake limitations (see above), suggesting a potential for oral toxicity underestimation. Furthermore, mixtures of MC congeners may dictate different uptake situations. Thus the 12.5, 50 and 150 µg MC-LR for toddlers, young children and adults as single doses can only serve as a rough approximation.

Subchronic to chronic intoxication scenario

I. The actual exposure of humans to MCs via food is rather difficult to estimate. Indeed, there is no general rule as to how much fish, shellfish, salad, rice, etc. is consumed daily per “international adult”. Thus, the guidance values for MC contamination of foods must be calculated based on the local proclivities in food consumption and consequently must be carried out by the authorities of the individual country. For the purpose of this review, the guidance values for MC-LR contamination of fish and shellfish were set according to the TDI calculated in Eqs. (1) and (2) (Table 3). From these calculations, it becomes obvious that children, as mentioned in the acute risk scenario, bear the highest risk of daily MC exposure. Additionally, some local populations largely dependant on one type of food source (e.g., fish, shellfish or crustaceans, rice) may be exposed either occasionally or chronically to high concentrations of MCs. However, in a worldwide view, the risk of uptake of MCs from contaminated foodstuffs appears low, at least for the general public.
II. High and therefore visible densities of cyanobacteria should prevent most people from swimming in such

"contaminated" water bodies. However, according to correspondence with colleagues from all over the world, sometimes even major cyanobacterial blooms do not prevent water bodies from being used for recreational purposes. The possible ingestion of cyanobacterial toxins via this route is limited to the summer and fall season in more temperate climates. In contrast, in tropical regions a non-seasonal (all-year round) exposure of swimming and playing children is a distinct possibility. In several countries, warning signs have been installed at water bodies used for recreation to inform the public about the potential danger. However, these warning signs should not simply ban bathing (and the public do not know the reason), detailed information about the reason for the ban and the possible consequences of ignoring of the recommendation for humans and animals should be listed. This would enhance the success of these warning signs.

III. Contrary to the situation for food or water intake, where a natural limitation can be assumed, daily consumption of BGAS are largely dependent on the individual (0.25–20 g) (Gilroy et al., 2000; Schaeffer et al., 1999). In addition, water and food are usually consumed together, that is, in a meal, providing for a different gastro-intestinal uptake scenario than for BGAS, which can be taken on an empty stomach. Thus, one can assume that self-medication or medication of children by their parents with BGAS is a scenario distinct from the typical variant exposure source scenario underlying the risk assessment calculation as foreseen in the IPCS (1995) documentation for the extrapolation of risk to humans from chemicals. Indeed, the BGAS uptake scenario is much more reminiscent of the administration of therapeutic compounds and should thus be treated entirely differently from the usual food and water-risk calculations. Similarly to the provisional WHO-guideline for drinking water, Gilroy et al. (2000) calculated a TDI of 0.04 μg MC-LR/kg day based on the MC-LR mouse NOAEL of 40 μg/kg day defined by Fawell et al. (1999) and the application of a total of 1000-fold uncertainty factor (Table 4), which resulted in a provisional tolerable level for MCs in BGAS of 1.0 μg MC-LR/g dry weight. This level was adopted by the Oregon Health Division as a provisional regulatory standard for BGAS products on 23/10/1997. However, as this provisional tolerable level was calculated for an adult only, children (e.g., 10–20 kg body weight) would be exposed to three to six times the MC-LR equivalent concentration per day. Thus, in the worst case (1.0 μg MC-LR/g dw BGAS and daily intake of 10 g BGAS), children could ingest up to a 30-fold amount of MC equivalents than that considered safe by the Oregon Health Division over weeks, months or even years. Moreover, as the levels of MCs in BGAS can exceed the provisional tolerable level for MCs in BGAS of 1.0 μg MC-LR/g dry weight by up to a factor of 10 (Gilroy et al., 2000; Hoeger, 2003; Hoeger and Dietrich, 2004; Lawrence et al., 2001), the level of safety (original uncertainty factor of 1000 by Gilroy et al. (2000)) can shrink to 3. However, if a more conservative TDI is employed the guidance value for MC-LR, even for adults of varying weight, falls below the 1 μg/g dw level even if only 2 g of BGAS are consumed per day. The guidance values for MC-LR are not only driven by the amount of BGAS taken up daily but also by the body weight of the person consuming these products on a daily basis (Table 4). As BGAS are marketed with clear intention for supplementation of infants and children with ADHD (Lindermann, 1995), Table 4 clearly demonstrates that BGAS consumed at 2 g per day, even with a residual level of 1 μg MC-LR/g dw, would exceed the TDI of infants and children by a factor 10–33 and 2.5–7.7, depending on the TDI chosen. If higher amounts are ingested daily, the TDI is exceeded by a multiple of the factors calculated for 2 g/day and in a worst case scenario (8 g BGAS per day) can exceed the TDI by a factor 40–123 and 10–31 in infants and children. The fact that several independent analyses (Hoeger, 2003; Hoeger and Dietrich, 2004; Gilroy et al., 2000; Lawrence et al., 2001; Schaeffer et al., 1999) detected more than 1.0 μg MC-LR equiv./g dw in 50–100% of the BGAS tested strongly suggests that higher exposures of consumers must be assumed than implicated by mere application of the Oregon Health Division provisional regulatory standard for these products on 23/10/1997. Indeed, these data and the comparison of guidance values calculated for BGAS applied to infants and children at various daily doses strongly demonstrate that the value of such a guidance is questionable. Clearly, if BGAS cannot be completely banned from the market, more conservative guidance values for MC content as well as a restriction for application to infants and children would be advisable if long-term health problems (chronic liver injury and potentially neuropathies and nephropathies) are to be avoided. Interestingly, a similar situation is presented in the case of seafood-derived complementary medicines in Australia, where dried shellfish meat products are self-prescribed by the patients. These shellfish capsules are suspected to contain a variety of biotoxins, for example, okadaic acid, a toxin with similar mode of action as the MCs (Llewellyn et al., 2004).

IV. Based on the knowledge that the amount of daily water consumption is naturally limited, guidance values for MC contamination in drinking water can be easily established (Tables 2,3). As already demonstrated for food intake, infants and children, although consuming less water per day, are potentially the most exposed. Thus, the guidance values for drinking water should clearly consider infants and children and not assume that differences in weight and consumption

have already been factored into the uncertainty factors of the human intra-species variability (Fig. 1) as often purported. Indeed, the situation in Itaparica (Teixera et al., 1993), where a very high number of the cyanobacterial bloom and drinking water-associated mortalities were children, lends more support to the lowering of the guidance value for MCs in drinking water. Generally, the WHO guidance value for MC-LR in drinking water of 1.0 μg/l appears aiming to evaluate the risk of MC contamination in drinking water (Table 2), although small differences are associated with the different allocation of daily water intake and body weight. Although MCs can occur in high concentrations in raw waters, proper water treatment for drinking water purposes can reduce the MC content to a minimum (Hitzfeld et al., 2000a, 2000b; Hoeger, 2003; Hoeger et al., 2004a). Consequently, MC contamination of raw water is primarily a problem in those areas where no adequate water treatment is available. Thus, the WHO guideline value for drinking water of 1 μg MC-LR/l, also reflecting the consensus amongst scientists, can be viewed as provisionally acceptable.

Uncertainties in the detection methods

Prerequisite for a guideline value is the availability of reliable detection methods. For the detection of MCs in drinking water, several methods exist and provide reliable results. Problems may arise if the matrix is complex and thus false positive and negative results can occur. The levels of MCs, which can be measured by ELISA, protein phosphatase assays as well as HPLC-DAD or MS largely depend on the preparation and detection methods used. For example, the detection of low amounts (0.5–5.0 μg/g) of MC in BGAS requires a much more complex preparation than the detection of low levels of MCs in drinking water. The detection of MCs in fish muscle or liver remains problematic. Indeed, due to the formation of covalent bonds between MCs and protein phosphatases, it is at present impossible to estimate how much of the detected MC in fish muscle or tissue is transferred within the food web, that is, is bioavailable for the next trophic level.

Overall, considering the enormous importance of proper detections of cyanotoxin contamination, the aspect of the appropriate preparation and detections systems must not be forgotten in future discussion of guideline values.

Conclusions

The data summarised in this review demonstrate that the present provisional guidance values can be exceeded dramatically, especially when using the more conservative TDI, and emphasises the importance of (i) establishment and validation of detection methods for MCs in complex matrices (ii) testing food for MC contamination and (iii) adjusting the guidance values for infants or children rather than using the "international adult" as a calculation basis. Although the standardised detection of MCs (nearly 80 congeners) is still a matter for future development and despite the detection-limitations discussed above, the current detection methods in water, foodstuffs and BGAS allow estimation of the actual MC-LR equivalent concentrations present. Consequently, a perfectioning of the sample preparation and detection methods and subsequently a more thorough monitoring of the food, water and BGAS contamination is advisable. A high degree of uncertainty remains as to the sufficiency of the uncertainty factors applied during extrapolation from animals to humans (factor 10), when considering the observed species (human–animal) differences in organic anion transporter profile (Fischer et al., 2004) and hence kinetic and dynamic dissimilarities (Batista et al., 2003). Although most acute and subchronic animal experiments have reported primarily liver pathology, some have also demonstrated nephropathy (Fischer and Dietrich, 2000a; Milutinovic et al., 2002; Miura et al., 1991; Nobre et al., 1999; Stotts et al., 1997), while the potential occurrence of neuropathy has largely been ignored, despite clinical indications in the Caruaru incident (Azevedo et al., 2002; Fischer et al., 2004; Pouria et al., 1998). The question remains to be resolved whether the endpoint for the pivotal study for human risk assessment should only include chronic liver injury (Falconer et al., 1994; Fawell et al., 1994, 1999) or the presence of preneoplastic lesions (Charbonneau et al., 2004), or whether indeed the development of an improved data base including nephropathy and neuropathy is more advisable. Furthermore, as the current risk assessment model is based primarily on MC-LR, an improved risk assessment using a MC-LR equivalents scheme, similar to the schemes already in place for dioxins and PCBs, thus including other congeners and incorporating the respective toxicokinetics and dynamic properties, would greatly reduce the degree of uncertainty and improve the risk assessment process.

Despite the above caveats, it appears that for the time being, the WHO guidance value for drinking water with 1.0 μg MC-LR/l should provide for sufficient protection of the consumer. In contrast, the application of guidance values for BGAS (Gilroy et al., 2000) appears misguided as the TDIs of infants and children, as well as adult consumers, are readily exceeded due to repeated contamination of BGAS and consumer dependent variation in daily BGAS consumption.

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