THE USE OF RAPID, SENSITIVE AND COST-EFFECTIVE METHODS FOR THE BROAD DETECTION OF MICROCYSTIN CONGENERS

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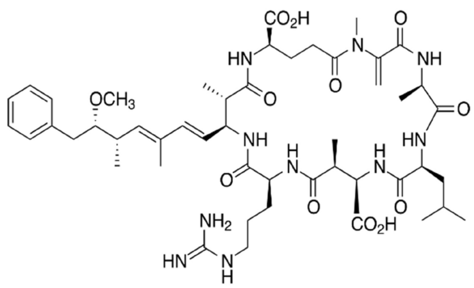
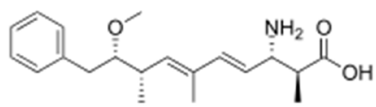
The need for reliable, near-real time detection methods for microcystin congeners in environmental samples is pressing. One of the main challenges when testing for this type of toxin is the many structural variants (congeners) of the toxin molecule. At present, at least 269 microcystins congeners have been reported, among these, about 20% appear to be the result of chemical or biochemical transformation of microcystins that can occur in the environment or during sample handling and extraction of cyanobacteria, including oxidation products, methyl esters, or post-biosynthetic metabolites.[1]



Microcystin-LR



ADDA

The toxicity of many microcystins have been studied using various approaches.[1] Table 1 lists toxicity in the mouse model as LD_{50}. Table 2 lists the IC_{50} inhibition value of protein phosphatases for various congeners.

Table 1. LD50 values (μ g kg-1 b.w., i.p. mouse) of microcystin congeners

MICROCYSTIN CONGENER	LD_{50}	MICROCYSTIN CONGENER	LD_{50}
MC-LA	50	[D-Asp^3,(*E*)Dhb^7]MC-RR	250
MC-AR	250	MC-M(O)R	700-800
MC-YA	60-70	MC-FR	250
[D-Asp^3,Dha^7]MC-LR	160-300	MC-YM(O)	56
[D-Asp^3]MC-LR	200-500	[ADMAdda^5]MC-LHar	60
[D-Asp^3,(E)-Dhb^7]MC-LR	70	[D-Leu^1]MC-LR	100
[Dha^7]MC-LR	250	MC-RR	500-800
[DMAdda^5]MC-LR	90-100	[(6Z)-Adda^5]MC-RR	1200
MC-LR	50	[D-Asp^3]MC-HtyR	80-100
[(6Z)-Adda^5]MC-LR	1200	[D-Asp^3, (*E*)-Dhb^7]MC-HtyR	70
MC-LY	90	MC-YR	70
[D-Asp^3,ADMAdda^5]MC-LR	160	[D-Asp^3, ADMAdda^5, Dhb^7]MC-RR	200
MC-HilR	100	[D-Glu(OC$_3$H$_7$O)^6]MC-LR	>1000
[D-Glu(OCH$_3$)^6]MC-LR	>1000	[D-Asp^3]MC-WR	95 ± 10

MICROCYSTIN CONGENER	LD_{50}	MICROCYSTIN CONGENER	LD_{50}
[$Mser^7$]MC-LR	150	MC-HtyR	160-300
[D-Asp^3]MC-FR	90 ± 10	MC-WR	150-200
[$ADMAdda^5$]MC-LR	60	[D-Asp^3, $ADMAdda^5$,Dhb^7]MC-HtyR	100
[D-Asp^3]MC-RR	350 ± 10	MC-LR Cys conjugate	1000
[Dha^7]MC-RR	180		

Table 2. IC_{50} (nM) Values for inhibition of serine/threonine protein phosphatases (PPs) by microcystin congeners

MICROCYSTIN CONGENER	LD_{50}	MICROCYSTIN CONGENER	LD_{50}
MC-LA	PP1	Rabbit muscle	2.3
	PP2A	Human hepatocytes	0.56
	PP2A	Rabbit muscle	0.05
	rPP2Ac	Recombinant human PP2A catalytic subunit	0.161 ± 0.002
MC-LV	PP1	Rabbit skeletal muscle	0.06–0.45
MC-LL	PP1	Rabbit skeletal muscle	0.06–0.45
[D-Asp^3,Dha^7]MC-LR	rPP2Ac	Recombinant human PP2A catalytic subunit	0.254 ± 0.004
MC-LM	PP1	Rabbit skeletal muscle	0.06–0.45
[D-Asp^3]MC-LR	PP2A	Rabbit skeletal muscle	0.09
[D-Asp^3,(E)-Dhb^7]MC-LR	rPP2Ac	Recombinant human PP2A catalytic subunit	0.201 ± 0.003
[D-Asp^3,(Z)-Dhb^7]MC-LR	rPP2Ac	Recombinant human PP2A catalytic subunit	0.16 ± 0.01
[Dha^7]MC-LR	PP1	Rabbit skeletal muscle	0.54-5
	PP2A	Bovine kidney	0.11 ± 0.04
	rPP2Ac	Recombinant human PP2A catalytic subunit	0.167 ± 0.003
[$DMAdda^5$]MC-LR	rPP1c	Recombinant rabbit skeletal muscle PP1	1.5
MC-LF	rPP1c	Recombinant rabbit skeletal muscle PP1	1.8
	PP1	Rabbit skeletal muscle	0.06-0.45
	PP2A	Human hepatocytes	0.57
	PP2A	Human red blood cells	1.1
	rPP2Ac	Recombinant human PP2A catalytic subunit	0.10 ± 0.02
MC-LR	PP1	Rabbit muscle	0.1-1.9
	PP1	Chicken gizzard myosin B	6
	PP1	Liver of grass carp	0.90
	rPP1c	Recombinant rabbit skeletal muscle PP1	1.2
	PP2A	Rabbit skeletal muscle	0.04-0.5
	PP2A	Human hepatocytes	0.46
	PP2A	Human erythrocytes	0.03-2.2
	PP2A	Bovine heart	0.05-2
	PP2A	Bovine kidney	0.2 ± 0.1
	PP2A	Mouse brain	0.28-3.15
	PP2A	Liver of grass carp	0.28

MICROCYSTIN CONGENER	LD_{50}	MICROCYSTIN CONGENER	LD_{50}
	rPP2Ac	Recombinant human PP2 catalytic subunit	0.032 ± 0.004
[(6Z)-Adda^{5}]MC-LR	PP2A	Mouse brain	80
	rPP1c	Recombinant rabbit skeletalmuscle PP1	> 100
MC-LY	PP2A	Human hepatocytes	0.34
[D-Asp^{3},ADMAdda^{5}]MC-LR	PP2Ab		4
[D-Glu(OMe)^{6}]MC-LR	rPP1c	Recombinant rabbit skeletal muscle PP1	> 100
[D-Asp^{3}(Dha)^{7}]MC-RR	rPP1c	Recombinant rabbit skeletal muscle PP1	0.22 ± 0.01
[D-Asp^{3}]MC-RR	PP2A	Human red blood cells	0.45-11.5
	rPP2Ac	Recombinant human PP2A catalytic subunit	0.30 ± 0.01
[Dha7]MC-RR	PP1	Rabbit muscle	8.3 ± 0.8
	PP2A	Bovine kidney	4 ± 1
	rPP2Ac	Recombinant human PP2A catalytic subunit	0.29 ± 0.01
	PP1	Rabbit skeletal muscle	2.6-5.7
[D-Asp^{3},(E)-Dhb^{7}]MC-RR	PP1	Rabbit skeletal muscle	1.8-56.4
	PP2A	Rabbit skeletal muscle	2.4
	PP2A	Human red blood cells	17.9-49.4
MC-LW	rPP1c	Recombinant rabbit skeletal muscle PP1	1.9
	PP2A	Human hepatocytes	0.29
	PP2A	Human red blood cells	1.1
	rPP2Ac	PP2A Recombinant human catalytic subunit	0.114 ± 0.003
MC-FR	rPP2Ac	PP2A Recombinant human catalytic subunit	0.069 ± 0.003
[Dha^{7}]MC-YR	rPP2Ac	PP2A Recombinant human catalytic subunit	0.379 ± 0.003
[D-Leu^{1}]MC-LR	PP1	Recombinant rabbit skeletal muscle PP1	0.5-4.43
MC-RR	PP1	Rabbit skeletal muscle	0.68
	PP1	Chicken gizzard myosin B	3
	PP1	Liver of gras carp	3.60
	rPP1c	Recombinant rabbit skeletal muscle PP1	1.5
	PP2A	Human red blood cells	0.241-175
	PP2A	Human hepatocytes	0.60
	PP2A	Mouse brain	0.72-1.4
	PP2A	Bovine cardiac muscle	1
	PP2A	Bovine kidney	10 ± 2
	PP2A	Rabbit skeletal muscle	0.1
	PP2A	Liver of gras carp	0.64
	rPP2Ac	Recombinant human PP2A catalytic subunit	0.056 ± 0.002
[(6Z)-Adda^{5}]MC-RR	rPP2Ac	Recombinant human PP2A catalytic subunit	10.1 ± 0.3
	PP2A	Mouse brain	80
[D-Asp^{3}]MC-HtyR	rPP2Ac	Recombinant human PP2A catalytic subunit	0.098 ± 0.006
[D-Asp^{3}],(E)-Dhb^{7}]MC-HtyR	rPP2Ac	Recombinant human PP2A catalytic subunit	0.122 ± 0.005
[D-Asp^{3}],(Z)-Dhb^{7}]MC-HtyR	rPP2Ac	Recombinant human PP2A catalytic subunit	0.110 ± 0.008

MICROCYSTIN CONGENER	LD_{50}	MICROCYSTIN CONGENER	LD_{50}
MC-YR	PP1	Rabbit skeletal muscle	1.0
	PP1	Liver of grass carp	0.90
	PP2A	Human red blood cells	0.26-9.0
	PP2A	Human hepatocytes	0.84
	PP2A	Bovine kidney	0.09 ± 0.02
	PP2A	Rabbit skeletal muscle	0.26
	PP2A	Mouse brain	0.39-1.3
	PP2A	Liver of grass carp	0.40
	rPPAc	Recombinant human PP2A catalytic subunit	0.125 ± 0.005
MC-WR	rPPAc	Recombinant human PP2A catalytic subunit	0.18 ± 0.01
[D-Asp3, ADMAdda^5, Dhb^7]MC-HtyR	PP1	Rabbit skeletal muscle	0.15-0,24
	PP2A	Human red blood cells	0.06
	PP2A	Bovine heart	0.06
	PP4	Porcine testis	0.04
	PP5	Recombinant human PP5 expressed in *E.coli*	0.5

Because of the toxicity exhibited by many of the tested congeners, it is important to quantify as many congeners as possible for risk assessment purposes since several microcystin congeners can be present in a single sample. As many as eight different congeners have been identified in a single sample[2] takenfrom Lake Erie surface water according to the Ohio EPA.

The two most common analytical methods for the analysis of microcystins are LC/MS and the ADDA-ELISA (EPA Method 546). Each technology has advantages and disadvantages: LC-MS technologies require sophisticated operating environments and are not amenable to the high-throughput analysis needed for environmental monitoring work. ELISA methods, because of their simplicity, ruggedness, and parallel sample analysis, are eminently more suitable as high-throughput methods and retain the high sensitivity and good specificity needed for this type of work, producing assays that are field portable, user-friendly, and cost-effective*.

The most common negative comment for LC/MS is that it can only measure toxins for which there is an analytical standard available (currently < 20), therefore underestimating total microcystin concentration in a sample. The most common negative comment for the ADDA-ELISA is that it can overestimate toxin concentration. The antibodies used in the ADDA-ELISA[3] have been developed against the ADDA portion of the molecule. This portion of the molecule plays an important role in the biological activity of the toxin since it is the part of the molecule that binds to the target enzyme protein phosphatase. ADDA and glutamine residues are essential for the toxicity of microcystins. Substitutions around the microcyclic part of the molecule will also confer more or less toxicity. Out of the 269 known microcystin congeners, 211 contain the ADDA residue (ref.) therefore they will be detected by the ELISA, potentially causing an overestimation of the concentration of microcystin congeners present in a sample when compared to LC/MS which can only detect those congeners for which analytical standards are available.

In view of the 2B carcinogenicity classification of MC-LR and the toxiciy of other congeners, using the ADDA-ELISA that offers the total sum of microcystin congeners in environmental samples represents a conservative approach to protect human health. LC/MS results potentially underestimate the total microcystin in a sample, representing a less conservative approach with a much lower likelihood of management action by authorities. The latter, is also associated with a higher likelihood of acute and chronic toxicity by persons exposed to surface waters if bodies of water are not closed for recreational or drinking water use when contaminated with microcystins toxins.

*The approximate cost of analysis by ELISA is as low as $11 per sample. The cost for LC-MS can be $150 or more per sample.

REFERENCES

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2. Luukkainen, Toxicon, Vol. 32, No. I, 133-139, 1994

3. USEPA Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by ADDA Enzyme-Linked Immunosorbent Assay

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