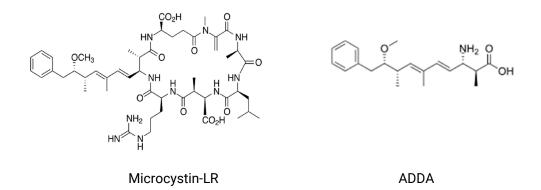


THE USE OF RAPID, SENSITIVE AND COST-EFFECTIVE METHODS FOR THE BROAD DETECTION OF MICROCYSTIN CONGENERS

By: Fernando M. Rubio, Gold Standard Diagnostics

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



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.

MICROCYSTIN CONGENER	LD ₅₀	MICROCYSTIN CONGENER	LD ₅₀
MC-LA	50	[D-Asp³,(<i>E</i>)Dhb ⁷]MC-RR	250
MC-AR	250	MC-M(0)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	[ADMAdda⁵]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
[DMAdda⁵]MC-LR	90-100	[(6Z)-Adda ⁵]MC-RR	1200
MC-LR	50	[D-Asp ³]MC-HtyR	80-100
[(6Z)-Adda⁵]MC-LR	1200	[D-Asp ³ , (<i>E</i>)-Dhb ⁷]MC-HtyR	70
MC-LY	90	MC-YR	70
[D-Asp ³ ,ADMAdda ⁵]MC-LR	160	[D-Asp³, ADMAdda⁵, 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-Asp³, ADMAdda⁵,Dhb7]MC-HtyR	100
[D-Asp ³]MC-RR	350 ± 10	MC-LR Cys conjugate	1000
[Dha ⁷]MC-RR	180		

Table 2. IC₅₀ (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
[DMAdda⁵]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

rP1cRecombinant rabbit skeletal muscle PP1> 100MCLYPP2AHuman hepatocytes0.34[D-Asp'ADMAdda']MC-LRPP2AKecombinant rabbit skeletal muscle PP1000[D-Asp'(Mo)]MC-RRPP1cRecombinant rabbit skeletal muscle PP10.30 ± 0.01[D-Asp'(Mo)]MC-RRPP2AHuman red blood cells0.45 ± 1.5[D-Asp'(Mo)]MC-RRPP2ARecombinant human PP2A catalytic subunit0.30 ± 0.01[D-Asp'(Mo)]MC-RRPP2ABoxine kidney4 ± 1[PP2AcRecombinant human PP2A catalytic subunit0.29 ± 0.01[D-Asp'(E)-Dhb']MC-RRPP1Rabbit skeletal muscle2.65.7[D-Asp'(E)-Dhb']MC-RRPP1Rabbit skeletal muscle2.4PP2AHuman red blood cells1.856.4[D-Asp'(E)-Dhb']MC-RRPP1Rabbit skeletal muscle2.4PP2AHuman red blood cells1.1PP2AHuman red blood cells1.1PP2AHuman red blood cells1.14 ± 0.03MC-LWPP1CRecombinant rabbit skeletal muscle PP10.69 ± 0.03[D-Lu']MC-LRPP2APP2A Recombinant human catalytic subunit0.314 ± 0.03MC-FRIPP2AcPP2A Recombinant human catalytic subunit0.379 ± 0.03[D-Lu']MC-LRPP1Rabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle PP10.54.14PP2ARabbit skeletal	MICROCYSTIN CONGENER	LD ₅₀	MICROCYSTIN CONGENER	LD ₅₀
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MC-LWrPP1cRecombinant rabbit skeletal muscle PP11.9PP2AHuman hepatocytes0.29PP2AHuman red blood cells1.1rP2AcPP2A Recombinant human catalytic subunit0.014 ± 0.003MC-FRrPP2AcPP2A Recombinant human catalytic subunit0.069 ± 0.003[Dha']MC-YRrPP2AcPP2A Recombinant human catalytic subunit0.379 ± 0.003[Dha']MC-IRPP1Recombinant human catalytic subunit0.379 ± 0.003[D-Leu']MC-LRPP1Recombinant rabbit skeletal muscle PP10.54.43MC-RRPP1Chicken gizzard myosin B3PP1Chicken gizzard myosin B3.60PP1Liver of gras carp3.60rPP1cRecombinant rabbit skeletal muscle PP11.5PP2AHuman red blood cells0.241-175PP2AHuman red blood cells0.241-175PP2ABovine cardiac muscle1.1PP2ABovine cardiac muscle1.1PP2ABovine cardiac muscle1.1PP2ARecombinant human PP2A catalytic subunit0.64PP2ALiver of gras carp0.64PP2ARecombinant human PP2A catalytic subunit0.65 ± 0.002[fc2)-Adda']MC-RRPP2AcRecombinant human PP2A catalytic subunit0.11 ± 0.3[fc2-Asp*]MC-HtyRIPP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006[rb4-Asp*]JMC-HtyRIPP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006[rb4-Asp*]JMC-HtyRIPP2AcRecombinant human PP		PP2A	Rabbit skeletal muscle	2.4
PP2A Human hepatocytes 0.29 PP2A Human red blood cells 1.1 iPP2A PP2A Recombinant human catalytic subunit 0.69 ± 0.003 [Dha [*]]MC-YR iPP2Ac PP2A Recombinant human catalytic subunit 0.69 ± 0.003 [D-Leu']MC-LR PP1Ac PP2A Recombinant human catalytic subunit 0.54.43 MC-RR PP1 Recombinant rabbit skeletal muscle PP1 0.54.43 MC-RR PP1 Recombinant rabbit skeletal muscle PP1 0.68 PP1 Chicken gizzard myosin B 3 PP1 Liver of gras carp 3.60 PP1 Recombinant rabbit skeletal muscle PP1 1.5 PP1 Liver of gras carp 3.60 PP1 Recombinant rabbit skeletal muscle PP1 1.5 PP2A Human hepatocytes 0.60 PP2A Human hepatocytes 0.211175 PP2A Human hepatocytes 0.60 PP2A Bovine cardiac muscle 0.1 PP2A Bovine cardiac muscle 1.1 PP2A Bovine kidney 0.56 ±		PP2A	Human red blood cells	17.9-49.4
PP2AHuman ed blood cells1.1rP2AcP2A Recombinant human catalytic subunit0.114 ± 0.003MC-FRrP2AcP2A Recombinant human catalytic subunit0.669 ± 0.003[bha*]MC-YRrPP2AcP2A Recombinant human catalytic subunit0.379 ± 0.003[b-Leu*]MC-LRP1Recombinant nabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle PP10.68PP1Chicken gizzard myosin B3.60PP1Liver of gras carp3.60PP1Recombinant rabbit skeletal muscle PP11.5PP2AHuman red blood cells0.241-175PP2AHuman red blood cells0.62PP2AHuman red blood cells0.62PP2ABovine cardiac muscle0.72-1.4PP2ABovine cardiac muscle1.1PP2ABovine kidney0.12PP2ARecombinant human PP2A catalytic subunit0.65 ± 0.002[fc2)-Adda*JMC-RRPP2ARecombinant human PP2A catalytic subunit0.55 ± 0.002[fc2)-Adda*JMC-RRIPP2ARecombinant human PP2A catalytic subunit0.12 ± 0.005[fc3-Aps]/fc1-Dth*JMC-HtyRIPP2ARecombinant human PP2A catalytic subunit0.98 ± 0.006[p-Aps]/fc1-Dth*JMC-HtyRIPP2ARecombinant human PP2A catalytic subunit0.98 ± 0.006	MC-LW	rPP1c	Recombinant rabbit skeletal muscle PP1	1.9
iPP2AcPP2A Recombinant human catalytic subunit0.114 ± 0.003MC-FRiPP2AcPP2A Recombinant human catalytic subunit0.609 ± 0.003[Dha']MC-YRiPP2AcPP2A Recombinant human catalytic subunit0.379 ± 0.003[D-Leu']MC-LRPP1Recombinant rabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle PP10.68PP1Chicken gizzard myosin B3.60iPP1Liver of gras carp3.60iPP1Recombinant rabbit skeletal muscle PP11.5iPP2AHuman red blod cells0.241-175iPP2AHuman red blod cells0.241-175iPP2ABovine cardiac muscle0.72-1.4iPP2ABovine cardiac muscle1.1iPP2ABovine cardiac muscle1.1iPP2ABovine cardiac muscle0.14iPP2ARobit skeletal muscle0.14iPP2ABovine kidney1.1iPP2ARecombinant human PP2A catalytic subunit0.056 ± 0.002i[C2)-Adda ³]MC-RRiPP2AcRecombinant human PP2A catalytic subunit0.11 ± 0.3iPP2AMouse brain0.056 ± 0.002iPP2ARecombinant human PP2A catalytic subunit0.11 ± 0.3iPP2ARecombinant human PP2A catalytic subunit0.091 ± 0.005iPP2ARecombinant human PP2A		PP2A	Human hepatocytes	0.29
MC-FRrPP2AcPP2A Recombinant human catalytic subunit0.069 ± 0.003[Dha']MC-YRrPP2AcPP2A Recombinant human catalytic subunit0.379 ± 0.003[D-Leu']MC-LRPP1Recombinant rabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle0.68PP1Chicken gizzard myosin B3PP1Liver of gras carp3.60rPP1cRecombinant rabbit skeletal muscle PP11.5PP2AHuman red blood cells0.241-175PP2AHuman hepatocytes0.60PP2ABovine cardiac muscle1PP2ABovine cardiac muscle1PP2ABovine cardiac muscle0.12PP2ARecombinant human PP2A catalytic subunit0.056 ± 0.002[f6Z)-Adda [*]]MC-RRrP2AcRecombinant human PP2A catalytic subunit0.11 ± 0.3[b-Asp [*]](E)-Dhb']MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006[b-Asp [*]](E)-Dhb']MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006		PP2A	Human red blood cells	1.1
[Dha7]MC-YRrPP2AcPP2A Recombinant human catalytic subunit0.379 ± 0.003[D-Leu']MC-LRPP1Recombinant rabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle0.68PP1Chicken gizzard myosin B3.60PP1Liver of gras carp3.60PP1Recombinant rabbit skeletal muscle PP11.5PP2ARecombinant rabbit skeletal muscle PP10.241.175PP2AHuman red blood cells0.241.175PP2AMouse brain0.72-1.4PP2ABovine cardiac muscle1.1PP2ABovine cardiac muscle1.1PP2ARebot skeletal muscle0.241.175PP2ABovine cardiac muscle0.21.175PP2ABovine cardiac muscle1.1PP2ABovine cardiac muscle0.1PP2ARecombinant human PP2A catalytic subunit0.56 ± 0.002[fc2)-Adda ³]MC-RRPP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[bAsp ³](E)-Dhb ³]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.981 ± 0.006[bAsp ³](E)-Dhb ³]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.121 ± 0.005		rPP2Ac	PP2A Recombinant human catalytic subunit	0.114 ± 0.003
[D-Leu']MC-LRPP1Recombinant rabbit skeletal muscle PP10.54.43MC-RRPP1Rabbit skeletal muscle0.68PP1Chicken gizzard myosin B3PP1Liver of gras carp3.60rPP1cRecombinant rabbit skeletal muscle PP11.5PP2AHuman red blood cells0.241-175PP2AHuman hepatocytes0.60PP2AMouse brain0.72-1.4PP2ABovine cardiac muscle1PP2ABovine kidney10 ± 2PP2ARecombinant human PP2A catalytic subunit0.56 ± 0.002[GZ)-Adda*]MC-RRrPP2AcRecombinant human PP2A catalytic subunit0.11 ± 0.3[D-Asp*](E)-Dhb*]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006	MC-FR	rPP2Ac	PP2A Recombinant human catalytic subunit	0.069 ± 0.003
MC-RRPP1Rabbit skeletal muscle0.68PP1Chicken gizzard myosin B3PP1Liver of gras carp3.60rP1cRecombinant rabbit skeletal muscle PP11.5PP2AHuman red blood cells0.241.175PP2AHuman hepatocytes0.60PP2ABovine cardiac muscle0.72.1.4PP2ABovine cardiac muscle1.9PP2ABovine cardiac muscle1.9PP2ABovine kidney0.12PP2ABovine kidney0.64PP2ALiver of gras carp0.64PP2ARecombinant human PP2A catalytic subunit0.65 ± 0.002[C62)-Adda ⁵]MC-RRPP2ARecombinant human PP2A catalytic subunit0.11 ± 0.3[D-Asps ⁵]MC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.908 ± 0.006[D-Asps ⁵],(E)-Dhb ⁵]MC-HtyRIP2AcRecombinant human PP2A catalytic subunit0.908 ± 0.006	[Dha ⁷]MC-YR	rPP2Ac	PP2A Recombinant human catalytic subunit	0.379 ± 0.003
P1Chicken gizzard myosin B3P1Liver of gras carp3.60P1Recombinant abbit skeletal muscle PP11.5P2AHuman red blood cells0.241.175P2AHuman hepatocytes0.60P2ABovine cardia cmuscle PD10.60P2ABovine cardia cmuscle PD10.72.1.4P2ABovine cardia cmuscle1.92P2ABovine cardia cmuscle0.12P2ABovine kindeng0.12P2ARebit skeletal muscle0.64P2ALiver of gras carp0.64P2ARecombinant PP2A catalytic subunit0.56 ± 0.02[f2-Adda ^{ci}]MC-RAPP2ARecombinant PP2A catalytic subunit0.91 ± 0.02[p2Asp ^{ci}]PP2AcRecombinant PP2A catalytic subunit0.91 ± 0.02[p3-Sp ^{ci}]PP2AcRecombinant PP2A catalytic subunit0.92 ± 0.02	[D-Leu ¹]MC-LR	PP1	Recombinant rabbit skeletal muscle PP1	0.5-4.43
PP1Liver of gras carp3.60rPP1cRecombinant rabbit skeletal muscle PP11.5PP2AHuman red blood cells0.241-175PP2AHuman hepatocytes0.60PP2AMouse brain0.72-1.4PP2ABovine cardiac muscle1PP2ABovine kidney10±2PP2ABovine kidney10±2PP2ARebit skeletal muscle0.1PP2ARecombinant human PP2A catalytic subunit0.66[fcZ)-Adda®MC-RRrPP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp®MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.981 ± 0.006[D-Asp®MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.981 ± 0.006	MC-RR	PP1	Rabbit skeletal muscle	0.68
rPP1cRecombinant rabbit skeletal muscle PP11.5PP2AHuman red blood cells0.241-175PP2AHuman hepatocytes0.60PP2AMouse brain0.72-1.4PP2ABovine cardiac muscle1PP2ABovine kidney10 ± 2PP2ARabbit skeletal muscle0.1PP2ARabbit skeletal muscle0.64PP2ARecombinant human PP2A catalytic subunit0.56 ± 0.002[fcZ)-Adda®MC-RRrP2AcRecombinant human PP2A catalytic subunit0.1 ± 0.3PP2AMouse brain80[fcAsp®MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006[pAsp®MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006[pAsp®MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.98 ± 0.006		PP1	Chicken gizzard myosin B	3
P2AHuma ned blood cells0.241-175P2AHuma ne patocytes0.60P2AMose brain0.721.4P2ABovine cardiac muscle1P2ABovine kidney0.1P2ARobit skeletal muscle0.1P2ARobit skeletal muscle0.1P2ARecombinant human PP2A catalytic subunit0.654.0002[G2-Adda ⁿ]MC-RRPP2AcRecombinant human PP2A catalytic subunit0.1[C4-SprightC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.984.0.006[D-AsprightC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.984.0.006[D-AsprightC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.984.0.006[D-AsprightC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.984.0.006[D-AsprightC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.984.0.006		PP1	Liver of gras carp	3.60
PP2AHuman hepatocytes0.60PP2AMouse brain0.721.4PP2ABovine cardiac muscle1PP2ABovine kidney10±2PP2ARabbit skeletal muscle0.1PP2ALiver of gras carp0.64PP2AcRecombinant human PP2A catalytic subunit0.1±0.3[G2-Adda ⁴]MC-RRPP2AcRecombinant human PP2A catalytic subunit0.1±0.3[D-Asp ³]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.98±0.006[D-Asp ⁴], (E)-Dhb ⁷]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.98±0.006[D-Asp ³], (E)-Dhb ⁷]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.12±0.005		rPP1c	Recombinant rabbit skeletal muscle PP1	1.5
PP2AMouse brain0.72-1.4PP2ABovine cardiac muscle1PP2ABovine kidney10 ± 2PP2ARabbit skeletal muscle0.1PP2ARabbit skeletal muscle0.64PP2AIver of gras carp0.64PP2AcRecombinant human PP2A catalytic subunit0.056 ± 0.002[6Z)-Adda ⁵]MC-RRrPP2AcRecombinant human PP2A catalytic subunit0.11 ± 0.3[D-Asp³]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp³],(E)-Dhb7]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp³],(E)-Dhb7]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.122 ± 0.005		PP2A	Human red blood cells	0.241-175
PP2ABovine cardiac muscle1PP2ABovine kidney10 ± 2PP2ARabbit skeletal muscle0.1PP2AKier of gras carp0.64PP2AcRecombinant human PP2A catalytic subunit0.056 ± 0.002[62)-Adda ⁵]MC-RRPP2AcRecombinant human PP2A catalytic subunit0.11 ± 0.3PP2ArPP2AcNouse brain0.098 ± 0.006[D-Asp ³]MC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.988 ± 0.006[D-Asp ³]MC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp ³]MC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.122 ± 0.005		PP2A	Human hepatocytes	0.60
PP2ABovine kidney10 ± 2PP2ARabbit skeletal muscle0.1PP2ALiver of gras carp0.64rP2AcRecombinant human PP2A catalytic subunit0.056 ± 0.002[62)-Adda ⁵]MC-RRPP2AcRecombinant human PP2A catalytic subunit0.1 ± 0.3PP2AMouse brain80[D-Asp ³]MC-HtyRPP2AcRecombinant human PP2A catalytic subunit0.998 ± 0.006[D-Asp ³], (E)-Dhb ⁷]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.122 ± 0.005		PP2A	Mouse brain	0.72-1.4
PP2A Rabbit skeletal muscle 0.1 PP2A Liver of gras carp 0.64 iPP2Ac Recombinant human PP2A catalytic subunit 0.056 ± 0.002 [(6Z)-Adda ⁵]MC-RR iPP2Ac PP2Ac Recombinant human PP2A catalytic subunit 10.1 ± 0.3 PP2A Mouse brain 80 [D-Asp ³]MC-HtyR iPP2Ac iPP2Ac Recombinant human PP2A catalytic subunit 0.098 ± 0.006 iPP2A Recombinant human PP2A catalytic subunit 0.122 ± 0.005		PP2A	Bovine cardiac muscle	1
PP2ALiver of gras carp0.64iP2ARecombinant PP2A catalytic subunit0.056 ± 0.002[6Z)-Adda ⁵]MC-RRrP2AcRecombinant human PP2A catalytic subunit10.1 ± 0.3P2AMouse brain80[D-Asp ³]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp ³],(E)-Dhb ⁷]MC-HtyRrP2AcRecombinant human PP2A catalytic subunit0.122 ± 0.005		PP2A	Bovine kidney	10 ± 2
rPP2AcRecombinant human PP2A catalytic subunit0.056 ± 0.002[(6Z)-Adda ⁵]MC-RRrPP2AcRecombinant human PP2A catalytic subunit10.1 ± 0.3PP2AMouse brain80[D-Asp ³]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp ³],(E)-Dhb ⁷]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.122 ± 0.005		PP2A	Rabbit skeletal muscle	0.1
[(6Z)-Adda ⁵]MC-RRrPP2AcRecombinant human PP2A catalytic subunit10.1 ± 0.3PP2AMouse brain80[D-Asp ³]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp ³],(E)-Dhb ⁷]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.122 ± 0.005		PP2A	Liver of gras carp	0.64
PP2A Mouse brain 80 [D-Asp³]MC-HtyR rPP2Ac Recombinant human PP2A catalytic subunit 0.098 ± 0.006 [D-Asp³],(E)-Dhb ⁷]MC-HtyR rPP2Ac Recombinant human PP2A catalytic subunit 0.122 ± 0.005		rPP2Ac	Recombinant human PP2A catalytic subunit	0.056 ± 0.002
[D-Asp³]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.098 ± 0.006[D-Asp³],(E)-Dhb7]MC-HtyRrPP2AcRecombinant human PP2A catalytic subunit0.122 ± 0.005	[(6Z)-Adda⁵]MC-RR	rPP2Ac	Recombinant human PP2A catalytic subunit	10.1 ± 0.3
[D-Asp ³],(E)-Dhb ⁷]MC-HtyR rPP2Ac Recombinant human PP2A catalytic subunit 0.122 ± 0.005		PP2A	Mouse brain	80
	[D-Asp ³]MC-HtyR	rPP2Ac	Recombinant human PP2A catalytic subunit	0.098 ± 0.006
[D-Asp ³],(Z)-Dhb ⁷]MC-HtyR rPP2Ac Recombinant human PP2A catalytic subunit 0.110 ± 0.008	[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, ADMAdda⁵, Dhb7]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 sample² 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-ELISA³ 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 toxicity 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

- **1.** Bouaicha *et al.* Structural Diversity, Characterization and Toxicology of Microcystins. doi:10.20944/preprints201910.0034.v1
- 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

