

Florida Department of Environmental
Protection

Bureau of Laboratories

Division of Environmental Assessment
and Restoration



2006 Microcystin Hold Time Study Results



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Analytical Laboratory Participants

- Florida Department of Environmental Protection, Central Chemistry Laboratory, Tallahassee, Florida (FDEP)
- South Florida Water Management District Laboratory, West Palm Beach, Florida (SFWMD)

Study design document

http://www.dep.state.fl.us/labs/biology/hab/docs/microcystin_ht.pdf

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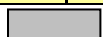
Summary Results for Experiment 1 (June 2006)

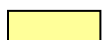
Analysis Day	Autoclave Day	MC-LR	MC-LA	MC-YR	MC_RR	Nodul- arin	FDEP LC Total Result	FDEP ELISA Result (Abraxis)	SFWMD ELISA Result (Envirologix)
0	0	20.80	6.11	0.84	7.39	0.00	35.1	82	
0	0	22.10	6.98	0.98	7.96	0.00	38.0	81	
0	0	24.30	7.54	0.96	7.83	0.00	40.6	80	
48 hrs	2	30.00	8.42	1.08	9.54	0.00	49.0	75	65
48 hrs	2	25.40	6.97	0.82	6.90	0.00	40.1	76	65
48 hrs	2	28.90	7.92	1.10	7.56	0.00	45.5	88	70
7	7	28.30	6.52	0.82	8.86	0.00	44.5	93	55
7	7	29.40	7.67	0.94	8.46	0.00	46.5	78	55
7	7	31.70	7.31	0.82	8.61	0.00	48.4	100	65
14	14	23.40	8.60	0.56	8.12	0.00	40.7	99	60
14	14	24.00	9.38	0.50	8.48	0.00	42.4	100	60
14	14	23.70	9.04	0.49	8.55	0.00	41.8	94	60
21	21	28.00	9.46	0.78	9.24	0.00	47.5	96	40
21	21	28.70	8.80	0.94	8.12	0.00	46.6	87	55
21	21	30.20	9.55	1.15	8.16	0.00	49.1	88	45
28	28	18.30	6.42	1.72	8.60	0.00	35.0	92	40
28	28	22.00	7.50	1.77	8.06	0.00	39.3	120	45
28	28	22.80	8.04	1.84	8.76	0.00	41.4	110	45
28	0	23.50	6.93	1.93	8.36	0.00	40.7	94	60
28	0	25.80	7.97	2.03	8.62	0.00	44.4	100	60
28	0	30.10	9.65	2.29	8.27	0.00	50.3	93	60

Sample was autoclaved on 0; others were autoclaved on Analysis Day

Summary Results for Experiment 2 (August 2006)

Analysis Day	Autoclave Day	MC-LR	MC-LA	MC-YR	MC-RR	Nodularin	FDEP LC Total Result	FDEP ELISA Results (Abraxis)	FDEP ELISA Results (Envirologix)	SFWMD ELISA Results (Envirologix)
0	0	2.80	0.34	0.00	0.88	0.00	4.0	15		
0	0	3.10	0.56	0.00	0.91	0.00	4.6	18		
0	0	3.10	0.65	0.00	0.95	0.00	4.7	14		
48 hrs	2	3.30	0.28	0.00	0.81	0.00	4.4	15	8	9
48 hrs	2	3.30	0.29	0.00	0.64	0.00	4.2	17	7.7	8.5
48 hrs	2	3.40	0.28	0.00	0.75	0.00	4.4	17	7.7	8.5
48 hrs	0	3.60	0.30	0.00	0.78	0.00	4.7	16	6.6	
48 hrs	0	3.30	0.25	0.00	0.70	0.00	4.3	12	6.9	
48 hrs	0	3.60	0.33	0.00	0.75	0.00	4.7	17	8	
7	7	3.64	0.63	0.00	0.77	0.00	5.0	16.5	7.16	7.5
7	7	3.98	0.51	0.00	0.81	0.00	5.3	13.9	6.47	7
7	7	3.54	0.66	0.00	0.80	0.00	5.0	15.7	6.49	7.5
14	14	3.48	0.53	0.00	0.65	0.00	4.7	12.4	5.60	7
14	14	3.87	0.64	0.00	0.79	0.00	5.3	13.2	7.10	6.5
14	14	4.10	0.82	0.00	0.91	0.00	5.8	11.8	7.70	8
21	21	3.31	0.67	0.00	0.88	0.00	4.9	15	6.1	7
21	21	3.10	0.67	0.00	0.92	0.00	4.7	13	6.7	6.5
21	21	3.42	0.69	0.00	0.91	0.00	5.0	13	6.8	6.5
28	28	3.03	0.79	0.00	0.89	0.00	4.7	14	5.1	7.5
28	28	3.58	0.86	0.00	1.00	0.00	5.4	18	6	8
28	28	3.61	0.74	0.00	1.00	0.00	5.3	16	5.9	5.5
28	0	4.29	0.93	0.00	0.96	0.00	6.2	16.1	5.7	9
28	0	3.57	0.87	0.00	0.85	0.00	5.3	14.9	6	9
28	0	4.26	0.92	0.00	0.95	0.00	6.1	14.3	6.6	9

 Analysis was not done

 Sample was autoclaved on 0; others were autoclaved on Analysis Day

Experiment 1 LC-MS/MS (FDEP)

Preparation Method = Autoclave; Calibration Range: Min=2, Max=50; No dilution was required for reported results.

Analysis Day	Bottle Number	LC-MS/MS Analysis						Preparation		Analysis		Recovery (%)for LCS
		MC-LR	MC-LA	MC-YR	MC_RR	Nodularin	Total Result in ug/L	Date	Time	Date	Time	
0	1	20.80	6.11	0.84	7.39	0.00	35.1	6/5/2006	11:00AM	6/5/2006	4:27PM	56.4 & 48.0
0	2	22.10	6.98	0.98	7.96	0.00	38.0				4:48PM	
0	3	24.30	7.54	0.96	7.83	0.00	40.6				5:09PM	
48 hrs	4	30.00	8.42	1.08	9.54	0.00	49.0	6/7/2006	10:00AM	6/7/2006	2:15PM	58.6 & 55.4
48 hrs	5	25.40	6.97	0.82	6.90	0.00	40.1				2:36PM	
48 hrs	6	28.90	7.92	1.10	7.56	0.00	45.5				2:58PM	
7	7	28.30	6.52	0.82	8.86	0.00	44.5	6/12/2006	7:30AM	6/12/2006	3:09PM	63.3 & 60.2
7	8	29.40	7.67	0.94	8.46	0.00	46.5				3:52PM	
7	9	31.70	7.31	0.82	8.61	0.00	48.4				4:13PM	
14	10	23.40	8.60	0.56	8.12	0.00	40.7	6/19/2006	11:15AM	6/21/2006	1:37PM	35.1 & 39.1
14	11	24.00	9.38	0.50	8.48	0.00	42.4				1:58PM	
14	12	23.70	9.04	0.49	8.55	0.00	41.8				2:19PM	
21	13	28.00	9.46	0.78	9.24	0.00	47.5	6/26/2006	8:30AM	6/26/2006	2:27PM	63.6 & 56.3
21	14	28.70	8.80	0.94	8.12	0.00	46.6				2:48PM	
21	15	30.20	9.55	1.15	8.16	0.00	49.1				3:10PM	
28	16	18.30	6.42	1.72	8.60	0.00	35.0	7/3/2006	8:30AM	7/3/2006	1:38PM	77.8 & 78.9
28	17	22.00	7.50	1.77	8.06	0.00	39.3				1:59PM	
28	18	22.80	8.04	1.84	8.76	0.00	41.4				2:42PM	
28	19	23.50	6.93	1.93	8.36	0.00	40.7	7/3/2006	8:30AM	7/3/2006	3:03PM	77.8 & 78.9
28	20	25.80	7.97	2.03	8.62	0.00	44.4				3:25PM	
28	21	30.10	9.65	2.29	8.27	0.00	50.3				3:46PM	

Bottles 19-21 were autoclaved on 0 and analyzed on 28

Sample analyzed on 0 without preparation - Only for information on the amount of dissolved microcystin

		9.61	3.55	0.61	2.57	0.00	16.3			6/5/2006	1:34PM
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Initial aliquot of the initial sample analyzed on 0 used to gauge concentration levels for LC/MS/MS

		22.70	7.31	1.02	8.12	0.00	39.2	6/5/2006	11:00AM	6/5/2006	1:55PM
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Experiment 1 Abraxis ELISA kit (FDEP)

Preparation Method = Autoclave, Calibration Range: Min=0.15, Max=5; See table for required dilutions.

Analysis Day	Bottle Number	Result in ug/L	Preparation		Analysis		Dilution Required for Reported Results	Recovery (%) for LCS
			Date	Time	Date	Time		
0	1	82	6/5/2006	11:00AM	6/5/2006	11:00AM	Yes, 10 x (high)	119 & 134
0	2	81						
0	3	80						
48 hrs	4	75	6/7/2006	10:00AM	6/7/2006	10:00AM	Yes, 50 x	96 & 115
48 hrs	5	76						
48 hrs	6	88						
7	7	93	6/15/2006	7:30AM	6/15/2006	7:30AM	Yes, 50 x	114 & 91.5
7	8	78						
7	9	100						
14	10	99	6/19/2006	11:15AM	6/19/2006	11:15AM	Yes, 50 x	98 & 83
14	11	100						
14	12	94						
21	13	96	6/26/2006	8:30AM	6/26/2006	8:30AM	Yes, 50 x	116 & 113
21	14	87						
21	15	88						
28	16	92	7/3/2006	8:30AM	7/3/2006	8:30AM	Yes, 50 x	157 & 154
28	17	120						
28	18	110						
28	19	94	7/3/2006	8:30AM	7/3/2006	8:30AM	Yes, 50 x	157 & 154
28	20	100						
28	21	93						

Bottles 19-21 were autoclaved on 0 and analyzed on 28

Experiment 1 Envirologix ELISA kit (SFWMD)

Preparation Method = Autoclave; Calibration Range: Min=0.15, Max=2.5; 50% dilution was required for reported results

Analysis Day	Bottle Number	Result in ug/L	Preparation		Analysis		% RPD for duplicates	% RPD for 2 wells	Recovery (%) for:	
			Date	Time	Date	Time			LCS	Matrix Spikes
48 hours	1	65	6/7/2006	8:05AM	6/7/2006	1:36PM	18	6	103.8, 113.2	95.2
48 hours	2	65						3		
48 hours	3	70						2		
7	4	55	6/12/2006	7:21AM	6/12/2006	11:54AM	0	10	103.8, 103.8	95.2
7	5	55						2		
7	6	65						3		
14	7	60	6/19/2006	8:00AM	6/19/2006	1:30PM	10.5	7	103.8, 103.8	85.7
14	8	60						19		
14	9	60						8		
21	10	40	6/26/2006	7:55AM	6/26/2006	12:26PM	9.5	13	103.8, 103.8	95.2
21	11	55						1		
21	12	45						2		
28	13	40	7/3/2006	7:30AM	7/3/2006	12:40PM	9.5	7	103.8, 94.3, 84.9	104.8
28	14	45						9		
28	15	45						3		
28	16	60	7/3/2006	7:30 AM	7/3/2006	12:40PM	9.5	6	103.8, 94.3, 84.9	104.8
28	17	60						11		
28	18	60						5		

Bottles 16-18 were autoclaved on 0 and analyzed on 28

Note: SFWMD could run Day 0 analyses because the samples had to be shipped to them.

Experiment 2 LC-MS/MS (FDEP)

Preparation Method = Autoclave; Calibration Range: Min=0.5, Max=10; No dilution was required for reported results.

Analysis Day	Bottle Number	LC-MS/MS Analysis						Preparation		Analysis		Recovery (%) for LCS	% RPD for dups
		MC-LR	MC-LA	MC-YR	MC_RR	Nodularin	Total Result in ug/L	Date	Time	Date	Time		
0	1	2.80	0.34	0.00	0.88	0.00	4.0	8/8/2006	12:30PM	8/8/2006	3:54PM	85/86.6	1.86
0	2	3.10	0.56	0.00	0.91	0.00	4.6				4:15PM		
0	3	3.10	0.65	0.00	0.95	0.00	4.7				4:36PM		
48 hours	4	3.30	0.28	0.00	0.81	0.00	4.4	8/10/2006	8:20AM	8/10/2006	1:24AP	88.4/91.8	3.77
48 hours	5	3.30	0.29	0.00	0.64	0.00	4.2				1:46PM		
48 hours	6	3.40	0.28	0.00	0.75	0.00	4.4				2:07PM		
48 hours	19	3.60	0.30	0.00	0.78	0.00	4.7	8/10/2006	8:20AM	8/10/2006	2:28PM	88.4/91.8	3.77
48 hours	20	3.30	0.25	0.00	0.70	0.00	4.3				2:50PM		
48 hours	21	3.60	0.33	0.00	0.75	0.00	4.7				3:11PM		
7	7	3.64	0.63	0.00	0.77	0.00	5.0	8/15/2006	7:30AM	8/15/2006	9:40AM	86.9/101	15
7	8	3.98	0.51	0.00	0.81	0.00	5.3				10:01AM		
7	9	3.54	0.66	0.00	0.80	0.00	5.0				10:23AM		
14	10	3.48	0.53	0.00	0.65	0.00	4.7	8/22/2006	7:30AM	8/22/2006	10:23AM	96.7/103	6.41
14	11	3.87	0.64	0.00	0.79	0.00	5.3				10:23AM		
14	12	4.10	0.82	0.00	0.91	0.00	5.8				10:23AM		
21	13	3.31	0.67	0.00	0.88	0.00	4.9	8/29/2006	7:30AM	8/29/2006	10:37AM	89/91	2.22
21	14	3.10	0.67	0.00	0.92	0.00	4.7				11:03AM		
21	15	3.42	0.69	0.00	0.91	0.00	5.0				11:24AM		
28	16	3.03	0.79	0.00	0.89	0.00	4.7	9/5/2006	8:30AM	9/5/2006	11:44AM	85.8/89.7	4.44
28	17	3.58	0.86	0.00	1.00	0.00	5.4				12:05PM		
28	18	3.61	0.74	0.00	1.00	0.00	5.3				12:27PM		
28	19	4.29	0.93	0.00	0.96	0.00	6.2	9/5/2006	8:30AM	9/5/2006	12:48PM	85.8/89.7	4.44
28	20	3.57	0.87	0.00	0.85	0.00	5.3				1:09PM		
28	21	4.26	0.92	0.00	0.95	0.00	6.1				1:31PM		

Bottles 19-21 were autoclaved on 0 and analyzed on 2 and 28

Experiment 2 Abraxis ELISA kit (FDEP)

Preparation Method = Autoclave; Calibration Range: Min=0.15, Max=3; 10 times dilution required

Analysis Day	Bottle Number	Result in ug/L	Preparation		Analysis		% RPD for dups	Recovery (%) for LCS
			Date	Time	Date	Time		
0	1	15	8/8/2006	12:30PM	8/8/2006	12:30PM	3.17	124/128
0	2	18						
0	3	14						
48 hours	4	15	8/10/2006	8:20AM	8/10/2006	8:20AM	6.4	136/145
48 hours	5	17						
48 hours	6	17						
48 hours	19	16	8/10/2006	8:20AM	8/10/2006	8:20AM	6.4	136/145
48 hours	20	12						
48 hours	21	17						
7	7	16.5	8/15/2006	7:30AM	8/15/2006	7:30AM	49	84/139
7	8	13.9						
7	9	15.7						
14	10	12.4	8/22/2006	7:30AM	8/22/2006	7:30AM	14.7	101/117
14	11	13.2						
14	12	11.8						
21	13	15	8/29/2006	7:30AM	8/29/2006	7:30AM	0.94	107/106
21	14	13						
21	15	13						
28	16	14	9/5/2006	8:30AM	9/5/2006	8:30AM	11.5	107/120
28	17	18						
28	18	16						
28	19	16.1	9/5/2006	8:30AM	9/5/2006	8:30AM	11.5	107/120
28	20	14.9						
28	21	14.3						

Bottles 19-21 were autoclaved on 0 and analyzed on 2 and 28

Experiment 2 Envirologix ELISA kit (FDEP)

Preparation Method = Autoclave; Calibration Range: See table; 10 times dilution required

Analysis Day	Bottle Number	Result in ug/L	Preparation		Analysis		% RPD for dupli cates	Recovery (%) for LCS	Calibration	
			Date	Time	Date	Time			Min	Max
48 hours	4	8	8/10/2006	8:20AM	8/10/2006	8:20AM	3.35	91/88	0.15	3
48 hours	5	7.7								
48 hours	6	7.7								
48 hours	19	6.6	8/10/2006	8:20AM	8/10/2006	8:20AM	3.35	91/88	0.15	3
48 hours	20	6.9								
48 hours	21	8								
7	7	7.16	8/15/2006	7:30AM	8/15/2006	7:30AM	1.08	93/92	0.16	2.5
7	8	6.47								
7	9	6.49								
14	10	5.60	8/22/2006	7:30AM	8/22/2006	7:30AM	4.94	79/83	0.16	2.5
14	11	7.10								
14	12	7.70								
21	13	6.1	8/29/2006	7:30AM	8/29/2006	7:30AM	1.29	78/77	0.16	2.5
21	14	6.7								
21	15	6.8								
28	16	5.1	9/5/2006	8:30AM	9/5/2006	8:30AM	6.99	74/69	0.16	2.5
28	17	6								
28	18	5.9								
28	19	5.7	9/5/2006	8:30AM	9/5/2006	8:30AM	6.99	74/69	0.16	2.5
28	20	6								
28	21	6.6								

Bottles 19-21 were autoclaved on 0 and analyzed on 2 and 28

Note: FDEP did not run the Envirologix kit test on Day 0

Experiment 2 Envirologix (SFWMD)

Preparation Method = Autoclave; Calibration Range: Min=0.15, Max=2.5; 5 times dilution required for reported results

Analysis Day	Bottle No.	Result in ug/L	Preparation		Analysis		% RPD for dups	% RPD for 2 wells	Recovery (%) for:	
			Date	Time	Date	Time			LCS	Matrix Spikes
48 hours	1	9	8/10/2006	7:00 AM	8/10/2006	4:57PM	7.4	4	113, 120	114
48 hours	2	8.5						3		
48 hours	3	8.5						6		
7	4	7.5	8/15/2006	8:30 AM	8/15/2006	1:14 PM	15.4	13	113, 120	114
7	5	7						1		
7	6	7.5						6		
14	7	7	8/22/2006	7:30 AM	8/22/2006	12:50 PM	9.5	5	94, 94	95
14	8	6.5						11		
14	9	8						14		
21	10	7	8/29/2006	7:30 AM	8/29/2006	12:23 PM	8.7	9	104, 113	114
21	11	6.5						4		
21	12	6.5						14		
28	13	7.5	9/5/2006	7:20 AM	9/5/2006	12:28 PM	9.5, 0	4	104, 104, 94	95, 105
28	14	8						3		
28	15	5.5						1		
28	16	9	9/5/2006	7:20 AM	9/5/2006	12:28 PM	9.5, 0	1	104, 104, 94	95, 105
28	17	9						3		
28	18	9						3		

Bottles 16-18 were autoclaved on 0 and analyzed on 28

Note: SFWMD could run Day 0 analyses because the samples had to be shipped to them.