

## **Supplementary Materials: Cylindrospermopsin-Microcystin-LR Combinations May Induce Genotoxic and Histopathological Damage in Rats**

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**Table S1.** Measurements (MN, alkaline comet and enzyme-modified comet assays) obtained in the target tissues (liver, stomach and blood) by dose and treatment assayed in male and female rats.

Genotoxicity Measurements (Males)										
Doses	Enzyme-Modified Comet Assay									
	% MN Test	Standard Comet Assay			Endo III-Sensitive Sites			Fpg-Sensitive Sites		
	Bone Marrow	Liver	Stomach	Blood	Liver	Stomach	Blood	Liver	Stomach	Blood
Negative control (water)	1.6	0.60	3.45	0.15	8.9	1.28	0.15	2.70	2.50	0.75
	0.22	0.00	0.01	0.25	1.2	9.43	4.22	10.10	12.00	0.35
	0.16	0.00	0.01	0.22	3.1	10.0	0.70	4.62	2.95	0.08
	0.16	0.05	4.50	0.12	10.0	8.4	1.10	10.60	4.50	0.05
	0.12	0.68	0.00	0.18	6.40	8.8	0.00	3.50	5.80	0.10
Solvent control (0.5% MeOH)	1.58	0.50	6.02	0.00	3.34	1.03	2.3	2.90	4.31	0.04
	1.10	0.00	4.00	3.20	0.44	0.16	0.03	2.95	1.15	0.45
	0.34	0.00	0.00	0.00	0.51	0.30	0.03	2.90	0.01	0.01
	0.48	0.20	0.00	0.05	0.74	0.53	0.70	8.00	0.00	0.10
	0.64	0.50	5.80	0.07	11.60	5.64	0.01	7.30	0.00	0.06
Positive Control	1.84	34.34	43.48	37.28	29.05	40.2	27.4	25.70	40.7	33.60
	2.10	16.52	17.42	15.70	25.35	32.9	30.4	23.15	35.0	25.80
	1.84	22.89	35.11	40.91	19.3	37.2	24.4	31.95	33.45	28.80
7.5 + 75 µg/kg b.w. CYN/MC-LR	2.40	0.50	1.50	0.01	12.9	12.6	0.41	9.18	8.30	0.07
	1.54	0.25	2.30	0.25	7.55	16.4	0.00	6.34	14.08	0.07
	1.95	0.15	2.10	0.40	7.19	9.85	1.99	5.19	6.35	0.08
	1.50	0.16	0.90	0.05	8.18	10.45	0.20	8.66	13.34	0.26
	1.36	0.80	1.50	0.22	9.34	12.54	0.42	14.55	14.68	0.49
23.7 + 237 µg/kg b.w. CYN/MC-LR	2.34	0.23	0.09	2.15	0.63	6.10	0.09	0.80	3.34	0.01
	1.28	0.33	0.06	0.1	3.11	0.35	0.06	0.60	2.95	0.01
	1.68	0.20	0.02	0.09	0.08	4.65	0.40	0.20	3.26	0.05
	2.10	0.27	0.05	0.04	0.76	8.80	0.02	0.35	10.98	0.02
	1.74	0.12	0.10	0.00	0.42	5.10	0.07	0.60	11.87	0.02
75 + 750 µg/kg b.w. CYN/MC-LR	2.68	2.18	9.70	0.65	9.0	6.20	0.00	15.45	12.95	0.07
	1.54	2.00	1.02	0.10	6.9	4.80	0.04	6.85	7.00	0.20
	1.48	1.23	0.50	0.00	4.7	4.20	0.04	8.70	4.70	0.13
	1.60	1.17	0.45	0.05	2.6	5.30	0.09	3.50	8.00	0.07
	2.12	1.82	9.20	6.90	1.7	4.75	0.58	3.75	5.70	3.64

  

Genotoxicity Measurements (Females)										
Doses	Enzyme-Modified comet assay									
	% MN Test	Standard Comet Assay			Endo III-Sensitive Sites			Fpg-Sensitive Sites		

	Bone Marrow	Liver	Stomach	Blood	Liver	Stomach	Blood	Liver	Stomach	Blood
Negative control (water)	0.84	0.59	0.00	0.75	0.17	11.4	0.64	1.85	6.60	2.59
	1.14	1.80	0.20	0.82	0.30	4.1	3.59	1.20	8.40	0.49
	0.48	0.17	0.45	0.08	0.82	12.7	0.00	3.85	12.90	0.01
	0.44	0.03	0.50	1.17	3.79	10.7	0.04	3.80	5.40	0.01
Solvent control (0.5% MeOH)	0.38	0.17	1.30	0.03	2.76	11.9	1.10	5.30	10.4	1.20
	0.70	1.43	0.07	0.02	0.60	5.79	1.10	2.16	7.36	1.60
	0.20	1.48	0.07	0.04	0.63	2.54	0.03	3.57	4.52	1.60
	0.84	0.28	0.05	0.05	0.46	3.06	2.05	1.56	11.22	2.15
Positive Control	0.54	0.44	0.06	0.04	2.03	6.19	0.35	2.27	7.83	0.01
	0.60	1.08	0.10	0.03	2.03	8.22	4.50	4.98	4.86	6.10
7.5 + 75 µg/kg b.w. CYN/MC-LR	3.28	23.80	37.50	29.35	23.2	46.95	30.0	24.01	37.90	31.30
	1.50	19.73	37.90	12.54	28.8	43.6	24.05	21.88	40.40	26.95
	2.64	23.44	39.00	36.22	29.7	49.7	29.2	27.57	35.50	25.00
23.7 + 237 µg/kg b.w. CYN/MC-LR	2.04	0.10	4.90	0.14	1.45	9.70	0.2	1.75	7.40	0.95
	2.30	0.93	5.90	0.16	7.38	14.40	3.9	8.70	10.20	0.08
	1.92	0.62	1.95	0.16	0.70	7.00	2.6	1.70	8.30	0.40
	1.92	0.03	2.10	0.03	1.80	11.5	2.7	2.60	10.50	2.80
	1.72	0.99	4.45	0.03	2.64	11.00	1.02	4.90	3.60	5.10
75 + 750 µg/kg b.w. CYN/MC-LR	2.02	0.48	1.90	0.70	0.41	6.90	0.02	0.00	2.00	0.02
	2.26	0.09	2.70	0.34	0.99	5.60	1.29	0.35	1.05	0.07
	2.12	2.71	2.21	0.06	2.05	8.90	0.07	0.40	7.40	0.00
	1.86	0.12	2.15	0.01	1.73	2.90	0.02	1.50	6.50	0.00
	2.18	0.11	2.95	0.53	1.49	3.70	0.01	4.00	3.60	0.00
75 + 750 µg/kg b.w. CYN/MC-LR	2.08	1.20	1.00	0.10	4.93	3.62	3.86	4.10	8.00	2.47
	2.34	0.05	1.15	1.76	0.43	1.95	0.44	1.00	5.00	0.57
	2.40	0.10	2.95	0.03	0.15	7.02	0.28	1.35	6.60	0.94
	1.80	0.95	1.60	1.38	1.93	1.93	0.64	0.05	11.40	0.30
	2.46	1.20	1.64	2.00	0.02	7.00	0.77	1.00	10.50	0.22

The values are expressed as individual measurements.

**Table S2.** Summary statistic for treatment groups including both sexes (male and female) for the standard and enzyme-modified comet assay in liver, stomach and blood cells.

	Enzyme-Modified Comet Assay								
	Standard Comet Assay			Endo-III Sensitives Sites			Fpg-Sensitives Sites		
	Liver (mean ± SD)	Stomach (mean ± SD)	Blood (mean ± SD)	Liver (mean ± SD)	Stomach (mean ± SD)	Blood (mean ± SD)	Liver (mean ± SD)	Stomach (mean ± SD)	Blood (mean ± SD)
Negative control (water)	0.41 ± 0.56	1.04 ± 1.62	0.38 ± 0.39	3.74 ± 3.56	8.87 ± 3.59	1.15 ± 1.52	4.75 ± 3.19	7.15 ± 3.65	0.56 ± 0.81

Solvent control (0.5% MeOH)	0.60 ± 0.58 (1.47-fold)	1.62 ± 2.58 (1.55-fold)	0.38 ± 1.06 (1.01-fold)	2.24 ± 3.43 (0.59-fold)	3.35 ± 2.92 (0.38-fold)	0.84 ± 1.21 (0.73-fold)	3.86 ± 2.20 (0.81-fold)	4.13 ± 3.87 (0.58-fold)	1.21 ± 1.90 (2.15-fold)
7.5 + 75 µg/kg CYN/MC-LR	0.45 ± 0.36 (1.11-fold)	2.76 ± 1.69 (2.65-fold)	0.15 ± 0.12 (0.38-fold)	5.91 ± 4.04 (1.58-fold)	11.54 ± 2.63 (1.30-fold)	1.34 ± 1.36 (1.16-fold)	6.36 ± 4.03 (1.34-fold)	9.68 ± 3.59 (1.35-fold)	1.03 ± 1.65 (1.83-fold)
23.7 + 237 µg/kg CYN/MC-LR	0.47 ± 0.80 (1.14-fold)	1.22 ± 1.26 (1.17-fold)	0.40±0.66 (1.07-fold)	1.17 ± 0.93 (0.31-fold)	5.3 ± 2.62 (0.60-fold)	0.21 ± 0.40 (0.18-fold)	0.88 ± 1.17 (0.19-fold)	5.30 ± 3.75 (0.74-fold)	0.02 ± 0.02 (0.04-fold)
75 + 750 µg/kg CYN/MC-LR	1.19 ± 0.71 (2.91-fold)	2.92 ± 3.51 (2.80-fold)	1.30±2.11 (3.44-fold)	3.24 ± 3.05 (0.86-fold)	4.68 ± 1.82 (0.53-fold)	0.67 ± 1.15 (0.58-fold)	4.58 ± 4.70 (0.96-fold)	7.99 ± 2.80 (1.12-fold)	0.86 ± 1.22 (1.53-fold)
Positive control (EMS)	23.45 ± 6.01*** (57.34-fold)	35.07 ± 9.07*** (33.65-fold)	28.67 ± 11.91*** (76.04-fold)	25.9 ± 4.10** (6.92-fold)	41.76 ± 6.25** (4.71-fold)	27.58 ± 2.79** (23.90-fold)	25.71 ± 3.65** (5.41-fold)	37.16 ± 2.99** (5.2-fold)	28.58 ± 3.34** (50.75-fold)

Negative control: Water; Solvent control: 0.5% MeOH; CYN: Cylindrospermopsin; MC-LR: Microcystin-LR; Positive control : Ethylmethanesulfonate (EMS). The levels of DNA strand breaks are expressed as % DNA in the tail of the comets. All values are represented as mean ± SD. Significantly different from control (\*\**p*<0.01; \*\*\**p* < 0.001).

**Table S3.** Relative weight (RW) (excised wet organ weight /animal weight) of liver and stomach from exposed male rats.

Male						
	Negative control (Water) <i>n</i> = 5 (mean ± SD)	Solvent Control (0.5% MeOH) <i>n</i> = 5 (mean ± SD)	7.5 + 75 µg/mL CYN/MC-LR <i>n</i> = 5 (mean ± SD)	23.7 + 237 µg/mL CYN/MC-LR <i>n</i> = 5 (mean ± SD)	75+750 µg/mL CYN/MC-LR <i>n</i> = 5 (mean ± SD)	Positive control (EMS) <i>n</i> = 3 (mean ± SD)
Body weight (g)	145.19 ± 27.56	156.54 ± 24.26	132.53 ± 12.18	164.75 ± 5.42	146.88 ± 15.75	138.38 ± 14.86
Liver	3.14 ± 0.03	3.22 ± 0.17	3.25 ± 0.11	3.02 ± 0.17	3.24 ± 0.25	3.52 ± 0.24
Stomach	0.49 ± 0.06	0.38 ± 0.05	0.52 ± 0.09	0.47 ± 0.09	0.46 ± 0.15	0.84 ± 0.30**

Negative control: water; Solvent control: 0.5% MeOH; CYN: Cylindrospermopsin; MC-LR: Microcystin-LR; Positive control : Ethylmethanesulfonate (EMS). The values are expressed as mean ± SD. Significantly different from the negative control (\*\**p* < 0.01).

**Table S4.** Relative weight (RW) (excised wet organ weight /animal weight) of liver and stomach from exposed female rats.

Female						
	Negative Control (Water) <i>n</i> = 5 (mean ± SD)	Solvent Control (0.5% MeOH) <i>n</i> = 5 (mean ± SD)	7.5 + 75 µg/mL CYN/MC-LR <i>n</i> = 5 (mean ± SD)	23.7 + 237 µg/mL CYN/MC-LR <i>n</i> = 5 (mean ± SD)	75 + 750 µg/mL CYN/MC-LR <i>n</i> = 5 (mean ± SD)	Positive Control (EMS) <i>n</i> = 3 (mean ± SD)
Body weight (g)	168.35 ± 15.57	173.53 ± 16.29	175.20 ± 7.28	165.46 ± 12.05	171.60 ± 9.44	172.30 ± 17.16
Liver	3.07 ± 0.17	2.97 ± 0.22	2.90 ± 0.19	2.96 ± 0.12	3.09 ± 0.16	3.56 ± 0.41*

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Stomach	0.39 ± 0.06	0.38 ± 0.06	0.33 ± 0.02	0.43 ± 0.09	0.52 ± 0.14	0.53 ± 0.26
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Negative control: Water; Solvent control: 0.5% MeOH; CYN: Cylindrospermopsin; MC-LR: Microcystin-LR; Positive control +: Ethylmethanesulfonate (EMS).  
The values are expressed as mean ± SD. Significantly different from the negative control (\**p* < 0.05)