

Acute Toxicity Tests of TEMPO-oxidized Cellulose Nano-fiber

Using *Daphnia magna* and *Oryzias latipes*

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Supplementary Information

Online Resource 1: Selection of a medium for ecotoxicity tests

Table S1 Compositions of the different media

Test medium	Ion concentration [mg/L]					
	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻
Very soft synthetic water	3.3	0.3	1.5	1.7	0.2	10.2
Blended water	8.0	1.2	2.4	4.9	8.0	13.0
Medium-hardness synthetic water	26.3	2.1	12.1	14.0	1.9	81.4
Dechlorinated water	25.3	4.5	6.5	16.6	37.4	27.1

Online Resource 2: Percentage deviation of the measured concentration from the nominal concentration and the percent change of the measured concentration observed in the evaluation of concentration uniformity

Table S2 Average percentage deviation of the measured concentration from the nominal concentration in ultrapure water

		0 h	24 h	48 h
Upper part	Average [%]	7.6	6.5	2.9
	SD	2.7	3.9	2.2
Lower part	Average [%]	10.1	5.0	2.8
	SD	3.6	4.2	2.0

Table S3 Average percent change in measured concentrations in ultra-pure water

		Temporal			Spatial			Spatiotemporal		
		0h→24h	24h→48h	0h→48h	0h→24h	24h→48h	0h→48h	0h→24h	24h→48h	0h→48h
Upper site	Average [%]	4.4	5.5	6.8	6.1	5.7	5.8	5.2	6.5	7.7
	SD	4.3	3.2	5.1	0.8	4.8	2.9	1.4	7.0	6.4
Lower sitepart	Average [%]	5.8	4.7	10.7	6.4	5.4	5.7	4.1	7.3	9.8
	SD	6.9	2.2	5.3	1.2	4.1	3.0	2.4	4.5	5.4

Table S4 Average percentage deviation of the measured concentration from the nominal concentration in very soft synthetic water

		0 h	24 h	48 h
Upper part	Average [%]	2.7	3.2	8.9
	SD	1.2	2.5	3.0
Lower part	Average [%]	1.8	11.9	7.0
	SD	1.7	6.2	6.1

Table S5 Average percent change in measured concentrations in very soft synthetic water

		Temporal			Spatial			Spatiotemporal		
		0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h
Upper part	Average [%]	1.7	6.0	7.7	1.5	15.3	7.1	13.3	7.2	8.4
	SD	1.0	2.6	1.6	1.3	2.9	6.2	3.1	3.8	4.5
Lower part	Average [%]	13.9	16.6	7.8	1.5	13.2	6.6	2.1	18.5	7.2
	SD	5.7	8.9	3.5	1.4	2.1	5.2	1.7	3.4	1.7

Table S6 Average percentage deviation of the measured concentration from the nominal concentration in blended water

		0 h	24 h	48 h
Upper part	Average [%]	5.9	1.2	2.8
	SD	5.3	0.7	2.8
Lower part	Average [%]	9.4	3.6	3.8
	SD	0.8	5.6	2.0

Table S7 Average percent change in measured concentrations in blended water

		Temporal			Spatial			Spatiotemporal		
		0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h
Upper part	Average [%]	6.1	3.6	3.0	4.1	4.3	6.3	8.8	3.0	9.0
	SD	4.4	1.5	4.9	3.8	5.5	4.4	7.3	3.2	4.7
Lower part	Average [%]	11.9	3.0	12.1	3.9	4.7	6.9	9.3	6.9	6.1
	SD	4.8	1.4	1.5	3.4	6.3	5.1	1.5	9.4	2.9

Table S8 Average percentage deviation of the measured concentration from the nominal concentration in dechlorinated tap water.

		0 h	24 h	48 h
Upper part	Average [%]	10.1	21.2	26.8
	SD	2.9	8.3	3.0
Lower part	Average [%]	3.8	19.1	24.5
	SD	4.0	6.3	1.7

Table S9 Average percent change in measured concentrations in dechlorinated tap water

		Temporal			Spatial			Spatiotemporal		
		0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h
Upper part	Average [%]	12.7	10.5	18.6	7.1	12.7	5.7	10.1	5.4	15.9
	SD	11.0	7.6	0.9	5.6	9.8	2.9	4.1	5.9	4.5
Lower part	Average [%]	15.9	7.0	21.4	6.5	12.0	5.4	18.1	9.3	23.9
	SD	6.5	7.8	3.4	4.8	8.3	2.5	7.8	3.1	3.8

Table S10 Average percentage deviation of the measured concentration from the nominal concentration in medium-hardness synthetic water.

		0 h	24 h	48 h
Upper part	Average [%]	24.3	33.8	15.8
	SD	1.5	2.6	5.0
Lower part	Average [%]	49.7	27.0	11.5
	SD	42.5	8.7	9.7

Table S11 Average percent change in measured concentrations in medium-hardness synthetic water

		Temporal			Spatial			Spatiotemporal		
		0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h	0 h→24 h	24 h→48 h	0 h→48 h
Upper part	Average [%]	12.4	27.5	11.3	55.1	13.0	15.8	8.5	45.5	27.0
	SD	4.7	12.7	6.0	90.1	14.4	11.0	6.8	29.3	22.2
Lower part	Average [%]	27.8	31.0	46.8	22.5	10.6	13.2	30.1	16.0	29.4
	SD	34.4	8.1	16.2	33.7	10.6	8.6	31.4	8.6	26.8

Online Resource 3: Ecotoxicity of TEMPO-CNF to test organisms

Table S12 Measured TEMPO-CNF concentrations during tests for *D. magna*

Nominal concentration [mg/L]	Measured concentration at 0 h [mg/L]	Measured concentration at 48 h [mg/L]	Percentage change in concentration from 0 to 48 h [%]
0	0.4	0.4	-
3.1	3.0	2.6	-13
6.3	6.6	4.5	-32
12.5	11.9	12.1	1.7
25	30	27	-10
50	45	50	11
100	103	101	-1.9

Table S13 Measured TEMPO-CNF concentrations during tests for *O. latipes*

Nominal concentration [mg/L]	Measured concentration at 0 h [mg/L]	Measured concentration at 48 h [mg/L]	Measured concentration of renewal water at 48 h [mg/L]	Measured concentration at 96 h [mg/L]	Percentage change in concentration from 0 to 48 h [%]	Percentage change in concentration from 48 to 96 h [%]
0	0.8	0.7	0.2	0.6	-	-
3.1	2.9	3	3.4	3.1	2.5	-6.8
6.3	6.6	5.9	6.4	6.4	-10	0.7
13	13	12	12	11	-10	-4.4
25	21	23	28	28	11	-2.9
50	44	51	48	48	15	1.2
100	97	98	100	105	1.6	5.1

Online Resource 4: Percentage deviation of the measured concentration from the nominal concentration observed in the evaluation of concentration uniformity of TEMPO-CNF in the test solutions during the *D. magna* acute toxicity test

Table S14 Average percentage deviation of the measured concentration from the nominal concentration (3 mg/L) in the *D. magna* acute toxicity test

		0 h	48 h
Upper part	Average [%]	11.1	13.3
	SD	5.1	0.0
Lower part	Average [%]	22.2	15.5
	SD	12.6	6.9

Table S15 Average percentage change in measured concentrations in the *D. magna* acute toxicity test (nominal concentration of 3 mg/L)

		Temporal 0 h→48 h	Spatial 0 h 48 h		Spatiotemporal 0 h→48 h
Upper part	Average [%]	18.0	15.0	5.1	19.9
	SD	9.7	11.1	5.9	14.3
Lower part	Average [%]	30.6	12.5	5.6	28.6
	SD	7.0	8.7	6.7	7.0

Table S16 Average percentage deviation of the measured concentration from the nominal concentration (100 mg/L) in the *D. magna* acute toxicity test

		0 h	48 h
Upper part	Average [%]	3.0	5.3
	SD	2.6	2.3
Lower part	Average [%]	1.7	3.0
	SD	2.9	1.7

Table S17 Average percentage change in measured concentrations in the *D. magna* acute toxicity test (nominal concentration of 100 mg/L).

		Temporal	Spatial		Spatiotemporal
		0 h→48 h	0 h	48 h	0 h→48 h
Upper part	Average [%]	8.1	3.2	4.6	4.5
	SD	1.9	2.4	3.1	5.4
Lower part	Average [%]	3.3	3.3	4.4	6.9
	SD	1.5	2.5	2.8	2.5

Online Resource 5: Concentration uniformity of TEMPO-CNF in the test solutions for the *O. latipes* acute toxicity test

The concentration uniformity of TEMPO-CNF in the test solutions over 48 h was investigated. In the present study, we used fish individuals from a population that was established from individuals purchased from AS ONE Corp. (Osaka, Japan) and bred in our laboratory. Individuals with an average body length of 29.7 ± 1.15 cm were selected as the test individuals and acclimated for one week under a light/dark cycle of 16/8 with the test medium set at 25 ± 1 °C. Test solutions with TEMPO-CNF concentrations of 3 mg/L and 100 mg/L were prepared by adding a stock solution of TEMPO-CNFs to the test medium. Each solution was replicated once. For each replicate, 7 fish individuals and 3 L of test solution were placed in a 3 L round water tank. After the CNF samples were collected, pH (AS800, ASONE Corp., Japan) and DO (HQ30d, HACH, U.S.) were measured at the start and end of the test. For the CNF analysis, 1 mL of the test solutions at two locations, 5 mm from the liquid surface and 5 mm from the bottom layer, were collected twice at the start and end of the test. Statistical analyses were performed according to the concentration uniformity of TEMPO-CNF in the test solutions for the *D. magna* acute toxicity test.

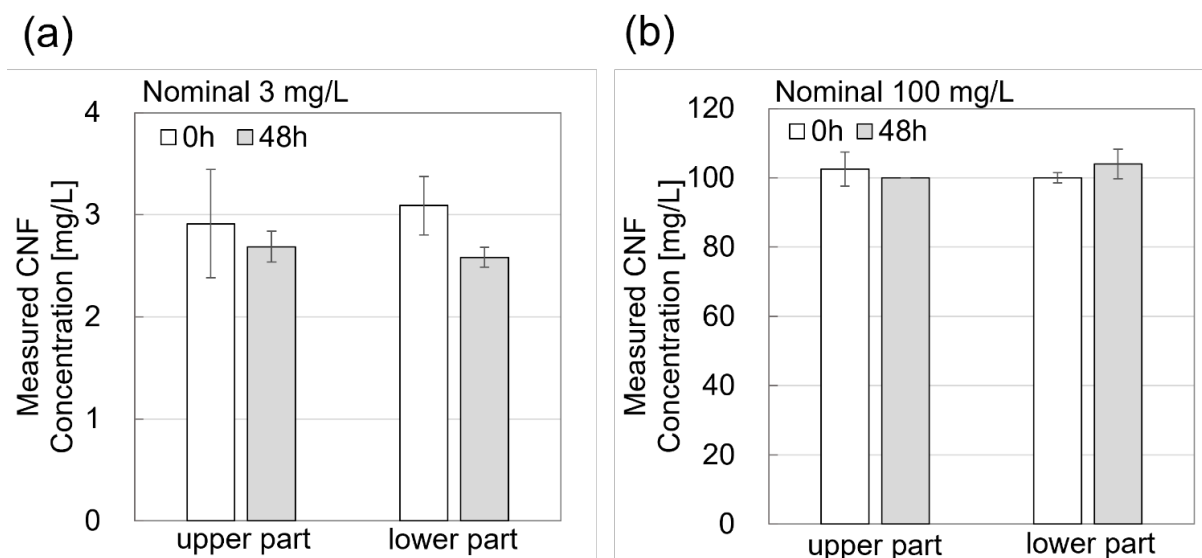


Fig. S4 Results of concentration uniformity tests of TEMPO-CNF in (a) 3 mg/L CNF and (b) 100 mg/L CNF solutions

Table S18 Average percentage of deviation of the measured concentration from the nominal concentration (3 mg/L) in the *O. latipes* acute toxicity test

		0 h	48 h
Upper part	Average [%]	3.3	10.0
Lower part	Average [%]	3.3	13.3

Table S19 Average percent change in measured concentrations in the *O. latipes* acute toxicity test (nominal concentration was 3 mg/L)

		Temporal	Spatial		Spatiotemporal
		0 h→48 h	0 h	48 h	0 h→48 h
Upper part	Average [%]	6.9	6.9	3.7	10.3
Lower part	Average [%]	16.1	6.5	3.8	12.9

Table S20 Average percentage of deviation of the measured concentration from the nominal concentration (100 mg/L) in the *O. latipes* acute toxicity test

		0 h	48 h
Upper part	Average [%]	2.5	0.0
Lower part	Average [%]	0.0	4.0

Table S21 Average percent change in measured concentrations in the *O. latipes* acute toxicity test (nominal concentration was 100 mg/L)

		Temporal	Spatial		Spatiotemporal
		0 h→48 h	0 h	48 h	0 h→48 h
Upper part	Average [%]	2.4	2.4	4.0	1.5
Lower part	Average [%]	4.0	2.5	3.8	0.0