

## **Supplementary Material**

# **Tail Coiling Assay in Zebrafish (*Danio rerio*) Embryos: Stage of Development, Promising Positive Control Candidates, and Selection of an Appropriate Organic Solvent for Screening of Developmental Neurotoxicity (DNT)**

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**Table S1:** Results from ANOVA on ranks (Kruskall–Wallis Test) for significant differences in percentage (%) of burst activity of embryos monitored from 18 to 54 hours post-fertilization (hpf) at 2 hours intervals (n = 40 embryos per group):

	ANOVA on ranks (Kruskall–Wallis Test) results – Burst activity (%)																		
hpf	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54
18	-	*	*	*	*	*	*	*	ns	*	*	ns							
20	-	ns	*	*	ns	ns	ns	*	*	*	*	*	*	*	*	*	ns	ns	*
22	-	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*
24	-	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*
26	-	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
28	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
30	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*
32	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	ns	ns	ns
34	-	ns	*	ns	ns	ns	ns	ns	ns	*	ns	*	ns						
36	-	ns	*	ns	ns														
38	-	ns	*	ns	ns														
40	-	ns	*	*	ns	ns													
42	-	ns	*	*	ns	ns													
44	-	ns	*	*	ns	ns													
46	-	ns	*	*	ns	ns													
48	-	ns	*	ns	ns	ns													
50	-	ns	*	ns	ns	ns													
52	-	ns	*	ns	ns	ns													
54	-	ns	*	ns	ns	ns													

Notes: hpf, hours post-fertilization; ns, non-significant; - not evaluated. Significant differences ( $p < 0.05$ ) are represented by \*.

**Table S2:** Results from ANOVA on ranks (Kruskall–Wallis Test) for significant differences in mean burst duration of embryos monitored from 18 to 54 hours post-fertilization (hpf) at 2 hours intervals (n = 40 embryos per group):

	ANOVA on ranks (Kruskall–Wallis Test) results – Mean burst duration																		
hpf	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54
18	-	*	*	*	*	*	*	*	*	ns	*	ns							
20	-	ns	*	*	*	*	ns	ns	ns	*	*	*	*	*	*	*	ns	ns	*
22	-	ns	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*
24	-	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
26	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
28	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
30	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
32	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*	
34	-	ns	*	*	*	*	ns	ns	ns	ns	ns								
36	-	ns																	
38	-	ns																	
40	-	ns																	
42	-	ns																	
44	-	ns	*	*	ns	ns	ns												
46	-	ns	*	*	ns	ns	ns												
48	-	ns																	
50	-	ns																	
52	-	ns																	
54	-	ns																	

Notes: hpf, hours post-fertilization; ns, non-significant; - not evaluated. Significant differences ( $p < 0.05$ ) are represented by \*.

**Table S3:** Results from ANOVA on ranks (Kruskall–Wallis Test) for significant differences in burst counts per minute of embryos monitored from 18 to 54 hours post-fertilization (hpf) at 2 hours intervals (n = 40 embryos per group):

	ANOVA on ranks (Kruskall–Wallis Test) results – Burst Counts per minute																		
hpf	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54
18	-	*	*	*	*	*	*	*	ns	*	*	ns							
20	-	ns	ns	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	ns	ns	ns
22	-	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	ns	*
24	-	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	ns	*	ns
26	-	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*	ns
28	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*	*
30	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*
32	-	ns	*	ns	*	*	*	*	*	*	*	*	*	ns	ns	ns	ns	ns	ns
34	-	ns	*	ns	ns														
36	-	ns	*	*	ns	ns													
38	-	ns	*	ns	ns														
40	-	ns	*	*	ns	ns													
42	-	ns	*	*	ns	ns													
44	-	ns	*	*	*	ns													
46	-	ns	*	*	ns	ns													
48	-	ns	*	ns	ns	ns													
50	-	ns	*	ns	ns	ns													
52	-	ns	*	-	*	ns													
54	-	ns	-	-	-	-													

Notes: hpf, hours post-fertilization; ns, non-significant; - not evaluated. Significant differences ( $p < 0.05$ ) are represented by \*.