Naphthenic acid mixtures and acid-extractable organics from oil sands process-affected water impair embryonic development of *Silurana (Xenopus) tropicalis*

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Supporting information

Number of pages: 14

Number of figures: 8

Number of tables: 4

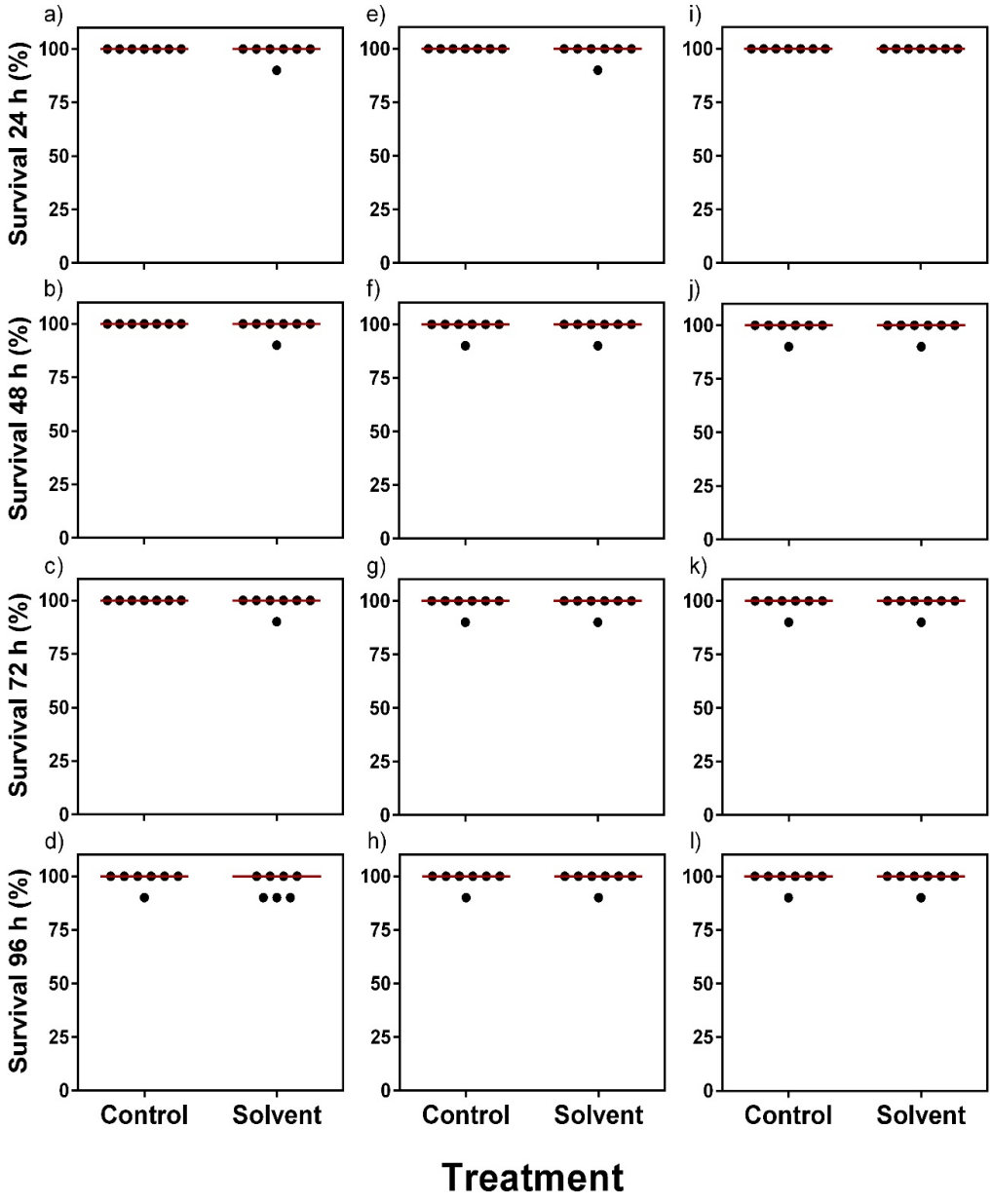

Figure S1. Survival rate of control against solvent treatments for 24, 48, 72, and 96 h, for the exposure of *S. tropicalis* embryos to S1M (a-d), S2M (e-h), and an AEOs (i-l). Ethanol at a final concentration of 0.0025% was used as a carrier solvent for S1M and S2M. NaOH at a final

concentration of 0.001 N was used as carrier solvent for AEOs. Each point represents a replicate and the red lines represent the median.

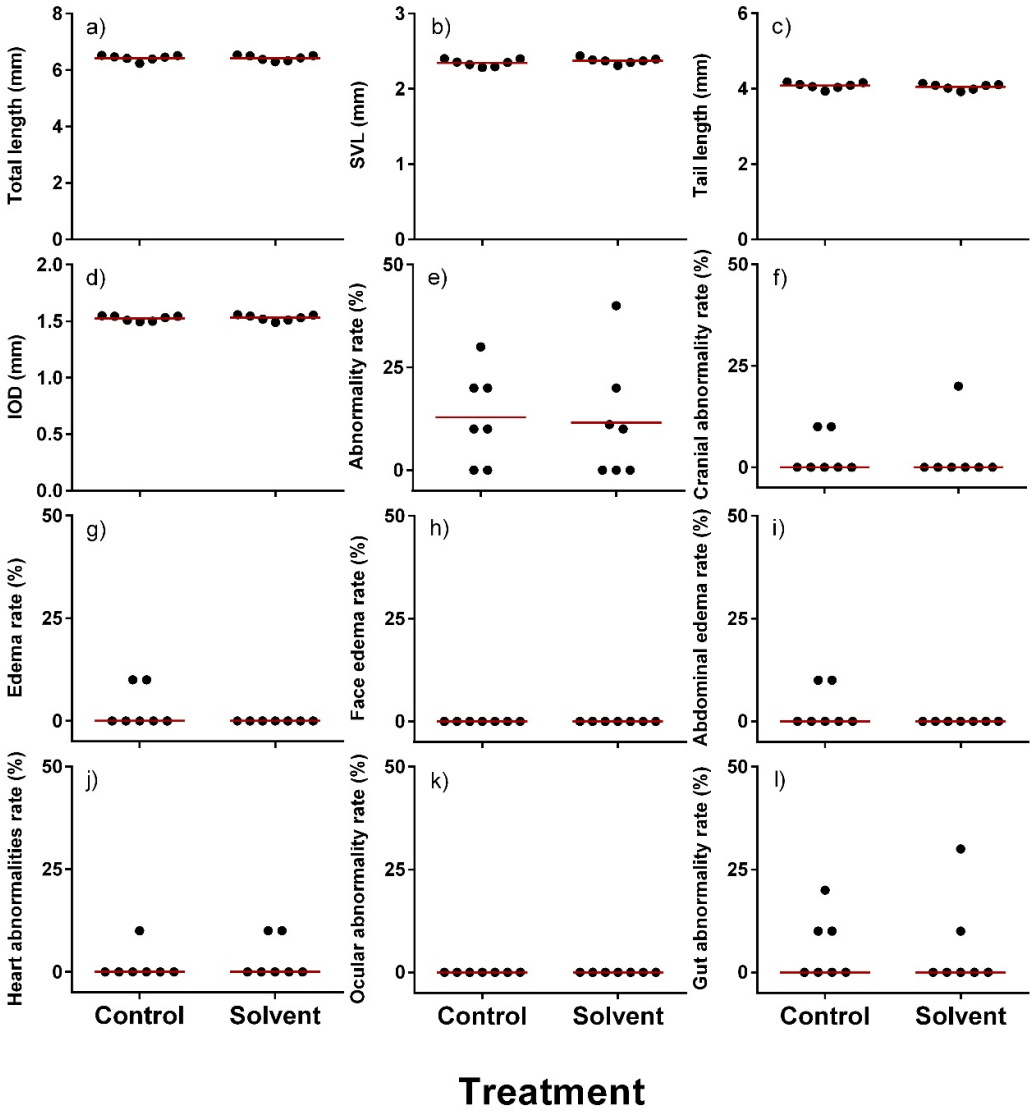

Figure S2. Effects of the solvent carrier on teratogenic endpoints in *S. tropicalis* embryos exposed to S1M. The teratogenic endpoints were TL (a), SVL (b), TaL (c), IOD (d), abnormality rate (e), cranial abnormality (f), edema (g), face edema (h), abdominal edema (i), heart abnormality (j),

ocular abnormality (k), and gut abnormality (l). Ethanol at a final concentration of 0.0025% was used as a carrier solvent. Each point represents a replicate. Red line represents the mean in a-e or the median in f-l.

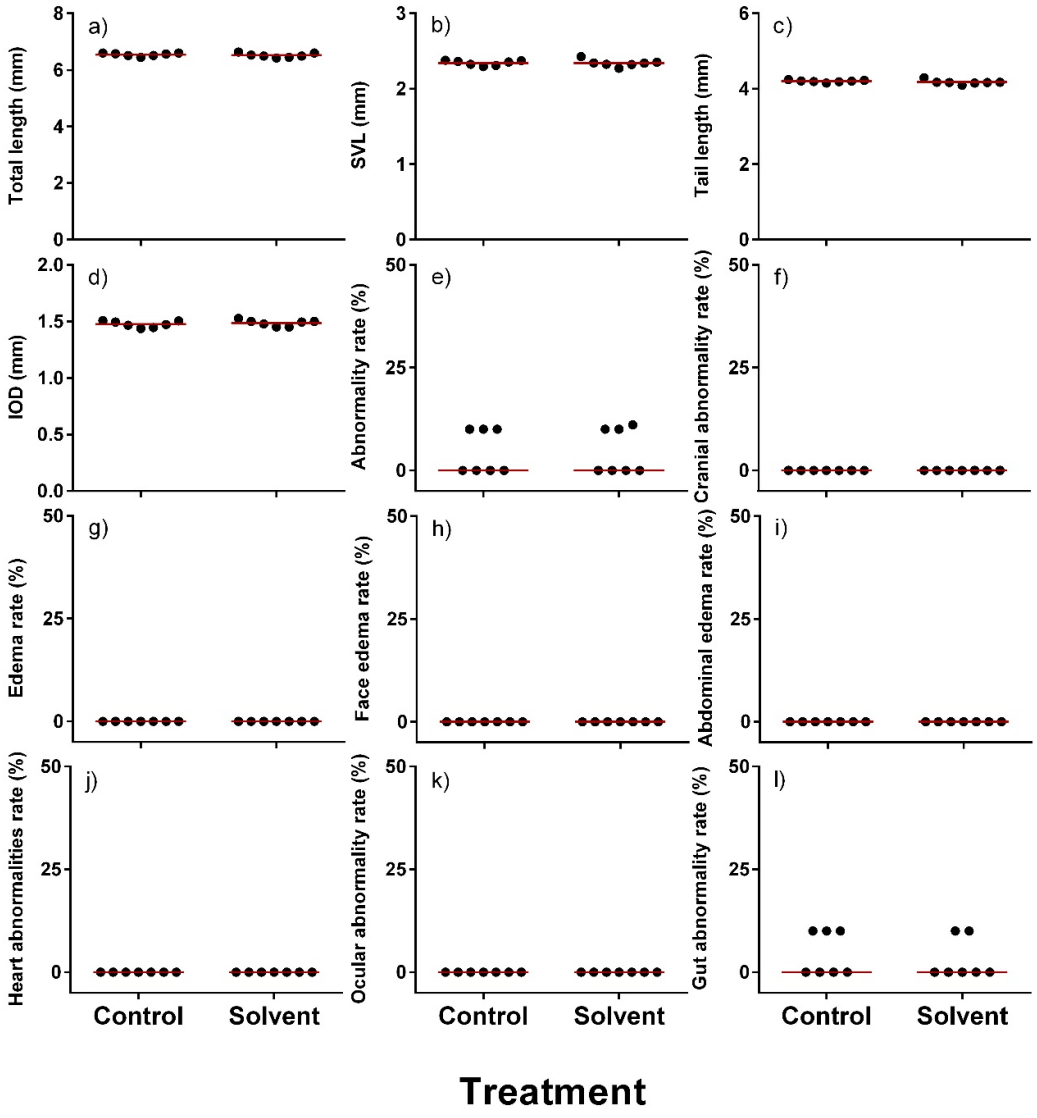

Figure S3. Effects of the solvent carrier on teratogenic endpoints in *S. tropicalis* embryos exposed to S2M. The teratogenic endpoints were TL (a), SVL (b), TaL (c), IOD (d), abnormality rate (e),

cranial abnormality (f), edema (g), face edema (h), abdominal edema (i), heart abnormality (j), ocular abnormality (k), and gut abnormality (l). Ethanol at a final concentration of 0.0025% was used as a carrier solvent. Each point represents a replicate. Red line represents the mean in a-d or the median in e-l.

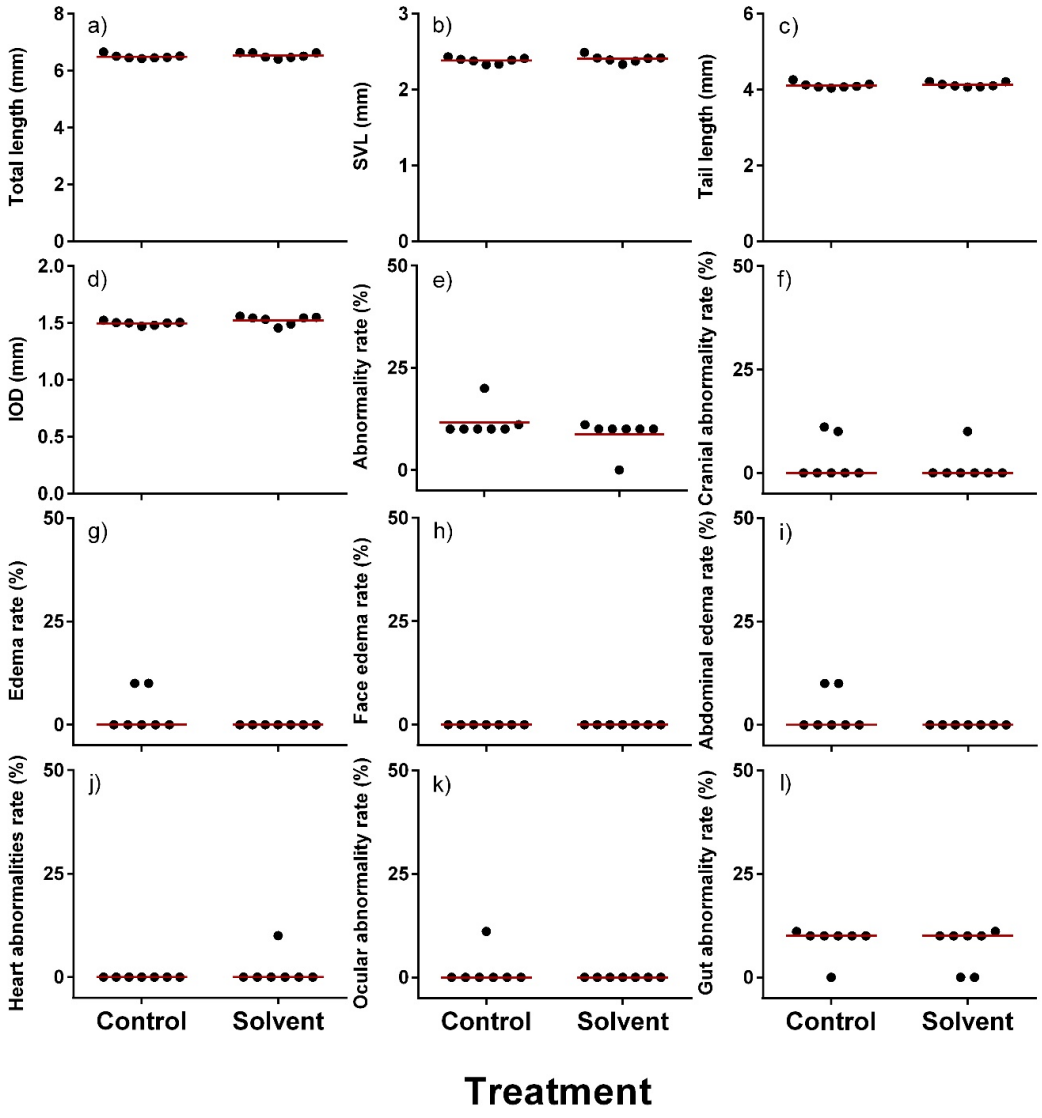

Figure S4. Effects of the solvent carrier on teratogenic endpoints in *S. tropicalis* embryos exposed to AEOs. The teratogenic endpoints were TL (a), SVL (b), TaL (c), IOD (d), abnormality rate (e), cranial abnormality (f), edema (g), face edema (h), abdominal edema (i), heart abnormality (j), ocular abnormality (k), and gut abnormality (l). NaOH at a final concentration of 0.001 N was used as carrier solvent. Each point represents a replicate. Red line represents the mean in a-d or the median in e-l.

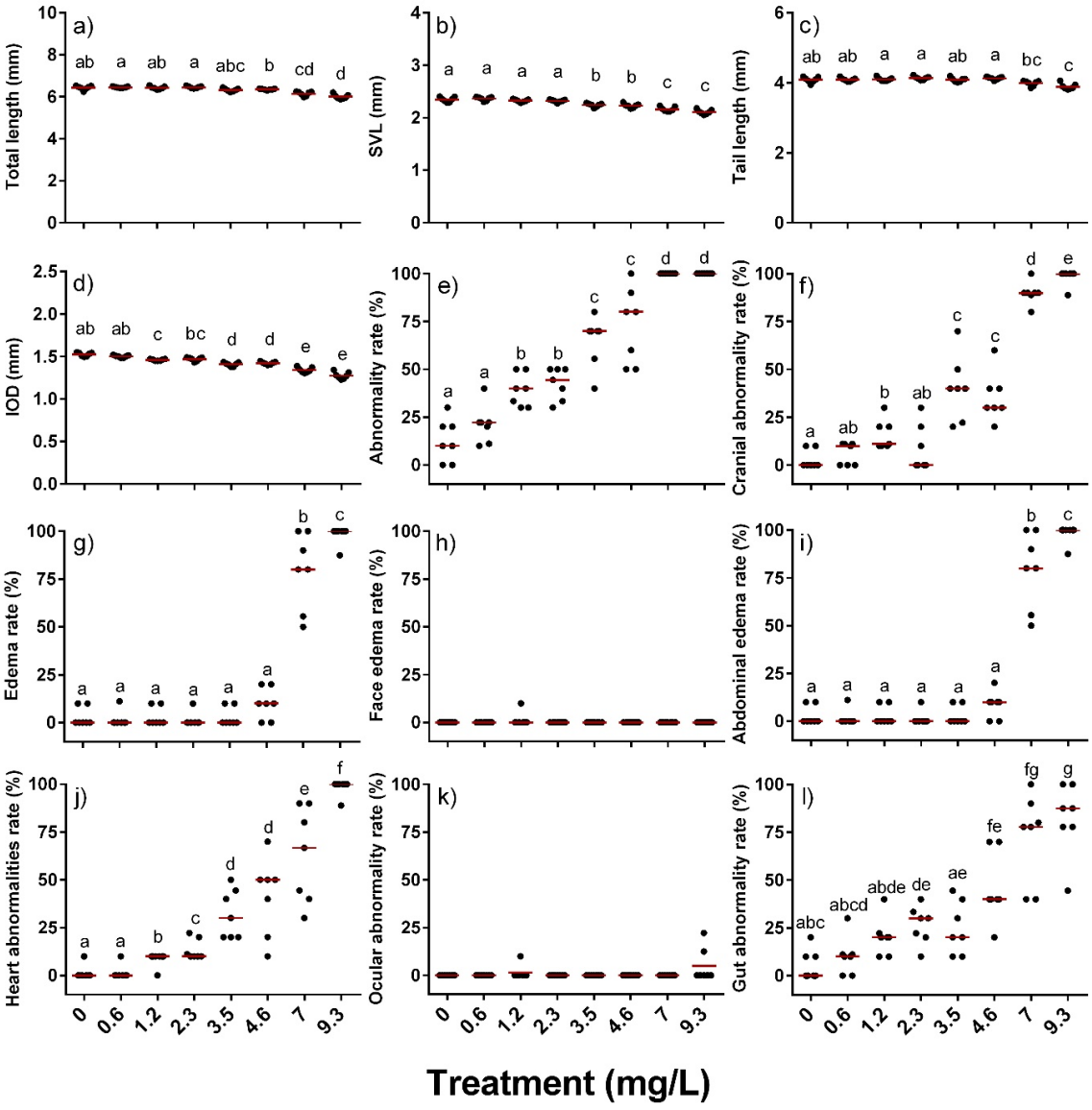

Figure S5. Teratogenic effects observed in *S. tropicalis* embryos exposed to S1M. The teratogenic endpoints were TL (a), SVL (b), TaL (c), IOD (d), abnormality rate (e), cranial abnormality rate (f), edema rate (g), face edema rate (h), abdominal edema rate (i), heart abnormality rate (j), ocular abnormality rate (k), and gut abnormality rate (l). Different letters indicate significant difference ($p < 0.05$). Each point represents a replicate. Red line indicates the mean in a-d and l or the median in e-k.

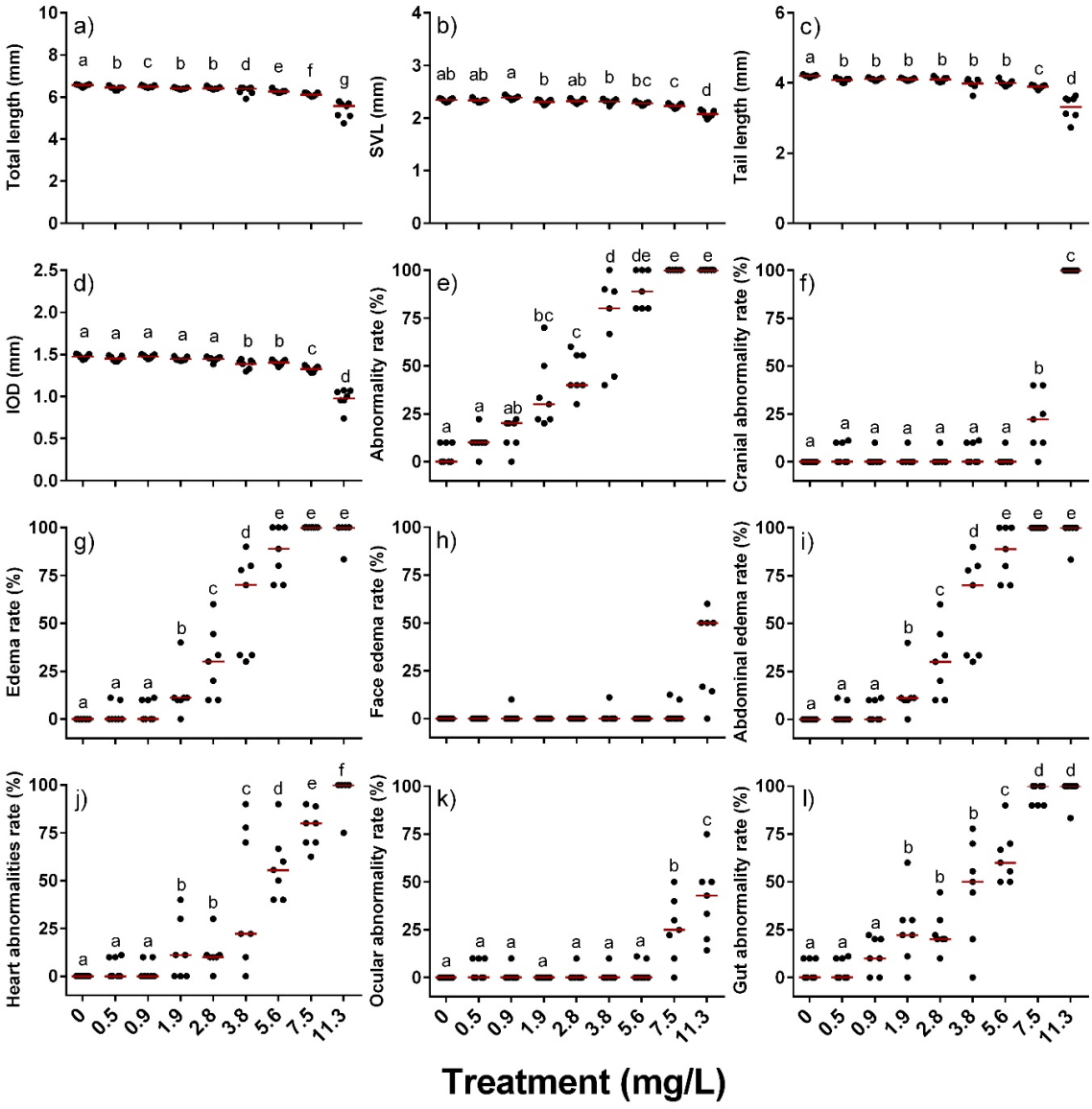

Figure S6. Teratogenic effects observed in *S. tropicalis* embryos exposed to S2M. The teratogenic endpoints were TL (a), SVL (b), TaL (c), IOD (d), abnormality rate (e), cranial abnormality rate (f), edema rate (g), face edema rate (h), abdominal edema rate (i), heart abnormality rate (j), ocular abnormality rate (k), and gut abnormality rate (l). Different letters indicate significant difference ($p < 0.05$). Each point represents a replicate. Red line represents the mean in b and e or median in a, c, d, and f-l.

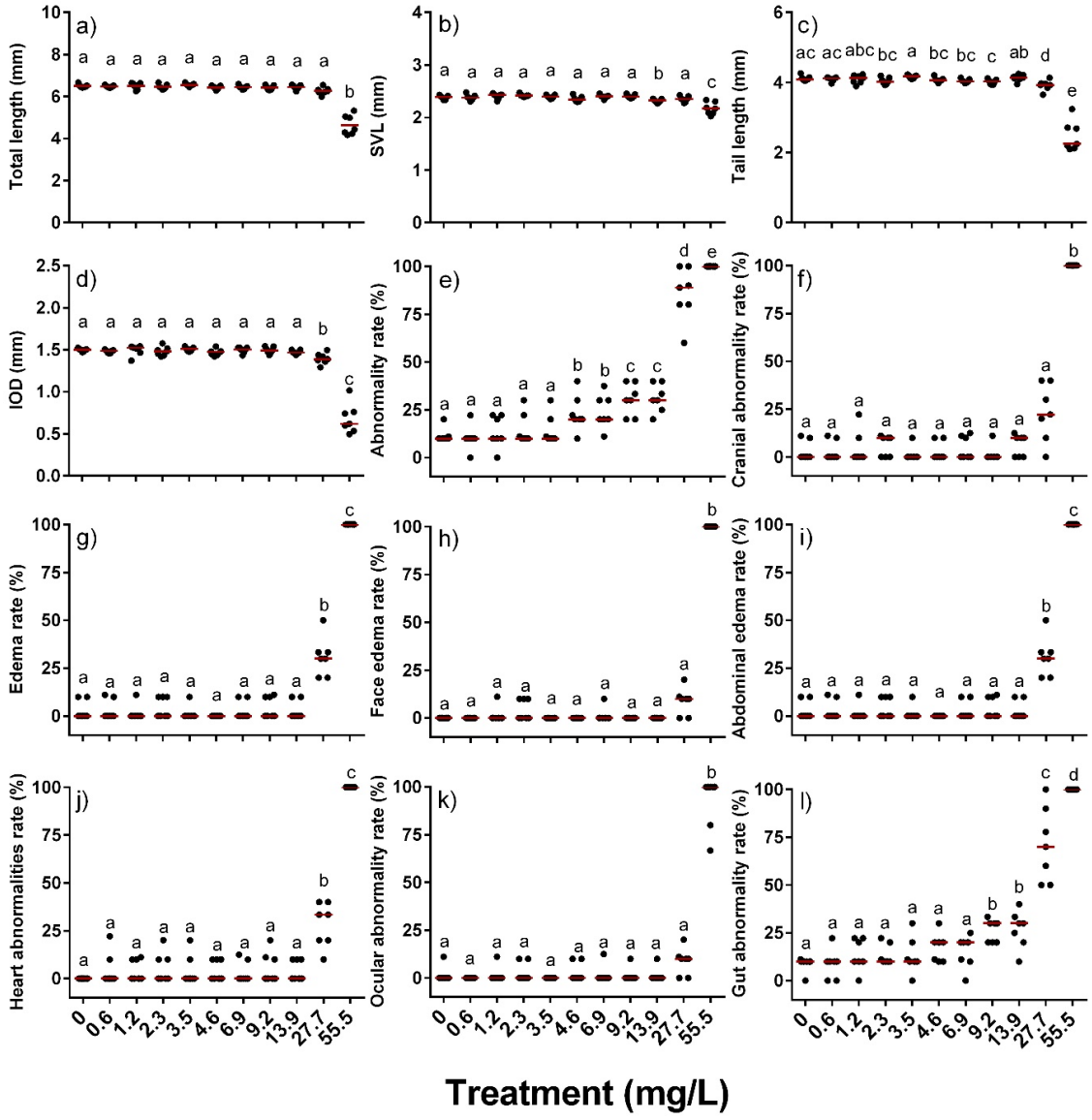

Figure S7. Teratogenic effects observed in *S. tropicalis* embryos exposed to AEOs. The teratogenic endpoints were TL (a), SVL (b), TaL (c), IOD (d), abnormality rate (e), cranial abnormality rate (f), edema rate (g), face edema rate (h), abdominal edema rate (i), heart abnormality rate (j), ocular abnormality rate (k), and gut abnormality rate (l). Different letters indicate significant difference ($p < 0.05$). Each point represents a replicate. Red line represents the mean in a or the median in b-l.

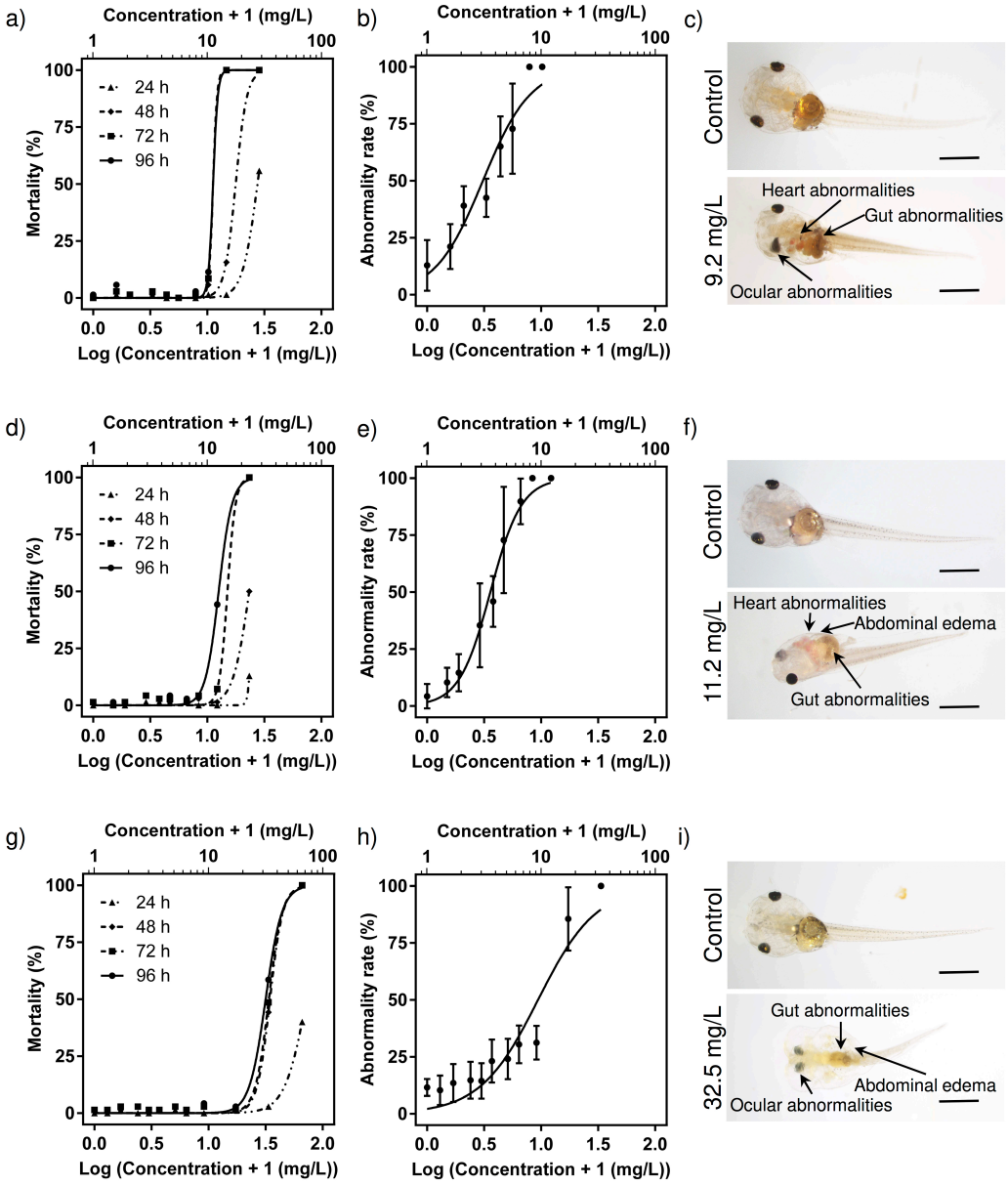

Figure S8. Dose-response curves for mortality, abnormality rate and main teratogenic effects induced by exposure of *S. tropicalis* embryos to S1M (a-c), SM2 (d-f), and AEOs (g-i). Concentrations were corrected based on O_2 family proportion. Each point represents the average of seven replicates and bars represent the standard deviation of the mean. The most commonly observed abnormalities were: edema, head malformation, gut malformation, heart abnormalities, and ocular malformation, as indicated by arrows. Scale bar is equal to 1 mm.

Table S1. Comparison of the EC_{50} and EC_{20} based on cranial abnormality rates of the 2 NA extracts (S1M and S2M) and an AEO extract on *S. tropicalis* after a 4-day exposure, based on the nominal concentration, measured concentration, and O_2 family concentration. Confidence interval 95% indicated between brackets. The coefficient of determination (R^2) calculated in GraphPad is also indicated. Different letters indicate values that are significantly different ($p < 0.05$).

	S1M EC_{50}	S2M EC_{50}	AEOs EC_{50}	S1M EC_{20}	S2M EC_{20}	AEOs EC_{20}
Nominal concentration (mg/L)	3.9^a [3.6, 4.3] $R^2 = 0.872$	8.7^b [8.3, 10.0] $R^2 = 0.960$	27.5^c [25.4, 35.3] $R^2 = 0.935$	2.5^a [2.2, 2.9] $R^2 = 0.872$	8.0^b [7.5, 9.5] $R^2 = 0.960$	23.5^c [20.6, 31.6] $R^2 = 0.935$
Measured concentration (mg/L)	4.5^a [4.2, 4.9] $R^2 = 0.875$	8.1^b [7.8, 9.4] $R^2 = 0.960$	31.7^c [29.3, 40.7] $R^2 = 0.935$	2.9^a [2.5, 3.3] $R^2 = 0.875$	7.5^b [7.0, 8.9] $R^2 = 0.960$	27.1^c [23.8, 36.4] $R^2 = 0.935$
Concentration based on the O_2 families (mg/L)	4.5^a [4.1, 4.9] $R^2 = 0.869$	8.1^b [7.7, 9.3] $R^2 = 0.960$	18.6^c [17.3, 24.0] $R^2 = 0.935$	2.9^a [2.5, 3.3] $R^2 = 0.869$	7.0^b [7.4, 8.7] $R^2 = 0.960$	16.0^c [14, 21.4] $R^2 = 0.935$

Table S2. Comparison of the EC_{50} and EC_{20} based on edema rates of the 2 NA extracts (S1M and S2M) and an AEO extract on *S. tropicalis* after a 4-day exposure, based on the nominal concentration, measured concentration, and O_2 family concentration. Confidence interval 95% indicated between brackets. The coefficient of determination (R^2) calculated in GraphPad is also indicated. Different letters indicate values that are significantly different ($p < 0.05$).

	S1M EC_{50}	S2M EC_{50}	AEOs EC_{50}	S1M EC_{20}	S2M EC_{20}	AEOs EC_{20}
Nominal concentration (mg/L)	5.2^b [4.9, 5.3] $R^2 = 0.954$	3.7^a [3.4, 3.9] $R^2 = 0.918$	26.5^c [25.1, 32.2] $R^2 = 0.964$	4.4^b [4.1, 4.7] $R^2 = 0.954$	2.5^a [2.2, 2.8] $R^2 = 0.918$	22.3^c [19.6, 23.3] $R^2 = 0.964$
Measured concentration (mg/L)	6.0^b [5.7, 6.2] $R^2 = 0.954$	3.5^a [3.2, 3.7] $R^2 = 0.918$	30.5^c [29.0, 37.2] $R^2 = 0.964$	5.1^b [4.8, 4.4] $R^2 = 0.954$	2.3^a [2.1, 2.6] $R^2 = 0.918$	25.8^c [22.6, 26.9] $R^2 = 0.964$
Concentration based on the O_2 families (mg/L)	5.9^b [5.7, 6.2] $R^2 = 0.954$	3.4^a [3.2, 3.6] $R^2 = 0.918$	18.0^c [17.1, 21.9] $R^2 = 0.964$	5.0^b [4.7, 5.3] $R^2 = 0.954$	2.3^a [2.1, 2.6] $R^2 = 0.918$	15.2^c [13.3, 15.8] $R^2 = 0.964$

Table S3. Comparison of the EC_{50} and EC_{20} based heart abnormality rates of the 2 NA extracts (S1M and S2M) and an AEO extract on *S. tropicalis* after a 4-day exposure, based on the nominal concentration, measured concentration, and O_2 family concentration. Confidence interval 95% indicated between brackets. The coefficient of determination (R^2) calculated in GraphPad is also indicated. Different letters indicate values that are significantly different ($p < 0.05$).

	S1M EC_{50}	S2M EC_{50}	AEOs EC_{50}	S1M EC_{20}	S2M EC_{20}	AEOs EC_{20}
Nominal concentration (mg/L)	4.3[a] [3.9, 4.7] $R^2 = 0.850$	5.1[a] [4.6, 5.7] $R^2 = 0.830$	26.9[b] [25.2, 34.2] $R^2 = 0.931$	2.5[a] [2.1, 2.9] $R^2 = 0.850$	3.0[a] [2.5, 3.5] $R^2 = 0.830$	22.7[b] [19.5, 23.7] $R^2 = 0.931$
Measured concentration (mg/L)	5.0[a] [4.5, 5.5] $R^2 = 0.850$	4.8[a] [4.3, 5.3] $R^2 = 0.831$	31.1[b] [29.1, 39.4] $R^2 = 0.931$	2.9[a] [2.4, 3.4] $R^2 = 0.850$	2.8[a] [2.3, 3.3] $R^2 = 0.831$	26.2[b] [22.6, 27.3] $R^2 = 0.931$
Concentration based on the O_2 families (mg/L)	4.9[a] [4.5, 5.4] $R^2 = 0.848$	4.7[a] [4.3, 5.3] $R^2 = 0.829$	18.3[b] [17.1, 23.2] $R^2 = 0.931$	2.9[a] [2.4, 3.3] $R^2 = 0.848$	2.8[a] [2.3, 3.3] $R^2 = 0.829$	15.4[b] [13.3, 16.1] $R^2 = 0.931$

Table S4. Comparison of the EC_{50} and EC_{20} based on gut abnormality rates of the 2 NA extracts (S1M and S2M) and an AEO extract on *S. tropicalis* after a 4-day exposure, based on the nominal concentration, measured concentration, and O_2 family concentration. Confidence interval 95% indicated between brackets. The coefficient of determination (R^2) calculated in GraphPad is also indicated. Different letters indicate values that are significantly different ($p < 0.05$).

	S1M EC_{50}	S2M EC_{50}	AEOs EC_{50}	S1M EC_{20}	S2M EC_{20}	AEOs EC_{20}
Nominal concentration (mg/L)	4.1[a] [3.6, 4.7] R^2= 0.740	4.2[a] [3.8, 4.7] R^2= 0.852	15.2[b] [13.4, 17.4] R^2= 0.842	1.7[a] [1.2, 2.2] R^2= 0.740	2.3[a] [1.9, 2.7] R^2= 0.852	6.4[b] [5.3, 7.6] R^2= 0.842
Measured concentration (mg/L)	4.7[a] [4.1, 5.5] R^2= 0.739	4.0[a] [3.6, 4.4] R^2= 0.853	17.5[b] [15.5, 20.1] R^2= 0.840	2.0[a] [1.4, 2.5] R^2= 0.739	2.1[a] [1.7, 2.6] R^2= 0.853	7.4[b] [6.2, 8.7] R^2= 0.840
Concentration based on the O_2 families (mg/L)	4.7[a] [4.1, 5.4] R^2= 0.739	3.9[a] [3.5, 4.4] R^2= 0.852	10.3[b] [9.1, 11.8] R^2= 0.848	1.9[a] [1.4, 2.5] R^2= 0.739	2.1[a] [1.7, 2.5] R^2= 0.852	4.3[b] [3.6, 5.0] R^2= 0.848