

SUPPLEMENTARY MATERIAL

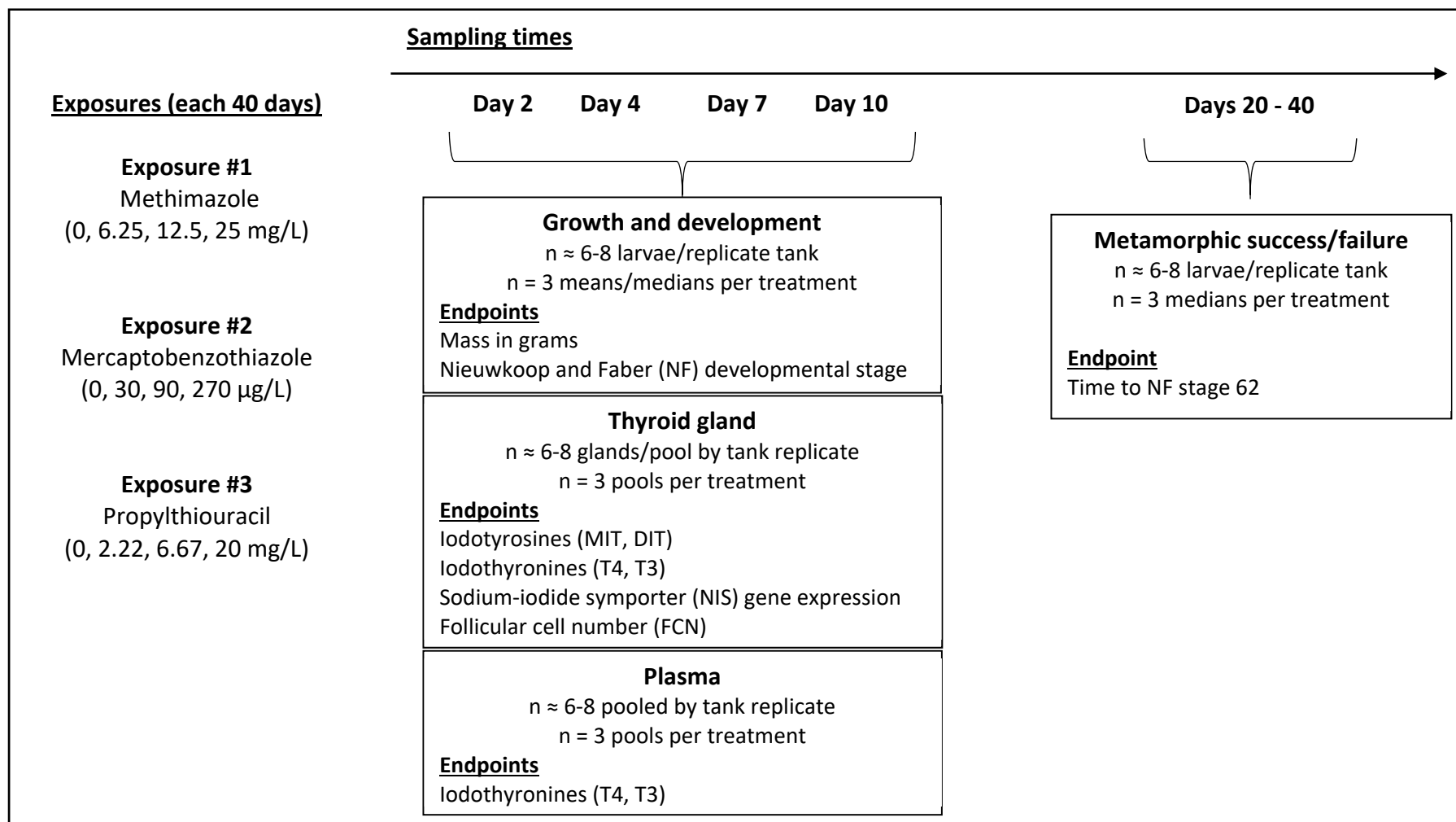


Figure S.1. Study design for pathway-based concentration-response and time-course aqueous exposures using three thyroperoxidase inhibitors starting with pro-metamorphic (Nieuwkoop and Faber stage 53/54) *Xenopus laevis* larvae.

Table S.1. Water chemistry summary for all tanks across each of three separate exposures.

| | Temp. (°C) | D.O. (mg L ⁻¹) | % O ₂ Sat. | pH | Conductivity (μS cm ⁻¹) | Alkalinity (CaCO ₃ L ⁻¹) | Hardness (CaCO ₃ L ⁻¹) |
|------|---------------|-------------------------------|-----------------------|-----|--|--|--|
| MMI | | | | | | | |
| Avg. | 21.3 | 6.7 | 76.7 | 7.4 | 109.0 | 45.8 | 46.3 |
| SD | 0.2 | 0.6 | 4.8 | 0.1 | 2.2 | 0.7 | 0.7 |
| Min. | 20.6 | 3.7 | 67.7 | 7.2 | 104.3 | 45.0 | 45.0 |
| Max. | 21.8 | 8.1 | 92.4 | 7.7 | 112.4 | 47.0 | 47.0 |
| MBT | | | | | | | |
| Avg. | 21.3 | 7.0 | 78.9 | 7.1 | 102.6 | 47.0 | 46.2 |
| SD | 0.2 | 0.3 | 3.8 | 0.3 | 3.6 | 0.7 | 0.7 |
| Min. | 20.9 | 6.4 | 73.0 | 6.1 | 98.8 | 46.0 | 45.0 |
| Max. | 21.9 | 7.7 | 87.0 | 7.4 | 109.7 | 48.0 | 47.0 |
| PTU | | | | | | | |
| Avg. | 21.0 | 6.6 | 74.3 | 7.4 | 101.6 | 43.8 | 44.9 |
| SD | 0.2 | 0.3 | 3.0 | 0.1 | 4.2 | 0.8 | 1.4 |
| Min. | 20.2 | 6.0 | 67.0 | 7.2 | 95.2 | 43.0 | 43.0 |
| Max. | 21.5 | 7.2 | 81.0 | 7.6 | 108.4 | 45.0 | 47.0 |

D.O. = dissolved oxygen; MMI, methimazole; MBT, 2-mercaptobenzothiazole; PTU, propylthiouracil

Table S.2. Analytical verification summary of chemical exposure concentrations by high-performance liquid chromatography with diode array detection.

| Trt. | Rep. tank | MMI | | | | | | MBT | | | | | | PTU | | | | | |
|---------------------|-----------|---------------|----------------------------|------|-----|----------|--------------|---------------|----------------------------|------|-----|----------|--------------|---------------|----------------------------|------|-----|----------|--------------|
| | | Nominal conc. | Mean measured conc. (mg/L) | SD | CV | <i>n</i> | % of nominal | Nominal conc. | Mean measured conc. (µg/L) | SD | CV | <i>n</i> | % of nominal | Nominal conc. | Mean measured conc. (mg/L) | SD | CV | <i>n</i> | % of nominal |
| Control | 1 | 0 mg/L | ND | 0 | 0 | 6 | n/a | 0 µg/L | ND | 0 | 0 | 4 | n/a | 0 mg/L | ND | 0 | 0 | 5 | n/a |
| | 2 | 0 mg/L | ND | 0 | 0 | 6 | n/a | 0 µg/L | ND | 0 | 0 | 4 | n/a | 0 mg/L | ND | 0 | 0 | 5 | n/a |
| | 3 | 0 mg/L | ND | 0 | 0 | 6 | n/a | 0 µg/L | ND | 0 | 0 | 4 | n/a | 0 mg/L | ND | 0 | 0 | 5 | n/a |
| Low | 1 | 6.25 mg/L | 7.4 | 0.4 | 5.4 | 6 | 118 | 30 µg/L | 27.0 | 2.0 | 7.4 | 4 | 90 | 2.22 mg/L | 2.47 | 0.05 | 1.9 | 5 | 111 |
| | 2 | 6.25 mg/L | 7.3 | 0.4 | 5.2 | 6 | 118 | 30 µg/L | 27.3 | 1.8 | 6.5 | 4 | 91 | 2.22 mg/L | 2.47 | 0.05 | 1.8 | 5 | 111 |
| | 3 | 6.25 mg/L | 7.4 | 0.4 | 5.6 | 6 | 118 | 30 µg/L | 27.6 | 2.2 | 8.0 | 4 | 92 | 2.22 mg/L | 2.47 | 0.05 | 1.9 | 5 | 111 |
| Grand mean | | | 7.4 | 0.01 | 0.2 | 3 | | 27.3 | 0.3 | 1.2 | 3 | | 2.47 | 0.00 | 0.03 | 3 | | | |
| Ungrouped trt. mean | | | 7.4 | 0.4 | 5.1 | 18 | | 27.3 | 1.8 | 6.7 | 12 | | 2.47 | 0.04 | 1.7 | 15 | | | |
| Medium | 1 | 12.5 mg/L | 14.7 | 0.8 | 5.7 | 6 | 118 | 90 µg/L | 79.9 | 5.8 | 7.2 | 4 | 89 | 6.67 mg/L | 7.48 | 0.16 | 2.1 | 5 | 112 |
| | 2 | 12.5 mg/L | 14.8 | 0.8 | 5.7 | 6 | 118 | 90 µg/L | 82.3 | 6.2 | 7.5 | 4 | 91 | 6.67 mg/L | 7.50 | 0.15 | 2.0 | 5 | 112 |
| | 3 | 12.5 mg/L | 14.8 | 0.8 | 5.5 | 6 | 118 | 90 µg/L | 83.0 | 6.0 | 7.3 | 4 | 92 | 6.67 mg/L | 7.49 | 0.16 | 2.1 | 5 | 112 |
| Grand mean | | | 14.8 | 0.03 | 0.2 | 3 | | 81.7 | 1.6 | 2.0 | 3 | | 7.49 | 0.01 | 0.1 | 3 | | | |
| Ungrouped trt. mean | | | 14.8 | 0.8 | 5.3 | 18 | | 81.7 | 5.6 | 6.9 | 12 | | 7.49 | 0.15 | 1.9 | 15 | | | |
| High | 1 | 25 mg/L | 29.5 | 1.5 | 5.0 | 6 | 118 | 270 µg/L | 264.3 | 10.4 | 3.9 | 6 | 98 | 20 mg/L | 22.93 | 0.48 | 2.1 | 5 | 115 |
| | 2 | 25 mg/L | 29.5 | 1.5 | 5.0 | 6 | 118 | 270 µg/L | 261.9 | 10.0 | 3.8 | 6 | 97 | 20 mg/L | 22.95 | 0.50 | 2.2 | 5 | 115 |
| | 3 | 25 mg/L | 29.5 | 1.4 | 4.9 | 6 | 118 | 270 µg/L | 264.5 | 9.8 | 3.7 | 6 | 98 | 20 mg/L | 22.94 | 0.48 | 2.1 | 5 | 115 |
| Grand mean | | | 29.5 | 0.03 | 0.1 | 3 | | 263.6 | 1.4 | 0.5 | 3 | | 22.94 | 0.01 | 0.05 | 3 | | | |
| Ungrouped trt. mean | | | 29.5 | 1.4 | 4.7 | 18 | | 263.6 | 9.5 | 3.6 | 18 | | 22.94 | 0.45 | 2.0 | 15 | | | |

MMI, methimazole; MBT, mercaptobenzothiazole; PTU, propylthiouracil; ND, not detected; SD, standard deviation; CV, coefficient of variation; n/a, not applicable; Rep., replicate; Trt., treatment; Conc., concentration; Grand mean, mean of replicate means; Ungrouped treatment mean, mean of all measurements across replicates.

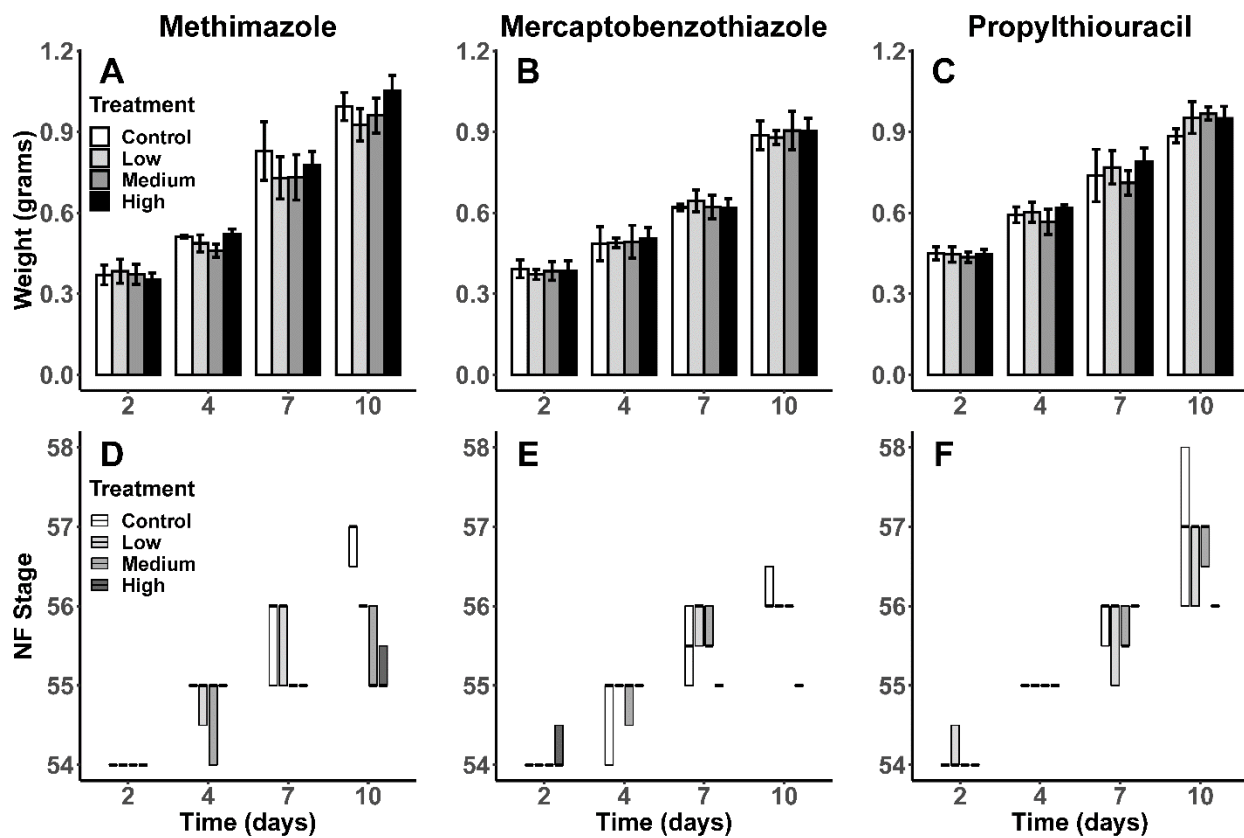


Figure S.2. Mean (\pm SD) temporal growth (A-C) and median (min-max) metamorphic development by NF stage (D-F) in three separate chemical exposure studies. There was no evidence of an effect on growth and the developmental data were not analyzed statistically. Sample sizes are $n = 3$ except for the high PTU treatment which has a sample size of $n = 2$. MMI, methimazole; MBT, mercaptobenzothiazole; PTU, propylthiouracil; MMI:Low, $55 \mu\text{M}$; MMI:Med, $110 \mu\text{M}$; MMI:High, $220 \mu\text{M}$; MBT:Low, $0.18 \mu\text{M}$; MBT:Med, $0.54 \mu\text{M}$; MBT:High, $1.6 \mu\text{M}$; PTU:Low, $13 \mu\text{M}$; PTU:Med, $39 \mu\text{M}$; PTU:High, $117.5 \mu\text{M}$.