

Supporting Information:

Development of a thyroperoxidase inhibition assay for high through-put screening

Katie B. Paul^{1,2}, Joan M. Hedge², Daniel M. Rotroff³, Michael W. Hornung⁴, Kevin M. Crofton²,
Steven O. Simmons²

¹Oak Ridge Institute for Science Education Postdoctoral Fellow; ²Integrated Systems Toxicology Division, ⁴Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, National Center for Computational Toxicology³, Office of Research and Development, U.S. Environmental Protection Agency

Table S1: Summary of selected optimization experiments for the 96-well format AUR-TPO assays

Condition#	Microsome Lot#	Protein/Reaction (μg)	AUR substrate concentration (μM) ^b	Dynamic Range ^a	IC ₅₀ (μM)	Z'
1^c	10	12.5	25	5.79	0.050	0.98
2	10	50	25	7.36	0.13	0.92
1^c	11	12.5	25	6.29	0.078	0.89
2	11	50	25	4.93	0.28	0.84
3	10	12.5	50	3.27	0.063	0.96
4	10	50	50	4.49	0.16	0.92
3	11	12.5	50	3.55	0.05	0.96
4	11	50	50	3.74	0.26	0.77
5	10	12.5	100	2.73	1.0	0.97
6	10	50	100	3.61	0.62	0.98
5	11	12.5	100	2.40	0.24	0.93
6	11	50	100	2.76	0.91	0.93

^aCalculated using response data from 100 μM MMI as the maximum response compared to vehicle control.

^bFinal concentration in the assay.

^cBolded condition one was selected for the 96-well format assay procedure.

Figure S1: Example Amplex UltraRed (AUR) rat thyroperoxidase inhibition assay profile by time

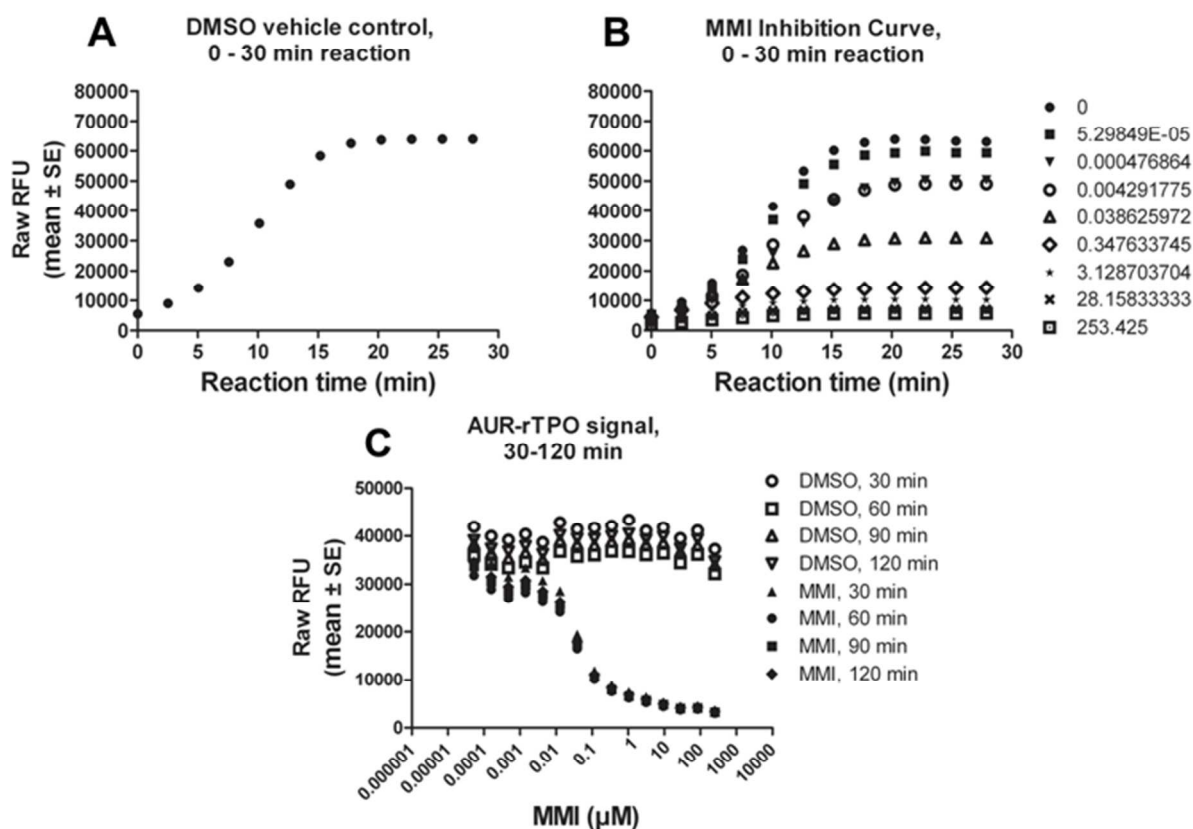
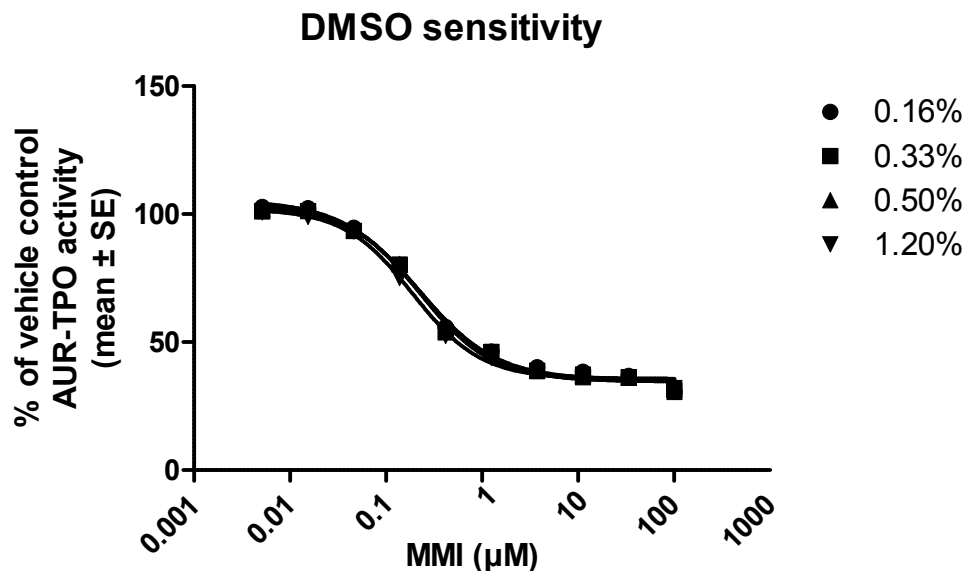


Figure S1A-C. AUR-rTPO signal over time. A. The DMSO vehicle control AUR-rTPO signal reaches a maximum stable signal between 15 and 20 min post-initiation of the peroxidase reaction; note that the maximum RFU that can be detected is 65000. B. The AUR-rTPO signal for a MMI concentration response curve stabilizes between 15 and 20 min post-initiation of the peroxidase reaction, and a concentration-dependent response is evident as increasing MMI concentration corresponds to decreased stable RFU. C. The AUR-rTPO signal for DMSO vehicle and MMI concentration response can be read with stable signal for at least 2 hr post-initiation of the peroxidase reaction without loss or change in signal. RFU = raw fluorescence units; DMSO = dimethyl sulfoxide, vehicle control data; MMI = methimazole concentration response data.

Figure S2: DMSO sensitivity of the AUR-TPO assay in a 96-well format



DMSO concentration (%)	MMI IC ₅₀ (µM)	95% confidence interval for IC ₅₀
0.16	0.2192	0.1521 to 0.3158
0.33	0.2271	0.1608 to 0.3207
0.50	0.2315	0.1640 to 0.3267
1.20	0.1895	0.1468 to 0.2445

Figure S2. DMSO sensitivity of the AUR-TPO assay in a 96-well format (n=2). MMI concentration-response curves in the AUR-TPO assay are shown with 0.16%, 0.33%, 0.50%, and 1.20% DMSO included in the reaction. The AUR-TPO assay does not appear sensitive to DMSO as assessed by comparing positive control MMI curves across increasing concentrations of solvent. The 96- and 384-well formats of the assay are routinely performed with 0.16 and 0.41% of DMSO, respectively