Supplemental Materials

**Supplemental Figure 1**. Design for fadrozole (FAD, µg/L)-trenbolone (TRB, ng/L) mixture study with female fathead minnows aimed at assessing the predictive ability of an AOP network.

**Supplemental Table 1.** Fish loading and sampling scheme for the 48 and 96 h exposures to 17β-trenbolone and fadrozole.

| **Test Day** | **Fish Loading** | **48 h Samples** | **96 h Samples** |
|---|---|---|---|
| 1 | Load 8 fish per tank (4 on each side of divider) into rep 1 (AM) | | |
| 2 | Load 8 fish per tank (4 on each side of divider) into reps 2 (AM) and 3 (PM) | | |
| 3 | | Sample half of the fish (n=4/tank) from rep 1 (AM) | |
| 4 | | Sample half of the fish (n=4/tank) from reps 2 (AM) and 3 (PM) | |
| 5 | | | Sample remaining fish (n=4/tank) from rep 1 (AM) |
| 6 | | | Sample remaining fish (n=4/tank) from reps 2 (AM) and 3 (PM) |

**Supplemental Table 2**. Fathead minnow gene-specific primer and probe sequences for quantitative polymerase chain reaction analyses. *vtg* = vitellogenin; *cyp19a1a* = aromatase; *cyp11a* = cholesterol side chain cleavage; FW = forward primer; RV = reverse primer; PB = probe

| Gene | Amplicon size (bp) | Accession # | Primer /Probe | Sequence (5′→′3) | Reference |
|---|---|---|---|---|---|
| *vtg* | 69 | AF130354 | FW | TCACCACATACGCCAAAAAGC | Cavallin et al. 2016 |
| | | | RV | CAAGTCTAAAGCCCGTCTGGTT | |
| | | | PB[a] | TCTAAGCACATTCCTATGGCGGCT | |
| *cyp19a1a* | 77 | AF288755 | FW | TGCTGACACATGCAGAAAAACTC | Villeneuve et al. 2006 |
| | | | RV | CAGCTCTCCGTGGCTCTGA | |
| | | | PB[b] | CCAGCTCGACTTCACAGCAGAGTTGATATTC | |
| *cyp11a* | 72 | DQ360498 | FW | CGACACCCGGACTTGCA | Villeneuve et al. 2007 |
| | | | RV | CACGTCTCCTTTAGAGGTGATACG | |
| | | | PB[b] | CCGTGCGGAAATCTCGGCTGC | |

[a] Dual-labeled DNA probe with 5' 6-FAM™/ZEN/3' Iowa Black® FQ Quencher (Integrated DNA Technologies)
[b] Dual-labeled DNA probe with 5'-FAM™ (6-carboxyl-fluorescein), 3'-Black Hole Quencher-1® (Integrated DNA Technologies)

References

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