Derivation and Evaluation of Adverse Outcome Pathways for the Effects of Cyclooxygenase Inhibitors on Reproductive Processes in Fish

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Abstract (currently 2309 characters out of 2500)

Cyclooxygenase (COX) inhibition is of concern in fish because COX inhibitors (e.g., ibuprofen) are ubiquitous in aquatic systems/fish tissues, and can disrupt synthesis of prostaglandins that modulate a variety of essential biological functions (e.g., reproduction). This study utilized newly generated high content (transcriptomic and metabolomic) empirical data in combination with existing high throughput (ACTOR, epa.gov) toxicity data to facilitate development of adverse outcome pathways (AOPs) for molecular initiating event (MIE) of COX inhibition. We examined effects of a waterborne, 96h exposure to three COX inhibitors (indomethacin (IN; 100 µg/L), ibuprofen (IB; 200 µg/L) and celecoxib (CX; 20 µg/L) on the liver metabolome and ovarian gene expression (using oligonucleotide microarray 4 x15K platform) in sexually mature fathead minnows (n=8). Differentially expressed genes were identified (t-test, $p < 0.01$), and functional analyses performed to determine enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways ($p < 0.05$). Principal component analysis indicated that liver metabolomics profiles of IN, IB and CX were not significantly different from control or one another. When compared to control, exposure to IB and CX resulted in differential expression of comparable numbers of genes (IB = 433, CX= 545). In contrast, 2558 genes were differentially expressed in IN-treated fish. KEGG pathway analyses show that IN had extensive effects on oocyte meiosis and muscle contraction processes, which is consistent with physiological roles of prostaglandins in the fish ovary. Transcriptomic data was congruent with apical endpoint data - IN caused significant suppression in plasma prostaglandin F2 alpha concentrations, and reduced ovarian COX activity, whereas IB and CX did not. Ovulation status, chemical treatment, and

their interaction did not have statistically significant effect on circulating vitellogenin. These findings were used to develop AOPs for COX inhibition (MIE) leading to reproductive failure (adverse outcome) via two series of key events – one associated with inhibition of reproductive behavior, and another with the inhibition of oocyte maturation/ovulation. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

Keywords: cyclooxygenase inhibition, prostaglandin, reproduction, fish, adverse outcome pathway, non-steroidal anti-inflammatory drugs