Methods:

Animals: For all steps, eggs and tadpoles were housed in tap water that was filtered using sediment and carbon filters and irradiated. Containers were plastic polypropylene bins with a 15 L capacity. Prior to use, the bins were scrubbed with ethanol, soaked in 10% bleach, rinsed in diluted vinegar, and were rinsed multiple times with tap water. Room temperatures ranged from 20-23C, and water temperatures ranged from 18-19C. *L. pipiens* eggs were purchased from Nasco and placed in 5 L of water in the plastic bins. After one week, the eggs hatched. After hatching, larvae were fed every other day as needed. Food was XXX? Water was changed 2 times per week and waste removed by suctioning if needed between water changes.

Treatments: Stock CPF was prepared by dissolving 0.1 mg of CPF into 1 mL of ethanol. Once larvae reached approximately stage 25 (were free swimming), we haphazardly assigned tadpoles to either control (0.001% ethanol) or CPF (1 ppb CPF in 0.001% ethanol) treatments. To do so, we first added 8 tadpoles to each of two bins. Three days after adding tadpoles to the bins, we added 50 ul of the 0.1 mg/mL stock CPF to one bin to achieve a 1 ppb concentration of CPF. To the other bin, we added 50 uL of ethanol as a control. At the same time, we set up another 10 bins of water, each with 1 ppb CPF, by adding 50 ul of the 0.1 mg/mL stock to 5 L of water. We also set up another 10 control bins by adding 50 uL of ethanol to 5 L of water. These extra bins were used for full water changes, which were done twice a week. In this way, we were able to continuously expose tadpoles to CPF (or control) as it broke down naturally over time.

Tissue Processing: After 39 days of treatment, tadpoles were euthanized by immersing in a xxx solution of MS222. They were rinsed in water, dabbed dry, and weighed. Using sterile tools and aseptic methods, we removed the brain and placed it immediately into 600 ul RNA later. We took about 6 minutes from immersion in MS222 to placement of the brain into RNA later. Developmental stage was also recorded (Gosner). Brains were stored at 4C for 30 days, and then placed at -20C. After one week at -20C, we extracted the RNA following kit directions using an illustra RNAspin Mini RNA isolation kit. We measured the amount and purity of RNA that was recovered using a nanodrop spectrophotometer. RNA concentration in ranged from 63.5 to 201.7 ng/uL in a 100 uL elution volume. The 260:280 ratio ranged from 2.12 to 2.24.

RNA Isolation, Library Preparation, and Sequencing: We performed RNA-Seq on 6 brains from the CPF tadpoles and 6 from the control tadpoles. mRNA was purified from total RNA using magnetic oligo (dT) beads that enrich for poly (A) tails. Fragmentation of the purified mRNA and cDNA synthesis were combined by heating the mRNA at 95C for 2 min in a buffer that contained 5X 1st Strand synthesis reaction buffer and RT-PE6 series. Each sample had a different RT-PE so that unique barcodes were added to the cDNA. Template switching and second strand cDNA was made using SMARTScribe RT, SMART7.5, and dNTP. Ds DNA fragments that were 400-700 bp in length were selected using AMPure (SPRI beads). PCR amplification using PE-PCR1 and PE-PCR2 was performed to add adaptors to facilitate the Illumina sequencing. Single end sequencing was done using an Illumina HiSeq 2500 instrument, in Rapid Run mode.