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Exposure to Environmentally Relevant Concentrations of Acetaminophen Increases the Physiological Stress Response in Juvenile Zebrafish (*Danio rerio*)

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Abstract

Non-steroidal anti-inflammatory drug, acetaminophen is the most popular over-the-counter medicine. Extensive usage and production have made acetaminophen the most abundant pharmaceutical pollutant in aquatic ecosystems. Studies have acclaimed acetaminophen-mediated negative health impacts on organisms which makes acetaminophen a potential physiological stressor for aquatic organisms. Behavioral assessments such as swimming activity, mirror biting frequency, and ammonia excretion can be used to assess fish's stress response to environmental insults. In this context, this study was conducted to investigate the effects of long-term juvenile exposure to environmentally relevant concentrations of acetaminophen on physiological stress response in model organisms, zebrafish. Zebrafish of 25 days post fertilization were maintained under environmentally relevant acetaminophen concentrations (10 µg/L, 75 µg/L) and in control tanks for 60 days in triplicate, according to OECD guidelines. The mean maximum swimming speed, mirror biting frequency, and ammonia excretion were analyzed using one-way ANOVA. According to the results, 10 µg/L acetaminophen-treated fish showed a significantly higher (0.026 cm/ms, $p<0.05$) mean maximum swimming speed, compared to the control (0.019 cm/ms, $p<0.05$). The mean maximum swimming speed of 75 µg/L acetaminophen-treated fish (0.021 cm/ms) was statistically insignificant but higher than the control treatment $(p\geq0.05)$. The mirror-biting frequency of 10 µg/L acetaminophen-treated fish was significantly higher (81.167/min) than the 75 µg/L acetaminophen-treated fish (36.084/min) and the control treatment (21.834/min) $(p<0.05)$. These results indicate that acetaminophen has caused physiological stress in zebrafish, and zebrafish are trying to maintain homeostasis by increasing stress responses. Significantly higher excretion of ammonia was observed in 10 µg/L acetaminophen-treated fish (1.0578 ppm, $p<0.05$) compared to the control treatment (0.6623 ppm). Higher but statistically insignificant ammonia excretion was observed in fish treated with 75 µg/L acetaminophen (0.8523 ppm, $p>0.05$). The highest ammonia excretion in 10 µg/L acetaminophen-treated fish confirms the increased metabolic activity. In 75 µg/L acetaminophen-treated fish even though a stress response can be observed this is lower than the 10 µg/L acetaminophen-treated fish. This may be due to the toxicity of acetaminophen which has overridden the stress response in zebrafish. The lowered ammonia excretion in 75 µg/L acetaminophen-treated fish further confirms that high toxicity and interrupted metabolism in higher acetaminophen doses have overridden the stress responses. This analysis implies that even environmental concentration of acetaminophen possesses a significant level of stress in aquatic organisms. However, more comprehensive assays are recommended in the analysis of higher dose consequences.

Keywords: *Acetaminophen, Ammonia excretion, Environmentally relevant doses, Juvenile zebrafish, Stress response*