# TFM toxicity in planaria: opportunity for validation of a surrogate species for rapid TFM toxicity testing

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**ABSTRACT:**

Sea lampreys (*Petromyzon marinus*) are an invasive fish species in the North American Great Lakes. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is the primary population control method, acting as an uncoupler of mitochondrial respiration and thus depleting internal energy reserves in sea lamprey. While non-target fish species are minimally impacted due to their TFM detoxification abilities, the effects of the lampricide on aquatic invertebrates have not been thoroughly explored. Using the brown flatworm/planarian (*Dugesia dorotocephala*), we hypothesized that TFM exposure will negatively impact flatworms, resulting in mortality, reduced locomotion, and a reduction in whole body energy reserves (glucose and glycogen). In addition, we also tested whether abiotic factors, such as temperature, pH and alkalinity, have an effect on TFM toxicity in this species. To examine this, *D. dorotocephala* were first exposed to target concentration of 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 16.0, and 25.0mg/L TFM, in triplicate, in soft water (alkalinity 100mg/L $CaCO_3$, pH 7.59, 20.2°C). Locomotion was recorded before and 5 minutes after TFM addition, while mortality was recorded at 30-minute intervals over 3h. The worm 2h TFM LC-50 was 2.83mg/L, approximately 2X the 9h LC-99 for the larval sea lampreys. Locomotion was significantly reduced compared to controls at concentrations >3.42 mg/L TFM. Exposure of the worms to their 2h TFM LC-50 for 0, 0.5, 1.0, 1.5 and 2.0h led to significant reductions in whole body glycogen in the first hour, with whole body lactate and glucose were unaffected. When paired with the lower whole body glycogen levels, we believe that reductions in locomotion could be a result of reduced ability to produce ATP to sustain exploratory behavior. The experiments were then repeated at three different temperatures, to establish whether temperature has an impact on whole body energy reserves and, thus, TFM toxicity. We found that acclimation of brown flatworms to 15 and 25 °C leads to higher whole body glycogen reserves than acclimation to 20 °C, which minimizes the effects of TFM in this species at the two temperatures. At 20 °C, however, glycogen levels were reduced with an increase in exposure time to TFM. We concluded that: 1) TFM toxicity changes with temperature in brown flatworms; 2) high and low temperature does have a protective effect in planaria against TFM toxicity, with the animals being most sensitive at 20 °C; and 3) the planarians respond to TFM differently compared to fishes. Lastly, we also tested whether water pH and alkalinity influences TFM toxicity in planarians and determined that, indeed, exposure to TFM at lower pH and alkalinity does increase sensitivity to the pesticide. When exposed to TFM at 80mg/L $CaCO_3$ alkalinity and changing pH, the 2h TFM LC-50 increased from 2.57mg/L at pH 7.0, to 2.81mg/L at pH 9; the 2h LC-99 followed a similar trend, increasing from 3.35mg/L to 3.67mg/L at the two respective pHs. The effect of water alkalinity followed a similar trend, with the lowest 2h LC-50 and LC-99 of 1.79mg/L and 2.44mg/L, respectively, at the lower alkalinity (40mg/L $CaCO_3$) and pH (6.5). Taken together, this work shows that TFM exposure impacts energy reserves in brown flatworms in a temperature-dependent manner, with the animals being most sensitive at 20 °C. In addition,

the toxicity of TFM is dependent on water pH and alkalinity, much like it is in fishes. Lastly, TFM appears to induce mortality in planarians, in part, by reducing energy reserves. This work has led us to explore the effects of TFM in this species further, by conducting time-course exposures at the 2h LC-99, to determine how energy reserves are impacted by this high level of TFM. We are also looking at whether planaria are able to detoxify TFM and what the genetic mechanisms are behind this, as well as looking at mitochondria function. Since flatworms are used extensively in research looking at not only environmental toxicology, but also neural and tissue regeneration, this work extends beyond its environmental applications.