MECHANISM(S) OF NICLOSAMIDE TOXICITY & ITS INTERACTIONS WITH TFM

Project ID – 2018_WIL_54074

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March 2025

ABSTRACT:

Populations of invasive sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes are controlled using the pesticide 3-trifluoromethyl-4-nitrophenol (TFM), which selectively targets larval sea lamprey in their nursery streams. Small amounts (0.5-2.0 %) of niclosamide (2′,5-dichloro-4′-nitrosalicylanilide), a molluscicide, are often applied with TFM to enhance its toxicity, which results in lower TFM consumption and cost-savings, without increasing toxicity to non-target fishes. A bottom-acting formulation of niclosamide, granular Bayluscide® (gB), is also used on its own to control larval sea lamprey populations in lentic waters and in high discharge, fast-flowing rivers. On its own, niclosamide is much more toxic than TFM, and far less selective to sea lamprey, with greater risks to non-target fishes. TFM interferes with mitochondrial ATP production by uncoupling oxidative phosphorylation, leading to deficits in energy reserves that eventually cause death. Niclosamide is thought to act in a similar manner, but relatively few studies have addressed its mechanism(s) or mode of action in sea lamprey or non-target fishes. The objectives of this research project were to: **(i)** determine if niclosamide interferes with mitochondrial function in a similar fashion to TFM, **(ii)** ascertain how niclosamide and TFM interact with one another to interfere with mitochondrial ATP production and physiological processes by sea lamprey, rainbow trout (*Oncorhynchus mykiss*), and lake sturgeon (*Acipenser fulvescens*), **(iii)** characterize how niclosamide is distributed within the body and detoxified by sea lamprey and trout, and **(iv)** determine if niclosamide alters gill function and structure in sea lamprey and rainbow trout. Isolated mitochondria, that were harvested from the liver of adult sea lamprey and exposed to niclosamide alone increased rates of state IV mitochondrial (leak) respiration, indicating that niclosamide uncoupled oxidative phosphorylation in an identical manner to the mechanism of action of TFM. However, niclosamide was a 30- to 60-times more potent uncoupler of oxidative phosphorylation than TFM, as demonstrated by a much lower (97-98 %) EC50, the amount of lampricide required to inhibit mitochondrial function by 50 %. Niclosamide interfered with mitochondrial ATP production in a similar fashion in rainbow trout, demonstrating the highly conserved nature of its mechanism of action across species. Predicted decreases in tissue energy stores were observed in the brain of all three species as the animals were forced to rely more on anaerobic pathways of ATP production when exposed to niclosamide. Marked reductions in brain phosphocreatine and glycogen stores, with lactate accumulation, were observed in larval sea lamprey. Reductions in brain ATP and glycogen, and greater lactate, were observed in trout. But there was no change in glycogen in the brain of lake sturgeon, which had very low basal concentrations of this energy store. Depleted liver glycogen stores were observed in all three species and likely related to the need to maintain glucose supply to the brain. Despite these severe homeostatic disturbances, larval sea lamprey, rainbow trout and lake sturgeon that survived 12 h sub-lethal exposure to niclosamide readily recovered and restored energy stores in brain, liver and muscle within 24 h. When exposed to environmentally relevant concentrations of TFM plus niclosamide (1%; TFM/Nic) greater TFM accumulation was observed in the muscle of larval sea lamprey than with TFM alone, suggesting that niclosamide interfered with TFM detoxification and elimination by the liver. At lower concentrations, the toxic interactions between TFM and niclosamide appeared to be strictly additive, but at higher

concentrations of TFM and niclosamide, in the range used for lampricide applications, toxicity was greater than additive (synergistic). Exposure of larval sea lamprey to niclosamide alone or in combination with TFM had minimal effect on plasma Na^+ or Cl^- concentration, and did not adversely affect gill Na^+/K^+-ATPase activity, a key metric of gill function. Nor did TFM/niclosamide mixtures or niclosamide alone substantially alter plasma Na^+ or Cl^- concentration in rainbow trout or lake sturgeon. We conclude that niclosamide is a more potent inhibitor of mitochondrial ATP production than TFM, which leads to depletion of tissue energy stores and toxicity at much lower exposure concentrations. However, the ability of larval sea lamprey to recover from TFM-niclosamide exposure or niclosamide alone suggests that interruptions to lampricide application regimens could result in residual lamprey that survive treatments. On the other hand, the resilience of non-target fishes such as endangered juvenile lake sturgeon and rainbow trout to sub-lethal niclosamide or TFM/niclosamide mixtures suggest that typical lampricide treatments are unlikely to have long-term impacts on the populations of fishes that survive the initial treatment.