Forensic Markers of Lampricide Toxicity & Mortality in Non-Target Fishes

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ABSTRACT:

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is applied to streams infested with larval sea lamprey (Petromyzon marinus) to control populations of this invasive species in the Great Lakes. Normally, TFM selectively targets sea lamprey due to their relatively low capacity, compared to non-target fishes, to detoxify TFM to the less toxic metabolite TFM-glucuronide. However, if lampricide uptake overwhelms a fish's detoxification capacity, non-target mortality can result. Non-target mortality following lampricide treatments can result from sudden drops in water pH or changes in water flow, but may go unobserved due to time of day and/or treatment location. The goal of this project was to develop reliable and efficient methods for the collection, preservation, storage and toxicological analysis of fish tissues to investigate incidents of non-target mortality that might be due to lampricide treatment. Objectives were to determine: (i) the concentrations of internal TFM that were fatal in non-target fishes; (ii) which tissues, handling and storage methods were most suitable for measuring post-mortem TFM and its metabolites, and (iii) if TFM and its metabolites could be measured in decomposing fishes. Accordingly, rainbow trout (Oncorhynchus mykiss) and white sucker (Catostomus commersonii) were exposed TFM in the lab, and different tissues (liver, white muscle, blood) were collected for TFM and TFM-metabolite analyses by the Upper Midwest Environmental Sciences Center (UMESC) using liquid chromatography-mass spec/mass spec (LC-MS/MS).

Following exposure to sub-lethal concentrations of TFM, TFM accumulation was greatest in liver, in which concentrations were 14-fold and 4-fold higher than in the whole blood of rainbow trout and white sucker, respectively. The concentration of TFM in muscle was usually slightly less than in blood. TFM-glucuronide was found in all tissues, with highest relative amounts in liver, followed by blood, with much less in muscle. A second metabolite, TFM-sulfate, was also detected at relatively high amounts in the liver and blood, indicating there is a second, functional TFM-detoxification pathway in fishes. Because liver TFM concentrations overlapped with those measured in rainbow trout that survived TFM exposure, it was not possible to establish a threshold concentration of TFM that could cause death using this tissue. However, TFM concentrations were notably higher in the blood (> 50%) and white muscle (~ 30 %) of rainbow trout that died from TFM exposure compared to individuals that survived. In rainbow trout, if blood TFM concentrations exceed 70nmol mL-1 and white muscle concentration exceed 28 nmol g -1 wet tissue weight (ww) death is likely to have been caused by TFM exposure.

The stability of TFM in tissues was affected by different post-collection handling methods. When samples were kept on ice for 1 h, prior to preservation in liquid N2, TFM concentrations were relatively stable in white muscle and blood of white sucker and rainbow trout, compared to control samples that had been immediately "snap-frozen" in liquid N2. However, TFM decreased by approximately 30 % in trout liver. If samples were kept at room temperature for relatively short periods of time (< 1 h), TFM concentrations were surprisingly stable (unchanged) compared to controls in rainbow trout, but slightly more variable in the white sucker, in which muscle TFM decreased by 30 % and blood TFM increased by approximately 20 %. Short-term storage conditions (1 week) also influenced liver TFM concentrations. When samples were quickly transferred to a -20°C freezer, TFM was stable in blood and muscle after 1 week compared to controls. In liver, however, TFM tended to increase in liver, and TFM-glucuronide and TFM-sulfate decreased. Refrigeration (4°C) for 1 week effectively preserved muscle TFM, which increased in blood. However, TFM was more than 95 % lower in liver, making it unusable. Treatment of tissues or blood with different preservatives such as heparin, sodium fluoride, or sodium citrate plus EDTA, had limited effects. After decomposition in water for 3 d or in air for 1 d, there was characteristic liquefaction of tissues and bloating in rainbow trout due to putrefactive processes. Blood collection was impossible, but TFM could still be quantified in muscle and liver, depending on temperature. In cooler (4°C) water, liver and muscle TFM were unchanged after 3 d of decomposition, whereas at 15°C and 20°C TFM concentrations were 50 % lower after 24 h in liver. In contrast, concentrations of TFM in muscle were more or less the same as in controls at 15°C, but declined by approximately after 24 h at 20°C. We conclude that forensically relevant post-mortem TFM and TFM-metabolite data can be obtained using tissues collected from fish that have died following TFM exposure, even after early stage decomposition. In cases of suspected lampricide toxicity we recommend that white muscle filets and liver (where possible) be collected from affected fish and frozen as quickly as possible using dry ice, or immediately transferred to a standard (-20°C) freezer. When rapid freezing is not possible in the field, samples should be kept on ice until transferred to a -20°C freezer. Unless collected near the time of death, blood sampling is not recommended. In all investigations, time of sampling, suspected time of death, water and air temperatures, water chemistry, location (immersed or on shoreline), and carcass condition should be noted. The chain of custody of samples also requires tracking. Within 1 week, samples packed in dry ice should be delivered to an accredited lab (e.g. UMESC) for subsequent measurement of TFM and TFM-metabolites to determine if fish were exposed to lampricides, and where possible, if measured concentrations were sufficient to cause death.