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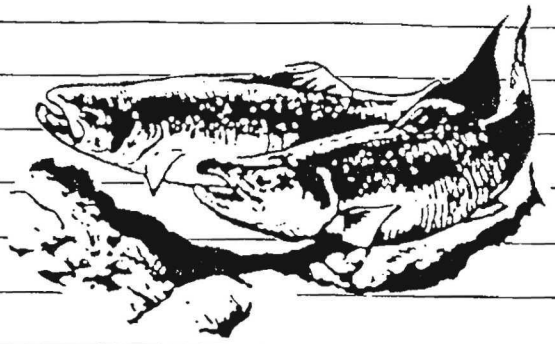

Harmful Residues From Eggs Exposed to Malachite Green Eliminated by Fry

Malachite green is a triphenylmethane dye that has been used extensively in fish culture throughout the world. In the United States, malachite green was used without restriction by operators of private and federal aquaculture facilities as an effective fungicide and ectoparasiticide on fish and fish eggs until it was found to be teratogenic in rainbow trout (*Oncorhynchus mykiss*). Thereafter, the U.S. Food and Drug Administration (FDA) regulated the use of malachite green on federal hatcheries until 1993, then banned its use on most of those hatcheries. Malachite green use, however, remains unregulated in private aquaculture.

The FDA is concerned about potential human exposure from various sources to malachite green—including food fish cultured on private hatcheries—and recently chose malachite green as a priority chemical for study. Because of this FDA scrutiny and because there is a potential for malachite green to bioaccumulate in fish, we conducted this study to describe the relative uptake and depuration rates of total malachite green residues in rainbow trout eggs and fry after exposure consistent with a treatment method used in aquaculture. We also describe the residue profiles in eggs and fry based on high pressure liquid chromatography (HPLC).

Analysis of Malachite Green Residues in Treated Eggs and Fry

Fertilized 1-day-old rainbow trout eggs were exposed to [methano-^{14}C] malachite green chloride for 1 h at a nominal concentration of 1.0 µg/mL on day 0 and every third day thereafter through day 24. All eggs hatched between days 25 and 31. On day 31, fry were exposed to a final treatment of [methano-^{14}C] malachite green chloride. Eggs or fry were sampled before each treatment; fry were sampled at selected times during the 28-day washout period after the last treatment. Malachite green-equivalent ^{14}C activity in individual eggs and fry were determined by sample oxidation and liquid scintillation counting. Malachite green-equivalent concentrations in samples increased throughout the exposure period to 0.271 µg/g before the final treatment on day 31. Twenty-eight days after the final treatment, malachite green-equivalent concentrations in fry declined to 0.055 µg/g. Malachite green residues decreased monoexponentially with a half-life of 13.3 days during the depuration phase for the absolute amount (µg/sample) and a half-life of 9.7 days for the concentration of malachite green residues. Growth dilution was responsible for the 27% shorter half-life for the concentration of malachite green residues.

Pooled egg and fry samples were analyzed by reverse-phase HPLC. Malachite green residues in extracts from eggs included chromatic malachite green, leuco malachite green, and an unknown polar metabolite. Only leuco malachite green and the unknown polar metabolite were resolved in extracts from fry. The most prominent residue in all samples was leuco malachite green.

Abatement of Malachite Green Concentrations

Adult fish reared from eggs treated with malachite green during incubation should contain virtually no residues. Malachite green residue concentrations will decrease in developing fish from elimination and growth dilution. The data presented could assist the FDA in establishing standards for malachite green use and may determine the fate of malachite green in aquaculture.

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