

# LC<sub>50</sub> determination for malachite green and formalin on rainbow trout (*Oncorhynchus mykiss*) juveniles

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## Abstract

Two relatively inexpensive and generally available prophylactic and therapeutic agents, commonly used against a wide spectrum of fish diseases by fish farmers throughout the world, namely zinc-free malachite green and formalin were investigated. The specific aim was the determination of the 96 h LC<sub>50</sub> values for rainbow trout juveniles. These LC<sub>50</sub> values are useful measurements of relative acute lethal toxicity to test organisms under specified conditions. From the results obtained during these experiments, it is recommended that *O. mykiss* juveniles can be bathed in malachite green concentrations of 1.25 x 10<sup>-4</sup> g·l<sup>-1</sup> to 2.25 x 10<sup>-4</sup> g·l<sup>-1</sup> or formalin concentrations of 0.0625 mg·l<sup>-1</sup> to 0.1 mg·l<sup>-1</sup> for as long as 24 h without mortalities.

## Introduction

Aquaculture is at present an important and rapidly expanding industry in many countries of the world (Anon, 1986; Liao, 1988). Increased fish production in fisheries is often accompanied by an increased incidence of more severe fish diseases caused by parasites and thus requires the use of therapeutic or prophylactic drugs. Formalin and malachite green have a long history of use in hatcheries for the control of fungal infections and external parasites of fish and fish eggs (Bills et al., 1977a; b).

Malachite green is effective for the treatment of external parasites e.g. *Argulus* (Hublou, 1958; Leteux and Meyer, 1972; Clifton - Hadley and Alderman, 1987) and protozoan e.g. *Ambiphyra*, *Chilodonella*, *Costia*, *Cryptocaryon irritans*, *Epistylis*, *Ichthyophthirius*, *Leptomitosis*, *Oodinium ocellatum*, *O. pillularis*, *Pythium*, *Trichodina* and *Trichophyrea* (Lanzing, 1965; Leteux and Meyer, 1972; Hoffman and Meyer, 1974; Nelson, 1974; Bills et al., 1977a; Herwig et al., 1979; Paperna, 1980; Alderman, 1985; Alderman and Clifton - Hadley, 1988). Malachite green has also been used in fish culture as a fungicide e.g. *Achyla*, *Aphanomyces*, *Ichthyophonus hoferi*, *Saprolegnia* (Lanzing, 1965; Martin, 1968; Bills et al., 1977a; Herwig et al., 1979; Hall and Unestam, 1980; Paperna, 1980; Pickering and Pottinger, 1985; Ingram, 1986; Clifton - Hadley and Alderman, 1987; Shepherd and Bromage, 1988; Alderman and Clifton - Hadley, 1988) and for the control of bacteria e.g. *Columnaris* (Lanzing, 1965; Herwig et al., 1979) and monogenetic trematodes e.g. *Gyrodactylus* (Herwig et al., 1979). In a recent paper Clifton - Hadley and Alderman (1987) reported that this dye can also control a systemic protozoan disease, proliferative kidney disease (PKD) in rainbow trout under laboratory conditions. These results indicate that both the development of clinical proliferative kidney disease and the presence of the protozoan parasite PKX in the trout kidney can be controlled by the application of malachite green at intervals throughout the summer.

To reduce labour, the managers of some hatcheries prefer to control fungal infection of eggs by chemical treatment instead of by removing dead eggs regularly (Edwards, 1978). Fungal infection is a persistent problem in hatcheries as dead eggs quickly become a focus of fungal proliferation which can then spread to adjacent healthy eggs. The most common species involved is *Saprolegnia*, which will infect fish tissue only in freshwater and at water temperatures usually below about 18°C. Malachite green is the most common and cost-effective means of controlling external saprolegniasis (Leteux and Meyer, 1972; Shepherd and Bromage, 1988).

Research has shown that malachite green possesses teratological properties, causing abnormalities in the development of fish eggs (Meyer and Jorgenson, 1983) and deformities amongst larvae so treated (Schnick and Meyer, 1978) as well as affecting man by causing tumors (Werth, 1958; Werth, 1960). Hatchery staff should therefore wear protective clothing and avoid contact with the chemical (Leteux and Meyer, 1972; Meyer and Jorgenson, 1983). As a result, it was banned in the USA after it had come under scrutiny during a program of registration of fisheries' chemicals (Schnick and Meyer, 1978). Despite these negative qualities, no effective alternatives have yet been found to replace malachite green as a medicament for treatment against certain fish fungi and ectoparasites (Scott and Eschmeyer, 1982; Schnick, 1988). It is, therefore, not surprising that the use of malachite green in aquaculture is frequently recorded in the most recent aquaculture-related literature (Srivastava and Srivastava, 1978; Alderman, 1985; Singhal et al., 1986; Alderman and Clifton - Hadley, 1988). Wedemeyer (1968) reports that malachite green increases the permeability of the vitelline membrane of fish for zinc, thus creating the need for a zinc-free form of the dye if used as a fish medicament.

Malachite green (zinc-free) has been generally used to control ectoparasites either as a dip at 1 : 15 000 (66.7 mg·l<sup>-1</sup>) with fish immersed for 10 to 30 s (Herwig et al., 1979; Stevenson, 1987; Sedgwick, 1990) or as a bath (1 : 500 000 = 2 mg·l<sup>-1</sup>) for fry and parr (Ingram, 1986; Sedgwick, 1990) or given a bath at 1 : 200 000 (5 mg·l<sup>-1</sup>) for 1 h (Stevenson, 1987). The bath concentration can also be used as treatment for incubating eggs after they have reached the eyed stage (Shepherd and Bromage, 1988; Sedgwick, 1990).

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