LC_{50} 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_{50} values for rainbow trout juveniles. These LC_{50} 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^{-4} g·ℓ^{-1} to 2.25 x 10^{-4} g·ℓ^{-1} or formalin concentrations of 0.0625 mℓ·ℓ^{-1} to 0.1 mℓ·ℓ^{-1} 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, Leptomitus, Oodinium ocellatum, O. pillularis, Pythium, Trichodina* and *Trichophyra* (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; Häll 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^{-1}) 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^{-1}) for fry and parr (Ingram, 1986; Sedgwick, 1990) or given a bath at 1 : 200 000 (5 mg·ℓ^{-1}) 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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Received 11 March 1994; accepted in revised form 5 October 1994.

Alternatively 0.1 mg·ℓ$^{-1}$ malachite green may be applied for one hour (and up to 2.2 to 5 mg·ℓ$^{-1}$ for trout eggs), by administration of stock solution to the water flowing into the tank or incubation facilities at a rate which will maintain this desired concentration (Paperna, 1980). Paperna (1980) also suggested that malachite green oxalate (zinc - free) must be applied to ponds at a dose of 0.15 mg·ℓ$^{-1}$ and to holding tanks at a dose of 0.10 to 0.20 mg·ℓ$^{-1}$ for one hour and recommends it as both a therapeutic and preventive treatment for fungal infections in fish. The authors have found the tolerance limit of trout to malachite green to be around 1.1 mg·ℓ$^{-1}$ per 6 h.

Although the use of malachite green as a therapeutant in fish culture has many advantages, it also poses various potential problems (Nelson, 1974), like toxicity to fishes (Bills et al., 1977a; Ross et al., 1985; Pickering and Pottinger, 1985; Clifton - Hadley and Alderman, 1987) and stress-induced during and after the treatment of fry of certain fishes (Glagoleva and Malikova, 1968; Bills and Hunn, 1976). Carcinogenesis, mutagenesis, chromosomal fractures, teratogenicity, or reduced fertility have been reported in rainbow trout (Amlacher, 1961; Lieder, 1961; Steffens et al., 1961; Nelson, 1974; Bills et al., 1977a; Schnick and Meyer, 1978; Meyer and Jorgenson, 1983), following treatment with malachite green.

Rainbow trout exposed to different concentrations of malachite green (0.05; 0.1; 0.15; 0.2 mg·ℓ$^{-1}$) showed an almost immediate increase in ventilation rate, cough rate and rate of oxygen consumption (Ross et al., 1985). The increased ventilation rate and respiration rate consistently observed by these authors were probably due to particulate clogging of the gills, mucus production and epithelial damage leading to an increased cough rate. These factors substantiate the necessity to provide supplementary aeration or oxygenation when treating fish in ponds or cages, especially at higher temperatures or in sea water, where dissolved oxygen levels are reduced (Ross et al., 1985).

Analysis of the tissue levels of malachite green by Clifton - Hadley and Alderman (1987) indicated that the dye both enters and accumulates in exposed fish to levels greater than the initial exposure concentration. The fact that malachite green can persist in fish tissue for some time after treatment has already been proven by Lanzing (1965) and Poe and Wilson (1983). This indicates a risk of long - term chronic toxicity plus a risk of sudden acute toxicity due to the fact that acute lethal tissue levels are exceeded with repetitive treatments.

To conclude, if malachite green is carefully used in aquaculture and according to prescribed procedures, there is little chance that it will pollute the environment after having been applied for the prescribed purposes. The active ingredient is neutralised in various ways e.g. by conversion of the ion to carbinol, and its adsorption onto organic particles as well as oxidation processes (Alderman, 1985).

Formalin, an aldehyde, was first discovered by the Russian chemist A M Butlerov, and first commercially manufactured in Germany in 1889. Formalin is the most widely used therapeutant and disinfectant in fish culture, controlling fungi, external protozoans, monogenetic trematodes and larvae of parasitic crustaceans (Hoffman and Meyer, 1974).

The efficacy of formalin is affected by the natural or developed resistance of a parasite (Schnick, 1973). Resistance to formalin by parasites, noted by Meyer (1968) and Van Duijn (1973) may in fact be due to other factors. Temperature, water chemistry, organic matter and other compounds in the water may play a role here (Rucker et al., 1963). This had already been confirmed by Kumar (1958), who showed a lower rate of control of fish parasites in ponds containing large amounts of suspended organic matter in the water. Temperature, water quality and organic material in water may influence its effectiveness. Most of these factors, however, contribute more towards toxicity to the host than to the parasite.

Formalin has had a long history of use in hatcheries for the control of such external parasites of fishes as *Costia* sp. and *Gyrodactylus* spp. (Fish, 1940; Rucker et al., 1963; Rucker, 1970; Wedemeyer, 1971; Wedemeyer and Yasutake, 1974; Edwards, 1978). Protozoans such as *Trichodina* spp.; *Ichthyobodo* sp; *Epistylis* spp.; *Ambiphrya* spp., *Apiosoma* spp.; *Chilodonella* spp. as well as *Ichthyophthirius multifiliis* can be eradicated effectively by a single application of 15 to 25 mg·ℓ$^{-1}$ formalin as an indefinite treatment applied directly to fish ponds (Reichenbach - Klinke and Elkan, 1965; Van Duijn, 1967; Dogiel et al., 1970; Goldstein; 1971; Paperna, 1980; Ross et al., 1985). According to Paperna (1980) the treatment of *Chilodonella* requires a higher concentration, 40 to 50 mg·ℓ$^{-1}$, of formalin. Testing the efficiency of low formalin concentration in fish ponds in Israel, Lahav and Sarig (1972) concluded that 30 to 40 mg·ℓ$^{-1}$ is sufficient to eradicate *Chilodonella* spp., *Ichthyobodo* sp. and *Trichodina* spp.. In these tests, the fish were parasite - free within 6 h after treatment (Lahav and Sarig, 1972).

Formalin added to the water is a universally accepted treatment, and can be administered in one of three ways , as a dip, a bath or as a flush. Dip treatments, using a high concentration of formalin, can only be used effectively where fish can be handled readily and returned to fresh, parasite-free water (Hoffman and Meyer, 1974). The main advantage of the dip treatment is that it requires comparatively small quantities of the substance used. This procedure should, however, be applied with caution, as the difference in concentration between a safe prophylactic or therapeutic treatment and one where it becomes toxic to the fish, is often very small (Piper et al., 1982).

Bath treatment varies with concentrations between 167 and 250 mg·ℓ$^{-1}$ (Wedemeyer, 1971; Wedemeyer and Yasutake, 1974; Edwards, 1978; Stuart, 1983; Stevenson, 1987; Collins, 1987) for up to 1 h. The treatment rate depends on fish species, fish size, fish condition, disease organisms and water exchange rate. The higher the stocking rate in tanks, the greater the chance of stress during treatment. Mortality is more likely to occur during treatment if the parasite load on the fish is heavy (Collins, 1987). However, the recommended 1 h treatment at concentrations of between 167 and 250 mg·ℓ$^{-1}$ can be unexpectedly toxic and substantial mortalities may occur (Wood, 1968). Experience has shown that rainbow trout (*O. mykiss*) are among the most sensitive of the salmonid fishes in this regard (Rucker et al., 1963; Wood, 1968; Smith and Piper, 1972). A concentration of about 15 to 25 mg·ℓ$^{-1}$ is required in ponds with normal algal blooms (Paperna, 1980; Stuart, 1983; Collins, 1987). Treatment can be given by dipping the fish or fry for 10 to 15 min in formalin at 1 : 2 500 (400 mg·ℓ$^{-1}$) (Stevenson, 1987).

Indiscriminate application and the lack of compensation for variables affecting the use of formalin are usually the major causes of fish mortalities. A wide range of tolerances for formalin exists for different fish species (Schnick, 1973), with the salmonids and centrarchids being the least sensitive, showing 24 h LC_{50}s for concentrations varying between 135 and 325 mg·ℓ$^{-1}$. Where comparative tests were carried out, formalin was shown to become more toxic with increasing water temperature (Schnick, 1973).

Care must be taken during dilution and the mixing in of a strong solution of formalin, so that areas of toxic hyper-concentrations do not develop (Hughes, 1971; Sedgwick, 1990).

TABLE 1
EXPERIMENTAL DATA FROM THE MALACHITE GREEN TOXICITY TEST ON JUVENILE *ONCORHYNCHUS MYKISS*

Concentration of malachite green g/ℓ x 10^{-4}	Total no. of fish	% Survival of fish											
	N	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	24 h	48 h	72 h	96 h
0.00	20	100	100	100	100	100	100	100	100	100	100	100	100
1.25	20	100	100	100	100	100	100	100	100	100	100	100	90
2.50	20	100	100	100	100	100	100	100	100	90	85	80	60
3.13	20	100	100	100	100	100	100	100	90	85	80	60	40
3.75	20	100	100	100	100	100	100	90	70	5	5	0	0
5.00	20	100	100	100	95	80	60	40	25	0	0	0	0
Dosage survival curve:													
For Y = a + bx ; a					102.75	110.99	121.99	133.49	140.74	152.22	148.98	143.01	120.52
b					-11994	-47974	-95949	-151910	-203916	-307814	-303833	-303922	-263987
LC_{50} (g/ℓ malachite green) estimated from graph					$4.40x10^{-3}$	$1.27x10^{-3}$	$7.50x10^{-4}$	$5.50x10^{-4}$	$4.45x10^{-4}$	$3.32x10^{-4}$	$3.26x10^{-4}$	$3.06x10^{-4}$	$2.67x10^{-4}$
					$4.79x10^{-3}$	$1.70x10^{-3}$	$1.15x10^{-3}$	$1.04x10^{-3}$	$9.25x10^{-4}$	$7,82x10^{-4}$	$7.26x10^{-4}$	$6.77x10^{-4}$	$5.14x10^{-4}$
95% confidence limits					$4.01x10^{-3}$	$8.38x10^{-4}$	$3.49x10^{-4}$	$5.86x10^{-5}$	$3.50x10^{-5}$	$1.18x10^{-4}$	$7.40x10^{-5}$	$6.54x10^{-5}$	$2.00x10^{-5}$

Fish (1940) and Van Duijn (1973) are of the opinion that proper aeration should be maintained when adding the formalin to the water. Sedgwick (1990) also stated that formalin reduces the oxygen in the water and that aeration should be provided during treatment.

Previous studies have shown formalin to cause damage to gill tissue (Wedemeyer and Yasutake, 1974; Ross et al., 1985), therefore the possibility of disrupting normal gill functions should be considered. Formalin has also been shown to cause disruption in homeostatic mechanisms in rainbow trout (Wedemeyer, 1971; Wedemeyer and Yasutake, 1974).

The aim of the study was to determine the 96 h LC_{50} values for rainbow trout juveniles exposed to malachite green and formalin, because of the rising concern about the potential hazards that might be associated with their application in South Africa.

These values do not necessarily have any direct meaning in terms of "safe" or "hazardous" conditions in natural water. Long - term exposure to much lower concentrations may be lethal to fish and other organisms and / or may cause nonlethal impairment of their function. Similarly, short - term exposure to these or higher values of total contaminants may cause no discernible effect (Franson, 1989).

Materials and methods

The LC_{50} experiments were conducted with *O. mykiss* juveniles of the same age and a mean mass of 0.3755 g (range of 0.233g to 0.531 g), which were bred and reared in an environmentally controlled laboratory at Rand Afrikaans University. The temperature was maintained at 10 ± 1°C throughout the experiments. A photoperiod of 24 h [12 : 12 (L : D)] was maintained. The continuous flow system used to determine the LC_{50} values, consisted of a reservoir tank (20 ℓ) which supplied water to an aerated exposure tank, (9ℓ glass aquarium). The overflow water from each exposure tank was discarded (Fig. 1).

The test juveniles were allowed to acclimate in the exposure tanks for 3 d before the addition of either malachite green or formalin solutions. The juveniles were not fed during this acclimation period and also not during the 96 h exposure period. Ten fish were used for each exposure test. The numbers of fish that survived each exposure were determined

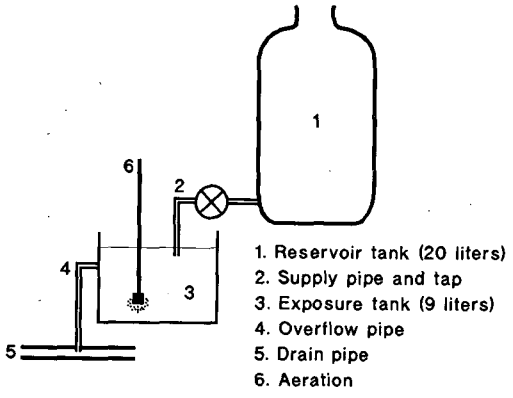

Figure 1
Continuous flow system used for toxicity tests

TABLE 2
EXPERIMENTAL DATA FROM THE FORMALIN TOXICITY TEST ON JUVENILE *ONCORHYNCHUS MYKISS*

Concentration of formalin mℓ/ℓ	Total no. of fish N	% Survival of fish											
		1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	24 h	48 h	72 h	96 h
0.00	20	100	100	100	100	100	100	100	100	100	100	100	100
0.0625	20	100	100	100	100	100	100	100	100	100	100	100	90
0.1	20	100	100	100	100	100	100	100	100	100	90	70	65
0.15	20	100	100	100	100	100	100	100	100	95	90	55	20
0.2	20	100	100	100	100	100	100	100	100	85	20	0	0
0.25	20	100	100	100	100	100	100	100	85	55	0	0	0
Dosage survival curve:													
For $Y = a + bx$; a									135.73	148.34	144.49	137.98	114.50
b									-385.08	-605.53	-593.37	-590.06	-501.66
LC_{50} (mℓ/ℓ formalin) estimated from graph									0.223	0.162	0.159	0.149	0.129
95% confidence limits									0.318	0.243	0.247	0.239	0.226
									0.128	0.081	0.071	0.059	0.032

at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 24, 48, 72 and 96 h, respectively.

The method of Franson (1989) was used to determine the LC_{50} values and 95% confidence intervals. Lethal concentration (LC) can be defined as the toxicant concentration producing death of the test organism. Usually defined as median (50%) lethal concentration, LC_{50}, i.e. concentration killing 50% of exposed organisms at a specific time of observation, for example, 96 h LC_{50} (Franson, 1989). The 96 hour LC_{50} values produced from standard acute toxicity tests are useful measures of relative acute lethal toxicity to test organisms under specified conditions (Franson, 1989).

Test solutions of the desired concentrations were added to the reservoir tanks, and the resulting mixtures were stirred to ensure homogeneity. The reservoir tanks were then connected to the test tanks by means of a pipe with a tap at the end. The flow from the reservoir tank was calibrated to replace the entire volume of the test tank at least twice daily.

Juveniles were exposed to malachite green concentrations ranging from 0 to 0.0025 g·ℓ^{-1}, but the final concentrations for determining the LC_{50} were between 0 and 0.0005 g·ℓ^{-1}. Juveniles were also exposed to between 0 to 0.4 m$\ell\cdot\ell^{-1}$ formalin, but the final range was between 0 and 0.25 m$\ell\cdot\ell^{-1}$. The LC_{50} determination procedure was done twice within the final concentration ranges. The mean values of these repetitions were plotted in Fig. 2 and 4 and also Tables 1 and 2.

The behaviour of the juveniles was continuously monitored during the first 8 h and thereafter 3 times per day. Dead fish were immediately removed from the exposure tanks. The water that was used in the study had the following qualities : pH = 6.95; conductivity (mS·m^{-1}) = 16.4 and total alkalinity ($CaCO_3$ mg·ℓ^{-1}) = 52.

Results

Environmental data obtained during the exposure of *O. mykiss* juveniles to malachite green and formalin after different time intervals are displayed in Tables 1 and 2. These data show that some *O. mykiss* juveniles died after only 4 h when exposed to the highest malachite green concentration, while some juveniles exposed to the highest formalin concentration only died after 8 h.

The 96 h LC_{50} value of *O. mykiss* juveniles for malachite green is 2.67 x 10^{-4} g·ℓ^{-1} and the 24-; 48- and 72 h LC_{50} values are 3.32 x 10^{-4}; 3.26 x 10^{-4} and 3.06 x 10^{-4} g·ℓ^{-1} respectively (Fig. 2).

The 96 h LC_{50} value of *O. mykiss* juveniles for formalin is 0.129 m$\ell\cdot\ell^{-1}$ and the 24 -; 48 - and 72 h LC_{50} values are 0.162; 0.159 and 0.149 m$\ell\cdot\ell^{-1}$ respectively (Fig. 4).

The toxicity curves of malachite green and formalin on the determined LC_{50} values for juvenile *O. mykiss* are shown in Figs. 3 and 5.

Behavioral changes recorded in *O. mykiss* juveniles were in order of their agitated swimming rates, increased rates of operculum movement and coughing movements. These reactions to malachite green and formalin were more pronounced in tanks containing the higher levels and less conspicuous at lower concentrations.

Discussion

The present study endeavoured to evaluate two widely used chemical agents, malachite green and formalin, as prophylactic treatments for juveniles of *O. mykiss*. Different methods of disease prevention and control can be employed in practice, if the presence of parasites and/or disease in fish is diagnosed. However, chemical control methods are usually preferred and a considerable

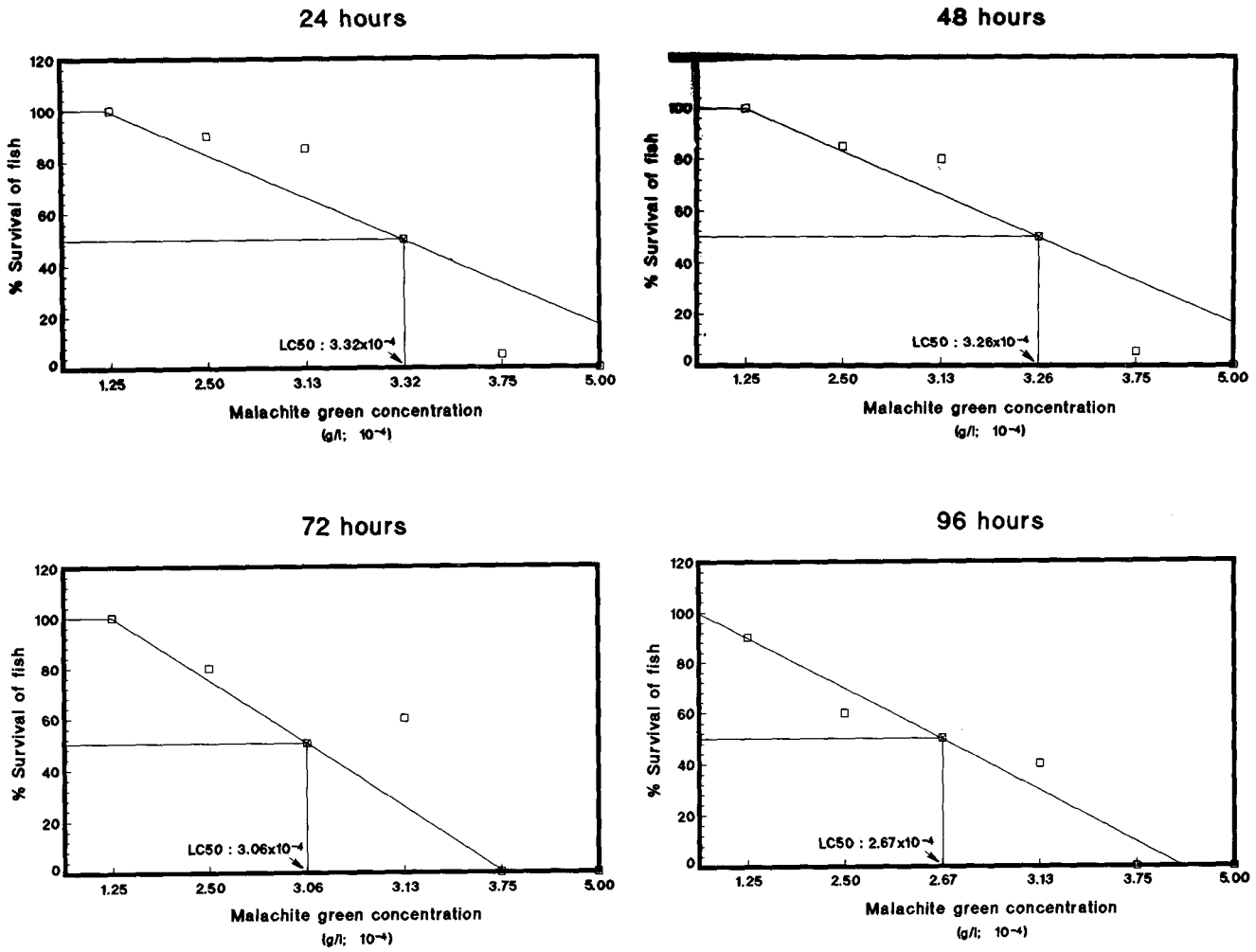

Figure 2
Dosage survival curves of malachite green for juvenile O. mykiss

amount of published data on disease control by this method is available as reviewed by Hoffman and Meyer (1974); Bills et al. (1977a; b); Herwig et al. (1979); Piper et al. (1982) and Clifton - Hadley and Alderman (1987).

Under local environmental conditions the recommendations in the literature are not always proven effective. This is largely as a result of different physical and chemical conditions of water used in the production of fish as well as differences in the species composition of the fish of the Northern and Southern Hemispheres.

The primary goal in the design and use of toxicity tests in biomonitoring is to predict, in combination with other environmental factors, with known accuracy, a concentration of a specific toxicant that will not harm an entire system and to make this prediction in a responsible and cost effective manner. In any bioassay, it is important to consider the water quality used in the experimental system. Modifying factors, such as water hardness, pH, alkalinity, and temperature, can affect the toxicant, and thus alter their toxicity (Chakoumakos et al., 1979). The LC_{50} values provide a useful means of comparing the relative acute lethal toxicity of specific toxicants to organisms under specified conditions.

A $TILC_{50}$ (lethal concentration producing 50% mortality independent of time) of 9.98 x 10^{-5} g·ℓ^{-1} was determined with malachite green for rainbow trout, as compared with LC_{50} of 2.8

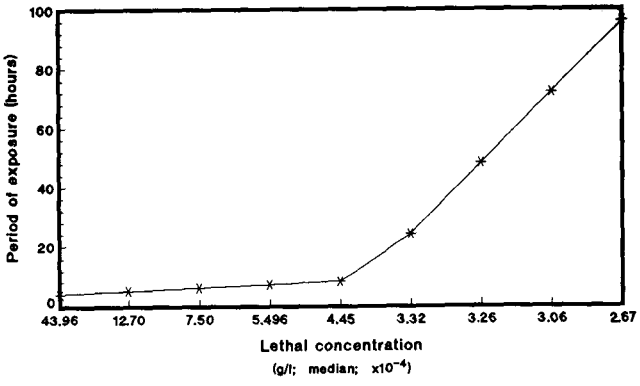

Figure 3
Toxicity curve of malachite green on the determined LC_{50} values for juvenile O. mykiss

x 10^{-4} g·ℓ^{-1} at 96 h at a temperature of 12°C and a pH of 6.5 (Bills et al., 1977a), which is slightly higher than the 2.67 x 10^{-4} g·ℓ^{-1} that was obtained in this study. The difference could have been due to the fact that Bills et al. (1977a) conducted their study on bigger rainbow trout (0.5 to 1.5 g), and at a higher water temperature than

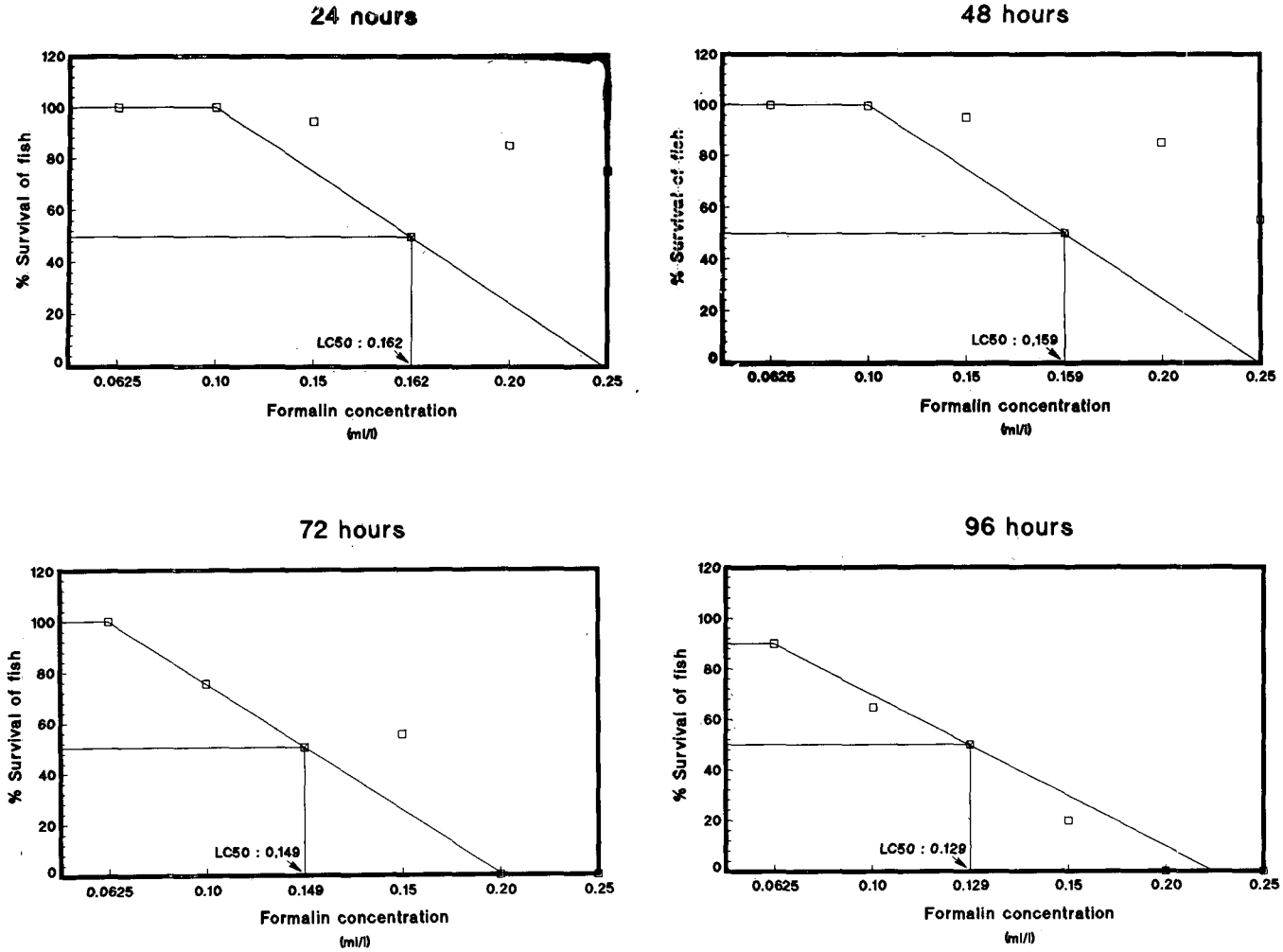

Figure 4
Dosage survival curves of formalin for juvenile O. mykiss

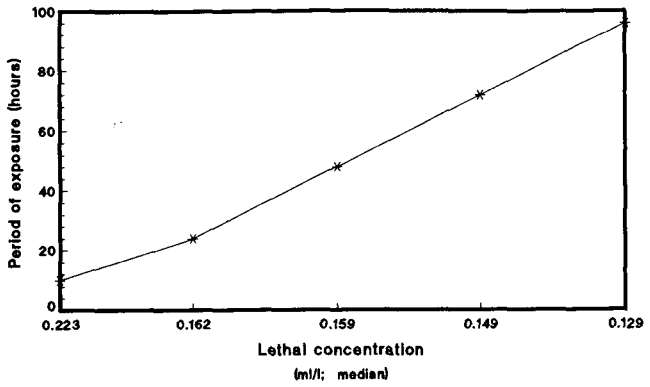

Figure 5
Toxicity curve of formalin on the determined LC_{50} values for juvenile O. mykiss

our study. Other parameters (pH, alkalinity etc.) might also have differed.

Willford (1967) reported 24 h LC_{50}s at 12°C for bluegills, rainbow-, brook-, brown- and lake trout of 6 x 10^{-4} g·ℓ^{-1} malachite green or less. Alderman (1985) found that the LT_{50} (exposure time producing 50% mortality) of a single concentration of 5 x 10^{-3} g·ℓ^{-1} malachite green for rainbow trout fingerlings varies with temperature. At lower temperature the fish tolerance was good. At 8°C the fingerlings were not significantly harmed by 1 h exposure to 5 x 10^{-3} g·ℓ^{-1} malachite green, but at 16°C this fell to 50 min, and to 40 min at 20°C. The majority of malachite green fish treatment will take place in the hatchery at the lower end of the temperature range. Nevertheless, it is clear that a relatively slight increase in temperature, such as might be encountered during treating fry in the spring, can convert an otherwise safe time/concentration regime to one capable of producing major mortalities.

Paperna (1980) recommended that malachite green applied to ponds at a dose of 1.5 x 10^{-4} g·ℓ^{-1} and to holding tanks at a dose of 2 x 10^{-4} g·ℓ^{-1} for one hour is high enough both for therapeutic and preventive treatments for fungal infections in fish. According to these recommendations *O. mykiss* juveniles can be bathed in malachite concentrations of 1.25 x 10^{-4} g·ℓ^{-1} to 2.25 x 10^{-4} g·ℓ^{-1}. These concentrations will be capable of killing the parasites and still be low enough not to cause any mortalities. The malachite green concentrations of 2 x 10^{-3} and 5 x 10^{-3} g·ℓ^{-1} recommended by Ingram (1986), Stevenson (1987) and Sedgwick (1990) is too high for use under Southern Africa conditions.

Higher malachite green concentrations can be used as a dip treatment, but we do not recommend dip treatment, because of the

stress caused by the continual handling of the fish.

Bills et al. (1977b) reported a $TILC_{50}$ of rainbow trout for formalin, of 72.0 $\mu l \cdot l^{-1}$, as compared with LC_{50} of 131.0 $\mu l \cdot l^{-1}$ at 96 h. The 96 h LC_{50} of 129 $\mu l \cdot l^{-1}$ obtained for *O. mykiss* juveniles in this study compares favourably with the findings of Bills et al. (1977b).

Oncorhynchus mykiss juveniles can therefore be bathed in formalin concentrations of 0.0625 $ml \cdot l^{-1}$ (25 $mg \cdot l^{-1}$) to 0.1 $ml \cdot l^{-1}$ (40 $mg \cdot l^{-1}$). Lahav and Sarig (1972); Paperna (1980) and Ross et al. (1985) recommend formalin concentrations of 15 to 50 $mg \cdot l^{-1}$ to eradicate most parasites. The recommended concentrations kill the parasites without causing any fish mortalities. In cases where higher formalin concentrations are used, the exposure time must be reduced.

Chemotherapy should be undertaken at the time of day when the water temperature is at its lowest and adequate oxygen levels must be maintained. It is important to monitor fish behaviour continuously during treatment and the fish farmer must be ready to switch on aerators or to flush the tanks with freshwater if the fish become stressed (Shepherd and Bromage, 1988). A calculated "drip" of the chemical at the correct concentration can be added to the water supply when the fish are in tanks or raceways. This is expensive but treatment can usually be given in freshwater, without the stress associated with capturing the fish. Low-level disinfection can be provided over a longer period (Sedgwick, 1990). Care must be taken that the effluent water containing the chemical solution does not reach rivers, to prevent killing of non-target animals in the rivers. The effluent water must be diluted before being released into rivers. Present governmental control on the use of chemicals in the environment necessitates counteraction of persistent compounds after they have served their purpose (Dawson, 1975). The two most commonly used techniques for removal of such compounds are chemical oxidation/reduction or adsorption on activated carbon. Bills et al. (1977b) reported that both these techniques failed to neutralise the toxicity of formalin. In fact, it was found that, under oxidative conditions, the solutions became more toxic. In contrast with formalin, activated carbon is an excellent procedure for removing malachite green from water (Bills et al., 1977a).

Acknowledgements

This research would not have been possible without the financial backing of the Foundation for Research Development (FRD) and the Rand Afrikaans University. Special acknowledgement is given to Mrs A Vorster for editing the manuscript; Department of Medical Illustrations and Audio Visual Services at MEDUNSA for assistance in the preparation of the graphical material and Mrs C. Göldner for typing the tables.

References

ALDERMAN, DJ (1985) Malachite green : a review. *J. Fish Dis.* **8** 289 - 298.

ALDERMAN, DJ and CLIFTON - HADLEY, RS (1988) Malachite green therapy of proliferative kidney disease in rainbow trout : Field trials. *Vet. Record* January **30** 103 - 106.

AMLACHER, E (1961) The effects of malachite green of fish, fish parasites (*Ichthyophthirius*, *Trichodina*), small crustaceans, and water plants. *Deutsche Fischerei Zeitung* **8** 12 - 15.

ANON, (1986) A second look at Asian and world aquaculture production. *Aquacult. Mag.* **12**(5) 6 - 16.

BILLS, TD and HUNN, JB (1976) Changes in the blood chemistry of coho salmon exposed to malachite green. *Prog. Fish-Cult.* **38**(4) 214 - 216.

BILLS, TD; MARKING, LL and CHANDLER, JH (1977a) Malachite green : Its toxicity to aquatic organisms, persistence and removal with activated carbon. *US Fish Wildl. Serv., Invest. Fish Control* **73** 1 - 6.

BILLS, TD; MARKING, LL and CHANDLER, JH (1977b) Formalin : Its toxicity to nontarget aquatic organisms, persistence, and counteraction. *US Fish Wildl. Serv., Invest. Fish Control* **73** 1 - 7.

CHAKOUMAKOS, C; RUSSO, RCA and THURSTON, RV (1979) Toxicity of copper to cutt-throat trout (*Salmo clarkii*) under different conditions of alkalinity, pH and water hardness. *Env. Sci. Technol.* **13** 213 - 219.

CLIFTON - HADLEY, RS and ALDERMAN, DJ (1987) The effects of malachite green upon proliferative kidney disease. *J. Fish Dis.* **10** 101 - 107.

COLLINS, C (1987) Review of a new fishery chemicals handbook. *Aquacult. Mag.* **13**(2) 52 - 54.

DAWSON, VK (1975) Counteracting chemicals used in fishery operations: Current technology and research. In : PH Eschmeyer (ed.), Rehabilitation of fish populations with toxicants : a symposium. *North Cent. Div., Am. Fish. Soc. Spec. Publ.* **4** 32 - 40.

DOGIEL, VA; PETRUSHEVSKI, GK and POLYANSKI, YuI (1970) *Parasitology of Fishes*. TFH Publications Ltd., Surrey. 384 pp.

EDWARDS, DJ (1978) *Salmon and Trout Farming in Norway*. Fishing News Books Ltd., Farnham, England, 195 pp.

FISH, FF (1940) Formalin for external protozoan parasites. *Prog. Fish - Cult.* **48** 1 - 10.

FRANSON, M-AH (1989) *Standard Methods For the Examination of Water and Wastewater*. CLESCERI, LS; GREENBERG, AE and TRUSSELL, RR (eds.) (17 th edn.) APHA, AWWA and WPCF.

GLAGOLEVA, TP and MALIKOVA, EM (1968) The effect of malachite green on the composition of blood in young Baltic salmon. *Ryb. Khoz.* **44** 15 - 18 (In Russian).

GOLDSTEIN, RJ (1971) *Cichlids*. TFH Publications, New York, p. 254.

HäLL, L and UNESTAM, T (1980) The effect of fungicides on survival of the crayfish plague fungus *Aphanomyces astaci*, Oomycetes, growing on fish scales. *Mycopathol.* **72**(3) 131 - 134.

HERWIG, N; GARIBALDI, L and WOLKE, RE (1979) *Handbook of Drugs and Chemicals used in the Treatment of Fish Diseases. A Manual of Fish Pharmacology and Material Medica*. Charles C Thomas, Publisher, Springfield. 272 pp.

HOFFMAN, GL and MEYER, FP (1974) *Parasites of Freshwater Fishes*. TFH Publications, Neptune, New Jersey.

HUBLOU, WF (1958) The use of malachite green to control *Trichodina*. *Prog. Fish - Cult.* **20** (3) 129 - 132.

HUGHES, JS (1971) Tolerance of striped bass, *Morone saxatilis* (Walbaum), larvae and fingerlings to nine chemicals used in pond culture. *Proc. Annu. Conf. Southeast. Game Fish Comm.* **24** 431 - 438.

INGRAM, M (1986) *Clearwater Guide to Ova and Milt : High Technology Broodstock Management*. Clearwater Publishing Ltd., 111 pp.

KUMAR, AKG (1958) Control of *Gyrodactylus* sp. on goldfish. M.Sc. Thesis, Alabama Polytechnic Institute, Auburn, Alabama, 47 pp.

LAHAV, M and SARIG, S (1972) Control of unicellular parasitic fauna. *Acta Parasitol. Pol.* **22**(6) 49 - 66.

LANZING, WJR (1965) Observations on malachite green in relation to its application to fish diseases. *Hydrobiologia* **25** 426 - 441.

LETEUX, F and MEYER, FP (1972) Mixtures of malachite green and formalin for controlling Ichthyophthirius and other protozoan parasites of fish. *Prog. Fish - Cult.* **34**(1) 21 - 26.

LIAO, I - CHIU (1988) East meets West : An eastern perspective of aquaculture. *19th Ann. Meeting World Aquacult. Soc.* Honolulu, Hawai, 4 - 8 January, 1988, 21 pp.

LIEDER, U (1961) Zur Wirkung des Cancerogens und Mutagens Malachitgrun (p - Dimethylaminofuchson - Dimethylimino - Oxalat [sulfat]) auf Mitosen bei Fischen und Fischeiern. *Naturwissenschaften* **48** 437 - 438.

MARTIN, RL (1968) Comparison of effects of concentrations of malachite green and acriflavine on fungi associated with diseased fish. *Prog. Fish - Cult.* **30** 153 - 158.

MEYER, FP (1968) A review of the parasites and diseases of fishes in warmwater ponds in North America. *FAO Fish. Report* **44** 290 - 318.

MEYER, FP and JORGENSON, TA (1983) Teratological and other effects of malachite green on development of rainbow trout and rabbits. *Trans. Am. Fish. Soc.* **112** 818 - 824.

NELSON, NC (1974) A review of the literature on the use of malachite green in fisheries. US Nat. Tech. Inform. Serv. Washington, DC, Doc. No. PB235 450. 79 pp.

PAPERNA, I (1980) *Parasites, Infections and Diseases of Fish in Africa.* Food and Agriculture Organization of United Nations, Rome, 1980.

PICKERING, AD and POTTINGER, TG (1985) Acclimation of the brown trout, *Salmo trutta* L., to the stress of daily exposure to malachite green. *Aquacult.* **44** 145 - 152.

PIPER, RG; McELWAIN, IB; IBORME, LE; McCRAREN, JP; FOWLER, LG and LEONARD, JR (1982) Fish hatchery management. *U.S, Dept. Interior, Fish and Wildlife Serv.* Washington, DC, 517 pp.

POE, WE and WILSON, RP (1983) Absorption of malachite green by channel catfish. *Prog. Fish - Cult.* **45** 228 - 229.

REICHENBACH - KLINKE, HH and ELKAN, E (1965) *The Principal Diseases of Lower Vertebrates.* Book I. *Diseases of Fishes.* TFH Publications, New York. 200 pp.

ROSS, LG; WARD, KMH and ROSS, B (1985) The effects of formalin, malachite green and suspended solids on the respiratory activity of rainbow trout, *Salmo gairdneri* Richardson. *Aquacult. Fish. Management* **16** 129 - 138.

RUCKER, R; TAYLOR, W and TONEY, D (1963) Formalin in the hatchery. *Prog. Fish - Cult.* **25** 203 - 207.

RUCKER, R (1970) Fish therapy : past, present and future. *Univ. Wash. Coll. Fish. 50th Anniv. Symp. Seattle.*

SCHNICK, RA (1973) Formalin as a therapeutant in fish culture. *US Fish Wildlife Serv.* Literature Review 74 - 09. National Technical Information Service, No. PB 237 198. 131 pp.

SCHNICK, RA (1988) Information on fish chemicals and drugs. *Aquacult. Mag.* **14**(3) 6 - 13.

SCHNICK, RA and MEYER, FP (1978) Registration of thirty - three fishery chemicals : status of research and estimated costs of required contract studies. *Invest. Fish Control* **86** 19.

SCOTT, TG and ESCHMEYER, PH (1982) *Fisheries and Wildlife Research, 1981.* U.S. Dept. Int., Fish Wildlife Serv. Denver, Colorado.

SEDGWICK, SD (1990) *Trout Farming Handbook.* (5th edn.) Fishing News Books. 164 pp.

SHEPHERD, CJ and BROMAGE, NR (1988) *Intensive Fish Farming.* BSP Professional Books, London. 404 pp.

SINGHAL, RN; JEET, S and DAVIES, RW (1986) Chemotherapy of six ectoparasitic diseases of cultured fish. *Aquacult.* **54** 165 - 171.

SMITH, CE and PIPER, RG (1972) Pathological effects in formalin - treated rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.* **29**(3) 328 - 329.

SRIVASTAVA, GC and SRIVASTAVA, RC (1978) A note on the potential applicability of malachite green oxalate in combating fish mycoses. *Mycopathologia* **64**(3) 169 - 171.

STEFFENS, W; LIEDER, U; NEHRING, D and HATTOP, HW (1961) Möglichkeiten und Gefahren der Anwendung von Malachitgrün in der Fischerei. *Zeitschrift Für Fischerei und deren Hilfswissenschaften* **10** 745 - 771.

STEVENSON, JP (1987) *Trout Farming Manual.* (2nd edn.) Fishing News Books Lim., Farnham, Surrey, England. 259 pp.

STUART, NC (1983) Treatment of fish diseases. *Vet. Rec.* **112** 173 - 177.

VAN DUIJN, C (1967) *Diseases of Fishes.* (2nd edn.) Iliffe Books Ltd., London. p. 309.

VAN DUIJN, C (1973) *Diseases of Fishes.* (2nd edn.) Iliffe Books, London. 372 pp.

WEDEMEYER, GA (1968) Uptake and distribution of zinc - 65 in the coho salmon egg (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* **26** 271 - 279.

WEDEMEYER, G (1971) The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Board Can.* **28** 1899 - 1904.

WEDEMEYER, G and YASUTAKE, WT (1974) Stress of formalin treatment in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.* **31** 179 - 184.

WERTH, G (1958) Die Erzeugung von Störungen im Erbgefüge und von Tumoren durch Experimentelle Gewebsanoxie. *Arzneimittelforschung* **8** 735 - 744.

WERTH, G (1960) Schädigungstumoren bei Ratten durch Behandlung einer Vorfahrengeneration mit Malachitgrün. *Deutsche Akademie für Wissenschaften, Berlin, Klässe für Medizin* **3** 24 - 28.

WILLFORD, WA (1967) Toxicity of 22 therapeutic compounds to six fishes. *U.S. Bureau of Sport Fish. and Wildlife, Invest. Fish Control* **18** 10.

WOOD, JW (1968) *Diseases of Pacific Salmon : Their Prevention and Treatment.* State of Washington, Dept. Fish. Hatch. Div. 74 pp.