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(54) **USE OF BRONOPOL FOR THE TREATMENT OF DISEASES IN FISH**

VERWENDUNG VON BRONOPOL ZUR BEHANDLUNG VON KRANKHEITEN BEIM FISCH
EMPLOI DE BRONOPOL POUR LE TRAITEMENT DE MALADIES CHEZ LE POISSON

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Description

[0001] This invention relates to the treatment of fungal infections (especially *Saprolegnia parasitica*) of fish, particularly, but not exclusively, trout, salmon, and salmonids in general. The invention also provides treatments of other fish diseases such as bacterial infections (for example bacterial gill disease *Flavobacterium branchiophilum* and *Cytophaga psychrophila*), protozoan infections such as ciliates (for example *Ichthyophthirius multifiliis*) and flagellates (for example *Ichthyobodo necatrix*).

[0002] *Saprolegnia parasitica* is a rapidly spreading and fatal fungal parasite affecting both fish and fish eggs. It is conventionally treated with malachite green (diamino-triphenylmethane), but though this treatment is highly effective, the use of malachite green carries with it a number of potential problems: the compound has been suggested as a possible carcinogen and teratogen, though these effects are as yet unproven; being a strong dye, it tends to discolour water, and can under certain conditions cause staining of fish which have been treated; it has a relatively long withholding period, so that significant residues can be present in treated fish when they are harvested and sold for consumption; and the compound is not licensed as a veterinary medicament, and is actually banned from use in US Federal hatcheries. The article by D.J. Alderman in *Journal of Fish Diseases* 8 (1985) 289-298 gives a review of the use of malachite green in treating fish diseases, and discusses some of the problems associated therewith.

[0003] One currently available alternative to malachite green is formalin, which is the substance which is now used in US Federal hatcheries. However, due to the irritancy of this substance, it can only be used under strictly controlled conditions. There is therefore a need for an improved treatment for fungal and other diseases of fish, particularly one which combines high efficacy with low toxicity. Many attempts to identify new treatments have been made in the past and the results of the screening of 40 potential alternative substances are set out in the article by D.J. Alderman appearing at *Journal of Fish Diseases* 5 (1982) 113-123, which also sets down standard protocols for the testing of candidate treatments. However, despite this and other work (such as that described in the article by T.A. Bailey appearing at *Aquaculture* 38 (1984) 97-104), the applicants are not aware of any suitable alternative treatments to malachite green and formalin having reached the marketplace.

[0004] GB 22263063 discloses an anti ulcer composition for fish based on a mixture of formaldehyde, a quaternary ammonium salt having bactericidal activity and a brominated alkanediol itself having anti bacterial activity.

[0005] The abstract of HU38833 refers to an antifungicide and antimicrobiological product comprising 0.5% bradophen, 0.05% bronopol and 5% hydrophilated silicon, for uses including veterinary applications.

[0006] It has now been found that bronopol (2-bromo-2-nitropropane-1,3-diol) has good activity at relatively low concentrations against *Saprolegnia parasitica*, and is safe to use. Bronopol is a known compound, and is used in concentrations of between 0.01 and 0.2% as an antimicrobial preservative and antiseptic in topical pharmaceutical formulations, cosmetics, and toiletries. It is stated however in "Handbook of Pharmaceutical Excipients" published by The Pharmaceutical Press (1994) that one of bronopol's major disadvantages is its relatively poor activity against yeasts and moulds. It is therefore surprising that bronopol is effective at relatively low concentrations against *Saprolegnia*.

[0007] As will be described in more detail below, bronopol has been shown to be effective against *Saprolegnia parasitica* infection in salmon, trout and trout eggs. It is envisaged however that the treatment will be effective against the same infection in other salmonid species, and indeed in other fish (for example ornamental or pet fish), fish eggs and other aquatic creatures (such as shrimps or prawns) in general. It is expected that there will also be efficacy against other fungal infections. Furthermore, as bronopol has been shown in the trials described below to prevent or slow the spread of infection, it may also be used as a prophylactic.

[0008] Bronopol is a commercial product available from a number of sources. It may be manufactured by reacting nitromethane with paraformaldehyde in an alkaline environment, followed by bromination. It is also known that some compounds (such as 5-bromo-5-nitro-1,3-dioxane) break down to release bronopol, and the invention therefore also extends to the use of such compounds in the place of bronopol.

[0009] Thus, the invention provides, in one aspect, the use of bronopol (2-bromo-2-nitropropane-1,3-diol) **[deletion(s)]** in the manufacture of a medicament for the treatment or prophylaxis of any of the following diseases in fish: fungal infections, flagellate protozoan infections, ciliate protozoan infections, bacterial gill disease, and myxobacterial infections, wherein said medicament contains bronopol **[deletion(s)]**, as its sole or principal active ingredient against the aforementioned diseases. The diseases may be caused by any of the following causative organisms: *Saprolegnia parasitica*, *Ichthyobodo necatrix*, *Ichthyophthirius multifiliis*, *Flavobacterium branchiophilum* and *Cytophaga psychrophila*. The diseases may be diseases of salmonid fish, in particular diseases of trout or salmon.

[0010] In another aspect, the invention provides a treatment bath for the treatment or prophylaxis of any of the following diseases in fish: fungal infections, flagellate protozoan infections, ciliate protozoan infections, bacterial gill disease, and myxobacterial infections, the treatment bath containing bronopol **[deletion(s)]**, at a concentration in the range 1 to 1000mg.l⁻¹ (ppm) as the sole or principal active ingredient against such disease.

[0011] Preferably, the concentration of bronopol in the treatment bath is in the range of 5mg.l⁻¹ to 200mg.l⁻¹, and ideally 10mg.l⁻¹ to 100mg.l⁻¹. Bronopol is a solid crystalline substance, and may conveniently be prepared as a solution

in a polar solvent, such as water or Dowanol (dipropylene glycol monoethylether).

[0012] Trial 1 (below) was conducted in mildly alkaline conditions (approximately pH 7.4 to 7.5) and it is possible that these conditions are favourable for the anti-fungal activity demonstrated by bronopol.

[0013] During the trials, bronopol was also tested against various other fish diseases, and was found to be effective against *Ichthyobodo necatrix*, which is an ectoparasite flagellate protozoan, *Flavobacterium branchiophilum* (the causative organism of bacterial gill disease), and *Cytophaga psychrophila*, which is a myxobacterium and the causative organism of Rainbow Trout Syndrome, Coldwater disease, Saddleback and Peduncle disease in salmonid fish. The activity against *Cytophaga* indicates possible efficacy against myxobacteria in general, and the activity against *Ichthyobodo* suggests efficacy against flagellates in general, as well as ciliate protozoans such as *Ichthyophthirius multifiliis*.

[0014] The main practical use of the activity against *Cytophaga* is likely to be in the disinfection of tanks and equipment and the invention therefore encompasses a method of disinfecting fish tanks and/or instruments for use in the husbandry of fish, the method comprising the step of exposing the tank/equipment to a solution containing bronopol [deletion(s)], as the sole a principal active disinfecting ingredient. Preferably, the bronopol is present in a concentration of between approximately 1 and 2000 mg.l⁻¹, more preferably 5 to 1000 mg.l⁻¹. The exposure time is preferably between 2 and 40 mins .

[0015] The invention is hereinafter described in more detail by way of example only, with reference to the following experimental trials:

TRIAL 1

In vitro efficacy of bronopol against *Saprolegnia parasitica*

[0016] This trial was carried out using the procedures described in Journal of Fish Diseases (1982) 5, 113-123, cited above.

Preparation of Inocula

[0017] A culture of *Saprolegnia parasitica* was maintained at 16°C on river water, glucose, yeast extract agar (RGY), consisting of yeast extract (1 g) , D(+)glucose (5 g), and agar (12 g) in 11 river water. Plates of RGY were seeded with the test organism and incubated at 25°C until growth just covered the full diameter of the dish (approximately 72 hours). Discs were cut from the outer 10 mm of the culture, using a 4-mm diameter punch (adapted from a gel chromatography well punch by welding-on a handle) and then used as standard inocula for testing.

Test 1A

Method

[0018] Bronopol was tested for activity against the *Saprolegnia parasitica* cultures at different concentrations ranging from 50mg.l⁻¹ to 100mg.l⁻¹, using Protocol II of the aforementioned J. Fish Diseases article, as follows. Polycarbonate filter membranes of 0.2-µm porosity and 25-mm diameter (Nuclepore; Sterlin) were sterilized by autoclaving and then placed on the surface of RGY plates (7 per 90-mm petri-dish). A standard 4-mm disc inoculum was then placed, inverted, at the centre of the filter. The dishes containing the filters were then incubated until the resulting mycelial mat had almost reached the edge of the filters. The original inocula were then clipped off (as far as practical) using hot forceps tips to avoid disturbing the loosely adherent mycelial mat. The mycelial mats together with their supporting filters were lifted off the agar surface on the filters, transferred to empty, sterile, petri-dishes and completely submerged in the bronopol solution at selected concentrations. At the end of the 1 hour exposure period the bronopol solution was removed by aspiration and replaced by two washes of sterile river water (for 5 and 30 min, respectively) before the mycelial mat and filter were transferred, filter uppermost, to the surface of a fresh RGY plate. After incubation at 16°C for 24 h, any new growth beyond the edge of the filter was measured at four points around the filter, at 90° intervals.

[0019] The experiment was performed using six separate culture samples at each concentration of bronopol, and was repeated using malachite green in place of bronopol. A negative control test using no active agent was also performed. The results are set out in the table below:

Table 1A

5 Radial growth in mm at 24h after exposure to bath of test concentration

BRONOPOL								MALACHITE GREEN					
Conc mg.l ⁻¹	Time (min)	1	2	3	4	5	6	1	2	3	4	5	6
1000	10	0	0	0	0	0	0	0	0	0	0	0	0
500	10	0	0	0	0	0	0	0	0	0	0	0	0
100	10	3	3	2	2	1	2	0	0	0	0	0	0
50	10	3	3	3	3	2	2	0	0	0	0	0	0
50	5	5	2	2	3	3	4	0	0	0	0	0	0
10	10	6	7	7	6	7	6	0	0	0	0	0	0
10	5	7	7	7	6	8	8	0	0	0	0	0	0
Control	10	7	7	7	6	7	7						

Discussion

[0020] In this test naked fungal hyphae are exposed to bronopol, which is shown to have an inhibitory effect as low as 50 ppm for 5 minutes against subsequent vegetative growth. Ten minutes' exposure at 500 ppm prevents all subsequent vegetative growth with this test method.

Test 1B

Method

[0021] In this test, bronopol was tested for efficacy against *Saprolegnia parasitica* using the more demanding Protocol III of the aforementioned J. Fish Diseases article, the method being as follows:

[0022] Four disc inocula were placed in each compartment of a sterile replidish (25 compartments in five rows; Sterilin). Five different test concentrations applied for five different exposure times could thus be carried out in quadruplicate in one dish. The test concentrations were added aseptically in volumes of 2.5 ml to each compartment for the standard exposure times, which were 5, 10, 20, 40 and 80 mins. At the end of the individual exposure times, the test solutions were removed from the compartments by aspiration. Each set of discs was then washed *in situ* in two changes of sterile river water (for 5 and 30 mins, respectively) and then incubated in a further 2.5 ml of sterile river water, *in situ*, for 72 h at 16°C. Subsequently, the discs were examined under a stereomicroscope, using transmitted dark-ground illumination, and scored for presence or absence of new growth on the agar disc surface. In all cases of doubt, particularly at the borderline between growth and no growth, discs were then transferred to RGY for a further 72 h at 16°C to determine accurately the viability of the mycelium within the disc. The test was repeated, substituting malachite green oxalate for bronopol.

[0023] The results were expressed both in terms of effect on zoosporulation and in terms of effect on vegetative growth, and are set out in the tables below:

Table 1B(i)

Bronopol - Effect on zoosporulation (percentage inhibition)

100%	100%	0	0	0	80	T
100%	100%	0	0	0	40	i
100%	100%	0	0	0	20	m
100%	100%	0	0	0	10	e
100%	100%	0	0	0	5	(min)
1000	100	10	1	0		

Concentration (mg.l⁻¹)

Table 1B(ii)

Malachite green oxalate - Effect on zoosporulation (percentage inhibition)

100%	100%	100%	100%	0	80	T
100%	100%	100%	100%	0	40	i
100%	100%	100%	100%	0	20	m
100%	100%	100%	40-50%	0	10	e
100%	100%	100%	40-50%	0	5	(min)
1000	100	10	1	0		

Concentration (mg.l⁻¹)

Table 1B(iii)

Bronopol - Effect on vegetative growth (percentage inhibition)

100%	10-20%	0	0	0	80	T
100%	0	0	0	0	40	i
100%	0	0	0	0	20	m
10-20%	0	0	0	0	10	e
10-20%	0	0	0	0	5	(min)
1000	100	10	1	0		

Concentration (mg.l⁻¹)

Table 1B(iv)

Malachite green oxalate - Effect on vegetative growth (percentage inhibition)

100%	100%	100%	0	0	80	T
100%	100%	100%	0	0	40	i
100%	100%	100%	0	0	20	m
100%	100%	100%	0	0	10	e
100%	100%	100%	0	0	5	(min)
1000	100	10	1	0		

Concentration (mg.l⁻¹)

Discussion

[0024] Bronopol has a marked effect on immediate zoosporulation with exposures as little as 5 minutes at concentrations of 100 ppm or (possibly) less. This test uses an agar plug method, in which the agar containing the mycelium obviously offers protection to the hyphae, but bronopol still penetrates and a 20 minute 1,000 ppm exposure prevents all subsequent growth, and this concentration also has a significant effect in as short as 5 minutes. Furthermore, the lower 100 ppm exposure for 80 minutes also appears to have some inhibitory effect.

TRIAL 2

In vivo efficacy of bronopol against Saprolegnia parasitica infection in Salmon

Method

[0025] 105 Atlantic salmon (*Salmon salar*) of mixed sex aged approximately 8 months and weighing approximately 30g on average were divided into three treatment groups, each of 35 fish. Each group was kept in fresh water at 12.5°C in a 210 litre fibreglass tank. The fish were fed daily with 2% body weight of "Biomar" production feed. After two weeks' acclimatisation, all three groups were artificially infected with *Saprolegnia parasitica*, the date of infection being designated 'day 0' of the trial. The groups were then treated, on days 1, 3 and 5, as follows:

Groups	Treatment	Treatment concentration	Treatment Duration
1	None	-	-
2	Malachite green	2mg.l ⁻¹	60 min bath
3	Bronopol	30mg.l ⁻¹	15 min bath

[0026] On day 8 of the trial, all fish were culled, and the degree of infection of each fish assessed according to the following scoring system:

Degree of fungal infection	Score
None	1
Mild (fungus visible on <25% of fish's surface)	2
Moderate (fungus visible on 50% of fish's surface)	3
Severe (Lesion(s) on fish)	4
Mortality*	5

(*Dead fish were removed as mortalities occurred, to avoid contamination of water.)

Results

[0027] The number of fish in each category of the above scoring system, and the total score for each group, were as follows:

SCORE	CONTROL	Malachite Green	Bronopol
None 1	18	25	23
Mild 2	3	1	6
Moderate 3	3	1	2
Severe 4	3	0	0
Mortality 5	8	8	4
TOTAL SCORE	85	70	61

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[0028] The cumulative numbers of mortalities for each day of the trial are illustrated graphically in Fig. 1.

Conclusion

5 **[0029]** Bronopol proved effective against *Saprolegnia* infection, and also reduced mortalities by 50% compared to both the non-treatment and the malachite green treatment groups.

TRIAL 3

10 In vivo efficacy of bronopol against *Ichthyobodo necatrix* infection in rainbow trout

Method

15 **[0030]** Rainbow trout (*Oncorhynchus mykiss*) having a mean weight of 15g were used in this trial, and prior to treatment were confined in a single pond with 6 individuals heavily infected with *Ichthyobodo*. Infection of the previously healthy individuals was confirmed after 5 days by microscopy of mounted gill specimens. Bronopol treatment was administered to a group of 20 fish using the same method as in Trial 2. The Trial was repeated using a group of 19 infected fish treated with formalin (a standard treatment for *Ichthyobodo*), with a further group of 20 infected fish receiving no treatment and acting as a control. At 7 days after treatment the mean number of parasites per gill filament was assessed in each group (by counting the number of parasites on 10 filaments of each of 5 fish), and at 14 days a further count was conducted, with individual fish being either categorised as having no infection, or being placed in one of four infected categories, according to the degree of infection.

Results

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[0031]

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Days Post Treatment	Infection Level	Bronopol	GROUP No treatment Control	Formalin
7	mean numbers of parasites per gill filament	<0.5	2.0	0
14	percentage in each category:			
	-	50	0	100
	+	43	29	0
	++	7	29	0
	+++	0	18	0
	++++	0	23	0
No mortalities occurred.				

40

Conclusion

45 **[0032]** Bronopol was shown to have good activity against *Ichthyobodo* infection in rainbow trout. Although less effective than formalin, its use carries less risk. It may be inferred that bronopol will have similar efficacy against *Ichthyobodo* infection in other salmonids, and in fish and aquatic organisms in general, and will also be useful in the prophylaxis of the disease.

TRIAL 4

50

In vitro efficacy of bronopol against *Cytophaga Psychrophila*

Method

55 **[0033]** First, the Minimal Bactericidal Concentration (MBC) for bronopol against *Cytophaga Psychrophila* was established by growing the organism in broth, and then adding different dilutions of bronopol to establish what concentration would kill the *Cytophaga*.

[0034] Then based on the MBC results, the contact times required to kill the *Cytophaga* were investigated. A known-

number of viable bacteria were added to distilled water and exposed to various concentrations of bronopol for 2-40 minutes. Then the bacteria were extracted by filtration, and the extracted bacteria tested for viability by investigating their growth in culture medium by measuring optical density at 520nm.

5 Results

[0035] The results are set out graphically in Fig. 2. The minimum test concentration which was shown to be effective was 5 mg.l⁻¹ (ppm), but it is likely that the actual minimum effective concentration lies between 1 mg.l⁻¹ and 5 mg.l⁻¹. It was also found that an exposure time of up to 40 mins was required to prevent bacterial growth after re-inoculation onto fresh media when concentrations of between 5 mg.l⁻¹ and 400 mg.l⁻¹ were used, with the necessary exposure time being reduced to around 4 minutes at a concentration of 1000 mg.l⁻¹ and around 2 minutes at 2000 mg.l⁻¹.

Conclusion

15 [0036] Bronopol was shown to be active against Cytophaga psychrophila at concentrations as low as 5 mg.l⁻¹. Cytophaga is an example of the group of bacteria known as myxobacteria, which are characterised by a protective mucopolysaccharide layer, and which are generally resistant to disinfectants (for example, the dose of iodophor disinfectant required to kill Cytophaga is approximately 2000 mg.l⁻¹). Based on the known uses of bronopol, this activity is therefore surprising, and may indicate activity against myxobacteria in general. Bronopol is likely to prove a suitable disinfectant treatment for fish tanks and equipment, and may also be of use in the treatment and/or prophylaxis for the various diseases caused by this organism in trout and other salmonids, and also (when these occur) in other fish and aquatic organisms.

25 TRIAL 5

In vivo efficacy of bronopol against Flavobacterium branchophilum infection in rainbow trout

[0037] Seven tanks of 150 rainbow trout each, at a stocking density of 100 g.l⁻¹, were included in this experiment. The fish averaged 15g in size. Water temperature was 11°C and turned over at a rate of once per hour.

30 [0038] One tank of fish was maintained as a negative control and was neither challenged or treated. An additional tank was challenged, but not treated, to act as a positive control. Two tanks were treated with chloramine-T (a conventional treatment) at a concentration of 10mg.l⁻¹. Three tanks were treated with bronopol, one each at the following concentrations: 5, 25 and 50 mg.l⁻¹.

35 [0039] Fish were challenged on day 0, and tanks were treated with either chloramine-T or bronopol on days 2 and 4 of the experiment. All six tanks which were experimentally infected with F. branchiophilum were treated with the respective therapeutants simultaneously for one hour in a static bath with aeration.

[0040] The efficacy of each treatment was evaluated by clinical signs, gill bacterial antigen levels measured by enzyme immunoassay (EIA), mortality rates and cumulative mortality and finally by histological examination.

40 [0041] Gill samples for EIA were collected from five fish in each tank before experimental infection, and before treatment. Further gill samples were collected 6 and 36 hours after the initial treatment, after which the groups were retreated with identical concentrations of the same therapeutant, in an identical manner. Gill samples from five fish from each tank were again collected 6 and 36 hours after the second treatment. Mortality in each tank was recorded each day. The results are presented graphically in Figs. 3 to 5.

45 [0042] Based on the mortality and EIA data, the lowest concentration of bronopol (5 mg.l⁻¹, Fig. 5) was found to be ineffective. Mortality was inversely related to the dose of bronopol (Fig. 3).

[0043] It is evident that mortality corresponds closely with the bacterial antigen levels detected on the gills, and that the EIA is a useful tool for monitoring the efficacy of a therapeutant's ability to eliminate bacteria from the gill surface. As with mortality, the bacterial gill antigen concentration at most sample times, is inversely proportional to the dose of therapeutant (Fig. 5). The two highest concentrations of bronopol effectively eliminated the bacteria from the gill surface after the second treatment (Fig. 5). It is clear that bronopol is effective against Flavobacterium branchophilum, and that in the trial bronopol at 25 mg.l⁻¹ and 50 mg.l⁻¹ reduced mortalities among the infected fish, both compared to the untreated fish, and to the fish treated with chloramine-T.

TRIAL 6

In vivo efficacy of bronopol against Saprolegnia parasitica in brown trout

5 Method

[0044] This trial set out to assess the efficacy of bronopol in the treatment of brown trout naturally infected with Saprolegnia parasitica. 40 fish were used in the trial, and these were divided equally into two groups held in separate tanks. A moribund fish heavily infected with Saprolegnia parasitica was placed in each tank, with the intention of infecting the other fish naturally. On days 1, 3 and 5 of the trial the fish in one group were treated to a 50mg.l⁻¹ bath of bronopol for 15 min, while the fish in the second group were left untreated and acted as a control. The degree of infection of the fish in both groups was assessed on day 7 of the trial.

15 Results

[0045] In both groups the moribund fish died on day 1 of the trial, but in each case was left in the tank to ensure a good challenge to the remaining fish. On day 7 of the trial all of the fish in the control group were noted to be severely affected by Saprolegnia, and were killed on humanitarian grounds. In contrast, the fish in the bronopol treatment group were all healthy and had no visual signs of Saprolegnia infection.

20 Conclusion

[0046] Repeated treatment with bronopol at a concentration of 50mg.l⁻¹ appears to be highly effective as a treatment for Saprolegnia infection in brown trout, and/or as a prophylactic to prevent infection.

25

Claims

- 30 1. The use of bronopol (2-bromo-2-nitropropane-1,3-diol) [**deletion(s)**], in the manufacture of a medicament for the treatment or prophylaxis of any of the following diseases in fish: fungal infections, flagellate protozoan infections, ciliate protozoan infections, bacterial gill disease, and myxobacterial infections, wherein said medicament contains bronopol, as its sole or principal active ingredient against the aforementioned diseases.
- 35 2. The use as claimed in claim 1, for the treatment or prophylaxis of diseases caused by any of the following causative organisms: *Saprolegnia parasitica*, *Ichthyobodo necatrix*, *Ichthyophthirius multifiliis*, *Flavobacterium branchiophilum* and *Cytophaga psychrophila*.
- 40 3. The use according to claim 1 or claim 2 for the treatment or prophylaxis of diseases of salmonid fish.
- 45 4. The use according to claim 3, for the treatment or prophylaxis of diseases of trout or salmon.
- 50 5. A treatment bath for the treatment or prophylaxis of any of the following diseases in fish: fungal infections, flagellate protozoan infections, ciliate protozoan infections, bacterial gill disease, and myxobacterial infections, the treatment bath containing bronopol, at a concentration in the range 1 to 1000mg.l⁻¹ (ppm) as the sole or principal active ingredient against such disease.
- 55 6. A treatment bath according to claim 5, wherein the concentration of bronopol is in the range 5 to 200 mg.l⁻¹ (ppm).
7. A treatment bath according to claim 6, wherein the concentration of bronopol is in the range 10 to 100 mg.l⁻¹ (ppm).
8. A method of disinfecting fish tanks and/or equipment for use in the husbandry of fish, the method comprising the step of exposing the tank or equipment to a solution containing bronopol (2-bromo-2-nitropropane-1,3-diol), as the sole or principal active disinfecting ingredient.
9. The method of claim 8, wherein the concentration of bronopol [**deletion(s)**], is in the range 1 to 2000 mg.l⁻¹ (ppm).
10. The method of claim 9, wherein the concentration of bronopol [**deletion(s)**], is in the range 5 to 1000 mg.l⁻¹ (ppm).

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11. The method of any of claims 8 to 10, wherein the tank or equipment is exposed to the solution for a period of time in the range 2 to 40 minutes.

5 Patentansprüche

1. Verwendung von Bronopol (2-Brom-2-nitropropan-1,3-diol) bei der Herstellung eines Arzneimittels zur Behandlung oder Prophylaxe einer beliebigen der nachfolgenden Krankheiten beim Fisch: Pilzinfektionen, Geißeltierchen-Infektionen, Flimmertierchen-Infektionen, bakterielle Kiemenschwellung und mykobakterielle Infektionen, wobei das Arzneimittel Bronopol als seinen alleinigen oder hauptsächlichen Wirkstoff gegen die vorstehend erwähnten Krankheiten enthält.
- 10
2. Verwendung nach Anspruch 1 zur Behandlung von oder Prophylaxe von Krankheiten, hervorgerufen durch die nachfolgenden verursachenden Organismen: *Saprolegnia parasitica*, *Ichthyobodo necatrix*, *Ichthyophthirius multifiliis*, *Flavobacterium branchiophilum* und *Cytophaga psychrophila*.
- 15
3. Verwendung nach Anspruch 1 oder Anspruch 2 zur Behandlung oder Prophylaxe von Krankheiten bei forellentartigem Fisch.
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4. Verwendung nach Anspruch 3 zur Behandlung oder Prophylaxe von Krankheiten bei Forelle oder Lachs.
5. Behandlungsbad zur Behandlung oder Prophylaxe einer beliebigen der nachfolgenden Krankheiten beim Fisch: Pilzinfektionen, Geißeltierchen-Infektionen, Flimmertierchen-Infektionen, bakterielle Kiemenschwellung und mykobakterielle Infektionen, wobei das Behandlungsbad Bronopol in einer Konzentration im Bereich von 1 bis 1000 mg.l⁻¹ (ppm) als seinen alleinigen oder hauptsächlichen Wirkstoff gegen derartige Krankheiten enthält.
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6. Behandlungsbad nach Anspruch 5, wobei die Konzentration von Bronopol im Bereich von 5 bis 200 mg.l⁻¹ (ppm) liegt.
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7. Behandlungsbad nach Anspruch 6, wobei die Konzentration von Bronopol im Bereich von 10 bis 100 mg.l⁻¹ (ppm) liegt.
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8. Verfahren zur Desinfektion von Behältern für Fische und/oder Ausrüstung zur Anwendung bei der Fischhaltung, wobei das Verfahren den Schritt der Exposition des Behälters oder der Ausrüstung einer Lösung, enthaltend Bronopol (2-Brom-2-nitropropan-1,3-diol) als den alleinigen oder hauptsächlichen desinfizierenden Wirkstoff, umfasst.
9. Verfahren nach Anspruch 8, wobei die Konzentration von Bronopol im Bereich von 1 bis 2000 mg.l⁻¹ (ppm) liegt.
10. Verfahren nach Anspruch 9, wobei die Konzentration von Bronopol im Bereich von 5 bis 1000 mg.l⁻¹ (ppm) liegt.
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11. Verfahren nach einem der Ansprüche 8 bis 10, wobei der Behälter oder die Ausrüstung über einen Zeitraum im Bereich von 2 bis 40 Minuten der Lösung ausgesetzt wird.

45 Revendications

1. Utilisation de bronopol (2-bromo-2-nitropropane-1,3-diol) dans la fabrication d'un médicament destiné au traitement ou à la prophylaxie de l'une quelconque des maladies suivantes chez le poisson: infections fongiques, infections par des protozoaires flagellés, infections par des protozoaires ciliés, pourriture bactérienne des branchies et infections par des mycobactéries, ledit médicament contenant du bronopol comme seul ou principal ingrédient actif contre les maladies susmentionnées. 1, pour
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2. Utilisation telle que revendiquée dans la revendication 1, pour le traitement ou la prophylaxie de maladies causées par l'un quelconque des agents pathogènes suivants: *Saprolegnia parasitica*, *Ichthyobodo necatrix*, *Ichthyophthirius multifiliis*, *Flavobacterium branchiophilum* et *Cytophaga psychrophila*.
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3. Utilisation selon la revendication 1 ou la revendication 2 pour le traitement ou la prophylaxie des maladies d'un salmonidé.

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4. Utilisation selon la revendication 3, pour le traitement ou la prophylaxie des maladies de la truite ou du saumon.
5. Bain de traitement pour le traitement ou la prophylaxie de l'une quelconque des maladies suivantes chez le poisson: infections fongiques, infections par des protozoaires flagellés, infections par des protozoaires ciliés, pourriture bactérienne des branchies et infections par des myxobactéries, ledit bain de traitement contenant du bronopol à une concentration comprise dans l'intervalle de 1 à 1000 mg.l⁻¹ (ppm) comme seul ou principal ingrédient actif contre une telle maladie.
6. Bain de traitement selon la revendication 5, dans lequel la concentration de bronopol est comprise dans l'intervalle de 5 à 200 mg.l⁻¹ (ppm).
7. Bain de traitement selon la revendication 6, dans lequel la concentration de bronopol est comprise dans l'intervalle de 10 à 100 mg.l⁻¹ (ppm).
8. Procédé de désinfection des réservoirs à poissons et/ou d'un équipement utilisé dans l'élevage des poissons, le procédé comprenant l'étape consistant à exposer le réservoir ou l'équipement à une solution contenant du bronopol (2-bromo-2-nitropropane-1,3-diol) comme seul ou principal ingrédient actif désinfectant.
9. Procédé selon la revendication 8, dans lequel la concentration de bronopol est comprise dans l'intervalle de 1 à 2000 mg.l⁻¹ (ppm).
10. Procédé selon la revendication 9, dans lequel la concentration de bronopol est comprise dans l'intervalle de 5 à 1000 mg.l⁻¹ (ppm).
11. Procédé selon l'une quelconque des revendications 8 à 10, dans lequel le réservoir ou l'équipement est exposé à la solution pendant une période de temps comprise dans l'intervalle de 2 à 40 minutes.

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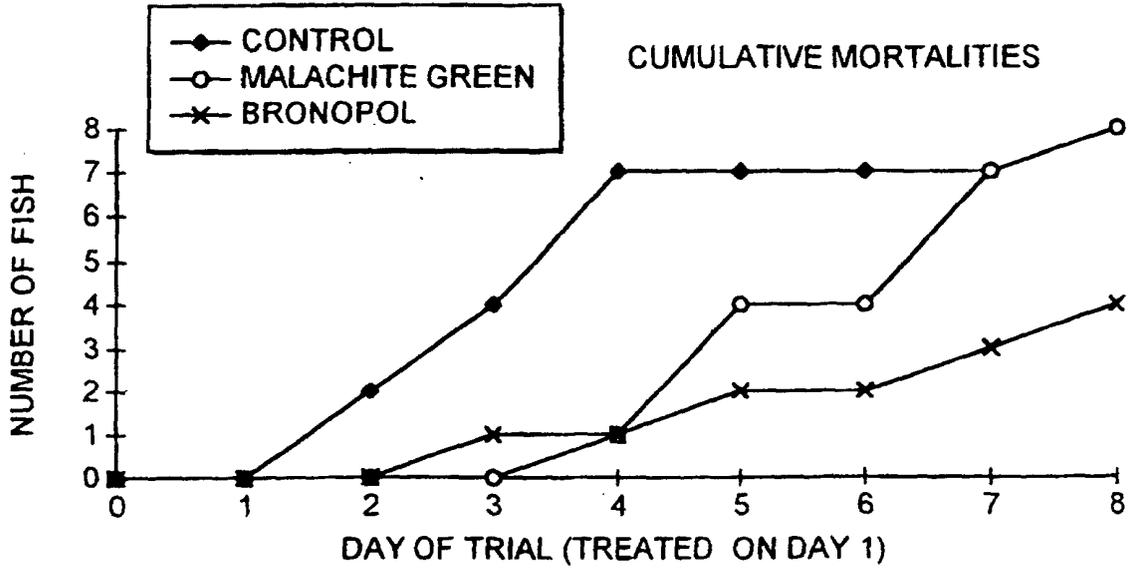


FIG. 1

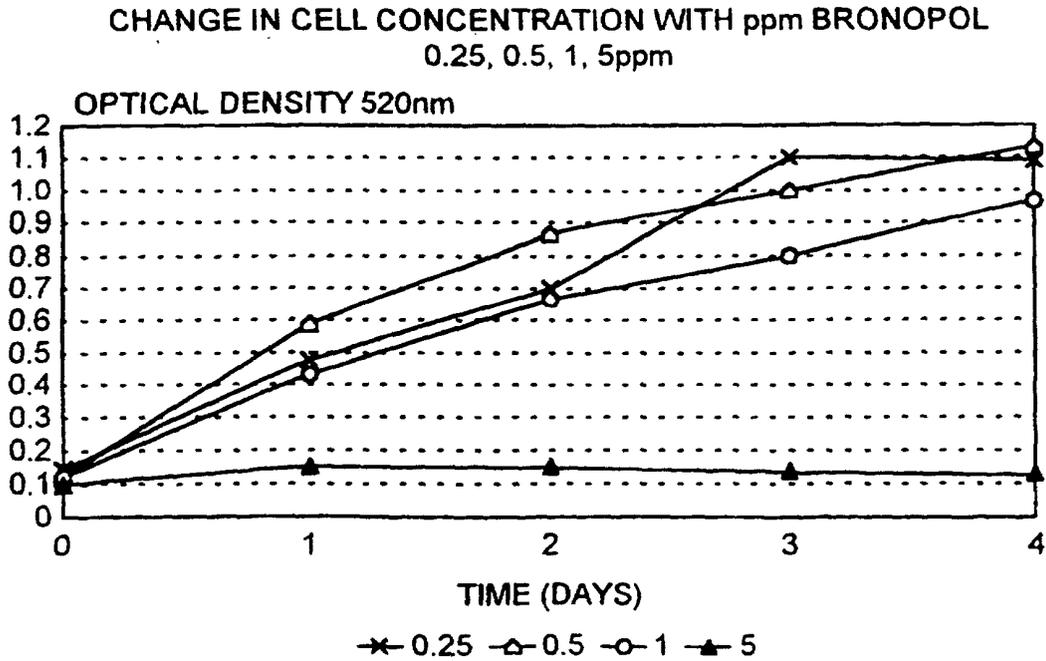


FIG. 2

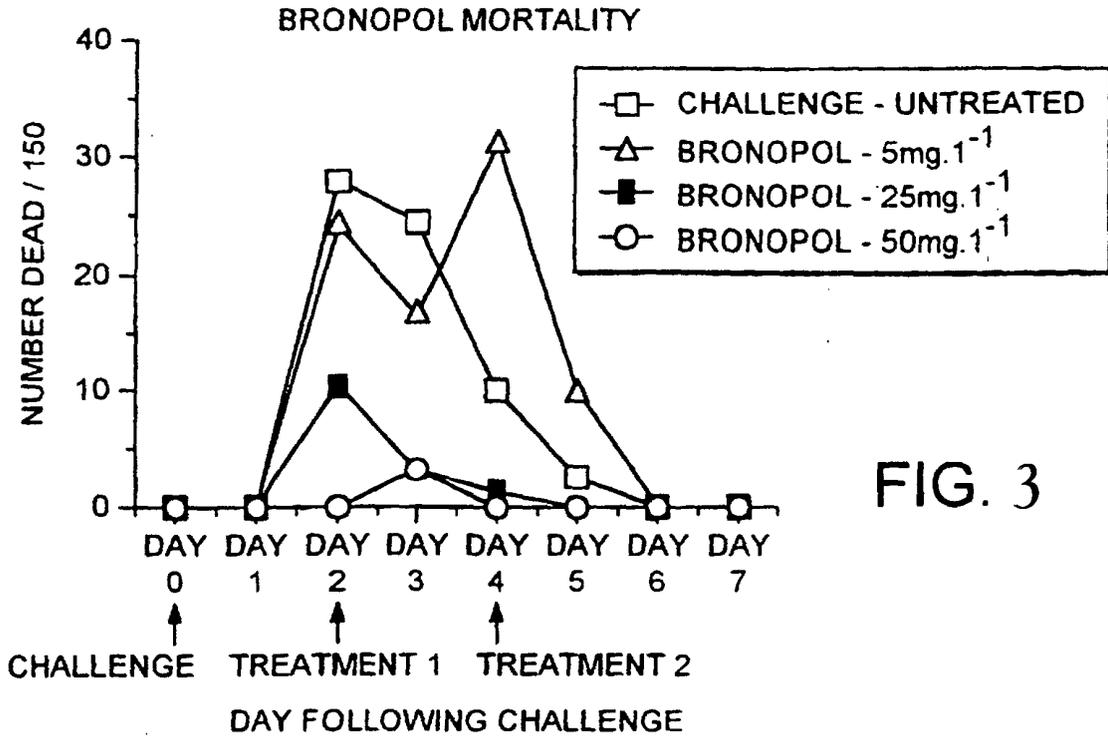


FIG. 3

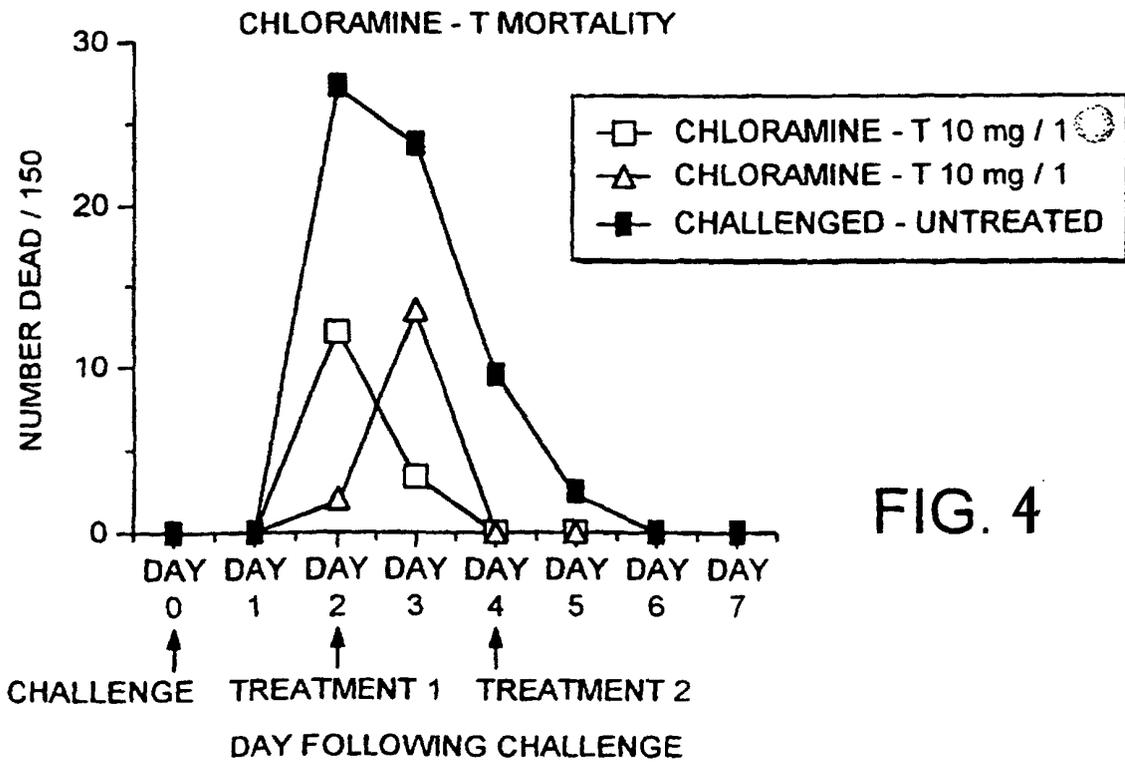


FIG. 4

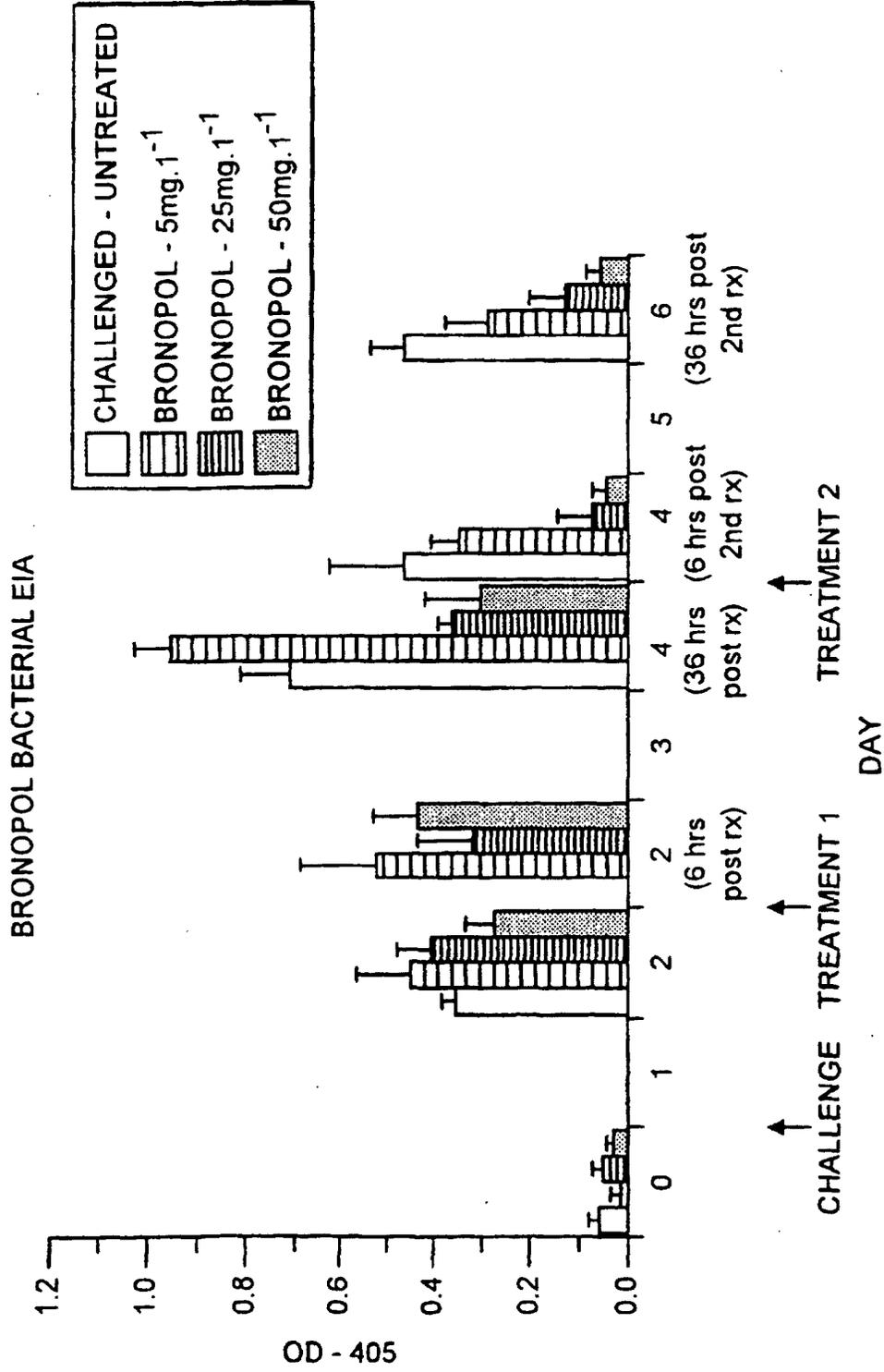


FIG. 5