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(54) METHODE POUR ELIMINER LES POUX DE POISSONS

(54) PROCESS FOR THE CONTROL OF SEA LICE

(57) The newly discovered property of the compound S-6-chloro-2,3- dihydro-2-oxo- 1,3-oxazol[4,5-b]pyridin-3-ylmethyl-O,O-dimethylphosphorothioate for controlling the salmon louse is disclosed, as are also methods of control and compositions for controlling sea lice.

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Process for the control of sea lice

Abstract of the Disclosure

The newly discovered property of the compound S-6-chloro-2,3- dihydro-2-oxo-1,3-oxazol[4,5-b]pyridin-3-yl-methyl-O,O-dimethylphosphorothioate for controlling the salmon louse is disclosed, as are also methods of control and compositions for controlling sea lice.

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Method of controlling sea lice

The present invention relates to the compound S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazol-[4,5-b]pyridin-3-ylmethyl-O,O-dimethylphosphorothioate known under the common name Azamethiphos for controlling sea lice in fish belonging to the group of the salmonids. The invention further relates to a method of controlling sea lice in salmonids, to the use of the above compound for controlling sea lice in the commercial farming of salmon and trout, and to the use thereof for the preparation of compositions for controlling sea lice.

For the sake of simplicity, the common name Azamethiphos will be used throughout this specification instead of the chemical name cited above.

For some years, salmon and trout have been among the most popular edible fish with consumers on account of their fine taste and low fishbone content. Demand has increased to such a degree that traditional fishing in open waters has for long been unable to satisfy it. In recent years, therefore, fish farms have been established, especially in the Northern European countries, with the object of artificially breeding these fish.

The term "salmon" within the scope of this invention will be understood as comprising all representatives of the family Salmonidae, especially of the subfamily Salmonini and, preferably, the following species: Salmon salar (Atlantic salmon); Salmon trutta (brown or sea trout); Salmon gairdneri (rainbow trout); and the Pacific salmon (Oncorhynchus): O. gorbuscha; O. keta; O. nekra; O. kisutch; O. tshawytscha and O. mason. Also comprised are artificially propagated species such as Salvelinus species and Salmo clarkii.

Preferred objects of the present invention are the Atlantic and Pacific salmon and the sea trout.

In present-day salmon and trout farming, juvenile fish are transferred in the smolt stage from fresh-water tanks to sea water cages. These latter are cubic, rectangular or also round cages having a metal frame which is covered with a fairly fine-meshed net. These cages are lowered into the sea until they are 9/10 submerged and then so anchored that they are

accessible from the top.

In another variant, the fish are kept in sea water tanks of different shape. The cages are moored in sea inlets such that a constant flow of water passes through them in order to ensure a sufficient supply of oxygen. A constant flow of salt water in the sea water tanks is also maintained along with a supply of oxygen. In this artifical environment the fish are fed and, if necessary, provided with medication until they mature sufficiently for marketing as edible fish or are selected for further breeding.

Extremely intensive cage stocking is maintained in these fish farms. The fish density reaches orders of magnitude of 10 to 25 kg of fish/m³. In this pure monoculture, the exceedingly high fish densities coupled with the other stress factors cause the caged fish to become in general markedly more susceptible to disease, epidemics and parasites than their free-living cospecifics. In order to maintain healthy populations, the caged fish must be treated regularly with bactericides and permanently monitored.

Besides infectious diseases, the prime threat in commercial salmon farming is, however, attack by parasites. In particular, two representatives of the class of Crustaceae (crustaceans) cause substantial losses in yield. These parasites are popularly known as sea lice. The one species is Lepeophtheirus, L. salmonis; the other is Caligus, C. elongatus. They are easily recognised. The former has a brown, horseshoe-shaped dorsal shell; the latter is also brown, but much smaller. These sea lice injure the fish by feeding on the scales, epithelium and the mucosa. When infestation is severe, these parasites also damage underlying dermis. If, moreover, infected salmon are kept in cooler waters, then they are normally no longer able to protect themselves from these pests. As a consequence, secondary infections and water-logging will occur, even if the sea lice are removed. In extreme cases, severe wounding resulting from infestation by these parasites leads to further tissue damage caused by ultraviolet radiation (McArdle & Bullock, 1987) or to the death of the fish from osmotic shock or the secondary infections (Saward et al., 1982; Tully, 1988a).

Sea lice are meanwhile widely prevalent and encountered in all fish farms. Severe infestation kills the fish. Mortality rates of over 50%, based on sea lice infestation, have been reported from Norwegian fish farms (Needham, 1978, cited in Steward et al., 1982). The extent of the damage depends on the time of year and on environmental factors, for example the salinity of the water (Rae, 1979) and average water temperature (Tully,

1988b). In a first phase, sea lice infestation is seen in the appearance of the parasites attached to the fish and later - even more clearly - from the damage caused to skin and tissue. The most severe damage is observed in smolts which are just in the phase in which they change from fresh water to sea water. The situation is made even worse by the specific conditions in the fish farms, where salmon of different age groups but of the same weight class are kept together; where fouled nets or cages are used; where high salt concentrations are to be found; where flow through the nets and cages is minimal and the fish are kept in a very narrow space.

Fish farmers who are confronted with this parasite problem have to suffer substantial financial losses and to carry additional expense. On the one hand, their fish are debilitated and damaged by the lice, resulting in lower rates of growth increase, and on the other, secondary infections have to be controlled with expensive medicaments and labour-intensive measures. The fish can often no longer be sold, as the consumer will reject the damaged goods. This parasitic infestation can pose a threat to the salmon farmer's livelihood.

The worst damage is caused by Lepeophtheirus, as even a few parasites cause widespread tissue damage. The life cycle of Lepeophtheirus consists substantially of two free-swimming larval stages (naupilus and copepodid stages), four chalimus stages, a pre-adult stage and the actual adult stage (Institute of Aquaculture, 1988). The chalimus, pre-adult and adult stages are host-dependent.

The most dangerous stages, because they cause the greatest damage, are all those parasiticising on the fish, especially the actual adult stages.

Pest control agents which can be used to combat sea lice are commercially available, for example Trichlorfon (dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate), which requires concentrations of 300 ppm in sea water, and Dichlorphos (2,2-dichloroethenyl dimethyl phosphate), which is effective from a concentration of 1 ppm. The shortcoming of these compounds is, however, the high concentrations in which they have to be used and the ecological problems associated therewith, which are of even greater consequence on account of the high half-life times.

Surprisingly, in Azamethiphos, S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazol[4,5-b]pyridin-3-ylmethyl-O,O-dimethylphosphorothioate, a representative of an entirely different class of

compounds, a substance has been found which, while having very low toxicity to fish, is even more effective and, in particular, whose photolytic and hydrolytic degradability is more rapid by about a factor of 3 as compared with the known sea lice control agents and, furthermore, which can be successfully used against all pre-adult and adult stages of sea lice on fish.

Thus, for example, in direct comparison with the known control agents mentioned above, Azamethiphos is still fully effective (100% control) in sea water having a salinity of 23-33% at the low concentration of 0.1 ppm, i.e. with 10 times less than Dichlorovos and 3000 times less than Trichlorfon. Furthermore, the <u>in vitro</u> test in the temperature range from 4 to 15°C shows that Azamethiphos is 100% effective at a concentration of 0.1 ppm even within 1 hour, whereas it is necessary to use the 20-fold amount of Dichlorvos and the more than 1000-fold amount of Trichlorfon. Moreover, Azamethiphos is markedly more effective against the very resistant chalimus stages, so that the number of applications can be reduced.

A further advantageous property of Azamethiphos is that, at the proposed concentrations, other marine animals such as lobsters, oysters, crustaceans (except the sea lice), fish and marine plants do not suffer injury. The degradation products of Azamethiphos are in any case non-injurious to marine fauna and flora.

Azamethiphos and its preparation and activity against representatives of insects and arthropods is disclosed in German Offenlegungsschrift 2 131 734. There are, however, no hints in scientific or patent literature that Azamethiphos might also be outstandingly effective against representatives of the class of the Crustaceae.

Compared with other organophosphorus insecticides, Azamethiphos is very readily soluble in water and can therefore be used undiluted. More easy to handle, however, are compositions containing the compound in dilute form. Suitable diluents for fish and other marine animals and plants are non-toxic substances which may be liquid or solid and, directly prior to the use of this invention, also water.

For ease of handling, the size of the commercial packs is such that they can be added undiluted to specific volumes of water. The size of the packs is governed by the average dimensions of the cage, so that packs can, for example, be provided for addition to 10 m^3 , 50 m^3 , 100 m^3 , 500 m^3 or 1000 m^3 of water. Thus, for example, for addition to a cage of

 520 m^3 , it would be possible to combine suitable unit dose packs, in this case (1 x 500) and (2 x 10).

For use in actual practice, foils are also suitable which contain the pest control agent in a readily water-soluble matrix.

The unit dose packs contain Azamethiphos, undiluted or in conjunction with non-toxic diluents, in a formulation ready for addition to specific volumes of water. The packs contain Azamethiphos in a concentration of 0.005 to 2 ppm, more conveniently from 0.01 to 1 ppm and, most preferably, from 0.05 to 0.5 ppm, when they are added to a specific volume of water.

Useful concentrations are in the range from 0.005 to 2 g AS/m³, preferably from 0.01 to 1 g AS/m³, more particularly from 0.05 to 0.5 g AS/m³ (AS = active substance).

The dilute formulations of this invention are prepared by mixing the active substance with liquid and/or solid formulation assistants by stepwise mixing and/or grinding such that the ready for use formulation so obtained exerts an optimum antiparasitic activity.

The formulation steps can be extended to include kneading, granulating (to granular formulations) and, if appropriate, compressing (to pellets, tablets).

Suitable formulation assistants are, typically, solid carriers, solvents and, where appropriate, surfactants, which are non-toxic to marine flora and fauna.

The following formulation assistants are used for preparing the formulations: solid carriers such as kaolin, talcum, bentonite, sodium chloride, calcium phosphate, carbohydrates, cellulose powder, cotton seed meal, polyethylene glycol ether, if necessary binders such as gelatin, soluble cellulose derivatives, if desired with the addition of surface-active compounds such as ionic or nonionic dispersants; also natural mineral fillers such as calcite, montmorillonite or attapulgite. To improve the physical properties it is also possible to add highly dispersed silicic acid or highly dispersed absorbent polymers. Suitable granulated adsorptive carriers are porous types, for example pumice, broken brick, sepiolite or bentonite; and suitable nonsorbent carriers are materials such as calcite or sand. In addition, a great number of pregranulated materials of inorganic or organic nature can be used, e.g. especially dolomite or pulverised plant residues.

Suitable solvents are: aromatic hydrocarbons, preferably the fractions containing 8 to 12 carbon atoms, e.g. xylene mixtures or substituted naphthalenes, phthalates such as dibutyl phthalate or dioctyl phthalate, aliphatic hydrocarbons such as cyclohexane or paraffins, alcohols and glycols and their ethers and esters, such as ethanol, ethylene glycol, ethylene glycol monomethyl or monoethyl ether, ketones such as cyclohexanone, strongly polar solvents such as N-methyl-2-pyrrolidone, dimethyl sulfoxide or dimethyl formamide, as well as vegetable oils or epoxidised vegetable oils such as epoxidised coconut oil or soybean oil; and water.

Depending on the type of formulation, suitable surface-active compounds are non-ionic, cationic and/or anionic surfactants having good emulsifying, dispersing and wetting properties. The term "surfactants" will also be understood as comprising mixtures of surfactants.

Suitable anionic surfactants can be both water-soluble soaps and water-soluble synthetic surface-active compounds.

Suitable soaps are the alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammonium salts of higher fatty acids (C_{10} - C_{22}), for example the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures which can be obtained, for example, from coconut oil or tallow oil.

Frequently so-called synthetic surfactants are used, especially fatty sulfonates, fatty sulfates, sulfonated benzimidazole derivatives or alkylarylsulfonates.

The fatty sulfonates or sulfates are usually in the form of alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammonium salts and generally contain a C₈-C₂₂-alkyl radical which also includes the alkyl moiety of acyl radicals, e.g. the sodium or calcium salt of lignosulfonic acid, of dodecylsulfate, or of a mixture of fatty alcohol sulfates obtained from natural fatty acids. These compounds also comprise the salts of sulfated and sulfonated fatty alcohol/ethylene oxide adducts. The sulfonated benzimidazole derivatives preferably contain 2 sulfonic acid groups and one fatty acid radical containing about 8 to 22 carbon atoms. Examples of alkylarylsulfonates are the sodium, calcium or triethanolamine salts of dodecylbenzenesulfonic acid, dibutylnaphthalenesulfonic acid, or of a condensate of naphthalenesulfonic acid and formaldehyde. Also suitable are corresponding phosphates, e.g. salts of the phosphated adduct of p-nonyl-

phenol with 4 to 14 moles of ethylene oxide.

Non-ionic surfactants are preferably polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, or saturated or unsaturated fatty acids and alkylphenols, said derivatives containing 3 to 30 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenols.

Further suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, ethylenediaminopolypropylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethylene glycol ether groups and 10 to 100 propylene glycol ether groups. These compounds usually contain 1 to 5 ethylene glycol units per propylene glycol unit.

Representative examples of non-ionic surfactants are nonylphenolpolyethoxyethanols, castor oil thioxilate, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethylene glycol and octylphenoxypolyethoxyethanol. Fatty acid esters of polyoxyethylene sorbitan, e.g. polyoxyethylene sorbitan trioleate, are also suitable non-ionic surfactants.

Cationic surfactants are preferably quaternary ammonium salts which contain, as N-substituent, at least one C_8 - C_{22} alkyl radical and, as further substituents, unsubstituted or halogenated lower alkyl, benzyl or hydroxy-lower alkyl radicals. The salts are preferably in the form of halides, methylsulfates or ethylsulfates, e.g. stearyltrimethylammonium chloride or benzyl bis(2-chloroethyl)ethylammonium bromide.

The surfactants customarily employed in the art of formulation are described e.g. in the following publications:

"Mc Cutcheon's Detergents and Emulsifiers Annual", MC Publishing Corp., Ridgewood, NJ USA, 1981",

Helmut Stache, "Tensid-Taschenbuch" (Handbook of Surfactants), 2nd. ed., C. Hanser Verlag Munich/Vienna 1981.

Suitable binders for water-soluble granules or tablets are chemically modified polymeric natural substances which are soluble in water or alcohol, for example starch, cellulose or

protein derivatives (e.g. methyl cellulose, carboxymethyl cellulose, ethyl hydroxyethyl cellulose, proteins such as zein, gelatin and the like) as well as synthetic polymers such as polyvinyl alcohol, polyvinyl pyrrolidone and the like. Tablets also contain fillers (e.g. starch, microcrystalline cellulose, sugar, lactose and the like), glidants and disintegrators.

The application of the compositions of this invention to the parasites can be made by introducing the compositions in the form of solutions, emulsions, suspensions (drenches), powders or tablets to the cage, where they are rapidly dissolved and distributed by the movement of the fish and the water. Concentrated solutions can also be diluted before addition to cages containing larger volumes of sea water. Concentration problems in the pens do not arise, as the fish thresh about vigorously in expectation of feed each time the cage is opened and so ensure rapid dilution.

The antiparasitic compositions normally contain 0.1 to 99 %, preferably 0.1 to 95 %, of Azamethiphos, and to 99.9 % to 1%, preferably 99.9 to 5% by weight, of a solid or liquid adjuvant, and 0 to 25 %, preferably 0.1 to 25 %, of a surfactant.

Whereas commercial products are preferably formulated as concentrates, the end user will normally employ dilute formulations obtainable by diluting the commercial product with water.

The compositions can also contain further ingredients such as antifoams, preservatives, viscosity regulators, binders, tackifiers and fertilisers or other chemical agents to obtain special effects.

Formulation Examples (throughout, percentages are by weight)

| F1. Emulsifiable concentrates | a) | b) | c) |
|------------------------------------|------|------|------|
| Azamethiphos | 25 % | 40 % | 50 % |
| calcium dodecylbenzenesulfonate | 5 % | 8 % | 6 % |
| castor oil polyethylene glycol | | | |
| ether (36 mol of ethylene oxide) | 5 % | - | _ |
| tributylphenol polyethylene glycol | | | |
| ether (30 mol of ethylene oxide) | - | 12 % | 4 % |
| cyclohexanone | _ | 15 % | 20 % |
| xylene mixture | 65 % | 25 % | 20 % |

Emulsions of any required concentration can be produced from such concentrates by dilution with water.

| F2. Solutions | a) | b) | c) d) | |
|----------------------------------|------|------|-------|------|
| Azamethiphos | 80 % | 10 % | 5 % | 95 % |
| ethylene glycol monomethyl ether | 20 % | •= | _ | _ |
| polyethylene glycol 400 | - | 70 % | _ | _ |
| N-methyl-2-pyrrolidone | - | 20 % | | - |
| epoxidised coconut oil | _ | - | 1 % | 5 % |
| ligroin (boiling range 160-190°) | _ | - | 94 % | _ |

These solutions are suitable for application in the form of microdrops.

| F3. Granulates | a) | b) |
|-------------------------------|------|------|
| Azamethiphos | 5 % | 10 % |
| kaolin | 94 % | - |
| highly dispersed silicic acid | 1 % | *** |
| attapulgite | | 90 % |

The active substance is dissolved in methylene chloride, the solution is sprayed onto the carrier, and the solvent is subsequently removed by evaporation under vacuum. Such granular formular formulations can be mixed with the feed.

| F4. <u>Dusts</u> | a) | b) |
|-------------------------------|------|------|
| Azamethiphos | 2 % | 5 % |
| highly dispersed silicic acid | 1 % | 5 % |
| talcum | 97 % | |
| kaolin | _ | 90 % |

Ready for use dusts are obtained by intimately mixing the carriers with the active substance.

| F5. Water dispersible powder mixture | | | a) | b) c) |
|---------------------------------------|-----|------|------|-------------|
| (I) Azamethiphos | | 25 % | 50 % | 75 % |
| sodium ligninsulfonate | | 5 % | 5 % | _ |
| oleic acid | | 3 % | | 5 % |
| sodium diisobutylnaphthalenesulfonate | | - | 6 % | 10 % |
| octylphenol polyethylene glycol ether | | | | |
| (7-8 mol of ethylene oxide) | | | 2 % | _ |
| highly dispersed silicic acid | | 5 % | 10 % | 10 % |
| kaolin | | 62 % | 27 % | |
| | | | | |
| | a) | b) | c) | d) |
| (Ia) Azamethiphos | 5% | 10% | 20% | 50% |
| sugar | 95% | 90% | 80% | 50% |
| | | | | |
| | | | | d) |
| (Ib) Azamethiphos (tech. ca. 53.8%) | | | | 53.8% |
| condensate of formaldehyde | | | | |
| and the sodium salt of | | | | |
| naphthalenephenolsulfonic | | | | |
| acid | | | | 4% |
| fatty alcohol sulfates and | | | | |
| alkylarylsulfonates | | | | 2% |
| kaolin | | | | 16% |
| sodium aluminium silicate | | | | 24.2% |
| | | | | |
| (Ic) Azamethiphos | | | | 50% |
| sodium lauryl sulfate | | | | 0.5% |
| dispersant H granulate | | | | 2% |
| kaolin | | | | 16% |
| silica | | | | 31.5% |
| | | | | |

The active substance is thoroughly mixed with the adjuvants and the mixture is thoroughly ground in a suitable mill, affording wettable powders which can be diluted with water to give suspensions of any desired concentration.

| F6. Emulsifiable concentrate | a) | b) | c) |
|---------------------------------------|------|------|------|
| Azamethiphos | 10 % | 8 % | 60 % |
| octylphenol polyethylene glycol ether | | | |
| (4-5 mol of ethylene oxide) | 3 % | 3 % | 2 % |
| castor oil polyglycol ether | | | |
| (36 mol of ethylene oxide) | 4 % | 5 % | 4 % |
| cyclohexanone | 30 % | 40 % | 15 % |
| xylene mixture | 50 % | 40 % | 15 % |

Emulsions of any required concentration can be obtained from this concentrate by dilution with water.

| F7. <u>Dusts</u> | a) | b) |
|------------------|------|------|
| Azamethiphos | 5 % | 8 % |
| talcum | 95 % | ₩. |
| kaolin | _ | 92 % |

Ready for use dusts are obtained by mixing the active ingredient with the carrier, and grinding the mixture in a suitable mill.

F8. Granulate

| Azamethiphos | 10 % |
|-------------------------|------|
| sodium ligninsulfonate | 2 % |
| carboxymethyl cellulose | 1 % |
| kaolin | 87 % |

The active substance is mixed and ground with the adjuvants, and the mixture is subsequently moistened with water. The mixture is extruded, granulated and then dried in a stream of air.

F9. Granulate

| Azamethiphos | | 3 % |
|-------------------------|---|------|
| polyethylene glycol 200 | | 3 % |
| kaolin | • | 94 % |

The finely ground active substance is uniformly applied, in a mixer, to the kaolin moistened with polyethylene glycol. Non-dusty coated granulates are obtained in this manner.

F10. Suspension concentrate

| Azamethiphos | 40 % |
|---------------------------------------|-------|
| ethylene glycol | 10 % |
| nonylphenol polyethylene glycol ether | |
| (15 mol of ethylene oxide) | 6 % |
| sodium ligninsulfonate | 10 % |
| 37 % aqueous formaldehyde solution | 0.2 % |
| silicone oil in the form of a 75 % | |
| aqueous emulsion | 0.8 % |
| water | 32 % |

The finely ground active substance is homogeneously mixed with the adjuvants, giving a suspension concentrate from which suspensions of any desired concentration can be obtained by dilution with water.

The use of Azamethiphos for the preparation of a composition for controlling Crustaceae, especially sea lice, which parasiticise on fish, is an object of this invention.

Biological Examples

1. Toxicity to salmon lice (in vitro test)

a) Collecting and cultivating the salmon lice

Adult and pre-adult stages of the salmon louse are gently removed with broad forceps from naturally infected Atlantic salmon which have been kept in fish farms, separated according to stage and sex, and kept in sea water tanks at 10°C and under continuous aeration. The sea water used for cultivating the lice comes from the fish farm from which the infected salmon have been taken. The tests themselves are carried out over 48 hours after collecting the lice.

b) In vitro test for determining the contact action of the control agent

Plastic containers are filled with 50 ml of sea water (10°C). Into each container are put 5 female and 5 male adults as well as 5 pre-adult salmon lice. The sea water is rapidly

decanted through a sieve and replaced by 50 ml of the test solution (sea water of 10°C containing the test compound). The lice are treated in this solution for 1 hour, as this corresponds more or less to the conditions in the fish pens. Each container is then flushed with fresh sea water and the lice are kept in fresh sea water. The test is evaluated by making a mortality count of the lice in accordance with sex, stage and concentration of test compound. The count is repeated hourly until there are no more lice surviving. All test are carried out in triplicate.

b1) Range-finding test

The lice are treated according to b) with active substance concentrations of 0.001 to 1.0 ppm for a period of 1 hour, and the mortality rate is determined by counting the dead parasites. Mortality is found to be 100% at 0.1 ppm after 1 hour, and at 0.01 ppm after 2 hours. Below 0.01 ppm, the parasites survive for longer than 24 hours.

b2) Effect of temperature and salinity on the toxicity of the active substance The effect of temperature is determined at values from 4° to 16°C at an active substance concentration of 0.01 ppm and a treatment time of 1 hour. The following results are obtained:

| Temperature [°C] | 4 | 8 | 10 | 12 | 16 |
|------------------------|----|----|----|----|----|
| parasite mortality [%] | 68 | 60 | 57 | 65 | 78 |

Above and below 10°C a slight increase in mortality is observed. In contrast, an increase in the salinity from 23% to 30%C at the same concentration of active substance and for the same treatment time has no significant effect on mortality.

2. Toxicity against salmon lice (in vitro test)

Five naturally infected Atlantic salmon are taken from the pen and transferred to well aerated sea water tanks. They remain there for 48 hours for acclimatisation and feed is withheld for 24 hours before the addition of test compound. A group of 5 salmon is treated for 1 hour at a concentration of 1.0 ppm of test compound, and a second group of 5 salmon is treated at a concentration of 0.1 ppm. The fish are kept for 24 hours in fresh sea water (without test compound) and a count is then made of dead and still living parasites. An untreated group of fish is also included in the evaluation. The test is carried out in

triplicate.

The evaluation shows that all adult and pre-adult stages are killed at both concentrations of 0.1 and 1.0 ppm. Thus in the <u>in vitro</u> and <u>in vivo</u> tests, matching results are obtained with the test compound.

What is claimed is:

- 1. S-6-Chloro-2,3-dihydro-2-oxo-1,3-oxazol[4,5-b]pyridin-3-yl-methyl-O,O-dimethyl-phosphorothioate for use in a method of controlling pests of the class of Crustaceae which parasiticise on fish of the family Salmonidae.
- 2. The compound of claim 1 for treating representatives of the family Salmonidae selected from the group consisting of: Salmon salar; Salmon trutta; Salmon gairdneri; Oncorhynchus gorbuscha; Oncorhynchus keta; Oncorhynchus nekra; Oncorhynchus kisutch; Oncorhynchus tshawytscha and Oncorhynchus mamson; Salvelinus species and Salmo clarkii.
- 3. The compound of claim 1 for controlling sea lice in active substance concentrations of 0.005 to 2 ppm.
- 4. The compound of claim 1 for controlling sea lice in concentrations from 0.01 to 1 ppm.
- 5. The compound of claim 1 for controlling sea lice in a concentration of 0.05 to 0.5 ppm.
- 6. A method of controlling sea lice of the class of Crustaceae, which comprises treating said pests at a concentration of 0.05 to 2 ppm with S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazol[4,5-b]pyridin-3-yl-methyl-O,O-dimethylphosphorothioate.
- 7. A method according to claim 6, wherein treatment is carried out at an active substance concentration of 0.005 to 2 ppm.
- 8. A method according to claim 7, wherein treatment is carried out at an active substance concentration of 0.1 to 1 ppm.
- 9. A method according to claim 8, wherein treatment is carried out at an active substance concentration of 0.05 to 0.5 ppm.
- 10. A method according to claim 6, wherein the sea lice are Lepeophtheirus or Caligus species.

- 11. A method according to claim 10, wherein the sea lice are the species Lepeophtheirus salmonis or Caligus elongatus.
- 12. A method according to claim 6, wherein the active substance is dissolved in the ambient water of the sea lice.
- 13. A method according to claim 6, wherein the active substance is added in dilute form to the ambient water of the sea lice.
- 14. A method according to claim 6, wherein the sea lice are in a pre-adult or adult stage.
- 15. Use of S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazol[4,5-b]pyridin-3-yl-methyl-O,O-dimethylphosphorothioate for pest control in commercial salmon and trout farming.
- 16. Use of S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazol[4,5-b]pyridin-3-yl-methyl-O,O-dimethylphosphorothioate for the preparation of compositions for controlling sea lice.

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