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(54) Title: FORMULATIONS OF BROAD SPECTRUM BIOCIDES AND THEIR USE IN IMPROVED METHODS FOR THE TREATMENT OF BALLAST WATER

(57) Abstract: The present invention relates to a method for the treatment of ballast water on board seagoing vessels including the use of formulations of sparingly soluble biocides.
FORMULATIONS OF BROAD SPECTRUM BIOCIDES AND THEIR USE IN
IMPROVED METHODS FOR THE TREATMENT OF BALLAST WATER

TECHNICAL FIELD

The invention relates to formulations of sparingly soluble
broad spectrum biocides and their use in the treatment of
ballast water. In particular, the invention relates to
formulations of 2-methyl-1,4-naphthalenedione and their use in
improved treatments of ballast water.

BACKGROUND ART

The introduction of harmful aquatic organisms and pathogens to
new environments, including via ships' ballast water, has been
identified as one of the four greatest threats to the world's
oceans.

It is estimated that a foreign marine species is introduced to
a new environment somewhere in the world every nine weeks.
Human health, ecological and economic impacts can be severe.
(Raaymakers (2002))

Shipping moves over 8 % of the world's commodities and
transfers approximately 3 to 5 billion tonnes of ballast water
internationally each year. Ballast water is essential to the
safe and efficient operation of modern shipping, providing
balance and stability to unladen ships.

Ballast water is carried by ships to ensure stability, trim
and structural integrity. Ships have carried solid ballast,
in the form of rocks, sand or metal for thousands of years.
In modern times, ships use water as ballast.
There are thousands of marine species that may be carried in ships ballast water. Anything that is small enough to pass through a ship's ballast water intake ports and pumps may be carried. This includes various propagules of bacteria and other microbes, small invertebrates and the eggs, cysts and larvae of various species.

The problem is compounded by the fact that virtually all marine species have life cycles that include a planktonic stage or stages. Even species in which the adults are unlikely to be taken on or in ballast water, for example, because they are too large or live attached to the sea bed, may be transferred in ballast water during their planktonic phase.

Natural barriers, such as temperature and land masses, have prevented many species from dispersing into certain areas. This has resulted in the natural patterns of biogeography observed in the oceans today. In particular, the pan-global tropical zone has separated the northern and southern temperate and cold waters zone. This has allowed many species to evolve quite independently in these latter zones, resulting in quite different marine biodiversity between the northern and southern hemispheres.

The commencement of the use of water as ballast, and the development of larger, faster ships completing their voyages in even shorter times, combined with rapidly increasing world trade, means that the natural barriers to the dispersal of species across the oceans are being reduced.
It is estimated that at least 7,000 different species are being carried in ships ballast tanks around the world. When factors are favourable a species introduced by the discharge of ballast water may survive and establish a reproductive population in the new host environment. The introduced species may even become invasive, out competing native species and multiplying into pest proportions.

In the USA the European zebra mussel Dreissena polymorpha has infested over 40% of internal waterways and they have required between US$ 750 million and US$ 1 billion in expenditure on control measures between 1989 and 2000.

In southern Australia, the Asian kelp and Undaria pinnatifida is invading new areas rapidly, displacing the native’s seabed communities.

In the black sea, the filter feeding North American jellyfish Mnemiopsis leidyi has occasionally reached densities of 1 kg of biomass/m². It has depleted native plants and stocks to such an extent that it has contributed to the collapse of the entire black sea commercial fisheries.

In several countries introduced, microscopic, "red-tide" algae (toxic dinoflagellates) have been absorbed by filter-feeding shellfish, such as oysters. When eaten by humans, these contaminated shellfish can cause paralysis and even death.

In response to these ecological threats states are introducing mandatory requirements concerning the discharge of ballast water. The threat was recognised in the report of the United Nations Conference on Environment and Development (UNCED). In the section on prevention, reduction and control of
degradation of the marine environment from sea-based activities it is stated:

17.30. States, acting individually, bilaterally, regionally or multilaterally and within the frame work of IMO and other relevant international organizations, whether sub-regional, regional or global, as appropriate, should assess the need for additional measures to address degradation of the marine environment from shipping by considering the adoption of appropriate rules on ballast water discharge to prevent the spread of non-indigenous organisms.

In November 1997 the International Maritime Organisation (IMO) adopted resolution A.868 (20) entitled "Guidelines for the control and management of ships ballast water to minimise a transfer of harmful aquatic organisms and pathogens".

In a report prepared for the Northeast-Midwest Institute and entitled "Global market analysis of ballast water treatment technology" it was anticipated that after 2008 all international trading vessels that use sea water as ballast are likely to fall under the IMO convention (Tjallingii et al (2001)).

In this report it was also anticipated that ballast water exchange (BWE) would remain an option open to ship owners for some time to come. The option for ship owners to choose between on board ballast water treatment (BWT) and ballast water exchange would therefore remain.

It was noted in the report that ballast water treatment (BWT) still faces technical challenges before it could provide a sound alternative for BWE. The most important difficulties were recognised as those relating to retrofitting equipment into existing ships, especially where the flow rates are high.
and available space for equipment is limited. The report stated that there was no doubt that a strong demand for an environmentally sound and effective ballast water treatment technology would emerge.

Ballast water exchange (BWE) cannot be utilised in near shore voyages and was noted in the report as being dangerous to ships under certain circumstances due to excessive stresses and strains. A technical constraint on the ready adoption of ballast water treatment as opposed to ballast water exchange is the "translation" of existing and field tested "on-shore technology and equipment" to the "on-board environment" with its different characteristics.

Any ballast water treatment adopted by the shipping industry must be safe for ship and crew, environmentally acceptable, practicable, economical, and biologically effective. Various options are available for the treatment of ballast water, including:

- Mechanical treatment methods such as filtration and separation;
- Physical treatment methods such as sterilization by ozone, ultraviolet light, electric currents and heat treatment; and
- Chemical treatment methods such as adding biocides to ballast water to kill organisms.

Mechanical treatment methods seek to remove organisms from the ballast water. Filtration and separation methods include
coagulation/flocculation, cyclonic separation, and self cleaning screens.

Physical treatment methods seek to kill the organisms in the ballast water. These include the use of ultraviolet light, ozone, heat treatment, oxygen deprivation, high frequency ultrasound, electro-ionization, gas super-saturation, and low frequency sonics.

Chemical treatment methods also seek to kill, or at least devitalize, the organisms in the ballast water. These methods include pH adjustment and the use of biocidal chemicals.

For a chemical treatment to be effective a minimum aquacidal concentration must be maintained throughout the volume of the ballast water for a specified period of time. The cost effectiveness of these methods of treatment will be enhanced if the minimum amount of chemical required to achieve the minimum aquacidal concentration throughout the volume of the ballast water is added. Environmental impacts when the treated ballast water is discharged also need to be minimised.

Menadione (2-Methyl-1,4-naphthalenedione, Vitamin K3) is a quinone derivative of naphthalene with a predicted solubility in water (pH 7, 25 °C) (calculated using Advanced Chemistry Development Software V9.04) of less than 400 μmol/L. Despite this low solubility, menadione was determined to be effective in devitalizing a broad range of harmful aquatic organisms and pathogens that may be present in ballast water (Cutler et al (2001)).

The low solubility of menadione has been perceived as a limitation in its use in the treatment of ballast water on
board ships. As noted above, to be effective the active ingredient must be present at a concentration effective to devitalize the harmful aquatic organisms and pathogens throughout a large, enclosed volume of water. To solve this problem one may resort to using suitable organic solvents in which the quinone is soluble to promote the dispersion of the compound in the water to be treated. The solubility of menadione in various solvent combinations has been determined by Song et al (2007).

As a consequence of this perceived limitation, water-soluble derivatives of menadione have been considered for use in the treatment of ballast water. Menadione sodium bisulfite (MSB) is one such water-soluble derivative of menadione, and by contrast with menadione, is relatively stable to photodegradation (Song et al (2008)). Other water-soluble derivatives of menadione that have been considered for use include the nicotinamide salt of menadione bisulphite (MNB) (Manzotti and Monteleone (2004)).

As stated in Manzotti and Monteleone (2004):

[An] object is to provide a method for treatment preferably of either fresh or salt water in water basins of any size, waters in conduits for civil and industrial use, and ballast water, said method using highly water soluble compounds in order to allow the desired concentration be obtained through the use of the least amount as possible of said compounds.

As further stated in Manzotti and Monteleone (2004):

... with those biocides which dissolve poorly in the water pool, the inhibition of the target organisms only takes place in a restricted area surrounding the biocide particle whereas a substantial survival of said organisms is to be expected in
the great part of the bulk of treated water. This is extremely important whenever water of water basins of any size, water from civil and industrial conduits and ballast water should be treated because of the huge quantity of liquid to be effectively disinfested.

The improved rate of biodegradation of MNB relative to that of menadione and MSB was presented in Manzotti and Monteleone (2004) as a particular advantage favoring the use of this water soluble menadione derivative over menadione.

It is an object of the invention to provide an improved method of ballast water treatment.

It is an object of the invention to provide formulations of 2-methyl-1,4-naphthalenedione that are particularly useful in the treatment of ballast water.

These objects are to be read disjunctively with the object of to at least provide a useful choice.

DISCLOSURE OF INVENTION

In a first aspect the invention provides a flowable concentrate formulation of a sparingly soluble biocide for use in the treatment of ballast water.

Preferably, the sparingly soluble biocide is 2-methyl-1,4-naphthalenedione.

Preferably, the flowable concentrate is a wettable powder or a suspension concentrate.
In a first embodiment, the flowable concentrate is a suspension concentrate. Preferably, the flowable concentrate is a suspension concentrate with a particle size distribution substantially equivalent to that provided in Figure 1. More preferably, the suspension concentrate comprises 40 to 85 % of the sparingly soluble biocide and 7 to 9 % of a starch or dihydric alcohol.

In a second embodiment, the flowable concentrate is a wettable powder. Preferably, the wettable powder comprises greater than 50% by weight of the sparingly soluble biocide. More preferably, the wettable powder comprises at least 80% by weight of the sparingly soluble biocide.

In an option, the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 5 to 1000. Preferably, the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 11 to 1000. More preferably, the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 20 to 1000.

In an embodiment of the first aspect the invention provides a suspension concentrate formulation consisting of:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (% w/w)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methyl-1,4-naphthalenedione</td>
<td>44.1</td>
<td>Active</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>6.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>TENSIOFIX™ SC</td>
<td>1.5</td>
<td>Formulant</td>
</tr>
<tr>
<td>TENSIOFIX™ XD23</td>
<td>0.5</td>
<td>Formulant</td>
</tr>
<tr>
<td>SAG 30</td>
<td>0.5</td>
<td>Antifoam</td>
</tr>
</tbody>
</table>
In an embodiment of the first aspect the invention provides a wettable powder formulation consisting of:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (% w/w)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXEL™ GXL</td>
<td>0.1</td>
<td>Preservative</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>0.05</td>
<td>Formulant</td>
</tr>
<tr>
<td>Water</td>
<td>45.25</td>
<td>Carrier</td>
</tr>
</tbody>
</table>

In a second aspect the invention provides a method of treating ballast water in a tank of a seagoing vessel comprising the steps of:

1. Adding an effective amount of a flowable concentrate formulation of a sparingly soluble biocide to the tank;

2. Maintaining the ballast water in the tank for a time and at a temperature sufficient to devitalize harmful aquatic organisms and pathogens that may be present in the ballast water; and then

3. Expelling the ballast water from the tank to the exterior of the vessel.

Preferably, the sparingly soluble biocide is the compound 2-methyl-1,4-naphthalenedione.

In a first embodiment, the flowable concentrate is a suspension concentrate. Preferably, the flowable concentrate
is a suspension concentrate with a particle size distribution substantially equivalent to that provided in Figure 1. More preferably, the suspension concentrate comprises 40 to 85% of the sparingly soluble biocide and 7 to 9% of a starch or dihydric alcohol.

In a second embodiment, the flowable concentrate is a wettable powder. Preferably, the wettable powder comprises greater than 50% by weight of the sparingly soluble biocide. More preferably, the wettable powder comprises at least 80% by weight of the sparingly soluble biocide.

In an option, the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 5 to 1000. Preferably, the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 11 to 1000. More preferably, the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 20 to 1000.

Preferably, the effective amount is sufficient to provide an initial concentration of the sparingly soluble biocide in the ballast water of at least 800 ppb. More preferably, the effective amount is sufficient to provide an initial concentration of the sparingly soluble biocide in the ballast water of at least 1.6 ppm. Most preferably, the effective amount is sufficient to provide an initial concentration of the sparingly soluble biocide in the ballast water of at least 5 ppm.
Preferably, the time sufficient to devitalize harmful aquatic organisms and pathogens is less than 48 hours. More preferably, the time sufficient to devitalize harmful aquatic organisms and pathogens is less than 24 hours.

In a third aspect the invention provides a suspension concentrate formulation of a sparingly soluble biocide packaged with directions for use in the treatment of ballast water in a tank of a seagoing vessel where the sparingly soluble biocide has a median particle size of 2.0 to 3.5 µm and a maximum particle size of 9.0 µm.

Preferably, the suspension concentrate formulation is a suspension concentrate formulation of the compound 2-methyl-1,4-naphthalenedione.

In a fourth aspect the invention provides a wettable powder formulation of a sparingly soluble biocide packaged with directions for use in the treatment of ballast water in a tank of a seagoing vessel where the wettable powder comprises at least 80% by weight of the sparingly soluble biocide.

Preferably, the suspension concentrate formulation is a suspension concentrate formulation of the compound 2-methyl-1,4-naphthalenedione.

In a fifth aspect the invention provides a seagoing vessel containing ballast water to which a flowable concentrate formulation of a sparingly soluble biocide of the first aspect of the invention has been added.

In the description and claims the following acronyms, terms and phrases have the meaning provided:
"Benthic" means normally living at the soil-water interface at the bottom of a sea or lake.

"Biocide" means an active ingredient to which the devitalization of a population of organisms or pathogens is attributed.

"Devitalisation" means the depriving of strength and vigour of a population of organisms or pathogens so that substantially no viable individuals remain and "devitalize" has a corresponding meaning.

"Flowable concentrate" means a concentrated formulation of active ingredient(s) in a liquid or solid form that is capable of being poured from a container.

"Harmful aquatic organisms and pathogens" means the plurality of species recognized by the International Maritime Organization (IMO) to present a threat to marine ecology.

"Pelagic" means normally living in the middle depths and surface waters a sea or lake.

"Population" means a collection of individuals of the same species.

"ppb" means parts per billion ($10^9$) on a per weight basis.

"ppm" means parts per million ($10^6$) on a per weight basis.

"Suspension concentrate" means a flowable concentrate that is a stable suspension of active ingredient(s) in water.
"Sparingly soluble" means having a solubility in water (pH 7, 25 °C) of less than 500 µmol/L.

"Treatment of ballast water" means the devitalization of harmful aquatic organisms and pathogens that may be present in the water and "treating of ballast water" has a corresponding meaning.

"Viable" means capable of growth and reproduction.

"Wettable powder" means a formulation that forms a suspension of active ingredient(s) on addition to water.

Unless stated otherwise percentages (%) used to define formulations and mixtures are calculated on a per weight basis.

Exemplary embodiments of the invention will now be described in detail with reference to the Figures of the accompanying drawings pages.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. Particle size distribution of the suspension concentrate designated SEAKLEEN® 500SC.

DETAILED DESCRIPTION

The inventor has determined that contrary to the assertions made in the prior art, formulations of menadione (2-methyl-1,4-naphthalenedione) can be used effectively in the treatment of ballast water. Indeed, the limited solubility of the active ingredient may be of particular advantage where the
harmful aquatic organisms and pathogens reside in the sediments that accumulate and are retained in the ballast water tanks of seagoing vessels.

The formulations are prepared as a flowable concentrate of the sparingly soluble biocide. The flowable concentrate may be in the form of a wettable powder or suspension concentrate, although the latter is particularly preferred due to the convenience with which an effective amount of the active ingredient may be delivered into the ballast water tank.

A flowable concentrate formulation of a sparingly soluble biocide that provides a suspension with a median particle size of 2.0 to 3.5 μm and a maximum particle size of 9.0 μm when added to the ballast water in a tank of a seagoing vessel is believed to be optimal to devitalize the harmful aquatic organisms and pathogens both suspended in the ballast water and residing in the residula sediment. Over time a portion of the sparingly soluble biocide is anticipated to concentrate in the sediment augmenting the efficacy of the ballast water treatment.

The efficacy of the treatment may be further augmented by the addition of a biocidal quaternary ammonium and/or phosphonium compound. The use of such compounds in combination with an anionic surfactant in a manner that prevents foaming is described in Sweeny et al (2008).

Biocidal quaternary ammonium and/or phosphonium compounds such as alkyl(dimethyl-benzylammonium chloride or tributyl-tetradecylphosphonium chloride may be used. Anionic surfactants may be selected from the group consisting of
diamyl sulfo succinate, dihexyl sulfo succinate, dioctyl sulfo succinate, ditridecyl sulfo succinate, and their salts.

In use it is recognized that the formulations may be dispensed directly into the ballast water enclosed in the tank while the ship is in transit. Alternatively, the formulations could be dispensed into the ballast water tank prior to the tank being filled with ballast water. A further option is for metered delivery of the formulations into the flow of ballast water as it is pumped into the tanks.

It will be apparent to those skilled in the art that formulants with undesirable environmental impacts, such as ethoxylated alkyl phenols, should be excluded from the formulations. For example, linear alcohols or ethylene oxide/propylene oxide block polymers are preferred for use as surfactants in addition to the biocidal quaternary ammonium and/or phosphonium compounds.

It will also be apparent from the results presented that efficacy of the method of the invention is enhanced by maintaining the ballast water in total darkness for a sufficient period of time prior to expelling the ballast water from the tank to the exterior of the vessel. The susceptibility of menadione to photo-degradation makes the sparingly soluble biocide particularly suitable for the treatment of ballast water.
EXAMPLES

Preparation of SEAKLEEN™ 800WP

5 Batches of a wettable powder formulation of 2-methyl-1,4-naphthalenedione were prepared. The ingredients listed in Tables 1a or 1b were mixed at the concentrations indicated and blended in a Brinkman Hammer Mill (1 x 2.0 mm, 2 x 0.75 mm) to provide the wettable powder.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (% w/w)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methyl-1,4-naphthalenedione</td>
<td>80.0</td>
<td>Active</td>
</tr>
<tr>
<td>Pure-Dent B700</td>
<td>8.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>STEPSPERSE™ DF-200</td>
<td>7.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>STEPSPERSE™ DF-500</td>
<td>3.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>STEPWET™ DF-90</td>
<td>1.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>HiSil 233</td>
<td>1.0</td>
<td>Formulant</td>
</tr>
</tbody>
</table>

Table 1a. Formulation designated SEAKLEEN™ 800WP.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (% w/w)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methyl-1,4-naphthalenedione</td>
<td>80.0</td>
<td>Active</td>
</tr>
<tr>
<td>Paragon Clay</td>
<td>8.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>STEPSPERSE™ DF-200</td>
<td>7.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>STEPSPERSE™ DF-500</td>
<td>3.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>STEPWET™ DF-90</td>
<td>1.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>HiSil 233</td>
<td>1.0</td>
<td>Formulant</td>
</tr>
</tbody>
</table>

Table 1b. Formulation designated SEAKLEEN™ 800WP.

Suspensibility of the wettable powder formulations was evaluated over a 3 month period at an elevated temperature of 50°C. The results for each formulation are presented in Tables 2a and 2b.
Table 2a. Suspensibility of the wettable powder formulation designated SEAKLEEN™ 800WP (Table 1a).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Suspensibility (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>1 month</td>
<td>3 month</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>90.7</td>
<td>79.9</td>
<td>79.9</td>
</tr>
<tr>
<td>2000 ppm</td>
<td>94.3</td>
<td>75.8</td>
<td>81.6</td>
</tr>
</tbody>
</table>

Table 2b. Suspensibility of the wettable powder formulation designated SEAKLEEN™ 800WP (Table 1b).

The formulations were determined to have a measured activity (%) of 95.5 ± 5.5 relative to the theoretical activity (mean of 2 batches).

Preparation of SEAKLEEN™ 500SC

Batches of a suspension concentrate (SC) formulation of 2-methyl-1,4-naphthalenedione were prepared. The ingredients listed in Tables 2a or 2b were blended to provide the suspension concentrate.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (% w/w)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methyl-1,4-naphthalenedione</td>
<td>44.1</td>
<td>Active</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>8.0</td>
<td>Formulant</td>
</tr>
<tr>
<td>TENSIOFIX™ SC</td>
<td>1.5</td>
<td>Formulant</td>
</tr>
<tr>
<td>TENSIOFIX™ XD23</td>
<td>0.5</td>
<td>Formulant</td>
</tr>
<tr>
<td>SAG 30</td>
<td>0.5</td>
<td>Antifoam</td>
</tr>
<tr>
<td>PROXEL™ GXL</td>
<td>0.1</td>
<td>Preservative</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>0.05</td>
<td>Formulant</td>
</tr>
<tr>
<td>Water</td>
<td>45.25</td>
<td>Carrier</td>
</tr>
</tbody>
</table>

Table 3. Formulation designated SEAKLEEN™ 500SC.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purity (% w/w)</td>
<td>44 ±0.2</td>
</tr>
<tr>
<td>Density (g/ml)</td>
<td>1.146 ±0.02</td>
</tr>
<tr>
<td>pH</td>
<td>6.42 ±0.01</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>95.2 ±1.0</td>
</tr>
</tbody>
</table>

Table 4. Characteristics of the formulation designated SEAKLEEN™ 500SC (mean of 2 batches).

5 Laboratory testing of the efficacy of formulations

The SEAKLEEN™ formulations were evaluated for use in the treatment of ballast water. Efficacy of the SEAKLEEN™ formulations (the compound 2-methyl-1,4-naphthalenedione as active ingredient) was determined with or without the inclusion of the water soluble menadione sodium bisulfite (MSB).

Assays were performed using an average initial count of 247 Mytilus galloprovincialis larvae per 10 ml sample. The results of the assays employing different light conditions for the aging of test solutions and bioassay are presented in Tables 5 to 9.

<table>
<thead>
<tr>
<th>Concentration (ppb)</th>
<th>% Mortality</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total darkness</td>
<td>Constant light</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
<td>100*</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td>1.6</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
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Table 5. Efficacy of a mixture of 80% MSB and 20% SEAKLEEN™ 800WP against Mytilus galloprovincialis larvae following aging of the test solutions for 48 hours in total darkness and conducting the bioassay under the light conditions indicated. *p ≤ 0.05
<table>
<thead>
<tr>
<th>Concentration (ppb)</th>
<th>% Mortality</th>
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</thead>
<tbody>
<tr>
<td>500</td>
<td>100*</td>
</tr>
<tr>
<td>200</td>
<td>4.3</td>
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<tr>
<td>100</td>
<td>1.6</td>
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<tr>
<td>50</td>
<td>0</td>
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<tr>
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Table 6. Efficacy of a mixture of 80% MSB and 20% SEAKLEEN™ 800WP against *Mytilus galloprovincialis* larvae following aging of the test solutions for 48 hours and conducting the bioassay under constant light. *p ≤ 0.05

<table>
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<th>Concentration (ppb)</th>
<th>% Mortality</th>
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<td>500</td>
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<tr>
<td>200</td>
<td>25.3*</td>
</tr>
<tr>
<td>100</td>
<td>1.7</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
</tr>
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</tbody>
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Table 7. Efficacy of the formulation SEAKLEEN™ 800WP against *Mytilus galloprovincialis* larvae following aging of the test solutions for 48 hours and conducting the bioassay in total darkness. *p ≤ 0.05

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<th>Concentration (ppb)</th>
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<td>500</td>
<td>94.4*</td>
</tr>
<tr>
<td>200</td>
<td>3.7*</td>
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<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
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Table 8. Efficacy of the formulation SEAKLEEN™ 800WP against *Mytilus galloprovincialis* larvae following aging of the test solutions for 48 hours in total darkness and conducting the bioassay under constant light. *p ≤ 0.05
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<td>500</td>
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</table>

*Table 9. Efficacy of the formulation SEAKLEEN™ 800WP against Mytilus galloprovincialis larvae following aging of the test solutions for 48 hours and conducting the bioassay under constant light. *p ≤ 0.05

Notably the formulation of SEAKLEEN™ 800WP demonstrated the highest efficacy at 200 ppm against the test organism Mytilus galloprovincialis larvae when both the aging of the test solutions and conducting of the bioassay were performed under total darkness. Efficacy of SEAKLEEN™ 800WP was reduced by either aging the test solutions or conducting the bioassay under constant light.

These results indicate the suitability of SEAKLEEN™ 800WP for use in the treatment of ballast water enclosed in tanks that is to be expelled to the exterior of the ship following treatment.

**Shipboard testing of the efficacy of formulations**

The efficacy of the formulation SEAKLEEN™ 800WP was evaluated in a shipboard trial. The trial was conducted aboard the tanker Seabulk Mariner as detailed in the publication of Wright et al (2009). The disclosures of this publication are incorporated in their entirety.

briefly, the formulation, SEAKLEEN™ 800WP was mixed as a slurry and the seawater in ballast water tanks was treated at a rate equivalent to a nominal concentration of 2-methyl-1,4-
naphthalenedione of 0.8 (tank 5S) or 1.6 (tank 5P) mg/L. Untreated water in a third tank partially filled at the commencement of the ballasting operation and completely filled at the end of the ballasting operation was used as a control (untreated).

Water samples were collected from the tanks containing treated seawater at 2 hours, 15 hours, 48 hours, 49.5 hours and 52.5 hours after treatment. The third, fourth and fifth samples were collected during and following the flow-through ballast water exchange that commenced just prior to collection of the third set of samples. The fifth set of samples were collected following a nominal 300% flow-through exchange. Collection of samples of treated ballast water was immediately followed by samples of control, untreated, ballast water.

Following a target dose of 0.8 mg/L of 2-methyl-1,4-naphthalenedione the third set of samples (48 hours) were determined to contain a concentration of 0.49 mg/L. Following a target dose of 1.6 mg/L of 2-methyl-1,4-naphthalenedione the third set of samples (48 hours) were determined to contain a concentration of 1.32 mg/L. The mortality of zooplankton (including benthic species) in these treated ballast water samples is presented in Table 10.

As stated by Wright et al (2009):

Following 48 h exposure to SEAKLEEN® in both the high-dose tank [1.6 mg/L] and the low-dose tank [0.8 mg/L] no surviving zooplankton were found.

With reference to the publication of Raikow et al (2006) the publication of Wright et al (2009) states:
While SEAKLEEN™ shows good efficacy against pelagic organisms, it is less effective against some benthic vegetative stages and eggs.

It should be noted that in the studies of Raikow et al. (2006) menadione sodium bisulfite (MSB), and not the formulations used in the present invention, was employed as the biocide. The advantageous properties of the flowable concentrate formulations would not therefore have been realised in the studies of Raikow et al. (2006).

Acknowledging the observed inefficiencies in achieving complete ballast water exchange, the retention in the ballast water tanks of an undischarged residue of the biocide in the sediment may therefore be desirable to the long-term efficacy of ballast water treatments employing the use of sparingly soluble broad spectrum biocides.

Although the invention has been described by way of exemplary embodiments it should be appreciated that variations and modifications may be made without departing from the scope of the invention. Furthermore where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification.

In particular, the ingredients of the formulations are identified by trade names. It will be recognized that ingredients that are functionally equivalent to these trade name products may be substituted at the same concentration for those specifically referenced.
<table>
<thead>
<tr>
<th>Species</th>
<th>Untreated (control) samples</th>
<th>2-methyl-1,4-naphthalenedione measured at 1.32 mg L$^{-1}$</th>
<th>2-methyl-1,4-naphthalenedione measured at 0.49 mg L$^{-1}$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Live</td>
<td>Dead</td>
<td>Live</td>
</tr>
<tr>
<td>Copepods adults (80-120 μm x 200-450 μm)</td>
<td>7273 ± 1575</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Copepodsites adults (60-90 μm x 150-250 μm)</td>
<td>1691 ± 422</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nauplii (50-70 μm x 80-90 μm)</td>
<td>7101 ± 2118</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bivalve larvae (70 μm x 70 μm)</td>
<td>474 ± 469</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protozoans (60 μm x 70 μm), (60 μm x 120 μm)</td>
<td>879 ± 652</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zoea (50-80 μm)</td>
<td>68 ± 117</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rotifers (40-50 μm)</td>
<td>1150 ± 468</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean no. live zooplankton &gt;50 μm</td>
<td>17,585 ± 2132</td>
<td>0</td>
<td>0</td>
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Table 10. Zooplankton counts in control (untreated) and treated samples (Wright et al. (2009)).
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CLAIMS

1) A flowable concentrate formulation of a sparingly soluble biocide for use in the treatment of ballast water.

2) The flowable concentrate formulation of claim 1 where the sparingly soluble biocide is 2-methyl-1,4-naphthalenedione.

3) The flowable concentrate formulation of claim 1 where the flowable concentrate is a wettable powder or a suspension concentrate.

4) The flowable concentrate formulation of claim 1 where the flowable concentrate is a suspension concentrate.

5) The flowable concentrate formulation of claim 4 where the suspension concentrate has a particle size distribution substantially equivalent to that provided in Figure 1.

6) The flowable concentrate formulation of claim 4 where the suspension concentrate comprises 40 to 85 % of the sparingly soluble biocide and 7 to 9 % of a starch or dihydric alcohol.

7) The flowable concentrate formulation of claim 1 where the flowable concentrate is a wettable powder.

8) The flowable concentrate formulation of claim 7 where the wettable powder comprises greater than 50% by weight of the sparingly soluble biocide.
9) The flowable concentrate formulation of claim 7 where the wettable powder comprises at least 80% by weight of the sparingly soluble biocide.

5 10) The flowable concentrate formulation of any one of claims 1 to 9 where the formulation further comprises at least one biocidal quaternary ammonium and/or phosphonium compound and at least one anionic surfactant in a molar ratio of from 5 to 1000.

10 11) The flowable concentrate formulation of any one of claims 1 to 9 where the formulation further comprises at least one biocidal quaternary ammonium and/or phosphonium compound and at least one anionic surfactant in a molar ratio of from 11 to 1000.

12) The flowable concentrate formulation of any one of claims 1 to 9 where the formulation further comprises at least one biocidal quaternary ammonium and/or phosphonium compound and at least one anionic surfactant in a molar ratio of from 20 to 1000.

13) A wettable powder formulation consisting of 80% 2-methyl-1,4-naphthalenedione, 8% Pure-Dent B700, 7% STEPSPERSE DF-200, 3% STEPSPERSE DF-500, 1% STEPWET™ DF-90 and 1% HiSil 233.

14) A suspension concentrate formulation consisting of 44% 2-methyl-1,4-naphthalenedione, 8% 1,2-Propanediol, 1.5% TENSIOFIX™ SC, 0.5% TENSIOFIX™ XD23, 0.5% SAG 30, 0.1% PROXEL™ GXL, 0.05% Xanthan gum and 45% water.
15) A method of treating ballast water in a tank of a seagoing vessel comprising the steps of:

- Adding an effective amount of a flowable concentrate formulation of a sparingly soluble biocide to the tank;

- Maintaining the ballast water in the tank for a time and at a temperature sufficient to devitalize harmful aquatic organisms and pathogens that may be present in the ballast water; and then

- Expelling the ballast water from the tank to the exterior of the vessel.

16) The method of claim 15 where the sparingly soluble biocide is the compound 2-methyl-1,4-naphthalenedione.

17) The method of claim 16 where the flowable concentrate is a suspension concentrate.

18) The method of claim 17 where the flowable concentrate is a suspension concentrate with a particle size distribution substantially equivalent to that provided in Figure 1.

19) The method of claim 18 where the suspension concentrate comprises 40 to 85 % of the sparingly soluble biocide and 7 to 9 % of a starch or dihydric alcohol.

20) The method of claim 15 where the flowable concentrate is a wettable powder.
21) The method of claim 20 where the wettable powder comprises greater than 50% by weight of the sparingly soluble biocide.

22) The method of claim 21 where the wettable powder comprises at least 80% by weight of the sparingly soluble biocide.

23) The method of claim 15 where the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 5 to 1000.

24) The method of claim 15 where the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 11 to 1000.

25) The method of claim 15 where the formulation further comprises at least one biocidal quaternary ammonium and/or phosponium compound and at least one anionic surfactant in a molar ratio of from 20 to 1000.

26) The method of claim 15 where the effective amount is sufficient to provide an initial concentration of the sparingly soluble biocide in the ballast water of at least 800 ppb.

27) The method of claim 26 where the effective amount is sufficient to provide an initial concentration of the sparingly soluble biocide in the ballast water of at least 1.6 ppm.

28) The method of claim 27 where the effective amount is sufficient to provide an initial concentration of the
sparingly soluble biocide in the ballast water of at least 5 ppm.

29) The method of claim 15 where the time sufficient to devitalize harmful aquatic organisms and pathogens is less than 48 hours.

30) The method of claim 29 where the time sufficient to devitalize harmful aquatic organisms and pathogens is less than 24 hours.

31) A suspension concentrate formulation of a sparingly soluble biocide packaged with directions for use in the treatment of ballast water in a tank of a seagoing vessel where the sparingly soluble biocide has a median particle size of 2.0 to 3.5 μm and a maximum particle size of 9.0 μm.

32) The suspension concentrate of claim 31 where the sparingly soluble biocide is the compound 2-methyl-1,4-naphthalenedione.

33) A wettable powder formulation of a sparingly soluble biocide packaged with directions for use in the treatment of ballast water in a tank of a seagoing vessel where the wettable powder comprises at least 80% by weight of the sparingly soluble biocide.

34) The wettable powder formulation of claim 33 where the sparingly soluble biocide is the compound 2-methyl-1,4-naphthalenedione.

35) A seagoing vessel containing ballast water to which a flowable concentrate formulation of a sparingly
soluble biocide of any one of claims 1 to 14 has been added.
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/068729

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.
A01N 25/14 (2006.01) A01N 35/06 (2006.01)
A01N 25/04 (2006.01) C02F 1/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPODOC, WPI, CAPLUS, AGRICOLA (Keywords - menadione, 2-Methyl-1,4-naphthalenedione, 2-Methyl-1,4-naphthoquinone, Vitamin K3, SeaKleen, ballast water, aquatic, biocide, wettable powder, suspension concentrate)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WRIGHT, D.A. et al. “Naphthoquinones as broad spectrum biocides for treatment of ship’s ballast water: Toxicity to phytoplankton and bacteria” Water Research, 2007, Vol 41, No. 6, pages 1294-1302 See abstract, sections 2.1.3-3.1.1 and Table 1</td>
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* Further documents are listed in the continuation of Box C
X See patent family annex

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search
28 January 2010

Date of mailing of the international search report
4 FEB 2010

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Form PCT/ISA/210 (second sheet) (July 2009)
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This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

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