

ELIMINATION AND INHIBITION OF
BIVALVE MOLLUSK ATTACHMENTS

IR 3453B

BACKGROUND OF THE INVENTION

This Application claims the benefit of United States
Provisional Applications 60/011,485 filed February 12,
1996 and 60/030,921 filed November 14, 1996.

This invention is a process for the eradication and
inhibition of bivalve mollusk attachments to solid
5 surfaces immersed in water inhabited thereby. More
particularly, the process involves the introduction into
water inhabited by bivalve mollusks, in various stages of
development, of a specific tertiary amine salt in an
amount at least sufficient to inhibit settlement of the
10 mollusks to any proximate solid surface.

One form of bivalve mollusks, Zebra mussels, are
believed to have been first delivered to North America

5 from Europe in the ballast of a ship. On discharge of
the ballast in Canadian waters, colonies of these mussels
quickly developed and spread through the Great Lakes and
surrounding rivers and lakes.

Zebra mussels, in both the mature and larval
10 (veliger) stages, cause serious fouling or obstruction
problems by attachment to subsurface solids and then to
the shells of each other to build clumps of the mollusks
across openings in conduits, e.g., cooling water intake
and effluent pipes of industrial and electrical
15 generating plants, and intakes of water supply
facilities. Additionally, the mollusks excessively
weight stress or foul tank and cooling tower walls,
underwater equipment, ship bottoms and ballast
compartments, oil rigs, locks, docks, pilings, dams,
20 linings of canals, anchors, gratings, valves and various
other subsurface structures and equipment in water
infested with these organisms.

For the purpose of this invention, the term bivalve
mollusks includes Zebra mussels (*Dreissena polymorpha*)
25 and other bivalve mollusks having larval (veliger) stages
which are free to swim and attach to (settle on) solid

5 surfaces, e.g., Blue mussels, Quagga mussels (*Dreissena*
bugenis) and Asiatic clams (*Carbicula fluminea*). Zebra
and Quagga mussels have fibrous tentacles called byssal
threads which enable firm attachment to underwater solid
surfaces.

10

PRIOR ART

There are various prior publications disclosing the
chemical treatment of water inhabited by mollusks to
prevent settlement on or to effect detachment of the
mollusks from solid subsurfaces. Some of these prior
15 references are directed to quaternary ammonium salts and
polymers thereof, and mixtures of the salts and polymers,
e.g., U.S. Patent Nos. 4,857,209; 5,015,395; 5,062,967;
5,096,601; and 5,290,805. Other prior publications teach
the use of water soluble C₈-C₁₈ alkyl guanidine salts to
20 control mollusk fouling, e.g., U.S. Patent No. 4,816,163.
A literature reference, Public Health Report, Vol. 82,
No. 9, 9/67, pp. 833-9, "Tests of 15 Experimental
Molluscicides Against *Australorbis Glabratus*" teaches
that both the mono and di (N,N-dimethyltridecylamine)
25 salts of Endothall are effective at 10 and 5 ppm

5 respectively to kill Puerto Rican snails. Chlorine,
generated, for example, by the introduction of chlorine
gas or sodium hypochlorite into the infested water, has
been the prevalent control agent for mollusks. While the
known chemical treatments for the control of Zebra
10 mussels are generally effective, their use can result in
undesirable environmental consequences, particularly with
respect to persistence and toxicity to nontarget
organisms.

 Tertiary amine salts of 3,6 endoxohydro-
15 orthophthalic acid are disclosed in U.S. Patent No.
3,207,593, issued September 21, 1965, as effective for
the control of submersed aquatic plant life.

STATEMENT OF THE INVENTION

 This invention is a method for the control of
20 bivalve mollusks to effect detachment from and inhibit
settlement on a solid immersed in water inhabited by said
mollusks comprising introducing into said water a
tertiary amine salt of an inorganic or organic acid, the

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5 amine radicals of said salt derived from an amine having the formula:



where R is an aliphatic hydrocarbon group containing from about 6 to about 20 carbon atoms and R' and R'' are the same or different alkyl radicals having from 1 to about 20 carbon atoms, and mixtures of said amine radicals, said amine salt being introduced at a rate and for a length of time sufficient to at least inhibit settlement of the larva stage of mollusks on said solid.

This invention also includes a method comprising first eradicating, by any means, bivalve mollusk attachments to a solid immersed in water. Preferably, a tertiary amine salt as defined above is introduced into the mollusk infested water at a rate and for a time sufficient to cause detachment of said mollusk attachments. Then, introducing into said water the above-described tertiary amine salt but at a rate at least sufficient to inhibit the settlement of larva stage

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5 bivalve mollusks to said solid.

DETAILED DESCRIPTION OF THE INVENTION

Bivalve mollusks in various stages of maturity are eradicated in accordance with this invention by exposure for a period of time to tertiary amine salts wherein the
10 amine radical is derived from an amine of the following formula:



where R is an aliphatic hydrocarbon group containing from
20 about 1 to about 20 carbon atoms and R' and R'' are the same or different alkyl radicals having from 1 to about 20, preferably from 1 to 8 carbon atoms. Examples of these alkyl radicals include methyl, ethyl, propyl, butyl, hexyl, octyl, decyl, dodecyl, octadecyl, eicosyl,
25 and isomers thereof. The aliphatic hydrocarbons represented by R include, for example, the C₁-C₂₀ alkyl radicals described above for R' and R'' as well as those groups on amines obtained by reductive amination of the acids from animal fats and vegetable oils, particularly

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5 tallow and coconut acids which have predominantly 12 to
18 carbon atoms in the chains. Of particular value for
this invention are coconut oil amines (predominantly
twelve and 14 carbon atoms) which have been converted to
tertiary amines by alkylation. However, other tertiary
10 amines within the above structural configuration are also
operable in this invention and such amines may be
obtained by the amination and subsequent alkylation of,
e.g., caproleic, oleic, linoleic, tallow, and soya acids.
Alternatively, these tertiary amines may be obtained by
15 conversion of the acids to an amide with a di-lower alkyl
secondary amine followed by reduction of the carbonyl
group. Specific amines which are preferably used to form
the salts useful in this invention include N,N-
dimethylcaprylamine, N,N-dimethylaurylamine, N,N-
20 diethylmyristylamine, N,N-dipropylstearylamine and the
like. However, because of availability and cost,
mixtures of amines are most preferably used such as the
mixtures found in N,N-dimethylcocoalkylamines, N,N-
dimethylsoyaamine, synthetic fatty acid amines, and the
25 like. The methods of making these amines are well known,
the various processes being disclosed in the text by

5 Astle entitled Industrial Organic Nitrogen Compounds
(Reinhold, 1961).

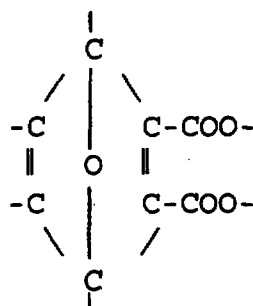
 The tertiary amine salts of this invention contain
amine radicals as previously described while the acid or
anhydride useful for the formation of the salt with the
10 tertiary amine may be any inorganic or organic acid or
anhydride thereof which does not appreciably decrease the
activity of the tertiary amine moiety. The inorganic
acids will include, for example, compounds of the formula
HX where X equals chlorine, bromine, iodine, $-\text{NO}_3$, $-\text{HSO}_4$,
15 $-\text{H}_2\text{PO}_4$ and the like. The organic acids will include, for
example, di- or polybasic carboxylic acids containing 1
to 24 carbon atoms, e.g., 3,6-endoxohydrophthalic acids
and its anhydride (see U.S. Patent 2,576,082).

 A tertiary amine salt of preference is the inorganic
20 acid salt of mono- or di- N,N-dimethylcocoamine, based on
cost and commercial availability, or the inorganic acid
salt of a synthesized chemical equivalent thereof.

 The 3,6-endoxohydroorthophthalic acid salts of this
invention will have less than three double bonds in the
25 endoxocarboxylic acid ring, but may have all three

5 degrees of ring saturation and thus will include 3,6-
 endoxodihydroorthophthalic acids, (e.g.

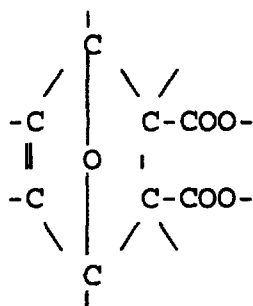
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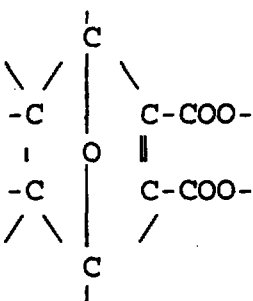
3,6-endoxotetrahydroorthophthalic acids, (e.g.

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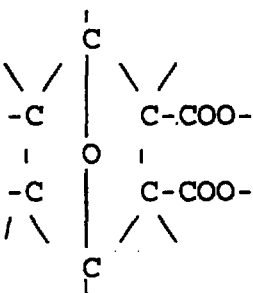
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and 3,6-endoxohexahydroorthophthalic acids (e.g.

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5 In this group, the latter compounds (i.e., the hexahydro compounds or endothalls) are the preferred anions to be used.

Where anhydrides are used, a mole of water is required, of course, to cause hydrolysis to the dibasic
10 acid. In addition to the unsubstituted acids, monovalent radical substituted derivatives may be used and such substituents will include halogens (e.g., chlorine, bromine, etc.), lower alkyl, lower alkoxy, lower aryl, lower aryloxy, nitro, cyano, haloalkyl (e.g.,
15 trifluoromethyl) and like groups. The substituted acids from which the salts useful in this invention are derived, are described and their preparation given in U.S. 2,576,080.

Examples of the techniques which may be used to
20 prepare these salts are disclosed in U.S. 2,576,082.

The compounds used in this invention when made from commercial amines are generally clear or slightly turbid tan or brown oils or syrups which do not readily lend themselves to crystallization. This resistance to
25 crystallization is probably due to the fact that the commercial amines used to form the salts are comprised of

5 more than one discrete amine species and such a mixture makes crystallization impossible. However, this is not important because the amine salt oil obtained as product is simply dissolved in a suitable solvent for use. When individual specific amines are used for salt formation, 10 however, the products are waxy or soap-like solids. The products used in this invention are readily soluble in water, ethanol and other alcohols, benzene, toluene, xylene and other aromatic hydrocarbons, diethyl ether, diacetone alcohol, etc.

15 The active tertiary amine salt is usually used in the process of this invention as a water-based formulation containing, for example, from 25 to 75 weight percent of the active salt. Other ingredients may also be incorporated in the formulation including effective 20 amounts of one or more dispersants, for example, isopropyl alcohol, diacetone alcohol, other water soluble alcohols and/or ketones, up to about 40 weight percent of the formulation. Surfactants for improving the dispersibility or suspensibility of active agents in 25 water may be included in the formulation. Anionic and/or nonionic surface active agents may be employed including,

5 for example, sodium lauryl sulfate, sodium alkylbenzene-sulfonate, sodium sulfosuccinate, sodium salt of ligninsulfonic acid, polyvinylpyrrolidone and its water-soluble alkylated derivatives, polyoxyethylene fatty alcohol ethers and other well-known surfactants.

10 Antifoam agents, e.g., ecologically safe defoamers meeting federal regulations, may be included. Various inert carriers or diluents are useful for the formulations including water-soluble or dispersible solids, e.g., sucrose, soluble starches, sugars,

15 absorbent graft copolymers of polyhydroxy polymers, acrylonitrile and acrylic comonomers. Other adjuvants well known in the agricultural formulation field may also be used in the formulation of this invention to improve or hasten the effectiveness of bivalve mollusk control in

20 the process of this invention.

It is further contemplated that other molluscicides and aquatic pesticides may be incorporated in the formulations containing the amine salts used for bivalve mollusk control and control of other aquatic pests.

25 Granular formulations may be prepared using the active tertiary amine of this invention along with the

5 usual adjuvants and carriers, i.e., clays and
surfactants.

The toxicity of the amine salt of this invention to
bivalve mollusks is dependent on the rate of application
and the time period of exposure. Exposure of attached
10 bivalve mollusks under static conditions to from more
than 0.5 up to about 10.0 parts per million (ppm) of the
active amine salt for a time period of from about 1 to 24
hours (the lower the concentration, the longer the time)
will serve to release or cause detachment of substantial
15 amounts of bivalve mollusks from the solid to which they
are attached, generally by lethal reaction. Preferably,
a rate of about 1.5 to 3 ppm over about 4 to about 8
hours, more preferably, a rate of about 1.5 to 2 ppm for
about 6 to 8 hours, is applied to effect clean out of the
20 mollusk fouled zone. Under dynamic conditions, for
example, when a discharge or intake pipe is obstructed
with clumps of bivalve mollusks, the amine salt is
incorporated into the moving or running water at a rate
which will substantially continuously subject the
25 mollusks to exposure to the toxic amine salt in the
amounts stated for the static conditions and for the same

5 length of time. That is, sufficient active agent is
dripped, pumped or otherwise injected into the flowing
water of the water-way to be treated to produce a
substantially continuous concentration of the agent,
proximate the attached mollusks of over 0.5 up to about
10 10.0 ppm for from 1 to 20 hours, preferably about 1.5 to
3 ppm for 4 to 8 hours, more preferably about 1.5 to 2
ppm for 6 to 8 hours. On completion of this treatment, a
substantial amount of the mollusks will have detached by
release of their attachment means from the solid surface
15 to which they were attached, and, if the water flow rate
is sufficient and unrestricted, will have washed away.

After clean out or removal of the obstructing or
fouling mollusks, the area may be maintained in
substantially clean condition by a maintenance dosage
20 procedure involving the introduction of the molluscicide
into the channel, conduit or other confined waterway, or
into a large body of water at least proximate the solid
surface sought to be maintained free of attachment of
veligers, juveniles and mature bivalve mollusks, at a
25 rate and time sequence effective to accomplish this. A
rate of introduction of about 0.2 to about 1 ppm

5 continuously over from about 1 to 4 hours, and then the
dose repeated every 8 to 16 hours. Preferably, this
dosage procedure is for from about 0.4 to about 0.6 ppm
continuously for 1.5 to about 2.5 hours repeated every 10
to 14 hours. Of course, these prescribed rates and times
10 relate to the degree of toxicity of the active
molluscicide. For example, if one employs a ditertiary
amine salt rather than the more active monotertiary amine
salt, reduced amounts and shorter times will be
applicable and these are to be determined empirically for
15 each amine salt disclosed by referencing the amounts
prescribed for the preferred compound.

While the preferred method for maintaining an
underwater free of bivalve mollusks in an infested area
is to use the clean-out process of this invention by
20 introducing into the water to flow proximate to a solid
to which the mollusks are attached, an effective amount
of the prescribed tertiary amine salt at a rate and
length of time sufficient to cause detachment of the
mussels. For freeing underwater solid surfaces from
25 mollusk attachment, other chemical, physical and
mechanical, or combinations of these treatments, may be

5 used for first eradicating the mollusks prior to using
the prescribed maintenance dosage program for maintaining
an area free of bivalve mollusk settlement. Mechanical
scrapers and reamers may be used to clean the mollusks
from the subsurface to which they are attached. Heat
10 treatment of the underwater solids at temperatures in
excess of 35° C for several hours is lethal to mollusks
of the Zebra mussel variety. Shock generated by an
electrical charge in the infested area, while expensive,
will detach the mollusks and veligers from an underwater
15 solid.

 An advantage for the amine salt of this invention is
that, particularly for concentrations below about 0.5
ppm, it does not require a holding period or deactivation
after use. It does not hydrolyze or photolyze in an
20 aqueous environment. Additionally, the tertiary amine,
at the prescribed treatment concentration, cleans scale,
slime, algae and other deposits from intake and outlet
conduits through which it passes.

 There are at least three areas which can be compared
25 that clearly set the amine salt of this invention, apart
from commercially used quaternary ammonium compounds,

5 i.e., quats. These are explained in the following
numbered paragraphs 1 - 3.

1. Aquatic Species Toxicity

LC50/EC50 values are the concentration of a
material required to cause 50% mortality in a test
10 population. The numbers below were taken from 96-hour
flow through studies which is the standard test required
by EPA. This means that the test material* was injected
as a flow through (constant level of exposure) treatment
for a period of 96 hours. It is believed that this type
15 of testing produces biased (less favorable) results for
the amine salt because the half life of the amine salt is
considerably less than 96 hours and the species exposed
are subject to one short exposure followed by rapid
dissipation, not a constant level of exposure. It is not
20 expected that there will be much difference in the
toxicity level of the quats when comparing 24- and 96-
hour exposures.

5 Results of 96-hour flow through aquatic studies

LC50/EC50 values expressed as mg/l:

	<u>Test species</u>	<u>TD Amine Salt*</u>	<u>Quat</u>
	Daphnid	.32	.02
10	Shrimp	2.4	.08
	Minnow	3.5	.36
	Bluegill	1.7	.88
	Trout	1.0	1.0
15	Differences vary from a factor of 30 to 1 for the Daphnid and Shrimp down to 2 to 1 for the Bluegill. The difference of 0 for the Trout is believed to be an aberration.		

20 * -mono-tertiary-dimethylcocoamine salt of endothall

2. Aquatic Half Life

From biological observations, the half life of the preferred amine salt of this invention is in the 24-hour range. The half life of a commercially used quat, available as CP-4 and CT-2 (Clam-trol®) composed of three cationic surfactants (N-alkyl dimethyl-benzyl ammonium chloride), is believed to be 28 days (Technical Session - International Zebra Mussel Conference in Toronto, Canada,

25

5 1995). The half life difference between the amine salt
of this invention and CP-4 is 28 fold.

3. Release Rates of Zebra Mussels

Plant operators who have used the amine salt of
this invention in an experimental program, e.g., as
10 reported in the following examples, and who have used
quats in commercial applications to eliminate Zebra
mussel attachments from plant water systems, indicate
that release of the mollusks with the use of quats is
from 24 to 48 hours after treatment while they
15 consistently report an 8 to 12 hour release period for
the mollusks after treatment with the amine salt of this
invention.

The plant effluent containing the quats must be
expensively treated for decontamination by passing it
20 through beds of bentonite clay to deactivate before
discharge.

The process of this invention is most effective in
waters in the temperature range of about 18° C up to
about 25° C, preferably 20-22° C. for the control of
25 Zebra mussel.

5 The following examples are set forth to demonstrate
the effectiveness of the present invention.

EXAMPLE 1

A synthetic tertiary, mixed C₈ - C₂₀ alkyldimethyl
amine* salt of hydrochloric acid, at a formulation
10 concentrating of above 53% by weight, was tested for its
effectiveness for the elimination of Zebra mussels by an
independent university laboratory experienced in the
testing of molluscicides. The toxicity of the above
described amine salt to Dreissena polymorpha (Zebra
15 mussels) was tested using both the Spearman-Kärber (S-K)
method () and the Litchfield-Wilcoxon (L-W)
method (), both at 17°C. The results of
the tests, which report lethal concentration (LC50) for
large and small mussels, are set forth in the following
20 Table 1.

*-ADMA™ WC Alkyldimethylamine for Albemarle Corporation

5

Table 1

	<u>S-K Method</u>	<u>L-W Method</u>
<u>MUSSEL SHELL LENGTH, 5-8 mm</u>		
24 hours - LC50*, ppm	1.17 (0.89-1.55)**	1.44 (1.06-1.96)
48 hours - LC50, ppm	0.44 (0.32-0.59)	0.51 (0.33-0.78)
<u>MUSSEL SHELL LENGTH, 20-25 mm</u>		
24 hours - LC50, ppm	2.03 (1.67-2.46)	2.0 (1.45-2.75)
48 hours - LC50, ppm	1.37 (1.19-1.57)	1.35 (1.16-1.58)

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* - Concentration of molluscicides which is lethal to 50% of the test species in period of test time.

** - Range in brackets is the 95% confidence limits of lethal ppm.

30

Additional experiments were conducted by the same independent laboratory referred to above in which Zebra mussels were exposed to a single concentration of the hydrochloric acid amine salt for varying periods of time to determine toxicity results (lethal times) for large and small mussels.

35

5 The data developed are summarized in Tables 2 and 3
below.

Table 2

Mortality %

10

Time

(hours)

6 24 30 48 72 96 120

Amine Salt Conc., ppm

Mussel shell length, 0.5 - 0.8 cm

15

0	0	0	0	0	0	0	0
0.25	0	0	0	0	2	4	14
0.50	6	12	20	36	50	62	66
1.00	6	76	82	96	100		

20

Mussel shell length, 2.0 - 2.5 cm

0	0	0	0	0	0	0	13
0.25	0	0	0	0	2	4	6
0.50	0	0	2	4	10	20	32
1.00	4	32	38	80	100		

25

Chemical solutions were renewed every 24 hours (5 replicates, 10 mussels per replicate).

30

The mean lethal time (LT50) of the hydrochlorine
acid salt of tertiary C₈ - C₂₀ alkyldimethylamine to Zebra

5 mussels of the different sizes was tested in accordance with the method of Litchfield and Wilcoxon (1949) by the independent research laboratory producing the results of Table 3 below.

Table 3

	<u>Mussel shell length</u>	<u>t-amine salt conc.</u>	<u>LT50(hr)</u>	<u>95% Confidence limits</u>
	0.5 - 0.8 cm	0.5	72	60 - 87
	0.5 - 0.8 cm	1.0	15.5	12 - 20
15	2.0 - 2.5 cm	1.0	33	30 - 37

Independent Testing Laboratory Comments: It was not possible to estimate an LT50 for each exposure concentration because, in several cases, mortality was either too high or too low. However, at an exposure concentration of 1.0 ppm, LT50's of 33 hours and 15.5 hours were estimated for large and small mussels, respectively, as shown in Table 3.

Furthermore, an LT50 of 72 hours was estimated for small mussels at an exposure concentration of 0.5 ppm. Thus, it should take exposure times of 72 hours to result in 100% mortality of both large and small mussels at an exposure concentration of 1.0 ppm.

Based on a battery of 30 or so chemicals tested by the independent testing laboratory, it observed that the hydrochlorine acid salt of the tertiary C_8 - C_{20}

5 alkyldimethylamine employed in this example is about as toxic
as chlorine and substantially more toxic to Zebra mussels than
certain other molluscicides which are commercially available,
e.g., Clam-Trol® CT1 and Calgon® H-130.

10

EXAMPLE 2

A field efficacy study was conducted to evaluate the
appropriate concentration of mono(N,N-dimethylcocoamine)
salt of endothall (3,6-endoxohexahydroorthophthalic acid)
(HYDROTHOL® 191 - a.i. 53 wt. %) required to control
15 Zebra mussels in the Cleveland Electric Eastlake Power
Plant on Lake Erie.

Clumps of viable Zebra mussels were added to each of
four (4) bioboxes, which had inside dimensions of 16-3/4
x 10-3/4 x 9 inches, constructed of plexiglass and
20 silicone adhesive with a 2-1/2 inches high water intake
tube. The water depth was maintained at 7-1/2 inches by
a 7-1/2 inches high drain. Two (2) of these bioboxes
were connected into the house service water system and
two (2) bioboxes were connected into the low pressure
25 service water system of the plant. Injection points for
a formulation of the above-identified endothall salt (53%

5 active ingredient) were located on the suction side of
the eight (8) house service water pumps used in the
system. The injection pumps were calibrated to deliver
an endothall salt concentration of 2 ppm. The low
pressure service water system was not treated, the two
10 bioboxes attached to this part of the system serving as
untreated controls.

At six (6) hours after injection began, mussels
within the treated bioboxes appeared to be gapped open,
not filtering and nonresponsive. After eight hours, the
15 treated bioboxes installed in the house service system
were opened and Zebra mussels were removed for further
evaluation. All mussels from the treated bioboxes were
considered nonviable. Mussels from the untreated
bioboxes installed in the low pressure service system
20 appeared normal, showed active filtering and responded to
gentle probing.

Analytical results showed an average concentration
of endothall salt in the first treated biobox of 0.70 ppm
and in the second treated biobox of 1.72 ppm over the
25 eight hour treatment period. Based on the analytical
results of the filter house samples of the power plant

5 system during the test, the overall concentration of
endothall salt in the plant discharge channel was
calculated to be 0.014 ppm.

The effective concentration of endothall salt for
the control of Zebra mussels in the above test during a 6
10 to 8 hour treatment period is 1.0 to 1.5 ppm.

EXAMPLE 3

Another field efficacy study with the mono(N,N-
dimethylcocoamine) salt of endothall (as in Example 2)
was conducted at the Toledo Edison Bay Shore Station,
15 which is about five miles east of downtown Toledo, Ohio,
at the western end of Lake Erie at the mouth of the
Maumee River.

Clumps of viable Zebra mussels were added to each of
three bioboxes, as described in Example 2, that were
20 connected to the water flow pipes at separate sites
within the power plant. The bioboxes were equilibrated
for several days prior to the initiation of study.
Injection points for the endothall salt formulation (53%
active) were located on the suction side of the raw water
25 pumps and on a service water header. The injection pumps

5 were calibrated to deliver a constant endothall salt
concentration of 3 ppm. The study was planned for a 48
hour exposure.

Water samples were taken from the bioboxes, the
cooling water tank and two (2) Maumee River effluent
10 sites. Concentrations of the endothall salt were
analytically measured for each of these sites at various
intervals by taking samples from the first four mentioned
sites at 0, 1, 2, 4 and 8 hours while samples were taken
from the two Maumee River effluent sites at 0, 4, 8, 12,
15 16, 24, 28, 32 and 36 hours. Additionally, water samples
were taken from the second Maumee River site at 44, 52,
60, 68, 76 and 84 hours.

At 1, 2 and 4 hours after initiation of the
treatment, the Zebra mussels in the bioboxes appeared
20 viable. At the 8 hour interval, all mussels within
bioboxes 1, 2 and 3 were gapped open and nonresponsive to
external stimuli. The bioboxes were opened and the
mussels removed for further evaluation. All mussels from
biobox 2 were considered nonviable while other mussels
25 showed slow response and were not expected to survive.

Static bioassays were conducted on the samples taken

5 from the Maumee River sites. These tests were standard
48 hour *Daphnia magna* and 96 hour fathead minnow studies
as specified under the U.S. Environmental Protection
Agency. The tests were conducted with 100% effluents.
None of the bioassay samples was acutely toxic to
10 *Ceriodaphnid* or fathead minnows.

EXAMPLE 4

Still another field study was performed to determine
the efficacy of low-level intermittent treatments of
mono(N,N- dimethylcocoamine) salt of endothall to inhibit
15 primary and secondary settlement of Zebra mussels under
field conditions.

The study was conducted at Ontario Hydro's Zebra
Mussel Research Facility on Lake Erie at Nanticoke,
Ontario. This site has large numbers of veligers and
20 translocating juveniles (larva stages of mussels). Tests
were performed in 16 continuous flow through cells (91 cm
length x 13 cm diameter) with nominal residence times of
30 minutes to simulate worst-case settlement conditions
in generating station service water systems. Triplicate
25 treatments consisted of controls and intermittent dosing

5 of the cells with a formulation of the endothall salt
(53% active - available as HYDROTHOL® 191 Aquatic
Algicide and Herbicide from Elf Atochem North America,
Inc.) at a target level of 0.5 ppm (mg/L-active
ingredient) continuously for 2 hours and a repetition
10 every 12 hours to provide increasing daily intermittent
dosages of 0, 2, 4, 6 and 8 ppm. Over the 10-week
treatment period, measured endothall salt concentrations
were within 20% of the target amount. Inlet lake water
temperatures ranged from 18 to 27° C, the average veliger
15 density was 2130 per cubic centimeter and the average
flow rate through the test cells was 0.73 L/min.

Compared to untreated controls, the various treated
cells demonstrated a 71 to 100% reduction in the numbers
of live mussels attached to flow cell surfaces after a
20 10-week treatment period. One-way analysis of variance
(ANOVA) indicated that this difference was significant.
Further analysis using the LSD multiple range test at the
95% confidence level showed that each of the treatments
(2, 4, 6 and 8 ppm/hour) had resulted in significantly
25 fewer numbers of attached live mussels than the untreated
control and that different intermittent doses had similar

5 efficacy. Most live Quagga mussels were less than 2.5
mm. in length indicating likely attachment within the 2
to 3 weeks prior to trail completion. Translocating
mussels were not evident in the treated cells. The
unexpectedly low degree of settlement in control cells,
10 43 per square meter, accompanied by normally high natural
mortality may have contributed to the inability to
distinguish between treatments of increasing intermittent
dosage. When the present data was compared to a previous
trial carried out at daily intermittent dosages ranging
15 from 0.5 to 2 ppm/hour, a minimum value of about 2 ppm/h.
(0.5 mg/L for 2 hours every 6 hours) is suggested for
efficacious control of mussels.

Example 5

A comparative study of the efficacy of several
20 molluscicides including those meeting the description of
those claimed herein was performed by a commercial,
independent testing laboratory having expertise in this
field. The purpose of these test was to compare LT50's
and the times-to-an-effective-kill (80-90%) of the Zebra
25 mussel exposed to the test molluscicides currently being
used commercially, and several experimental materials,

5 for a total of eight (8) tests. Twenty-four hour static
exposure here conducted with adult Zebra mussels
(approximately 10 - 20 minutes). The physical system and
procedure used in carrying out the tests is described
below:

10 The test chambers were 90 X 50 mm glass
crystallizing dishes containing 200 mL of test solution.
Three replicates of 10 Zebra mussels each were maintained
for each exposure concentration and control. Mussels
were added impartially by introducing the mussels, two at
15 a time, to each dish until all dishes contained 10
mussels.

Dilution water was unadulterated water from a 100
meter bedrock well supplemented on demand with untreated
town well water characterized as soft water with a
20 typical total hardness of 20 - 40 mg/L as CaCO_3 and an
alkalinity of 20 - 35 mg/L CaCO_3 . The pH range is from
6.9 to 7.5 and the specific conductance range is 80 - 150
micromhos/cm. These parameters were monitored weekly to
assure that they remained in the typical ranges. Total
25 hardness and alkalinity were determined according to
Standard Methods for the Examination of Water and
Wastewater (APHA, 1989). Periodic analysis of

5 representative samples were conducted to assure the
absence of substances harmful to aquatic organisms.

The water temperature of the test solutions was
maintained at approximately 20°C by ambient laboratory
(controlled) temperature. The photoperiod was maintained
10 at 16 hours light, 8 hours darkness using an automatic
timer. Total dissolved oxygen concentration was >80% of
saturation during the tests. The Zebra mussels were
placed into the exposure solutions within 30 minutes of
their preparation.

15 At test initiation, duplicate water samples of an
appropriate volume were taken from each concentration and
control solution (before distribution to the exposure
vessels) for determination of test material
concentration. Ion chromatography was used to analyze
20 Endothall (Tests #1 - #3). Amperometric titration was used
for chlorine (Test #4). Spectrophotometric methods were
used for poly DMDAAC (polyquat) and Clam-Trol® (Tests #5
and #6) and GC-NPD method was used for the amines (Tests
#7 and #8). All samples were taken from a point
25 approximately midway between the surface, bottom and
sides of each test vessel.

5 Biological observations were made for mortality and sublethal effects (e.g., closed or gaped shells).

Mortalities were recorded and removed when observed.

LT50 results were determined by observations made and recorded at 0,2,4,6,8,10,12,16,20 and 24 hours post-treatment. Mortality data derived from the tests were used to statistically estimate at median lethal time (LT50) and the 95% confidence interval for each concentration of each material tested. The LT50 is the time (hours) which produces 50% mortality in the tests organism population at the stated test material concentration. A computer program used to calculate LC50 (lethal concentration to produce 50% mortality in the test organism) values was used to calculate the LT50 values.

20 The results are summarized in the following table.

Table 5

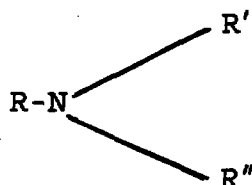
<u>Active Test Material</u>	<u>Measured Concentration</u> <u>(mg/L)</u>	<u>LT50</u> <u>(hrs.)</u>
25 1. Cocoamine-Endothall salt of Example 2 (5ppm acid eqv.)	6.5	6.0
30 2. Cocoamine HCl salt (5ppm acid equiv.)	9.9	5.8

5	3. Synthetic mixed t-C ₈ -C ₂₀ Alkyl dimethylamine	7.4	5.5
	-Endothall salt (5 ppm acid eqv.)	4.5	10.9
10	4. Synthetic mixed t-C ₂ -C ₂₀ alkyl dimethylamine - HCl salt of Example 1 (5 ppm acid eqv.)	11	5.2
	5. Dipotassium Salt of Endothall (AQUATHOL®k)	460	8.5
15		250	13.0
	6. Clam-Trol® CT-2 (Betz Labs) (N-alkyl dimethylbenzyl ammonium chloride)	6.6	13.0
		4.0	>24.0
20	7. Polyquat (Calgon Corporation) [poly (dimethyldiallyl ammonium chloride)]	8.3	>24.0
25	8. Chlorine	2.5	>24.0

The nominal concentrations of the Endothall salts were intended to be 5ppm as Endothall acid. The amine-hydrochloride salt concentrations were calculated to be the equivalent amount of amine at 5 ppm Endothall acid in the amine-Endothall salt. Clam-Trol® and Polyquat concentrations are based on total product. It is apparent from the above-reported static tests that the t-amine salts of the present invention are significantly effective against Zebra mussel infestation and substantial more effective than commercial molluscicides particularly at lower dosage.

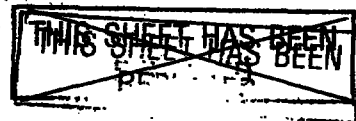
5 CLAIMS

1. A method for the control of bivalve mollusks to effect detachment from and to inhibit settlement on solids immersed in water inhabited by said mollusks comprising introducing into said water an amine salt of
10 an inorganic or organic acid, the amine radical of said salt derived from an amine having the formula:



where R is an aliphatic hydrocarbon group containing from about 1 to about 20 carbon atoms and R' and R'' are the same or different alkyl radicals having from 1 to about
20 20 carbon atoms, and mixtures of said amine radicals, said amine salt being introduced at a rate and length of time to at least inhibit settlement of the larva stage of said mollusk.

2. The method of claim 1 wherein said inorganic
25 acid has the formula HX where X is chlorine, bromine, iodine, $-\text{HSO}_4$, $-\text{NO}_3$ or H_2PO_4 .





5 3. The method of claim 1 wherein said acid is a
mono- or polybasic carboxylic acid containing from 1 to
24 carbon atoms.

 4. The method of claim 1 wherein R ranges from 8
to 20 carbon atoms and R' and R'' range from 1 to 8 carbon
10 atoms.

 5. The method of claim 1 wherein said rate ranges
from about 0.25 to about 4 ppm and said length of time is
from about 1 to 16 hours.

 6. The method of claim 4 wherein said amine salt
15 is the monoamine salt.

 7. The method of claim 4 wherein said amine salt
is the diamine salt.

 8. The method of claim 6 wherein said rate ranges
from about 1 to 4 ppm and the length of time is from
20 about 6 to about 8 hours.

 9. The method of claim 1 wherein said rate ranges
from about 0.25 to about 1 ppm and said length of time is
from about 1 to 3 hours and this dosage is repeated every
8 to 16 hours.

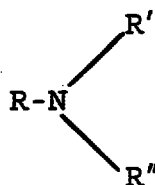


5 10. The method of claim 1 wherein R is cocoamine, R' and R'' are each methyl and the amine salt is a monoamine salt.

11. The method of claim 10 wherein said rate is about 1.5 to about 2 ppm for a length of time of about 6
10 to 8 hours.

12. A method for the continuous control of bivalve mollusks to effect detachment from and inhibit settlement on a solid immersed in water inhabited by said mollusks comprising the steps of (a) removing bivalve mollusk
15 attachments from said solid by any means, and (b) thereafter introducing into said water an amine salt of an acid, the amine radical of said salt derived from an amine having the formula:

20



where R is an aliphatic hydrocarbon group containing from about 1 to about 20 carbon atoms and R' and R'' are the
25 same or different alkyl radical having 1 to about 20 carbon atoms, and mixtures of said amine radicals, the



5 introduction of said amine salt being at a rate and for a
length of time at least sufficient to inhibit the
settlement of larva stage mollusks on said solid.

13. The method of claim 12 wherein said acid has
the general formula HX where X is chlorine, bromine,
10 iodine, $-\text{HSO}_4$, $-\text{NO}_3$ or $-\text{H}_2\text{PO}_4$.

14. The method of claim 12 wherein said acid is a
mono- or polybasic carboxylic acid containing 1 to 24
carbon atoms.

15. The method of claim 12 wherein step (a) is
15 accomplished by the introduction into said water of the
amine salt introduced in step (b) but at a dosage rate
and length of time at least sufficient to cause the
attached mollusks to detach.

16. The method of claim 12 wherein said acid is
20 3,6-endoxohydroorthophthalic acid.

17. The method of claim 16 wherein R is cocoamine,
R' and R'' are each methyl and said amine salt is a
monoamine salt.





5 18. The method of claim 17 wherein step (a) is accomplished by the introduction into said water of the amine salt introduced in step (b) but at a dosage rate and length of time at least sufficient to cause said attached mollusks to detach.

10 19. The method of claim 12 wherein said rate is from about 0.25 to about 1 ppm, said length of time ranges from about 1 to 3 hours, and the dosage is repeated every 8 to 16 hours.

15 20. The method of claim 17 wherein the dosage rate is about 1 to 4 ppm and said length of time is from about 6 to about 8 hours.

20 21. A method for the continuous control of bivalve mollusks to effect detachment and inhibit settlement on a solid immersed in water inhabited by said mollusks comprising the steps of (a) removing bivalve mollusk attachments from said solid by any means, and b) thereafter introducing into said water a bivalve mulluscicide at a rate and for a length of time at least sufficient to inhibit the settlement of larva stage
25 bivalve mollusks on said solid.

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- 5 22. The method of claim 21 wherein step (a) is accomplished by the introduction into said water of a bivalve molluscicide in an amount and for a length of time sufficient to cause the attached mollusks to detach.

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ABSTRACT OF THE DISCLOSURE

A process is disclosed for the eradication of and subsequent inhibition of bivalve mollusk settlement on solid surfaces immersed in water inhabited by said mollusk comprising introducing into said water, a specified tertiary amine salt at a rate and for a time at least sufficient to inhibit settlement of said mollusk.

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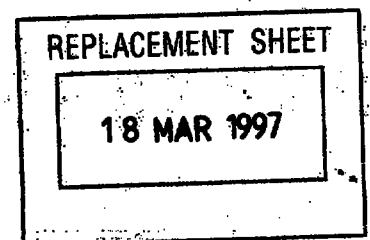
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ELIMINATION AND INHIBITION OF BIVALVE MOLLUSK
ATTACHMENTSABSTRACT OF THE DISCLOSURE

A process is disclosed for the eradication of and subsequent inhibition of bivalve mollusk settlement on solid surfaces immersed in water inhabited by said mollusk comprising introducing into said water, a specified tertiary amine salt at a rate and for a time at least sufficient to inhibit settlement of said mollusk.



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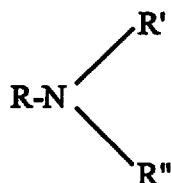
respectively to kill Puerto Rican snails. Chlorine, generated, for example, by the introduction of chlorine gas or sodium hypochlorite into the infested water, has been the prevalent control agent for mollusks. While the known chemical treatments for the control of Zebra mussels are generally effective, their use can result in undesirable environmental consequences, particularly with respect to persistence and toxicity to nontarget organisms.

Tertiary amine salts of 3,6 endoxhydroorthophthalic acid are disclosed in U.S. Patent No. 3,207,593, issued September 21, 1965, as effective for the control of submersed aquatic plant life.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

STATEMENT OF THE INVENTION

In one embodiment the present invention provides a method for the control of bivalve mollusks to effect detachment from and inhibit settlement on solids immersed in water inhabited by said mollusks comprising introducing into said water an amine salt of an inorganic or organic acid, the amine radical of said salt derived from an amine having the formula:

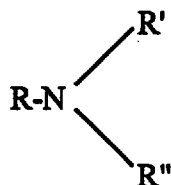


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where R is an aliphatic hydrocarbon group containing from about 1 to about 20 carbon atoms and R' and R'' are the same or different alkyl radicals having from 1 to about 20 carbon atoms, and mixtures of said amine radicals, said amine salt
5 being introduced at a rate and length of time to at least inhibit settlement of the larva stage of said mollusk; with the proviso that the organic acid is not a boric acid.

According to another embodiment of the invention there is provided a method for the continuous control of bivalve
10 mollusks to effect detachment from and inhibit settlement on a solid immersed in water inhabited by said mollusks comprising the steps of (a) removing bivalve mollusk attachments from said solid by any means, and (b) thereafter introducing into said water an amine salt of an acid, the amine radical of said
15 salt derived from an amine having the formula:



20 where R is an aliphatic hydrocarbon group containing from about 1 to about 20 carbon atoms and R' and R'' are the same or different alkyl radical having 1 to about 20 carbon atoms, and
25 mixtures of said amine radicals, the introduction of said amine salt being at a rate and for a length of time at least sufficient to inhibit the settlement of larva stage mollusks on said solid; with the proviso that the acid is not a boric acid.

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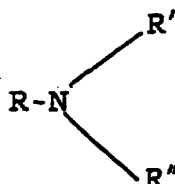
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DETAILED DESCRIPTION OF THE INVENTION

Bivalve mollusks in various stages of maturity are eradicated in accordance with this invention by exposure for a period of time to tertiary amine salts wherein the amine radical is derived from an amine of the following formula:

10

15



20

25

where R is an aliphatic hydrocarbon group containing from about 1 to about 20 carbon atoms and R' and R'' are the same or different alkyl radicals having from 1 to about 20, preferably from 1 to 8 carbon atoms. Examples of these alkyl radicals include methyl, ethyl, propyl, butyl, hexyl, octyl, decyl, dodecyl, octadecyl, eicosyl, and isomers thereof. The aliphatic hydrocarbons represented by R include, for example, the C₁-C₂₀ alkyl radicals described above for R' and R'' as well as those groups on amines obtained by reductive amination of the acids from animal fats and vegetable oils, particularly

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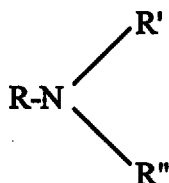
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CLAIMS

1. A method for the control of bivalve mollusks to effect detachment from and to inhibit settlement on solids
5 immersed in water inhabited by said mollusks comprising introducing into said water an amine salt of an inorganic or organic acid, the amine radical of said salt derived from an amine having the formula:

10



15 where R is an aliphatic hydrocarbon group containing from about 1 to about 20 carbon atoms and R' and R'' are the same or different alkyl radicals having from 1 to about 20 carbon atoms, and mixtures of said amine radicals, said amine salt
20 being introduced at a rate and length of time to at least inhibit settlement of the larva stage of said mollusk; with the proviso that the inorganic acid is not a boric acid.

2. The method of claim 1 wherein said inorganic acid has the formula HX where X is chlorine, bromine, iodine, -
25 HSO₄, -NO₃ or H₂PO₄.

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introduction of said amine salt being at a rate and for a length of time at least sufficient to inhibit the settlement of larva stage mollusks on said solid; with the proviso that the acid is not a boric acid.

5

13. The method of claim 12 wherein said acid has the general formula HX where X is chlorine, bromine, iodine, $-HSO_4$, $-NO_3$ or $-H_2PO_4$.

10

14. The method of claim 12 wherein said acid is a mono- or polybasic carboxylic acid containing 1 to 24 carbon atoms.

15. The method of claim 12 wherein step (a) is accomplished by the introduction into said water of the amine salt introduced in step (b) but at a dosage rate and length of time at least sufficient to cause the attached mollusks to detach.

16. The method of claim 12 wherein said acid is 3,6-20 endooxohydroorthophthalic acid.

17. The method of claim 16 wherein R is a cocoamine, R' and R'' are each methyl and said amine salt is a monoamine salt.

25

18. The method of claim 17 wherein step (a) is accomplished by the introduction into said water of the amine salt introduced in step (b) but at a dosage rate and length of time at least sufficient to cause said attached mollusks to 30 detach.

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19. The method of claim 12 wherein said rate is from about 0.25 to about 1 ppm, said length of time ranges from about 1 to 3 hours, and the dosage is repeated every 8 to 16 hours.

5

20. The method of claim 17 wherein the dosage rate is about 1 to about 4 ppm and said length of time is from about 6 to about 8 hours.

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