Image Cover Sheet

CLASSIFICATION	SYSTEM NUMBER 507720
UNCLASSIFIED	

TITLE

A SUMMARY OF BIOCIDE EFFICACY TRIALS ON CANDIDATE BIOCIDES FOR THE CONTROL OF MICROBIOLOGICAL CONTAMINATION IN DISTILLATE FUELS

System Number:

Patron Number:

Requester:

Notes:

DSIS Use only:

Deliver to:

National Defence
Research and Development Branch

Défense nationale
Bureau de recherche et développement

TECHNICAL MEMORANDUM 98/209
February 1998

A SUMMARY OF BIOCIDE EFFICACY TRIALS ON CANDIDATE BIOCIDES FOR THE CONTROL OF MICROBIOLOGICAL CONTAMINATION IN DISTILLATE FUELS

R. D. Haggett and R. M. Morchat

Defence Research Establishment Atlantic

Centre de Recherches pour la Défense Atlantique

DEFENSIO PER SCIENTIAM

Canada

DEFENCE RESEARCH ESTABLISHMENT ATLANTIC

9 GROVE STREET

P.O. BOX 1012
DARTMOUTH, N.S.
B2Y 3Z7

TELEPHONE
(902) 426-3100

CENTRE DE RECHERCHES POUR LA DÉFENSE ATLANTIQUE

9 GROVE STREET

C.P. BOX 1012
DARTMOUTH, N.É.
B2Y 3Z7

National Defence
Research and Development Branch

Défense nationale
Bureau de recherche et développement

A SUMMARY OF BIOCIDE EFFICACY TRIALS ON CANDIDATE BIOCIDES FOR THE CONTROL OF MICROBIOLOGICAL CONTAMINATION IN DISTILLATE FUELS

R. D. Haggett and R. M. Morchat

February 1998

Approved by R. M. Morchat: R Morchat
Acting Head/ Dockyard Laboratory (Atlantic)

TECHNICAL MEMORANDUM 98/209

Defence Research Establishment Atlantic

Centre de Recherches pour la Défense Atlantique

DEFENSIO PER SCIENTIAM

Canada

ABSTRACT

This report summarizes the results of biocide efficacy trials conducted over an eight year period (1984-1992) by Defence Research Establishment Atlantic, Dockyard Laboratory. The purpose of these trials was to determine which biocide or biocidal agent was most effective in the control or elimination of microbiological contamination (MBC) in distillate fuel systems.

Growth conditions, culture population (mixed or pure), percent free water present and sampling techniques were all found to have an effect on the measured efficacy of a biocide. In general the efficacy trial results indicated that biocides containing chlorinated and non-chlorinated isothiazolin-3 ones as the active ingredients were the most effective growth inhibitors of pure and mixed microbial cultures. Biocides containing organ-borate, as the active ingredient, exhibited the lowest efficacy. The effectiveness of the organo-borates was greatly influenced by the amount of free water present. The routine use of diethylene glycol monomethyl ether (DiEGME) combined with the practice of good fuel husbandry appears to be a suitable alternative to biocide use in systems where large volumes of free water are unlikely to be present.

Although biocides are an effective means to control or eliminate MBC in distillate fuel systems and, in some cases, may be the only available course of action, careful consideration must be given to alternative programs as well as all health and environmental risks associated with biocide use before a treatment program is initiated.

RÉSUMÉ

Ce rapport est un résumé des résultats d'essais d'efficacité de biocides réalisés sur une période de huit ans (1984 - 1992) par le laboratoire de l'arsenal maritime du Centre de recherches pour la Défense (Atlantique). Ces essais avaient pour objet de déterminer quel était le biocide ou agent biocide le plus efficace pour réduire ou éliminer la contamination microbiologique (MBC) dans les mazouts légers.

Il s'est avéré que les conditions de croissance, la population cultivée (mixte ou pure), le pourcentage d'eau libre présent et les techniques d'échantillonnage avaient tous un effet sur l'efficacité mesurée des biocides. En général, les résultats des essais d'efficacité indiquent que les biocides dans lesquels des isothiazolin-3-ones chlorées ou non chlorées étaient l'ingrédient actif inhibitaient le mieux la croissance des cultures microbiennes mixtes ou pures. Les biocides dont l'ingrédient actif était un organoborate étaient les moins efficaces. L'efficacité des organoborates variait fortement en fonction de la quantité d'eau libre présente. Une utilisation de routine d l'éther monométhylique du diéthylèneglycol (DiEGME) réunie à la pratique d'une bonne gestion du carburant semble être une solution de rechange acceptable à l'utilisation de biocides dans les systèmes où de gros volumes d'eau libre ne risquent pas d'être présents.

Même si les biocides sont une méthode efficace de réduire ou d'éliminer la MBC dans les mazouts légers et qu'ils peuvent être la seule solution possible dans certains cas, il faut, avant de commencer un traitement, bien envisager les programmes de rechange ainsi que les risques pour la santé et l'environnement liés à l'utilisation de biocides.

Executive Summary

DREA TM/98/209

A Summary of Biocide Efficacy Trials on Candidate Biocides for the Control of Microbiological Contamination in Distillate Fuels
by
R. D. Haggett and R. M. Morchat

This report summarizes the results of biocide efficacy trials conducted over an eight year period (1984-1992) by Defence Research Establishment Atlantic, Dockyard Laboratory. The original work was initiated as a result of microbial contamination in the fuel tanks of Canadian Navy vessels. The purpose of these trials was to determine which biocide or biocidal agent was most effective in the control or elimination of microbiological contamination (MBC) in distillate fuel systems. It is well documented that MBC will have deleterious effects in a fuel system. The most dramatic are filter plugging, coalescer malfunction, degradation of protective coatings and gaskets, and corrosion of exposed metal surfaces. Eliminating fuel tank water bottoms and practicing proper fuel husbandry are the preferred and recommended means by which to prevent and control MBC. However, in cases of severe contamination, treatment of the affected fuel systems with a biocidal agent may be the only successful course of action .

Throughout the duration of this project an attempt was made to evaluate selected biocides and biocidal agents which were reported in the literature to be the most effective in controlling or eliminating MBC in distillate fuel. Chemicals with reported biocidal or biostatic effects were also evaluated to determine how effective they were compared to true biocides.

The results of these studies have indicated that the presence of mixed microbial populations poses entirely different implications for the addition of biocides than the single-species inocula. Tests of biocides on pure cultures of *H. resinae* or other monocultures are unreliable when determining biocide efficacy for any given fleet of vessels because they do not accurately reflect microbial growth in fuel tanks. Biocide tests on real tank MBC samples are the most realistic manner to evaluate effectiveness. Growth conditions, culture population (mixed or pure), percent free water present and sampling techniques were all found to have an effect on the measured efficacy of a biocide. In general the efficacy trial results indicated that biocides containing chlorinated and non-chlorinated isothiazolin-3-ones as the active ingredients were the most effective growth inhibitors of pure and mixed microbial cultures. Biocides containing organo-borate, as the active ingredient, exhibited the lowest efficacy. The effectiveness of the organo-borates was greatly influenced by the amount of free water present. The routine use of diethylene glycol monomethyl ether (DiEGME) combined with the practice of good fuel husbandry appears to be a suitable alternative to biocide use in systems where large volumes of free water are unlikely to be present.

The primary concerns with the use of any biocide are the effects of the active ingredients on the aquatic environment, when present in discharged water bottoms, as well as the health of personnel who may come in contact with treated fuel. Although biocides are an effective means to control or eliminate MBC in distillate fuel systems and, in some cases, may be the only available course of action, careful consideration must be given to alternative programs as well as all health and environmental risks associated with biocide use before a treatment program is initiated.

TABLE OF CONTENTS

1.0 INTRODUCTION

Microbiological contamination (MBC) of hydrocarbon fuels is a very general and widespread phenomenon. It has been reported (1) that very few stocks of uncontaminated kerosene-fraction fuels exist anywhere in the world. A variety of microorganisms have been identified in kerosene-fraction and distillate fuels. Traditionally the dominant organism has been *Hormoconis resinae* (formerly known as *Cladosporium resinae*) which is able to metabolize the C_9 - C_{19} carbon chains in fuel oils and grow profusely at the fuel/water interface to produce thick microbial mats and sludges. Other microorganisms such as the yeast *Candida sp.* are also common fuel contaminants. Numerous other fungi and bacteria (mainly *Pseudomonas*) have also been isolated from aerobic water layers. Under anaerobic conditions the bacterium *Desulfovibrio* can proliferate and induce pitting and corrosion in uncoated fuel tanks through the production of sulfides. The proliferation of microorganisms, and the relative dominance of fungi, yeast, aerobic bacteria or sulfate reducing bacteria, depends on many factors including; the presence of water, nutrients, pH, oxygen levels, salinity and ambient temperature.

Over the past two decades some Canadian Navy ships have had significant, albeit infrequent, problems with microbiological contamination in their shipboard fuel systems including the F-44 (JP-5) aviation turbine fuel system. It is well documented (1, 2) that MBC will have deleterious effects in a fuel system. The most dramatic are filter plugging, coalescer malfunction, degradation of protective coatings and gaskets, and corrosion of exposed metal surfaces. Eliminating fuel tank water bottoms and practicing proper fuel husbandry are the preferred and recommended means by which to prevent and control MBC. However, in cases of severe contamination, treatment of the affected fuel systems with a biocidal agent may be the only successful course of action.

Defence Research Establishment Atlantic (DREA) has been involved, since 1984, with research into the control and/or elimination of MBC in distillate fuels and fuel systems. The original work was initiated as a result of microbial contamination in the fuel tanks of Canadian Navy vessels. The purpose of this research was to determine the most effective way to combat MBC and included investigation of the use of biocides and biocidal agents and alternative methods of control. The results of efficacy studies conducted, under contract to DREA, on 12 biocides and biocidal agents (3, 4, 5, 6, 7) are reported in this paper.

2.0 BIOCIDES

Throughout the duration of this project (1984 to 1992) an attempt was made to evaluate selected biocides and biocidal agents which were reported in the literature to be the most effective in controlling or eliminating MBC in distillate fuel. The biocides and biocidal agents evaluated in this study are listed in Table 1 and are identified by their active ingredients. It can be seen from Table 1 that several of the biocides contain the same active ingredients. Biocides manufactured by different companies, but containing the same active ingredients (Biocides D and I and Biocides F, K and L), were included in this evaluation because the active ingredients were either present in different concentrations, in different matrices or were applied at different recommended dosages.

Chemicals with reported biocidal or biostatic effects were also evaluated to determine how effective they were compared to true biocides. One such chemical, diethylene glycol monomethyl ether (DiEGME), which is a common additive to military aviation turbine fuels as a fuel system icing inhibitor, is listed as Biocide G in Table 1 . DiEGME is reported to be a biostat (an agent which will inhibit growth but cannot eliminate growth once it has been initiated). There is no consensus as to how DiEGME inhibits MBC. Some feel there is a chemical inhibition of the microorganism while others suggest DiEGME combines with free water present thus preventing it from becoming a source of oxygen. Although oxygen is essential for growth, most mat forming fungi are able to satisfy their oxygen requirement by extracting oxygen from the water molecule. These organisms are known as facultative anaerobes. It was determined, based on experiments conducted with ethylene glycol monomethyl ether (EGME), that the effectiveness of EGME and DiEGME is inversely proportional to the amount of free water present (8). These findings support the theory that EGME and DiEGME have a preferential solubility for water and their biostatic effect is primarily due to combining with the free water to reduce available oxygen.

Other biocides which were evaluated were the organo-borate based compounds which are widely used by the commercial aviation industry to prevent MBC in aircraft fuel tanks. These chemicals were included because of their common usage in spite of literature reports which question their effectiveness when treated fuel is exposed to increased amounts of free water. It has been reported (8) that a change in the fuel : water ratio from 100:1 to 10:1 is sufficient to change the effect of 0.02 percent (200 ppm) organo-borates from growth inhibiting to growth stimulating.

Table 1

Identification of Biocides Used in Efficacy Studies by Active Ingredient

BIOCIDE	ACTIVE INGREDIENT	%
A	Tert-butylamine Omadine	NS
B	Formaldehyde (unreacted) 1-(2-Hydroxyethyl)-2-Alkyl(C_{18})-2-Imidazoline	0.12 25
C	Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate	NS
D	2,2'-oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane) 2,2'(1-methyltrimethylenedioxy) bis-(4-methyl 1-1,3,2-dioxaborinane)	95 (Total)
E	Poly [oxyethylene (dimethyliminio) ethylene (dimethyliminio)-ethylene dichloride]	NS
F	5-Chloro-2-methyl-4-isothiazolin-3-one 2-Methyl-4-isothiazolin-3-one	8.6 2.6
G	Diethylene glycol momomethyl ether	100
H	4-(2-Nitrobutyl) morpholone 4,4'-(2-Ethyl-2-nitrotrimethylene) dimorpholine 1-nitropropane	70 20 1.7
I	2,2'(1-methyltrimethylenedioxy) bis-(4-methyl 1-1,3,2-dioxaborinane) 2,2'-oxybis (4,4,6-trimethyl 1-1,3,2-dioxaborinane)	67.6 27.4
J	2,2'-dithio-bis (pyridine-1-oxide)	NS
K	5-Chloro-2-methyl-4-isothiazolin-3-one 2-Methyl-4-isothiazolin-3-one	1.15 0.35
L	5-Chloro-2-methyl-4-isothiazolin-3-one 2-Methyl -4-isothiazolin-3-one	1.15 0.35

NS = Not stated, assumed 100%

3.0 METHODS

3.1 Biocide Efficacy Laboratory Scale Trials

Laboratory scale trials were carried out to evaluate the effect of biocides and biocidal agents on ships-derived mixed microbial populations and pure cultures of *Hormoconis resinae.* Biocide A (Table 1) was used throughout this project as a biocide control.

The laboratory scale trials were carried out in 250 ml milk dilution bottles containing 95 ml of sterile Naval Distillate Fuel and 5 ml of sterile synthetic seawater (Instant Ocean - Aquarium Systems, Mentor, Ohio) supplemented with 0.05% of each of KH_2PO_4, $MGSO_4 \cdot 7H_2O$, $(NH_4)_2SO_4$, K_2HPO_4, and yeast extract.

The bottles were inoculated with 0.5 ml of either a pure culture of H. resinae or an inoculum derived from the MBC contaminated tanks of various marine vessels. The inocula were cultured for six weeks prior to use to get sufficient mass of MBC. All bottle tests were run in parallel. For each biocide 36 bottles (Triplicate x 3 concentrations x 4 intervals) were inoculated at time zero and nine bottles were tested at each interval (2, 4, 6, and 8 weeks). The biocides were administered at three concentrations based on total biocide product to fuel (by volume) recommended by the manufacturer. The concentrations used were; half maintenance dose, maintenance dose and twice maintenance dose. All bottles were incubated at 13-16°C in the dark. At each interval (2, 4, 6 and 8 weeks) the fuel layer was carefully decanted leaving 5 ml water, the Microbial mat and a minimum amount of fuel. Growth was determined as Total Protein (mg/bottle) by Sigma Procedure No. TPRO-562 (Sigma Chemical Company, St. Louis, Missouri, USA) as used in previous studies.

3.2 Biocide Efficacy Drum Scale Trials

During 1991/1992 trials were carried out over six months in 205 liter (45 gallon) drum scale tests in an effort to confirm that efficacy results observed in laboratory-scale short-term (8 week) trials could be reproduced in larger, longer term trials.

Six drums were loaded with 136 Liter of waste naval distillate from a wheeler barge. The fuel was not sterilized prior to use. Six Liters (5% by volume) of Halifax harbour seawater was added to each drum. The drums were then inoculated with a microbial mat which was cultured from growth taken from the fuel tank of a naval vessel.

The most effective biocidal agents (chlorinated and non-chlorinated isothiazolin-3-one), as determined from the laboratory studies, were then added to the fuel/water/MBC mixture at maintenance dosages. Two drums containing fuel and seawater but no biocide were used as controls.

The drums were held at 13-16°C and sampled at 2 weeks, 6 weeks, 10 weeks, etc., for the duration of the trial. The drums were sampled by three techniques; Bacon bomb sampler, Vampire pump and Kaliwassa tube. The Bacon bomb sampled the bottom of the drum, the Vampire pump removed a bottom sample by suction and the Kaliwassa tube provided an integrated drum sample. Once taken the samples were immediately transferred to 250 ml milk dilution bottles, allowed to settle and handled as per the laboratory scale trials.

Water bottom samples taken using the Bacon Bomb sampler were tested for sulfate (9), dissolved oxygen (10) and ammonia (10).

At the conclusion of the six month drum scale trial a portion of the contents of each drum was pumped through a Racor fuel filter unit used on most Canadian Forces ships. The maximum back pressure was noted and the filter was tested for microbial mass (as protein). The microbial mass was also cultured in an effort to identify and quantitate the resident species.

4.0 RESULTS

Because the most effective biocidal agents from each year were retested the following year a comparison may be made, with reasonable accuracy, of the overall effectiveness of all biocides tested. For the purpose of this summary, it is useful to compare laboratory bottle scale tests as percent effectiveness when compared to the control (no biocide). This is expressed as a percent of control bottle growth and the lower the percent growth the more effective the biocide. The relative biocide efficacies at half maintenance dose, maintenance dose and twice maintenance dose levels are shown in Table 2.

Table 2

Relative Biocide Efficacy at Maintenance Dose Levels

YEAR	TRIAL	BIOCIDE	CONC. (PPM)	EFFICACY	GROWTH AS % OF CONTROL AT 8 WEEKS
1985	*H. resinae*	A	1.0	minor inhibition	39
			1.5	minor inhibition	55
			2.1	major inhibition	0
		B	40	minor inhibition	106
			70	minor inhibition	37
			100	major inhibition	19
		C	5.0	major inhibition	0
			10	major inhibition	0
			20	major inhibition	0
		D	120	stimulation	111
			174	stimulation	105
			305	stimulation	116
	Mixed Tank Scale	A	1.0	no measurable inhibition	94
			1.5	" "	104
			2.1	" "	49
		B	40	no measurable inhibition	60
			70	" "	---
			100	" "	87
		C	5.0	no measurable inhibition	75
			10	" "	95
			20	" "	164
		D	120	no measurable inhibition	93
			174	" "	162
			305	" "	92

Table 2 - Continued

YEAR	TRIAL	BIOCIDE	CONC. (PPM)	EFFICACY	GROWTH AS % OF CONTROL AT 8 WEEKS
1986	*H. resinae*	A	2.0	inhibition	48 (6 weeks)
		E	125	minor inhibition	92 (6 weeks)
		F	10	total inhibition	0 (6 weeks)
		G	500	inhibition	29 (6 weeks)
	Destroyer Inoculum	A	2.0	total inhibition	81 (6 weeks)
		E	125	stimulation	120 (6 weeks)
		F	10	inhibition	62 (6 weeks)
		G	500	stimulation	110 (6 weeks)
1988	*H. resinae*	A	2.0	total inhibition	0
		F	10	total inhibition	0
		H	250	no effect	100
	Coast Guard Inoculum	A	2.0	inhibition	49
		F	10	total inhibition	0
		H	250	no effect	96
	C10/Y10	A/F	2.0/10	total inhibition	0
	C90/Y10	"	"	"	0
	C50/Y50	"	"	"	0
	C10/P90	"	"	"	0
	C90/P10	"	"	"	0
	C50/P50	"	"	"	0
	Y10/P90	"	"	"	0
	Y90/P10	"	"	"	0
	Y50/P50	"	"	"	0

Table 2 - Continued

YEAR	TRIAL	BIOCIDE	CONC. (PPM)	EFFICACY	GROWTH AS % OF CONTROL AT 8 WEEKS
1992	*H. resinae*	A	33	stimulation	119
		I	68	inhibition	49
			135	inhibition	16
			270	inhibition	7
		J	4	inhibition	28
			8	inhibition	28
			17	no effect	97
		K	5	inhibition	3
			10	inhibition	6
			20	inhibition	5
		L	35	inhibition	10
			70	inhibition	5
			140	inhibition	23
	Riverton Inoculum	A	33	no effect	91
		I	68	no effect	98
			135	no effect	100
			270	no effect	93
		J	4	no effect	106
			8	stimulation	120
			17	stimulation	143
		K	5	stimulation	110
			10	no effect	90
			20	inhibition	44
		L	35	inhibition	35
			70	inhibition	24
			140	inhibition	34

Table 2 - Continued

YEAR	TRIAL	BIOCIDE	CONC. (PPM)	EFFICACY	GROWTH AS % OF CONTROL AT 8 WEEKS
	Protecteur Inoculum	A	33	minor inhibition	71
		I	68	stimulation	120
			135	stimulation	124
			270	major stimulation	152
		J	4	no effect	89
			8	minor inhibition	84
			17	no effect	106
		K	5	no effect	106
			10	no effect	105
			20	inhibition	54
		L	35	inhibition	30
			70	inhibition	22
			140	inhibition	32
	Terra Nova Inoculum	A	33	minor inhibition	86
		I	68	stimulation	124
			135	stimulation	115
			270	no effect	100
		J	4	minor inhibition	78
			8	minor inhibition	78
			17	minor inhibition	85
		K	5	stimulation	111
			10	inhibition	53
			20	inhibition	64
		L	35	inhibition	27
			70	inhibition	23
			140	inhibition	27

Interpretation of the trial results indicate that the order of effectiveness of the biocides evaluated, from most to least effective would be: L-F-K-A-C-B-J-E-H-G-I-D as shown in Table 3. The results of both short and long term laboratory trials indicated that the most effective biocide overall was Biocide L followed by Biocides F and K. All three of these products contained chlorinated and non-chlorinated isothiazolin-3-one as the active ingredients but either in different concentrations or matrices (Table 4). It can be seen from Table 4 that Biocides L and K contain the same percent weight of active ingredient but Biocide L uses 90.0 weight percent dipropylene glycol as the matrix while Biocide K uses 95.63 weight percent water. Even though Biocide K is administered at a maintenance dosage seven times that of Biocide L (Table 2) it was not as effective during the trial period. This difference in the efficacy of these two biocides could be attributed to a natural biostatic effect of the dipropylene glycol matrix used in Biocide L. The same argument can be used to explain the efficacy of Biocide F, compared to Biocide L. Biocide F is administered at a maintenance dosage equivalent to Biocide L but contains approximately ten times the active ingredient in a matrix of 60 percent water

The least effective biocide overall was determined to be Biocide D which contains 95 weight percent organic-borates as the active ingredients. This product not only appeared to be ineffective against pure and mixed cultures but, actually enhanced growth if used in concentrations greater than the recommended dosage. These results agree with the findings of other researchers who have evaluated the efficacy of biocides containing these compounds (8).

It is interesting to note that Biocide G, which is the fuel system icing inhibitor DiEGME, performed slightly better than organo-borate compounds as a growth inhibitor.

Table 3

Ranking of Biocides Used in Efficacy Studies by Effectiveness

RANK	ACTIVE INGREDIENT	BIOCIDE
1	5-Chloro-2-methyl-4-isothiazoline-3-one 2-Methyl -4-isothiazoline-3-one	L
2	5-Chloro-2-methyl-4-isothiazolin-3-one 2-Methyl-4-isothiazoline-3-one	F
3	5-Chloro-2-methyl-4-isothiazoline-3-one 2-Methyl-4-isothiazoline-3-one	K
4	Tert-butylamine Omadine	A
5	Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate	C
6	Formaldehyde (unreacted) 1-(2-Hydroxyethyl)-2-Alkyl(C_{18})-2-Imidazoline	B
7	2,2'-dithio-bis (pyridine-1-oxide)	J
8	Poly [oxyethylene (dimethyliminio) ethylene (dimethyliminio)- ethylene dichloride]	E
9	4-(2-Nitrobutyl) morpholone 4,4'-(2-Ethyl-2-nitrotrimethylene) dimorpholine 1-nitropropane	H
10	Diethyleneglycol momomethyl ether	G
11	2,2'(1-methyltrimethylenedioxy) bis-(4-methyl 1-1,3,2-dioxaborinane) 2,2'-oxybis (4,4,6-trimethyl 1-1,3,2-dioxaborinane)	I
12	2,2'-oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane) 2,2'(1-methyltrimethylenedioxy) bis-(4-methyl 1-1,3,2-dioxaborinane)	D

Table 4

Chemical Composition of Most Effective Biocides as Tested

INGREDIENT	PERCENT (BY WEIGHT)		
	BIOCIDE		
	F	K	L
5-Chloro-2-methyl-4-isothiazolin-3-one	10-11	1.19	1.15
2-Methyl-4-isothiazolin-3-one	3-4	0.35	0.35
Dipropylene Glycol	0.0	0.0	90.0
Water	60-62	95.63	5.85
Magnesium Chloride	8-9	0.95	2.65
Magnesium Nitrate	15-17	1.78	0.0
Copper Nitrate	0.0	0.1	0.0

5.0 DISCUSSION

The results of these studies have indicated that the presence of mixed microbial populations poses entirely different implications for the addition of biocides than the single-species inocula. Tests of biocides on pure cultures of *H. resinae* or other monocultures are unreliable when determining biocide efficacy for any given fleet of vessels because they do not accurately reflect microbial growth in fuel tanks. Biocide tests on real tank MBC samples are the most realistic manner to evaluate effectiveness. Drum trials indicate that, at present, sampling variability can overwhelm data trends for establishing relative biocide effectiveness. These trial results also suggest that factors such as percent water to fuel have a large effect on biocide effectiveness and can render a biocide ineffective.

One way to inhibit MBC in distillate fuels, particularly in small systems, such as aircraft fuel tanks, is the addition of 0.15-0.2 percent (by volume) Diethylene glycol monomethlyl ether (Di-EGME). This additive is used in most military aviation turbine fuels to prevent suspended water, which can drop out of suspension at low fuel temperatures as free water, from freezing and blocking fuel systems. This is accomplished by combining with the free water, preventing it from freezing and allowing the water to be carried through the engine. A beneficial side effect of Di-EGME is that it will inhibit the growth of MBC by not allowing the free water to become available as an oxygen source for the organism. It is also believed that this chemical is mildly toxic to microorganisms. This additive could be very effective, when combined with the practice of good fuel husbandry, in fuel systems where a large amount of water would not normally be present. Such a combination would negate the need for routine use of biocides.

There is no doubt that at present the only effective way to eliminate MBC is the use of a biocidal agent. Even though a biocide containing chlorinated and non-chlorinated isothiazolin-3-one is approved for use in the Canadian Forces it can only be used, with the permission of National Defence Headquarters, as a last resort and under strictly controlled conditions. The primary concerns with the use of any biocide are the effects of the active ingredients on the aquatic environment, when present in discharged water bottoms, as well as the health of personnel who may come in contact with treated fuel. The Material Safety Data Sheets for isothiazolin based biocides state that, as supplied, it can cause corneal damage in the eyes and produce severe skin irritation. Diluted isothiazolins may also cause allergic skin reactions and irritation to mucus membranes in the nose and throat, and may be fatal if swallowed.

The preferred method to control microbiological contamination, in distillate fuel systems in the Canadian Forces, is the practice of good fuel husbandry and proper fuel handling. However, it must be noted that good fuel handling practices will only control, not eliminate, MBC. The regular stripping of water bottoms from fuel tanks will help minimize microbiological contamination, as organisms can grow only if water is present.

6.0 CONCLUSIONS

The results obtained from efficacy trials of such a long duration lead to some very interesting conclusions as to the effectiveness of biocides in the inhibition or prevention of microorganisms in distillate fuel systems.

Biocides containing chlorinated and non-chlorinated isothiazolin-3-ones as the active ingredients have been shown to be effective in controlling MBC in pure and mixed cultures. Organo-borates have been shown to be ineffective in inhibiting MBC particularly in mixed cultures and systems were large water bottoms are present.

Efficacy trials must be conducted under conditions that are a true representation of actual fuel tank growth conditions. This would mean the inclusion of large scale trials (drum size) on mixed microbial populations.

Close attention must be paid to sampling methods and growth conditions (temperature, water content) as slight variations in these variables can have dramatic effects on the effectiveness of a biocide.

Questions must be asked when the routine addition of a biocide is being considered as necessary to maintain the integrity of a fuel supply. It has been shown that the practice of good fuel husbandry and/or the routine addition of DiEGME in fuel system, where large water bottoms are not likely to occur, are effective in controlling MBC. This is particularly true with aviation turbine fuel supplies.

There is no doubt that biocides are an effective means to control or eliminate MBC in distillate fuel systems and, in some cases, may be the only available alternative. However, careful consideration should be given to alterative programs as well as all health and environmental risks before proceeding with a routine biocide treatment program.

REFERENCES

1. May, M. E. and Neihof, R. A., "Growth of *Cladosporium resinae* in Seawater/Fuel Systems", Developments in Industrial Microbiology, 22: 781-787, 1981.

2. Haggett, R.D., Morchat, R.M., "Microbiological Contamination: Biocide Treatment in Naval Distillate Fuel", August 1990, International Biodeterioration, Volume **29**, No. 1, 87-99, 1992.

3. Hebda, A. J., MacGregor, C. D. and Brown, R. G., "A Report on Testing and the Use of Biocides to Control Microbiological Contamination in Naval Distillate Fuel", DREA Contractor Report CR/85/412, July 1985.

4. Hebda, A. J., Jones, G. M., "Conditions and Modification to Conditions for Growth of *C. resinae* in Canadian Naval Vessels", DREA Contractor Report CR/86/412, April 1986.

5. Hebda, A. J., Jones, G. M. and Brown, R. G., "Efficacy Trials on Candidate Biocides for Microbiological Control Using Variable Cultures/Types in Bottle Scale Tests", DREA Contractor Report CR/87/431, May 1987.

6. Painter, H. G., MacGregor, C. D., "Research into the Control/Elimination of Microbiological Contamination in Naval Distillate Fuel", DREA Contractor Report CR/88/422, May 1988.

7. MacGregor, C., Devitt, S., "Efficacy Trials on Candidate Biocides Proposed for Use by the Canadian Forces", DREA Contractor Report CR/92/459, September 1992.

8. Hill, E. C., "The Control of Microorganisms in Aircraft Fuel Systems", Journal of the Institute of Petroleum, Volume **56**, No. 549, May 1970.

9. APHA-AWWA-WPCF, "Standard Methods for the Examination of Water and Waste Water"; 17th Edition, 1989.

10. Strickland J. D., Parsons, T. R., " A Practical Handbook of Seawater Analysis, Bulletin 167, Fisheries Research Board of Canada, Ottawa, 1972.

UNCLASSIFIED

SECURITY CLASSIFICATION OF FORM
(highest classification of Title, Abstract, Keywords)

DOCUMENT CONTROL DATA

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1. ORIGINATOR (The name and address of the organization preparing the document. Organizations for whom the document was prepared, e.g. Establishment sponsoring a contractor's report, or tasking agency, are entered in section 8.) Defence Research Establishment Atlantic, Dockyard Laboratory, P.O. Box 1012, Dartmouth, N.S., Canada B2Y 3Z7	2. SECURITY CLASSIFICATION (Overall security of the document including special warning terms if applicable.) UNCLASSIFIED

3. TITLE (The complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S,C,R or U) in parentheses after the title.)

A Summary of Biocide Efficacy Trials on Candidate Biocides for the Control of Microbiological Contamination in Distillate Fuels

4. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)

HAGGETT, Randy D. and MORCHAT, Richard M.

5. DATE OF PUBLICATION (Month and year of publication of document.) February 1998	6a. NO. OF PAGES (Total containing information. Include Annexes, Appendices, etc.) 21	6b. NO. OF REFS. (Total cited in document.) 10

6. DESCRIPTIVE NOTES (The category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

Technical Memorandum

8. SPONSORING ACTIVITY (The name of the department project office or laboratory sponsoring the reseach and development. include the address.)

Defence Research Establishment Atlantic, Dockyard Laboratory
P.O. Box 1012, Dartmouth, N.S., Canada B2Y 3Z7

9a. PROJECT OR GRANT NUMBER (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.) Project 1gi-11	9b. CONTRACT NUMBER (If appropriate, the applicable number under which the document was written.)
10a. ORIGINATOR'S DOCUMENT NUMBER (The official document number by which the document is identified by the originating activity. This number mrst be unique to this document.) DREA Technical Memorandum 98/209	10b. OTHER DOCUMENT NUMBERS (Any other numbers which may be assigned this document either by the originator or by the sponsor.)

11. DOCUMENT AVAILABILITY (Any limitations on further dissemination of the document, other than those imposed by security classification)

(X) Unlimited distribution
( ) Distribution limited to defence departments and defence contractors; further distribution only as approved
( ) Distribution limited to defence departments and Canadian defence contractors; further distribution only as approved
( ) Distribution limited to government departments and agencies; futher distribution only as approved
( ) Distribution limited to defence departments; further distribution only as approved
( ) Other (please specify):

12. DOCUMENT ANNOUNCEMENT (Any limitation to the bibliographic annoucement of this document. This will normally correspond to the Document Availability (11). However, where futher distribution (beyond the audience specified in 11) is possible, a wider announcement audience may be selected.)

Unlimited

UNCLASSIFIED

SECURITY CLASSIFICATION OF FORM

DCD03 2/06/87

13. ABSTRACT (a brief and factual summary of the document. It may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall begin with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (S), (C), (R), or (U). It is not necessary to include here abstracts in both official languages unless the text is bilingual).

This report summarizes the results of biocide efficacy trials conducted over an eight year period (1984-1992) by Defence Research Establishment Atlantic, Dockyard Laboratory. The purpose of these trials was to determine which biocide or biocidal agent was most effective in the control or elimination of microbiological contamination (MBC) in distillate fuel systems.

Growth conditions, culture population (mixed or pure), percent free water present and sampling techniques were all found to have an effect on the measured efficacy of a biocide. In general the efficacy trial results indicated that biocides containing chlorinated and non-chlorinated isothiazolin-3 ones as the active ingredients were the most effective growth inhibitors of pure and mixed microbial cultures. Biocides containing organ-borate, as the active ingredient, exhibited the lowest efficacy. The effectiveness of the organo-borates was greatly influenced by the amount of free water present. The routine use of diethylene glycol monomethyl ether (DiEGME) combined with the practice of good fuel husbandry appears to be a suitable alternative to biocide use in systems where large volumes of free water are unlikely to be present.

Although biocides are an effective means to control or eliminate MBC in distillate fuel systems and, in some cases, may be the only available course of action, careful consideration must be given to alternative programs as well as all health and environmental risks associated with biocide use before a treatment program is initiated.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS (technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus. e.g. Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus-identified. If it not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title).

Biocide
Microbiological Contamination
Fuel Quality
Biocide Efficacy

#507720

D
R
E
A

DEFENSIO PER SCIENTIAM

C
R
D
A