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Behaviour, Occurrence, and Aquatic Toxicity of New Antifouling Biocides and Preliminary Assessment of Risk to Aquatic Ecosystems

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Abstract　Paints containing biocides prevent the adhesion of organisms to the hulls of ships, and are hazardous to aquatic ecosystems. I reviewed published literature reporting the behaviour, occurrence, and toxicity in aquatic environments of the organotin-free new antifouling biocides. Our analysis included the representative alternative new biocides; 2,4,5,6-tetrachloro-isophthalonitrile (chlorothalonil), N'-dimethyl-N-phenylsulphamide (dichlofluanid), 1-(3,4-dichlorophenyl)-3,3-dimethylurea (diuron), 2-methylthio-4-t-butylamino-6-cyclopropylamino-s-triazine (Irgarol 1051), 4,5-dichloro-2-(n-octyl)-4-isothiazoline (Sea Nine 211), 2-(thiocyanomethylthio)benzothiazole (TCMTB), bis-(1-hydroxy-2(1H)-pyridinethionate-O,S) zinc (zinc pyrithione, ZnPT), zinc ethylene bis-(dithiocarbamate) (zineb), pyridinetriphenylboron (PK), and bis- (1-hydroxy-2(1H)-pyridinethionate-O,S) copper (copper pyrithione, CuPT). These new biocides are classified as "toxic to very toxic" according to guidelines of the Organization for Economic Co-operation and Development, with toxicity values similar to those of TBT. The new biocides are decomposed by hydrolysis, photolysis, and biological activity and thus are less persistent in the environment than TBT, but low vapour pressure limits their evaporation. Because of medium octanol-water coefficient (Kow) and organic carbon partitioning coefficient (Koc) values, these new biocides, except for zineb, ZnPT, and CuPT, tend to be distributed in both seawater and sediments but have low bioaccumulation in aquatic organisms. Chlorothalonil, dichlofluanid, diuron, Irgarol 1051 and its degradation product, 2-methylthio-4-tert-butylamino-6-amino-s-triazine (M1), and Sea Nine 211 have been found in natural waters, with higher concentrations in marinas and small harbours for fishing boats than in coastal waters, but insufficient data are available to assess contamination by these biocides. The reported concentrations of Irgarol 1051, M1, and Sea Nine 211 exceed their respective predicted no-effect concentration values, especially in marinas and fishery harbours, suggesting that these new biocides are already harming aquatic ecosystems in some areas. Further research is needed to assess the ecotoxicological risks of these biocides and to develop methods for estimation of their concentrations in ambient water.

Key words : TBT-free antifouling paint, biocide, fate in environment, toxicity, risk assessment, aquatic organisms

Introduction

Prevention of biofouling, the adhesion of organisms to the hulls of ships, is a crucial aspect of ship maintenance and the effective operation of marine vessels. Many antifouling paints have been developed

and widely used. Cuprous oxide-based paints were used until around 1965, when they were replaced with paints containing organotin compounds, most commonly tributyltin (TBT) or triphenyltin (TPT), which effectively control biofouling by a wide variety of organisms. It has become apparent, however, that organotin compounds are hazardous to aquatic environments. These organotin compounds are poorly biodegradable and thus remain in the environment for a long time. Moreover, these compounds are highly toxic to aquatic organisms, so bioaccumulation is a concern.

In Japan, the use of organotin compounds in antifouling paints has been regulated by the Chemical Substances Control Law, which prohibits the manufacture, import, and sale of organotin compounds, and by notifications and other forms of administrative guidance together with controls on use that are voluntarily imposed by various organizations. The Marine Environment Protection Committee of the International Maritime Organization has been exploring a world-wide prohibition of the application of organotins as antifouling biocides and in October 2001 adopted the International Convention on the Control of Harmful Antifouling Systems on Ships, which resulted in a ban on the application of organotin-based antifouling paints. Therefore, a number of organotin-free antifouling paints have been developed. In addition to cuprous oxide, several organic chemical substances, such as 2-mercaptopyridine N-oxide zinc salt, are used as biocides in organotin-free antifouling paints. Few studies have been undertaken on either the fate in the aquatic environment or the harmful effects on aquatic organisms of these new antifouling biocides.

Author investigated ecotoxicological information available in published literature to estimate the harmful effects of these new biocides on aquatic organisms and the marine environment.

Physicochemical properties

Two studies (Thomas 2001a; Harino 2004) provided data on representative new biocides (Table 1). Thomas (2001a) listed 8 representative new biocides (Fig. 1), 2,4,5,6-tetrachloro-isophth alonitrile (chlorothalonil), N'-dimethyl-N-phenyl-sulphamide (dichlofluanid), 1-(3,4-dichlorophenyl)-3,3-dimethylurea (diuron), 2-methylthio-4-t-butyl-amino-6-cyclopropylamino-s-triazine (Irgarol 1051), 4,5-dichloro-2-(n-octyl)-4-isothiazoline (Sea Nine 211), 2-(thiocyanomethylthio)benzothiazole (TCMTB), bis-(1-hydroxy-2(1H)-pyridinethionate-O,S) zinc (zinc pyrithione, ZnPT), and zinc ethylene bis-(dithiocarbamate) (zineb). Reported values of octanol-water coefficient (Kow), solubility, and estimated organic carbon partitioning coefficient (Koc) for these compounds are summarized in Table 1. Harino (2004) summarized the physicochemical properties of the new biocides pyridinetriphenylboron (PK) and bis-(1-hydroxy-2 (1H)-pyridinethionate-O,S) copper (copper pyrithione, CuPT) (Table 1). Most of these new biocides have been used as pesticides (herbicides and fungicides); however, Sea Nine 211, CuPT, and PK were produced for use as antifouling biocides (Fig. 1).

PK (Hokko Chemical Industry) was introduced commercially as an alternative biocide in 1993. Limited physicochemical data are available for this compound; therefore, it is difficult to assess its impact on aquatic environments.

Vapour pressures of chlorothalonil, dichlofluanid, Irgarol 1051, diuron, and Sea Nine 211 are 0.076 mPa (25 ℃), 0.016 mPa (20 ℃), 0.089 mPa, 0.41 mPa (50 ℃), and 0.98 mPa (25 ℃), respectively. These values are lower than that of TBTO, and indicating that these new biocides tend to mainly remain in the liquid phase.

Diuron is the most soluble of the new biocides (35 mg/L). The solubilities of Sea Nine 211, TCMTB, ZnPT, and Irgarol 1051 are 14 mg/L, 10.4 mg/L, 8 mg/L, and 7 mg/L, respectively; these compounds have greater solubility than chlorothalonil, dichlofluanid, and CuPT.

The log Kow values of the new biocides ranged from 0.6 to 4.0. Chlorothalonil has the highest (log Kow of 4.0), and zineb has the lowest (log Kow of 0.6). In spite of the low solubilities of ZnPT and CuPT, both have low log Kow values of 0.9 (Table 1), suggesting that these new biocides may not be bioconcentrated to high concentrations in aquatic organisms.
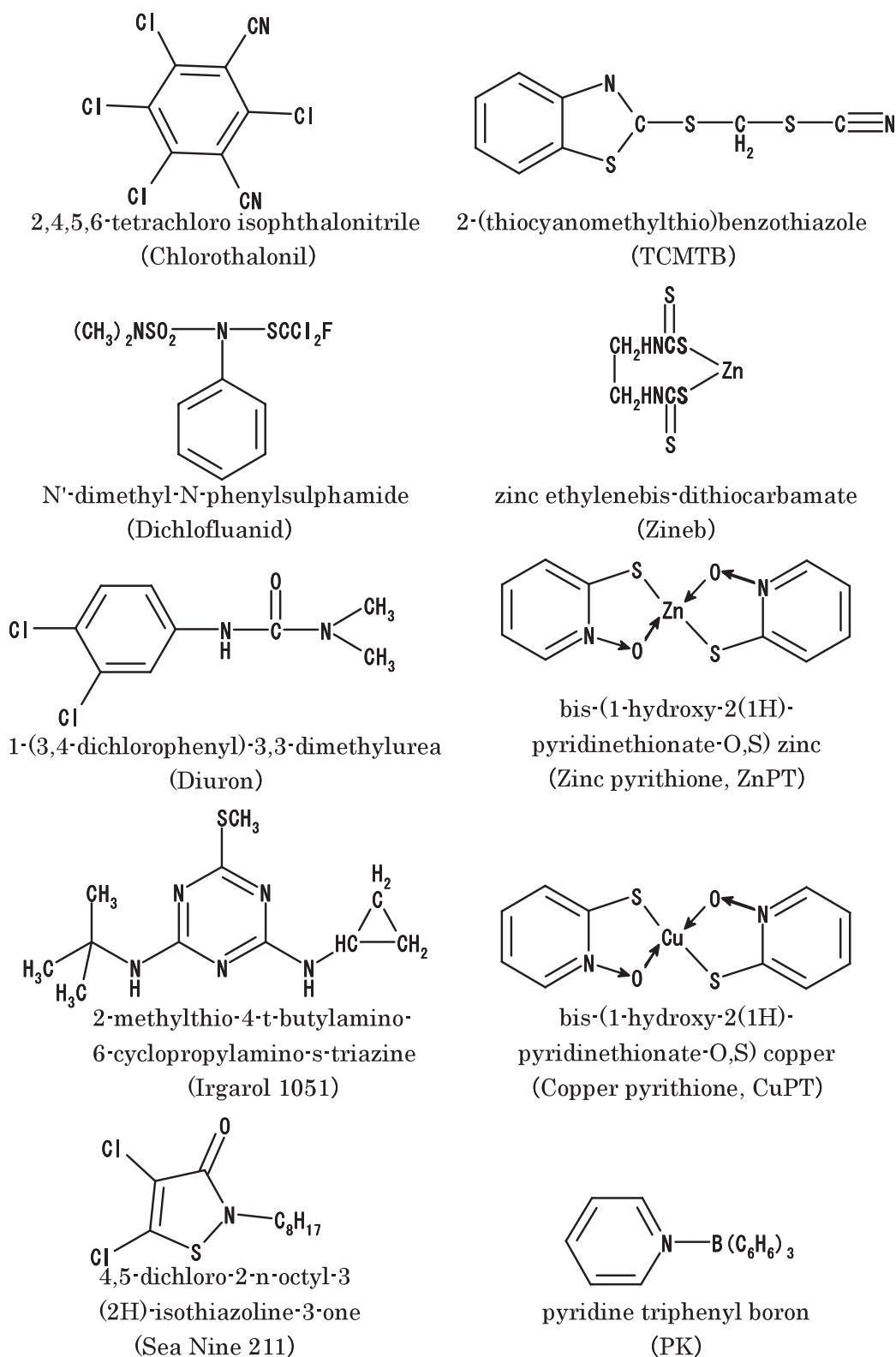
2,4,5,6-tetrachloro isophthalonitrile
(Chlorothalonil)

2-(thiocyanomethylthio)benzothiazole
(TCMTB)

N'-dimethyl-N-phenylsulphamide
(Dichlofluanid)

zinc ethylenebis-dithiocarbamate
(Zineb)

1-(3,4-dichlorophenyl)-3,3-dimethylurea
(Diuron)

bis-(1-hydroxy-2(1H)-
pyridinethionate-O,S) zinc
(Zinc pyrithione, ZnPT)

2-methylthio-4-t-butylamino-
6-cyclopropylamino-s-triazine
(Irgarol 1051)

bis-(1-hydroxy-2(1H)-
pyridinethionate-O,S) copper
(Copper pyrithione, CuPT)

4,5-dichloro-2-n-octyl-3
(2H)-isothiazoline-3-one
(Sea Nine 211)

pyridine triphenyl boron
(PK)

Fig. 1. The constitutional formula of representative new biocides.

Table 1. Physico-chemical properties of new biocides[1*] and bis (tributyltin) oxide (TBTO)[2*]

Biocide	Solubility (mg/L)	LogKow	Estimated LogKoc[3*]	Vapour Pressure (mPa)
Chlorothalonil	0.9	4.0	3.8	0.076(25℃)
Dichlofluanid	1.3	3.7	3.5	0.016(20℃)
Diuron	35	2.8	2.6	0.41(50℃)
Irgarol 1051	7	3.95	3.7	0.089
Sea Nine 211	14	2.8	2.6	0.98(25℃)
TCMTB	10.4	3.3	3.1	—
Zineb	0.07-10	0.8	0.6	—
ZnPT	8	0.9	0.7	—
CuPT	<1	0.9	0.7	—
PK	1	—	—	＜133(20℃)
TBTO	<1−>100	3.19−3.84	2.98−3.63	1.0(20℃)

[1*]:   Reported by Thomas (2001a) and Harino (2004).

[2*]:   Reported by UNEP, ILO and WHO(1990)

[3*]:   Koc was estimated by using the equation (logKoc = logKow − 0.21) presented by Karickhoff (1981).

Log Koc values estimated by the equation (log Koc = log Kow‑0.21) (Karickhoff, 1981) are also shown in Table 1. Log Koc values of chlorothlonil, dichlofluanid, diuron, Irgarol 1051, Sea Nine 211, and TCMTB ranged from 2.9 to 3.8, and were same order of TBTO. The log Koc values of zineb (0.6), ZnPT (0.7), and CuPT (0.7), were comparatively low. The medium Koc values of the new biocides such as chlorothalonil suggest that these biocides as well as TBTO may exist in both water and sediments; whereas biocides with low Koc values, such as zineb, ZnPT, and CuPT, may exist mainly in water. Galvin *et al*. (1998) reported that ZnPT and CuPT exist in sediments as copper and manganese complexes.

Degradation

Author reviewed degradation processes, including photolysis, hydrolysis, and biodegradation, for the new biocides.

Chlorothalonil

Chlorothalonil is rapidly photodegraded by sunlight, with a half-life ranging from 1 to 48 h according to photodegradation measurements in several natural waters. Chloro-1,3-dicyanobenzene and benzamide are produced as photodegradation products (Sakkas *et al*., 2002a). Biodegradation was found to be slower than photodegradation, with a half-life of 8 to 9 days (Walker *et al*. 1988).

Dichlofluanid

Thomas (2001a) reviewed the degradation of dichlofluanid in the natural environment, and reported a half-life of 18 h, which is relatively rapid degradation.

Diuron

Harino (2004) reported that no half-life data were available on diuron. Ellis and Camper (1982) reported that more than 80% of diuron was degraded in seawater within 27 days, with most of the degradation due to biological activity; photodegradation had only a minor effect. Under aerobic conditions, the primary degradation products of diuron are 3-(3,4-dichlorophenyl)-1-methyl urea,

3,4-dichlorophenyl urea, and 3-(3,4-dichloroaniline), whereas the primary anaerobic degradation product is 3-(4-chlorophenyl)-1,1'-dimethylurea.

Irgarol 1051

Many researchers (Liu *et al.*, 1997; Okamura, 2002a; Okamura and Sugiyama, 2004) have studied the degradation of Irgarol 1051. Liu *et al.* (1997) demonstrated that Irgarol 1051 is degraded by white rot fungus (*Phanerochaete chrysosporium*) to 2-methylthio-4-tert-butylamino-6-amino-s-triazine (M1) through N-dealkylation of a cyclopropyl group from the cyclopropylamino side-chain at the 6-position of the s-triazine ring. M1 is not degraded further by *P. chrysosporium,* and appears to accumulate as an end degradation product in aquatic environments. Irgarol 1051 is also transferred to M1 by Hg-catalyzed hydrolysis (Liu *et al.,* 1999a) and by photolysis (Okamura, 2002a). The photodegradation of Irgarol 1051 is stimulated by dissolved organic materials such as humic and fulvic substances (Okamura and Sugiyama, 2004). The Irgarol 1051 half-life (Harino 2004) ranged from 36 to 273 days. These results suggest that Irgarol 1051 is fairly stable in aquatic environments.

Sea Nine 211

Sea Nine 211 undergoes hydrolysis, photolysis, and biodegradation. Shade *et al.* (1993) reported that Sea Nine 211 is stable in sterile water at pH 7 but is hydrolyzed under acidic and alkaline conditions. Harino (2004) reported that biodegradation is rapid in seawater, with a half-life ranging from <0.5 to >30 days; N-octyl ozamic acid, 4,5-dichlorothiazole, and N-octyl carbamic acid are produced as the primary degradation products.

TCMTB

Laboratory results by Brownlee *et al.* (1992) indicated that TCMTB is rapidly degraded into 2-mercaptobenzothiazole and 2-(methylthio) benzothiazole. TCMTB is easily degraded by photolysis, with a reported half-life of <0.5 h, and has a reported biodegradation half-life of 740 h in natural waters.

Zineb

Zineb is known to degrade rapidly through hydrolysis to 5,6-dihydro-3H-imidazo-(2,1-c) -1,2,4-dithiazole-3-thion, ethylene diisothiocyanate, and ethylenethiourea (Hunter and Evans, 1991).

A half-life of 96 h was observed in hydrolysis experiments at pH 10 and 20 ℃ .

ZnPT and CuPT

Comprehensive studies on degradation of ZnPT and CuPT (Turley *et al.* 2000) found that the hydrolysis half-life of CuPT in sterilized artificial seawater is 12.9 days. Results of a seawater die-away experiment indicated that at an initial CuPT concentration of 52 μg/L, the half-life with respect to biodegradation is approximately 4 days in seawater and 7 h in river water. CuPT is also sensitive to light, with a reported photolysis half-life of 29.1 min.

The reported hydrolysis half-life of ZnPT is >90 days, and ZnPT also biodegrades in seawater, with a half-life of approximately 4 days. Like CuPT, ZnPT has high sensitivity to light; its reported half-life is 17.5 min under a filtered xenon arc lamp and <2 min under natural sunlight.

These results suggest that ZnPT and CuPT are mainly degraded by photolysis in aquatic environments. Pyridine sulfonic acid is produced from ZnPT and CuPT as a degradation products.

PK

There are no available data on PK hydrolysis or biodegradation in the aquatic environment. Photodegradation of PK dissolved in artificial seawater and subjected to UV radiation (254 nm, 5.5 W) (Amey and Waldron, 2004) was very rapid and in <1 h led to boric acid production by elimination of pyridine and phenol.

In summary, the information on the degradation of these new antifouling biocides suggests that these new biocides are not stable compared with the previously used TBT. Because of their more rapid degradation, these new biocides pose less of an environmental risk than TBT.

Concentrations in the Aquatic Environment

Chlorothalonil

Chlorothalonil concentrations in seawater were studied in coastal areas of the United Kingdom (Thomas *et al.* 2002; Voulvoulis *et al.* 2000). Chlorothalonil was not detected in seawater from Southampton (Thomas *et al.,* 2002). In seawater of Blackwater Estuary, however, concentrations

<0.0002-0.00138 μg/L were reported by Voulvoulis *et al.*（2000）.

Dichlofluanid

Dichlofluanid was detected in coastal waters in Greece (Sakkas *et al., 2002b*) and Spain (Martinez *et al., 2001*) in concentrations ranging from <0.001 to 0.284 μg/L and from <0.002 to 0.76 μg/L, respectively.

Diuron

The concentration of diuron in seawater was determined by Thomas *et al.* （2001b）at the south coast of the United Kingdom（Southampton and Sutton Harbour）. In Southampton, the concentration in seawater ranged from 0.004 to 6.742 μg/L in marinas and harbours and from <0.001 to 0.465 μg/L in estuarine water. The concentrations in Sutton Harbour ranged from 0.001 to 0.334 μg/L. Harino *et al.* （2005）measured concentrations of diuron in seawater in Osaka Port, Japan, that ranged from 0.013 to 0.35 μg/L.

Irgarol 1051

The concentrations of Irgarol 1051 in the coastal waters of Japan, Canada, France, the United Kingdom, Germany, and Sweden and in the lakes of Switzerland are summarized in Table 2. Irgarol 1051 was not detected in seawater samples taken from 4 locations in Canada, including Vancouver. It was detected, however, in the coastal waters of France, the United Kingdom, Germany, Sweden, and Japan and in samples taken from marinas in the lakes of Switzerland.

The concentrations in seawater samples taken in France ranged from below the detection limit to 1.7 μg/L, with the highest values being measured for samples taken in the marinas of the Cote d'Azur region（Readan *et al., 1993*）. Irgarol 1051 was measured by Tolosa *et al.* （1996）at marinas, ports, and beaches; the concentrations were 0.022-0.64 μg/L, 0.0138-0.264 μg/L, and <0.0015-0.017 μg/L, respectively. The concentrations at marinas were higher than those at ports and beaches. The concentrations determined by Readan *et al.*（1993）did not differ from those reported by Tolosa *et al.* （1996）. These results suggest that the annual variation of concentrations is not significant.

In a United Kingdom survey covering 7 coastal areas（Gough *et al.*; 1994; Zhou *et al.*; 1996; Scarlett *et al.*; 1997; Thomas *et al., 2001b*）, the Irgarol 1051 concentration in seawater ranged from <0.001 to 0.682 μg/L. The concentrations were higher in the marinas than in coastal waters. The highest concentration of 0.682 μg/L was measured in a marina in Humber.

The Irgarol 1051 concentrations measured at marinas in the North Sea and the Baltic Sea were 0.011-0.17 μg/L and 0.08-0.44 μg/L, respectively （Biselli *et al., 2000*）. In general, concentrations were higher in the Baltic Sea than in the North Sea. The concentrations measured in marinas in Germany were similar to those in the marinas of France and the United Kingdom.

The Irgarol 1051 concentrations from 0.03 to 0.4 μg/L were reported at marinas in estuarine areas of Sweden. This substance has been detected not only in seawater but in terrestrial water samples taken from marinas in the lakes of Switzerland.

In Japan, Okamura *et al.* （2000a）and Liu *et al.* （1999b）conducted surveys of environmental contamination in the Seto Inland Sea. Irgarol 1051 and its degradation product M1 were detected in the seawater but not in sediment. Irgarol 1051 concentrations in seawater ranged from below the detection limit to 0.296 μg/L, with the higher concentrations being detected in marinas and small fishing ports located in the prefectures of Hiroshima, Okayama, Hyogo, and Kagawa （Table 5）. M1 has been detected in seawater taken in the Seto Inland Sea, and M1 concentrations in seawater ranged from below the detection limit to 1.27 μg/L. These results indicate that MI concentrations tend to be higher than those of Irgarol 1051 and that M1 remains in aquatic environments for a longer period than Irgarol 1051.

Irgarol 1051 concentrations in seawater were found to be higher in marinas and small fishing ports where pleasure boats and small fishing vessels are moored than in large ports used by ocean-going vessels. Irgarol 1051 concentrations are also higher in Europe （France, the United Kingdom, Germany, and Sweden）than in Japan. These results indicate that antifouling paints containing Irgarol 1051 are predominantly used on pleasure boats and small fishing vessels, and they are used in greater volumes in the European countries than in Japan.

Table 2. Concentration of Irgarol 1051 and its degradation products (M1) in water at various regions.

Country and Location		Concentration (μ g/L)		Reference
		Irgarol 1051	M1	
Japan				
Seto Inland Sea	marina	nd – 0.0853	0.0288 – 1.27	Okamura *et al.* (2000a)
	fishery harbour	nd – 0.296	nd – 1.21	Okamura *et al.* (2000a)
	Kojima port	nd – 0.0195		Liu *et al.* (1999b)
	Kobe port	nd		Liu *et al.* (1999b)
Canada				Liu *et al.* (1999b)
Vancouver, Tront	marina	nd		
Montreal & Halifax	port	nd		
France				
Coate d'Azur	marina	0.11 – 1.7		Readan *et al.* (1993)
	port	<0.005 – 0.28		
	marina	0.022 – 0.64		Tolosa *et al.* (1996)
	port	0.0138 – 0.264		
	beach	<0.0015 – 0.017		
United Kingdom				
Hamble estuary		0.012 – 0.19		Gough *et al.* (1994)
Medway estuary		0.004 – 0.018		
NRA southern region	marina	0.052 – 0.5		
	estuary	<0.002 – 0.014		
	river	<0.002		
Humber	marina	0.169 – 0.682		Zhou *et al.* (1996)
Plymouth	marina	0.028 – 0.127		Scarlett *et al.* (1997)
Southampton	marina & harbour	0.007 – 0.403		Thomas *et al.* (2001b)
	estuary water	<0.001 – 0.047		
Sutton harbour		<0.001 – 0.084		
Germany				
North Sea	marina	0.011 – 0.17		Biselli *et al.* (2000)
Baltic Sea	marina	0.08 – 0.44		
Sweden				
Fiskebackski	marina	0.03 – 0.4		Tolosa *et al.* (1996)
Switzerland				
Port d'Ouchy	marina	0.0025 – 0.145		Toth *et al.* (1996)

Irgarol 1051: 2-methylthio-4-t-butylamino-6-cyclopropylamino-s-triazine
M1: 2-methylthio-4-t-butylamino-6-amino-s-triazine
nd: below the detection limit

Irgarol 1051 concentrations in sediments were measured by Biselli *et al.*（2000）in the North Sea and Baltic Sea and by Albanis *et al.*（2002）in the coastal area of Greece. The concentrations ranged from 3 to 25 μg/kg dry weight in the North Sea, 4 to 220 μg/kg dry weight in the Baltic Sea, and less than the detection limit to 338 μg/kg dry weight in Greece. Concentrations were particularly high in the sediments of marinas, a tendency similar to that observed in seawater.

Sea Nine 211

Thomas *et al.*（2001b）reported that Sea Nine 211 was not detected in seawater and sediments collected at marinas on the south coast of the United Kingdom in 1998. On the other hand, Martinez *et al.*（2001）reported that Sea Nine 211 was detected at concentrations ranging from 0.26 to 3.7 μg/L in seawater from the Spanish Mediterranean. Sea Nine 211 was detected in the coastal areas of Greece at concentrations ranging from <0.0063 to 0.049 μg/L （Sakkas *et al.,* 2002b）and in Osaka Port in Japan （Harino *et al.,* 2005）from <0.0003 to 0.0055 μg/L.

TCMTB, ZnPT, CuPT, and PK

Thomas *et al.*（2004）reported that their monitoring studies did not detect chlorothalonil, dichlofluanid, ZnPT, Sea Nine 211, or TCMTB in seawater in the United Kingdom. Harino *et al.* （2005）also reported that ZnPT and CuPT were not detected in seawater collected in Osaka Port, Japan. I found no literature reporting concentration of PK in aquatic environments.

Harmful effects of new biocides on aquatic organisms

Chlorothalonil

Acute toxicity of chlorothalonil to *Daphnia magna, Onchorhynchus mykiss,* and *Gasterosteus aculeatus* are summarized in Table 3. The 48 h EC50 to *D. magna* ranged from 97 to 129 μg/L, and the 96 h LC50 to fish species ranged from 69 to 76 μg/L. Acute toxicities to crustacean and fish species are significant, and are "very toxic" according to Organization for Economic Co-operation and Development（OECD）ecotoxicity classification guidelines（OECD, 1996）.

Sea Nine 211

Sea Nine 211 is acutely toxic to phytoplankton at concentrations of 13.9-32 μg/L, to crustacean species at concentrations of 4.7-1,312 μg/L, to bivalves at a concentration of 850 μg/L, and to fish species at concentrations of 2.7-20.5 μg/L（Table 3）. From these data, author conclude that this compound exhibits weaker acute toxicity to bivalves than other aquatic organisms such as phytoplankton and fish. Based on these acute toxicity values, Sea Nine 211 is classified as "very toxic to toxic" according to the OECD guidelines.

Chronic toxicities of Sea Nine 211 to *D. magna* and sheepshead minnows（*Cyprinodon variegatus*）are reported to occur at concentrations of 1.2 μg/L and 6 μg/L, respectively（Table 4）. The 28 d LC50 to rainbow trout larvae is 14 μg/L（Okamura, *et al.,* 2002）, leading to fears that the chronic toxicity of Sea Nine 211 is equivalent to that of other antifouling compounds such as ZnPT, CuPT, and PK.

ZnPT and CuPT

Published literature contains very little data on the acute toxicity of ZnPT and CuPT to aquatic organisms（Table 3）. The respective toxicity values of ZnPT are reported to be 2.06-28 μg/L for phytoplankton（Shipbuilding Research Association of Japan, 2002）, 29-34 μg/L for *D. magna*（Shipbuilding Research Association of Japan, 2002）, and 2.6-400 μg/L for fish species（Shipbuilding Research Association of Japan, 2002）. CuPT exposure causes acute toxicity leading to decreased phytoplankton growth at concentrations of 2.8-35 μg/L （Shipbuilding Research Association of Japan, 2002）, *D. magna* immobilization at 22 μg/L（Shipbuilding Research Association of Japan, 2002）, and decreased survival of fish species at 4.3-7.67 μg/L（Shipbuilding Research Association of Japan, 2002）. Mochida *et al.* （2006）recently studied the acute toxicity of CuPT and ZnPT to toy shrimp（*Heptacarpus futilirostris*）and red sea bream（*Pagrus major*）; 96 h LC50s to red sea bream were 9.3 μg/L for CuPT and 98.2 μg/L for ZnPT. On the other hand, 96 h LC50s of CuPT and ZnPT were 2.5 and 120 μg/L, respectively, for toy shrimp. Although the available toxicity data are limited, they indicate that CuPT exhibits greater acute toxicity to fish and shrimp species than does ZnPT. Toxicological data on ZnPT and CuPT evaluated on the basis of the OECD

classification guidelines correspond to the "very toxic" definition, suggesting that both compounds are highly toxic to aquatic organisms.

In organotin-free antifouling paints, the pyrithione compounds (ZnPT and CuPT) are usually used in combination with cuprous oxide, and their acute toxicity may change with the presence of copper.

Mochida *et al.* (2006) examined the toxicities of pyrithione compounds (ZnPT and CuPT) in the presence of copper ions. The presence of Cu^{2+} did not change the acute toxicity of CuPT but significantly increased the acute toxicity of ZnPT. According to Mochida *et al.* (2006), the enhancement of toxicity by Cu^{2+} is attributable to

Table 3. Acute toxicity of new biocides to aquatic organisms

Aquatic organisms	Endpoint	Acute toxicity					
		ZnPT[1]* (μg/L)	CuPT[2]* (μg/L)	Pyridine sulfonic acid(μg/L)	PK[3]* (μg/L)	Sea Nine 211[4]* (μg/L)	Chlorothalonil[5]* (μg/L)
Micro algae							
Selenastrum capricornutum	72-120hEC50	28(2)[6]*	35(2)			32(4)	
Skeletonema costatum	72hEC50	2.06(2)	28.4(2)		2.15(1)	13.9(4)	
freshwater algae	120hLC50			28,900(2)			
Crustacean							
Daphnia magna	48hEC50	29—34(2)	22(2)	>122,000(2)			97—129(5)
mysid shrimp	96hLC50	6.3(2)		71,600(2)		4.7(4)	
toy shrimp	96hLC50	120(6)	2.5(6)				
brown shrimp	96hLC50					12.4(4)	
Uca pugilator	96hLC50					1,312(4)	
Penaeus japonicus	96hLC50	1,780(2)	43.6(2)		149(1)	12.6(1)	
Tigriopus japonicus	24hEC50	160(3)	31(3)		16(3)	30(2)	
Tigriopus japonicus	24hLC50	>500(3)	41(3)		110(3)	77(2)	
Mussel							
bay mussel	96hLC50					850(4)	
Fish							
Lepomis macrochirus	96hLC50					14(4)	
Pimephales promelas	96hLC50	2.6(2)	4.3(2)	68,500(2)			
Oncorhynchus mykiss	96hLC50	3.2(2)		57,100(2)		2.7(4)	69—76(5)
Cyprinodon variegatus	96hLC50	400(2)		>127,000(2)		20.5(4)	
Pagrus major	96hLC50	273(2)	7.67(2)		242(1)		
Pagrus major	96hLC50	98.2(6)	9.3(6)				
Gasterosteus aculeatus	96hLC50						73(5)

1*: Bis-(1-hydroxy-2(1H)-pyridinethionate-O,S)zinc, 2*: Bis-(1-hydroxy-2(1H)-pyridinethionate-O,S)copper,

3*: Pyridinetriphenylboron, 4*: 4,5-dichloro-2-n-octyl-3-isothiazolone(Sea Nine 211), 5*: 2,4,5,6-tetrachloro-isophthalonitrile

6*: Figure in the parenthesis exhbit the reference.　(1): Shipbuilding Research Association of Japan (1999),(2) Shipbuilding Research Association of Japan (2001), (3): Shipbuilding Research Association of Japan (2002), (4): Shade *et al.* (1994), (5):Ernst *et al.* (1991), (6):Mochida *et al.* (2006)

Table 4. Chronic toxicity of new biocides to aquatic organisms

Aquatic organisms	Endpoint	Chronic toxicity				Reference
		ZnPT[1]* (μg/L)	CuPT[2]* (μg/L)	PK[3]* (μg/L)	Sea Nine 211[4]* (μg/L)	
Crustacean						
Daphnia magna	21day chronic EC50				1.2	Shade *et al.* (1993)
Fish						
Cyprinodon variegatus	early life NOEC				6	Shade *et al.* (1993)
Oncorhynchus tshawytcha	7dLC50	8.4	7.6	140	14	Okamura *et al.* (2002b)
	14dLC50	5.6	3.0	84	14	
	21dLC50	4.9	1.7	61	14	
	28dLC50	4.6	1.3	42	14	

1*: Bis-(1-hydroxy-2(1H)-pyridinethionate-O,S)zine, 2*: Bis-(1-hydroxy-2(1H)-pyridinethionate-O,S)copper,

3*: Pyridinetriphenylboron, 4*:4,5-dichloro-2-n-octyl-3-isothiazolone (Sea Nine 211)

conversion of ZnPT to more toxic CuPT.

ZnPT has significant effects on the embryogenesis of fish species, and an early life-stage toxicological study in which fertilized eggs were exposed to ZnPT confirmed deformities in the spinal curvature of fish larva after hatching (Goka, 1999). For rainbow trout larvae, Okamura *et al.* (2002b) obtained 28 d LC50 values of 4.6 μg/L for ZnPT and 1.3 μg/L for CuPT (Table 4). These toxicity values raise the concern that both compounds may exhibit high chronic toxicity to fish species.

ZnPT and CuPT are degraded in the aquatic environment to pyridine sulfonic acid. The acute toxicities of pyridine sulfonic acid were as follows; 120 h LC50 to freshwater algae was 28,900 μg/L, 48 h EC50 to *D. magna* was >122,000 μg/L, and 96 h LC50 to fish species was 57,100->127,000 μg/L (Table 3). The acute toxicities of pyridine sulfonic acid were weaker than those of the parent compounds, ZnPT and CuPT. These results suggest that toxicities of ZnPT and CuPT decrease in the process of degradation.

PK

Because of its recent development for use as a biocide in antifouling paints, only limited data are available on the harmful effects of PK on aquatic organisms (Table 3) : the 72 h EC50 to the phytoplankton *Skeletonema costatum* is 2.15 μg/L (Shipbuilding Research Association of Japan,1999), the 96 h LC50 to shrimp (*Penaeus japonicus*) is 149 μg/L (Shipbuilding Research Association of Japan, 1999), and the 96 h LC50 to red sea bream (*Pagrus major*) is 242 μg/L (Shipbuilding Research Association of Japan, 1999). Although the acute toxicity of PK is weaker than that of ZnPT and CuPT, it is classified as "very toxic" according to the OECD toxicity guidelines.

The 28 d LC50 of PK is 42 μg/L based on 28-day rearing experiments using rainbow trout larvae (Okamura, *et al.,* 2002). This value is equivalent to that of ZnPT and CuPT. These results raise a great concern of this compound's harmful effect on aquatic organisms.

Irgarol 1051

Acute toxicity of Irgarol 1051 and its degradation product M1 to various aquatic organisms are summarized in Table 5. Irgarol 1051 does not affect bacterial growth even at a concentration of 50,000 μg/L (Okamura *et al.,* 2000a). The 24 h LC50 of Irgarol 1051 to crustacean species is reported to range from 5,700 to 12,000 μg/L (Okamura *et al.,* 2000a). It inhibits mobilization (48 h EC50) at a concentration of 8,100 μg/L (Ciba-Geigy, 1995). The 48 h LC50 to oyster larvae is 3,200 μg/L, whereas the 96 h LC50 to fish species ranges from 790 μg/L for rainbow trout to 4,000 μg/L for zebrafish (Hall Jr. *et al.,* 1999).

The EC50 for inhibition of phytoplankton growth ranges from 0.1 to 2.3 μg/L. Irgarol 1051 is very toxic to seaweed; germination and development of spores is inhibited at concentrations ranging from 0.6 to 5.9 μg/L (Okamura *et al.,* 2000b). The constitutional formula of Irgarol 1051 resembles that of triazine herbicides; therefore, it seems reasonable that Irgarol 1051 is remarkably more toxic to aquatic vegetation such as phytoplankton and seaweed than to marine animals such as crustaceans and fish.

Based on the ecotoxicity classification guidelines (OECD, 1996), Irgarol 1051 acute toxicity values to phytoplankton and aquatic animals (crustaceans and fish) correspond to the definition of "very toxic" and "toxic", respectively.

As shown in Table 5, the 72 h EC50 of M1 to phytoplankton growth ranged from 1.9 to 46 μg/L (Okamura *et al.,* 2000a). The 96 h EC50 of M1 to the germination and development of the spores of seaweed ranged from 1.7 to 130 μg/L (Okamura *et al.*, 2000b), whereas it is acutely toxic to crustacea (*Daphnia magna*) species at concentrations of 11,000 μg/L (Okamura *et al.,* 2000a). EC50 and LC50 values of M1 are 10 times greater than those of the parent compound. Although the toxicity of Irgarol 1051 decreases as the compound is degraded, its degradation product M1 is classified as "toxic" according to the OECD ecotoxicity classifications.

The chronic toxicity of Irgarol 1051 was evaluated by an early life-stage toxicity test using rainbow trout (embryo to larval stage) (Table 6). A no observed effect concentration of 4.02 μg/L was determined by the inhibition of larval growth during 60 days after hatching (Hall Jr. *et al.,* 1999). Chronic toxicity and acute toxicity values revealed that the acute/chronic toxicity ratios of Irgarol 1051 to rainbow trout ranged from 3 to 100. This

Table 5. Acute toxicity of Irgarol 1051 and its biodegradation products (M1) to aquatic organisms

Aquatic organisms		Endpoint	Toxicity index	Acute toxicity(μ g/L)		Reference
Class	Species			Irgarol 1051 [1*]	M1 [2*]	
Bacteria	Vibrio fisheri	bioluminescence	30min.EC50	>50,000	>50,000	Okamura et al. (2000a)
Micro algae	Selenastrum capricornutum	biomass	72hEbC50	1.6	19	Okamura et al. (2000a)
	Selenastrum capricornutum	growth rate	72hErC50	2.3	46	Okamura et al. (2000a)
	Senedesmus sp.		72hEC50	1.4		Ciba-Geigy (1995)
	Raphidocelis subcapitata		EC50	1		Ciba-Geigy (1995)
	Anabaena flos-aquae		EC50	2		Ciba-Geigy (1995)
	Navicula pelliculata		EC50	0.1		Ciba-Geigy (1995)
	Skeletonema costatum		EC50	0.4		Ciba-Geigy (1995)
Seaweed	Porphyra yezoensis	conchospore survival	96hLC50	5,000	6,500	Okamura et al. (2000b)
		conchospore germination	96hEC50	4.1	130	Okamura et al. (2000b)
		conchospore growth	96hEC50	0.6	17	Okamura et al. (2000b)
	Eisenia bicylis	gamatephyte growth	96hEC50	5.9	>32	Okamura et al. (2000b)
Crustacean	Daphnia magna	lethality	24hLC50	16,000	17,000	Okamura et al. (2000a)
			48hLC50	8,300	11,000	Okamura et al. (2000a)
	Daphnia pulex	lethality	24hLC50	5,700	27,000	Okamura et al. (2000a)
	Thamnocepharus platyurus	lethality	24hLC50	12,000	19,000	Okamura et al. (2000a)
	Daphnia magna		48hEC50	8,100		Ciba-Geigy (1995)
	Mysidopsis bahia	lethality	96hLC50	40		Hall Jr. et al. (1999)
	Artemia salina	lethality	24hLC50	>4,000	>4,000	Okamura et al. (2000a)
Bivalves	oyster larvae	lethality	48hLC50	3,200		Ciba-Geigy (1995)
Fish	Cyprinodon variegatus	lethality	96hLC50	3,500		Hall Jr. et al. (1999)
	Barachydanio rerio	lethality	96hLC50	4,000		Ciba-Geigy (1995)
	Oncorhynchus mykiss	lethality	96hLC50	790		Hall Jr. et al. (1999)
	Menidia beryllina	lethality	96hLC50	1,580		Hall Jr. et al. (1999)
	Lepomis macrochirus	lethality	96hLC50	2,600		Hall Jr. et al. (1999)

1*:2-methylthio-4-t-butylamino-6-cyclopropylamino-s-triazine,　2*: 2- methylthio-4-t-butylamino-6-amino-s-triazine

Table 6. Chronic toxicity of Irgarol 1051 to shrimp and fish.

Aquatic organisms		Endpoint	Exposure period	Chronic toxicity (μ g/L)	Reference
Class	Species				
Crustacean	Mysidopsis bahia	growth	28day	110 (LOEC: 260)	Hall Jr. et al. (1999)
	shrimp	survival	28day	260 (LOEC: 490)	Hall Jr. et al. (1999)
Fish	Oncorhynchus mykiss	hatchability		184	Hall Jr. et al. (1999)
		survival	60day after hatch	184	Hall Jr. et al. (1999)
		growth	60day after hatch	4.02 (LOEC:9.14)	Hall Jr. et al. (1999)
		reproduction	28day	260 (LOEC: 490)	Hall Jr. et al. (1999)
	Oncorhynchus tshawytcha	LC50	28day	88	Okamura et al. (2002b)

LOEC: lowest observed effect concentration

Irgarol 1051: 2-methylthio-4-t-butylamino-6-cyclopropylamino-s-triazine

ratio is similar to that of numerous other chemical substances such as lindane, PCP, DDT, and dieldrin.

In summary, despite the paucity of data on the toxicity of the new antifouling biocides to aquatic organisms, available published data suggest that Irgarol 1051 is toxic to phytoplankton and seaweed and that CuPT and ZnPT are highly toxic to marine animals. The acute toxicity of these new biocides is found to be in the order of micrograms per liter, which is equivalent to that of the TBT (Koyama and Shimizu, 1992; Horiguchi and Shimizu, 1992; IMO, 1997).

Preliminary risk assessment

The predicted no effect concentration (PNEC) was tentatively estimated from the lowest acute toxicity value by the OECD method. In accordance

with OECD method, the uncertainty factor of 100 was used to calculate PNEC. The values of PNEC for several new biocides are shown in Table 7 together with the observed concentrations of these biocides in natural waters.

The highest concentration found in natural water (EC) and PNEC of chlorothalonil, was 0.00138 μg/L and 0.69 μg/L, respectively. EC / PNEC ratio was 0.002, and EC was two orders of magnitude lower than the PNEC value. Although the available data on chlorothalonil concentrations in natural waters are very limited, these results suggest that its hazardous impact on the aquatic environments can be considered small.

The PNEC was 0.016 μg/L for Irgarol 1051, 0.19 μg/L for M1 , and 0.027 μg/L for Sea Nine 211. The ECs of Irgarol 1051 ranged from 0.264 to 1.7 μg/L, and EC / PNEC ratio fell between 5.33 and 106.25. The EC was one or two orders magnitude higher than PNEC in marinas. The ECs of 1.21 and 1.27 μg/L were observed in M1, and was EC / PNEC ratio was estimated as 6.37 or 6.68. The EC / PNEC ratio of Sea Nine 211 ranged from <0.01 to137.

The higher EC / PNEC was recognized in marinas and fisheries harbours, raising concerns that these biocides are already harming the aquatic ecosystem, especially in marinas and fisheries harbours. The concentrations of Irgarol 1051, M1, and Sea Nine 211 in seawater collected at coastal areas such as beaches were significantly lower than PNEC values. Hazardous impacts by these new biocides seem restricted to particular areas such as marinas and fisheries harbours, where many pleasure boats and/or fishing boats are moored.

Harino *et al*. (2005) did not detect pyrithione compounds (ZnPT and CuPT) in the seawater of Osaka Port, Japan. These Osaka port results indicate that hazardous impact of pyrithione compounds are not yet a concern. The limited published data on the concentrations of PK preclude evaluation of the hazardous impact of PK on the marine ecosystem, and further studies are needed.

Conclusion

The low vapour pressure of the new biocides in

Table 7. Preliminary risk assessment of several new biocides to aquatic organisms by comparing PNEC with the highest concentration in natural waters.

Biocide		Lowest value of acute toxicity data (μg/L)		PNEC 1* (μg/L)	Highest concentration in natural water(μg/L) (EC)	EC / PNEC
Chlorothalonil	*D. magna,*	EC50	97	0.69	0.00138(Black water estuary)	0.002
	O. mykiss,	LC50	69			
Irgarol 1051	*S. Capricornutum*	EC50	1.6		0.0853(marina, Japan)	5.33
	D. magna	EC50	8,100	0.016	0.296(Fisheries harbour, Japan)	18.50
	O. mykiss	LC50	790		1.7(marina, France)	106.25
					0.264(port, France)	16.50
					0.682(marina, south coast of UK)	42.63
					0.44(marina, the Baltic Sea)	27.50
					0.4(marina, Sweden)	25.00
M1	*S. capricornutum*	EC50	19	0.19	1.27(marina, Japan)	6.68
	D. magna	EC50	27,000		1.21(fishery harbour, Japan)	6.37
Sea Nine 211	*S. capricornutum*	EC50	32		0.26－3.7(Mediterranean Sea, Spain)	9.62－137.00
	mysid shrimp	LC50	4.7	0.027	<0.0063－0.049(Greece)	<0.23－1.81
	O. mykiss	LC50	2.7		<0.0003－0.00055(Osaka port, Japan)	<0.01－0.02
Zn PT	*S. capricornutum*	E C50	28		is not detected in Osaka port, Japan.	
	D. magna	E C50	29	0.026	The contamination is not exactly	－
	P. promelas	LC50	2.6		demonstrated.	
Cu PT	*S. capricornutum*	EC50	35		is not detected in Osaka port, Japan.	
	D. magna	EC50	22	0.025	The contamination is not exactly	－
	H. futilirostris	LC50	2.5		demonstrated.	
	P.promelas	EC50	4.3			
PK	*S. costatum*	EC50	2.2		There are not available information on	
	T. japonicus	EC50	16	0.022	the concentration in seawater.	－
	P. major	LC50	242			

1*: PNEC was tentatively estimated from the lowest acute toxicity value by the OECD method using uncertainty factor of 100.

water limits their evaporation. Because of medium Kow and Koc values, the new biocides, except zineb, ZnPT, and CuPT, tend to be distributed in both seawater and sediments, and they are hardly bioconcentrated in aquatic organisms.

The new biocides are decomposed by hydrolysis, photolysis, and biological activity. Thus they are less stable than TBT and do not persist for a long time in aquatic environments. These new biocides are classified as "toxic" or "very toxic" by the OECD classification guideline, and show almost the same toxicity as TBT.

Chlorothalonil, dichlofluanid, diuron, Irgarol 1051 and its degradation product M1, and Sea Nine 211 have been detected in natural waters, with higher concentrations in marinas and small harbours for fishing boat than in coastal waters. Research on the occurrence in seawater in Japan was carried out only by Harino *et al.,* who did not detect pyrithione compounds. Detailed surveys are needed to clarify the present extent of contamination by the new biocides.

The reported concentrations of Irgarol 1051, M1, and Sea Nine 211 in natural waters exceed the PNEC values, particularly in marinas and fishery harbours. Igarol 1051, M1, and Sea Nine 211 are causing a hazardous impact on the aquatic ecosystem in marinas and fishery harbours. To assess the ecotoxicological risk of the new biocides in detail, further research on toxicity and the development of methods for estimating concentrations in natural waters are needed.

Acknowledgements

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