Remarkable Synergistic Effects in Antifouling Chemicals against *Vibrio fischeri* in a Bioluminescent Assay

Xiaojian Zhou,[a] Hideo Okamura,[b] and Shinichi Nagata*,[a]

[a]*Environmental Biochemistry Division, Research Center for Inland Seas and* [b]*Faculty of Maritime Sciences, Kobe University, 5–1–1 Fukaeminami, Higashinada, Kobe 658–0022, Japan*

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A bioluminescent assay using *Vibrio fischeri* Deutsche Sammlung von Mikroorganismen (DSM) 7151 was applied to evaluate the toxicities of some new antifouling chemicals. First, the efficient concentration, led to 50% inhibition of bioluminescence (EC_{50}) values of the single antifouling chemicals zinc 2-pyridinethiol-1-oxide (Zn-pt), copper 2-pyridinethiol-1-oxide (Cu-pt), $CuSO_4$, zinc bis(N,N′-dimethyl)-dithiocarbamate (Ziram), 4,5-dichloro-2-(n-octyl)-3(2H)-isothiazolone (SeaNine 211), pyridine triphenylboron (PTPB), 3-iodo-2-propynyl butylcarbamate (IPBC), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron), dichlofluanid (N-dichlorofluoromthylthio-N′,N′-dimethyl-N-phynylsulfamide) (DCF), and 2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-s-triazine (Irgarol 1051) were determined to be 0.08, 0.12, 0.22, 0.31, 0.35, 0.75, 8.49, 12.74, 39 and > 40 mg/l at 30 min of incubation, respectively. Then, 45 different combinations composed of two antifoulants each were evaluated. Based on the EC_{50} values at 30 min of incubation, typical patterns of interaction for the combinations were classified into three groups based on the comparison of inhibition difference between single chemicals and their mixtures. Mixture toxicity indices were also introduced to examine the interaction effect of each combination. The results showed that most combinations were partially additive, and there was no antagonistic effect among the present combinations of chemicals. Additive effects were observed in the case of Diuron, PTPB, or SeaNine 211 when mixed with IPBC. Marked synergistic effects were observed for Irgarol 1051, Ziram, Zn-pt, and Cu-pt when mixed with Cu^{2+}, which will make these chemicals more toxic against organisms in marine environments.

Key words —— antifoulant, synergistic effect, bioluminescent assay, *Vibrio fischeri*

INTRODUCTION

Many types of chemicals, which may be toxic to organisms and communities, have been discharged into various environments through human activities. Numerous antifouling chemicals used for controlling the growth of marine organisms on submerged structures such as hulls of ships have been released into marine environments, while more vessels are sailing around the world.[1)] Under the International Maritime Organization, since 2003 organotin-based antifoulants, which have been widely used for many years in antifouling paints, have been strictly regulated and their use prohibited because of their severe negative impacts on marine organisms.[1–3)] The ecotoxicologic behaviors of new antifoulants used in place of organotin compounds are poorly understood. In particular, their toxicities when used as mixtures have not been evaluated preciously regardless of the high possibility of serious impacts on the environment.[4,5)] Estimation of the toxicity of mixtures of these chemicals is important due to various antifoulants that are mixed in paint products.

Bioluminescence occurs mainly (although not exclusively) in species living in marine environments.[6)] Importantly, light-emitting bacteria are the most abundant and widespread among luminescent organisms.[7)] *Vibrio fischeri* (*V. fischeri*) is a symbiotic bacterium living in the light organs of fish of the family Monocentridae, as well as the cephalopods *Sepiola* and *Euprymna*, and its bioluminescence intensity (BLI) is sensitive to environmental changes.[8,9)] The BLI of cells in certain phases is strictly related to cell activities, which ensures that

*To whom correspondence should be addressed: Environmental Biochemistry Division, Research Center for Inland Seas, Kobe University, 5–1–1 Fukaeminami, Higashinada, Kobe 658–0022, Japan. Tel. & Fax: +81-78-431-6342; E-mail: nagata@maritime.kobe-u.ac.jp