# Acute Toxicity Assessment of New Algicides of Thiazolidinediones Derivatives, TD53 and TD49, Using *Ulva pertusa* Kjellman

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## ABSTRACT

**Objectives** : This study was aimed to examine the acute toxicity assessment of two new algicides, thiazolidinediones derivatives (TD53 and TD49), which were synthesized to selectively control red tide, to the marine ecosystem.

**Methods** : The assessment employed by a new method using *Ulva pertusa* Kjellman which has been recently accepted as a standard method of ISO. The toxicity was assessed by calculating the $EC_{50}$ (Effective Concentration of 50%), NOEC (No Observed Effect Concentration) and PNEC (Predicted No Effect Concentration) using acute toxicity data obtained from exposure experiments. $EC_{50}$ value of TD49 and TD53 was examined by 96-hrs exposure together with Solutol as a TD49 dispersing agent and DMSO as a TD53 solvent.

**Results** : $EC_{50}$ value of TD53 was 1.65 μM. From the results, values of NOEC and PNEC were calculated to be 0.63 μM and 1.65 nM, respectively. DMSO under the range of 0~10 μM, which is same solvent concentration used in examining TD53, showed no toxic effect. $EC_{50}$ value of TD49 was 0.18 μM and that of Solutol was 1.70 μM. NOEC and PNEC of TD49 were 0.08 μM and 0.18 nM, respectively and those for Solutol were 1.25 μM and 1.25 nM, respectively.

**Conclusions** : From the values of NOEC, PNEC of TD53 and TD49, TD49 showed 9 times stronger toxicity than TD53. On the other hand, DMSO showed no toxicity on the *Ulva pertusa* Kjellman, but Solutol was found to be a considerable toxicity by itself.

**Key words** : Acute toxicity, *Ulva pertusa* Kjellman, Algicides, $EC_{50}$, PNEC, NOEC

## INTRODUCTION

A drastic increase of red tide (harmful algal blooms),

due to eutrophication and changes in environmental conditions around coastal waters, has caused abnormal mortality of aquatic organisms and thus, has brought about serious damage to the fishery industry [1]. Many methods have been studied to effectively eliminate red tide organisms during harmful algal blooms, including physical methods, chemical methods using

chemical algicides, clay or red clay spraying, as well as other methods using flocculants or additives, such as magnesium hydroxide, quicklime, shell powder and zeolites [2]. Currently, spraying of clay or red clay is used to remove red tide organisms [3]. However, this method can cause the secondary problem of inhibiting the respiration or metabolism of benthos [4]. Although chemical agents, such as copper sulfate, hydrogen peroxide and triosin, have been reported to effectively eliminate red tide in a relatively short time [5], the application of such chemical agents to ecological systems has potential risks [6].

This study was conducted to develop red tide removal technology that is in harmony with the natural ecosystem and friendly to the environment, causing minimum toxic effects to the marine ecosystem and free from secondary pollution. The algicide development team focused on the development of an algicide that has weak toxicity to the marine ecosystem, but is highly specific to the harmful algae [7]. The basic framework of the new algicide was thiazolidinedione (TD). TD, a group of molecular receptors in neurons, was introduced in the late 1990s for the treatment of diabetes-related diseases, and has activity when bound to PPARs (peroxisome proliferator-activated receptors), a group of receptor molecules inside the cell nucleus [8]. The algicide activity of the recently developed TD49 was compared with that of DCMU (3-(3, 4-dichlorophenyl)-1, 1-dimethylurea) [9,10], a well-known, commercially available photosynthesis inhibitor of plants and phytoplankton, which showed that TD49 was more selective to the harmful algae than DCMU [11]. However, although TD49 has high toxicity to the harmful algae, it also has low solubility. Thus, TD53 was designed and synthesized to improve the

solubility, which was also verified to have excellent algicide activity to harmful algae, such as *Heterosigma akashiwo*, *Chattonella marina* and *Cocholodium polykrikoides*, even at low concentrations of 0.1-2 μM [11].

In this study, a toxicity assessment was performed using *Ulva pertusa* Kjellman to assess the toxic effects of the new algicides, TD53 and TD49, on the aquatic ecosystem. The ecological toxicity assessment technique, using the Korean domestic organism *U. pertusa* Kjellman, has been adopted as the ISO international standard (2009). *U. pertusa* Kjellman is considered an ideal target species for toxicity assessments, since it satisfies the requirements of being widely distributed, convenient to collect, simple to culture and store, and is sensitive to toxicity, in addition to being applicable to both freshwater and seawater [12]. The toxicity was assessed by calculating the $EC_{50}$ (Effective Concentration of 50%), NOEC (No Observed Effect Concentration) and PNEC (Predicted No Effect Concentration) using acute toxicity data collected from exposure experiments of the two new algicides to *U. pertusa* Kjellman.

## MATERIALS AND METHODS

### 1. Preparation of the new algicides

The newly synthesized TD53 and TD49 as shown in Fig. 1 were provided by Lee *et al*. [7]. Since TD53 (MW: 303.38) is almost insoluble in distilled water, it was dissolved in DMSO (dimethyl sulfoxide) in which the solubility is 12 mg in 100 mL of the solvent (396 μM). TD49 (MW: 337.8) was completely dissolved with the dispersing agent, Solutol® HS15 (MW: 960; BASF, Ludwigshafen, Germany) in 100% etha-
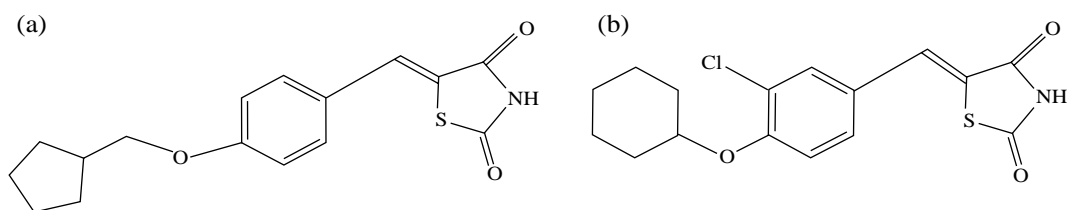


**Fig. 1.** Chemical structures of TD53 (a) and TD49 (b).

nol. Ethanol in the mixture was then removed under vacuum at room temperature using a vacuum pump. The solid dispersing agent including TD49 was scraped out with a spatula and completely dried under vacuum for 12 hours. The dried TD49 was re-dissolved in distilled water using an ultrasonic wave and used as the stock solution (296 μM) for the exposure experiments.

For dilution of the TD53 and TD49 to the desired treatment concentrations, Coralife® culture medium was used, which was prepared by the addition of 35 g of Coralife®, 5 mL of $K_2HPO_4$ (20 mM) and 1 mL of $KNO_3$ (1 M) to 1 L of distilled water.

## 2. Method of the acute toxicity assessment using *U. pertusa* Kjellman

In the test method using *U. pertusa* Kjellman, which is adopted as the ISO international standard method (2009), toxicity is assessed in terms of the area of color change using a diagnosis kit, similar to the litmus paper technique [12-14]. *U. pertusa* Kjellman is a representative algae that is distributed in the south, east and west coasts of Korea, which has the advantages that it can be easily collected, stored and cultured for 2-3 months in the laboratory, and various measurement elements, such as sporulation rate, germination rate, growth rate, photosynthesis rate and respiration rate, can be chosen as the endpoint [12,14,15]. Particularly, the reaction of *U. pertusa* Kjellman to toxic substances is known to be very sensitive when assessing the reproduction process; therefore, the test endpoint of this study was the sporulation rate, which is part of the reproduction process. Thus, the excellence of this method has already been verified in terms of sensitivity to toxic substances [12,14].

### 1) Preparation of test organisms
Green and healthy foliage (longer than 10 cm) of *U. pertusa* Kjellman was taken from the culture medium and prepared as circular fragments using a cork borer with a diameter of 6 mm. The circular fragments were taken from within 10-20 mm of the foliage edge.

The test solutions, with different concentrations, were divided into 10 mL aliquots in a 6-well cell plate. For the dilution experiment, a control group was set for each concentration of the targeted test substance solutions.

For each test well, eight circular fragments of the foliage were exposed. For this, the foliage fragments were put into the well 4 times, with 2 fragments per time.

### 2) Toxicity experiment
The culture temperature, salinity, light radiation and photoperiod conditions were 15°C, 30 psu, 80-100 μmol · $m^{-2}$ · $S^{-1}$ and 12 : 12 h light/dark cycle, respectively. The TD53 concentrations for the experiment were 0, 0.32, 0.63, 1.25, 2.5, 5.0 and 10 μM, with the same concentrations used when DMSO was the solvent in the experiment. The TD49 concentrations were 0, 0.04, 0.08, 0.16, 0.31, 0.63 and 1.25 μM, with concentrations of Solutol, the solvent, of 0, 0.32, 0.63, 1.25, 2.5, 5.0 and 10 μM. The toxicities of DMSO and the dispersing agent Solutol were also investigated as indications of the background toxicities. The test was finished after 96 hours of exposure, with the entire foliage and sporulation areas then measured using image analysis equipment.

### 3) Calculation of $EC_{50}$ of TD53 and TD49
Steel's many-one rank test, which is a nonparametric statistical method that can be used when the repeated numbers of the experimental group and the control group are the same, was used to analyze the data. The calculation was performed by linear interpolation using the statistical software, TOXCALC 5.0 (Tidepool Scientific Software, USA).

## 3. Calculation of NOEC and PNEC

PNEC (Predicted No Effect Concentration) was calculated based on the NOEC and $EC_{50}$, which are the results from the acute toxicity assessment. According to the OECD guidelines [16], if only limited toxicity data are acquired from the assessment of the toxicity of chemicals, a constant assessment factor is applied during each step of the extrapolation to calculate the PNEC to the ecosystem. Since the acute assessment

was performed for one species of *U. pertusa* Kjellman, a factor value of 1,000 was applied to calculate the PNEC.

## RESULTS

### 1. The acute toxicity exposure assessment to TD53

The TD53 treatment concentrations were 0, 0.32, 0.63, 1.25, 2.5, 5.0 and 10 μM, and the $EC_{50}$ of TD53 after 96 hours was 1.65 μM, ranging 0.93-3.01 μM at the 95% confidence limit (Fig. 2). The NOEC calculated from the experimental results was 0.63 μM, and the PNEC calculated by applying the factor 1,000 to the $EC_{50}$ was 1.65 nM.

DMSO was considered to have no toxic effects at the concentration of less 0.1% used in the experiment. Thus, to understand the solvent toxicity, concentrations within the range 0 to 10 μM TD53, i.e. the treatment concentrations, were investigated, and the results showed that DMSO had almost no toxicity, as shown in Fig. 2.

### 2. The acute toxicity exposure assessment to TD49

The TD49 treatment concentrations were 0, 0.04, 0.08, 0.16, 0.31, 0.63 and 1.25 μM, and the $EC_{50}$ after 96 hours was 0.18 μM, ranging 0.12-0.27 μM at the 95% confidence limit (Fig. 3).
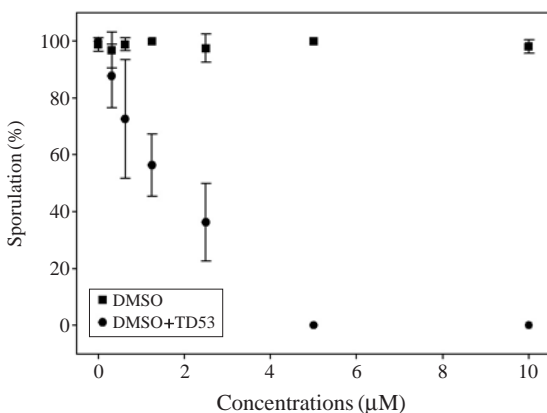
The background toxicity of Solutol, the dispersing agent used for TD49, was also investigated. The Solutol toxicity was investigated to a concentration higher than the TD49 treatment concentration range (10 μM). The $EC_{50}$ after 96 hours was 1.70 μM, ranging 1.16-1.98 μM at the 95% confidence limit (Fig. 3).

The NOEC of TD49 was 0.08 μM and the PNEC was 0.18 nM when the factor 1,000 was applied to the $EC_{50}$. The Solutol of TD49 was 1.25 μM and the PNEC was 1.70 nM when the factor 1,000 was applied to the $EC_{50}$.

### 3. Comparison of the acute toxicity exposure assessment results of TD53 and TD49

The results of the acute toxicities of the two new

**Table 1.** Comparison of $EC_{50}$, NOECs and PNECs of TD53 and TD49 in toxic assessment using *Ulva pertusa* Kjellman

|  | $EC_{50}$ (μM) | NOEC (μM) | Factor | PNEC (nM) |
|---|---|---|---|---|
| DMSO | NA | NA | 1000 | NA |
| TD53+DMSO | 1.65 | 0.63 | 1000 | 1.65 |
| Solutol | 1.7 | 1.25 | 1000 | 1.70 |
| TD49+Solutol | 0.18 | 0.08 | 1000 | 0.18 |

NOEC: no observed effect concentration.
PNEC: predicted no effect concentration.
NA: not affected.



**Fig. 2.** Sporulation ratio of *Ulva pertusa* Kjellman exposed to different concentrations of TD53+DMSO and DMSO.
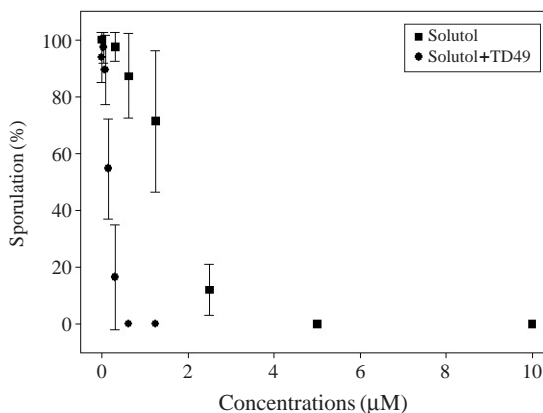


**Fig. 3.** Sporulation ratio of *Ulva pertusa* Kjellman exposed to different concentrations of TD49+Solutol and Solutol.

algicides to *U. pertusa* Kjellman showed that the $EC_{50}$, NOEC and PNEC of TD53 were 1.65 μM, 0.63 μM and 1.65 nM, respectively. The $EC_{50}$, NOEC and PNEC of TD49 were 0.18 μM, 0.08 μM and 0.18 nM, respectively (Table 1), indicating higher toxicity than that of TD53.

## DISCUSSION

Considering the $EC_{50}$ of TD53 and TD49, the toxicity of TD49 was about 9 times stronger than that of TD53. From the toxicity to *U. pertusa* Kjellman, based only on the PNEC values from this experiment, TD53 was deemed to be safe 9 times at the same concentration with TD49. The calculated PNEC of TD49 was 0.18 nM, which is a measure of non-toxicity to the surrounding environment. Although the algicide spray concentration may be higher than the PNEC, the reason why as follow: the algicide has high specificity to the harmful algae, and considering the spraying target in the sea is a water level between 1 and 5 m, where the red tide is generated, the algicide would be rapidly spread by the tidal current due to the deep water depth and wide breadth of the sea, therefore, the algicide should not have a considerable effect on the environment.

Although DMSO, the solvent used for TD53, did not show any toxicity to *U. pertusa* Kjellman, the $EC_{50}$ of Solutol, used as the dispersing agent for TD49, was 1.70 μM, which corresponds to 10% of the toxicity of the TD49 and Solutol mixture. The toxicity of the dispersing agent might have partially contributed to the toxicity of TD49. From the result, it can be presumed that the toxicity of Solutol may have strengthened the toxicity of TD49. Therefore, additional study is required to improve the solubility of TD49 to reduce its toxicity to the marine ecosystem and increase the algicide specificity to the harmful algae.

## CONCLUSIONS

In this study, a toxicity assessment was carried out

using *U. pertusa* Kjellman to assess the toxic effects of two newly designed algicides, TD53 and TD49, on the aquatic ecosystem. The results of the acute toxicity exposure assessment showed that the toxicities were in the order: TD49＞TD53＞Solutol (dispersing agent), on comparison of the $EC_{50}$ values, indicating that TD49 had the highest toxicity. The NOEC and PNEC were in the order: Solutol＞TD53＞TD49. Considering the toxic effects to *U. pertusa* Kjellman, TD49 may have a strong toxic effect also on the surrounding environment, although it had highly selective algicide capacity to the harmful algae. However, it can be assumed that the concentration of the new algicides may have little effect on the marine ecosystem, considering the dilution effect of the marine tidal current. However, the assessment of the toxicity to only one species, *U. pertusa* Kjellman, may not be sufficient to assess the toxic effect on the aquatic ecosystem. Therefore, further acute toxicity assessments are currently being conducted with respect to several species related to the food chain in the aquatic ecosystem.

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