Presumption of toxic mechanism of tributyltin on photosystem in marine diatoms by comparison to diuron as a reference agent through chlorophyll a fluorescence transient analysis

Mst Ruhina Margia Khanam¹, Yohei Shimasaki¹*, Michito Tsuyama², Hiroshi Goto¹, Xuchun Qiu³, Koki Mukai¹, Yuji Oshima¹, ⁴

¹Laboratory of Marine Environmental Science, Faculty of Agriculture, Kyushu University
744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
²Laboratory of Silviculture, Faculty of Agriculture, Kyushu University
744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
³Institute of Environmental Health and Ecological Security, School of Environment and Safety Engineering, Jiangsu University
Zhenjiang, Jiangsu 212013, P.R. China
⁴Institute of Nature and Environmental Technology, Kanazawa University,
Kakuma-machi, Kanazawa, 920-1192, Japan

ABSTRACT
In this study, an OJIP-test of two marine diatom species, Thalassiosira pseudonana (single-celled species) and Skeletonema marinoi-dohrnii complex (chain-forming species), were exposed to EC10 and EC50 levels of diuron and tributyltin (TBT) for 72 h. Increased tendency of relative fluorescence intensity (Ft/Fo value) at 300 μs and J step (2 ms) and a significantly (P < 0.05) increased Mo value (initial slope of fluorescence curve) were observed during 72 of diuron exposure in both species, whereas TBT did not increase those. These results strongly suggest that diuron efficiently blocked photosystem II-catalyzed photosynthetic electron transport at the secondary electron acceptor Qb, which is original biological activity of diuron in diatoms. However, some parameters (e.g., ϕPo and ϕEo, Fv/Fo and PI ABS) were significantly (P < 0.05) decreased by TBT treatment. Although the mechanism responsible for the decrease is not clear, relatively severe reductions in levels of Fv/Fo (an indicator of water-splitting activity) by TBT treatment than diuron treatment suggest that TBT inhibits photosynthetic function via inhibition of photosynthetic oxygen evolving systems, a different mechanism than that of diuron. Moreover, present study suggested that PIABS derived from OJIP-test is a high sensitive biological marker for detecting the toxic effect of pollutants which inhibit photosynthetic function.

Key Words: Antifouling agents, Diatoms, Photosystem inhibition, Chlorophyll a Fluorescence transient, OJIP-test

*Corresponding author, Email: simasaki@agr.kyushu-u.ac.jp; Tel: +81-92-802-4606, Fax: +81-92-802-4606
1. INTRODUCTION

Biofouling in the sea is a widespread phenomenon that impacts many structures submerged in water including ship's hulls, oil and gas installations and fishing nets and cages. This phenomenon leads to a loss of industrial efficiency in various ways. For example, biofouling of ships' hulls increases fuel consumption via increased friction against water, increases cleaning costs and decreases the durability of the hulls. Indeed, a 1 mm thick layer of algal slime may increase hull friction by 80% and fuel consumption by 17% (Evans et al. 2000). Bio-films in tubes and pipes in plants can also increase resistance to flow and cause clogging and reduced pressure.

Conventional methods for controlling biofouling involve the application of paint containing antifouling reagents, which plays an important role in preventing the settlement and growth of marine organisms on submerged structures (Boxall et al. 2000 ; Evans et al. 2000). However, if these antifouling reagents are released into marine environments, they can cause adverse effects on marine organisms. Indeed, tributyltin (TBT) containing antifouling paints, which have been commonly used worldwide from the mid-1960s to the 1970s (Yi et al. 2014), had serious adverse effects on many marine organisms in the 1970s (Alzieu 2000). This compound was found to have bio-concentration factors as high as 10,000 (Jacobson and Willingham 2000) because of its log Kow values near 4.4 at pH 8 (Meador 2000). Because of its high bio-concentration factor and harmful effects to marine organisms, the application of TBT as an antifouling paint has been regulated in the European Union (EU) since the late 1980s and the use of antifouling paints containing TBT has been banned in many countries along with the International Maritime Organization (IMO) since 2008. However, TBT is used in anti-fouling paints in some countries of the Caribbean and Asia (Caribbean Environment Programme), especially in developing countries and countries that do not belong to the IMO. As a result, TBT has been detected in sea water at 23.9 ng Sn L$^{-1}$ in Korean bays (Kim et al. 2014) and in sediment at 4.25 µg g$^{-1}$ wet weight in Manado, Indonesia, 0.21 µg g$^{-1}$ wet weight in Benoa Port in Bali, Indonesia and 0.63 µg g$^{-1}$ wet weight in Hakata Port, Japan (Undap et al. 2013). Moreover, the average concentration of butyltin from the Turkish Aegean Sea coastline was reported to be 1091.5 ng Sn g$^{-1}$ (Yozukmaz et al. 2011) after its worldwide ban, and TBT is still a matter of concern for marine organisms.

Some antifouling agents, such as 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (diuron), are used as alternatives to TBT. Diuron, which is a widely used antifouling agent in the marine environment, is also used as an herbicide in agricultural fields worldwide. As a result, diuron has been detected at levels over the maximum allowable concentration of the environmental quality standard (MAC-EQS; 1.8 µg L$^{-1}$, Sjollema et al. 2014) in coastal areas of some countries (e.g., 2.18 µg L$^{-1}$ in Japan, Kaonga et al. 2015).

Because diuron and TBT have been used all over the world, many studies have investigated the harmful effects of these chemicals on non-target marine organisms (Nebeker and Schuytema 1998 ; Alzieu 2000), including microalgae, which are important primary producers and the base of the aquatic food web. Diatoms are the most successful phytoplankton in aquatic ecosystems, contributing about 40% of the organic carbon produced in the ocean each year (45 to 50 billion metric tons). The presence of antifouling agents in marine environments may affect the productivity of microalgae by interrupting their photosynthetic activity, which could in turn affect the total marine ecology. The photosynthetic process is a key phase of plant metabolism; therefore, it is important to ensure that antifouling agents
do not adversely impact photosynthetic function when evaluating the risks associated with these compounds.

Diuron is a typical photosystem II (PSII) inhibitor that blocks electron transfer from Q$_A$ to Q$_B$ by binding to the D1 protein in the PSII complex of higher plants, thereby preventing the production of high-energy compounds such as adenosine triphosphate (ATP), reductant potential and CO$_2$ fixation. Tributyltin is generally known to inhibit ATPase and ATP synthase activity in various organisms, including unicellular green algae (Catt et al. 1984; Moreland 1980; Watling-Payne and Selwyn 1974). Some studies have investigated the inhibitory effects of TBT on photosynthesis related parameters in microalgae, such as the carbon fixation rate in the Cryptophytes Rhodomonas salina and Skeletonema costatum (Petersen and Kusk 2000) and oxygen production in some phytoplankton species (Nicolas and Nakahara 2002). However, Shimasaki et al. (2015) suggested that TBT has a different toxic mechanism than that of a PSII inhibitor Irgarol via chlorophyll a fluorescence response in the Skeletonema marinoi-dohrnii complex. Nevertheless, information regarding the mechanism of TBT toxicity on the photosystems of unicellular algae is limited; therefore, additional study is needed to clarify its effects on photosynthetic activity and the mechanism of these effects.

Chlorophyll a fluorescence is often proposed as a simple, rapid and sensitive indicator (Strasser 2004; Kuster and Altenburger 2007) for investigation of the effects of pollutants or environmental changes on the photosynthetic apparatus. Chlorophyll a fluorescence transient analysis provides a number of useful parameters to quantify energy flow through the reaction center (RC) of PSII (Force et al. 2003; Strasser 2004). Among various approaches to analysis of chlorophyll a fluorescence transient, the OJIP-test (Strasser et al. 2000; Strasser 2004) is frequently used in different areas of plant biology (Gilmore et al. 1998; Yusuf et al. 2010). The OJIP-test is based on the theory of energy flow in thylakoid membranes (Force et al. 2003), which enables understanding of the relationships among PSII activity, fluorescence signals and their analytical expressions (Strasser et al. 2000; Bussotti et al. 2007). However, the OJIP-test approach has not been fully investigated in marine planktonic diatoms to elicit the toxic effects of pollutants on their photosystems.

Therefore, this study was conducted to investigate the response of OJIP parameters in two marine planktonic diatom species, Thalassiosira pseudonana (single-celled species) and Skeletonema marinoi-dohrnii complex (chain-forming species), exposed to diuron and TBT and to elucidate the mechanisms of the toxic effects of TBT in these species.

2. MATERIALS AND METHODS

2.1 Algal culture

Axenic strains of Thalassiosira pseudonana (CCMP 1335) was obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota, USA (formerly CCMP, East Boothbay, ME) and Skeletonema marinoi-dohrnii complex (NIES-324) was obtained from the National Institute for Environmental Science (NIES), Tsukuba, Japan. The cultures were maintained in glass vials with screw caps (35 mm × 78 mm; Maruemu Corporation, Osaka, Japan) containing 10 mL modified SWM-3 medium (Yamasaki et al. 2007) (pH 7.9, salinity 30 PSU). The cells were grown at 25°C under 80–90 μmol photons m$^{-2}$ s$^{-1}$ light-emitting diodes (LED) (LDA7N, Toshiba, Kanagawa, Japan) on a 12/12 h light/dark cycle in a growth chamber (Eyelatron FLI-160, Tokyo Rikakikai, Tokyo, Japan). Irradiance in the growth chamber was measured with a quantum scalar laboratory irradiance sensor (Biospherical Instruments, USA).
2.2 Determination of the effective concentration of TBT for growth of *T. pseudonana*

The 10% and 50% effective concentrations (EC10 and EC50) of diuron for growth of *T. pseudonana* and *S. marinoi-dohrnii* (Khanam et al. 2017) and TBT in *S. marinoi-dohrnii* (Shimasaki et al. 2015) were determined in previous studies. To estimate the EC values of TBT for growth of *T. pseudonana*, TBT (TBT chloride, 95% purity, Tokyo Kasei Kogyo, Tokyo, Japan) exposure tests were conducted. Tributyltin was dissolved in dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries, Osaka, Japan) as stock solution (1 mg mL\(^{-1}\) DMSO). The nominal concentration of TBT in the test medium was adjusted to 0, 0.5, 1, 2, 4, and 8 µg L\(^{-1}\) (n=6 for the treatment groups and control) by dilution of the stock solution in DMSO. The final DMSO concentration in each test tube was 0.0001%, including a 0 µg L\(^{-1}\) (control) group. The initial cell densities of *T. pseudonana* were adjusted to 1 × 10^4 cells mL\(^{-1}\) in each test tube (13 mm × 100 mm; Maruemu Corporation, Osaka, Japan) in the early stationary phase. The cultures were then incubated at 25°C under 80–90 μmol photons m\(^{-2}\) s\(^{-1}\) LED on 12/12 h light/dark cycle in a growth chamber and each test tube was mixed twice daily. Growth of *T. pseudonana* in each tube was monitored using an *in vivo* fluorometer (Model 10-AU, Turner Designs, CA, USA) at 0, 24, 48, and 72 h from the beginning of exposure. Before measuring the *in vivo* fluorescence, the cell suspension was kept in the dark for 40 min. The algal growth was also monitored by direct cell counting with microscopic observation at 0 and 72 h of exposure. The growth rate (divisions day\(^{-1}\)) in each treatment group was calculated by the method described by Guillard (1973) using cell count data of 0 and 72 h as follows:

\[ \mu = \frac{(\ln N_t - \ln N_0)}{t} \]  
\[ \text{Growth rate (divisions/day)} = \frac{\mu}{\ln 2} \]  
where \( \mu \) is the specific growth rate, \( t \) is the time in days, \( N_t \) is the cell density (cells mL\(^{-1}\)) after \( t \) days from the beginning of exposure, and \( N_0 \) is the initial cell density in equation (1). The effective concentrations for growth rate (EC10 and EC50) were then estimated by probit analysis using the statistical software SPSS version 10.0 (SPSS, Inc., IL, USA).

2.3 Chlorophyll a fluorescence transient analysis (OJIP-test)

After determination of EC values (EC10 and EC50), aliquots of the diatom culture in the early stationary phase were resuspended in 5 mL of modified SWM-3 medium at an initial cell density of 1 × 10^4 cells mL\(^{-1}\) in glass tubes (13 mm × 100 mm; Maruemu Corporation, Osaka, Japan) containing diuron at 0, 1.4, or 5.9 µg L\(^{-1}\) (control, EC10, and EC50, respectively, 0.0001% DMSO in each glass vial, n=5) for *T. pseudonana* and 0, 0.8, or 4.0 µg L\(^{-1}\) (control, EC10, and EC50, respectively, 0.0001% of DMSO in each glass vial, n=5) for *S. marinoi-dohrnii*. In the case of TBT, aliquots of the diatom culture in the early stationary phase were resuspended in 5 mL of modified SWM-3 medium at an initial cell density of 1 × 10^4 cells mL\(^{-1}\) in glass tubes containing TBT at 0, 1, or 1.7 µg L\(^{-1}\) (control, EC10, and EC50, respectively, 0.0001% DMSO in each glass vial, n=5) for *T. pseudonana* and 0, 0.68, or 2.14 µg L\(^{-1}\) (control, EC10, and EC50, respectively, 0.0001% of DMSO in each glass vial, n=5) for *S. marinoi-dohrnii*.

These samples were then incubated under the conditions described above for the acute toxicity test. Subsequently, at 24, 48, and 72 h after diuron and TBT exposure, the culture was subjected to chlorophyll *a* fluorescence measurement at room temperature using an Aquapen-C AP-C 100 (Photon Systems Instruments, Czech Republic). All samples were dark-adapted for 40 min prior to the measurements. Some parameters were derived from the fluorescence transient to quantify the flow of energy through the reaction center of PSII (Straar 1995; Force et al. 2003; Strasser...
et al. 2004). When dark adapted photosynthetic organisms are illuminated with high light, chlorophyll \(a\) fluorescence rises from an initial low value (the minimal fluorescence: \(F_0\)) at 50 \(\mu\)s to the fluorescence value at 2 ms (\(F_t\)), 60 ms (\(F_j\)) and a peak of fluorescence at 1000 ms (\(F_{m}\)) occurs (Fig. 1). The OJ including 300 \(\mu\)s is the phase leading to the reduction of \(Q_A\) to \(Q_A^-\). The JI phase parallels the reduction of the PQpool (Tóth et al. 2007), and the IP phase is correlated with the reduction of ferredoxin in the presence of inactive ferredoxin: NADP\(^+\) oxidoreductase (Schansker et al. 2005). Some parameters are derived from the fluorescence transient, which facilitates understanding how the flow of energy through the reaction center of PSII is distributed among absorption, trapping, electron transport and dissipation (Force et al. 2003; Strasser et al. 2004). The energy fluxes can be thought of as an absorbed flux (ABS), a trapping flux (TR), an electron transport flux (ET) and a flux defining the dissipation of non-trapped energy as heat and some fluorescence, or transferred to other systems (DI). Table 1 summarizes the parameters extracted and calculated from the OJIP-test.

2.4 Statistical analysis

Statistical differences between treatment groups and the control were analyzed by Dunnett’s test after assumptions of homogeneity of variance which had been tested by Levene’s test. All statistical analyses were performed using SPSS version 10.0 (SPSS, Inc., IL, USA).

3. RESULTS

3.1 Effects of diuron on photosynthetic energy flux of diatoms

In both diatom species, the \(F_t/F_0\) values showed temporal increases at 300 \(\mu\)s and J step and decreased at I and P steps in response to diuron treatment especially at the EC50 at 24, 48 and 72 of exposure when compared with control (Fig. 2). Among parameters calculated from chlorophyll \(a\) fluorescence transient analysis, significant increases \((P < 0.05)\) were observed in ABS/RC, TR/RC, DI/RC and Mo (Fig. 3). Conversely, ET/RC and \(F_v/F_o\) decreased significantly in the EC50 exposure group, and some parameters were significantly \((P < 0.05)\) changed in the EC10 exposure groups when compared with each control. All flux ratios indicated significant decreases in
EC50 in response to diuron treatment (Fig. 4), especially ETo related parameters (Ψo and Ψ0) showed large decreases in response to diuron treatment. The PI ABS value, which expressed three overall functional steps (energy absorption, energy trapping and energy conversion into electron transport) decreased significantly ($P < 0.05$) in the EC10 and EC50 group of diuron treated *S. marinoi-dohrnii* and *T. pseudonana* at 24, 48 and 72 h (Fig. 5).

### 3.2 Effect of TBT on photosynthetic energy flux of diatoms

The Ft/Fo values of the TBT exposure groups, except for EC10 in *T. pseudonana*, showed decreased tendencies through steps from 300 µs to P compared with the control (Fig. 6). In both species, two parameters (ABS/RC and DIo/RC) significantly ($P < 0.05$) increased, but Fv/Fo significantly ($P < 0.05$) decreased in the EC50 exposure group (Fig. 7). The TRo/RC, ETa/RC and Mo values did not change significantly in response to almost all the TBT treatment (Fig. 7). Two flux ratio parameters (ϕPo and ϕEo) indicated a significant ($P < 0.05$) decrease in TBT in response to both the EC10 and EC50 treatment or the EC50 treatment alone when compared with the control (Fig. 8). However, unlike diuron treatment, Ψo, which reflects the flux ratio between ETo/RC and TRo/RC, did not decrease significantly in response to TBT treatment. Finally, the PI ABS decreased significantly ($P < 0.05$) in the EC10 and EC50 group of TBT treated *S. marinoi-dohrnii* and *T. pseudonana* at 24, 48 and 72 h (Fig. 9).
4. DISCUSSION
4.1 Characterization/evaluation of diuron mechanism by OJIP-test

Diuron, a representative PSII inhibitor, is known to have toxic effects on plant photosynthesis by binding to the $Q_o$ binding site of the PSII photosynthetic complex with high affinity (Chesworth et al. 2004). The increase in relative fluorescence value at 300 µs and J-step at 24, 48 and 72 h in diuron treated diatoms (Fig. 2) strongly supported that diuron inhibits electron transport on the acceptor side of PSII to reaction centers in diatoms since a rise in the J-step level is a marker of large amounts of...
accumulation of reduced QA (QA\(^{-}\)) (Strasser 1992; Strasser 2004). As expected from the results of significant increase of \(F_{300\mu s}/Fo\) and \(F_{J}/Fo\), the OJIP parameters reflecting the electron transport after QA (i.e., ET\(_{O}/RC\), \(\Psi_o\) and \(\phi_{Eo}\)) were also decreased by diuron treatment. Moreover, PI\(_{ABS}\), which is calculated using \(\phi_{Po}\), \(\Psi_o\) and ABS/RC, significantly decreased by change of all these three parameters in response to diuron treatment (EC10 and EC50) in both species, demonstrating diminution of the PSII activity. These results indicate that diuron binds the QB binding site of the PSII photosynthetic complex in diatoms as in higher plants.

4.2 Characterization/evaluation of TBT mechanism by OJIP test

Different from diuron, the shape of Ft/Fo curves in two diatom species exposed to TBT showed significant decrease of relative fluorescence value in all steps after O-step except for EC10 in \(T.\) pseudonana compared with the control (Fig. 6), and consequently significant decrease were found in \(\phi_{Po}\) and PI\(_{ABS}\). However, since there was no significant increase in the Ft/Fo value at 300\(\mu s\) and J step unlike in the case of diuron treatment, the values of TRo/RC, ET\(_{O}/\) RC, \(\Psi_o\) and Mo were not significantly changed by TBT treatment (Table 2). These findings
indicated that TBT did not block the electron transport chain at the acceptor site of PSII. This was also suggested by Shimasaki et al. (2015), who found that Irgarol (a PSII inhibitor similar to diuron) increased chlorophyll fluorescence Fo, but TBT did not. Conversely, the present study showed that $\phi_{Po}$ and PI$_{ABS}$ (by change of $\phi_{Po}$ and ABS/RC) were significantly decreased by TBT exposure. The decrease of $\phi_{Po}$, which are the representative parameters reflecting the activity of PSII, was induced by decreased ratio of $F_m$ to $F_o$. These results indicate that TBT could induce photosystem inhibition through different mechanisms than diuron.

In general, TBT is known to inhibit ATPase/ATP synthase activity in various organisms, including unicellular green algae (Catt et al. 1984; Moreland 1980; Watling-Payne and Selwyn 1974). von Ballmoos et al. (2004) reported that TBT inhibits ion channel of F-type ATP synthase. If TBT inhibits F-type ATP synthase activity in chloroplasts of diatoms, the protons concentration in the thylakoid lumen should increase via decreased flow of protons from the thylakoid lumen to the stroma (Kramer et al. 2004). An increased proton concentration induces acidification of the intrathylakoid pH that should be eased by decreased oxidation.

Fig. 4 Effects of different concentrations of diuron on calculated parameters (flux ratio) based on the chlorophyll fluorescence transient curves of S. marinoi-dohnii and T. pseudonana. $\phi_{Po}$: TRo/ABS ($F_v$/ $F_o$), $\Psi_o$: ET$_o$/TR$_o$, $\phi_{EO}$: ET$_o$/ABS. *significant change of each parameter compared with the control ($P < 0.05$).
Toxic mechanism of tributyltin on photosystem in marine diatoms

of PQH$_2$, which is known as a photosynthetic control (Harbinson et al. 1990). As a result of this scenario, reduction power accumulates in the electron transport chain and TRo/RC and ETo/RC might increase because of TBT exposure. However, these parameters were not changed by TBT exposure. Thus, we couldn’t obtain data supporting the inhibition of F-type ATP synthase activity by TBT in the present study. Additional inspection is necessary to confirm this.

A likely explanation of other toxic mechanisms on photosynthesis is inhibition of the oxygen-evolving system by TBT. The ratio of Fv and Fo has been used as a parameter of oxygen-evolving systems (Kriedemann et al., 1985). Moreover, there have been some reports using the Fv/Fo ratio as an indicator of water-splitting complexes on the donor side of PSII (Starck et al. 2000, Jajoo et al. 2014, Kalaji et al. 2011). In the present study, the Fv/Fo ratios were decreased by TBT treatment (Fig. 7) in both diatom species due to decrease of relative F$_m$ value (F$_m$/Fo). Decrease in the F$_m$ value potentially is caused by inhibition of oxygen-evolving complexes (Pospíšil and Dau 2000, Tóth et al. 2009). The inhibition levels of Fv/Fo compared with the control were more severe in the TBT treatment (around or less than 50% of the control value by EC50) than the diuron treatment (more than 80% of the control value by EC50). These observations suggest that TBT

Fig. 5 Effects of different concentrations of diuron on calculated parameter PI$_{ABS}$ based on the chlorophyll fluorescence transient curves. *significant change of each parameter compared with control (P < 0.05).
may influence the oxygen-evolving system. However, further study is needed to clarify the mechanism of TBT for photosystem inhibition in algae.

4.3 Deduced mechanism of increase of ABS/RC by either treatments

Our results also showed that ABS/RC increased in the EC50 group of both species in response to both diuron and TBT treatment at 24, 48 and 72 h (Figs. 3 and 7). This parameter is calculated as the sum of trapped energy flux (TRo/RC) and dissipated energy flux (untrapped energy flux, DIo / RC) or calculated by dividing TRo/RC by $\phi_{Po}$. It has been reported that after most environmental stresses, a portion of the
reaction centers (RCs) are transformed to inactive reaction centers (Strasser et al. 2004). Thus, the increased expression ABS/RC might be because of the decreased number of RCs per absorption (RC/ABS). On the other hand, no change in TRo/RC (especially in TBT treatment group) and an increase in ABS/RC is an indication of an increase in the PSII antenna size (Chalifour 2011). Wilson and Huner (2000) suggested that transthylakoid delta-pH adjusts the size of the LHCII antenna complex. Shimasaki et al. (2015) reported that TBT didn’t affect the content ratio of chlorophyll a and c, which is an indicator of the amount ratio of reaction center and light-harvesting antenna, in S. marinoi-dohrnii exposed to TBT. Further study (e.g., investigating the protein expression level) is needed to explain the mechanism responsible for increases in the ABS/RC of diatoms exposed to TBT and diuron.

4.4 Potential of OJIP-test for toxicity evaluation
The present results showed that some parameters detected the adverse effects of diuron and TBT at EC10 or EC50 levels. These parameters evaluate local or whole conditions of PSII, from the light-harvesting to electron transport chain after electron acceptor (Qa). Among them, the $\phi_{ps}$ (generally known as Fv/Fm), which reflects the PSII activity based on the following simple formula $\phi_{ps} = (F_M - F_o)/F_M$. 

---

Fig. 7 Effects of different concentrations of TBT on calculated parameters based on the chlorophyll fluorescence transient curves in S. marinoi-dohrnii and T. pseudonana. *significant change of each parameter compared with control ($P < 0.05$).
Has been frequently used for evaluating the effects of pollutants or environmental stress on photosynthetic activity of algae and plant. This parameter should be significantly decreased due to the over-reduction of electron transport chain. Beside the direct inhibition of PSII, the decrease in \( \phi_{Po} \) can also be induced by the decrease in Calvin cycle activity and/or consumption rate of ATP and NADPH, which are essential for various energy metabolic system. Thus, variations in the \( \phi_{Po} \) may reflect various cellular physiological changes induced by pollutants and/or environmental stress. In addition, the PI_{ABS} showed drastic decreased values in diuron and/or TBT exposure groups.

**Fig. 8** Effects of different concentrations of TBT on calculated parameters (flux ratio) based on the chlorophyll fluorescence transient curves in *S. marinoi-dohrnii* and *T. pseudonana*. \( \phi_{Po}: TR_o/ABS \) (Fv/Fo), \( \Psi_{O}: ET_o/TR_o \), \( \phi_{EO}: ET_o/ABS \), *significant change of each parameter compared with control \( (P < 0.05) \).

Because the PI_{ABS} is a multi-parametric expression of three independent steps in PSII (i.e., ABS, TR, and ET), this index can give a more detailed examination of the fluorescence signal reflecting the activity of PSII. Thus, \( \phi_{Po} \) and PI_{ABS} is thought to be useful parameters in the eco-toxicological studies, which aimed at detecting the adverse effects of pollutants and/or environmental stress on photosynthesis of algae and high plants.

**5. CONCLUSIONS**

The results of this study demonstrated the mechanism of diuron and TBT toxicity in marine diatoms and compared it with the
Toxic mechanism of tributyltin on photosystem in marine diatoms

Diuron decreased $\phi_{Po}$ and PI$_{ABS}$ values accompanied by over-reduction of electron transport chain, which was affirmed by the increase of Mo value based on the increase of $F_{300\mu s}/F_0$ and decrease of $F_M/F_0$. Thus Mo value is a useful parameter for evaluating over-reduction of until the electron acceptor, $Q_A$ in electric transport chain. The results of this study also indicated that TBT decreased $\phi_{Po}$ and PI$_{ABS}$ values mainly due to the decrease of $F_M/F_0$ with no occurrence of over-reduction in electron transport chain, which was affirmed by the lack of a significant change in Mo value. Although this study couldn’t detect any evidence supporting inhibiting effect of TBT on ATP metabolism which is generally known activity of TBT, the decreased Fv/Fo value may be the leading effect of TBT treatment on oxygen-evolving systems. However, further molecular investigation is needed to confirm the direct action site of TBT on diatoms.

ACKNOWLEDGEMENT

This study was partially supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (18K05787). We thank Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.
### REFERENCES


Caribbean Environment Programme (CEP, 4/1/2014), Persistent Organic Pollutants (POPs) and Pesticides


Gilmore AM, Shinkarev VP, Hazlett TL, Govindjee (1998) Quantitative analysis of the effects of intrathylakoid pH and xanthophyll cycle pigments on chlorophyll a fluorescence lifetime distributions and

---

**TABLE 2** Summary of OJIP test in two diatom species, Skeletonema marinoi-dohrnii complex (S. m.d.) and Thalassiosira pseudonana (T. p.), exposed to diuron or TBT. Up-pointing arrows indicate parameters significantly increased by chemical treatment at the EC50 level only (↑) or both EC10 and 50 (↑↑) compared with the control at 72 h. Down-pointing arrows indicate parameters significantly decreased at the EC50 level only (↓) or both EC10 and 50 (↓↓) compared with the control at 72 h. ‘nc’ indicates no significant change by chemical treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Response at 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diuron</td>
</tr>
<tr>
<td>ABS / RC</td>
<td>↑</td>
</tr>
<tr>
<td>TRo / RC</td>
<td>↑</td>
</tr>
<tr>
<td>ETo / RC</td>
<td>↓↓</td>
</tr>
<tr>
<td>DIo/ RC</td>
<td>↑</td>
</tr>
<tr>
<td>Fv /Fo</td>
<td>nc</td>
</tr>
<tr>
<td>Mo</td>
<td>↑↑</td>
</tr>
<tr>
<td>φo (TRo/ ABS)</td>
<td>↓</td>
</tr>
<tr>
<td>Ψo (ETo/ TRo)</td>
<td>↓↓</td>
</tr>
<tr>
<td>φEo (ETo/ ABS)</td>
<td>↓↓</td>
</tr>
<tr>
<td>PI_{ABS}</td>
<td>↓↓</td>
</tr>
</tbody>
</table>
intensity in thylakoids. Biochem 37:13582-13593
Kim NS, Shim WJ, Yim UH, Hong SH, Ha SY, Han GM, Shin KH (2014) Assessment of TBT and organic booster biocide contamination in seawater from coastal areas of South Korea. Mar Pollut Bull 78(1-2):201-208
parameters in the marine diatom sp. exposed to tributyltin and Irgarol. Jpn J Environ Toxicol 18(1):1-10


Straaer B (1995) Measuring fast fluorescence transients to address environmental questions L; the JIP-test. Photosynthesis: from light to biosphere. 977-980


(受付：2019年6月23日；受理2019年10月10日)