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TOXICITY REDUCTION OF METAL PYRITHIONES BY NEAR ULTRAVIOLET
IRRADIATION

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ABSTRACT

Zinc pyrithione (ZnPT) or copper pyrithione (CuPT) has been used as one of the effective ship-antifouling agents alternative to organotin compounds. There have been a few data on their residue levels in environmental matrices due to their unstability to light and a lack of suitable analytical procedure. It is possible to investigate the fate of such compounds by toxicity alteration with certain treatment. The purpose of this study was to evaluate the degradation of the pyrithiones through toxicity reduction by near ultraviolet (UV-A) irradiation. Metal pyrithiones dissolved in acetonitrile were irradiated with UV-A lamp for 0, 0.5, 1, and 2 hours, and were subjected for UV spectral measurement and for toxicity evaluation by both sea urchin and freshwater rotifer bioassays. For bioassays, photolysed samples were dissolved in dimethyl sulfoxide after evaporation of acetonitrile. The changes in UV spectra of photolysed ZnPT or CuPT showed time-dependent degradation and the UV spectra at 2 hours irradiation suggested substantial decomposition. Toxicities of ZnPT and CuPT were 12 µg/l and 5 µg/l as 24-hour LC50 to the survival of rotifer; 10×10^{-6} ng/l and 2.3 ng/L as 27-hour EC50 to the normal pluteus formation, respectively. By evaporation of acetonitrile, the EC50 of ZnPT became 2.2 ng/l, which was the same value as CuPT. The EC50s of ZnPT or CuPT to both species increased with longer irradiation time. Photolyzed ZnPT or CuPT demonstrated substantial degradation in the UV spectra, but possessed marked toxicity. One reason why photolyzed CuPT was toxic to rotifer was due to high toxicity of copper ion formed by UV-A irradiation.

Keywords: antifouling, copper, egg development, pyrithiones, photodegradation, rotifer, sea urchin, UV-A, zinc,

INTRODUCTION

The antifouling compounds, which are alternatives to organotins, are intended to be used worldwide. There have been limited data available on the toxic effects of them towards environmentally relevant marine species, specially non-fouling organisms. Using a sea urchin egg bioassay, we reported the toxicity of alternative antifouling compounds such as Irgarol 1051, Diuron, triphenyl borane pyridine, Sea Nine-211, copper pyrithione, zinc pyrithione, and along with tributyltin oxide (TBTO) (Kobayashi and Okamura, 2002). The previous investigation reported that zinc pyrithione inhibited sea urchin egg development at extremely low concentration than the other antifoulant did. No observed effect concentrations (NOEC) of zinc pyrithione and copper pyrithione were estimated as 10 pg/L and 10 µg/L, respectively. Fish toxicity of zinc pyrithione are reported as a 28-day LC50 of 1 µg/L for juvenil rainbow trout and a 24-hour EC50 of 1 mg/L for fish cell line (Okamura et al., 2002). Copper pyrithione showed higher toxicity to both fish species than zinc pyrithione did. On the other hand, it is reported that the metal pyrithiones rapidly degrade in the water column to form less toxic compounds by photodegradation (Turley et al., 2000). It is, therefore, interested in the degradation of zinc pyrithione in aquatic environments. Analytical procedure of metal pyrithiones has not been fully established although stable transformation of zinc pyrithione by copper chelation is reported (Thomas 2000). On this situation, it is possible to apply a bioassay to delineate the relationship between degradation and toxicity of the compound.

In the present study, we planned to observe the effects of zinc pyrithione and copper pyrithione treated with UV-A irradiation, using a sea urchin egg bioassay. The aim of this work is to clarify the effects of UV-A irradiation on the toxicity of metal pyrithione. UV-A region is a major portion of ultra-violet irradiation in natural sunlight which can penetrate to the earth ().

MATERIALS AND METHODS

Preparation of Test Samples

Zinc pyrithione (2-mercaptopyridine *N*-oxide zinc salt, 98%, ZnPT) was purchased from Tokyo

Chemical Industry Co.Ltd., Japan and Copper pyriothione (2-mercaptopyridine *N*-oxide copper salt, 98.7%, CuPT) from Hayashi Pure Chemical Industries, Ltd., Japan. ZnPT or CuPT was dissolved in acetonitrile (MeCN, Wako Pure Chemical Industries, Ltd, Japan, HPLC grade) to be 100 mg/L. Three ml of the soln in a quartz cell (1 cm light pass) tightly capped was irradiated horizontally with an UV-A lamp (20 W, National Co. Ltd., Japan). UV-A intensity at the center of the cell was adjusted to be 2000 $\mu\text{W}/\text{cm}^2$ ($= 20 \text{ W}/\text{m}^2$) using a digital UV meter attached with an UV-A detector (Model UV-103, Macam). The UV-A intensity is within the values of solar irradiation observed in summer at west Japan (Okamura et al., 1999). The solns were irradiated for 0, 0.5, 1, and 2 hours at ambient temperature. A portion (0.03 mL) was added to MeCN (2.97 mL) to be nominal concentration of 1 mg/L to determine its UV spectrum. The solvent MeCN in the irradiated sample (2.97 mL) was evaporated by nitrogen stream below 40°C in a hot block bath. The resultant residue was dissolved in 2.97 mL of dimethyl sulfoxide (DMSO, Kanto Chemicals, spectroscopy grade). The following five samples dissolved in DMSO were prepared for a bioassay. The concentration in the sample ZnPT-0 or CuPT-0 must be 100 mg/L if no loss or no reaction occurs during the treatment process.

1. ZnPT-control or CuPT-control : no evaporation and no irradiation, just dissolved in DMSO
2. ZnPT-0 or CuPT-0 : with evaporation after no irradiation
3. ZnPT-0.5 or CuPT-0.5 : with evaporation after 0.5-hour irradiation
4. ZnPT-1 or CuPT-1 : with evaporation after 1-hour irradiation
5. ZnPT-2 or CuPT-2 : with evaporation after 2-hour irradiation

Toxicity on Embryo Development of Sea Urchin

Bioassay using sea urchin *Anthocidaris crassispina* was performed in the same manner as shown in our previous study (Kobayashi and Okamura, 2002). The specimen was obtained near the Seto Marine Biological Laboratory, Wakayama Prefecture, Japan, August 2002. Eggs and sperm were mixed and inseminated in the test samples. Rates of first cleavage, formation of plutei, and of any anomalies in their development were monitored.

Stock solutions of the test chemicals dissolved in DMSO were made up into a series of seawater

solutions by successive 10 times dilution with standard seawater. The first cleavage occurred in most cases 50-60 min after fertilization at 26°C. The rate and nature of the first cleavage, namely the proportions of undivided cells and normal doubled cells in each test water set, were checked after some adequate time. After successive divisions and developmental changes, the proportions of normal plutei, retarded, malformed and pre-pluteus embryos or larvae were checked at 27 h after fertilization. Two hundred eggs or embryos were fixed with 5% formaldehyde at the time of these examinations. The tests were repeated three times on different three batches.

Lethal Toxicity on Freshwater Rotifer

A standardized bioassay was performed using juveniles of the freshwater rotifer *Brachionus calyciflorus*, supplied as dormant eggs in the Rotoxkit F (Microbiotest Inc., Deinze, Belgium). Cysts were hatched in moderately hard water at 25°C under continuous illumination. The neonates were collected within two hours after hatching and used immediately in the experiments using a 48-well microplate, which allowed testing of seven concentrations and one control with five replicates. A 24-hour LC50 with 95% confidence intervals was calculated from the data obtained by 30 individuals at one concentration.

RESULTS AND DISCUSSION

UV Spectra of the Metal Pyrithiones Irradiated by UV-A

Prior to redissolving into DMSO, the UV spectra of ZnPT or CuPT in acetonitrile after 0, 0.5, 1, 2-hour irradiation were measured with an UV spectrophotometer (UV-1650PC, Shimadzu). The concentration of the original sample without irradiation was 1.0 mg/L (Fig.1). The UV spectrum of ZnPT exhibited absorption maxima at 243, 273, and 325 nm. The absorption maxima decreased up to one-hour irradiation and the spectrum of two-hour irradiated sample was totally different from the others. The UV spectrum of CuPT exhibited absorption maxima at 251 and 320 nm (Fig.1). The absorption maxima at a shorter wavelength 251 nm moved to shorter depending on the irradiation time, although there was no change in the absorption maxima at longer wavelength 320 nm. The spectrum of two-hour irradiated

sample was totally different from the others. It was suggested that two-hour irradiation substantially degraded ZnPT or CuPT molecule in acetonitrile.

Effects on Sea Urchin Embryos

ZnPT treatment without UV irradiation on sea urchin eggs was shown the similar results as shown in the previous report (Kobayashi and Okamura, 2002). It revealed in the higher concentration than in the concentration of this report (Fig.3).

Zinc pyrithione: Fertilization and cleavage rates were somewhat high at almost concentrations, however, delay in development occurred at higher concentrations.

Various abnormal plutei occurred at medium concentrations, and normal development rates decreased or increased depending on the concentration.

Developmental delays and abnormal developments also appeared to be similarly affected. At lower concentrations, normal development rates increased.

As for the UV-light treatment, the effects of zinc pyrithione on the eggs or embryos were decreased with the increased duration time of the treatment in all cases, especially in higher concentrations (see Table, right side and Figure).

Okamura

Toxicity of five samples for ZnPT is shown in Fig.3. This graph clearly shows that the inhibition on sea urchin egg development is dependent on the duration of UV-A irradiation. This is responded to the 27-hour EC50s summarized in Table 2. Toxicity of ZnPT-control was much weaker than the other previous study. ZnPT-0 was much weaker.

Copper pyrithione: Fertilization and cleavage rates were low at high and medium concentrations. However, delay in development occurred at their concentrations also. Various abnormal plutei occurred at high concentrations, and normal development rates were decreased or increased depending on the concentrations. Developmental delays and abnormal developments also appeared to be similarly affected. At lower concentrations, normal development rates were increased. As for the UV-light

treatment, the effects of copper pyrrithione on the eggs or embryos were decreased with the increased duration time of the treatment in all cases, especially in higher concentrations (Fig.4).

DISCUSSION

Effects of zinc pyrrithione (without UV-radiation) on sea urchin development was shown the similar severe results as in the case of our previous report (Kobayashi and Okamura, 2002). The environmental fate studies reported that pyrrithiones rapidly degraded in the water to less toxic compounds (Turley et al., 2000). However, it was the absence of ecological effect researches. On the present study, UV irradiation for pyrrithionrs were shown the degradation of their effects upon the sea urchin development depending on the irradiating duration time. Their photodegradation effects upon the pyrrithiones by UV light are evident.

To compare with their degraded effects upon sea urchin development between Zn-pyrrithione and Cu-pyrrithione, Zn-pyrrithione effects is stronger than Cu-pyrrithione. On the other word, degradation of Zn-pyrrithione with UV light is faster than that of Cu-pyrrithione. It is shown in Figure, on the effects of pyrrithiones on sea urchin development treated with UV light reveals as follows. The normal sea urchin development rates are increased with decreased pyrrithions concentration and increased UV-treated durations.

Okamura:

The structural change of both metal pyrrithione molecules by UV-A irradiation was evident, however, it was suggested that heavy metal ion (zinc or copper) which was derived from the mother moiety caused toxicity to sea urchin at the concentration. Thus, sea urchin is very sensitive to the metals. It is needed to clarify the effects of photodegraded pyrrithiones to the other organisms.

CONCLUSIONS

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Table 1. Toxicity of metal pyrithiones on embryo development of sea urchin *Anthocidaris crass*

test sample	treatment	27-hour EC50 (ng/L) with 95% confidence intervals	
	UVA-evaporation	Me=Zn	Me=Cu
MePT-control 1	no-no	7.1x10 ⁻⁶ (5.3~9.8x10 ⁻⁶)	nd
MePT-control 2	no-no	10x10 ⁻⁶ (7.6~14x10 ⁻⁶)*	2.3 (1.7~3.2)#
MePT-0	no-yes	2.2 (1.7~2.7)	1.8 (1.3~2.5)
MePT-0.5	0.5h-yes	3.0 (2.4~4.0)	9.1 (6.7~12)
MePT-1	1h-yes	14 (11~17)	24 (19~32)
MePT-2	2h-yes	28 (21~36)	60 (46~79)
metal ion	no-no	8,500 (8,100~9,000)	13,000 (12,000~14,000)#

Data obtained by using *Hemicentrotus pulcherrimus*

* Kobayashi and Okamura, 2002

Table 2. Toxicity of metal pyrithiones on the lethality of freshwater rotifer *Brachionus calyciflorus* .

test sample	treatment	24-hour LC50 (μg/L) with standard deviation			
	UVA-evaporation	Me=Zn	DAP*	Me=Cu	DAP*
MePT-control 1	no-no	12±3.6	11~43	5.2±0.3	0~34
MePT-control 2	no-no	11±2.4	94~114	4.6±0.7	96~123
MePT-0	no-yes	12±5.3	87~114	6.0±2.3	96~123
MePT-0.5	0.5h-yes	14±3.8	87~114	7.6±0.9	96~123
MePT-1	1h-yes	29±11	94~120	11±0.8	100~135
MePT-2	2h-yes	>100	94~120	29±7.8	100~135
metal ion	no-no	3100 (1300-5100)#	0.0	5.3 (4.6-6.1)#	0.0

* days after preparation of MePT standard solution

LC50 with 95% confidence intervals in parentheses

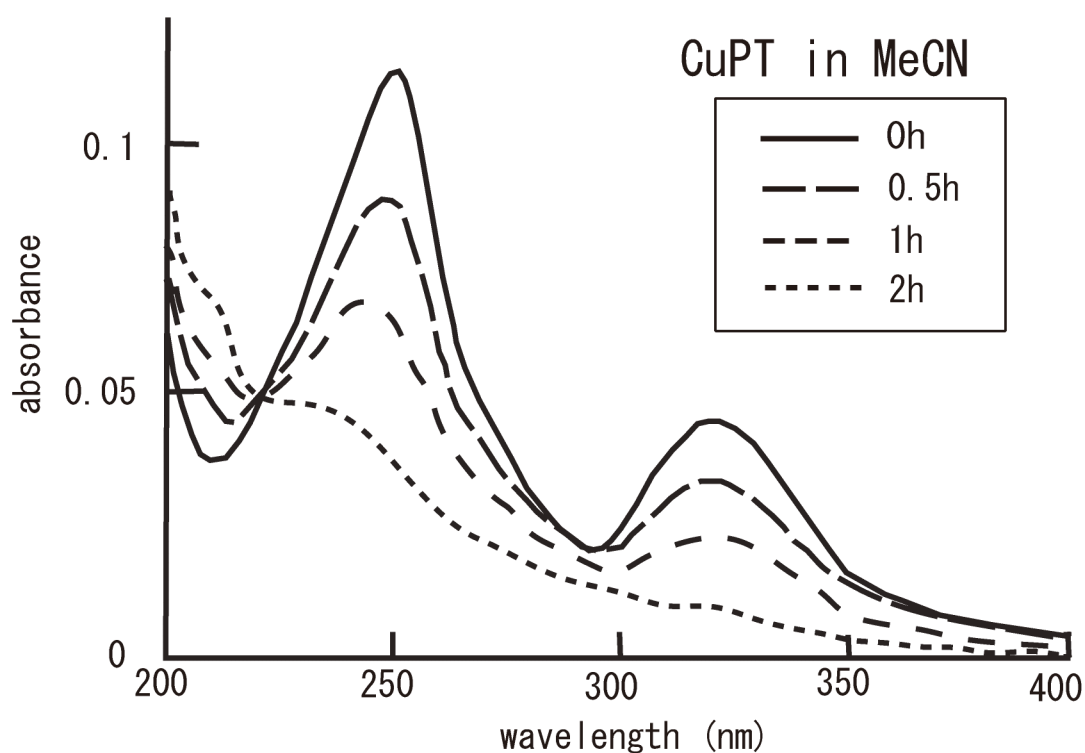
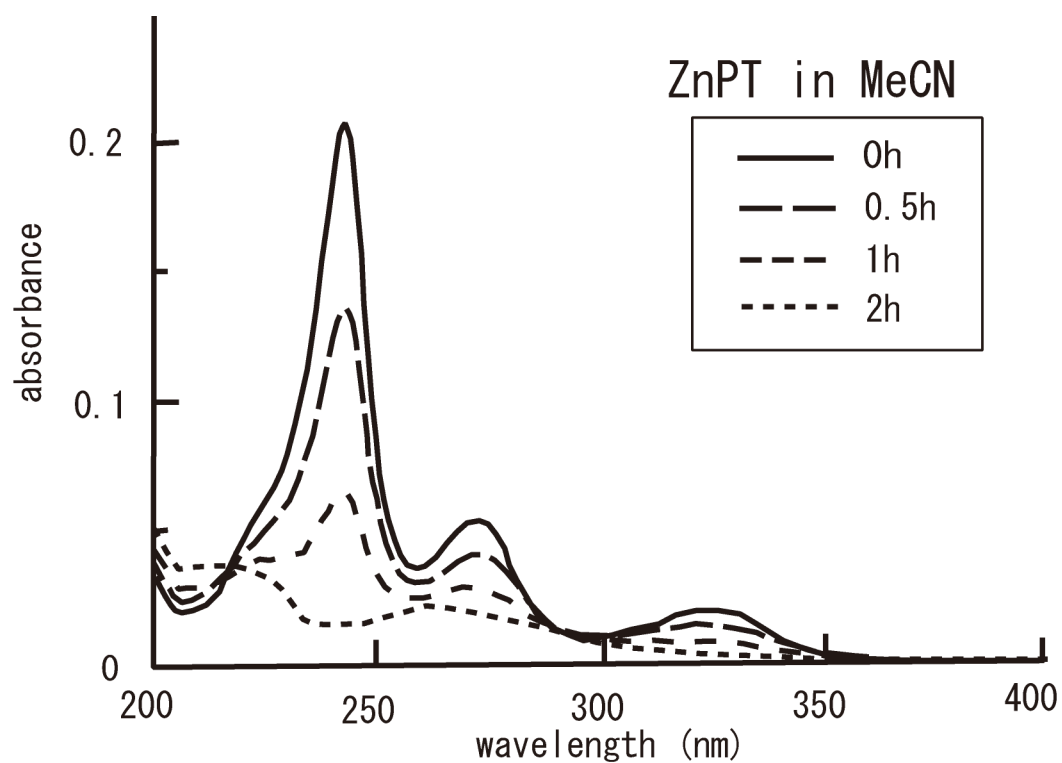


Figure 1. UV spectra of zinc pyrithione and copper pyrithione irradiated by UV-A lamp.

Nominal concentration: 1.0 mg/L, UV-A intensity at the center of quartz cell: 20 W/m²

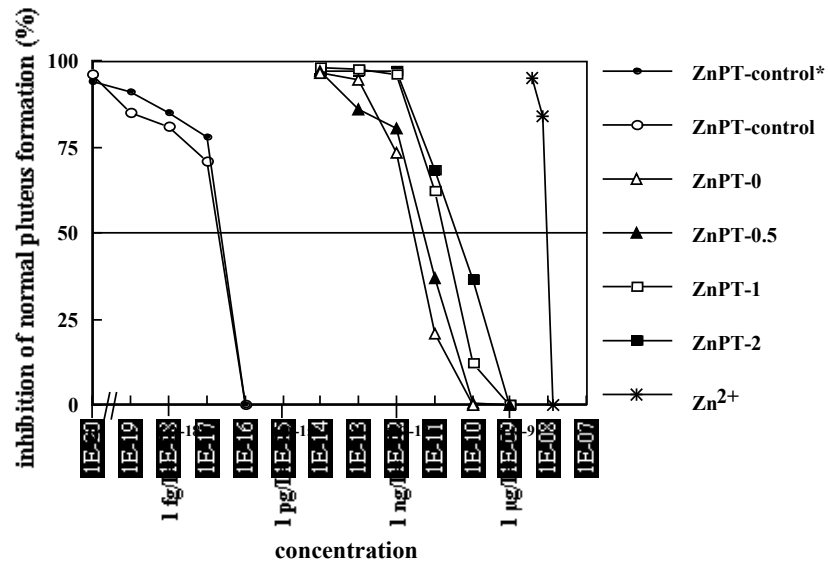


Figure 2. Effects of zinc pyriethione on sea urchin egg development.

* reference data in "Kobayashi and Okamura, 2002"

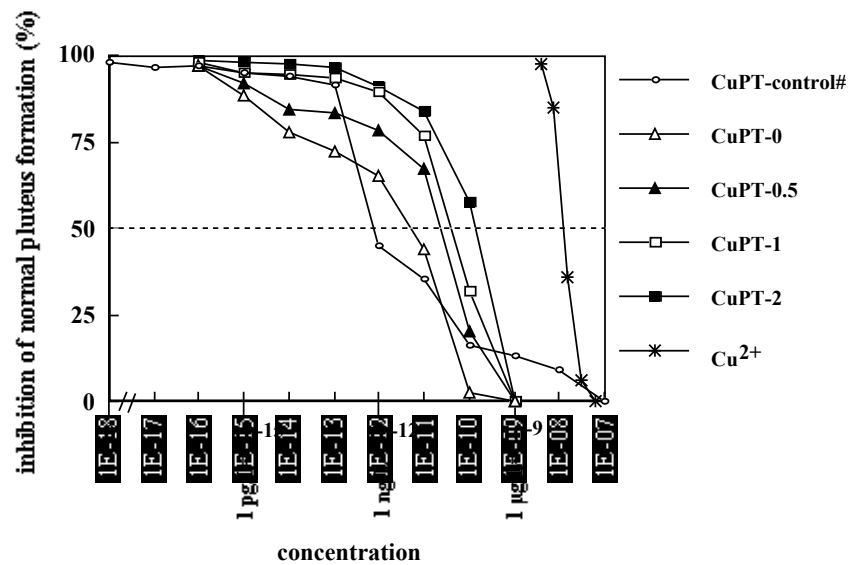


Figure 3. Effects of copper pyriethione on sea urchin egg development.