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(Citation)

Chemosphere, 74(9):1275-1278

(Issue Date)

2009-03

(Resource Type)

journal article

(Version)

Accepted Manuscript

(URL)

<https://hdl.handle.net/20.500.14094/90000898>



Ecotoxicity of the degradation products of triphenylborane pyridine (TPBP) antifouling agent

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Abstract

Triphenylborane pyridine (TPBP) is an alternative to organotin antifouling compounds. This work aimed to identify the unknown Peak #1, and to evaluate the ecotoxicity of TPBP and its degradation products. Peak #1 was produced from TPBP dissolved in acetonitrile under UV–A photolysis using a high-pressure mercury lamp. The Peak #1 fraction was purified using two-step column chromatography from a TPBP–acetonitrile solution. The major compound of the fraction was identified as being biphenyl from the ^1H –NMR and ^{13}C –NMR spectra. The ecotoxicity of four degradation products (diphenylborane hydroxide, phenylborane dihydroxide, phenol, and biphenyl) and TPBP towards two marine planktons were assessed. The 48 h LC_{50} values of the crustacean, *Artemia salina*, were 0.13 mg/L for TPBP, 14 mg/L for biphenyl, 17 mg/L for phenol, and >50 mg/L for the other degradation products. The 72 h EC_{50} values of the diatom, *Skeletonema costatum*, were 0.0022 mg/L for TPBP, 1.2 mg/L for biphenyl, and > 2 mg/L for the other degradation products. Thus, the ecotoxicity of biphenyl and the other degradation products were not high compared to the parent compound, TPBP.

Keywords: *Artemia*, bioassay, biocide, biphenyl, marina, *Skeletonema*

1. Introduction

Antifouling compounds are used to prevent biofouling on ship's hulls, with minimum risk to aquatic environments. Triphenylborane pyridine (TPBP) is an alternative to organotin antifouling compounds, whose use has been prohibited in Japan since the late 1980s. The usage of TPBP is increasing in popularity in some Asian countries due to the effectiveness of the Antifouling System Convention that banned organotin compounds on ship's hulls. A

summary of TPBP as an antifouling biocide, including the environmental aspects, has been published by Amey and Waldron (2004). An analytical method using ion pair HPLC to determine TPBP residues was reported recently by Takahashi et al. (2005). However, analysis of TPBP residues in seawater, sediments, and biota has not been reported on to date. The environmental effect of TPBP on fish and fish cell lines (Okamura et al., 2002), sea urchin embryos (Kobayashi and Okamura, 2002), some plant species (Okamura et al., 2003), and marine planktons (Mieno et al., 2004) have been reported, based on nominal concentrations of TPBP. There is little information on the fate of TPBP in water, except for the hydrolysis and photolysis study carried out by Zhou et al. (2007). In that report, a persistent unknown compound, Peak #1, was found in both hydrolysis and photolysis products of TPBP in water. The aims of this work were: (1) to identify Peak #1, and (2) to evaluate the ecotoxicity of TPBP and its degradation products to estimate the environmental effect of TPBP in aquatic environments.

2. Materials and methods

2.1 Chemicals

The triphenylborane pyridine (TPBP), diphenylborane hydroxide (DPB) and phenylborane dihydroxide (MPB) used were donated by the Hokko Chemical Industry Co. Ltd., Japan. The phenol, benzene, biphenyl (pesticide grade), pyridine (spectroscopy grade), acetonitrile (HPLC grade), dimethyl sulfoxide (DMSO, spectroscopy grade), methanol (HPLC grade), and dichloromethane (HPLC grade) used were purchased from Wako Pure Chemical Industries, Ltd., Japan. The tetrabutyl ammonium phosphate (TBAP, 0.5 mol/L) was used as an ion pair reagent and was purchased from the Tokyo Kasei Kogyo Co. Ltd., Japan. The water used to prepare all the aqueous solutions was obtained using an Aquarius RFU554CA

ultrapure water system (Advantec, Japan). In the toxicity tests, all the compounds tested were dissolved in DMSO at 5,000 mg/L for TPBP and at 10,000 mg/L for DPB, MPB, benzene, phenol, and biphenyl.

2.2 Irradiation of the TPBP solution

A TPBP solution (1,000 mg per liter of acetonitrile) was ultrasonically treated to dissolve any particulate matter. The TPBP solution (330 mL) in a glass bottle of a photochemical device (Model UVL-100HA-300, Riko Kagaku Sangyo Co., Japan) was irradiated using a 100 W high-pressure mercury lamp located inside the device. The lamp emitted UV-A radiation at a maximum wavelength of $\lambda = 365$ nm, with the operating wavelength in the range 312–577 nm. The maximum UV-A intensity on the outside of the glass bottle surface was 7,000 $\mu\text{W}/\text{cm}^2$. The irradiated TPBP solution (5 mL) was sampled periodically over a period of up to 48 h, and then, pyridine (50 μL) was added to stabilize the TPBP. The samples were directly subjected to HPLC analysis to determine the concentration of TPBP and its degradation products. The dilution obtained by adding 1% pyridine was ignored so that the concentrations of the target compounds could be calculated.

2.3 Isolation and identification of Peak #1

A TPBP acetonitrile solution (330 mL, concentration = 1,000 mg/L) was irradiated using a high-pressure mercury lamp for a period of 48 h. This was repeated three times on different days. A total volume of 1,000 mL of the above solution, corresponding to 1.0 g of TPBP, was irradiated. The unknown Peak #1 in the reaction mixture (1,000 mL) was isolated using two-step column chromatography. The acetonitrile in the reaction mixture was evaporated *in vacuo* at 40 °C, and the resultant solution (*ca.* 60 mL) was subjected to silica column

chromatography (50 g, Bond Elute) that had been precleaned using dichloromethane (DCM, 200 mL). Fresh DCM (200 mL) was added to the Si column, and the DCM in the eluted fraction was evaporated to dryness *in vacuo*. The resultant residue was mixed with water (50 mL), and subjected to an ODS column (10 g, Bond Elute) that had been preconditioned by subsequent elution of acetonitrile (60 mL) and water (120 mL). The ODS column was the eluted using fresh water (100 mL), 50% acetonitrile in water (100 mL), and acetonitrile (100 mL).

Peak #1 was only found in the acetonitrile fraction, and the residue after evaporation weighed 67 mg. According to this procedure, the yield of the fraction containing Peak #1 was 6.7% of the original 1,000 mg of TPBP. The fraction was analyzed using ^1H -NMR spectroscopy and UV spectroscopy. The ^1H -NMR spectra were measured using a Varian Gemini-300 spectrometer operating at 300 MHz in CDCl_3 . The UV spectra were measured using a Hitachi U-3000 spectrometer with a 10 mm cell in CHCl_3 .

2.4 HPLC analysis

TPBP and its degradation products were characterized using ion pair HPLC (Zhou et al., 2007) according to the procedure of Takahashi et al. (2005). The TPBP, DPB, MPB, phenol, benzene, and biphenyl in the test samples were analyzed using a semi-micro HPLC system (LaChrom Elite, Hitachi, Japan) equipped with a photodiode array detector (Model L-2450) and a fluorescence detector (Model L-2480), installed in tandem after the outlet of the column (Develosil ODSUG5, 2.0 mm \times 150 mm, particle size = 5 μm , pore size = 14 nm, Nomura Chemicals, Japan), and mounted on an ODS UGS pre-column and pre-filter. The solvents used were: (A) 5 mM of TBAP in water, and (B) acetonitrile at a flow rate of 0.2 mL/min with a column oven temperature of 40 $^{\circ}\text{C}$. A linear gradient elution was employed with

programming from an initial 30% B to 100% B in a 20 min run. A constant flow at 30% B was achieved from 20.1 min to 40 min to equilibrate the column for further analysis. A volume of 2 μ L of the test sample was injected into the column using an auto-sampler (Model L-2200). The fluorescence intensity at the excitation wavelength of $E_x = 275$ nm and the emission wavelength of $E_m = 295$ nm were used to quantify the TPBP and phenol. The UV absorption at a wavelength of $\lambda = 210$ nm was used for the other compounds. Both DPB and MPB were eluted at the same retention time, and therefore, the concentration was calculated as the sum of DPB and MPB.

2.5 Ecotoxicity testing

To carry out ecotoxicity assessment of the test compounds, Artoxkit M (Microbiotest Inc., Belgium) was used in a crustacean mortality test. The cysts of *Artemia salina* in artificial seawater (ASPM water) were placed in an incubator at 25 °C under a fluorescent lamp, and the neonates that hatched within 24 h were used in the toxicity tests. The toxicity tests were performed using a 24-well microplate (Iwaki, Japan) with ten individuals per 1 mL in a well. The test chemicals were prepared in a five concentration series in ASPM water. A total of thirty neonates in three wells were used for each test chemical concentration. The neonates were exposed to the test chemicals for a period of 48 h at 25 °C in the dark. A 48 h LC_{50} value (50% of the lethal concentration) was calculated from the number of living crustaceans counted using a binocular microscope employing the Ecotox-Statics v2.2 software package, released by the Japanese Society of Environmental Toxicology.

The algal growth inhibition test using the marine diatom *Skeletonema costatum* (NIES-323) was performed according to the ISO standard method (1995). The culture medium was fabricated by adding nutrient stock solutions into natural seawater collected from the Pacific

Ocean, and was sterilized using membrane filtration (pore size = 0.2 μm). A glass tube with a silicon plug was used as the culture vessel, and this was also used as a measurement vessel to determine the concentration of chlorophyll as algal biomass *in vivo*. The test chemical solutions were prepared in a five concentration series with the culture medium in the test tube. A total of three tubes were used for each concentration of the test chemical. Algal cells of *S. costatum* were counted using a hematometer employing a microscope, and the initial algal density was adjusted to 10^4 cells/mL. The test tubes (5 mL with the test chemical and algae in the medium) were placed in an incubator at 20 °C under a continuous fluorescent lamp. The algal biomass was measured using a fluorometer (Model TD-700, Turner Designs Inc.) each 24 h period up to 72 h. A 72 h EC_{50} value (50% of the effective concentration) and a no observed effective concentration (NOEC) value were calculated from the area under the growth curves using the Ecotox-Statics v2.2 software package.

3. Results and discussion

3.1 Identification of Peak #1

The ^1H -NMR spectral data of the isolated fraction were as follows: ^1H -NMR (CDCl_3) δ = 7.35 (2H, t, J = 7.8 Hz), 7.44 (4H, t, J = 7.8 Hz), 7.59 (4H, d, J = 7.8 Hz). The NMR and UV spectral data were identical to those of an authentic sample of biphenyl. Thus, Peak #1 was identified as being biphenyl.

3.2 Photodegradation of TPBP in acetonitrile

The photodegradation of TPBP in acetonitrile is shown with the formation of some

degradation products in Fig. 1. The initial TPBP concentration was measured as being 820 mg/L and decreased to *ca.* 100 mg/L after 24 h, and 0.82 mg/L after 48 h of UV–A irradiation. The concentration of phenol, benzene, and biphenyl increased from the onset of irradiation up to a period of 6 h, and then remained constant up to 48 h. The sum of the initial concentration of MPB + DPB (7.1 mg/L) increased to 70.2 mg/L after 6 h irradiation, and then decreased to 3.8 mg/L after 48 h. The initial concentration of benzene (13.2 mg/L) increased to 63 mg/L after 6 h irradiation, and remained stable up to 48 h. The initial concentration of phenol (19.3 mg/L) increased to 124 mg/L after 24 h irradiation, and increased to 152 mg/L after 48 h irradiation. The initial concentration of biphenyl (0.84 mg/L) increased to 8.2 mg/L after 48 h irradiation. The observation that all the degradation products were found in the test sample before irradiation indicates that they were produced on dissolving under ultrasonication without any photochemical reactions taking place. The concentrations of the degradation products after 48 h irradiation decreased in the following order: phenol > benzene >> biphenyl > MPB + DPB.

3.3 Ecotoxicity of TPBP and its degradation products

The ecotoxicity of the test compounds and the Peak #1 fraction on the crustacean *Artemia salina* and the algae *Skeletonema costatum* are shown in Table 1. The test samples are arranged in decreasing order of toxicity based on the 48 h LC₅₀ value of the crustacean, as follows: TPBP >> Peak #1 = biphenyl = phenol > DPB = MPB.

The 48 h LC₅₀ value of TPBP for *A. salina* was of the same order of magnitude as found in our former study (0.23 mg/L by Mieno et al., 2004). The 48 h LC₅₀ value of phenol found in this study (17 mg/L) was lower than the reference values of 28 mg/L (24 h LC₅₀ value (Crisinel et al., 1994)) and 175 mg/L (24 h LC₅₀ value (Calleja et al., 1994)). The 48 h LC₅₀

value of biphenyl in this study (14 mg/L) was slightly higher than the 24 h LC₅₀ value of *A. salina* from Abernethy et al. (1986) (4.0 mg/L).

The test compounds are arranged in decreasing order of toxicity based on the 72 h EC₅₀ value for algae as follows: TPBP > Peak #1 >> biphenyl > DPB > MPB > phenol. The 72 h EC₅₀ value of TPBP for *S. costatum* was of the same order of magnitude as found in our former study (0.0034 mg/L by Mieno et al., 2004). The 72 h EC₅₀ value of phenol (15 mg/L) was lower than the reported value (50 mg/L as 120 h EC₅₀) for the same test species (Cowgill et al., 1989). The 72 h EC₅₀ value of biphenyl (1.2 mg/L) was not comparable with any literature values. The 72 h EC₅₀ value of biphenyl in this study was much higher than that of the Peak #1 fraction (0.070 mg/L), and this suggests that the Peak #1 fraction possibly contains other compounds that are more toxic to algae than biphenyl.

Biphenyl is suspected to be the most toxic compound among the degradation products of TPBP. The amount of biphenyl produced was the least among the degradation products (Fig. 1). The environmental toxicity of biphenyl towards *Daphnia magna* has been reported to be 0.36 mg/L from 48 h LC₅₀ values in a flow through test (Gersich et al., 1989) and 2.1 mg/L from the 48 h LC₅₀ value in a static acute test (Dill et al., 1982). The toxicity of biphenyl towards several fish species is in the range 1.4–4.6 mg/L from the 96 h LC₅₀ values from a static test (Dill et al., 1982). Thus, biphenyl has a moderately acute toxicity, but not a chronic toxicity towards fish and crustaceans. The residue concentration of biphenyl in aquatic environments is not available in the open literature. Our experimental results indicate the degradation products of TPBP are less toxic to the planktonic species tested than TPBP.

4. Conclusions

The ecotoxicity of triphenylborane pyridine (TPBP) and its degradation products was investigated, and the following conclusions are noted:

- 1) The unknown compound, Peak #1, is one of the photodegradation products of TPBP in water, and is photochemically produced from TPBP in acetonitrile. The Peak #1 fraction was purified from a photochemical reaction mixture in acetonitrile using column chromatography. The major compound in the Peak #1 fraction was identified as being biphenyl using NMR spectral analysis.
- 2) Phenol, benzene, and biphenyl produced from the photolysis of TPBP in acetonitrile were stable against UV-A for a period of 48 h. Once formed from the photolysis of TPBP in acetonitrile, both DPB and MPB were significantly degraded against UV-A within a period of 48 h.
- 3) The parent compound, TPBP, exhibited a more severe toxicity to the crustacean *Artemia salina* and the diatom *Skeletonema costatum* than the other degradation products, such as biphenyl, phenol, benzene, DPB, and MPB. These results suggest that some degradation products have a less toxic effect on marine ecosystems than the parent compound does.

Acknowledgements

The triphenylborane pyridine, diphenylborane hydroxide, and phenylborane dihydroxide were kindly donated by the Hokko Chemical Industry Co. Ltd., Japan. Marine diatom *Skeletonema costatum* (NIES-323) was obtained from Microbial Culture Collection, National Institute for Environmental Studies, Japan. This work was partly supported by a Grant-in-Aid for Scientific Research (Monbu Kagakusyo, B20310019).

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Figure captions

Fig. 1. Photodegradation of TPBP and the formation of degradation products in acetonitrile.

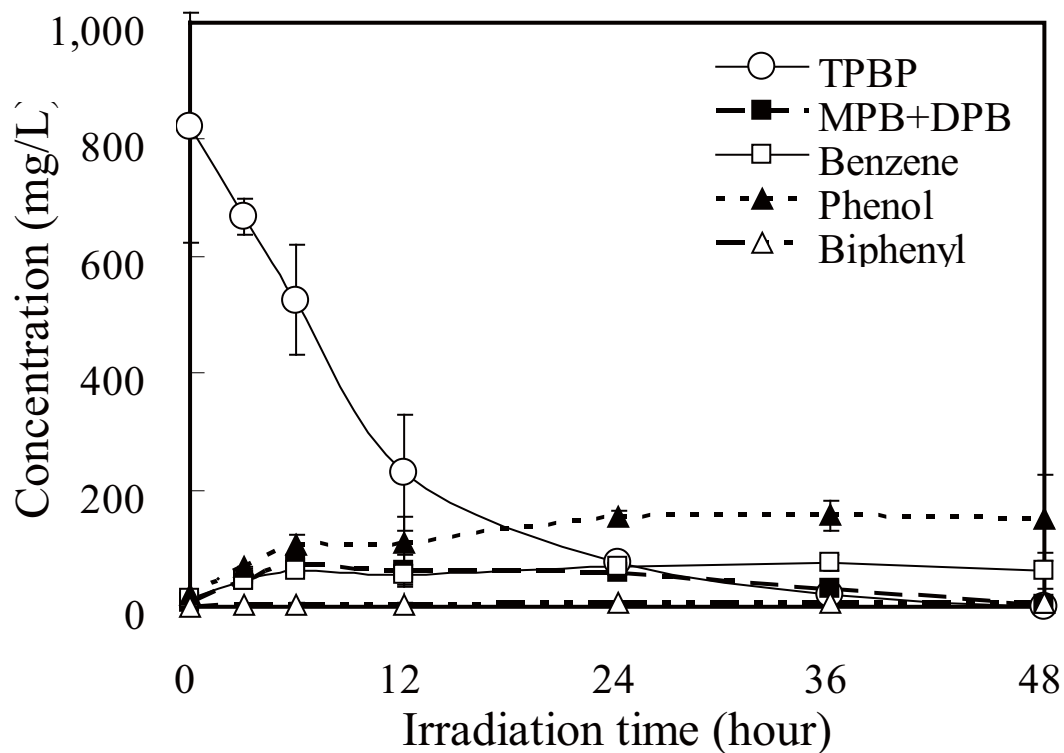


Table 1 Ecotoxicity of TPBP and its degradation products.				
	<i>Artemia salina</i>		<i>Skeletonema costatum</i>	
	LC ₁₀ (mg/L)	48-h LC ₅₀ (mg/L)	NOEC (mg/L)	72-h EC ₅₀ (mg/L)
TPBP	0.054	0.13 (0.083-0.18)	0.00031	0.0022 (0.0020-0.0025)
DPB	8.8	50 (30-100)	0.63	2.4 (2.2-2.7)
MPB	9.9	67 (36-100)	0.63	5.0 (4.4-5.7)
Phenol	5.4	17 (10-25)	6.3	15 (14-17)
Biphenyl	5.0	14 (8.4-20)	< 0.63	1.2 (1.0-1.3)
Peak #1	5.0	8.9 (5.9-12)	0.0078	0.070 (0.064-0.076)