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RESEARCH NOTE

TOXICITY OF BENZOTRIAZOLE AND BENZOTRIAZOLE DERIVATIVES TO THREE AQUATIC SPECIES

DAVID A. PILLARD^{1*}, JEFFREY S. CORNELL², DOREE L. DUFRESNE¹ and MARK T. HERNANDEZ³

¹ENSR, 4303 West LaPorte Avenue, Fort Collins, Colorado 80521, USA; ²US Air Force Center for Environmental Excellence, HQ AFCEE/ERT, 3207 North Road, Brooks AFB, Texas 78235, USA and

³Department of Civil, Environmental, and Architectural Engineering, University of Colorado at Boulder, Campus Box 428, Boulder, Colorado 80309, USA

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Abstract—Benzotriazole and its derivatives comprise an important class of corrosion inhibitors, typically used as trace additives in industrial chemical mixtures such as coolants, deicers, surface coatings, cutting fluids, and hydraulic fluids. Recent studies have shown that benzotriazole derivatives are a major component of aircraft deicing fluids (ADFs) responsible for toxicity to bacteria (Microtox[®]). Our current research compared the toxicity of benzotriazole (BT), two methylbenzotriazole (MeBT) isomers, and butylbenzotriazole (BBT). Acute toxicity assays were used to model the response of three common test organisms: Microtox[®] bacteria (*Vibrio fischeri*), fathead minnow (*Pimephales promelas*) and water flea (*Ceriodaphnia dubia*). The response of all the three organisms varied over two orders of magnitude among all compounds. *Vibrio fischeri* was more sensitive than either *C. dubia* or *P. promelas* to all the test materials, while *C. dubia* was less sensitive than *P. promelas*. The response of test organisms to unmethylated benzotriazole and 4-methylbenzotriazole was similar, whereas 5-methylbenzotriazole was more toxic than either of these two compounds. BBT was the most toxic benzotriazole derivative tested, inducing acute toxicity at a concentration of ≤ 3.3 mg/l to all organisms. © 2000 Elsevier Science Ltd. All rights reserved

Key words—benzotriazole, deicers, glycol, toxicity

INTRODUCTION

Corrosion prevention of metal components is important in many industrial and commercial applications. Benzotriazole (BT) (Fig. 1a) and its derivatives are commonly used to inhibit copper and other “yellow metal” corrosion in many applications and are included in numerous domestic and industrial products, such as deicing fluids, automotive coolants, antifreezes, cutting fluids, hydraulic brake fluids, and coating materials. They are also used as a brightening agent in the metal plating industry.

Methyl-substituted benzotriazoles are known as tolyltriangles or methyl-benzotriazoles (MeBT). MeBT is used as an additive to most commercially available aircraft deicing fluids (ADFs). Modern glycol-based commercial ADFs are typically

composed of ethylene or propylene glycol (50–90%), 4(5)-methylbenzotriazole (0.5–0.6% of the 4 and 5 isomer mixture), and a proprietary mixture of other materials that differ among manufacturers and that influence performance characteristics (e.g., viscosity, wettability) of the ADF. MeBT is usually found in ADFs as a 45:55% mixture (by mass) of the 4-MeBT isomer and the 5-MeBT isomer, respectively (Fig. 1b and c). Butylbenzotriazole (BBT) (Fig. 1d) is considered as an industry standard for corrosion control in heat exchangers.

Studies have indicated that glycol-based ADF formulations are significantly more toxic to aquatic organisms than can be explained by the presence of propylene or ethylene glycol alone (Fisher *et al.*, 1995; Hartwell *et al.*, 1995; Pillard, 1995). Recent investigations indicate that BT and MeBT are toxic to the luminescent bacteria used in the Microtox[®] assay (Cancilla *et al.*, 1997; Cornell *et al.*, 1998). In addition, MeBT inhibited the biodegradation of propylene glycol during laboratory microcosm

*Author to whom all correspondence should be addressed.
Tel: +1-970-416-0916; fax: +1-970-493-8935; e-mail: dpillard@ensr.com

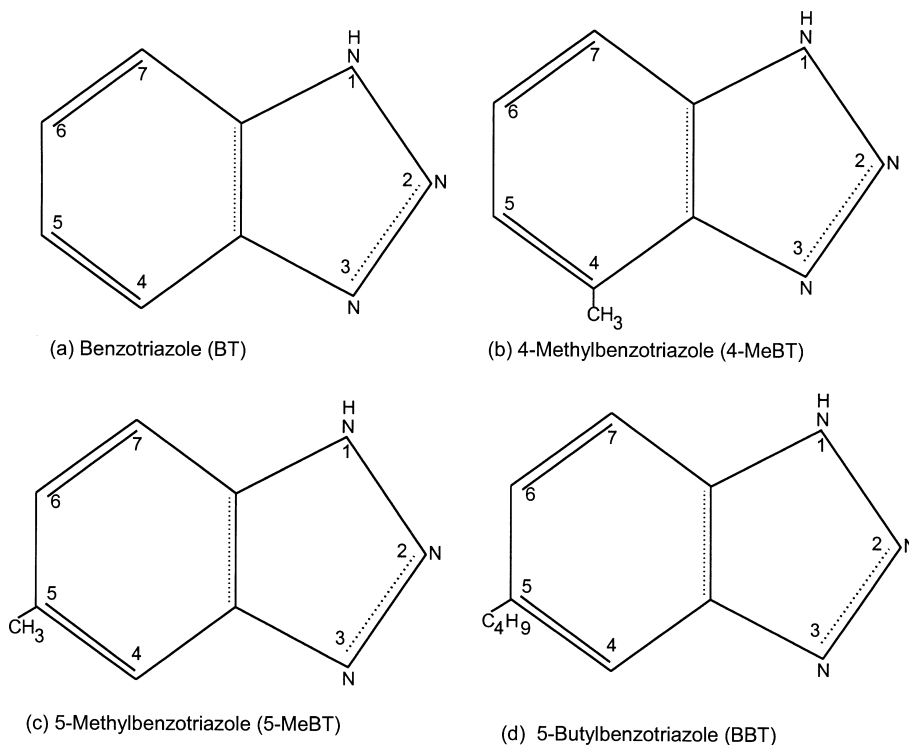


Fig. 1. Chemical structure of (a) benzotriazole, (b) 4-methylbenzotriazole, (c) 5-methylbenzotriazole, and (d) 5-butylbenzotriazole.

studies using ADF-contaminated soils (Cornell *et al.*, 1998). There are few data on the toxicity of MeBT to macroorganisms such as fish and zooplankton (Cornell *et al.*, 1998). Samples collected from a perched water monitoring well at an international airport were found to have estimated BT and MeBT concentrations of 126 and 215 mg/L, respectively (Cancilla *et al.*, 1998). These concentrations are several times greater than concentrations known to be toxic to bacteria, fish, and invertebrates (Cancilla *et al.*, 1997; Cornell *et al.*, 1998). The goal of the present study was to compare the relative toxicity of different BT derivatives to bacteria and standard aquatic laboratory test organisms.

METHODS

Preparation of test solutions

All BT solutions were obtained from Cincinnati Specialties, Inc. (Cincinnati, OH, USA). Stock solutions of BT, 4-MeBT, 5-MeBT, and 4(5)-MeBT (45:55% mixture) for tests with *C. dubia* and fathead minnows were prepared by mixing each compound with 1 l of laboratory-prepared, USEPA, moderately hard water (Weber, 1993) to make the high test concentration. Serial dilutions were prepared by mixing the high test concentration of BT, 4(5)-MeBT, 4-MeBT, and 5-MeBT with moderately hard water to make each subsequent test concentration. Because of BBT's poor water solubility, methanol (99.98% HPLC grade, EM Science, Gibbstown, NJ) was used as a carrier liquid to produce a BBT-containing test solution. The methanol/BBT solution was mixed with moderately hard water to produce

a nominal high concentration of approximately 7,000 mg/l methanol and 50 mg/l BBT. A methanol control treatment was prepared that contained a nominal concentration of 7,000 mg/l methanol (but no BBT) in moderately hard water. For the Microtox[®] studies, stock solutions were adjusted with granular NaCl to a salinity of 20‰ to accommodate the marine bacterium used in Microtox[®] studies.

Chemical measurements

All solutions were analyzed via direct aqueous injection into a Shimadzu high-pressure liquid chromatograph (HPLC) fitted with a UV detector (λ_{254}). BT (and all derivatives) separation was achieved isocratically using an Inertsill ODS-3 250 × 4.6 mm column and an eluent comprising of 50% methanol, 49% de-ionized water and 1% acetic acid. The method detection limit for all BT derivatives varied from 0.5 to 1.0 ppm v/v.

Toxicity tests

Ceriodaphnia dubia and *Pimephales promelas* (fathead minnows) were obtained from in house cultures at ENSR's Fort Collins Environmental Toxicology Laboratory (FCETL) in Fort Collins, CO, USA. *C. dubia* were <24 h old and fathead minnows were 5–7 days old at the time of test initiation. Desiccated Microtox[®] bacteria (*Vibrio fischeri*, formerly *Photobacterium phosphoreum*) were obtained from Azur Environmental (Carlsbad, CA). Tests with *C. dubia* and *P. promelas* were acute studies conducted according to USEPA test methods (Weber, 1993). *C. dubia* tests were 48 h in length, conducted static (no renewal) in 30 ml plastic cups; *P. promelas* tests were 96 h in length, conducted in 500 ml glass beakers, and were renewed with freshly-prepared solutions at 48 h. Both tests were conducted with a 16 h:8 h light:dark cycle in a 20°C

Table 1. LC₅₀s and EC₅₀s calculated for three species exposed to four benzotriazole derivatives

| Compound | Microtox 15 min EC ₅₀ (95% CL) (mg/l) | <i>C. dubia</i> 48 h LC ₅₀ (95% CL) (mg/l) | <i>P. promelas</i> 96 h LC ₅₀ (95% CL) (mg/l) |
|---|---|--|---|
| Benzotriazole | NM ^a | 102 (86–120) | 65 (38–75) ^b |
| 4-Methylbenzotriazole | 21 (9.0–47) | 118 (104–134) | 63 (57–69) |
| 5-Methylbenzotriazole | 8.7 (8.2–9.2) | 79 (69–91) | 22 (18–26) |
| 1:1 mixture of 4- and 5-Methylbenzotriazole | 7.3 (6.9–7.7) | 108 (76–152) ^b | 38 (32–46) |
| Butylbenzotriazole | 0.88 (0.71–1.1) | 1.1 (0.89–1.3) | 3.3 (2.4–4.8) ^b |

^aUnmethylated benzotriazole was not tested using Microtox.

^bReliable confidence limits could not be calculated; measured test concentrations that bracket the LC₅₀ are shown.

Notes: EC₅₀ = concentration causing a 50% reduction in light production by the Microtox bacteria; LC₅₀ = concentration causing 50% mortality in the test organisms; CL = confidence limits.

Table 2. *Ceriodaphnia dubia* (48 h) and *Pimephales promelas* (96 h) NOAECs and LOAECs^a

| Compound | <i>C. dubia</i> (48 h endpoints) (mg/l) | | <i>P. promelas</i> (96 h endpoints) (mg/l) | |
|---|---|-------|--|-------|
| | NOAEC | LOAEC | NOAEC | LOAEC |
| Benzotriazole | 92 | 184 | 46 | 92 |
| 4-Methylbenzotriazole | 95 | 190 | 47 | 95 |
| 5-Methylbenzotriazole | 47 | 94 | 11 | 24 |
| 1:1 Mixture of 4- and 5-Methylbenzotriazole | 76 | 152 | 19 | 38 |
| Butylbenzotriazole | 0.57 | 1.14 | 2.38 | 4.75 |

^aNotes: NOAEC = No Observed Adverse Effect Concentration; LOAEC = Lowest Observed Adverse Effect Concentration.

environmental chamber. *C. dubia* were not fed during the test; *P. promelas* were fed 100 µl of brine shrimp nauplii per test chamber at 48 h (2 h prior to renewal). Microtox[®] tests were conducted according to the basic method described in Beckman Instruments (Beckman Instruments Inc., 1985). Bacterial luminescence was measured at 5 and 15 min.

The time-dependent median lethal concentrations (LC₅₀s) for *C. dubia* and *P. promelas* were calculated using either probit, Spearman-Kärber, Trimmed Spearman-Kärber, or graphical methods according to the flow-chart in Weber (1993). Where appropriate, USEPA software was used (USEPA, 1994). Five- and 15-min median effective concentrations (EC₅₀s) were calculated for the Microtox[®] studies according to Azur Environmental statistical analysis methods (Azur Environmental, 1997). Toxstat 3.4 (West, Inc. and Gulley, 1994) was used to calculate the No Observed Adverse Effect Concentrations (NOAECs) and Lowest Observed Adverse Effect Concentrations (LOAECs) for the *C. dubia* and *P. promelas* studies. Proportional survival data were transformed using the arcsine square root transformation before analysis. Shapiro Wilk's test and Bartlett's test were used to determine normality and homogeneity of variance of the data ($\alpha = 0.01$). Significant differences were determined using the nonparametric, Steel's Many One Rank Test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Measured concentrations of all BT, 4-MeBT, 5-MeBT, and 4(5)-MeBT were within 8% of the nominal test concentrations; the measured concentration of BBT was within 24% of the nominal concentration. All statistical endpoints are based on the measured concentrations. There was no mortality in any concentration of methanol, indicating that

toxicity in the study with BBT was associated with the triazole and not the methanol carrier.

All test organisms were most sensitive to BBT; all assays conducted with this compound resulted in LC₅₀s or EC₅₀s \leq 3.3 mg/l (Table 1). All test organisms were more sensitive to 5-MeBT than to 4-MeBT; the 5-MeBT LC₅₀ or EC₅₀ was approximately 30% lower than the 4-MeBT LC₅₀ or EC₅₀ in all cases. For the multicellular organisms, the LC₅₀s of the 4(5)-MeBT mixture were approximately equal to the geometric mean of the LC₅₀s of each isomer when tested separately. In the Microtox[®] tests, the 15-min EC₅₀s of both 5-MeBT and 4(5)-MeBT were similar to the EC₅₀ (5.91 mg/l) reported by Cancilla *et al.* (1997). The same relative toxicity can be seen in the hypothesis testing endpoints (NOAECs and LOAECs) (Table 2).

P. promelas were more sensitive than *C. dubia* to all of the BTs, except BBT, and Microtox[®] bacteria were more sensitive than *C. dubia* or fathead minnows to all BT formulas. Considering previous observations regarding the toxicity of BT derivatives (Cancilla *et al.*, 1997), and those reported here, it appears that the toxicity of these compounds is strongly associated with the number, position, and length of the alkanes attached to the phenyl moiety.

Waste water from aircraft deicing operations may reach surface water streams and lakes, and may also percolate through soil horizons into groundwater. BT and its derivatives have been reported in groundwater at a major North American airport at concentrations > 100 mg/l (Cancilla *et al.*, 1998).

Based upon the results of this investigation, BT concentrations in excess of 100 mg/l are high enough to cause acute toxicity to prokaryotic and eukaryotic organisms. In addition, BT concentrations in excess of 60 mg/l have been found to significantly inhibit biodegradation of otherwise readily degradable propylene glycol, which is the active deicing ingredient of several aircraft deicing formulations (Cornell *et al.*, 1998). It is possible, therefore, that deicers may persist in the environment for a longer period due to the presence of additives such as BT.

CONCLUSIONS

- Butylbenzotriazole was the most toxic of the tested compounds.
- Microtox bacteria (*Vibrio fischeri*) were more sensitive than either *C. dubia* or *P. promelas* to the benzotriazole derivatives.
- *P. promelas* was more sensitive than *C. dubia* to the benzotriazole derivatives, except butylbenzotriazole.
- The concentration of benzotriazoles found to be toxic to standard laboratory organisms is lower than the concentration of benzotriazoles identified in groundwater at a major North American airport. However, additional laboratory and field studies are needed to determine if the presence of additives contributes to the persistence of deicers, and other BT-containing commercial products, in the environment.

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