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Comparative Aquatic Toxicity Evaluation of 2-(Thiocyanomethylthio)benzothiazole and Selected Degradation Products Using *Ceriodaphnia dubia*

S. T. Nawrocki,[1] K. D. Drake,[2] C. F. Watson,[3] G. D. Foster,[4] K. J. Maier[5]

[1] Department of Cancer Biology, University of Texas-Houston, M.D. Anderson Cancer Center, Houston, Texas 77001, USA
[2] Microban Products Co., 11515 Vanstory Dr., Huntersville, North Carolina 28078, USA
[3] Buckman Laboratories International, Inc., 1256 N. McLean Blvd., Memphis, Tennessee 38018, USA
[4] Department of Chemistry, George Mason University, MSN 3E2, Fairfax, Virginia 22030, USA
[5] Department of Environmental Health, East Tennessee State University, Box 70682, Johnson City, Tennessee 37614-1176, USA

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Abstract. 2-(Thiocyanomethylthio)benzothiazole (TCMTB) is a biocide used in the leather, pulp and paper, and water-treatment industries. TCMTB may enter aquatic ecosystems during its manufacture and use. TCMTB is environmentally unstable; therefore, it is important to evaluate the toxicity of the more persistent degradation products. This study compared the toxicity of TCMTB with its degradation products 2-mercaptobenzothiazole (2-MBT), 2-(methylthio)benzothiazole (MTBT), benzothiazole (BT), and 2-hydroxybenzothiazole (HOBT). Toxicity was determined using *Ceriodaphnia dubia* 48-hour acute and 7-day chronic test protocols. TCMTB was the most toxic compound evaluated in both the acute and chronic tests with EC50s of 15.3 and 9.64 μg/L, respectively. 2-MBT, the first degradation product, was the second most toxic compound with acute and chronic EC50s of 4.19 and 1.25 mg/L, respectively. The toxicity of MTBT and HOBT were similar with acute EC50s of 12.7 and 15.1 mg/L and chronic EC50s of 6.36 and 8.31 mg/L, respectively. The least toxic compound was BT with acute and chronic EC50s of 24.6 and 54.9 mg/L, respectively. TCMTB was orders of magnitude more toxic than its degradation products. Toxicity data on these benzothiazole degradation products is important because of concerns regarding their release, degradation, persistence, and non–target organism effects in aquatic ecosystems.

Benzothiazoles and benzothiazole-based formulations are manufactured for a variety of industrial applications including use as vulcanizing agents, fungicides, algicides, and corrosion inhibitors in antifreeze (Reemtsma *et al.* 1995). Benzothiazoles appear in the environment primarily as a result of their production and use as rubber vulcanization accelerators (De Wever and Verachtert 1997). These compounds can also enter the environment by water and air emissions, in rubber dust from tires, in pesticide application, and with releases of cooling water or antifreeze (Brownlee *et al.* 1992).

2-(Thiocyanomethylthio)benzothiazole (TCMTB) is a benzothiazole derivative specifically developed for use as a biocide. TCMTB is used as a slimicide and fungicide in the lumber, pulp and paper, paints and coatings, and leather industries (Aguera *et al.* 2000; Meding *et al.* 1993; Ward 1989). TCMTB-based compounds and formulations have been used globally for approximately 20 years as an alternative to chlorophenol compounds such as pentachlorophenol, as an antisapstain agent, and as a microbicide in the lumber industry (Brownlee *et al.* 1992). Use of chlorophenol compounds has been banned because of concerns about their toxicity, persistence, occupational impacts, and hazardous impurities (Ward 1989). TCMTB is also used as a substitute for tri-*n*-butyltin as the active ingredient in antifouling paints (Aguera *et al.* 2000). Potential routes of entry of TCMTB into the environment are from its use as a pesticide or from emissions during its manufacture.

TCMTB is not a naturally occurring compound; therefore, the sources of this substance are anthropogenic in origin. However, 2-mercaptobenzothiazole (2-MBT), 2-(methylthio)benzothiazole (MTBT), benzothiazole (BT), and 2-hydroxybenzothiazole (HOBT) can be produced naturally in a few organisms (De Wever and Verachtert 1997). TCMTB is susceptible to rapid degradation based on a number of environment fate studies. Brownlee *et al*. (1992) formulated a partial aquatic environmental-fate pathway for TCMTB (Fig. 1). Hydrolysis and/or photolysis of TCMTB results in 2-MBT, which can photolyze to BT and HOBT or undergo biomethylation to MTBT. At pH 5 TCMTB is stable; at pH 7 TCMTB is slowly hydrolyzed; and at pH 9 the hydrolysis is rapid (Meding *et al.* 1993). The major breakdown pathway of TCMTB results from photolysis. Breakdown of TCMTB is rapid exhibiting a DT_{50} (50% degradation time) <1 day with

Correspondence to: K. J. Maier; *email:* maier@etsu.edu

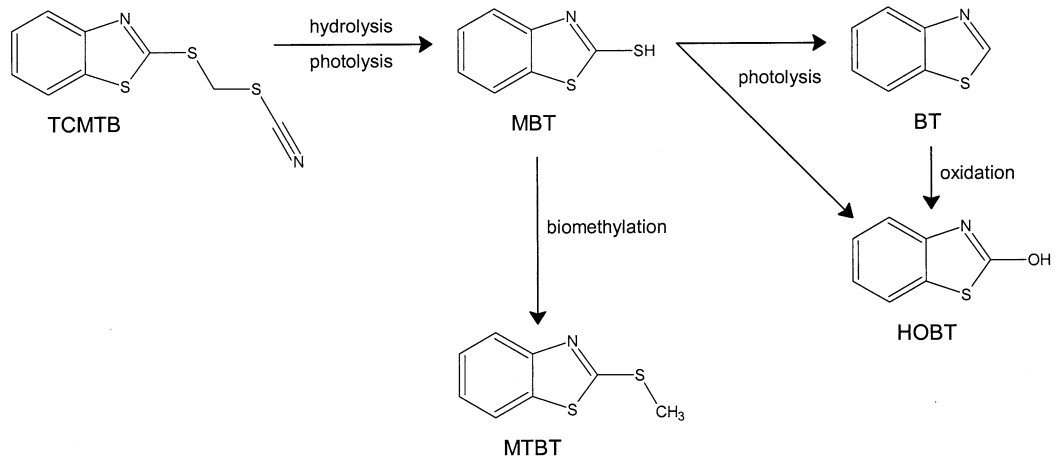

Fig. 1. Proposed degradation and transformation pathway of TCMTB in the aquatic environment (adapted from Brownlee *et al.* 1992). TCMTB = 2-(thiocyanomethylthio)benzothiazole

irradiation by full sunlight (Brownlee *et al.* 1992). The half-life of TCMTB in degradation studies ranges between <24 hours to 9 days (Fathulla *et al.* 1995; Wenell 1994; Brownlee *et al.* 1992). TCMTB is nonvolatile because of its low vapor pressure and partial water solubility. Mineralization of TCMTB is unlikely because the compound appears to break down into the relatively stable molecules—BT, HOBT, and MTBT—which have been reported in aquatic systems (Brownlee *et al.* 1992). The four selected degradation products of TCMTB were selected because of their potential presence in aquatic systems.

Although the main source of 2-MBT to the environment is through rubber production, it also is the initial transformation product of TCMTB. 2-MBT is rapidly photolyzed in water to yield BT as the major degradate and HOBT. 2-MBT may also undergo biomethylation to form MTBT, a compound resistant to sunlight photolysis (Brownlee *et al.* 1992). When exposed to chlorine in drinking water or sewage disinfectants, BT may undergo oxidation by hypochlorite to yield HOBT (Brownlee *et al.* 1992). Because TCMTB may potentially transform rapidly in the environment, it is important to study the major breakdown products. Because the degradation products MTBT, BT, and HOBT may be persistent in the environment, they may be present in high concentrations. However, previous studies have only found these three compounds existing at very low concentrations, part per trillion to low part per billion levels, in the aquatic or marine environment (Bester *et al.* 1997; Reddy and Quinn 1997).

TCMTB and its transformation products have a high acute toxicity to algae, fish, and daphnia (Kruzynski and Birtwell 1994; Wenell 1994; MacKinnon and Farrell 1992). Concentrations of TCMTB or its degradation products have been found in surface waters, drinking waters, sediments, and in biota (Wenell 1994; Spies *et al.* 1987; Crathorne *et al.* 1984; Coleman *et al.* 1980). Benzothiazole compounds present in industrial wastewater are not completely removed by standard anaerobic and aerobic wastewater treatment (Reemtsma *et al.* 1995). Literature is available on the production and use of benzothiazole compounds, but data on the toxicity and environmental fate of these compounds is limited (Wenell 1994; MacKinnon and Farrell 1992). Some studies on the environmental toxicity and fate of TCMTB and 2-MBT are available because these are the predominant benzothiazole-based compounds in industry. However, research on the environmental toxicity of the breakdown products of TCMTB and 2-MBT is lacking. The comparative aquatic toxicity of these compounds is of interest because benzothiazole compounds may be potentially harmful to the environment.

The objective of this study was to determine and compare the acute and chronic toxicities of TCMTB and its major aquatic breakdown products (2-MBT, BT, HOBT, and MTBT) to *Ceriodaphnia dubia*. Previous reviews have summarized the toxicity and degradation of TCMTB and 2-MBT (De Wever *et al.* 2001; De Wever and Verachtert 1997; Hanssen *et al.* 1991; Hanssen and Henderson 1991). This research is focused on the three degradation products, BT, HOBT, and MTBT, for which there are no available chronic toxicity data. The organism *C. dubia* was chosen as a test species because it is used in whole-effluent toxicity (WET) testing and has been determined to be a sensitive species in effluent toxicity assays (Hemming *et al.* 2002; Middaugh *et al.* 1997). WET testing plays an important role in industries receiving their National Pollutant Discharge Elimination System permits (Grothe *et al.* 1996). The significance of this research was to produce toxicity data on these benzothiazole compounds, which may be used for risk assessment purposes when examining the impact of selected industrial effluents to aquatic environments.

Material and Methods

Experiments were performed using the cladoceran *C. dubia*. Neonates (<24 hours old) were obtained from laboratory cultures maintained at 25°C ± 1°C in moderately hard water and reared according to guidelines recommended by the U.S. Environmental Protection Agency (USEPA 1993, 1994). The cultures were fed a combination of the green alga *Selenastrum capricornutum* and YCT (a combination of yeast, Cerophyll, and trout chow) as recommended by the USEPA. The algae were cultured in Woods Hole algal growth media to the desired cell density (USEPA 1994).

Buckman Laboratories International Inc. (Memphis, TN) provided TCMTB (99.4% pure; lot 946-13). 2-MBT (98% pure; lot 05426DN) and BT (96% pure, lot 04030TY) were obtained from Aldrich Chemical Company (St. Louis, MO). MTBT (98% pure; lot A010106901) and HOBT (99% pure; lot A008333701) were purchased from Acros Chemical Company (Pittsburgh, PA). Because of the low water solubility of these compounds, stocks were prepared by dissolving the compounds in dimethylformamide (DMF) (99.95% pure; lot 37H3649) (Sigma Chemical Company, St. Louis, MO). The stocks were stored in foil-covered glass beakers and kept at 4°C. Test concentrations were prepared from these stocks using moderately hard dilution water. Dilutions were prepared fresh daily to limit possible degradation. Five nominal concentrations (Tables 1 and 2) were selected based on preliminary experiments for each chemical. Two controls—a control plus DMF and a dilution water control—were also prepared. The DMF control was formulated by adding an amount of DMF equal to the greatest test concentration to the dilution water. The largest concentration of DMF used in preparing test concentrations was 2 ml/L.

The protocols for the toxicity tests were performed as recommended by the USEPA. Exposures were conducted in 30-ml plastic cups containing 20 ml solution. In the 48-hour acute tests, 4 replicates containing 5 neonates each were used for each exposure concentration. Neonates <24 hours old were pipetted into the exposure chambers. The 48-hour acute tests were a static nonrenewal exposure, and the organisms were not fed during this period. Survival counts were made after 24- and 48-hour periods in accordance with standard USEPA protocols (1993). In the chronic toxicity tests, each concentration had 10 replicates (1 daphnid/container,) and the organisms were fed daily with a combination of *Selenastrum capricornutum* and YCT, as recommended by the USEPA (1994). The chronic tests were static renewal, where concentrations were renewed daily for a period of 7 days, which is sufficient time for 1 organism to produce 3 broods. Mortality and reproduction were recorded every 24 hours for each organism. Two reference toxicant tests were performed using sodium dodecyl sulfate and NaCl to assure that the organisms were responding appropriately to toxicant exposure. All toxicity tests were conducted at 25°C ± 1°C with a 16 hours light/8 hours dark photoperiod.

Standard water-quality parameters were measured including dissolved oxygen, pH, conductivity, temperature, alkalinity, hardness, ammonia, and chlorine. Dissolved oxygen, pH, conductivity, and temperature were measured daily using probes and meters (Yellow Springs Instrument Co., Yellow Springs, OH). Alkalinity and hardness were measured at the initiation and termination of each test using Hach titrant kits (Hach Co., Loveland, CO). Ammonia and chlorine levels were similarly determined using a Hach colorimetry kit.

Measured concentrations were determined by high-pressure liquid chromatography (HPLC) analysis at George Mason University, Fairfax, VA. To limit possible degradation, samples were shipped overnight on ice in amber glass bottles. 2-MBT, MTBT, BT, and HOBT samples were prepared by using 5 ml sample water diluted with 5 ml acetonitrile. This diluted sample was spiked with 119 µg 2-phenylbenzothiazole, the internal standard. HPLC analyses were performed using 2 ml of each prepared sample. Because of the low concentration of TCMTB, 500 to 700 ml of sample were extracted using C18-bonded phase-extraction cartridges. The eluates for each sample were combined and concentrated to 1 ml using dry nitrogen gas blow-down. The sample was spiked with the internal standard and analyzed using HPLC. TCMTB extraction yielded recoveries of 86% ± 15% for 2 µg/L and 99% ± 10% for 10 µg/L TCMTB. A previous study indicated that gas chromatography is not a preferred technique because TCMTB is not stable at high temperatures (Daniels and Swan 1987). For the acute tests, 2 L of each concentration were prepared to ship two 800-ml volumes of sample as initial and final concentrations to be analyzed by HPLC. The final 800 ml was exposed to the same conditions as the acute test. This procedure allowed detection of any degradation that may have occurred during the 48-hour static nonrenewal period. The detection limit for TCMTB was 0.45 µg/L, for 2-MBT was 0.35 µg/L, for MTBT was 0.17 µg/L, for BT was 0.11 µg/L, and for HOBT was 0.13 µg/L. Similar steps were performed for the chronic tests; however, additional samples were analyzed because of the length of the test. Concentrations were taken on days 0, 4, and 7 to be analyzed. Averages were performed using the measured concentrations to determine each mean for statistical analyses. In addition, several spiked samples were analyzed to ensure quality control.

For each chemical, measured concentration averages were used to calculate EC50 estimates and associated 95% confidence intervals using the probit analysis where applicable. Where the probit analysis was not applicable because of a lack of two partial mortalities, either the graphical method, the Spearman-Karber, or the trimmed Spearman-Karber method was used to determine EC50 values as recommended by the USEPA (1993, 1994). Sublethal effects were also examined by observing the number of neonates produced during the 7-day period in each concentration and the control. Statistical

Table 1. Acute 48-hour mortality responses of *C. dubia* exposed to analytically determined concentrations of TCMTB, 2-MBT, MTBT, BT, and HOBT

Compound	Nominal (mg/L)	Measured ± SD (mg/L)	% Mortality
TCMTB	0	BD	0
	0.00125	0.00105[a]	0
	0.0025	0.00308 ± 0.0007	0
	0.0050	0.0055 ± 0.0016	0
	0.010	0.0119 ± 0.0032	25
	0.020	0.0230 ± 0.0043	100
2-MBT	0	BD	0
	0.625	0.59 ± 0.23	0
	1.25	2.07 ± 0.13	5
	2.5	2.38 ± 0.18	25
	5.0	6.54[a]	90
	10.0	10.11 ± 2.53	100
MTBT	0	BD	0
	1.0	1.31 ± 0.36	0
	3.0	4.19 ± 0.39	0
	6.0	6.77 ± 0.24	5
	9.0	13.88 ± 1.13	60
	12.0	19.90 ± 4.84	100
BT	0	BD	0
	5.0	5.36 ± 0.21	0
	10.0	10.62 ± 0.71	0
	20.0	18.60 ± 1.92	35
	40.0	40.85 ± 4.21	95
	80.0	85.83 ± 6.46	100
HOBT	0	BD	0
	2.5	2.72 ± 0.83	0
	5.0	4.77 ± 0.04	0
	10.0	9.93 ± 2.08	30
	20.0	19.11 ± 4.51	90
	40.0	35.80 ± 1.63	100

[a] Measurement of a single sample.
BD = Below analytic detection limits.
BT = Benzothiazole.
HOBT = 2-Hydroxybenzothiazole.
MTBT = 2-(Methylthio)benzothiazole.
2-MBT = 2-Mercaptobenzothiazole.
TCMBT = 2-(Thiocyanomethylthio)benzothiozole.

Table 2. Chronic 7-day mortality and reproduction responses of *C. dubia* exposed to analytically determined concentrations of TCMTB, 2-MBT, MTBT, BT, and HOBT

Compound	Nominal (mg/L)	Measured ± SD (mg/L)	% Mortality	Average no. of Neonates ± SD
TCMTB	0	BD	0	21.8 ± 1.40
	0.000625	0.00084 ± 0.00033	0	21.6 ± 1.95
	0.00125	0.00153 ± 0.00021	0	21.2 ± 1.23
	0.0025	0.0025 ± 0.00038	0	21.6 ± 1.43
	0.005	0.0056 ± 0.0020	0	11.8 ± 2.48*
	0.010	0.012 ± 0.0040	80	0.0*
2-MBT	0	BD	0	21.1 ± 1.20
	0.3125	0.302 ± 0.17	0	20.9 ± 1.29
	0.625	0.839 ± 0.19	0	21.2 ± 1.47
	1.25	1.43 ± 0.40	70	0.0*
	2.5	1.61[a]	100	0.0*
	5.0	3.82[a]	100	0.0*
MTBT	0	BD	0	22.1 ± 2.08
	1.0	1.21 ± 0.051	0	21.2 ± 1.40
	3.0	2.79 ± 0.37	0	13.0 ± 1.76*
	6.0	4.36 ± 0.98[b]	0	0.4 ± 0.70*
	9.0	7.76 ± 1.60[b]	90	0.0*
	12.0	10.40[a]	100	0.0*
BT	0	BD	0	21.0 ± 1.05
	5.0	6.58 ± 1.36[b]	0	21.5 ± 1.71
	10.0	11.92 ± 5.93[b]	0	21.3 ± 1.83
	20.0	24.28 ± 3.63	0	8.3 ± 2.58*
	40.0	39.11 ± 1.44	10	0.0*
	80.0	75.23[a]	100	0.0*
HOBT	0	BD	0	21.5 ± 1.58
	1.25	2.37 ± 0.82	0	20.8 ± 1.23
	2.5	2.74 ± 0.74	0	21.4 ± 1.51
	5.0	5.81 ± 2.14	0	10.7 ± 2.06*
	10.0	10.81 ± 3.44	100	0.0*
	15.0	13.62 ± 0.75[b]	100	0.0*

[a] Measurement of a single sample.
[b] Measurement of two samples.
* Significant difference from control determined by Dunnett's Test.
BD = Below analytic detection limits.
BT = Benzothiazole.
HOBT = 2-Hydroxybenzothiazole.
MTBT = 2-(Methylthio) benzothiazole.
2-MBT = 2-Mercaptobenzothiazole.
TCMBT = 2-(Thiocyanomethylthio)benzothiazole.

differences were determined using Dunnett's Test. Differences were considered significant at $p \leq 0.05$. All statistical analyses were determined using the Toxstat (West, Inc., Cheyenne, WY) software package.

Results

Dilution water and DMF control survival was 100% in all toxicity tests. Water quality was within the guidelines established by the USEPA in all tests. The ranges of water-quality parameters were temperature 24.1°C to 25.0°C, dissolved oxygen 7.88 to 9.40 mg/L, pH 7.35 to 8.35, conductivity 310 to 338 µS, hardness 85 to 100 mg/L, and alkalinity 70 to 85 mg/L. Ammonia and chlorine concentrations were below detectable limits in all tests.

The 48-hour acute EC50 (95% confidence interval) for TCMTB was 15.3 µg/L (13.7 to 16.9 µg/L) using the Spearman-Karber method. The four degradate acute EC50s were all determined using probit analysis. The 48-hour acute probit transformation concentration-mortality data are presented in Figure 2a. The acute EC50 for 2-MBT was 4.19 mg/L (3.52 to 5.09 mg/L), for MTBT was 12.7 mg/L (11.1 to 14.3 mg/L), for BT was 24.6 mg/L (20.9 to 29.7 mg/L), and for HOBT was 15.1 mg/L (12.9 to 17.7 mg/L). Table 1 presents the mean measured concentrations and 48-hour mortality data for these compounds.

The 7-day chronic EC50 and 95% confidence interval for TCMTB were calculated by the trimmed Spearman-Karber method because of a single partial mortality and the lack of 100% mortality in the highest concentration. The 7-day chronic probit transformation concentration-mortality data are presented in Figure 2b. The chronic EC50 for TCMTB was 9.64 µg/L (8.38 to 10.93 µg/L). The chronic EC50s and 95% confidence intervals for 2-MBT, MTBT, and BT were determined using the Spearman-Karber method. The EC50 for 2-MBT was 1.25 mg/L (1.14 to 1.36 mg/L), for MTBT was 6.36 mg/L (5.80 to 6.92 mg/L), and for BT was 54.9 mg/L

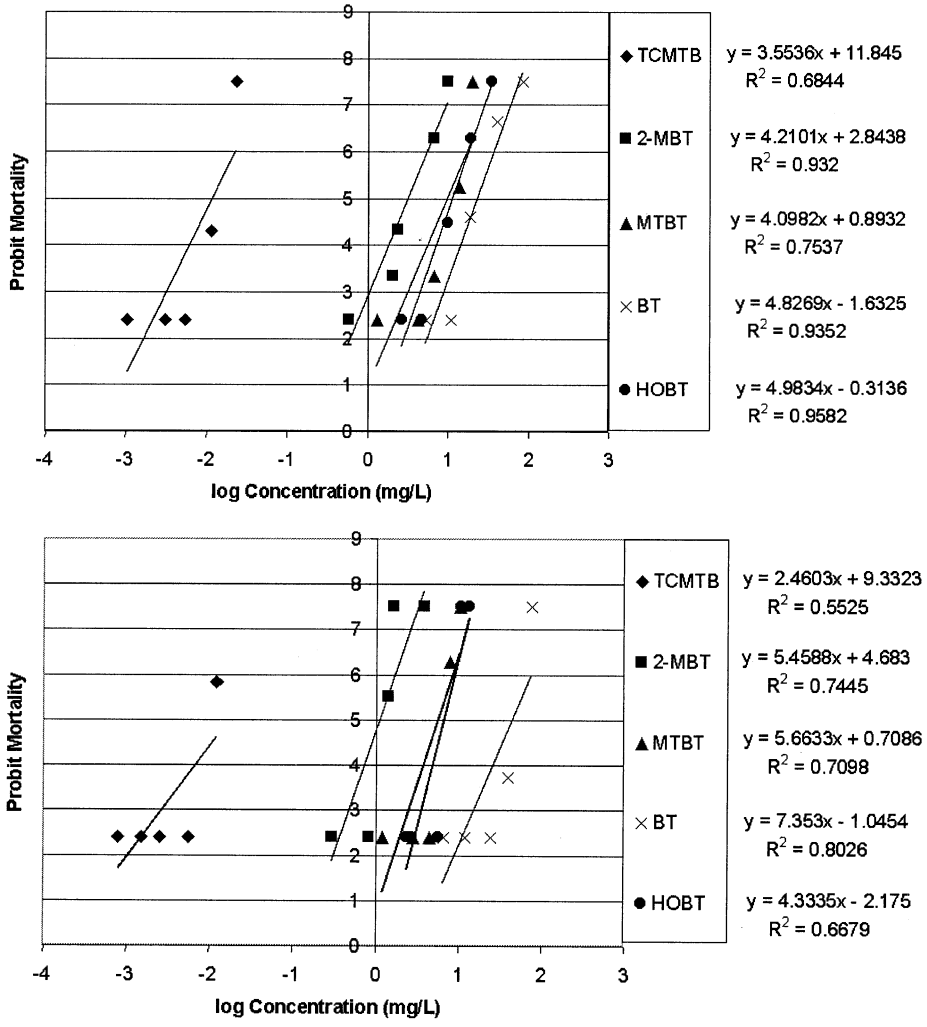

Fig. 2. Survival of *C. dubia* exposed to TCMTB and selected breakdown products. (**a**) Acute (48-hour) mortality for TCMTB and breakdown products with probit transformation. (**b**) Chronic (7-day) effects of TCMTB and selected breakdown products with probit transformation. TCMTB = 2-(thiocyanomethylthio)benzothiazole

(50.1 to 59.6 mg/L). The graphic method was employed to determine the chronic EC50 for HOBT because no partial mortalities existed. The chronic EC50 for HOBT was 8.31 mg/L. The 7-day chronic mean measured concentrations and mortality data are presented in Table 2.

No observed–effect concentrations (NOEC) were determined for all the benzothiazole compounds using Dunnett's Test. The NOEC for TCMTB was 2.5 µg/L, for 2-MBT was 0.84 mg/L, for MTBT was 1.21 mg/L, for BT was 11.9 mg/L, and for HOBT was 2.74 mg/L. The NOEC values are listed in Table 3. The average number of neonates and the significant differences from the control group as determined by the Dunnett's test are listed in Table 2.

Discussion

TCMTB was determined to be several orders of magnitude more toxic than its degradation products in both 48-hour and 7-day chronic toxicity tests (Table 3). The 48-hour EC50, 7-day EC50, and NOEC for TCMTB were all in the part per billion range, whereas the degradation products exhibited toxicity in the part per million range.

The 48-hour acute toxicity test yielded an EC50 of 15.3 µg/L for TCMTB (13.7 to 17.7 µg/L). This EC50 is similar to that reported in a previous study in which the 48-hour EC50 of TCMTB to *Daphnia magna* was 22 µg/L (18 to 25µg/L) (Wenell 1994). 2-MBT, the second most toxic compound tested in this study, was determined to have an acute EC50 of 4.19 mg/L (3.52 mg/L to 5.09 mg/L). This concentration is also similar to a *D. magna* study where the 48-hour EC50 for 2-MBT was reported to be 4.1 mg/L (Hanssen and Henderson 1991). One of the more stable degradation products, MTBT was found to be less toxic with an acute EC50 of 12.7 mg/L (11.1 to 14.3 mg/L). BT was the least toxic compound tested with an acute EC50 of 24.6 mg/L (20.89 to 29.73 mg/L). HOBT was expected to have a similar toxicity to BT based on the structural similarities; however, it was determined to be closer to MTBT with an acute EC50 of 15.1 mg/L (12.9 to 17.7 mg/L). Little literature is available on the aquatic toxicity of these compounds to provide data comparison.

In the 7-day chronic toxicity test, the benzothiazole compounds followed the same relative ranking of toxicity as observed from the acute toxicity tests. TCMTB was more toxic than its degradation products with a chronic EC50 of 9.64 µg/L (8.38 to 10.93 µg/L). 2-MBT was the most toxic of the

Table 3. Comparative toxicity of TCMTB, 2-MBT, MTBT, BT, and HOBT based on acute (48-hour EC50), chronic (7-day EC50), and NOEC determination

Compound	48-hr EC50 (mg/L)	7-day EC50 (mg/L)	NOEC (mg/L)
TCMTB	0.015 (0.014–0.017)[a]	0.0096 (0.0084–0.011)	0.0025
2-MBT	4.19 (3.52–5.09)	1.25 (1.14–1.36)	0.84
MTBT	12.7 (11.1–14.3)	6.36 (5.80–6.92)	1.21
BT	24.6 (20.9–29.7)	54.9 (50.1–59.6)	11.9
HOBT	15.1 (12.9–17.7)	8.31	2.74

[a] Values in parentheses are 95% confidence limits.
BT = Benzothiazole.
HOBT = 2-Hydroxybenzothiazole.
MTBT = 2-(Methylthio) benzothiazole.
2-MBT = 2-Mercaptobenzothiazole.
NOEC = No observed – effect concentration.
TCMBT = 2-(Thiocyanomethylthio)benzothiazole.

degradates at 1.25 mg/L (1.14 to 1.36 mg/L). MTBT and HOBT also resembled the acute toxicity pattern because they were found to be similar in chronic toxicity with 7-day EC50 values of 6.36 mg/L (5.80 to 6.92 mg/l) and 8.31 mg/L, respectively. Although BT was the least toxic compound as expected from the acute toxicity test (EC50 of 24.6 mg/L [20.89 to 29.73 mg/L]), the chronic EC50 of 54.9 mg/L (50.1 to 59.6 mg/L) was approximately two times greater than the acute value. Because of the length of the chronic toxicity test, *C. dubia* were fed to maintain survival. It is possible that BT may have bound to the added food, thus decreasing the bioavailability of the toxicant to the organism. Brownlee *et al*. (1992) reported that BT was able to partition onto suspended particles. Therefore, this could explain the lower toxicity observed in the chronic test; however, further studies would need to be conducted to examine this hypothesis.

The NOEC values followed a similar pattern of relative toxicity with the acute and chronic EC50 values. The NOEC for TCMTB was determined to be 2.5 μg/L, which was relatively close to the 8.7 μg/L NOEC previously reported for *D. magna* (Wenell 1994). 2-MBT represented the second lowest NOEC at 0.84 mg/L, which was close to the MTBT concentration of 1.21 mg/L. Although BT had an extremely high 7-day EC50 of 54.9 mg/L, the NOEC decreased considerably to 11.9 mg/L. As mentioned previously, the food in the BT test may have altered the mortality rate to produce the high EC50 of 54.9 mg/L, but it appears that reproduction is still affected, thus leading to the lower NOEC of 11.9 mg/L. The NOEC for HOBT was 2.74 mg/L, which was similar to the MTBT value. NOEC values for 2-MBT, MTBT, BT, and HOBT for daphnia have not been determined in previous studies.

It was surprising that very little or no degradation occurred during the 48-hour toxicity tests according to the analytic data. Although the intensity of light in the laboratory is not as intense as direct sunlight, it was expected that some degradation would occur through hydrolysis. No significant degradation appeared to occur during any of the tests.

Data on the biodegradation of TCMTB and other benzothiazoles are scarce at environmentally relevant parts per billion concentrations. Several studies have measured the concentrations of certain benzothiazole compounds in aquatic environments. A study in which TCMTB was incubated with suspended sediment from Canagagigue Creek (Ontario, Canada) determined that TCMTB was undetectable by 12 hours and that MTBT was the stable degradation product (Brownlee *et al.* 1992). In estuarine and marine waters, measured concentrations of MTBT were detected from 0.04 to 1.37 ng/L. BT was also detected in a range from 0.25 to 2.7 ng/L (Crathorne *et al.* 1984). In another study, BT and HOBT were detected in urban runoff in the parts per trillion to low parts per billion range (Coleman *et al.* 1980). Both of these studies show that the benzothiazoles may be present in the environment albeit at concentrations that are at least 1 order of magnitude below toxic levels as determined in this study.

Several laboratory studies have examined the toxicity of TCMTB to aquatic organisms and are reviewed in Wenell *et al*. (1994). These previous studies coincide with our conclusion, i.e., that the degradation products of TCMTB are much less toxic than the parent compound. The results of this study demonstrate that TCMTB is more toxic by several orders of magnitude than its degradation products in both acute and chronic exposures. The initial degradation product, 2-MBT, was the second most toxic compound according to this study. MTBT and HOBT were the next most toxic compounds possessing similar EC50 and NOEC values. BT was determined to be the least toxic compound in all of the toxicity tests. However, all of the compounds tested appeared to possess steep dose–response curves. The breakdown products are potentially more environmentally stable and may reach higher concentrations in the environment. One of the degradation products, BT, appears to decrease in toxicity when exposed to the presence of food in these toxicity evaluations. This suggests that BT may have the ability to bind to algae or organic particles, which may limit bioavailability. Because previous studies have demonstrated that TCMTB may break down rapidly in the environment, this research adds insight to the aquatic toxicity of these breakdown products, which may be useful for regulatory purposes. Future experiments must be conducted to examine the concentrations and potential toxicity of these compounds in selected industrial effluents.

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References

Aguera A, Piedra L, Hernando MD, Fernandez-Alba AR (2000) Multiresidue method for the analysis of five antifouling agents in marine and coastal waters by gas chromatography-mass spectrometry with large-volume injection. J Chromatogr A 889:261–269

Bester K, Huhnerfuss H, Lange W, Theobald N (1997) Results of non-target screening of lipophilic organic pollutants in the German Gight I: Benzothiazoles. Sci Total Environ 207:111–118

Brownlee BG, Carey JH, MacInnis GA, Pellizzari IT (1992) Aquatic environmental chemistry of 2-(thiocyanomethylthio)benzothiazole and related benzothiazoles. Environ Toxicol Chem 11:1153–1168

Coleman WE, Melton RG, Kopfler FC, Barone KA, Aurand TA, Jellison MG (1980) Identification of organic compounds in a mutagenic extract of a surface drinking water by a computerized gas chromatography mass spectrometry system (GC/MS/COM). Environ Sci Technol 14:576–588

Crathorne B, Fielding M, Steel CP, Watts CD (1984) Organic compounds in water: Analysis using coupled-column high-performance liquid chromatography and soft-ionization mass spectrometry. Environ Sci Technol 18:797–802

Daniels CR, Swan EP (1987) HPLC assay of the anti-stain chemical TCMTB applied to lumber surfaces. J Chromatogr Sci 25:43–45

De Wever H, Besse P, Verachtert H (2001) Microbial transformations of 2-substituted benzothiazoles. Appl Microbiol Biotechnol 57:620–625

De Wever HD, Verachtert H (1997) Biodegradation and toxicity of benzothiazoles. Water Res 31:2673–2684

Fathulla RN, Lawrie C, Drake K, Watson C (1995) Environmental fate of TCMTB: 1 Aquatic breakdown. Corning Hazelton, Madison, WI

Grothe DR, Dickson KL, Reed-Judkins DK (1996) Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts SETAC Press, Danvers, MA

Hanssen HW, Henderson ND (1991) A review of the environmental impact and toxic effects of 2-MBT Environmental Protection Division, Victoria, BC

Hanssen HW, Henderson ND, Ward JEH (1991) A review of the environmental impact and toxic effects of TCMTB Environmental Protection Division, Victoria, BC

Hemming HM, Turner PK, Brooks BW, Waller WT, La Point TW (2002) Assessment of toxicity reduction in wastewater effluent flowing through a treatment wetland using *Pimephales*, *Ceriodaphnia dubia*, and *Vibrio fischeri*. Arch Environ Contam Toxicol 42:9–16

Kruzynski GM, Birtwell IK (1994) A predation bioassay to quantify the ecological significance of sublethal responses of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) to the antisapstain fungicide TCMTB. Can J Fish Aquat Sci 51:1780–1790

MacKinnon DL, Farrell AP (1992) The effect of 2-(thiocyanomethylthio)benzothiazole on juvenile coho salmon (*Oncorhynchus Kisutch*): Sublethal toxicity testing. Environ Toxicol Chem 11:1541–1548

Meding B, Toren K, Karlberg A-T, Hagberg S, Wass K (1993) Evaluation of skin symptoms among workers at a Swedish paper mill. Am J Ind Med 23:721–728

Middaugh DP, Beckham N, Fournie JW, Deardorff TL (1997) Evaluation of bleached kraft mill process water using Microtox®, *Ceriodaphnia dubia*, and *Menidia beryllina* toxicity tests. Arch Environ Contam Toxicol 32:367–375

Reddy CM, Quinn JG (1997) Environmental chemistry of benzothiazoles derived from rubber. Environ Sci Technol 31:2847–2853

Reemtsma T, Fiehn O, Kalnowski G, Jekel M (1995) Microbial transformations and biological effects of fungicide-derived benzothiazoles determined in industrial wastewater. Environ Sci Technol 29:478–485

Spies RB, Andresen BD, Rice Jr DW (1987) Benzothiazoles in estuarine sediments as indicators of street runoff. Nature 327:697–699

U. S. Environmental Protection Agency(1993) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed (EPA/600/4-90/027F). Office of Research and Development U.S. Environmental Protection Agency, Cincinnati, OH

U. S. Environmental Protection Agency(1994) Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms, 3rd edition (EPA/600/4-91/002) Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH

Ward JEH (1989) The environmental impacts and behavior of TCMTB Waste Management Branch, Victoria, BC

Wenell T (1994) Ecotoxicological evaluation of 2-(thiocyanomethylthio)benzothiazole (TCMTB) KEMI National Chemical Inspectorate, Solna, Sweden